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

(19) World Intellectual Property Organization
International Bureau

(43) International Publication Date
6 August 2015 (06.08.2015)

WIPO | PCT

(10) International Publication Number
WO 2015/116868 A2

(51) International Patent Classification:
C12Q 1/68 (2006.01) *G01N 33/574* (2006.01)

(21) International Application Number:
PCT/US2015/013618

(22) International Filing Date:
29 January 2015 (29.01.2015)

(25) Filing Language: English

(26) Publication Language: English

(30) Priority Data:

61/933,268	29 January 2014 (29.01.2014)	US
61/935,825	4 February 2014 (04.02.2014)	US
61/971,506	27 March 2014 (27.03.2014)	US
61/989,419	6 May 2014 (06.05.2014)	US
61/991,346	9 May 2014 (09.05.2014)	US
62/002,118	22 May 2014 (22.05.2014)	US
62/032,455	1 August 2014 (01.08.2014)	US

(71) Applicant: **CARIS MPI, INC.** [US/US]; 6655 N. Macarthur Blvd., Irving, TX 75039 (US).

(72) Inventors: **GATALICA, Zoran**; 9243 N 81st Street, Scottsdale, AZ 85258 (US). **BASU, Gargi**; 21112 N 72nd Place, Scottsdale, AZ 85255 (US). **FELDMAN, Rebecca**; 3892 E Old Stone Circle, Chandler, AZ 85249 (US). **MILLIS, Sherri**; 1215 W. Rose Lane, Phoenix, AZ 85013 (US).

(74) Agent: **AKHAVAN, Ramin**; Caris MPI, INC., 6655 N. MacArthur Blvd., Irving, TX 75039 (US).

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

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

Published:

— *without international search report and to be republished upon receipt of that report (Rule 48.2(g))*

WO 2015/116868 A2

(54) Title: MOLECULAR PROFILING OF IMMUNE MODULATORS

(57) Abstract: Provided herein are methods and systems of molecular profiling of diseases, such as cancer. In some embodiments, the molecular profiling can be used to identify treatments for the disease, such as treatments that provide likely benefit or likely lack of benefit for the disease. The molecular profiling can include analysis of immune modulators such as PD-1 and/or its ligand PD-L1.

MOLECULAR PROFILING OF IMMUNE MODULATORS

CROSS-REFERENCE

[0001] This application claims the benefit of priority to United States Provisional Patent Application Serial Nos. 61/933,268, filed on January 29, 2014, 61/935,825, filed on February 4, 2014, 61/971,506, filed on March 27, 2014, 61/989,419, filed on May 6, 2014, 61/991,346, filed on May 9, 2014, 62/002,118, filed on May 22, 2014 and 62/032,455, filed on August 1, 2014; all of which applications are incorporated by reference herein in their entirety.

BACKGROUND

[0002] Disease states in patients are typically treated with treatment regimens or therapies that are selected based on clinical based criteria; that is, a treatment therapy or regimen is selected for a patient based on the determination that the patient has been diagnosed with a particular disease (which diagnosis has been made from classical diagnostic assays). Although the molecular mechanisms behind various disease states have been the subject of studies for years, the specific application of a diseased individual's molecular profile in determining treatment regimens and therapies for that individual has been disease specific and not widely pursued.

[0003] Some treatment regimens have been determined using molecular profiling in combination with clinical characterization of a patient such as observations made by a physician (such as a code from the International Classification of Diseases, for example, and the dates such codes were determined), laboratory test results, x-rays, biopsy results, statements made by the patient, and any other medical information typically relied upon by a physician to make a diagnosis in a specific disease. However, using a combination of selection material based on molecular profiling and clinical characterizations (such as the diagnosis of a particular type of cancer) to determine a treatment regimen or therapy presents a risk that an effective treatment regimen may be overlooked for a particular individual since some treatment regimens may work well for different disease states even though they are associated with treating a particular type of disease state.

[0004] Patients with refractory or metastatic cancer are of particular concern for treating physicians. The majority of patients with metastatic or refractory cancer eventually run out of treatment options or may suffer a cancer type with no real treatment options. For example, some patients have very limited options after their tumor has progressed in spite of front line, second line and sometimes third line and beyond) therapies. For these patients, molecular profiling of their cancer may provide the only viable option for prolonging life.

[0005] More particularly, additional targets or specific therapeutic agents can be identified assessment of a comprehensive number of targets or molecular findings examining molecular mechanisms, genes, gene expressed proteins, and/or combinations of such in a patient's tumor. Identifying multiple agents that can treat multiple targets or underlying mechanisms would provide cancer patients with a viable therapeutic alternative on a personalized basis so as to avoid standar therapies, which may simply not work or identify therapies that would not otherwise be considered by the treating physician.

[0006] There remains a need for better theranostic assessment of cancer vicitims, including molecular profiling analysis that identifies at least one individual profile to provide more informed and effective personalized treatment options, resulting in improved patient care and enhanced treatment outcomes. The present invention provides methods and systems for identifying treatments for these individuals by molecular profiling a sample from the individual. The molecular profiling can include analysis of immune modulators such as PD-1 and/or its ligand PD-L1.

SUMMARY OF THE INVENTION

[0007] The present invention provides methods and system for molecular profiling, using the results from molecular profiling to identify treatments for individuals. In some embodiments, the treatments were not identified initially as a treatment for the disease or disease lineage. The molecular profiling can include analysis of an immune checkpoint related gene or gene product. The immune checkpoint related gene or gene product can be PD-1 or a PD-1 ligand such as PD-L1 or PD-L2.

[0008] In an aspect, the invention provides a method of identifying at least one treatment associated with a cancer in a subject, comprising: a) determining a molecular profile for at least one sample from the subject by assessing a plurality of gene or gene products, wherein the plurality of gene or gene products comprises at least one of PD-1 and PD-L1; and b) identifying, based on the molecular profile, at least one of: i) at least one treatment that is associated with benefit for treatment of the cancer; ii) at least one treatment that is associated with lack of benefit for treatment of the cancer; and iii) at least one treatment associated with a clinical trial.

[0009] The plurality of gene or gene product may further comprise other immune modulating biomarkers as desired. For example, such immune modulating biomarkers can be selected from the group consisting of CTL4A, IDO1, COX2, CD80, CD86, CD8A, Granzyme A, Granzyme B, CD19, CCR7, CD276, LAG-3, TIM-3, and a combination thereof.

[0010] In an embodiment, the plurality of gene or gene products further comprises at least one gene or gene product selected from any of **Tables 2**, **6**, **7** or **10-17**. For example, the plurality of gene or gene products further comprises at least one, e.g., at least 1, 2, 3, 4, 5, 6, 7, 8, 9, 10, 11, 12, 13, 14, 15, 16, 17, 18, 19, 20, 21, 22, 23, 24, 25, 26, 27, 28, 29, 30, 31, 32, 33, 34, 35, 36, 37, 38, 39, 40, 41, 42, 43, 44, 45, 46, 47, 48, 49, 50, 51, 52 or 53, of 1p19q, ABL1, AKT1, ALK, APC, AR, ATM, BRAF, BRCA1, BRCA2, cKIT, cMET, CSF1R, CTNNB1, EGFR, EGFRvIII, ER, ERBB2 (HER2), FGFR1, FGFR2, FLT3, GNA11, GNAQ, GNAS, HER2, HRAS, IDH1, IDH2, JAK2, KDR (VEGFR2), KRAS, MGMT, MGMT-Me, MLH1, MPL, NOTCH1, NRAS, PDGFRA, Pgp, PIK3CA, PR, PTEN, RET, RRM1, SMO, SPARC, TLE3, TOP2A, TOPO1, TP53, TS, TUBB3 and VHL.

[0011] A variety of beneficial molecular characteristics and molecular technologies can be used to assess the plurality of gene or gene products to determine the molecular profile. For example, gene amplification assess for at least one, e.g., 1 or 2, of HER2 and cMET, and gene deletion can be assessed for at least 1p19q. ISH or other techniques to assess nucleic acid can be used for such analysis. Similarly, protein levels may be assessed for at least one, e.g., at least 1, 2, 3, 4, 5, 6, 7, 8, 9, 10, 11, 12, 13, 14, 15, 16, 17 or 18, of AR, cMET, EGFR, ER, HER2, MGMT, PD-1, PD-L1, Pgp, PR, PTEN, RRM1, SPARC, TLE3,

TOP2A, TOPO1, TS and TUBB3. IHC or other protein expression techniques can be used for such analysis. In some embodiments, SPARC is assessed with IHC performed with both monoclonal ("m") or polyclonal ("p") primary antibodies. Sequence analysis can be used to assess at least one, e.g., at least 1, 2, 3, 4, 5, 6, 7, 8, 9, 10, 11, 12, 13, 14, 15, 16, 17, 18, 19, 20, 21, 22, 23, 24, 25, 26, 27, 28, 29, 30, 31, 32, 33, 34, 35 or 36, of ABL1, AKT1, ALK, APC, ATM, BRAF, BRCA1, BRCA2, cKIT, cMET, CSF1R, CTNNB1, EGFR, ERBB2, FGFR1, FGFR2, FLT3, GNA11, GNAQ, GNAS, HRAS, IDH1, JAK2, KDR (VEGFR2), KRAS, MLH1, MPL, NOTCH1, NRAS, PDGFRA, PIK3CA, PTEN, RET, SMO, TP53 and VHL. In some embodiments, the sequence analysis further comprises that of at least one, e.g., at least 1, 2, 3, 4, 5, 6, 7, 8, 9, 10 or 11, of CDH1, ERBB4, FBXW7, HNF1A, JAK3, NPM1, PTPN11, RB1, SMAD4, SMARCB1 and STK11. Such sequence analysis may detect mutations, including without limitation point mutations, SNPs, insertions and deletions. Next generation sequencing (NGS), Sanger sequencing, PCR, RT-PCR, pyrosequencing or other sequencing methodology may be used for such analysis. In preferred embodiments, NGS is used to sequence most if not all genes in one assay.

[0012] Any useful combination of biomarkers and techniques can be used to determine the molecular profile. For example, assessing the plurality of gene or gene products can comprise using ISH to assess at least one, e.g., at least 1, 2, or 3, of HER2, 1p19q and cMET; using IHC to assess at least one, e.g., at least 1, 2, 3, 4, 5, 6, 7, 8, 9, 10, 11, 12, 13, 14, 15, 16, 17 or 18, of AR, cMET, EGFR, ER, HER2, MGMT, PD-1, PD-L1, Pgp, PR, PTEN, RRM1, SPARC, TLE3, TOP2A, TOPO1, TS and TUBB3; using sequence analysis to assess at least one, e.g., at least 1, 2, 3, 4, 5, 6, 7, 8, 9, 10, 11, 12, 13, 14, 15, 16, 17, 18, 19, 20, 21, 22, 23, 24, 25, 26, 27, 28, 29, 30, 31, 32, 33, 34, 35 or 36, of ABL1, AKT1, ALK, APC, ATM, BRAF, BRCA1, BRCA2, cKIT, cMET, CSF1R, CTNNB1, EGFR, ERBB2, FGFR1, FGFR2, FLT3, GNA11, GNAQ, GNAS, HRAS, IDH1, JAK2, KDR (VEGFR2), KRAS, MLH1, MPL, NOTCH1, NRAS, PDGFRA, PIK3CA, PTEN, RET, SMO, TP53 and VHL; and/or using sequence analysis to assess at least one, e.g., at least 1, 2, 3, 4, 5, 6, 7, 8, 9, 10 or 11, of CDH1, ERBB4, FBXW7, HNF1A, JAK3, NPM1, PTPN11, RB1, SMAD4, SMARCB1 and STK11.

[0013] The plurality of gene or gene products can comprise additional useful biomarkers, in some cases depending on the cancer lineage. In some embodiments, the plurality of gene or gene products further comprises at least one of MLH1, MSH2, MSH6, PMS2, microsatellite instability (MSI), ROS1 and ERCC1. Protein expression of MLH1, MSH2, MSH4, PMS2 can be assessed, e.g., by IHC. These markers may be examined for a colorectal cancer. Microsatellite instability (MSI) can be assessed using nucleic acid analysis methods, including without limitation fragment analysis. MSI may also be examined for a colorectal cancer. In some embodiments, at least one of ROS1 and ERCC1 are assessed for gene amplification or rearrangement. Such analysis can be performed by ISH.

[0014] In an embodiment, the plurality of gene or gene products is according to any of **Tables 7** or **10-16** herein.

[0015] Any number of useful biomarker-drug associations can be used by the methods of the invention. In some embodiments, the step of correlating the molecular profile with treatments comprises associating beneficial treatment of the cancer with immune modulating therapy targeting at least one of PD-1, PD-L1,

PD-L2, CTL4A, IDO1, COX2, CD80, CD86, CD8A, Granzyme A, Granzyme B, CD19, CCR7, CD276, LAG-3 or TIM-3, wherein the cancer is apoptotic or necrotic. The step of identifying based on the molecular profile can comprise correlating the molecular profile with treatments whose benefit has been assessed for cancers characterized by presence or level, overexpression, underexpression, copy number, mutation, deletion, insertion, translocation, amplification, rearrangement, or other molecular alteration in at least one member of the plurality of gene or gene products. In some embodiments, the step of correlating the molecular profile with treatments is according to at least one biomarker-drug association in any of **Tables 3-7**, **Table 8**, **Tables 11-17**, **Table 19**, **Tables 24-26** and **FIGs. 28D-E**. The step of correlating the molecular profile with treatments can be according to at least one biomarker-drug association rule, e.g., 1, 2, 3, 4, 5, 6, 7, 8, 9, 10, 11, 12, 13, 14, 15, 16, 17, 18, 19, 20, 21,22, 23, 24, 25, 26 or 27 associations, selected from: performing protein analysis on PD1 to determine likely benefit or lack of benefit from an PD-1 modulating therapy, PD-1 inhibitor, anti-PD-1 immunotherapy, anti-PD-1 monoclonal antibody, nivolumab, pidilizumab (CT-011, CureTech, LTD), pembrolizumab (lambrolizumab, MK-3475, Merck), a PD-1 antagonist, a PD-1 ligand soluble construct, and/or AMP-224 (Amplimmune); performing protein analysis on PD-L1 to determine likely benefit or lack of benefit from a PD-L1 modulating therapy, PD-L1 inhibitor, anti-PD-L1 immunotherapy, anti-PD-L1 monoclonal antibody, BMS-936559, MPDL3280A/RG7446, and/or MEDI4736 (MedImmune); performing protein analysis on RRM1 to determine likely benefit or lack of benefit from an antimetabolite and/or gemcitabine; performing protein analysis on TS to determine likely benefit or lack of benefit from a antimetabolite, fluorouracil, capecitabine, and/or pemetrexed; performing protein analysis on TOPO1 to determine likely benefit or lack of benefit from a TOPO1 inhibitor, irinotecan and/or topotecan; performing at least one of protein analysis on MGMT, analysis of MGMT promoter methylation, and sequencing on IDH1 to determine likely benefit or lack of benefit from an alkylating agent, temozolomide, and/or dacarbazine; performing protein analysis on AR to determine likely benefit or lack of benefit from an anti-androgen, bicalutamide, flutamide, abiraterone and/or enzalutamide; performing protein analysis on ER to determine likely benefit or lack of benefit from a hormonal agent, tamoxifen, fulvestrant, letrozole, and/or anastrozole; performing protein analysis on at least one of ER, PR and AR to determine likely benefit or lack of benefit from a hormonal agent, tamoxifen, toremifene, fulvestrant, letrozole, anastrozole, exemestane, megestrol acetate, leuprolide, goserelin, bicalutamide, flutamide, abiraterone, enzalutamide, triptorelin, abarelix, and/or degarelix; performing at least one of protein analysis on HER2 and gene amplification analysis on HER2 to determine likely benefit or lack of benefit from a tyrosine kinase inhibitor and/or lapatinib, pertuzumab, and/or ado-trastuzumab emtansine (T-DM1); performing at least one of protein analysis on HER2, gene amplification analysis on HER2, protein analysis on PTEN and sequencing on PIK3CA to determine likely benefit or lack of benefit from HER2 targeted therapy, and/or trastuzumab; performing at least one of gene amplification analysis on TOP2A, gene amplification analysis on HER2, protein analysis on TOP2A and protein analysis on PGP to determine likely benefit or lack of benefit from an anthracycline, doxorubicin, liposomal-doxorubicin, and/or epirubicin; performing sequencing on at least one of cKIT and PDGFRA to determine likely

benefit or lack of benefit from a tyrosine kinase inhibitor and/or imatinib; performing at least one of gene rearrangement analysis on ALK and gene rearrangement analysis on ROS1 to determine likely benefit or lack of benefit from a tyrosine kinase inhibitor and/or crizotinib; performing at least one of protein analysis on ER or sequencing on PIK3CA to determine likely benefit or lack of benefit from an mTOR inhibitor, everolimus, and/or temsirolimus; performing sequencing on RET to determine likely benefit or lack of benefit from a tyrosine kinase inhibitor, and/or vandetanib; performing protein analysis on at least one of TLE3, TUBB3 and PGP to determine likely benefit or lack of benefit from a taxane, paclitaxel, and/or docetaxel; performing protein analysis on SPARC to determine likely benefit or lack of benefit from a taxane, and/or nab-paclitaxel; performing at least one of PCR and sequencing on BRAF to determine likely benefit or lack of benefit from a tyrosine kinase inhibitor, vemurafenib, dabrafenib, and/or trametinib; performing at least one of sequencing on KRAS, sequencing on BRAF, sequencing on NRAS, sequencing on PIK3CA and protein analysis on PTEN to determine likely benefit or lack of benefit from an EGFR-targeted antibody, cetuximab, and/or panitumumab; performing sequencing on EGFR to determine likely benefit or lack of benefit from an EGFR-targeted antibody, and/or cetuximab; performing at least one of sequencing on EGFR, sequencing on KRAS, gene amplification analysis on cMET, sequencing on PIK3CA and protein analysis on PTEN to determine likely benefit or lack of benefit from a tyrosine kinase inhibitor, erlotinib, and/or gefitinib; performing sequencing on EGFR to determine likely benefit or lack of benefit from a tyrosine kinase inhibitor, and/or afatinib; performing sequencing on cKIT to determine likely benefit or lack of benefit from a tyrosine kinase inhibitor, and/or sunitinib; performing sequencing on at least one of BRCA1, BRCA2 and/or protein analysis on ERCC1 to determine likely benefit or lack of benefit from carboplatin, cisplatin, and/or oxaliplatin; performing gene rearrangement analysis on ALK to determine likely benefit or lack of benefit from ceritinib; and detecting 1p19q codeletion to determine likely benefit or lack of benefit from procarbazine, lomustine, and/or vincristine (PCV). Protein level can be determined using any useful protein analysis method, e.g., IHC. The sequencing can be any useful sequencing technique, e.g., next generation sequencing or Sanger sequencing. DNA methylation can be determined using any useful methylation analysis technique, e.g., pyrosequencing. Gene amplification can be determined using any useful copy number analysis method, e.g., ISH, FISH and/or CISH. Translocation can be determined using any useful analysis method, e.g., ISH, FISH and/or CISH. Gene rearrangement or deletion (e.g., 1p19q) can be determined using any useful analysis method, e.g., ISH, FISH and/or CISH. The relationship between biomarker status and likely benefit or lack of benefit can be determined as described herein (e.g., **Tables 6**, **8**, **17**). In an embodiment, the step of correlating the molecular profile with treatments comprises associating beneficial treatment of the cancer with immunotherapy targeting at least one of PD-1, PD-L1, CTLA-4, IDO-1, and CD276, wherein determining the molecular profile indicates that the cancer is AR-/HER2-/ER-/PR- (quadruple negative) and/or carries a mutation in BRCA1. In another embodiment, the step of correlating the molecular profile with treatments comprises associating beneficial treatment of the cancer with immunotherapy targeting PD-1 wherein determining the molecular profile indicates that the cancer carries a mutation in at least one cancer-related gene. The cancer-related gene can include at least one, e.g., at

least 1, 2, 3, 4, 5, 6, 7, 8, 9, 10, 11, 12, 13, 14, 15, 16, 17, 18, 19, 20, 21, 22, 23, 24, 25, 26, 27, 28, 29, 30, 31, 32, 33, 34, 35, 36, 37, 38, 39, 40, 41, 42, 43, 44, 45, 46 or 47, of ABL1, AKT1, ALK, APC, ATM, BRAF, BRCA1, BRCA2, cKIT, cMET, CSF1R, CTNNB1, EGFR, ERBB2, FGFR1, FGFR2, FLT3, GNA11, GNAQ, GNAS, HRAS, IDH1, JAK2, KDR (VEGFR2), KRAS, MLH1, MPL, NOTCH1, NRAS, PDGFRA, PIK3CA, PTEN, RET, SMO, TP53, VHL, CDH1, ERBB4, FBXW7, HNF1A, JAK3, NPM1, PTPN11, RB1, SMAD4, SMARCB1 and STK11. Other cancer related genes, such as those disclosed herein or in the COSMIC (Catalogue Of Somatic Mutations In Cancer) database (available at cancer.sanger.ac.uk/cancergenome/projects/cosmic/), can be assessed as well.

[0016] In some embodiments of the invention, the step of correlating the molecular profile with treatments comprises associating beneficial treatment of the cancer with immune modulating therapy targeting at least one of PD-1, PD-L1, PD-L2, CTL4A, IDO1, COX2, CD80, CD86, CD8A, Granzyme A, Granzyme B, CD19, CCR7, CD276, LAG-3 or TIM-3, wherein determining the molecular profile indicates that the cancer microenvironment expresses PD-L1. The expression of PD-L1 in the cancer microenvironment may be determined in at least one of tumor cells, T cells, natural killer (NK) cells, macrophages, dendritic cells (DCs), B cells, epithelial cells, and vascular endothelial cells. The benefit or lack of benefit of the immune modulating therapy can depend on which cell types express the PD-L1.

[0017] The methods of the invention can further comprise identifying at least one candidate clinical trial for the subject based on the molecular profiling. For example, the criteria for entry into the trial may include the status of at least one biomarker included within the molecular profile.

[0018] In some embodiments of the methods of the invention, the step of identifying based on the molecular profile comprises correlating the molecular profile with treatments whose benefit has been assessed for cancers characterized by presence or level, overexpression, underexpression, copy number, mutation, deletion, insertion, translocation, amplification, rearrangement, or other molecular alteration in PD-1 and/or PD-L1. The at least one treatment can be a therapy for PD-1 and/or PD-L1, such as an immune modulating therapy. In some embodiments, the inhibitor of PD-1 is selected from the group consisting of a PD-1 inhibitor, anti-PD-1 immunotherapy, anti-PD-1 monoclonal antibody, nivolumab, lambrolizumab, pidilizumab (CT-011, CureTech, LTD), pembrolizumab (MK-3475, Merck), a PD-1 antagonist, a PD-1 ligand soluble construct, AMP-224 (Amplimmune), and a combination thereof. The inhibitor of PD-L1 can be selected from the group consisting of a PD-L1 inhibitor, anti-PD-L1 immunotherapy, anti-PD-L1 monoclonal antibody, BMS-936559, MPDL3280A/RG7446, MEDI4736 (MedImmune), and a combination thereof. The at least one treatment may comprise multiple therapies for PD-1 and/or PD-L1, including without limitation combinations of treatments having different delivery or treatment modalities for the same biomarker, combinations of treatments for both PD-1 and/or PD-L1, or combinations of therapies for PD-1 and/or PD-L1 with treatments for other biomarkers such as any of those disclosed herein (e.g., in any of **Tables 3-7**, **Table 8**, **Tables 11-17**, **Table 19**, **Tables 24-26** and **FIGs. 28D-E**).

[0019] In certain embodiments, the inhibitor of PD-1 and/or PD-L1 is associated with benefit for treatment of the cancer if the sample expresses both PD-1 and another useful PD-1 ligand such as PD-L1.

[0020] The presence or level of PD-1 can be determined in tumor infiltrating lymphocytes (TILs). The presence or level of PD-L1 can be determined in various cells of the tumor microenvironment, including without limitation at least one of tumor cells, T cells, natural killer (NK) cells, macrophages, dendritic cells (DCs), B cells, epithelial cells, and vascular endothelial cells.

[0021] In various embodiments of the methods of the invention, the at least one sample comprises formalin-fixed paraffin-embedded (FFPE) tissue, fixed tissue, core needle biopsy, fine needle aspirate, unstained slides, fresh frozen (FF) tissue, formalin samples, tissue comprised in a solution that preserves nucleic acid or protein molecules, and/or a bodily fluid sample. In embodiments, the sample comprises tumor tissue or cells from a tumor. The bodily fluid may comprise a malignant fluid, a pleural fluid or peritoneal fluid. The bodily fluid can include without limitation peripheral blood, sera, plasma, ascites, urine, cerebrospinal fluid (CSF), sputum, saliva, bone marrow, synovial fluid, aqueous humor, amniotic fluid, cerumen, breast milk, broncheoalveolar lavage fluid, semen, prostatic fluid, cowper's fluid, pre-ejaculatory fluid, female ejaculate, sweat, fecal matter, tears, cyst fluid, pleural fluid, peritoneal fluid, pericardial fluid, lymph, chyme, chyle, bile, interstitial fluid, menses, pus, sebum, vomit, vaginal secretions, mucosal secretion, stool water, pancreatic juice, lavage fluids from sinus cavities, bronchopulmonary aspirates, blastocyst cavity fluid, or umbilical cord blood. The bodily fluid may comprise circulating tumor cells (CTCs). In some embodiments, the at least one sample comprises a microvesicle population. At least one member of the plurality of gene or gene products may be associated with the microvesicle population.

[0022] In some embodiments of the methods herein, the subject has not previously been treated for the cancer. In addition, the subject may not have previously been treated with the at least one treatment that is associated with benefit for treatment of the cancer.

[0023] The cancer can be a metastatic and/or recurrent cancer. The cancer may be refractory to a prior treatment. Such prior treatment may be the standard of care for the cancer. In certain embodiment, the cancer is refractory to all known standard of care treatments.

[0024] Based on the results of the methods of the invention, the caregiver, e.g., a treating physician such as an oncologist, may determine a treatment regimen to the subject. In preferred embodiments, progression free survival (PFS), disease free survival (DFS), or lifespan is extended by administration of the at least one treatment that is associated with benefit for treatment of the cancer to the subject.

[0025] The methods of the invention can be used to guide treatment for any appropriate cancer. In various embodiments, the cancer comprises an acute lymphoblastic leukemia; acute myeloid leukemia; adrenocortical carcinoma; AIDS-related cancer; AIDS-related lymphoma; anal cancer; appendix cancer; astrocytomas; atypical teratoid/rhabdoid tumor; basal cell carcinoma; bladder cancer; brain stem glioma; brain tumor, brain stem glioma, central nervous system atypical teratoid/rhabdoid tumor, central nervous system embryonal tumors, astrocytomas, craniopharyngioma, ependymoblastoma, ependymoma, medulloblastoma, medulloepithelioma, pineal parenchymal tumors of intermediate differentiation, supratentorial primitive neuroectodermal tumors and pineoblastoma; breast cancer; bronchial tumors; Burkitt lymphoma; cancer of unknown primary site (CUP); carcinoid tumor; carcinoma of unknown

primary site; central nervous system atypical teratoid/rhabdoid tumor; central nervous system embryonal tumors; cervical cancer; childhood cancers; chordoma; chronic lymphocytic leukemia; chronic myelogenous leukemia; chronic myeloproliferative disorders; colon cancer; colorectal cancer; craniopharyngioma; cutaneous T-cell lymphoma; endocrine pancreas islet cell tumors; endometrial cancer; ependymoblastoma; ependymoma; esophageal cancer; esthesioneuroblastoma; Ewing sarcoma; extracranial germ cell tumor; extragonadal germ cell tumor; extrahepatic bile duct cancer; gallbladder cancer; gastric (stomach) cancer; gastrointestinal carcinoid tumor; gastrointestinal stromal cell tumor; gastrointestinal stromal tumor (GIST); gestational trophoblastic tumor; glioma; hairy cell leukemia; head and neck cancer; heart cancer; Hodgkin lymphoma; hypopharyngeal cancer; intraocular melanoma; islet cell tumors; Kaposi sarcoma; kidney cancer; Langerhans cell histiocytosis; laryngeal cancer; lip cancer; liver cancer; malignant fibrous histiocytoma bone cancer; medulloblastoma; medulloepithelioma; melanoma; Merkel cell carcinoma; Merkel cell skin carcinoma; mesothelioma; metastatic squamous neck cancer with occult primary; mouth cancer; multiple endocrine neoplasia syndromes; multiple myeloma; multiple myeloma/plasma cell neoplasm; mycosis fungoides; myelodysplastic syndromes; myeloproliferative neoplasms; nasal cavity cancer; nasopharyngeal cancer; neuroblastoma; Non-Hodgkin lymphoma; nonmelanoma skin cancer; non-small cell lung cancer; oral cancer; oral cavity cancer; oropharyngeal cancer; osteosarcoma; other brain and spinal cord tumors; ovarian cancer; ovarian epithelial cancer; ovarian germ cell tumor; ovarian low malignant potential tumor; pancreatic cancer; papillomatosis; paranasal sinus cancer; parathyroid cancer; pelvic cancer; penile cancer; pharyngeal cancer; pineal parenchymal tumors of intermediate differentiation; pineoblastoma; pituitary tumor; plasma cell neoplasm/multiple myeloma; pleuropulmonary blastoma; primary central nervous system (CNS) lymphoma; primary hepatocellular liver cancer; prostate cancer; rectal cancer; renal cancer; renal cell (kidney) cancer; renal cell cancer; respiratory tract cancer; retinoblastoma; rhabdomyosarcoma; salivary gland cancer; Sézary syndrome; small cell lung cancer; small intestine cancer; soft tissue sarcoma; squamous cell carcinoma; squamous neck cancer; stomach (gastric) cancer; supratentorial primitive neuroectodermal tumors; T-cell lymphoma; testicular cancer; throat cancer; thymic carcinoma; thymoma; thyroid cancer; transitional cell cancer; transitional cell cancer of the renal pelvis and ureter; trophoblastic tumor; ureter cancer; urethral cancer; uterine cancer; uterine sarcoma; vaginal cancer; vulvar cancer; Waldenström macroglobulinemia; or Wilm's tumor. The cancer can include without limitation an acute myeloid leukemia (AML), breast carcinoma, cholangiocarcinoma, colorectal adenocarcinoma, extrahepatic bile duct adenocarcinoma, female genital tract malignancy, gastric adenocarcinoma, gastroesophageal adenocarcinoma, gastrointestinal stromal tumor (GIST), glioblastoma, head and neck squamous carcinoma, leukemia, liver hepatocellular carcinoma, low grade glioma, lung bronchioloalveolar carcinoma (BAC), non-small cell lung cancer (NSCLC), lung small cell cancer (SCLC), lymphoma, male genital tract malignancy, malignant solitary fibrous tumor of the pleura (MSFT), melanoma, multiple myeloma, neuroendocrine tumor, nodal diffuse large B-cell lymphoma, non epithelial ovarian cancer (non-EOC), ovarian surface epithelial carcinoma, pancreatic adenocarcinoma, pituitary carcinomas, oligodendroglioma, prostatic adenocarcinoma, retroperitoneal or peritoneal

carcinoma, retroperitoneal or peritoneal sarcoma, small intestinal malignancy, soft tissue tumor, thymic carcinoma, thyroid carcinoma, or uveal melanoma.

[0026] In some embodiments, the cancer comprises a breast cancer, triple negative breast cancer, metaplastic breast cancer (MpBC), head and neck squamous cell carcinoma (HNSCC), human papilloma virus (HPV)-positive HNSCC, HPV–negative/TP53-mutated HNSCC, metastatic HNSCC, oropharyngeal HNSCC, non-oropharyngeal HNSCC, a carcinoma, a sarcoma, a melanoma, a luminal A breast cancer, a luminal B breast cancer, HER2+ breast cancer, a high microsatellite instability (MSI-H) colorectal cancer, a microsatellite stable colorectal cancer (MSS), non-small cell lung cancer (NSCLC), chordoma, or adrenal cortical carcinoma. The carcinoma can be a carcinoma of the breast, colon, lung, pancreas, prostate, Merkel cell, ovary, liver, endometrial, bladder, kidney or cancer of unknown primary (CUP). The sarcoma can be a liposarcoma, chondrosarcoma, extraskeletal myxoid chondrosarcoma or uterine sarcoma. In some embodiments, the sarcoma comprises an alveolar soft part sarcoma (ASPS), angiosarcoma, breast angiosarcoma, chondrosarcoma, chordoma, clear cell sarcoma, desmoplastic small round cell tumor (DSRCT), epithelioid hemangioendothelioma (EHE), epithelioid sarcoma, endometrial stromal sarcoma (ESS), ewing sarcoma, fibromatosis, fibrosarcoma, giant cell tumour, leiomyosarcoma (LMS), uterine LMS, liposarcoma, malignant fibrous histiocytoma (MFH/UPS), malignant peripheral nerve sheath tumor (MPNST), osteosarcoma, perivascular epithelioid cell tumor (PEComa), rhabdomyosarcoma, solitary fibrous tumor (SFT), synovial sarcoma, fibromyxoid sarcoma, fibrous hamartoma of infancy, hereditary leiomyomatosis, angiomyolipoma, angiomyxoma, atypical spindle cell lesion (with fibrohistiocytic differentiation), chondroblastoma, dendritic cell sarcoma, granular cell tumor, high grade myxoid sarcoma, high-grade myoepithelial carcinoma, hyalinizing fibroblastic sarcoma, inflammatory myofibroblastic sarcoma, interdigitating dendritic cell tumor, intimal sarcoma, leiomyoma, lymphangitic sarcomatosis, malignant glomus tumor, malignant myoepithelioma, melanocytic neoplasm, mesenchymal neoplasm, mesenteric glomangioma, metastatic histocytoid neoplasm, myoepithelioma, myxoid sarcoma, myxoid stromal, neurilemmoma, phyllodes, rhabdoid, round cell, sarcoma not otherwise specified (NOS), sarcomatous mesothelioma, schwannoma, spindle and round cell sarcoma, spindle cell or spinocellular mesenchymal tumor.

[0027] In a related aspect, the invention provides a method of generating a molecular profiling report comprising preparing a report comprising results of the determining and identifying steps as described above. In some embodiments, the report further comprises a list of the at least one treatment that is associated with benefit for treatment of the cancer, a list of the at least one treatment that is associated with lack of benefit for treatment of the cancer, and/or a list of at least one treatment that is associated with indeterminate benefit for treating the cancer. The report can further comprise identification of the at least one treatment as standard of care or not for the cancer, e.g., using guidelines such as NCCN for the cancer's lineage. In some embodiments, the report further comprises a list of clinical trials for which the subject is indicated and/or eligible based on the molecular profile. **FIGs. 29A-X** present an illustrative report according to the invention.

[0028] The report may comprise various listings and descriptions of the molecular profiling that was performed. In some embodiments, the report further comprises a listing of at least one member of the plurality of genes or gene products assessed with description of the at least one member. For example, such descriptions can be as provided in **Table 6** or **Table 17** herein. In embodiments, the report comprises a listing of the laboratory techniques used to assess the members of the plurality of genes or gene products. For example, the report can specify whether each member was assessed by at least one of ISH, IHC, Next Generation sequencing, Sanger sequencing, PCR, pyrosequencing and fragment analysis. The report can provide an evidentiary level for each biomarker-drug association. For example, the report may comprises a list of evidence supporting the identification of certain treatments as likely to benefit the patient, not benefit the patient, or having indeterminate benefit. *See, e.g.,* **Table 8** and accompanying text herein.

[0029] The report can provide any desired combination of such information. In some embodiments, the report further comprises: 1) a list of the genes and/or gene products in the molecular profile; 2) a description of the molecular profile of the genes and/or gene products as determined for the subject; 3) a treatment associated with at least one of the genes and/or gene products in the molecular profile; and 4) and an indication whether each treatment is likely to benefit the patient, not benefit the patient, or has indeterminate benefit. The description of the molecular profile of the genes and/or gene products as determined for the subject may comprise the technique used to assess the gene and/or gene products and the results of the assessment.

[0030] In preferred embodiments, the report is computer generated. For example, the can be a printed report or a computer file. The report can be made accessible via a web portal.

[0031] In still another related aspect, the invention provides use of a reagent in carrying out the methods of the invention, and/or use of a reagent in the manufacture of a reagent or kit for carrying out the methods of the invention. Relatedly, the invention provides a kit comprising a reagent for carrying out the methods of the invention. The reagent can be any useful reagent for performing molecular profiling. For example, the reagent may comprise at least one of a reagent for extracting nucleic acid from a sample, a reagent for performing ISH, a reagent for performing IHC, a reagent for performing PCR, a reagent for performing Sanger sequencing, a reagent for performing next generation sequencing, a reagent for a DNA microarray, a reagent for performing pyrosequencing, a nucleic acid probe, a nucleic acid primer, an antibody, a reagent for performing bisulfite treatment of nucleic acid, and a combination thereof.

[0032] In yet another related aspect, the invention provides a report generated by the methods of the invention. The report can be a report as described above. For example, the can be a printed report or a computer file. The report can be made accessible via a web portal. The invention also provides a computer system for generating the report.

[0033] In an aspect, the invention provides a system for identifying at least one treatment associated with a cancer in a subject, comprising: a) a host server; b) a user interface for accessing the host server to access and input data; c) a processor for processing the inputted data; d) a memory coupled to the processor for storing the processed data and instructions for: accessing a molecular profile generated by

the methods of the invention and identifying, based on the molecular profile, at least one of: i) at least one treatment that is associated with benefit for treatment of the cancer; ii) at least one treatment that is associated with lack of benefit for treatment of the cancer; and iii) at least one treatment associated with a clinical trial; and e) a display for displaying the identified at least one of: i) at least one treatment that is associated with benefit for treatment of the cancer; ii) at least one treatment that is associated with lack of benefit for treatment of the cancer; and iii) at least one treatment associated with a clinical trial.

[0034] In an aspect, the invention provides a computer medium comprising at least one rule, e.g., 1, 2, 3, 4, 5, 6, 7, 8, 9, 10, 11, 12, 13, 14, 15, 16, 17, 18, 19, 20, 21, 22, 23, 24 or 25 rules, selected from **Table 8**. In a related aspect, the invention provides a computer medium comprising at least one rule, e.g., 1, 2, 3, 4, 5, 6, 7, 8, 9, 10, 11, 12, 13, 14, 15, 16, 17, 18, 19, 20, 21,22, 23, 24, 25, 26 or 27 rules, selected from: performing protein analysis on PD1 to determine likely benefit or lack of benefit from an PD-1 modulating therapy, PD-1 inhibitor, anti-PD-1 immunotherapy, anti-PD-1 monoclonal antibody, nivolumab, pidilizumab (CT-011, CureTech, LTD), pembrolizumab (lambrolizumab, MK-3475, Merck), a PD-1 antagonist, a PD-1 ligand soluble construct, and/or AMP-224 (Amplimmune); performing protein analysis on PD-L1 to determine likely benefit or lack of benefit from a PD-L1 modulating therapy, PD-L1 inhibitor, anti-PD-L1 immunotherapy, anti-PD-L1 monoclonal antibody, BMS-936559, MPDL3280A/RG7446, and/or MEDI4736 (MedImmune); performing protein analysis on RRM1 to determine likely benefit or lack of benefit from an antimetabolite and/or gemcitabine; performing protein analysis on TS to determine likely benefit or lack of benefit from a antimetabolite, fluorouracil, capecitabine, and/or pemetrexed; performing protein analysis on TOPO1 to determine likely benefit or lack of benefit from a TOPO1 inhibitor, irinotecan and/or topotecan; performing at least one of protein analysis on MGMT, analysis of MGMT promoter methylation, and sequencing on IDH1 to determine likely benefit or lack of benefit from an alkylating agent, temozolomide, and/or dacarbazine; performing protein analysis on AR to determine likely benefit or lack of benefit from an anti-androgen, bicalutamide, flutamide, abiraterone and/or enzalutamide; performing protein analysis on ER to determine likely benefit or lack of benefit from a hormonal agent, tamoxifen, fulvestrant, letrozole, and/or anastrozole; performing protein analysis on at least one of ER, PR and AR to determine likely benefit or lack of benefit from a hormonal agent, tamoxifen, toremifene, fulvestrant, letrozole, anastrozole, exemestane, megestrol acetate, leuprolide, goserelin, bicalutamide, flutamide, abiraterone, enzalutamide, triptorelin, abarelix, and/or degarelix; performing at least one of protein analysis on HER2 and gene amplification analysis on HER2 to determine likely benefit or lack of benefit from a tyrosine kinase inhibitor and/or lapatinib, pertuzumab, and/or ado-trastuzumab emtansine (T-DM1); performing at least one of protein analysis on HER2, gene amplification analysis on HER2, protein analysis on PTEN and sequencing on PIK3CA to determine likely benefit or lack of benefit from HER2 targeted therapy, and/or trastuzumab; performing at least one of gene amplification analysis on TOP2A, gene amplification analysis on HER2, protein analysis on TOP2A and protein analysis on PGP to determine likely benefit or lack of benefit from an anthracycline, doxorubicin, liposomal-doxorubicin, and/or epirubicin; performing sequencing on at least one of cKIT and PDGFRA to determine likely benefit or lack of benefit from a tyrosine kinase inhibitor and/or

imatinib; performing at least one of gene rearrangement analysis on ALK and gene rearrangement analysis on ROS1 to determine likely benefit or lack of benefit from a tyrosine kinase inhibitor and/or crizotinib; performing at least one of protein analysis on ER or sequencing on PIK3CA to determine likely benefit or lack of benefit from an mTOR inhibitor, everolimus, and/or temsirolimus; performing sequencing on RET to determine likely benefit or lack of benefit from a tyrosine kinase inhibitor, and/or vandetanib; performing protein analysis on at least one of TLE3, TUBB3 and PGP to determine likely benefit or lack of benefit from a taxane, paclitaxel, and/or docetaxel; performing protein analysis on SPARC to determine likely benefit or lack of benefit from a taxane, and/or nab-paclitaxel; performing at least one of PCR and sequencing on BRAF to determine likely benefit or lack of benefit from a tyrosine kinase inhibitor, vemurafenib, dabrafenib, and/or trametinib; performing at least one of sequencing on KRAS, sequencing on BRAF, sequencing on NRAS, sequencing on PIK3CA and protein analysis on PTEN to determine likely benefit or lack of benefit from an EGFR-targeted antibody, cetuximab, and/or panitumumab; performing sequencing on EGFR to determine likely benefit or lack of benefit from an EGFR-targeted antibody, and/or cetuximab; performing at least one of sequencing on EGFR, sequencing on KRAS, gene amplification analysis on cMET, sequencing on PIK3CA and protein analysis on PTEN to determine likely benefit or lack of benefit from a tyrosine kinase inhibitor, erlotinib, and/or gefitinib; performing sequencing on EGFR to determine likely benefit or lack of benefit from a tyrosine kinase inhibitor, and/or afatinib; performing sequencing on cKIT to determine likely benefit or lack of benefit from a tyrosine kinase inhibitor, and/or sunitinib; performing sequencing on at least one of BRCA1, BRCA2 and/or protein analysis on ERCC1 to determine likely benefit or lack of benefit from carboplatin, cisplatin, and/or oxaliplatin; performing gene rearrangement analysis on ALK to determine likely benefit or lack of benefit from ceritinib; and detecting 1p19q codeletion to determine likely benefit or lack of benefit from procarbazine, lomustine, and/or vincristine (PCV). Protein level can be determined using any useful protein analysis method, e.g., IHC. The sequencing can be any useful sequencing technique, e.g., next generation sequencing or Sanger sequencing. DNA methylation can be determined using any useful methylation analysis technique, e.g., pyrosequencing. Gene amplification can be determined using any useful copy number analysis method, e.g., ISH, FISH and/or CISH. Translocation can be determined using any useful analysis method, e.g., ISH, FISH and/or CISH. Gene rearrangement or deletion (e.g., 1p19q) can be determined using any useful analysis method, e.g., ISH, FISH and/or CISH. The relationship between biomarker status and likely benefit or lack of benefit can be determined as described herein (e.g., **Tables 6**, **8** or **17**).

INCORPORATION BY REFERENCE

[0035] All publications and patent applications mentioned in this specification are herein incorporated by reference to the same extent as if each individual publication or patent application was specifically and individually indicated to be incorporated by reference.

BRIEF DESCRIPTION OF THE DRAWINGS

[0036] A better understanding of the features and advantages of the present invention will be obtained by reference to the following detailed description that sets forth illustrative embodiments, in which the principles of the invention are used, and the accompanying drawings of which:

[0037] **FIG. 1** illustrates a block diagram of an exemplary embodiment of a system for determining individualized medical intervention for a particular disease state that utilizes molecular profiling of a patient's biological specimen that is non disease specific.

[0038] **FIG. 2** is a flowchart of an exemplary embodiment of a method for determining individualized medical intervention for a particular disease state that utilizes molecular profiling of a patient's biological specimen that is non disease specific.

[0039] **FIGS. 3A** through **3D** illustrate an exemplary patient profile report in accordance with step 80 of **FIG. 2**.

[0040] **FIG. 4** is a flowchart of an exemplary embodiment of a method for identifying a drug therapy/agent capable of interacting with a target.

[0041] **FIGS. 5-14** are flowcharts and diagrams illustrating various parts of an information-based personalized medicine drug discovery system and method in accordance with the present invention.

[0042] **FIGS. 15-25** are computer screen print outs associated with various parts of the information-based personalized medicine drug discovery system and method shown in **FIGS. 5-14**.

[0043] **FIGs. 26A-B** illustrate a diagram showing a biomarker centric (**FIG. 26A**) and therapeutic centric (**FIG. 26B**) approach to identifying a therapeutic agent.

[0044] **FIGs. 27A-27B** illustrate molecular intelligence (MI) profiles comprising biomarkers and associated therapeutic agents that can be assessed to identify candidate therapeutic agents. The indicated MI Plus profiles include additional cancer markers to be assessed by mutational analysis for diagnostic, prognostic and related purposes. NextGen refers to Next Generation Sequencing. PyroSeq refers to pyrosequencing. SangerSeq refers to Sanger dye termination sequencing. **FIG. 27A** and **FIG. 27B** illustrate an MI profile and and MI PLUS profile, respectively.

[0045] **FIGs. 28A-E** illustrate biomarkers assessed using a molecular profiling approach as outlined in **FIGs. 27A-B**, **Tables 7-17**, and accompanying text herein. **FIG. 28A** illustrates biomarkers that are assessed. The biomarkers that are assessed according to the Next Generation sequencing panel in **FIG. 28A** are shown in **FIG. 28B**. **FIG. 28C** illustrates sample requirements that can be used to perform molecular profiling on a patient tumor sample according to the panels in **FIGs. 28A-B**. **FIG. 28D** and **FIG. 28E** detail the biomarkers assessed, technology platforms utilized and associated therapies or clinical trials.

[0046] **FIGs. 29A-X** illustrate an exemplary patient report based on molecular profiling for a patient having a metastatic pancreatic adenocarcinoma.

[0047] **FIG. 30** illustrates progression free survival (PFS) using therapy selected by molecular profiling (period B) with PFS for the most recent therapy on which the patient has just progressed (period A). If

PFS(B) / PFS(A) ratio ≥ 1.3, then molecular profiling selected therapy was defined as having benefit for patient.

[0048] **FIG. 31** is a schematic of methods for identifying treatments by molecular profiling if a target is identified.

[0049] **FIG. 32** illustrates the distribution of the patients in the study as performed in Example 1.

[0050] **FIG. 33** is graph depicting the results of the study with patients having PFS ratio ≥ 1.3 was 18/66 (27%).

[0051] **FIG. 34** is a waterfall plot of all the patients for maximum % change of summed diameters of target lesions with respect to baseline diameter.

[0052] **FIG. 35** illustrates the relationship between what clinician selected as what she/he would use to treat the patient before knowing what the molecular profiling results suggested. There were no matches for the 18 patients with PFS ratio ≥ 1.3.

[0053] **FIG. 36** is a schematic of the overall survival for the 18 patients with PFS ratio ≥ 1.3 versus all 66 patients.

[0054] **FIG. 37** illustrates a molecular profiling system that performs analysis of a cancer sample using a variety of components that measure expression levels, chromosomal aberrations and mutations. The molecular "blueprint" of the cancer is used to generate a prioritized ranking of druggable targets and/or drug associated targets in tumor and their associated therapies.

[0055] **FIG. 38** shows an example output of microarray profiling results and calls made using a cutoff value.

[0056] **FIGs. 39A-N** illustrate results of molecular profiling of immune related biomarkers in a cohort of over 5000 breast cancer patients.

[0057] **FIGs. 40A-E** illustrate results of molecular profiling of a cohort of 126 Triple Negative (TN) Metaplastic Breast Cancers.

[0058] **FIG. 41** illustrates results of molecular profiling of PD1 and PDL1 in HPV+ and HPV-/TP53 mutated head and neck squamous cell carcinomas.

[0059] **FIGs. 42A-D** illustrates a case of endometrial adenocarcinoma (**FIG. 42A**, hematoxylin and eosin stained section) exhibiting microsatellite instability caused by the loss of MLH-1 protein [note retained MLH-1 protein expression in the nuclei of the tumor infiltrating lymphocytes] (**FIG. 42B**, immunohistochemical stain); PD-1+ Tumor-infiltrating lymphocytes (**FIG. 42C**, immunohistochemical stain); aberrant expression of PD-L1 in the tumor cells' basolateral membranes (**FIG. 42D**, immunohistochemical stain).

DETAILED DESCRIPTION OF THE INVENTION

[0060] The present invention provides methods and systems for identifying therapeutic agents for use in treatments on an individualized basis by using molecular profiling. The molecular profiling approach provides a method for selecting a candidate treatment for an individual that could favorably change the clinical course for the individual with a condition or disease, such as cancer. The molecular profiling approach provides clinical benefit for individuals, such as identifying drug target(s) that provide a longer

progression free survival (PFS), longer disease free survival (DFS), longer overall survival (OS) or extended lifespan. Methods and systems of the invention are directed to molecular profiling of cancer on an individual basis that can provide alternatives for treatment that may be convention or alternative to conventional treatment regimens. For example, alternative treatment regimes can be selected through molecular profiling methods of the invention where, a disease is refractory to current therapies, e.g., after a cancer has developed resistance to a standard-of-care treatment. Illustrative schemes for using molecular profiling to identify a treatment regime are shown in **FIGs. 2**, **49A-B** and **50**, each of which is described in further detail herein. Thus, molecular profiling provides a personalized approach to selecting candidate treatments that are likely to benefit a cancer. In embodiments, the molecular profiling method is used to identify therapies for patients with poor prognosis, such as those with metastatic disease or those whose cancer has progressed on standard front line therapies, or whose cancer has progressed on multiple chemotherapeutic or hormonal regimens.

[0061] Personalized medicine based on pharmacogenetic insights, such as those provided by molecular profiling according to the invention, is increasingly taken for granted by some practitioners and the lay press, but forms the basis of hope for improved cancer therapy. However, molecular profiling as taught herein represents a fundamental departure from the traditional approach to oncologic therapy where for the most part, patients are grouped together and treated with approaches that are based on findings from light microscopy and disease stage. Traditionally, differential response to a particular therapeutic strategy has only been determined after the treatment was given, i.e. a posteriori. The "standard" approach to disease treatment relies on what is generally true about a given cancer diagnosis and treatment response has been vetted by randomized phase III clinical trials and forms the "standard of care" in medical practice. The results of these trials have been codified in consensus statements by guidelines organizations such as the National Comprehensive Cancer Network and The American Society of Clinical Oncology. The NCCN Compendium™ contains authoritative, scientifically derived information designed to support decision-making about the appropriate use of drugs and biologics in patients with cancer. The NCCN Compendium™ is recognized by the Centers for Medicare and Medicaid Services (CMS) and United Healthcare as an authoritative reference for oncology coverage policy. On-compendium treatments are those recommended by such guides. The biostatistical methods used to validate the results of clinical trials rely on minimizing differences between patients, and are based on declaring the likelihood of error that one approach is better than another for a patient group defined only by light microscopy and stage, not by individual differences in tumors. The molecular profiling methods of the invention exploit such individual differences. The methods can provide candidate treatments that can be then selected by a physician for treating a patient. In a study of such an approach presented in Example 1 herein, the results were profound: in 66 consecutive patients, the treating oncologist never managed to identify the molecular target selected by the test, and 27% of patients whose treatment was guided by molecular profiling managed a remission 1.3x longer than their previous best response. At present, such results are virtually unheard of result in the salvage therapy setting.

[0062] Molecular profiling can be used to provide a comprehensive view of the biological state of a sample. In an embodiment, molecular profiling is used for whole tumor profiling. Accordingly, a number of molecular approaches are used to assess the state of a tumor. The whole tumor profiling can be used for selecting a candidate treatment for a tumor. Molecular profiling can be used to select candidate therapeutics on any sample for any stage of a disease. In embodiment, the methods of the invention are used to profile a newly diagnosed cancer. The candidate treatments indicated by the molecular profiling can be used to select a therapy for treating the newly diagnosed cancer. In other embodiments, the methods of the invention are used to profile a cancer that has already been treated, e.g., with one or more standard-of-care therapy. In embodiments, the cancer is refractory to the prior treatment/s. For example, the cancer may be refractory to the standard of care treatments for the cancer. The cancer can be a metastatic cancer or other recurrent cancer. The treatments can be on-compendium or off-compendium treatments.

[0063] Molecular profiling can be performed by any known means for detecting a molecule in a biological sample. Molecular profiling comprises methods that include but are not limited to, nucleic acid sequencing, such as a DNA sequencing or mRNA sequencing; immunohistochemistry (IHC); in situ hybridization (ISH); fluorescent in situ hybridization (FISH); chromogenic in situ hybridization (CISH); PCR amplification (e.g., qPCR or RT-PCR); various types of microarray (mRNA expression arrays, low density arrays, protein arrays, etc); various types of sequencing (Sanger, pyrosequencing, etc); comparative genomic hybridization (CGH); NextGen sequencing; Northern blot; Southern blot; immunoassay; and any other appropriate technique to assay the presence or quantity of a biological molecule of interest. In various embodiments of the invention, any one or more of these methods can be used concurrently or subsequent to each other for assessing target genes disclosed herein.

[0064] Molecular profiling of individual samples is used to select one or more candidate treatments for a disorder in a subject, e.g., by identifying targets for drugs that may be effective for a given cancer. For example, the candidate treatment can be a treatment known to have an effect on cells that differentially express genes as identified by molecular profiling techniques, an experimental drug, a government or regulatory approved drug or any combination of such drugs, which may have been studied and approved for a particular indication that is the same as or different from the indication of the subject from whom a biological sample is obtain and molecularly profiled.

[0065] When multiple biomarker targets are revealed by assessing target genes by molecular profiling, one or more decision rules can be put in place to prioritize the selection of certain therapeutic agent for treatment of an individual on a personalized basis. Rules of the invention aide prioritizing treatment, e.g., direct results of molecular profiling, anticipated efficacy of therapeutic agent, prior history with the same or other treatments, expected side effects, availability of therapeutic agent, cost of therapeutic agent, drug-drug interactions, and other factors considered by a treating physician. Based on the recommended and prioritized therapeutic agent targets, a physician can decide on the course of treatment for a particular individual. Accordingly, molecular profiling methods and systems of the invention can select candidate treatments based on individual characteristics of diseased cells, e.g., tumor cells, and other personalized

factors in a subject in need of treatment, as opposed to relying on a traditional one-size fits all approach that is conventionally used to treat individuals suffering from a disease, especially cancer. In some cases, the recommended treatments are those not typically used to treat the disease or disorder inflicting the subject. In some cases, the recommended treatments are used after standard-of-care therapies are no longer providing adequate efficacy.

[0066] The treating physician can use the results of the molecular profiling methods to optimize a treatment regimen for a patient. The candidate treatment identified by the methods of the invention can be used to treat a patient; however, such treatment is not required of the methods. Indeed, the analysis of molecular profiling results and identification of candidate treatments based on those results can be automated and does not require physician involvement.

Biological Entities

[0067] Nucleic acids include deoxyribonucleotides or ribonucleotides and polymers thereof in either single- or double-stranded form, or complements thereof. Nucleic acids can contain known nucleotide analogs or modified backbone residues or linkages, which are synthetic, naturally occurring, and non-naturally occurring, which have similar binding properties as the reference nucleic acid, and which are metabolized in a manner similar to the reference nucleotides. Examples of such analogs include, without limitation, phosphorothioates, phosphoramidates, methyl phosphonates, chiral-methyl phosphonates, 2-O-methyl ribonucleotides, peptide-nucleic acids (PNAs). Nucleic acid sequence can encompass conservatively modified variants thereof (e.g., degenerate codon substitutions) and complementary sequences, as well as the sequence explicitly indicated. Specifically, degenerate codon substitutions may be achieved by generating sequences in which the third position of one or more selected (or all) codons is substituted with mixed-base and/or deoxyinosine residues (Batzer et al., Nucleic Acid Res. 19:5081 (1991); Ohtsuka et al., J. Biol. Chem. 260:2605-2608 (1985); Rossolini et al., Mol. Cell Probes 8:91-98 (1994)). The term nucleic acid can be used interchangeably with gene, cDNA, mRNA, oligonucleotide, and polynucleotide.

[0068] A particular nucleic acid sequence may implicitly encompass the particular sequence and "splice variants" and nucleic acid sequences encoding truncated forms. Similarly, a particular protein encoded by a nucleic acid can encompass any protein encoded by a splice variant or truncated form of that nucleic acid. "Splice variants," as the name suggests, are products of alternative splicing of a gene. After transcription, an initial nucleic acid transcript may be spliced such that different (alternate) nucleic acid splice products encode different polypeptides. Mechanisms for the production of splice variants vary, but include alternate splicing of exons. Alternate polypeptides derived from the same nucleic acid by read-through transcription are also encompassed by this definition. Any products of a splicing reaction, including recombinant forms of the splice products, are included in this definition. Nucleic acids can be truncated at the 5' end or at the 3' end. Polypeptides can be truncated at the N-terminal end or the C-terminal end. Truncated versions of nucleic acid or polypeptide sequences can be naturally occurring or created using recombinant techniques.

[0069] The terms "genetic variant" and "nucleotide variant" are used herein interchangeably to refer to changes or alterations to the reference human gene or cDNA sequence at a particular locus, including, but not limited to, nucleotide base deletions, insertions, inversions, and substitutions in the coding and non-coding regions. Deletions may be of a single nucleotide base, a portion or a region of the nucleotide sequence of the gene, or of the entire gene sequence. Insertions may be of one or more nucleotide bases. The genetic variant or nucleotide variant may occur in transcriptional regulatory regions, untranslated regions of mRNA, exons, introns, exon/intron junctions, etc. The genetic variant or nucleotide variant can potentially result in stop codons, frame shifts, deletions of amino acids, altered gene transcript splice forms or altered amino acid sequence.

[0070] An allele or gene allele comprises generally a naturally occurring gene having a reference sequence or a gene containing a specific nucleotide variant.

[0071] A haplotype refers to a combination of genetic (nucleotide) variants in a region of an mRNA or a genomic DNA on a chromosome found in an individual. Thus, a haplotype includes a number of genetically linked polymorphic variants which are typically inherited together as a unit.

[0072] As used herein, the term "amino acid variant" is used to refer to an amino acid change to a reference human protein sequence resulting from genetic variants or nucleotide variants to the reference human gene encoding the reference protein. The term "amino acid variant" is intended to encompass not only single amino acid substitutions, but also amino acid deletions, insertions, and other significant changes of amino acid sequence in the reference protein.

[0073] The term "genotype" as used herein means the nucleotide characters at a particular nucleotide variant marker (or locus) in either one allele or both alleles of a gene (or a particular chromosome region). With respect to a particular nucleotide position of a gene of interest, the nucleotide(s) at that locus or equivalent thereof in one or both alleles form the genotype of the gene at that locus. A genotype can be homozygous or heterozygous. Accordingly, "genotyping" means determining the genotype, that is, the nucleotide(s) at a particular gene locus. Genotyping can also be done by determining the amino acid variant at a particular position of a protein which can be used to deduce the corresponding nucleotide variant(s).

[0074] The term "locus" refers to a specific position or site in a gene sequence or protein. Thus, there may be one or more contiguous nucleotides in a particular gene locus, or one or more amino acids at a particular locus in a polypeptide. Moreover, a locus may refer to a particular position in a gene where one or more nucleotides have been deleted, inserted, or inverted.

[0075] Unless specified otherwise or understood by one of skill in art, the terms "polypeptide," "protein," and "peptide" are used interchangeably herein to refer to an amino acid chain in which the amino acid residues are linked by covalent peptide bonds. The amino acid chain can be of any length of at least two amino acids, including full-length proteins. Unless otherwise specified, polypeptide, protein, and peptide also encompass various modified forms thereof, including but not limited to glycosylated forms, phosphorylated forms, etc. A polypeptide, protein or peptide can also be referred to as a gene product.

[0076] Lists of gene and gene products that can be assayed by molecular profiling techniques are presented herein. Lists of genes may be presented in the context of molecular profiling techniques that detect a gene product (e.g., an mRNA or protein). One of skill will understand that this implies detection of the gene product of the listed genes. Similarly, lists of gene products may be presented in the context of molecular profiling techniques that detect a gene sequence or copy number. One of skill will understand that this implies detection of the gene corresponding to the gene products, including as an example DNA encoding the gene products. As will be appreciated by those skilled in the art, a "biomarker" or "marker" comprises a gene and/or gene product depending on the context.

[0077] The terms "label" and "detectable label" can refer to any composition detectable by spectroscopic, photochemical, biochemical, immunochemical, electrical, optical, chemical or similar methods. Such labels include biotin for staining with labeled streptavidin conjugate, magnetic beads (e.g., DYNABEADS™), fluorescent dyes (e.g., fluorescein, Texas red, rhodamine, green fluorescent protein, and the like), radiolabels (e.g., ^{3}H, ^{125}I, ^{35}S, ^{14}C, or ^{32}P), enzymes (e.g., horse radish peroxidase, alkaline phosphatase and others commonly used in an ELISA), and calorimetric labels such as colloidal gold or colored glass or plastic (e.g., polystyrene, polypropylene, latex, etc) beads. Patents teaching the use of such labels include U.S. Pat. Nos. 3,817,837; 3,850,752; 3,939,350; 3,996,345; 4,277,437; 4,275,149; and 4,366,241. Means of detecting such labels are well known to those of skill in the art. Thus, for example, radiolabels may be detected using photographic film or scintillation counters, fluorescent markers may be detected using a photodetector to detect emitted light. Enzymatic labels are typically detected by providing the enzyme with a substrate and detecting the reaction product produced by the action of the enzyme on the substrate, and calorimetric labels are detected by simply visualizing the colored label. Labels can include, e.g., ligands that bind to labeled antibodies, fluorophores, chemiluminescent agents, enzymes, and antibodies which can serve as specific binding pair members for a labeled ligand. An introduction to labels, labeling procedures and detection of labels is found in Polak and Van Noorden Introduction to Immunocytochemistry, 2nd ed., Springer Verlag, NY (1997); and in Haugland Handbook of Fluorescent Probes and Research Chemicals, a combined handbook and catalogue Published by Molecular Probes, Inc. (1996).

[0078] Detectable labels include, but are not limited to, nucleotides (labeled or unlabelled), compomers, sugars, peptides, proteins, antibodies, chemical compounds, conducting polymers, binding moieties such as biotin, mass tags, calorimetric agents, light emitting agents, chemiluminescent agents, light scattering agents, fluorescent tags, radioactive tags, charge tags (electrical or magnetic charge), volatile tags and hydrophobic tags, biomolecules (e.g., members of a binding pair antibody/antigen, antibody/antibody, antibody/antibody fragment, antibody/antibody receptor, antibody/protein A or protein G, hapten/anti-hapten, biotin/avidin, biotin/streptavidin, folic acid/folate binding protein, vitamin B12/intrinsic factor, chemical reactive group/complementary chemical reactive group (e.g., sulfhydryl/maleimide, sulfhydryl/haloacetyl derivative, amine/isotriocyanate, amine/succinimidyl ester, and amine/sulfonyl halides) and the like.

[0079] The term "antibody" as used herein encompasses naturally occurring antibodies as well as non-naturally occurring antibodies, including, for example, single chain antibodies, chimeric, bifunctional and humanized antibodies, as well as antigen-binding fragments thereof, (e.g., Fab', $F(ab')_2$, Fab, Fv and rIgG). See also, Pierce Catalog and Handbook, 1994-1995 (Pierce Chemical Co., Rockford, Ill.). See also, e.g., Kuby, J., Immunology, 3.sup.rd Ed., W. H. Freeman & Co., New York (1998). Such non-naturally occurring antibodies can be constructed using solid phase peptide synthesis, can be produced recombinantly or can be obtained, for example, by screening combinatorial libraries consisting of variable heavy chains and variable light chains as described by Huse et al., Science 246:1275-1281 (1989), which is incorporated herein by reference. These and other methods of making, for example, chimeric, humanized, CDR-grafted, single chain, and bifunctional antibodies are well known to those skilled in the art. See, e.g., Winter and Harris, Immunol. Today 14:243-246 (1993); Ward et al., Nature 341:544-546 (1989); Harlow and Lane, Antibodies, 511-52, Cold Spring Harbor Laboratory publications, New York, 1988; Hilyard et al., Protein Engineering: A practical approach (IRL Press 1992); Borrebaeck, Antibody Engineering, 2d ed. (Oxford University Press 1995); each of which is incorporated herein by reference.

[0080] Unless otherwise specified, antibodies can include both polyclonal and monoclonal antibodies. Antibodies also include genetically engineered forms such as chimeric antibodies (e.g., humanized murine antibodies) and heteroconjugate antibodies (e.g., bispecific antibodies). The term also refers to recombinant single chain Fv fragments (scFv). The term antibody also includes bivalent or bispecific molecules, diabodies, triabodies, and tetrabodies. Bivalent and bispecific molecules are described in, e.g., Kostelny et al. (1992) J Immunol 148:1547, Pack and Pluckthun (1992) Biochemistry 31:1579, Holliger et al. (1993) Proc Natl Acad Sci USA. 90:6444, Gruber et al. (1994) J Immunol:5368, Zhu et al. (1997) Protein Sci 6:781, Hu et al. (1997) Cancer Res. 56:3055, Adams et al. (1993) Cancer Res. 53:4026, and McCartney, et al. (1995) Protein Eng. 8:301.

[0081] Typically, an antibody has a heavy and light chain. Each heavy and light chain contains a constant region and a variable region, (the regions are also known as "domains"). Light and heavy chain variable regions contain four framework regions interrupted by three hyper-variable regions, also called complementarity-determining regions (CDRs). The extent of the framework regions and CDRs have been defined. The sequences of the framework regions of different light or heavy chains are relatively conserved within a species. The framework region of an antibody, that is the combined framework regions of the constituent light and heavy chains, serves to position and align the CDRs in three dimensional spaces. The CDRs are primarily responsible for binding to an epitope of an antigen. The CDRs of each chain are typically referred to as CDR1, CDR2, and CDR3, numbered sequentially starting from the N-terminus, and are also typically identified by the chain in which the particular CDR is located. Thus, a V_H CDR3 is located in the variable domain of the heavy chain of the antibody in which it is found, whereas a V_L CDR1 is the CDR1 from the variable domain of the light chain of the antibody in which it is found. References to V_H refer to the variable region of an immunoglobulin heavy chain of an antibody, including the heavy chain of an Fv, scFv, or Fab. References to V_L refer to the variable region of an immunoglobulin light chain, including the light chain of an Fv, scFv, dsFv or Fab.

[0082] The phrase "single chain Fv" or "scFv" refers to an antibody in which the variable domains of the heavy chain and of the light chain of a traditional two chain antibody have been joined to form one chain. Typically, a linker peptide is inserted between the two chains to allow for proper folding and creation of an active binding site. A "chimeric antibody" is an immunoglobulin molecule in which (a) the constant region, or a portion thereof, is altered, replaced or exchanged so that the antigen binding site (variable region) is linked to a constant region of a different or altered class, effector function and/or species, or an entirely different molecule which confers new properties to the chimeric antibody, e.g., an enzyme, toxin, hormone, growth factor, drug, etc.; or (b) the variable region, or a portion thereof, is altered, replaced or exchanged with a variable region having a different or altered antigen specificity.

[0083] A "humanized antibody" is an immunoglobulin molecule that contains minimal sequence derived from non-human immunoglobulin. Humanized antibodies include human immunoglobulins (recipient antibody) in which residues from a complementary determining region (CDR) of the recipient are replaced by residues from a CDR of a non-human species (donor antibody) such as mouse, rat or rabbit having the desired specificity, affinity and capacity. In some instances, Fv framework residues of the human immunoglobulin are replaced by corresponding non-human residues. Humanized antibodies may also comprise residues which are found neither in the recipient antibody nor in the imported CDR or framework sequences. In general, a humanized antibody will comprise substantially all of at least one, and typically two, variable domains, in which all or substantially all of the CDR regions correspond to those of a non-human immunoglobulin and all or substantially all of the framework (FR) regions are those of a human immunoglobulin consensus sequence. The humanized antibody optimally also will comprise at least a portion of an immunoglobulin constant region (Fc), typically that of a human immunoglobulin (Jones et al., Nature 321:522-525 (1986); Riechmann et al., Nature 332:323-327 (1988); and Presta, Curr. Op. Struct. Biol. 2:593-596 (1992)). Humanization can be essentially performed following the method of Winter and co-workers (Jones et al., Nature 321:522-525 (1986); Riechmann et al., Nature 332:323-327 (1988); Verhoeyen et al., Science 239:1534-1536 (1988)), by substituting rodent CDRs or CDR sequences for the corresponding sequences of a human antibody. Accordingly, such humanized antibodies are chimeric antibodies (U.S. Pat. No. 4,816,567), wherein substantially less than an intact human variable domain has been substituted by the corresponding sequence from a non-human species.

[0084] The terms "epitope" and "antigenic determinant" refer to a site on an antigen to which an antibody binds. Epitopes can be formed both from contiguous amino acids or noncontiguous amino acids juxtaposed by tertiary folding of a protein. Epitopes formed from contiguous amino acids are typically retained on exposure to denaturing solvents whereas epitopes formed by tertiary folding are typically lost on treatment with denaturing solvents. An epitope typically includes at least 3, and more usually, at least 5 or 8-10 amino acids in a unique spatial conformation. Methods of determining spatial conformation of epitopes include, for example, x-ray crystallography and 2-dimensional nuclear magnetic resonance. See, e.g., Epitope Mapping Protocols in Methods in Molecular Biology, Vol. 66, Glenn E. Morris, Ed (1996).

[0085] The terms "primer", "probe," and "oligonucleotide" are used herein interchangeably to refer to a relatively short nucleic acid fragment or sequence. They can comprise DNA, RNA, or a hybrid thereof, or

chemically modified analog or derivatives thereof. Typically, they are single-stranded. However, they can also be double-stranded having two complementing strands which can be separated by denaturation. Normally, primers, probes and oligonucleotides have a length of from about 8 nucleotides to about 200 nucleotides, preferably from about 12 nucleotides to about 100 nucleotides, and more preferably about 18 to about 50 nucleotides. They can be labeled with detectable markers or modified using conventional manners for various molecular biological applications.

[0086] The term "isolated" when used in reference to nucleic acids (e.g., genomic DNAs, cDNAs, mRNAs, or fragments thereof) is intended to mean that a nucleic acid molecule is present in a form that is substantially separated from other naturally occurring nucleic acids that are normally associated with the molecule. Because a naturally existing chromosome (or a viral equivalent thereof) includes a long nucleic acid sequence, an isolated nucleic acid can be a nucleic acid molecule having only a portion of the nucleic acid sequence in the chromosome but not one or more other portions present on the same chromosome. More specifically, an isolated nucleic acid can include naturally occurring nucleic acid sequences that flank the nucleic acid in the naturally existing chromosome (or a viral equivalent thereof). An isolated nucleic acid can be substantially separated from other naturally occurring nucleic acids that are on a different chromosome of the same organism. An isolated nucleic acid can also be a composition in which the specified nucleic acid molecule is significantly enriched so as to constitute at least 10%, 20%, 30%, 40%, 50%, 60%, 70%, 80%, 90%, 95%, or at least 99% of the total nucleic acids in the composition.

[0087] An isolated nucleic acid can be a hybrid nucleic acid having the specified nucleic acid molecule covalently linked to one or more nucleic acid molecules that are not the nucleic acids naturally flanking the specified nucleic acid. For example, an isolated nucleic acid can be in a vector. In addition, the specified nucleic acid may have a nucleotide sequence that is identical to a naturally occurring nucleic acid or a modified form or mutein thereof having one or more mutations such as nucleotide substitution, deletion/insertion, inversion, and the like.

[0088] An isolated nucleic acid can be prepared from a recombinant host cell (in which the nucleic acids have been recombinantly amplified and/or expressed), or can be a chemically synthesized nucleic acid having a naturally occurring nucleotide sequence or an artificially modified form thereof.

[0089] The term "isolated polypeptide" as used herein is defined as a polypeptide molecule that is present in a form other than that found in nature. Thus, an isolated polypeptide can be a non-naturally occurring polypeptide. For example, an isolated polypeptide can be a "hybrid polypeptide." An isolated polypeptide can also be a polypeptide derived from a naturally occurring polypeptide by additions or deletions or substitutions of amino acids. An isolated polypeptide can also be a "purified polypeptide" which is used herein to mean a composition or preparation in which the specified polypeptide molecule is significantly enriched so as to constitute at least 10% of the total protein content in the composition. A "purified polypeptide" can be obtained from natural or recombinant host cells by standard purification techniques, or by chemically synthesis, as will be apparent to skilled artisans.

[0090] The terms "hybrid protein," "hybrid polypeptide," "hybrid peptide," "fusion protein," "fusion polypeptide," and "fusion peptide" are used herein interchangeably to mean a non-naturally occurring

polypeptide or isolated polypeptide having a specified polypeptide molecule covalently linked to one or more other polypeptide molecules that do not link to the specified polypeptide in nature. Thus, a "hybrid protein" may be two naturally occurring proteins or fragments thereof linked together by a covalent linkage. A "hybrid protein" may also be a protein formed by covalently linking two artificial polypeptides together. Typically but not necessarily, the two or more polypeptide molecules are linked or "fused" together by a peptide bond forming a single non-branched polypeptide chain.

[0091] The term "high stringency hybridization conditions," when used in connection with nucleic acid hybridization, includes hybridization conducted overnight at 42 °C in a solution containing 50% formamide, 5×SSC (750 mM NaCl, 75 mM sodium citrate), 50 mM sodium phosphate, pH 7.6, 5×Denhardt's solution, 10% dextran sulfate, and 20 microgram/ml denatured and sheared salmon sperm DNA, with hybridization filters washed in 0.1×SSC at about 65 °C. The term "moderate stringent hybridization conditions," when used in connection with nucleic acid hybridization, includes hybridization conducted overnight at 37 °C in a solution containing 50% formamide, 5×SSC (750 mM NaCl, 75 mM sodium citrate), 50 mM sodium phosphate, pH 7.6, 5×Denhardt's solution, 10% dextran sulfate, and 20 microgram/ml denatured and sheared salmon sperm DNA, with hybridization filters washed in 1×SSC at about 50 °C. It is noted that many other hybridization methods, solutions and temperatures can be used to achieve comparable stringent hybridization conditions as will be apparent to skilled artisans.

[0092] For the purpose of comparing two different nucleic acid or polypeptide sequences, one sequence (test sequence) may be described to be a specific percentage identical to another sequence (comparison sequence). The percentage identity can be determined by the algorithm of Karlin and Altschul, Proc. Natl. Acad. Sci. USA, 90:5873-5877 (1993), which is incorporated into various BLAST programs. The percentage identity can be determined by the "BLAST 2 Sequences" tool, which is available at the National Center for Biotechnology Information (NCBI) website. See Tatusova and Madden, FEMS Microbiol. Lett., 174(2):247-250 (1999). For pairwise DNA-DNA comparison, the BLASTN program is used with default parameters (e.g., Match: 1; Mismatch: -2; Open gap: 5 penalties; extension gap: 2 penalties; gap x_dropoff: 50; expect: 10; and word size: 11, with filter). For pairwise protein-protein sequence comparison, the BLASTP program can be employed using default parameters (e.g., Matrix: BLOSUM62; gap open: 11; gap extension: 1; x_dropoff: 15; expect: 10.0; and wordsize: 3, with filter). Percent identity of two sequences is calculated by aligning a test sequence with a comparison sequence using BLAST, determining the number of amino acids or nucleotides in the aligned test sequence that are identical to amino acids or nucleotides in the same position of the comparison sequence, and dividing the number of identical amino acids or nucleotides by the number of amino acids or nucleotides in the comparison sequence. When BLAST is used to compare two sequences, it aligns the sequences and yields the percent identity over defined, aligned regions. If the two sequences are aligned across their entire length, the percent identity yielded by the BLAST is the percent identity of the two sequences. If BLAST does not align the two sequences over their entire length, then the number of identical amino acids or nucleotides in the unaligned regions of the test sequence and comparison sequence is considered to be zero and the percent identity is calculated by adding the number of identical amino acids or nucleotides in

the aligned regions and dividing that number by the length of the comparison sequence. Various versions of the BLAST programs can be used to compare sequences, e.g., BLAST 2.1.2 or BLAST+ 2.2.22.

[0093] A subject or individual can be any animal which may benefit from the methods of the invention, including, e.g., humans and non-human mammals, such as primates, rodents, horses, dogs and cats. Subjects include without limitation a eukaryotic organisms, most preferably a mammal such as a primate, e.g., chimpanzee or human, cow; dog; cat; a rodent, e.g., guinea pig, rat, mouse; rabbit; or a bird; reptile; or fish. Subjects specifically intended for treatment using the methods described herein include humans. A subject may be referred to as an individual or a patient.

[0094] Treatment of a disease or individual according to the invention is an approach for obtaining beneficial or desired medical results, including clinical results, but not necessarily a cure. For purposes of this invention, beneficial or desired clinical results include, but are not limited to, alleviation or amelioration of one or more symptoms, diminishment of extent of disease, stabilized (i.e., not worsening) state of disease, preventing spread of disease, delay or slowing of disease progression, amelioration or palliation of the disease state, and remission (whether partial or total), whether detectable or undetectable. Treatment also includes prolonging survival as compared to expected survival if not receiving treatment or if receiving a different treatment. A treatment can include administration of a therapeutic agent, which can be an agent that exerts a cytotoxic, cytostatic, or immunomodulatory effect on diseased cells, e.g., cancer cells, or other cells that may promote a diseased state, e.g., activated immune cells. Therapeutic agents selected by the methods of the invention are not limited. Any therapeutic agent can be selected where a link can be made between molecular profiling and potential efficacy of the agent. Therapeutic agents include without limitation drugs, pharmaceuticals, small molecules, protein therapies, antibody therapies, viral therapies, gene therapies, and the like. Cancer treatments or therapies include apoptosis-mediated and non-apoptosis mediated cancer therapies including, without limitation, chemotherapy, hormonal therapy, radiotherapy, immunotherapy, and combinations thereof. Chemotherapeutic agents comprise therapeutic agents and combinations of therapeutic agents that treat, cancer cells, e.g., by killing those cells. Examples of different types of chemotherapeutic drugs include without limitation alkylating agents (e.g., nitrogen mustard derivatives, ethylenimines, alkylsulfonates, hydrazines and triazines, nitrosureas, and metal salts), plant alkaloids (e.g., vinca alkaloids, taxanes, podophyllotoxins, and camptothecan analogs), antitumor antibiotics (e.g., anthracyclines, chromomycins, and the like), antimetabolites (e.g., folic acid antagonists, pyrimidine antagonists, purine antagonists, and adenosine deaminase inhibitors), topoisomerase I inhibitors, topoisomerase II inhibitors, and miscellaneous antineoplastics (e.g., ribonucleotide reductase inhibitors, adrenocortical steroid inhibitors, enzymes, antimicrotubule agents, and retinoids).

[0095] A biomarker refers generally to a molecule, including without limitation a gene or product thereof, nucleic acids (e.g., DNA, RNA), protein/peptide/polypeptide, carbohydrate structure, lipid, glycolipid, characteristics of which can be detected in a tissue or cell to provide information that is predictive, diagnostic, prognostic and/or theranostic for sensitivity or resistance to candidate treatment.

Biological Samples

[0096] A sample as used herein includes any relevant biological sample that can be used for molecular profiling, e.g., sections of tissues such as biopsy or tissue removed during surgical or other procedures, bodily fluids, autopsy samples, and frozen sections taken for histological purposes. Such samples include blood and blood fractions or products (e.g., serum, buffy coat, plasma, platelets, red blood cells, and the like), sputum, malignant effusion, cheek cells tissue, cultured cells (e.g., primary cultures, explants, and transformed cells), stool, urine, other biological or bodily fluids (e.g., prostatic fluid, gastric fluid, intestinal fluid, renal fluid, lung fluid, cerebrospinal fluid, and the like), etc. The sample can comprise biological material that is a fresh frozen & formalin fixed paraffin embedded (FFPE) block, formalin-fixed paraffin embedded, or is within an RNA preservative + formalin fixative. More than one sample of more than one type can be used for each patient. In a preferred embodiment, the sample comprises a fixed tumor sample.

[0097] The sample used in the methods described herein can be a formalin fixed paraffin embedded (FFPE) sample. The FFPE sample can be one or more of fixed tissue, unstained slides, bone marrow core or clot, core needle biopsy, malignant fluids and fine needle aspirate (FNA). In an embodiment, the fixed tissue comprises a tumor containing formalin fixed paraffin embedded (FFPE) block from a surgery or biopsy. In another embodiment, the unstained slides comprise unstained, charged, unbaked slides from a paraffin block. In another embodiment, bone marrow core or clot comprises a decalcified core. A formalin fixed core and/or clot can be paraffin-embedded. In still another embodiment, the core needle biopsy comprises 1, 2, 3, 4, 5, 6, 7, 8, 9, 10 or more, e.g., 3-4, paraffin embedded biopsy samples. An 18 gauge needle biopsy can be used. The malignant fluid can comprise a sufficient volume of fresh pleural/ascitic fluid to produce a 5x5x2mm cell pellet. The fluid can be formalin fixed in a paraffin block. In an embodiment, the core needle biopsy comprises 1, 2, 3, 4, 5, 6, 7, 8, 9, 10 or more, e.g., 4-6, paraffin embedded aspirates.

[0098] A sample may be processed according to techniques understood by those in the art. A sample can be without limitation fresh, frozen or fixed cells or tissue. In some embodiments, a sample comprises formalin-fixed paraffin-embedded (FFPE) tissue, fresh tissue or fresh frozen (FF) tissue. A sample can comprise cultured cells, including primary or immortalized cell lines derived from a subject sample. A sample can also refer to an extract from a sample from a subject. For example, a sample can comprise DNA, RNA or protein extracted from a tissue or a bodily fluid. Many techniques and commercial kits are available for such purposes. The fresh sample from the individual can be treated with an agent to preserve RNA prior to further processing, e.g., cell lysis and extraction. Samples can include frozen samples collected for other purposes. Samples can be associated with relevant information such as age, gender, and clinical symptoms present in the subject; source of the sample; and methods of collection and storage of the sample. A sample is typically obtained from a subject.

[0099] A biopsy comprises the process of removing a tissue sample for diagnostic or prognostic evaluation, and to the tissue specimen itself. Any biopsy technique known in the art can be applied to the molecular profiling methods of the present invention. The biopsy technique applied can depend on the

tissue type to be evaluated (e.g., colon, prostate, kidney, bladder, lymph node, liver, bone marrow, blood cell, lung, breast, etc.), the size and type of the tumor (e.g., solid or suspended, blood or ascites), among other factors. Representative biopsy techniques include, but are not limited to, excisional biopsy, incisional biopsy, needle biopsy, surgical biopsy, and bone marrow biopsy. An "excisional biopsy" refers to the removal of an entire tumor mass with a small margin of normal tissue surrounding it. An "incisional biopsy" refers to the removal of a wedge of tissue that includes a cross-sectional diameter of the tumor. Molecular profiling can use a "core-needle biopsy" of the tumor mass, or a "fine-needle aspiration biopsy" which generally obtains a suspension of cells from within the tumor mass. Biopsy techniques are discussed, for example, in Harrison's Principles of Internal Medicine, Kasper, et al., eds., 16th ed., 2005, Chapter 70, and throughout Part V.

[00100] Standard molecular biology techniques known in the art and not specifically described are generally followed as in Sambrook et al., Molecular Cloning: A Laboratory Manual, Cold Spring Harbor Laboratory Press, New York (1989), and as in Ausubel et al., Current Protocols in Molecular Biology, John Wiley and Sons, Baltimore, Md. (1989) and as in Perbal, A Practical Guide to Molecular Cloning, John Wiley & Sons, New York (1988), and as in Watson et al., Recombinant DNA, Scientific American Books, New York and in Birren et al (eds) Genome Analysis: A Laboratory Manual Series, Vols. 1-4 Cold Spring Harbor Laboratory Press, New York (1998) and methodology as set forth in U.S. Pat. Nos. 4,666,828; 4,683,202; 4,801,531; 5,192,659 and 5,272,057 and incorporated herein by reference. Polymerase chain reaction (PCR) can be carried out generally as in PCR Protocols: A Guide to Methods and Applications, Academic Press, San Diego, Calif. (1990).

Vesicles

[00101] The sample can comprise vesicles. Methods of the invention can include assessing one or more vesicles, including assessing vesicle populations. A vesicle, as used herein, is a membrane vesicle that is shed from cells. Vesicles or membrane vesicles include without limitation: circulating microvesicles (cMVs), microvesicle, exosome, nanovesicle, dexosome, bleb, blebby, prostasome, microparticle, intralumenal vesicle, membrane fragment, intralumenal endosomal vesicle, endosomal-like vesicle, exocytosis vehicle, endosome vesicle, endosomal vesicle, apoptotic body, multivesicular body, secretory vesicle, phospholipid vesicle, liposomal vesicle, argosome, texasome, secresome, tolerosome, melanosome, oncosome, or exocytosed vehicle. Furthermore, although vesicles may be produced by different cellular processes, the methods of the invention are not limited to or reliant on any one mechanism, insofar as such vesicles are present in a biological sample and are capable of being characterized by the methods disclosed herein. Unless otherwise specified, methods that make use of a species of vesicle can be applied to other types of vesicles. Vesicles comprise spherical structures with a lipid bilayer similar to cell membranes which surrounds an inner compartment which can contain soluble components, sometimes referred to as the payload. In some embodiments, the methods of the invention make use of exosomes, which are small secreted vesicles of about 40–100 nm in diameter. For a review of membrane vesicles, including types and characterizations, see *Thery et al., Nat Rev Immunol. 2009 Aug;9(8):581-93*. Some properties of different types of vesicles include those in **Table 1**:

Table 1: Vesicle Properties

Feature	Exosomes	Microvesicles	Ectosomes	Membrane particles	Exosome-like vesicles	Apoptotic vesicles
Size	50-100 nm	100-1,000 nm	50-200 nm	50-80 nm	20-50 nm	50-500 nm
Density in sucrose	1.13-1.19 g/ml			1.04-1.07 g/ml	1.1 g/ml	1.16-1.28 g/ml
EM appearance	Cup shape	Irregular shape, electron dense	Bilamellar round structures	Round	Irregular shape	Heterogeneous
Sedimentation	100,000 g	10,000 g	160,000-200,000 g	100,000-200,000 g	175,000 g	1,200 g, 10,000 g, 100,000 g
Lipid composition	Enriched in cholesterol, sphingomyelin and ceramide; contains lipid rafts; expose PPS	Expose PPS	Enriched in cholesterol and diacylglycerol; expose PPS		No lipid rafts	
Major protein markers	Tetraspanins (e.g., CD63, CD9), Alix, TSG101	Integrins, selectins and CD40 ligand	CR1 and proteolytic enzymes; no CD63	CD133; no CD63	TNFRI	Histones
Intracellular origin	Internal compartments (endosomes)	Plasma membrane	Plasma membrane	Plasma membrane		

Abbreviations: phosphatidylserine (PPS); electron microscopy (EM)

[00102] Vesicles include shed membrane bound particles, or "microparticles," that are derived from either the plasma membrane or an internal membrane. Vesicles can be released into the extracellular environment from cells. Cells releasing vesicles include without limitation cells that originate from, or are derived from, the ectoderm, endoderm, or mesoderm. The cells may have undergone genetic, environmental, and/or any other variations or alterations. For example, the cell can be tumor cells. A vesicle can reflect any changes in the source cell, and thereby reflect changes in the originating cells, e.g., cells having various genetic mutations. In one mechanism, a vesicle is generated intracellularly when a segment of the cell membrane spontaneously invaginates and is ultimately exocytosed (see for example, *Keller et al., Immunol. Lett. 107 (2): 102–8 (2006)*). Vesicles also include cell-derived structures bounded by a lipid bilayer membrane arising from both herniated evagination (blebbing) separation and sealing of portions of the plasma membrane or from the export of any intracellular membrane-bounded vesicular structure containing various membrane-associated proteins of tumor origin, including surface-bound molecules derived from the host circulation that bind selectively to the tumor-derived proteins together with molecules contained in the vesicle lumen, including but not limited to tumor-derived microRNAs or intracellular proteins. Blebs and blebbing are further described in *Charras et al., Nature Reviews Molecular and Cell Biology, Vol. 9, No. 11, p. 730-736 (2008)*. A vesicle shed into circulation or bodily fluids from tumor cells may be referred to as a "circulating tumor-derived vesicle." When such vesicle is an exosome, it may be referred to as a circulating-tumor derived exosome (CTE). In some instances, a

vesicle can be derived from a specific cell of origin. CTE, as with a cell-of-origin specific vesicle, typically have one or more unique biomarkers that permit isolation of the CTE or cell-of-origin specific vesicle, e.g., from a bodily fluid and sometimes in a specific manner. For example, a cell or tissue specific markers are used to identify the cell of origin. Examples of such cell or tissue specific markers are disclosed herein and can further be accessed in the Tissue-specific Gene Expression and Regulation (TiGER) Database, available at bioinfo.wilmer.jhu.edu/tiger/; Liu et al. (2008) TiGER: a database for tissue-specific gene expression and regulation. BMC Bioinformatics. 9:271; TissueDistributionDBs, available at genome.dkfz-heidelberg.de/menu/tissue_db/index.html.

[00103] A vesicle can have a diameter of greater than about 10 nm, 20 nm, or 30 nm. A vesicle can have a diameter of greater than 40 nm, 50 nm, 100 nm, 200 nm, 500 nm, 1000 nm or greater than 10,000 nm. A vesicle can have a diameter of about 30-1000 nm, about 30-800 nm, about 30-200 nm, or about 30-100 nm. In some embodiments, the vesicle has a diameter of less than 10,000 nm, 1000 nm, 800 nm, 500 nm, 200 nm, 100 nm, 50 nm, 40 nm, 30 nm, 20 nm or less than 10 nm. As used herein the term "about" in reference to a numerical value means that variations of 10% above or below the numerical value are within the range ascribed to the specified value. Typical sizes for various types of vesicles are shown in **Table 1**. Vesicles can be assessed to measure the diameter of a single vesicle or any number of vesicles. For example, the range of diameters of a vesicle population or an average diameter of a vesicle population can be determined. Vesicle diameter can be assessed using methods known in the art, e.g., imaging technologies such as electron microscopy. In an embodiment, a diameter of one or more vesicles is determined using optical particle detection. See, e.g., U.S. Patent 7,751,053, entitled "Optical Detection and Analysis of Particles" and issued July 6, 2010; and U.S. Patent 7,399,600, entitled "Optical Detection and Analysis of Particles" and issued July 15, 2010.

[00104] In some embodiments, vesicles are directly assayed from a biological sample without prior isolation, purification, or concentration from the biological sample. For example, the amount of vesicles in the sample can by itself provide a biosignature that provides a diagnostic, prognostic or theranostic determination. Alternatively, the vesicle in the sample may be isolated, captured, purified, or concentrated from a sample prior to analysis. As noted, isolation, capture or purification as used herein comprises partial isolation, partial capture or partial purification apart from other components in the sample. Vesicle isolation can be performed using various techniques as described herein or known in the art, including without limitation size exclusion chromatography, density gradient centrifugation, differential centrifugation, nanomembrane ultrafiltration, immunoabsorbent capture, affinity purification, affinity capture, immunoassay, immunoprecipitation, microfluidic separation, flow cytometry or combinations thereof.

[00105] Vesicles can be assessed to provide a phenotypic characterization by comparing vesicle characteristics to a reference. In some embodiments, surface antigens on a vesicle are assessed. A vesicle or vesicle population carrying a specific marker can be referred to as a positive (biomarker+) vesicle or vesicle population. For example, a DLL4+ population refers to a vesicle population associated with DLL4. Conversely, a DLL4- population would not be associated with DLL4. The surface antigens can

provide an indication of the anatomical origin and/or cellular of the vesicles and other phenotypic information, e.g., tumor status. For example, vesicles found in a patient sample can be assessed for surface antigens indicative of colorectal origin and the presence of cancer, thereby identifying vesicles associated with colorectal cancer cells. The surface antigens may comprise any informative biological entity that can be detected on the vesicle membrane surface, including without limitation surface proteins, lipids, carbohydrates, and other membrane components. For example, positive detection of colon derived vesicles expressing tumor antigens can indicate that the patient has colorectal cancer. As such, methods of the invention can be used to characterize any disease or condition associated with an anatomical or cellular origin, by assessing, for example, disease-specific and cell-specific biomarkers of one or more vesicles obtained from a subject.

[00106] In embodiments, one or more vesicle payloads are assessed to provide a phenotypic characterization. The payload with a vesicle comprises any informative biological entity that can be detected as encapsulated within the vesicle, including without limitation proteins and nucleic acids, e.g., genomic or cDNA, mRNA, or functional fragments thereof, as well as microRNAs (miRs). In addition, methods of the invention are directed to detecting vesicle surface antigens (in addition or exclusive to vesicle payload) to provide a phenotypic characterization. For example, vesicles can be characterized by using binding agents (e.g., antibodies or aptamers) that are specific to vesicle surface antigens, and the bound vesicles can be further assessed to identify one or more payload components disclosed therein. As described herein, the levels of vesicles with surface antigens of interest or with payload of interest can be compared to a reference to characterize a phenotype. For example, overexpression in a sample of cancer-related surface antigens or vesicle payload, e.g., a tumor associated mRNA or microRNA, as compared to a reference, can indicate the presence of cancer in the sample. The biomarkers assessed can be present or absent, increased or reduced based on the selection of the desired target sample and comparison of the target sample to the desired reference sample. Non-limiting examples of target samples include: disease; treated/not-treated; different time points, such as a in a longitudinal study; and non-limiting examples of reference sample: non-disease; normal; different time points; and sensitive or resistant to candidate treatment(s).

[00107] In an embodiment, molecular profiling of the invention comprises analysis of microvesicles, such as circulating microvesicles.

MicroRNA

[00108] Various biomarker molecules can be assessed in biological samples or vesicles obtained from such biological samples. MicroRNAs comprise one class biomarkers assessed via methods of the invention. MicroRNAs, also referred to herein as miRNAs or miRs, are short RNA strands approximately 21-23 nucleotides in length. MiRNAs are encoded by genes that are transcribed from DNA but are not translated into protein and thus comprise non-coding RNA. The miRs are processed from primary transcripts known as pri-miRNA to short stem-loop structures called pre-miRNA and finally to the resulting single strand miRNA. The pre-miRNA typically forms a structure that folds back on itself in self-complementary regions. These structures are then processed by the nuclease Dicer in animals or

DCL1 in plants. Mature miRNA molecules are partially complementary to one or more messenger RNA (mRNA) molecules and can function to regulate translation of proteins. Identified sequences of miRNA can be accessed at publicly available databases, such as www.microRNA.org, www.mirbase.org, or www.mirz.unibas.ch/cgi/miRNA.cgi.

[00109] miRNAs are generally assigned a number according to the naming convention " mir-[number]." The number of a miRNA is assigned according to its order of discovery relative to previously identified miRNA species. For example, if the last published miRNA was mir-121, the next discovered miRNA will be named mir-122, etc. When a miRNA is discovered that is homologous to a known miRNA from a different organism, the name can be given an optional organism identifier, of the form [organism identifier]- mir-[number]. Identifiers include hsa for Homo sapiens and mmu for Mus Musculus. For example, a human homolog to mir-121 might be referred to as hsa-mir-121 whereas the mouse homolog can be referred to as mmu-mir-121.

[00110] Mature microRNA is commonly designated with the prefix "miR" whereas the gene or precursor miRNA is designated with the prefix "mir." For example, mir-121 is a precursor for miR-121. When differing miRNA genes or precursors are processed into identical mature miRNAs, the genes/precursors can be delineated by a numbered suffix. For example, mir-121-1 and mir-121-2 can refer to distinct genes or precursors that are processed into miR-121. Lettered suffixes are used to indicate closely related mature sequences. For example, mir-121a and mir-121b can be processed to closely related miRNAs miR-121a and miR-121b, respectively. In the context of the invention, any microRNA (miRNA or miR) designated herein with the prefix mir-* or miR-* is understood to encompass both the precursor and/or mature species, unless otherwise explicitly stated otherwise.

[00111] Sometimes it is observed that two mature miRNA sequences originate from the same precursor. When one of the sequences is more abundant that the other, a "*" suffix can be used to designate the less common variant. For example, miR-121 would be the predominant product whereas miR-121* is the less common variant found on the opposite arm of the precursor. If the predominant variant is not identified, the miRs can be distinguished by the suffix "5p" for the variant from the 5' arm of the precursor and the suffix "3p" for the variant from the 3' arm. For example, miR-121-5p originates from the 5' arm of the precursor whereas miR-121-3p originates from the 3' arm. Less commonly, the 5p and 3p variants are referred to as the sense ("s") and anti-sense ("as") forms, respectively. For example, miR-121-5p may be referred to as miR-121-s whereas miR-121-3p may be referred to as miR-121-as.

[00112] The above naming conventions have evolved over time and are general guidelines rather than absolute rules. For example, the let- and lin- families of miRNAs continue to be referred to by these monikers. The mir/miR convention for precursor/mature forms is also a guideline and context should be taken into account to determine which form is referred to. Further details of miR naming can be found at www.mirbase.org or Ambros et al., A uniform system for microRNA annotation, RNA **9**:277-279 (2003).

[00113] Plant miRNAs follow a different naming convention as described in Meyers et al., Plant Cell. 2008 20(12):3186-3190.

[00114] A number of miRNAs are involved in gene regulation, and miRNAs are part of a growing class of non-coding RNAs that is now recognized as a major tier of gene control. In some cases, miRNAs can interrupt translation by binding to regulatory sites embedded in the 3′-UTRs of their target mRNAs, leading to the repression of translation. Target recognition involves complementary base pairing of the target site with the miRNA's seed region (positions 2–8 at the miRNA's 5′ end), although the exact extent of seed complementarity is not precisely determined and can be modified by 3′ pairing. In other cases, miRNAs function like small interfering RNAs (siRNA) and bind to perfectly complementary mRNA sequences to destroy the target transcript.

[00115] Characterization of a number of miRNAs indicates that they influence a variety of processes, including early development, cell proliferation and cell death, apoptosis and fat metabolism. For example, some miRNAs, such as lin-4, let-7, mir-14, mir-23, and bantam, have been shown to play critical roles in cell differentiation and tissue development. Others are believed to have similarly important roles because of their differential spatial and temporal expression patterns.

[00116] The miRNA database available at miRBase (www.mirbase.org) comprises a searchable database of published miRNA sequences and annotation. Further information about miRBase can be found in the following articles, each of which is incorporated by reference in its entirety herein: Griffiths-Jones et al., miRBase: tools for microRNA genomics. NAR 2008 36(Database Issue):D154-D158; Griffiths-Jones et al., miRBase: microRNA sequences, targets and gene nomenclature. NAR 2006 34(Database Issue):D140-D144; and Griffiths-Jones, S. The microRNA Registry. NAR 2004 32(Database Issue):D109-D111. Representative miRNAs contained in Release 16 of miRBase, made available September 2010.

[00117] As described herein, microRNAs are known to be involved in cancer and other diseases and can be assessed in order to characterize a phenotype in a sample. See, e.g., Ferracin et al., Micromarkers: miRNAs in cancer diagnosis and prognosis, Exp Rev Mol Diag, Apr 2010, Vol. 10, No. 3, Pages 297-308; Fabbri, miRNAs as molecular biomarkers of cancer, Exp Rev Mol Diag, May 2010, Vol. 10, No. 4, Pages 435-444.

[00118] In an embodiment, molecular profiling of the invention comprises analysis of microRNA.

[00119] Techniques to isolate and characterize vesicles and miRs are known to those of skill in the art. In addition to the methodology presented herein, additional methods can be found in U.S. Patent Nos. 7,888,035, entitled "METHODS FOR ASSESSING RNA PATTERNS" and issued February 15, 2011; and 7,897,356, entitled "METHODS AND SYSTEMS OF USING EXOSOMES FOR DETERMINING PHENOTYPES" and issued March 1, 2011; and International Patent Publication Nos. WO/2011/066589, entitled "METHODS AND SYSTEMS FOR ISOLATING, STORING, AND ANALYZING VESICLES" and filed November 30, 2010; WO/2011/088226, entitled "DETECTION OF GASTROINTESTINAL DISORDERS" and filed January 13, 2011; WO/2011/109440, entitled "BIOMARKERS FOR THERANOSTICS" and filed March 1, 2011; and WO/2011/127219, entitled "CIRCULATING BIOMARKERS FOR DISEASE" and filed April 6, 2011, each of which applications are incorporated by reference herein in their entirety.

Circulating Biomarkers

[00120] Circulating biomarkers include biomarkers that are detectable in body fluids, such as blood, plasma, serum. Examples of circulating cancer biomarkers include cardiac troponin T (cTnT), prostate specific antigen (PSA) for prostate cancer and CA125 for ovarian cancer. Circulating biomarkers according to the invention include any appropriate biomarker that can be detected in bodily fluid, including without limitation protein, nucleic acids, e.g., DNA, mRNA and microRNA, lipids, carbohydrates and metabolites. Circulating biomarkers can include biomarkers that are not associated with cells, such as biomarkers that are membrane associated, embedded in membrane fragments, part of a biological complex, or free in solution. In one embodiment, circulating biomarkers are biomarkers that are associated with one or more vesicles present in the biological fluid of a subject.

[00121] Circulating biomarkers have been identified for use in characterization of various phenotypes, such as detection of a cancer. See, e.g., Ahmed N, et al., Proteomic-based identification of haptoglobin-1 precursor as a novel circulating biomarker of ovarian cancer. Br. J. Cancer 2004; Mathelin et al., Circulating proteinic biomarkers and breast cancer, Gynecol Obstet Fertil. 2006 Jul-Aug;34(7-8):638-46. Epub 2006 Jul 28; Ye et al., Recent technical strategies to identify diagnostic biomarkers for ovarian cancer. Expert Rev Proteomics. 2007 Feb;4(1):121-31; Carney, Circulating oncoproteins HER2/neu, EGFR and CAIX (MN) as novel cancer biomarkers. Expert Rev Mol Diagn. 2007 May;7(3):309-19; Gagnon, Discovery and application of protein biomarkers for ovarian cancer, Curr Opin Obstet Gynecol. 2008 Feb;20(1):9-13; Pasterkamp et al., Immune regulatory cells: circulating biomarker factories in cardiovascular disease. Clin Sci (Lond). 2008 Aug;115(4):129-31; Fabbri, miRNAs as molecular biomarkers of cancer, Exp Rev Mol Diag, May 2010, Vol. 10, No. 4, Pages 435-444; PCT Patent Publication WO/2007/088537; U.S. Patents 7,745,150 and 7,655,479; U.S. Patent Publications 20110008808, 20100330683, 20100248290, 20100222230, 20100203566, 20100173788, 20090291932, 20090239246, 20090226937, 20090111121, 20090004687, 20080261258, 20080213907, 20060003465, 20050124071, and 20040096915, each of which publication is incorporated herein by reference in its entirety. In an embodiment, molecular profiling of the invention comprises analysis of circulating biomarkers.

Gene Expression Profiling

[00122] The methods and systems of the invention comprise expression profiling, which includes assessing differential expression of one or more target genes disclosed herein. Differential expression can include overexpression and/or underexpression of a biological product, e.g., a gene, mRNA or protein, compared to a control (or a reference). The control can include similar cells to the sample but without the disease (e.g., expression profiles obtained from samples from healthy individuals). A control can be a previously determined level that is indicative of a drug target efficacy associated with the particular disease and the particular drug target. The control can be derived from the same patient, e.g., a normal adjacent portion of the same organ as the diseased cells, the control can be derived from healthy tissues from other patients, or previously determined thresholds that are indicative of a disease responding or not-responding to a particular drug target. The control can also be a control found in the same sample, e.g. a

housekeeping gene or a product thereof (e.g., mRNA or protein). For example, a control nucleic acid can be one which is known not to differ depending on the cancerous or non-cancerous state of the cell. The expression level of a control nucleic acid can be used to normalize signal levels in the test and reference populations. Illustrative control genes include, but are not limited to, e.g., β-actin, glyceraldehyde 3-phosphate dehydrogenase and ribosomal protein P1. Multiple controls or types of controls can be used. The source of differential expression can vary. For example, a gene copy number may be increased in a cell, thereby resulting in increased expression of the gene. Alternately, transcription of the gene may be modified, e.g., by chromatin remodeling, differential methylation, differential expression or activity of transcription factors, etc. Translation may also be modified, e.g., by differential expression of factors that degrade mRNA, translate mRNA, or silence translation, e.g., microRNAs or siRNAs. In some embodiments, differential expression comprises differential activity. For example, a protein may carry a mutation that increases the activity of the protein, such as constitutive activation, thereby contributing to a diseased state. Molecular profiling that reveals changes in activity can be used to guide treatment selection.

Methods of gene expression profiling include methods based on hybridization analysis of polynucleotides, and methods based on sequencing of polynucleotides. Commonly used methods known in the art for the quantification of mRNA expression in a sample include northern blotting and in situ hybridization (Parker & Barnes (1999) Methods in Molecular Biology 106:247-283); RNAse protection assays (Hod (1992) Biotechniques 13:852-854); and reverse transcription polymerase chain reaction (RT-PCR) (Weis et al. (1992) Trends in Genetics 8:263-264). Alternatively, antibodies may be employed that can recognize specific duplexes, including DNA duplexes, RNA duplexes, and DNA-RNA hybrid duplexes or DNA-protein duplexes. Representative methods for sequencing-based gene expression analysis include Serial Analysis of Gene Expression (SAGE), gene expression analysis by massively parallel signature sequencing (MPSS) and/or next generation sequencing.

[00123] ***RT-PCR***

[00124] Reverse transcription polymerase chain reaction (RT-PCR) is a variant of polymerase chain reaction (PCR). According to this technique, a RNA strand is reverse transcribed into its DNA complement (i.e., complementary DNA, or cDNA) using the enzyme reverse transcriptase, and the resulting cDNA is amplified using PCR. Real-time polymerase chain reaction is another PCR variant, which is also referred to as quantitative PCR, Q-PCR, qRT-PCR, or sometimes as RT-PCR. Either the reverse transcription PCR method or the real-time PCR method can be used for molecular profiling according to the invention, and RT-PCR can refer to either unless otherwise specified or as understood by one of skill in the art.

[00125] RT-PCR can be used to determine RNA levels, e.g., mRNA or miRNA levels, of the biomarkers of the invention. RT-PCR can be used to compare such RNA levels of the biomarkers of the invention in different sample populations, in normal and tumor tissues, with or without drug treatment, to characterize patterns of gene expression, to discriminate between closely related RNAs, and to analyze RNA structure.

[00126] The first step is the isolation of RNA, e.g., mRNA, from a sample. The starting material can be total RNA isolated from human tumors or tumor cell lines, and corresponding normal tissues or cell lines, respectively. Thus RNA can be isolated from a sample, e.g., tumor cells or tumor cell lines, and compared with pooled DNA from healthy donors. If the source of mRNA is a primary tumor, mRNA can be extracted, for example, from frozen or archived paraffin-embedded and fixed (e.g. formalin-fixed) tissue samples.

[00127] General methods for mRNA extraction are well known in the art and are disclosed in standard textbooks of molecular biology, including Ausubel et al. (1997) Current Protocols of Molecular Biology, John Wiley and Sons. Methods for RNA extraction from paraffin embedded tissues are disclosed, for example, in Rupp & Locker (1987) Lab Invest. 56:A67, and De Andres et al., BioTechniques 18:42044 (1995). In particular, RNA isolation can be performed using purification kit, buffer set and protease from commercial manufacturers, such as Qiagen, according to the manufacturer's instructions (QIAGEN Inc., Valencia, CA). For example, total RNA from cells in culture can be isolated using Qiagen RNeasy mini-columns. Numerous RNA isolation kits are commercially available and can be used in the methods of the invention.

[00128] In the alternative, the first step is the isolation of miRNA from a target sample. The starting material is typically total RNA isolated from human tumors or tumor cell lines, and corresponding normal tissues or cell lines, respectively. Thus RNA can be isolated from a variety of primary tumors or tumor cell lines, with pooled DNA from healthy donors. If the source of miRNA is a primary tumor, miRNA can be extracted, for example, from frozen or archived paraffin-embedded and fixed (e.g. formalin-fixed) tissue samples.

[00129] General methods for miRNA extraction are well known in the art and are disclosed in standard textbooks of molecular biology, including Ausubel et al. (1997) Current Protocols of Molecular Biology, John Wiley and Sons. Methods for RNA extraction from paraffin embedded tissues are disclosed, for example, in Rupp & Locker (1987) Lab Invest. 56:A67, and De Andres et al., BioTechniques 18:42044 (1995). In particular, RNA isolation can be performed using purification kit, buffer set and protease from commercial manufacturers, such as Qiagen, according to the manufacturer's instructions. For example, total RNA from cells in culture can be isolated using Qiagen RNeasy mini-columns. Numerous miRNA isolation kits are commercially available and can be used in the methods of the invention.

[00130] Whether the RNA comprises mRNA, miRNA or other types of RNA, gene expression profiling by RT-PCR can include reverse transcription of the RNA template into cDNA, followed by amplification in a PCR reaction. Commonly used reverse transcriptases include, but are not limited to, avilo myeloblastosis virus reverse transcriptase (AMV-RT) and Moloney murine leukemia virus reverse transcriptase (MMLV-RT). The reverse transcription step is typically primed using specific primers, random hexamers, or oligo-dT primers, depending on the circumstances and the goal of expression profiling. For example, extracted RNA can be reverse-transcribed using a GeneAmp RNA PCR kit (Perkin Elmer, Calif., USA), following the manufacturer's instructions. The derived cDNA can then be used as a template in the subsequent PCR reaction.

[00131] Although the PCR step can use a variety of thermostable DNA-dependent DNA polymerases, it typically employs the Taq DNA polymerase, which has a 5'-3' nuclease activity but lacks a 3'-5' proofreading endonuclease activity. TaqMan PCR typically uses the 5'-nuclease activity of Taq or Tth polymerase to hydrolyze a hybridization probe bound to its target amplicon, but any enzyme with equivalent 5' nuclease activity can be used. Two oligonucleotide primers are used to generate an amplicon typical of a PCR reaction. A third oligonucleotide, or probe, is designed to detect nucleotide sequence located between the two PCR primers. The probe is non-extendible by Taq DNA polymerase enzyme, and is labeled with a reporter fluorescent dye and a quencher fluorescent dye. Any laser-induced emission from the reporter dye is quenched by the quenching dye when the two dyes are located close together as they are on the probe. During the amplification reaction, the Taq DNA polymerase enzyme cleaves the probe in a template-dependent manner. The resultant probe fragments disassociate in solution, and signal from the released reporter dye is free from the quenching effect of the second fluorophore. One molecule of reporter dye is liberated for each new molecule synthesized, and detection of the unquenched reporter dye provides the basis for quantitative interpretation of the data.

[00132] TaqMan™ RT-PCR can be performed using commercially available equipment, such as, for example, ABI PRISM 7700™ Sequence Detection System™ (Perkin-Elmer-Applied Biosystems, Foster City, Calif., USA), or LightCycler (Roche Molecular Biochemicals, Mannheim, Germany). In one specific embodiment, the 5' nuclease procedure is run on a real-time quantitative PCR device such as the ABI PRISM 7700 Sequence Detection System. The system consists of a thermocycler, laser, charge-coupled device (CCD), camera and computer. The system amplifies samples in a 96-well format on a thermocycler. During amplification, laser-induced fluorescent signal is collected in real-time through fiber optic cables for all 96 wells, and detected at the CCD. The system includes software for running the instrument and for analyzing the data.

[00133] TaqMan data are initially expressed as Ct, or the threshold cycle. As discussed above, fluorescence values are recorded during every cycle and represent the amount of product amplified to that point in the amplification reaction. The point when the fluorescent signal is first recorded as statistically significant is the threshold cycle (Ct).

[00134] To minimize errors and the effect of sample-to-sample variation, RT-PCR is usually performed using an internal standard. The ideal internal standard is expressed at a constant level among different tissues, and is unaffected by the experimental treatment. RNAs most frequently used to normalize patterns of gene expression are mRNAs for the housekeeping genes glyceraldehyde-3-phosphate-dehydrogenase (GAPDH) and β-actin.

[00135] Real time quantitative PCR (also quantitative real time polymerase chain reaction, QRT-PCR or Q-PCR) is a more recent variation of the RT-PCR technique. Q-PCR can measure PCR product accumulation through a dual-labeled fluorigenic probe (i.e., TaqMan probe). Real time PCR is compatible both with quantitative competitive PCR, where internal competitor for each target sequence is used for normalization, and with quantitative comparative PCR using a normalization gene contained within the sample, or a housekeeping gene for RT-PCR. See, e.g. Held et al. (1996) Genome Research 6:986-994.

[00136] Protein-based detection techniques are also useful for molecular profiling, especially when the nucleotide variant causes amino acid substitutions or deletions or insertions or frame shift that affect the protein primary, secondary or tertiary structure. To detect the amino acid variations, protein sequencing techniques may be used. For example, a protein or fragment thereof corresponding to a gene can be synthesized by recombinant expression using a DNA fragment isolated from an individual to be tested. Preferably, a cDNA fragment of no more than 100 to 150 base pairs encompassing the polymorphic locus to be determined is used. The amino acid sequence of the peptide can then be determined by conventional protein sequencing methods. Alternatively, the HPLC-microscopy tandem mass spectrometry technique can be used for determining the amino acid sequence variations. In this technique, proteolytic digestion is performed on a protein, and the resulting peptide mixture is separated by reversed-phase chromatographic separation. Tandem mass spectrometry is then performed and the data collected is analyzed. See Gatlin et al., Anal. Chem., 72:757-763 (2000).

[00137] ***Microarray***

[00138] The biomarkers of the invention can also be identified, confirmed, and/or measured using the microarray technique. Thus, the expression profile biomarkers can be measured in cancer samples using microarray technology. In this method, polynucleotide sequences of interest are plated, or arrayed, on a microchip substrate. The arrayed sequences are then hybridized with specific DNA probes from cells or tissues of interest. The source of mRNA can be total RNA isolated from a sample, e.g., human tumors or tumor cell lines and corresponding normal tissues or cell lines. Thus RNA can be isolated from a variety of primary tumors or tumor cell lines. If the source of mRNA is a primary tumor, mRNA can be extracted, for example, from frozen or archived paraffin-embedded and fixed (e.g. formalin-fixed) tissue samples, which are routinely prepared and preserved in everyday clinical practice.

[00139] The expression profile of biomarkers can be measured in either fresh or paraffin-embedded tumor tissue, or body fluids using microarray technology. In this method, polynucleotide sequences of interest are plated, or arrayed, on a microchip substrate. The arrayed sequences are then hybridized with specific DNA probes from cells or tissues of interest. As with the RT-PCR method, the source of miRNA typically is total RNA isolated from human tumors or tumor cell lines, including body fluids, such as serum, urine, tears, and exosomes and corresponding normal tissues or cell lines. Thus RNA can be isolated from a variety of sources. If the source of miRNA is a primary tumor, miRNA can be extracted, for example, from frozen tissue samples, which are routinely prepared and preserved in everyday clinical practice.

[00140] Also known as biochip, DNA chip, or gene array, cDNA microarray technology allows for identification of gene expression levels in a biologic sample. cDNAs or oligonucleotides, each representing a given gene, are immobilized on a substrate, e.g., a small chip, bead or nylon membrane, tagged, and serve as probes that will indicate whether they are expressed in biologic samples of interest. The simultaneous expression of thousands of genes can be monitored simultaneously.

[00141] In a specific embodiment of the microarray technique, PCR amplified inserts of cDNA clones are applied to a substrate in a dense array. In one aspect, at least 100, 200, 300, 400, 500, 600, 700, 800, 900, 1,000, 1,500, 2,000, 3000, 4000, 5000, 6000, 7000, 8000, 9000, 10,000, 15,000, 20,000, 25,000, 30,000,

35,000, 40,000, 45,000 or at least 50,000 nucleotide sequences are applied to the substrate. Each sequence can correspond to a different gene, or multiple sequences can be arrayed per gene. The microarrayed genes, immobilized on the microchip, are suitable for hybridization under stringent conditions. Fluorescently labeled cDNA probes may be generated through incorporation of fluorescent nucleotides by reverse transcription of RNA extracted from tissues of interest. Labeled cDNA probes applied to the chip hybridize with specificity to each spot of DNA on the array. After stringent washing to remove non-specifically bound probes, the chip is scanned by confocal laser microscopy or by another detection method, such as a CCD camera. Quantitation of hybridization of each arrayed element allows for assessment of corresponding mRNA abundance. With dual color fluorescence, separately labeled cDNA probes generated from two sources of RNA are hybridized pairwise to the array. The relative abundance of the transcripts from the two sources corresponding to each specified gene is thus determined simultaneously. The miniaturized scale of the hybridization affords a convenient and rapid evaluation of the expression pattern for large numbers of genes. Such methods have been shown to have the sensitivity required to detect rare transcripts, which are expressed at a few copies per cell, and to reproducibly detect at least approximately two-fold differences in the expression levels (Schena et al. (1996) Proc. Natl. Acad. Sci. USA 93(2):106-149). Microarray analysis can be performed by commercially available equipment following manufacturer's protocols, including without limitation the Affymetrix GeneChip technology (Affymetrix, Santa Clara, CA), Agilent (Agilent Technologies, Inc., Santa Clara, CA), or Illumina (Illumina, Inc., San Diego, CA) microarray technology.

[00142] The development of microarray methods for large-scale analysis of gene expression makes it possible to search systematically for molecular markers of cancer classification and outcome prediction in a variety of tumor types.

[00143] In some embodiments, the Agilent Whole Human Genome Microarray Kit (Agilent Technologies, Inc., Santa Clara, CA). The system can analyze more than 41,000 unique human genes and transcripts represented, all with public domain annotations. The system is used according to the manufacturer's instructions.

[00144] In some embodiments, the Illumina Whole Genome DASL assay (Illumina Inc., San Diego, CA) is used. The system offers a method to simultaneously profile over 24,000 transcripts from minimal RNA input, from both fresh frozen (FF) and formalin-fixed paraffin embedded (FFPE) tissue sources, in a high throughput fashion.

[00145] Microarray expression analysis comprises identifying whether a gene or gene product is up-regulated or down-regulated relative to a reference. The identification can be performed using a statistical test to determine statistical significance of any differential expression observed. In some embodiments, statistical significance is determined using a parametric statistical test. The parametric statistical test can comprise, for example, a fractional factorial design, analysis of variance (ANOVA), a t-test, least squares, a Pearson correlation, simple linear regression, nonlinear regression, multiple linear regression, or multiple nonlinear regression. Alternatively, the parametric statistical test can comprise a one-way analysis of variance, two-way analysis of variance, or repeated measures analysis of variance. In other

embodiments, statistical significance is determined using a nonparametric statistical test. Examples include, but are not limited to, a Wilcoxon signed-rank test, a Mann-Whitney test, a Kruskal-Wallis test, a Friedman test, a Spearman ranked order correlation coefficient, a Kendall Tau analysis, and a nonparametric regression test. In some embodiments, statistical significance is determined at a p-value of less than about 0.05, 0.01, 0.005, 0.001, 0.0005, or 0.0001. Although the microarray systems used in the methods of the invention may assay thousands of transcripts, data analysis need only be performed on the transcripts of interest, thereby reducing the problem of multiple comparisons inherent in performing multiple statistical tests. The p-values can also be corrected for multiple comparisons, e.g., using a Bonferroni correction, a modification thereof, or other technique known to those in the art, e.g., the Hochberg correction, Holm-Bonferroni correction, Šidák correction, or Dunnett's correction. The degree of differential expression can also be taken into account. For example, a gene can be considered as differentially expressed when the fold-change in expression compared to control level is at least 1.2, 1.3, 1.4, 1.5, 1.6, 1.7, 1.8, 1.9, 2.0, 2.2, 2.5, 2.7, 3.0, 4, 5, 6, 7, 8, 9 or 10-fold different in the sample versus the control. The differential expression takes into account both overexpression and underexpression. A gene or gene product can be considered up or down-regulated if the differential expression meets a statistical threshold, a fold-change threshold, or both. For example, the criteria for identifying differential expression can comprise both a p-value of 0.001 and fold change of at least 1.5-fold (up or down). One of skill will understand that such statistical and threshold measures can be adapted to determine differential expression by any molecular profiling technique disclosed herein.

[00146] Various methods of the invention make use of many types of microarrays that detect the presence and potentially the amount of biological entities in a sample. Arrays typically contain addressable moieties that can detect the presence of the entity in the sample, e.g., via a binding event. Microarrays include without limitation DNA microarrays, such as cDNA microarrays, oligonucleotide microarrays and SNP microarrays, microRNA arrays, protein microarrays, antibody microarrays, tissue microarrays, cellular microarrays (also called transfection microarrays), chemical compound microarrays, and carbohydrate arrays (glycoarrays). DNA arrays typically comprise addressable nucleotide sequences that can bind to sequences present in a sample. MicroRNA arrays, e.g., the MMChips array from the University of Louisville or commercial systems from Agilent, can be used to detect microRNAs. Protein microarrays can be used to identify protein–protein interactions, including without limitation identifying substrates of protein kinases, transcription factor protein-activation, or to identify the targets of biologically active small molecules. Protein arrays may comprise an array of different protein molecules, commonly antibodies, or nucleotide sequences that bind to proteins of interest. Antibody microarrays comprise antibodies spotted onto the protein chip that are used as capture molecules to detect proteins or other biological materials from a sample, e.g., from cell or tissue lysate solutions. For example, antibody arrays can be used to detect biomarkers from bodily fluids, e.g., serum or urine, for diagnostic applications. Tissue microarrays comprise separate tissue cores assembled in array fashion to allow multiplex histological analysis. Cellular microarrays, also called transfection microarrays, comprise various capture agents, such as antibodies, proteins, or lipids, which can interact with cells to facilitate

their capture on addressable locations. Chemical compound microarrays comprise arrays of chemical compounds and can be used to detect protein or other biological materials that bind the compounds. Carbohydrate arrays (glycoarrays) comprise arrays of carbohydrates and can detect, e.g., protein that bind sugar moieties. One of skill will appreciate that similar technologies or improvements can be used according to the methods of the invention.

[00147] Certain embodiments of the current methods comprise a multi-well reaction vessel, including without limitation, a multi-well plate or a multi-chambered microfluidic device, in which a multiplicity of amplification reactions and, in some embodiments, detection are performed, typically in parallel. In certain embodiments, one or more multiplex reactions for generating amplicons are performed in the same reaction vessel, including without limitation, a multi-well plate, such as a 96-well, a 384-well, a 1536-well plate, and so forth; or a microfluidic device, for example but not limited to, a TaqMan™ Low Density Array (Applied Biosystems, Foster City, CA). In some embodiments, a massively parallel amplifying step comprises a multi-well reaction vessel, including a plate comprising multiple reaction wells, for example but not limited to, a 24-well plate, a 96-well plate, a 384-well plate, or a 1536-well plate; or a multi-chamber microfluidics device, for example but not limited to a low density array wherein each chamber or well comprises an appropriate primer(s), primer set(s), and/or reporter probe(s), as appropriate. Typically such amplification steps occur in a series of parallel single-plex, two-plex, three-plex, four-plex, five-plex, or six-plex reactions, although higher levels of parallel multiplexing are also within the intended scope of the current teachings. These methods can comprise PCR methodology, such as RT-PCR, in each of the wells or chambers to amplify and/or detect nucleic acid molecules of interest.

[00148] Low density arrays can include arrays that detect 10s or 100s of molecules as opposed to 1000s of molecules. These arrays can be more sensitive than high density arrays. In embodiments, a low density array such as a TaqMan™ Low Density Array is used to detect one or more gene or gene product in **Table 2**, **Table 6** or **Table 17**. For example, the low density array can be used to detect at least 1, 2, 3, 4, 5, 6, 7, 8, 9, 10, 15, 20, 25, 30, 40, 50, 60, 70, 80, 90 or 100 genes or gene products in **Table 2**, **Table 6** or **Table 17**.

[00149] In some embodiments, the disclosed methods comprise a microfluidics device, "lab on a chip," or micrototal analytical system (pTAS). In some embodiments, sample preparation is performed using a microfluidics device. In some embodiments, an amplification reaction is performed using a microfluidics device. In some embodiments, a sequencing or PCR reaction is performed using a microfluidic device. In some embodiments, the nucleotide sequence of at least a part of an amplified product is obtained using a microfluidics device. In some embodiments, detecting comprises a microfluidic device, including without limitation, a low density array, such as a TaqMan™ Low Density Array. Descriptions of exemplary microfluidic devices can be found in, among other places, Published PCT Application Nos. WO/0185341 and WO 04/011666; Kartalov and Quake, Nucl. Acids Res. 32:2873-79, 2004; and Fiorini and Chiu, Bio Techniques 38:429-46, 2005.

Any appropriate microfluidic device can be used in the methods of the invention. Examples of microfluidic devices that may be used, or adapted for use with molecular profiling, include but are not

limited to those described in U.S. Pat. Nos. 7,591,936, 7,581,429, 7,579,136, 7,575,722, 7,568,399, 7,552,741, 7,544,506, 7,541,578, 7,518,726, 7,488,596, 7,485,214, 7,467,928, 7,452,713, 7,452,509, 7,449,096, 7,431,887, 7,422,725, 7,422,669, 7,419,822, 7,419,639, 7,413,709, 7,411,184, 7,402,229, 7,390,463, 7,381,471, 7,357,864, 7,351,592, 7,351,380, 7,338,637, 7,329,391, 7,323,140, 7,261,824, 7,258,837, 7,253,003, 7,238,324, 7,238,255, 7,233,865, 7,229,538, 7,201,881, 7,195,986, 7,189,581, 7,189,580, 7,189,368, 7,141,978, 7,138,062, 7,135,147, 7,125,711, 7,118,910, 7,118,661, 7,640,947, 7,666,361, 7,704,735; U.S. Patent Application Publication 20060035243; and International Patent Publication WO 2010/072410; each of which patents or applications are incorporated herein by reference in their entirety. Another example for use with methods disclosed herein is described in *Chen et al., "Microfluidic isolation and transcriptome analysis of serum vesicles," Lab on a Chip, Dec. 8, 2009 DOI: 10.1039/b916199f.*

[00150] ***Gene Expression Analysis by Massively Parallel Signature Sequencing (MPSS)***

[00151] This method, described by Brenner et al. (2000) Nature Biotechnology 18:630-634, is a sequencing approach that combines non-gel-based signature sequencing with in vitro cloning of millions of templates on separate microbeads. First, a microbead library of DNA templates is constructed by in vitro cloning. This is followed by the assembly of a planar array of the template-containing microbeads in a flow cell at a high density. The free ends of the cloned templates on each microbead are analyzed simultaneously, using a fluorescence-based signature sequencing method that does not require DNA fragment separation. This method has been shown to simultaneously and accurately provide, in a single operation, hundreds of thousands of gene signature sequences from a cDNA library.

[00152] MPSS data has many uses. The expression levels of nearly all transcripts can be quantitatively determined; the abundance of signatures is representative of the expression level of the gene in the analyzed tissue. Quantitative methods for the analysis of tag frequencies and detection of differences among libraries have been published and incorporated into public databases for SAGE™ data and are applicable to MPSS data. The availability of complete genome sequences permits the direct comparison of signatures to genomic sequences and further extends the utility of MPSS data. Because the targets for MPSS analysis are not pre-selected (like on a microarray), MPSS data can characterize the full complexity of transcriptomes. This is analogous to sequencing millions of ESTs at once, and genomic sequence data can be used so that the source of the MPSS signature can be readily identified by computational means.

[00153] ***Serial Analysis of Gene Expression (SAGE)***

[00154] Serial analysis of gene expression (SAGE) is a method that allows the simultaneous and quantitative analysis of a large number of gene transcripts, without the need of providing an individual hybridization probe for each transcript. First, a short sequence tag (e.g., about 10-14 bp) is generated that contains sufficient information to uniquely identify a transcript, provided that the tag is obtained from a unique position within each transcript. Then, many transcripts are linked together to form long serial molecules, that can be sequenced, revealing the identity of the multiple tags simultaneously. The expression pattern of any population of transcripts can be quantitatively evaluated by determining the

abundance of individual tags, and identifying the gene corresponding to each tag. See, e.g. Velculescu et al. (1995) Science 270:484-487; and Velculescu et al. (1997) Cell 88:243-51.

DNA Copy Number Profiling

[00155] Any method capable of determining a DNA copy number profile of a particular sample can be used for molecular profiling according to the invention as long as the resolution is sufficient to identify the biomarkers of the invention. The skilled artisan is aware of and capable of using a number of different platforms for assessing whole genome copy number changes at a resolution sufficient to identify the copy number of the one or more biomarkers of the invention. Some of the platforms and techniques are described in the embodiments below. In some embodiments of the invention, ISH techniques as described herein are also used for determining copy number / gene amplification.

[00156] In some embodiments, the copy number profile analysis involves amplification of whole genome DNA by a whole genome amplification method. The whole genome amplification method can use a strand displacing polymerase and random primers.

[00157] In some aspects of these embodiments, the copy number profile analysis involves hybridization of whole genome amplified DNA with a high density array. In a more specific aspect, the high density array has 5,000 or more different probes. In another specific aspect, the high density array has 5,000, 10,000, 20,000, 50,000, 100,000, 200,000, 300,000, 400,000, 500,000, 600,000, 700,000, 800,000, 900,000, or 1,000,000 or more different probes. In another specific aspect, each of the different probes on the array is an oligonucleotide having from about 15 to 200 bases in length. In another specific aspect, each of the different probes on the array is an oligonucleotide having from about 15 to 200, 15 to 150, 15 to 100, 15 to 75, 15 to 60, or 20 to 55 bases in length.

[00158] In some embodiments, a microarray is employed to aid in determining the copy number profile for a sample, e.g., cells from a tumor. Microarrays typically comprise a plurality of oligomers (e.g., DNA or RNA polynucleotides or oligonucleotides, or other polymers), synthesized or deposited on a substrate (e.g., glass support) in an array pattern. The support-bound oligomers are "probes", which function to hybridize or bind with a sample material (e.g., nucleic acids prepared or obtained from the tumor samples), in hybridization experiments. The reverse situation can also be applied: the sample can be bound to the microarray substrate and the oligomer probes are in solution for the hybridization. In use, the array surface is contacted with one or more targets under conditions that promote specific, high-affinity binding of the target to one or more of the probes. In some configurations, the sample nucleic acid is labeled with a detectable label, such as a fluorescent tag, so that the hybridized sample and probes are detectable with scanning equipment. DNA array technology offers the potential of using a multitude (e.g., hundreds of thousands) of different oligonucleotides to analyze DNA copy number profiles. In some embodiments, the substrates used for arrays are surface-derivatized glass or silica, or polymer membrane surfaces (see e.g., in Z. Guo, et al., Nucleic Acids Res, 22, 5456-65 (1994); U. Maskos, E. M. Southern, Nucleic Acids Res, 20, 1679-84 (1992), and E. M. Southern, et al., Nucleic Acids Res, 22, 1368-73 (1994), each incorporated by reference herein). Modification of surfaces of array substrates can be accomplished by many techniques. For example, siliceous or metal oxide surfaces can be derivatized with

bifunctional silanes, i.e., silanes having a first functional group enabling covalent binding to the surface (e.g., Si-halogen or Si-alkoxy group, as in --$SiCl_3$ or --$Si(OCH_3)_3$, respectively) and a second functional group that can impart the desired chemical and/or physical modifications to the surface to covalently or non-covalently attach ligands and/or the polymers or monomers for the biological probe array. Silylated derivatizations and other surface derivatizations that are known in the art (see for example U.S. Pat. No. 5,624,711 to Sundberg, U.S. Pat. No. 5,266,222 to Willis, and U.S. Pat. No. 5,137,765 to Farnsworth, each incorporated by reference herein). Other processes for preparing arrays are described in U.S. Pat. No. 6,649,348, to Bass et. al., assigned to Agilent Corp., which disclose DNA arrays created by in situ synthesis methods.

[00159] Polymer array synthesis is also described extensively in the literature including in the following: WO 00/58516, U.S. Pat. Nos. 5,143,854, 5,242,974, 5,252,743, 5,324,633, 5,384,261, 5,405,783, 5,424,186, 5,451,683, 5,482,867, 5,491,074, 5,527,681, 5,550,215, 5,571,639, 5,578,832, 5,593,839, 5,599,695, 5,624,711, 5,631,734, 5,795,716, 5,831,070, 5,837,832, 5,856,101, 5,858,659, 5,936,324, 5,968,740, 5,974,164, 5,981,185, 5,981,956, 6,025,601, 6,033,860, 6,040,193, 6,090,555, 6,136,269, 6,269,846 and 6,428,752, 5,412,087, 6,147,205, 6,262,216, 6,310,189, 5,889,165, and 5,959,098 in PCT Applications Nos. PCT/US99/00730 (International Publication No. WO 99/36760) and PCT/US01/04285 (International Publication No. WO 01/58593), which are all incorporated herein by reference in their entirety for all purposes.

[00160] Nucleic acid arrays that are useful in the present invention include, but are not limited to, those that are commercially available from Affymetrix (Santa Clara, Calif.) under the brand name GeneChip™. Example arrays are shown on the website at affymetrix.com. Another microarray supplier is Illumina, Inc., of San Diego, Calif. with example arrays shown on their website at illumina.com.

[00161] In some embodiments, the inventive methods provide for sample preparation. Depending on the microarray and experiment to be performed, sample nucleic acid can be prepared in a number of ways by methods known to the skilled artisan. In some aspects of the invention, prior to or concurrent with genotyping (analysis of copy number profiles), the sample may be amplified any number of mechanisms. The most common amplification procedure used involves PCR. See, for example, PCR Technology: Principles and Applications for DNA Amplification (Ed. H. A. Erlich, Freeman Press, NY, N.Y., 1992); PCR Protocols: A Guide to Methods and Applications (Eds. Innis, et al., Academic Press, San Diego, Calif., 1990); Mattila et al., Nucleic Acids Res. 19, 4967 (1991); Eckert et al., PCR Methods and Applications 1, 17 (1991); PCR (Eds. McPherson et al., IRL Press, Oxford); and U.S. Pat. Nos. 4,683,202, 4,683,195, 4,800,159 4,965,188, and 5,333,675, and each of which is incorporated herein by reference in their entireties for all purposes. In some embodiments, the sample may be amplified on the array (e.g., U.S. Pat. No. 6,300,070 which is incorporated herein by reference)

[00162] Other suitable amplification methods include the ligase chain reaction (LCR) (for example, Wu and Wallace, Genomics 4, 560 (1989), Landegren et al., Science 241, 1077 (1988) and Barringer et al. Gene 89:117 (1990)), transcription amplification (Kwoh et al., Proc. Natl. Acad. Sci. USA 86, 1173 (1989) and WO88/10315), self-sustained sequence replication (Guatelli et al., Proc. Nat. Acad. Sci. USA,

87, 1874 (1990) and WO90/06995), selective amplification of target polynucleotide sequences (U.S. Pat. No. 6,410,276), consensus sequence primed polymerase chain reaction (CP-PCR) (U.S. Pat. No. 4,437,975), arbitrarily primed polymerase chain reaction (AP-PCR) (U.S. Pat. Nos. 5,413,909, 5,861,245) and nucleic acid based sequence amplification (NABSA). (See, U.S. Pat. Nos. 5,409,818, 5,554,517, and 6,063,603, each of which is incorporated herein by reference). Other amplification methods that may be used are described in, U.S. Pat. Nos. 5,242,794, 5,494,810, 4,988,617 and in U.S. Ser. No. 09/854,317, each of which is incorporated herein by reference.

[00163] Additional methods of sample preparation and techniques for reducing the complexity of a nucleic sample are described in Dong et al., Genome Research 11, 1418 (2001), in U.S. Pat. Nos. 6,361,947, 6,391,592 and U.S. Ser. Nos. 09/916,135, 09/920,491 (U.S. Patent Application Publication 20030096235), 09/910,292 (U.S. Patent Application Publication 20030082543), and 10/013,598.

[00164] Methods for conducting polynucleotide hybridization assays are well developed in the art. Hybridization assay procedures and conditions used in the methods of the invention will vary depending on the application and are selected in accordance with the general binding methods known including those referred to in: Maniatis et al. Molecular Cloning: A Laboratory Manual (2.sup.nd Ed. Cold Spring Harbor, N.Y., 1989); Berger and Kimmel Methods in Enzymology, Vol. 152, Guide to Molecular Cloning Techniques (Academic Press, Inc., San Diego, Calif., 1987); Young and Davism, P.N.A.S, 80: 1194 (1983). Methods and apparatus for carrying out repeated and controlled hybridization reactions have been described in U.S. Pat. Nos. 5,871,928, 5,874,219, 6,045,996 and 6,386,749, 6,391,623 each of which are incorporated herein by reference.

[00165] The methods of the invention may also involve signal detection of hybridization between ligands in after (and/or during) hybridization. See U.S. Pat. Nos. 5,143,854, 5,578,832; 5,631,734; 5,834,758; 5,936,324; 5,981,956; 6,025,601; 6,141,096; 6,185,030; 6,201,639; 6,218,803; and 6,225,625, in U.S. Ser. No. 10/389,194 and in PCT Application PCT/US99/06097 (published as WO99/47964), each of which also is hereby incorporated by reference in its entirety for all purposes.

[00166] Methods and apparatus for signal detection and processing of intensity data are disclosed in, for example, U.S. Pat. Nos. 5,143,854, 5,547,839, 5,578,832, 5,631,734, 5,800,992, 5,834,758; 5,856,092, 5,902,723, 5,936,324, 5,981,956, 6,025,601, 6,090,555, 6,141,096, 6,185,030, 6,201,639; 6,218,803; and 6,225,625, in U.S. Ser. Nos. 10/389,194, 60/493,495 and in PCT Application PCT/US99/06097 (published as WO99/47964), each of which also is hereby incorporated by reference in its entirety for all purposes.

Immuno-based Assays

[00167] Protein-based detection molecular profiling techniques include immunoaffinity assays based on antibodies selectively immunoreactive with mutant gene encoded protein according to the present invention. These techniques include without limitation immunoprecipitation, Western blot analysis, molecular binding assays, enzyme-linked immunosorbent assay (ELISA), enzyme-linked immunofiltration assay (ELIFA), fluorescence activated cell sorting (FACS) and the like. For example, an optional method of detecting the expression of a biomarker in a sample comprises contacting the sample

with an antibody against the biomarker, or an immunoreactive fragment of the antibody thereof, or a recombinant protein containing an antigen binding region of an antibody against the biomarker; and then detecting the binding of the biomarker in the sample. Methods for producing such antibodies are known in the art. Antibodies can be used to immunoprecipitate specific proteins from solution samples or to immunoblot proteins separated by, e.g., polyacrylamide gels. Immunocytochemical methods can also be used in detecting specific protein polymorphisms in tissues or cells. Other well-known antibody-based techniques can also be used including, e.g., ELISA, radioimmunoassay (RIA), immunoradiometric assays (IRMA) and immunoenzymatic assays (IEMA), including sandwich assays using monoclonal or polyclonal antibodies. See, e.g., U.S. Pat. Nos. 4,376,110 and 4,486,530, both of which are incorporated herein by reference.

[00168] In alternative methods, the sample may be contacted with an antibody specific for a biomarker under conditions sufficient for an antibody-biomarker complex to form, and then detecting said complex. The presence of the biomarker may be detected in a number of ways, such as by Western blotting and ELISA procedures for assaying a wide variety of tissues and samples, including plasma or serum. A wide range of immunoassay techniques using such an assay format are available, see, e.g., U.S. Pat. Nos. 4,016,043, 4,424,279 and 4,018,653. These include both single-site and two-site or "sandwich" assays of the non-competitive types, as well as in the traditional competitive binding assays. These assays also include direct binding of a labelled antibody to a target biomarker.

[00169] A number of variations of the sandwich assay technique exist, and all are intended to be encompassed by the present invention. Briefly, in a typical forward assay, an unlabelled antibody is immobilized on a solid substrate, and the sample to be tested brought into contact with the bound molecule. After a suitable period of incubation, for a period of time sufficient to allow formation of an antibody-antigen complex, a second antibody specific to the antigen, labelled with a reporter molecule capable of producing a detectable signal is then added and incubated, allowing time sufficient for the formation of another complex of antibody-antigen-labelled antibody. Any unreacted material is washed away, and the presence of the antigen is determined by observation of a signal produced by the reporter molecule. The results may either be qualitative, by simple observation of the visible signal, or may be quantitated by comparing with a control sample containing known amounts of biomarker.

[00170] Variations on the forward assay include a simultaneous assay, in which both sample and labelled antibody are added simultaneously to the bound antibody. These techniques are well known to those skilled in the art, including any minor variations as will be readily apparent. In a typical forward sandwich assay, a first antibody having specificity for the biomarker is either covalently or passively bound to a solid surface. The solid surface is typically glass or a polymer, the most commonly used polymers being cellulose, polyacrylamide, nylon, polystyrene, polyvinyl chloride or polypropylene. The solid supports may be in the form of tubes, beads, discs of microplates, or any other surface suitable for conducting an immunoassay. The binding processes are well-known in the art and generally consist of cross-linking covalently binding or physically adsorbing, the polymer-antibody complex is washed in preparation for the test sample. An aliquot of the sample to be tested is then added to the solid phase complex and

incubated for a period of time sufficient (e.g. 2-40 minutes or overnight if more convenient) and under suitable conditions (e.g. from room temperature to 40°C such as between 25°C and 32°C inclusive) to allow binding of any subunit present in the antibody. Following the incubation period, the antibody subunit solid phase is washed and dried and incubated with a second antibody specific for a portion of the biomarker. The second antibody is linked to a reporter molecule which is used to indicate the binding of the second antibody to the molecular marker.

[00171] An alternative method involves immobilizing the target biomarkers in the sample and then exposing the immobilized target to specific antibody which may or may not be labelled with a reporter molecule. Depending on the amount of target and the strength of the reporter molecule signal, a bound target may be detectable by direct labelling with the antibody. Alternatively, a second labelled antibody, specific to the first antibody is exposed to the target-first antibody complex to form a target-first antibody-second antibody tertiary complex. The complex is detected by the signal emitted by the reporter molecule. By "reporter molecule", as used in the present specification, is meant a molecule which, by its chemical nature, provides an analytically identifiable signal which allows the detection of antigen-bound antibody. The most commonly used reporter molecules in this type of assay are either enzymes, fluorophores or radionuclide containing molecules (i.e. radioisotopes) and chemiluminescent molecules.

[00172] In the case of an enzyme immunoassay, an enzyme is conjugated to the second antibody, generally by means of glutaraldehyde or periodate. As will be readily recognized, however, a wide variety of different conjugation techniques exist, which are readily available to the skilled artisan. Commonly used enzymes include horseradish peroxidase, glucose oxidase, β-galactosidase and alkaline phosphatase, amongst others. The substrates to be used with the specific enzymes are generally chosen for the production, upon hydrolysis by the corresponding enzyme, of a detectable color change. Examples of suitable enzymes include alkaline phosphatase and peroxidase. It is also possible to employ fluorogenic substrates, which yield a fluorescent product rather than the chromogenic substrates noted above. In all cases, the enzyme-labelled antibody is added to the first antibody-molecular marker complex, allowed to bind, and then the excess reagent is washed away. A solution containing the appropriate substrate is then added to the complex of antibody-antigen-antibody. The substrate will react with the enzyme linked to the second antibody, giving a qualitative visual signal, which may be further quantitated, usually spectrophotometrically, to give an indication of the amount of biomarker which was present in the sample. Alternately, fluorescent compounds, such as fluorescein and rhodamine, may be chemically coupled to antibodies without altering their binding capacity. When activated by illumination with light of a particular wavelength, the fluorochrome-labelled antibody adsorbs the light energy, inducing a state to excitability in the molecule, followed by emission of the light at a characteristic color visually detectable with a light microscope. As in the EIA, the fluorescent labelled antibody is allowed to bind to the first antibody-molecular marker complex. After washing off the unbound reagent, the remaining tertiary complex is then exposed to the light of the appropriate wavelength, the fluorescence observed indicates the presence of the molecular marker of interest. Immunofluorescence and EIA techniques are both very

well established in the art. However, other reporter molecules, such as radioisotope, chemiluminescent or bioluminescent molecules, may also be employed.

[00173] ***Immunohistochemistry (IHC)***

[00174] IHC is a process of localizing antigens (e.g., proteins) in cells of a tissue binding antibodies specifically to antigens in the tissues. The antigen-binding antibody can be conjugated or fused to a tag that allows its detection, e.g., via visualization. In some embodiments, the tag is an enzyme that can catalyze a color-producing reaction, such as alkaline phosphatase or horseradish peroxidase. The enzyme can be fused to the antibody or non-covalently bound, e.g., using a biotin-avadin system. Alternatively, the antibody can be tagged with a fluorophore, such as fluorescein, rhodamine, DyLight Fluor or Alexa Fluor. The antigen-binding antibody can be directly tagged or it can itself be recognized by a detection antibody that carries the tag. Using IHC, one or more proteins may be detected. The expression of a gene product can be related to its staining intensity compared to control levels. In some embodiments, the gene product is considered differentially expressed if its staining varies at least 1.2, 1.3, 1.4, 1.5, 1.6, 1.7, 1.8, 1.9, 2.0, 2.2, 2.5, 2.7, 3.0, 4, 5, 6, 7, 8, 9 or 10-fold in the sample versus the control.

[00175] IHC comprises the application of antigen-antibody interactions to histochemical techniques. In an illustrative example, a tissue section is mounted on a slide and is incubated with antibodies (polyclonal or monoclonal) specific to the antigen (primary reaction). The antigen-antibody signal is then amplified using a second antibody conjugated to a complex of peroxidase antiperoxidase (PAP), avidin-biotin-peroxidase (ABC) or avidin-biotin alkaline phosphatase. In the presence of substrate and chromogen, the enzyme forms a colored deposit at the sites of antibody-antigen binding. Immunofluorescence is an alternate approach to visualize antigens. In this technique, the primary antigen-antibody signal is amplified using a second antibody conjugated to a fluorochrome. On UV light absorption, the fluorochrome emits its own light at a longer wavelength (fluorescence), thus allowing localization of antibody-antigen complexes.

Epigenetic Status

[00176] Molecular profiling methods according to the invention also comprise measuring epigenetic change, i.e., modification in a gene caused by an epigenetic mechanism, such as a change in methylation status or histone acetylation. Frequently, the epigenetic change will result in an alteration in the levels of expression of the gene which may be detected (at the RNA or protein level as appropriate) as an indication of the epigenetic change. Often the epigenetic change results in silencing or down regulation of the gene, referred to as "epigenetic silencing." The most frequently investigated epigenetic change in the methods of the invention involves determining the DNA methylation status of a gene, where an increased level of methylation is typically associated with the relevant cancer (since it may cause down regulation of gene expression). Aberrant methylation, which may be referred to as hypermethylation, of the gene or genes can be detected. Typically, the methylation status is determined in suitable CpG islands which are often found in the promoter region of the gene(s). The term "methylation," "methylation state" or "methylation status" may refers to the presence or absence of 5-methylcytosine at one or a plurality of CpG

dinucleotides within a DNA sequence. CpG dinucleotides are typically concentrated in the promoter regions and exons of human genes.

[00177] Diminished gene expression can be assessed in terms of DNA methylation status or in terms of expression levels as determined by the methylation status of the gene. One method to detect epigenetic silencing is to determine that a gene which is expressed in normal cells is less expressed or not expressed in tumor cells. Accordingly, the invention provides for a method of molecular profiling comprising detecting epigenetic silencing.

[00178] Various assay procedures to directly detect methylation are known in the art, and can be used in conjunction with the present invention. These assays rely onto two distinct approaches: bisulphite conversion based approaches and non-bisulphite based approaches. Non-bisulphite based methods for analysis of DNA methylation rely on the inability of methylation-sensitive enzymes to cleave methylation cytosines in their restriction. The bisulphite conversion relies on treatment of DNA samples with sodium bisulphite which converts unmethylated cytosine to uracil, while methylated cytosines are maintained (Furuichi Y, Wataya Y, Hayatsu H, Ukita T. Biochem Biophys Res Commun. 1970 Dec 9;41(5):1185-91). This conversion results in a change in the sequence of the original DNA. Methods to detect such changes include MS AP-PCR (Methylation-Sensitive Arbitrarily-Primed Polymerase Chain Reaction), a technology that allows for a global scan of the genome using CG-rich primers to focus on the regions most likely to contain CpG dinucleotides, and described by Gonzalgo et al., Cancer Research 57:594-599, 1997; MethyLight™, which refers to the art-recognized fluorescence-based real-time PCR technique described by Eads et al., Cancer Res. 59:2302-2306, 1999; the HeavyMethyl™assay, in the embodiment thereof implemented herein, is an assay, wherein methylation specific blocking probes (also referred to herein as blockers) covering CpG positions between, or covered by the amplification primers enable methylation-specific selective amplification of a nucleic acid sample; HeavyMethyl™MethyLight™ is a variation of the MethyLight™ assay wherein the MethyLight™ assay is combined with methylation specific blocking probes covering CpG positions between the amplification primers; Ms-SNuPE (Methylation-sensitive Single Nucleotide Primer Extension) is an assay described by Gonzalgo & Jones, Nucleic Acids Res. 25:2529-2531, 1997; MSP (Methylation-specific PCR) is a methylation assay described by Herman et al. Proc. Natl. Acad. Sci. USA 93:9821-9826, 1996, and by U.S. Pat. No. 5,786,146; COBRA (Combined Bisulfite Restriction Analysis) is a methylation assay described by Xiong & Laird, Nucleic Acids Res. 25:2532-2534, 1997; MCA (Methylated CpG Island Amplification) is a methylation assay described by Toyota et al., Cancer Res. 59:2307-12, 1999, and in WO 00/26401A1.

[00179] Other techniques for DNA methylation analysis include sequencing, methylation-specific PCR (MS-PCR), melting curve methylation-specific PCR (McMS-PCR), MLPA with or without bisulfite treatment, QAMA, MSRE-PCR, MethyLight, ConLight-MSP, bisulfite conversion-specific methylation-specific PCR (BS-MSP), COBRA (which relies upon use of restriction enzymes to reveal methylation dependent sequence differences in PCR products of sodium bisulfite-treated DNA), methylation-sensitive single-nucleotide primer extension conformation (MS-SNuPE), methylation-sensitive single-strand conformation analysis (MS-SSCA), Melting curve combined bisulfite restriction analysis (McCOBRA),

PyroMethA, HeavyMethyl, MALDI-TOF, MassARRAY, Quantitative analysis of methylated alleles (QAMA), enzymatic regional methylation assay (ERMA), QBSUPT, MethylQuant, Quantitative PCR sequencing and oligonucleotide-based microarray systems, Pyrosequencing, Meth-DOP-PCR. A review of some useful techniques is provided in Nucleic acids research, 1998, Vol. 26, No. 10, 2255-2264; Nature Reviews, 2003, Vol.3, 253-266; Oral Oncology, 2006, Vol. 42, 5-13, which references are incorporated herein in their entirety. Any of these techniques may be used in accordance with the present invention, as appropriate. Other techniques are described in U.S. Patent Publications 20100144836; and 20100184027, which applications are incorporated herein by reference in their entirety.

[00180] Through the activity of various acetylases and deacetylylases the DNA binding function of histone proteins is tightly regulated. Furthermore, histone acetylation and histone deactelyation have been linked with malignant progression. See Nature, 429: 457-63, 2004. Methods to analyze histone acetylation are described in U.S. Patent Publications 20100144543 and 20100151468, which applications are incorporated herein by reference in their entirety.

Sequence Analysis

[00181] Molecular profiling according to the present invention comprises methods for genotyping one or more biomarkers by determining whether an individual has one or more nucleotide variants (or amino acid variants) in one or more of the genes or gene products. Genotyping one or more genes according to the methods of the invention in some embodiments, can provide more evidence for selecting a treatment.

[00182] The biomarkers of the invention can be analyzed by any method useful for determining alterations in nucleic acids or the proteins they encode. According to one embodiment, the ordinary skilled artisan can analyze the one or more genes for mutations including deletion mutants, insertion mutants, frame shift mutants, nonsense mutants, missense mutant, and splice mutants.

[00183] Nucleic acid used for analysis of the one or more genes can be isolated from cells in the sample according to standard methodologies (Sambrook et al., 1989). The nucleic acid, for example, may be genomic DNA or fractionated or whole cell RNA, or miRNA acquired from exosomes or cell surfaces. Where RNA is used, it may be desired to convert the RNA to a complementary DNA. In one embodiment, the RNA is whole cell RNA; in another, it is poly-A RNA; in another, it is exosomal RNA. Normally, the nucleic acid is amplified. Depending on the format of the assay for analyzing the one or more genes, the specific nucleic acid of interest is identified in the sample directly using amplification or with a second, known nucleic acid following amplification. Next, the identified product is detected. In certain applications, the detection may be performed by visual means (e.g., ethidium bromide staining of a gel). Alternatively, the detection may involve indirect identification of the product via chemiluminescence, radioactive scintigraphy of radiolabel or fluorescent label or even via a system using electrical or thermal impulse signals (Affymax Technology; Bellus, 1994).

[00184] Various types of defects are known to occur in the biomarkers of the invention. Alterations include without limitation deletions, insertions, point mutations, and duplications. Point mutations can be silent or can result in stop codons, frame shift mutations or amino acid substitutions. Mutations in and outside the coding region of the one or more genes may occur and can be analyzed according to the

methods of the invention. The target site of a nucleic acid of interest can include the region wherein the sequence varies. Examples include, but are not limited to, polymorphisms which exist in different forms such as single nucleotide variations, nucleotide repeats, multibase deletion (more than one nucleotide deleted from the consensus sequence), multibase insertion (more than one nucleotide inserted from the consensus sequence), microsatellite repeats (small numbers of nucleotide repeats with a typical 5-1000 repeat units), di-nucleotide repeats, tri-nucleotide repeats, sequence rearrangements (including translocation and duplication), chimeric sequence (two sequences from different gene origins are fused together), and the like. Among sequence polymorphisms, the most frequent polymorphisms in the human genome are single-base variations, also called single-nucleotide polymorphisms (SNPs). SNPs are abundant, stable and widely distributed across the genome.

[00185] Molecular profiling includes methods for haplotyping one or more genes. The haplotype is a set of genetic determinants located on a single chromosome and it typically contains a particular combination of alleles (all the alternative sequences of a gene) in a region of a chromosome. In other words, the haplotype is phased sequence information on individual chromosomes. Very often, phased SNPs on a chromosome define a haplotype. A combination of haplotypes on chromosomes can determine a genetic profile of a cell. It is the haplotype that determines a linkage between a specific genetic marker and a disease mutation. Haplotyping can be done by any methods known in the art. Common methods of scoring SNPs include hybridization microarray or direct gel sequencing, reviewed in Landgren et al., Genome Research, 8:769-776, 1998. For example, only one copy of one or more genes can be isolated from an individual and the nucleotide at each of the variant positions is determined. Alternatively, an allele specific PCR or a similar method can be used to amplify only one copy of the one or more genes in an individual, and the SNPs at the variant positions of the present invention are determined. The Clark method known in the art can also be employed for haplotyping. A high throughput molecular haplotyping method is also disclosed in Tost et al., Nucleic Acids Res., 30(19):e96 (2002), which is incorporated herein by reference.

[00186] Thus, additional variant(s) that are in linkage disequilibrium with the variants and/or haplotypes of the present invention can be identified by a haplotyping method known in the art, as will be apparent to a skilled artisan in the field of genetics and haplotyping. The additional variants that are in linkage disequilibrium with a variant or haplotype of the present invention can also be useful in the various applications as described below.

[00187] For purposes of genotyping and haplotyping, both genomic DNA and mRNA/cDNA can be used, and both are herein referred to generically as "gene."

[00188] Numerous techniques for detecting nucleotide variants are known in the art and can all be used for the method of this invention. The techniques can be protein-based or nucleic acid-based. In either case, the techniques used must be sufficiently sensitive so as to accurately detect the small nucleotide or amino acid variations. Very often, a probe is used which is labeled with a detectable marker. Unless otherwise specified in a particular technique described below, any suitable marker known in the art can be used, including but not limited to, radioactive isotopes, fluorescent compounds, biotin which is detectable using

streptavidin, enzymes (e.g., alkaline phosphatase), substrates of an enzyme, ligands and antibodies, etc. See Jablonski et al., Nucleic Acids Res., 14:6115-6128 (1986); Nguyen et al., Biotechniques, 13:116-123 (1992); Rigby et al., J. Mol. Biol., 113:237-251 (1977).

[00189] In a nucleic acid-based detection method, target DNA sample, i.e., a sample containing genomic DNA, cDNA, mRNA and/or miRNA, corresponding to the one or more genes must be obtained from the individual to be tested. Any tissue or cell sample containing the genomic DNA, miRNA, mRNA, and/or cDNA (or a portion thereof) corresponding to the one or more genes can be used. For this purpose, a tissue sample containing cell nucleus and thus genomic DNA can be obtained from the individual. Blood samples can also be useful except that only white blood cells and other lymphocytes have cell nucleus, while red blood cells are without a nucleus and contain only mRNA or miRNA. Nevertheless, miRNA and mRNA are also useful as either can be analyzed for the presence of nucleotide variants in its sequence or serve as template for cDNA synthesis. The tissue or cell samples can be analyzed directly without much processing. Alternatively, nucleic acids including the target sequence can be extracted, purified, and/or amplified before they are subject to the various detecting procedures discussed below. Other than tissue or cell samples, cDNAs or genomic DNAs from a cDNA or genomic DNA library constructed using a tissue or cell sample obtained from the individual to be tested are also useful.

[00190] To determine the presence or absence of a particular nucleotide variant, sequencing of the target genomic DNA or cDNA, particularly the region encompassing the nucleotide variant locus to be detected. Various sequencing techniques are generally known and widely used in the art including the Sanger method and Gilbert chemical method. The pyrosequencing method monitors DNA synthesis in real time using a luminometric detection system. Pyrosequencing has been shown to be effective in analyzing genetic polymorphisms such as single-nucleotide polymorphisms and can also be used in the present invention. See Nordstrom et al., Biotechnol. Appl. Biochem., 31(2):107-112 (2000); Ahmadian et al., Anal. Biochem., 280:103-110 (2000).

[00191] Nucleic acid variants can be detected by a suitable detection process. Non limiting examples of methods of detection, quantification, sequencing and the like are; mass detection of mass modified amplicons (e.g., matrix-assisted laser desorption ionization (MALDI) mass spectrometry and electrospray (ES) mass spectrometry), a primer extension method (e.g., iPLEX™; Sequenom, Inc.), microsequencing methods (e.g., a modification of primer extension methodology), ligase sequence determination methods (e.g., U.S. Pat. Nos. 5,679,524 and 5,952,174, and WO 01/27326), mismatch sequence determination methods (e.g., U.S. Pat. Nos. 5,851,770; 5,958,692; 6,110,684; and 6,183,958), direct DNA sequencing, fragment analysis (FA), restriction fragment length polymorphism (RFLP analysis), allele specific oligonucleotide (ASO) analysis, methylation-specific PCR (MSPCR), pyrosequencing analysis, acycloprime analysis, Reverse dot blot, GeneChip microarrays, Dynamic allele-specific hybridization (DASH), Peptide nucleic acid (PNA) and locked nucleic acids (LNA) probes, TaqMan, Molecular Beacons, Intercalating dye, FRET primers, AlphaScreen, SNPstream, genetic bit analysis (GBA), Multiplex minisequencing, SNaPshot, GOOD assay, Microarray miniseq, arrayed primer extension (APEX), Microarray primer extension (e.g., microarray sequence determination methods), Tag arrays,

Coded microspheres, Template-directed incorporation (TDI), fluorescence polarization, Colorimetric oligonucleotide ligation assay (OLA), Sequence-coded OLA, Microarray ligation, Ligase chain reaction, Padlock probes, Invader assay, hybridization methods (e.g., hybridization using at least one probe, hybridization using at least one fluorescently labeled probe, and the like), conventional dot blot analyses, single strand conformational polymorphism analysis (SSCP, e.g., U.S. Pat. Nos. 5,891,625 and 6,013,499; Orita et al., Proc. Natl. Acad. Sci. U.S.A. 86: 27776-2770 (1989)), denaturing gradient gel electrophoresis (DGGE), heteroduplex analysis, mismatch cleavage detection, and techniques described in Sheffield et al., Proc. Natl. Acad. Sci. USA 49: 699-706 (1991), White et al., Genomics 12: 301-306 (1992), Grompe et al., Proc. Natl. Acad. Sci. USA 86: 5855-5892 (1989), and Grompe, Nature Genetics 5: 111-117 (1993), cloning and sequencing, electrophoresis, the use of hybridization probes and quantitative real time polymerase chain reaction (QRT-PCR), digital PCR, nanopore sequencing, chips and combinations thereof. The detection and quantification of alleles or paralogs can be carried out using the "closed-tube" methods described in U.S. patent application Ser. No. 11/950,395, filed on Dec. 4, 2007. In some embodiments the amount of a nucleic acid species is determined by mass spectrometry, primer extension, sequencing (e.g., any suitable method, for example nanopore or pyrosequencing), Quantitative PCR (Q-PCR or QRT-PCR), digital PCR, combinations thereof, and the like.

[00192] The term "sequence analysis" as used herein refers to determining a nucleotide sequence, e.g., that of an amplification product. The entire sequence or a partial sequence of a polynucleotide, e.g., DNA or mRNA, can be determined, and the determined nucleotide sequence can be referred to as a “read” or “sequence read.” For example, linear amplification products may be analyzed directly without further amplification in some embodiments (e.g., by using single-molecule sequencing methodology). In certain embodiments, linear amplification products may be subject to further amplification and then analyzed (e.g., using sequencing by ligation or pyrosequencing methodology). Reads may be subject to different types of sequence analysis. Any suitable sequencing method can be used to detect, and determine the amount of, nucleotide sequence species, amplified nucleic acid species, or detectable products generated from the foregoing. Examples of certain sequencing methods are described hereafter.

[00193] A sequence analysis apparatus or sequence analysis component(s) includes an apparatus, and one or more components used in conjunction with such apparatus, that can be used by a person of ordinary skill to determine a nucleotide sequence resulting from processes described herein (e.g., linear and/or exponential amplification products). Examples of sequencing platforms include, without limitation, the 454 platform (Roche) (Margulies, M. et al. 2005 Nature 437, 376-380), Illumina Genomic Analyzer (or Solexa platform) or SOLID System (Applied Biosystems; see PCT patent application publications WO 06/084132 entitled “Reagents, Methods, and Libraries For Bead-Based Sequencing” and WO07/121,489 entitled “Reagents, Methods, and Libraries for Gel-Free Bead-Based Sequencing”), the Helicos True Single Molecule DNA sequencing technology (Harris TD et al. 2008 Science, 320, 106-109), the single molecule, real-time (SMRT™) technology of Pacific Biosciences, and nanopore sequencing (Soni G V and Meller A. 2007 Clin Chem 53: 1996-2001), Ion semiconductor sequencing (Ion Torrent Systems, Inc, San Francisco, CA), or DNA nanoball sequencing (Complete Genomics, Mountain View, CA), VisiGen

Biotechnologies approach (Invitrogen) and polony sequencing. Such platforms allow sequencing of many nucleic acid molecules isolated from a specimen at high orders of multiplexing in a parallel manner (Dear Brief Funct Genomic Proteomic 2003; 1: 397-416; Haimovich, Methods, challenges, and promise of next-generation sequencing in cancer biology. Yale J Biol Med. 2011 Dec;84(4):439-46). These non-Sanger-based sequencing technologies are sometimes referred to as NextGen sequencing, NGS, next-generation sequencing, next generation sequencing, and variations thereof. Typically they allow much higher throughput than the traditional Sanger approach. See Schuster, Next-generation sequencing transforms today's biology, *Nature Methods* **5**:16-18 (2008); Metzker, Sequencing technologies - the next generation. Nat Rev Genet. 2010 Jan;11(1):31-46. These platforms can allow sequencing of clonally expanded or non-amplified single molecules of nucleic acid fragments. Certain platforms involve, for example, sequencing by ligation of dye-modified probes (including cyclic ligation and cleavage), pyrosequencing, and single-molecule sequencing. Nucleotide sequence species, amplification nucleic acid species and detectable products generated there from can be analyzed by such sequence analysis platforms. Next-generation sequencing can be used in the methods of the invention, e.g., to determine mutations, copy number, or expression levels, as appropriate. The methods can be used to perform whole genome sequencing or sequencing of specific sequences of interest, such as a gene of interest or a fragment thereof.

[00194] Sequencing by ligation is a nucleic acid sequencing method that relies on the sensitivity of DNA ligase to base-pairing mismatch. DNA ligase joins together ends of DNA that are correctly base paired. Combining the ability of DNA ligase to join together only correctly base paired DNA ends, with mixed pools of fluorescently labeled oligonucleotides or primers, enables sequence determination by fluorescence detection. Longer sequence reads may be obtained by including primers containing cleavable linkages that can be cleaved after label identification. Cleavage at the linker removes the label and regenerates the 5' phosphate on the end of the ligated primer, preparing the primer for another round of ligation. In some embodiments primers may be labeled with more than one fluorescent label, e.g., at least 1, 2, 3, 4, or 5 fluorescent labels.

[00195] Sequencing by ligation generally involves the following steps. Clonal bead populations can be prepared in emulsion microreactors containing target nucleic acid template sequences, amplification reaction components, beads and primers. After amplification, templates are denatured and bead enrichment is performed to separate beads with extended templates from undesired beads (e.g., beads with no extended templates). The template on the selected beads undergoes a 3' modification to allow covalent bonding to the slide, and modified beads can be deposited onto a glass slide. Deposition chambers offer the ability to segment a slide into one, four or eight chambers during the bead loading process. For sequence analysis, primers hybridize to the adapter sequence. A set of four color dye-labeled probes competes for ligation to the sequencing primer. Specificity of probe ligation is achieved by interrogating every 4th and 5th base during the ligation series. Five to seven rounds of ligation, detection and cleavage record the color at every 5th position with the number of rounds determined by the type of library used. Following each round of ligation, a new complimentary primer offset by one base in the 5' direction is laid

down for another series of ligations. Primer reset and ligation rounds (5-7 ligation cycles per round) are repeated sequentially five times to generate 25-35 base pairs of sequence for a single tag. With mate-paired sequencing, this process is repeated for a second tag.

[00196] Pyrosequencing is a nucleic acid sequencing method based on sequencing by synthesis, which relies on detection of a pyrophosphate released on nucleotide incorporation. Generally, sequencing by synthesis involves synthesizing, one nucleotide at a time, a DNA strand complimentary to the strand whose sequence is being sought. Target nucleic acids may be immobilized to a solid support, hybridized with a sequencing primer, incubated with DNA polymerase, ATP sulfurylase, luciferase, apyrase, adenosine 5' phosphosulfate and luciferin. Nucleotide solutions are sequentially added and removed. Correct incorporation of a nucleotide releases a pyrophosphate, which interacts with ATP sulfurylase and produces ATP in the presence of adenosine 5' phosphosulfate, fueling the luciferin reaction, which produces a chemiluminescent signal allowing sequence determination. The amount of light generated is proportional to the number of bases added. Accordingly, the sequence downstream of the sequencing primer can be determined. An illustrative system for pyrosequencing involves the following steps: ligating an adaptor nucleic acid to a nucleic acid under investigation and hybridizing the resulting nucleic acid to a bead; amplifying a nucleotide sequence in an emulsion; sorting beads using a picoliter multiwell solid support; and sequencing amplified nucleotide sequences by pyrosequencing methodology (e.g., Nakano et al., "Single-molecule PCR using water-in-oil emulsion;" Journal of Biotechnology 102: 117-124 (2003)).

[00197] Certain single-molecule sequencing embodiments are based on the principal of sequencing by synthesis, and use single-pair Fluorescence Resonance Energy Transfer (single pair FRET) as a mechanism by which photons are emitted as a result of successful nucleotide incorporation. The emitted photons often are detected using intensified or high sensitivity cooled charge-couple-devices in conjunction with total internal reflection microscopy (TIRM). Photons are only emitted when the introduced reaction solution contains the correct nucleotide for incorporation into the growing nucleic acid chain that is synthesized as a result of the sequencing process. In FRET based single-molecule sequencing, energy is transferred between two fluorescent dyes, sometimes polymethine cyanine dyes Cy3 and Cy5, through long-range dipole interactions. The donor is excited at its specific excitation wavelength and the excited state energy is transferred, non-radiatively to the acceptor dye, which in turn becomes excited. The acceptor dye eventually returns to the ground state by radiative emission of a photon. The two dyes used in the energy transfer process represent the "single pair" in single pair FRET. Cy3 often is used as the donor fluorophore and often is incorporated as the first labeled nucleotide. Cy5 often is used as the acceptor fluorophore and is used as the nucleotide label for successive nucleotide additions after incorporation of a first Cy3 labeled nucleotide. The fluorophores generally are within 10 nanometers of each for energy transfer to occur successfully.

[00198] An example of a system that can be used based on single-molecule sequencing generally involves hybridizing a primer to a target nucleic acid sequence to generate a complex; associating the complex with a solid phase; iteratively extending the primer by a nucleotide tagged with a fluorescent molecule; and capturing an image of fluorescence resonance energy transfer signals after each iteration (e.g., U.S. Pat.

No. 7,169,314; Braslavsky et al., PNAS 100(7): 3960-3964 (2003)). Such a system can be used to directly sequence amplification products (linearly or exponentially amplified products) generated by processes described herein. In some embodiments the amplification products can be hybridized to a primer that contains sequences complementary to immobilized capture sequences present on a solid support, a bead or glass slide for example. Hybridization of the primer-amplification product complexes with the immobilized capture sequences, immobilizes amplification products to solid supports for single pair FRET based sequencing by synthesis. The primer often is fluorescent, so that an initial reference image of the surface of the slide with immobilized nucleic acids can be generated. The initial reference image is useful for determining locations at which true nucleotide incorporation is occurring. Fluorescence signals detected in array locations not initially identified in the "primer only" reference image are discarded as non-specific fluorescence. Following immobilization of the primer-amplification product complexes, the bound nucleic acids often are sequenced in parallel by the iterative steps of, a) polymerase extension in the presence of one fluorescently labeled nucleotide, b) detection of fluorescence using appropriate microscopy, TIRM for example, c) removal of fluorescent nucleotide, and d) return to step a with a different fluorescently labeled nucleotide.

[00199] In some embodiments, nucleotide sequencing may be by solid phase single nucleotide sequencing methods and processes. Solid phase single nucleotide sequencing methods involve contacting target nucleic acid and solid support under conditions in which a single molecule of sample nucleic acid hybridizes to a single molecule of a solid support. Such conditions can include providing the solid support molecules and a single molecule of target nucleic acid in a "microreactor." Such conditions also can include providing a mixture in which the target nucleic acid molecule can hybridize to solid phase nucleic acid on the solid support. Single nucleotide sequencing methods useful in the embodiments described herein are described in U.S. Provisional Patent Application Ser. No. 61/021,871 filed Jan. 17, 2008.

[00200] In certain embodiments, nanopore sequencing detection methods include (a) contacting a target nucleic acid for sequencing ("base nucleic acid," e.g., linked probe molecule) with sequence-specific detectors, under conditions in which the detectors specifically hybridize to substantially complementary subsequences of the base nucleic acid; (b) detecting signals from the detectors and (c) determining the sequence of the base nucleic acid according to the signals detected. In certain embodiments, the detectors hybridized to the base nucleic acid are disassociated from the base nucleic acid (e.g., sequentially dissociated) when the detectors interfere with a nanopore structure as the base nucleic acid passes through a pore, and the detectors disassociated from the base sequence are detected. In some embodiments, a detector disassociated from a base nucleic acid emits a detectable signal, and the detector hybridized to the base nucleic acid emits a different detectable signal or no detectable signal. In certain embodiments, nucleotides in a nucleic acid (e.g., linked probe molecule) are substituted with specific nucleotide sequences corresponding to specific nucleotides ("nucleotide representatives"), thereby giving rise to an expanded nucleic acid (e.g., U.S. Pat. No. 6,723,513), and the detectors hybridize to the nucleotide representatives in the expanded nucleic acid, which serves as a base nucleic acid. In such embodiments, nucleotide representatives may be arranged in a binary or higher order arrangement (e.g., Soni and Meller,

Clinical Chemistry 53(11): 1996-2001 (2007)). In some embodiments, a nucleic acid is not expanded, does not give rise to an expanded nucleic acid, and directly serves a base nucleic acid (e.g., a linked probe molecule serves as a non-expanded base nucleic acid), and detectors are directly contacted with the base nucleic acid. For example, a first detector may hybridize to a first subsequence and a second detector may hybridize to a second subsequence, where the first detector and second detector each have detectable labels that can be distinguished from one another, and where the signals from the first detector and second detector can be distinguished from one another when the detectors are disassociated from the base nucleic acid. In certain embodiments, detectors include a region that hybridizes to the base nucleic acid (e.g., two regions), which can be about 3 to about 100 nucleotides in length (e.g., about 4, 5, 6, 7, 8, 9, 10, 11, 12, 13, 14, 15, 16, 17, 18, 19, 20, 25, 30, 35, 40, 50, 55, 60, 65, 70, 75, 80, 85, 90, or 95 nucleotides in length). A detector also may include one or more regions of nucleotides that do not hybridize to the base nucleic acid. In some embodiments, a detector is a molecular beacon. A detector often comprises one or more detectable labels independently selected from those described herein. Each detectable label can be detected by any convenient detection process capable of detecting a signal generated by each label (e.g., magnetic, electric, chemical, optical and the like). For example, a CD camera can be used to detect signals from one or more distinguishable quantum dots linked to a detector.

[00201] In certain sequence analysis embodiments, reads may be used to construct a larger nucleotide sequence, which can be facilitated by identifying overlapping sequences in different reads and by using identification sequences in the reads. Such sequence analysis methods and software for constructing larger sequences from reads are known to the person of ordinary skill (e.g., Venter et al., Science 291: 1304-1351 (2001)). Specific reads, partial nucleotide sequence constructs, and full nucleotide sequence constructs may be compared between nucleotide sequences within a sample nucleic acid (i.e., internal comparison) or may be compared with a reference sequence (i.e., reference comparison) in certain sequence analysis embodiments. Internal comparisons can be performed in situations where a sample nucleic acid is prepared from multiple samples or from a single sample source that contains sequence variations. Reference comparisons sometimes are performed when a reference nucleotide sequence is known and an objective is to determine whether a sample nucleic acid contains a nucleotide sequence that is substantially similar or the same, or different, than a reference nucleotide sequence. Sequence analysis can be facilitated by the use of sequence analysis apparatus and components described above.

[00202] Primer extension polymorphism detection methods, also referred to herein as "microsequencing" methods, typically are carried out by hybridizing a complementary oligonucleotide to a nucleic acid carrying the polymorphic site. In these methods, the oligonucleotide typically hybridizes adjacent to the polymorphic site. The term "adjacent" as used in reference to "microsequencing" methods, refers to the 3' end of the extension oligonucleotide being sometimes 1 nucleotide from the 5' end of the polymorphic site, often 2 or 3, and at times 4, 5, 6, 7, 8, 9, or 10 nucleotides from the 5' end of the polymorphic site, in the nucleic acid when the extension oligonucleotide is hybridized to the nucleic acid. The extension oligonucleotide then is extended by one or more nucleotides, often 1, 2, or 3 nucleotides, and the number and/or type of nucleotides that are added to the extension oligonucleotide determine which polymorphic

variant or variants are present. Oligonucleotide extension methods are disclosed, for example, in U.S. Pat. Nos. 4,656,127; 4,851,331; 5,679,524; 5,834,189; 5,876,934; 5,908,755; 5,912,118; 5,976,802; 5,981,186; 6,004,744; 6,013,431; 6,017,702; 6,046,005; 6,087,095; 6,210,891; and WO 01/20039. The extension products can be detected in any manner, such as by fluorescence methods (see, e.g., Chen & Kwok, Nucleic Acids Research 25: 347-353 (1997) and Chen et al., Proc. Natl. Acad. Sci. USA 94/20: 10756-10761 (1997)) or by mass spectrometric methods (e.g., MALDI-TOF mass spectrometry) and other methods described herein. Oligonucleotide extension methods using mass spectrometry are described, for example, in U.S. Pat. Nos. 5,547,835; 5,605,798; 5,691,141; 5,849,542; 5,869,242; 5,928,906; 6,043,031; 6,194,144; and 6,258,538.

Microsequencing detection methods often incorporate an amplification process that proceeds the extension step. The amplification process typically amplifies a region from a nucleic acid sample that comprises the polymorphic site. Amplification can be carried out using methods described above, or for example using a pair of oligonucleotide primers in a polymerase chain reaction (PCR), in which one oligonucleotide primer typically is complementary to a region 3' of the polymorphism and the other typically is complementary to a region 5' of the polymorphism. A PCR primer pair may be used in methods disclosed in U.S. Pat. Nos. 4,683,195; 4,683,202, 4,965,188; 5,656,493; 5,998,143; 6,140,054; WO 01/27327; and WO 01/27329 for example. PCR primer pairs may also be used in any commercially available machines that perform PCR, such as any of the GeneAmp™ Systems available from Applied Biosystems.

[00203] Other appropriate sequencing methods include multiplex polony sequencing (as described in Shendure et al., Accurate Multiplex Polony Sequencing of an Evolved Bacterial Genome, Sciencexpress, Aug. 4, 2005, pg 1 available at www.sciencexpress.org/4 Aug. 2005/Page1/10.1126/science.1117389, incorporated herein by reference), which employs immobilized microbeads, and sequencing in microfabricated picoliter reactors (as described in Margulies et al., Genome Sequencing in Microfabricated High-Density Picolitre Reactors, Nature, August 2005, available at www.nature.com/nature (published online 31 Jul. 2005, doi:10.1038/nature03959, incorporated herein by reference).

[00204] Whole genome sequencing may also be used for discriminating alleles of RNA transcripts, in some embodiments. Examples of whole genome sequencing methods include, but are not limited to, nanopore-based sequencing methods, sequencing by synthesis and sequencing by ligation, as described above.

[00205] Nucleic acid variants can also be detected using standard electrophoretic techniques. Although the detection step can sometimes be preceded by an amplification step, amplification is not required in the embodiments described herein. Examples of methods for detection and quantification of a nucleic acid using electrophoretic techniques can be found in the art. A non-limiting example comprises running a sample (e.g., mixed nucleic acid sample isolated from maternal serum, or amplification nucleic acid species, for example) in an agarose or polyacrylamide gel. The gel may be labeled (e.g., stained) with ethidium bromide (see, Sambrook and Russell, Molecular Cloning: A Laboratory Manual 3d ed., 2001).

The presence of a band of the same size as the standard control is an indication of the presence of a target nucleic acid sequence, the amount of which may then be compared to the control based on the intensity of the band, thus detecting and quantifying the target sequence of interest. In some embodiments, restriction enzymes capable of distinguishing between maternal and paternal alleles may be used to detect and quantify target nucleic acid species. In certain embodiments, oligonucleotide probes specific to a sequence of interest are used to detect the presence of the target sequence of interest. The oligonucleotides can also be used to indicate the amount of the target nucleic acid molecules in comparison to the standard control, based on the intensity of signal imparted by the probe.

[00206] Sequence-specific probe hybridization can be used to detect a particular nucleic acid in a mixture or mixed population comprising other species of nucleic acids. Under sufficiently stringent hybridization conditions, the probes hybridize specifically only to substantially complementary sequences. The stringency of the hybridization conditions can be relaxed to tolerate varying amounts of sequence mismatch. A number of hybridization formats are known in the art, which include but are not limited to, solution phase, solid phase, or mixed phase hybridization assays. The following articles provide an overview of the various hybridization assay formats: Singer et al., Biotechniques 4:230, 1986; Haase et al., Methods in Virology, pp. 189-226, 1984; Wilkinson, In situ Hybridization, Wilkinson ed., IRL Press, Oxford University Press, Oxford; and Hames and Higgins eds., Nucleic Acid Hybridization: A Practical Approach, IRL Press, 1987.

[00207] Hybridization complexes can be detected by techniques known in the art. Nucleic acid probes capable of specifically hybridizing to a target nucleic acid (e.g., mRNA or DNA) can be labeled by any suitable method, and the labeled probe used to detect the presence of hybridized nucleic acids. One commonly used method of detection is autoradiography, using probes labeled with ^{3}H, ^{125}I, ^{35}S, ^{14}C, ^{32}P, ^{33}P, or the like. The choice of radioactive isotope depends on research preferences due to ease of synthesis, stability, and half-lives of the selected isotopes. Other labels include compounds (e.g., biotin and digoxigenin), which bind to antiligands or antibodies labeled with fluorophores, chemiluminescent agents, and enzymes. In some embodiments, probes can be conjugated directly with labels such as fluorophores, chemiluminescent agents or enzymes. The choice of label depends on sensitivity required, ease of conjugation with the probe, stability requirements, and available instrumentation.

[00208] In embodiments, fragment analysis (referred to herein as "FA") methods are used for molecular profiling. Fragment analysis (FA) includes techniques such as restriction fragment length polymorphism (RFLP) and/or (amplified fragment length polymorphism). If a nucleotide variant in the target DNA corresponding to the one or more genes results in the elimination or creation of a restriction enzyme recognition site, then digestion of the target DNA with that particular restriction enzyme will generate an altered restriction fragment length pattern. Thus, a detected RFLP or AFLP will indicate the presence of a particular nucleotide variant.

[00209] Terminal restriction fragment length polymorphism (TRFLP) works by PCR amplification of DNA using primer pairs that have been labeled with fluorescent tags. The PCR products are digested using RFLP enzymes and the resulting patterns are visualized using a DNA sequencer. The results are

analyzed either by counting and comparing bands or peaks in the TRFLP profile, or by comparing bands from one or more TRFLP runs in a database.

[00210] The sequence changes directly involved with an RFLP can also be analyzed more quickly by PCR. Amplification can be directed across the altered restriction site, and the products digested with the restriction enzyme. This method has been called Cleaved Amplified Polymorphic Sequence (CAPS). Alternatively, the amplified segment can be analyzed by Allele specific oligonucleotide (ASO) probes, a process that is sometimes assessed using a Dot blot.

[00211] A variation on AFLP is cDNA-AFLP, which can be used to quantify differences in gene expression levels.

[00212] Another useful approach is the single-stranded conformation polymorphism assay (SSCA), which is based on the altered mobility of a single-stranded target DNA spanning the nucleotide variant of interest. A single nucleotide change in the target sequence can result in different intramolecular base pairing pattern, and thus different secondary structure of the single-stranded DNA, which can be detected in a non-denaturing gel. See Orita et al., Proc. Natl. Acad. Sci. USA, 86:2776-2770 (1989). Denaturing gel-based techniques such as clamped denaturing gel electrophoresis (CDGE) and denaturing gradient gel electrophoresis (DGGE) detect differences in migration rates of mutant sequences as compared to wild-type sequences in denaturing gel. See Miller et al., Biotechniques, 5:1016-24 (1999); Sheffield et al., Am. J. Hum, Genet., 49:699-706 (1991); Wartell et al., Nucleic Acids Res., 18:2699-2705 (1990); and Sheffield et al., Proc. Natl. Acad. Sci. USA, 86:232-236 (1989). In addition, the double-strand conformation analysis (DSCA) can also be useful in the present invention. See Arguello et al., Nat. Genet., 18:192-194 (1998).

[00213] The presence or absence of a nucleotide variant at a particular locus in the one or more genes of an individual can also be detected using the amplification refractory mutation system (ARMS) technique. See e.g., European Patent No. 0,332,435; Newton et al., Nucleic Acids Res., 17:2503-2515 (1989); Fox et al., Br. J. Cancer, 77:1267-1274 (1998); Robertson et al., Eur. Respir. J., 12:477-482 (1998). In the ARMS method, a primer is synthesized matching the nucleotide sequence immediately 5' upstream from the locus being tested except that the 3'-end nucleotide which corresponds to the nucleotide at the locus is a predetermined nucleotide. For example, the 3'-end nucleotide can be the same as that in the mutated locus. The primer can be of any suitable length so long as it hybridizes to the target DNA under stringent conditions only when its 3'-end nucleotide matches the nucleotide at the locus being tested. Preferably the primer has at least 12 nucleotides, more preferably from about 18 to 50 nucleotides. If the individual tested has a mutation at the locus and the nucleotide therein matches the 3'-end nucleotide of the primer, then the primer can be further extended upon hybridizing to the target DNA template, and the primer can initiate a PCR amplification reaction in conjunction with another suitable PCR primer. In contrast, if the nucleotide at the locus is of wild type, then primer extension cannot be achieved. Various forms of ARMS techniques developed in the past few years can be used. See e.g., Gibson et al., Clin. Chem. 43:1336-1341 (1997).

[00214] Similar to the ARMS technique is the mini sequencing or single nucleotide primer extension method, which is based on the incorporation of a single nucleotide. An oligonucleotide primer matching the nucleotide sequence immediately 5' to the locus being tested is hybridized to the target DNA, mRNA or miRNA in the presence of labeled dideoxyribonucleotides. A labeled nucleotide is incorporated or linked to the primer only when the dideoxyribonucleotides matches the nucleotide at the variant locus being detected. Thus, the identity of the nucleotide at the variant locus can be revealed based on the detection label attached to the incorporated dideoxyribonucleotides. See Syvanen et al., Genomics, 8:684-692 (1990); Shumaker et al., Hum. Mutat., 7:346-354 (1996); Chen et al., Genome Res., 10:549-547 (2000).

[00215] Another set of techniques useful in the present invention is the so-called "oligonucleotide ligation assay" (OLA) in which differentiation between a wild-type locus and a mutation is based on the ability of two oligonucleotides to anneal adjacent to each other on the target DNA molecule allowing the two oligonucleotides joined together by a DNA ligase. See Landergren et al., Science, 241:1077-1080 (1988); Chen et al, Genome Res., 8:549-556 (1998); Iannone et al., Cytometry, 39:131-140 (2000). Thus, for example, to detect a single-nucleotide mutation at a particular locus in the one or more genes, two oligonucleotides can be synthesized, one having the sequence just 5' upstream from the locus with its 3' end nucleotide being identical to the nucleotide in the variant locus of the particular gene, the other having a nucleotide sequence matching the sequence immediately 3' downstream from the locus in the gene. The oligonucleotides can be labeled for the purpose of detection. Upon hybridizing to the target gene under a stringent condition, the two oligonucleotides are subject to ligation in the presence of a suitable ligase. The ligation of the two oligonucleotides would indicate that the target DNA has a nucleotide variant at the locus being detected.

[00216] Detection of small genetic variations can also be accomplished by a variety of hybridization-based approaches. Allele-specific oligonucleotides are most useful. See Conner et al., Proc. Natl. Acad. Sci. USA, 80:278-282 (1983); Saiki et al, Proc. Natl. Acad. Sci. USA, 86:6230-6234 (1989). Oligonucleotide probes (allele-specific) hybridizing specifically to a gene allele having a particular gene variant at a particular locus but not to other alleles can be designed by methods known in the art. The probes can have a length of, e.g., from 10 to about 50 nucleotide bases. The target DNA and the oligonucleotide probe can be contacted with each other under conditions sufficiently stringent such that the nucleotide variant can be distinguished from the wild-type gene based on the presence or absence of hybridization. The probe can be labeled to provide detection signals. Alternatively, the allele-specific oligonucleotide probe can be used as a PCR amplification primer in an "allele-specific PCR" and the presence or absence of a PCR product of the expected length would indicate the presence or absence of a particular nucleotide variant.

[00217] Other useful hybridization-based techniques allow two single-stranded nucleic acids annealed together even in the presence of mismatch due to nucleotide substitution, insertion or deletion. The mismatch can then be detected using various techniques. For example, the annealed duplexes can be subject to electrophoresis. The mismatched duplexes can be detected based on their electrophoretic

mobility that is different from the perfectly matched duplexes. See Cariello, Human Genetics, 42:726 (1988). Alternatively, in an RNase protection assay, a RNA probe can be prepared spanning the nucleotide variant site to be detected and having a detection marker. See Giunta et al., Diagn. Mol. Path., 5:265-270 (1996); Finkelstein et al., Genomics, 7:167-172 (1990); Kinszler et al., Science 251:1366-1370 (1991). The RNA probe can be hybridized to the target DNA or mRNA forming a heteroduplex that is then subject to the ribonuclease RNase A digestion. RNase A digests the RNA probe in the heteroduplex only at the site of mismatch. The digestion can be determined on a denaturing electrophoresis gel based on size variations. In addition, mismatches can also be detected by chemical cleavage methods known in the art. See e.g., Roberts et al., Nucleic Acids Res., 25:3377-3378 (1997).

[00218] In the mutS assay, a probe can be prepared matching the gene sequence surrounding the locus at which the presence or absence of a mutation is to be detected, except that a predetermined nucleotide is used at the variant locus. Upon annealing the probe to the target DNA to form a duplex, the E. coli mutS protein is contacted with the duplex. Since the mutS protein binds only to heteroduplex sequences containing a nucleotide mismatch, the binding of the mutS protein will be indicative of the presence of a mutation. See Modrich et al., Ann. Rev. Genet., 25:229-253 (1991).

[00219] A great variety of improvements and variations have been developed in the art on the basis of the above-described basic techniques which can be useful in detecting mutations or nucleotide variants in the present invention. For example, the "sunrise probes" or "molecular beacons" use the fluorescence resonance energy transfer (FRET) property and give rise to high sensitivity. See Wolf et al., Proc. Nat. Acad. Sci. USA, 85:8790-8794 (1988). Typically, a probe spanning the nucleotide locus to be detected are designed into a hairpin-shaped structure and labeled with a quenching fluorophore at one end and a reporter fluorophore at the other end. In its natural state, the fluorescence from the reporter fluorophore is quenched by the quenching fluorophore due to the proximity of one fluorophore to the other. Upon hybridization of the probe to the target DNA, the 5' end is separated apart from the 3'-end and thus fluorescence signal is regenerated. See Nazarenko et al., Nucleic Acids Res., 25:2516-2521 (1997); Rychlik et al., Nucleic Acids Res., 17:8543-8551 (1989); Sharkey et al., Bio/Technology 12:506-509 (1994); Tyagi et al., Nat. Biotechnol., 14:303-308 (1996); Tyagi et al., Nat. Biotechnol., 16:49-53 (1998). The homo-tag assisted non-dimer system (HANDS) can be used in combination with the molecular beacon methods to suppress primer-dimer accumulation. See Brownie et al., Nucleic Acids Res., 25:3235-3241 (1997).

[00220] Dye-labeled oligonucleotide ligation assay is a FRET-based method, which combines the OLA assay and PCR. See Chen et al., Genome Res. 8:549-556 (1998). TaqMan is another FRET-based method for detecting nucleotide variants. A TaqMan probe can be oligonucleotides designed to have the nucleotide sequence of the gene spanning the variant locus of interest and to differentially hybridize with different alleles. The two ends of the probe are labeled with a quenching fluorophore and a reporter fluorophore, respectively. The TaqMan probe is incorporated into a PCR reaction for the amplification of a target gene region containing the locus of interest using Taq polymerase. As Taq polymerase exhibits 5'-3' exonuclease activity but has no 3'-5' exonuclease activity, if the TaqMan probe is annealed to the target

DNA template, the 5'-end of the TaqMan probe will be degraded by Taq polymerase during the PCR reaction thus separating the reporting fluorophore from the quenching fluorophore and releasing fluorescence signals. See Holland et al., Proc. Natl. Acad. Sci. USA, 88:7276-7280 (1991); Kalinina et al., Nucleic Acids Res., 25:1999-2004 (1997); Whitcombe et al., Clin. Chem., 44:918-923 (1998).

[00221] In addition, the detection in the present invention can also employ a chemiluminescence-based technique. For example, an oligonucleotide probe can be designed to hybridize to either the wild-type or a variant gene locus but not both. The probe is labeled with a highly chemiluminescent acridinium ester. Hydrolysis of the acridinium ester destroys chemiluminescence. The hybridization of the probe to the target DNA prevents the hydrolysis of the acridinium ester. Therefore, the presence or absence of a particular mutation in the target DNA is determined by measuring chemiluminescence changes. See Nelson et al., Nucleic Acids Res., 24:4998-5003 (1996).

[00222] The detection of genetic variation in the gene in accordance with the present invention can also be based on the "base excision sequence scanning" (BESS) technique. The BESS method is a PCR-based mutation scanning method. BESS T-Scan and BESS G-Tracker are generated which are analogous to T and G ladders of dideoxy sequencing. Mutations are detected by comparing the sequence of normal and mutant DNA. See, e.g., Hawkins et al., Electrophoresis, 20:1171-1176 (1999).

[00223] Mass spectrometry can be used for molecular profiling according to the invention. See Graber et al., Curr. Opin. Biotechnol., 9:14-18 (1998). For example, in the primer oligo base extension (PROBE™) method, a target nucleic acid is immobilized to a solid-phase support. A primer is annealed to the target immediately 5' upstream from the locus to be analyzed. Primer extension is carried out in the presence of a selected mixture of deoxyribonucleotides and dideoxyribonucleotides. The resulting mixture of newly extended primers is then analyzed by MALDI-TOF. See e.g., Monforte et al., Nat. Med., 3:360-362 (1997).

[00224] In addition, the microchip or microarray technologies are also applicable to the detection method of the present invention. Essentially, in microchips, a large number of different oligonucleotide probes are immobilized in an array on a substrate or carrier, e.g., a silicon chip or glass slide. Target nucleic acid sequences to be analyzed can be contacted with the immobilized oligonucleotide probes on the microchip. See Lipshutz et al., Biotechniques, 19:442-447 (1995); Chee et al., Science, 274:610-614 (1996); Kozal et al., Nat. Med. 2:753-759 (1996); Hacia et al., Nat. Genet., 14:441-447 (1996); Saiki et al., Proc. Natl. Acad. Sci. USA, 86:6230-6234 (1989); Gingeras et al., Genome Res., 8:435-448 (1998). Alternatively, the multiple target nucleic acid sequences to be studied are fixed onto a substrate and an array of probes is contacted with the immobilized target sequences. See Drmanac et al., Nat. Biotechnol., 16:54-58 (1998). Numerous microchip technologies have been developed incorporating one or more of the above described techniques for detecting mutations. The microchip technologies combined with computerized analysis tools allow fast screening in a large scale. The adaptation of the microchip technologies to the present invention will be apparent to a person of skill in the art apprised of the present disclosure. See, e.g., U.S. Pat. No. 5,925,525 to Fodor et al; Wilgenbus et al., J. Mol. Med., 77:761-786 (1999); Graber et al., Curr. Opin. Biotechnol., 9:14-18 (1998); Hacia et al., Nat. Genet., 14:441-447 (1996); Shoemaker et al., Nat.

Genet., 14:450-456 (1996); DeRisi et al., Nat. Genet., 14:457-460 (1996); Chee et al., Nat. Genet., 14:610-614 (1996); Lockhart et al., Nat. Genet., 14:675-680 (1996); Drobyshev et al., Gene, 188:45-52 (1997).

[00225] As is apparent from the above survey of the suitable detection techniques, it may or may not be necessary to amplify the target DNA, i.e., the gene, cDNA, mRNA, miRNA, or a portion thereof to increase the number of target DNA molecule, depending on the detection techniques used. For example, most PCR-based techniques combine the amplification of a portion of the target and the detection of the mutations. PCR amplification is well known in the art and is disclosed in U.S. Pat. Nos. 4,683,195 and 4,800,159, both which are incorporated herein by reference. For non-PCR-based detection techniques, if necessary, the amplification can be achieved by, e.g., in vivo plasmid multiplication, or by purifying the target DNA from a large amount of tissue or cell samples. See generally, Sambrook et al., Molecular Cloning: A Laboratory Manual, 2nd ed., Cold Spring Harbor Laboratory, Cold Spring Harbor, N.Y., 1989. However, even with scarce samples, many sensitive techniques have been developed in which small genetic variations such as single-nucleotide substitutions can be detected without having to amplify the target DNA in the sample. For example, techniques have been developed that amplify the signal as opposed to the target DNA by, e.g., employing branched DNA or dendrimers that can hybridize to the target DNA. The branched or dendrimer DNAs provide multiple hybridization sites for hybridization probes to attach thereto thus amplifying the detection signals. See Detmer et al., J. Clin. Microbiol., 34:901-907 (1996); Collins et al., Nucleic Acids Res., 25:2979-2984 (1997); Horn et al., Nucleic Acids Res., 25:4835-4841 (1997); Horn et al., Nucleic Acids Res., 25:4842-4849 (1997); Nilsen et al., J. Theor. Biol., 187:273-284 (1997).

[00226] The Invader™ assay is another technique for detecting single nucleotide variations that can be used for molecular profiling according to the invention. The Invader™ assay uses a novel linear signal amplification technology that improves upon the long turnaround times required of the typical PCR DNA sequenced-based analysis. See Cooksey et al., Antimicrobial Agents and Chemotherapy 44:1296-1301 (2000). This assay is based on cleavage of a unique secondary structure formed between two overlapping oligonucleotides that hybridize to the target sequence of interest to form a "flap." Each "flap" then generates thousands of signals per hour. Thus, the results of this technique can be easily read, and the methods do not require exponential amplification of the DNA target. The Invader™ system uses two short DNA probes, which are hybridized to a DNA target. The structure formed by the hybridization event is recognized by a special cleavase enzyme that cuts one of the probes to release a short DNA "flap." Each released "flap" then binds to a fluorescently-labeled probe to form another cleavage structure. When the cleavase enzyme cuts the labeled probe, the probe emits a detectable fluorescence signal. See e.g. Lyamichev et al., Nat. Biotechnol., 17:292-296 (1999).

[00227] The rolling circle method is another method that avoids exponential amplification. Lizardi et al., Nature Genetics, 19:225-232 (1998) (which is incorporated herein by reference). For example, Sniper™, a commercial embodiment of this method, is a sensitive, high-throughput SNP scoring system designed for the accurate fluorescent detection of specific variants. For each nucleotide variant, two linear, allele-

specific probes are designed. The two allele-specific probes are identical with the exception of the 3'-base, which is varied to complement the variant site. In the first stage of the assay, target DNA is denatured and then hybridized with a pair of single, allele-specific, open-circle oligonucleotide probes. When the 3'-base exactly complements the target DNA, ligation of the probe will preferentially occur. Subsequent detection of the circularized oligonucleotide probes is by rolling circle amplification, whereupon the amplified probe products are detected by fluorescence. See Clark and Pickering, Life Science News 6, 2000, Amersham Pharmacia Biotech (2000).

[00228] A number of other techniques that avoid amplification all together include, e.g., surface-enhanced resonance Raman scattering (SERRS), fluorescence correlation spectroscopy, and single-molecule electrophoresis. In SERRS, a chromophore-nucleic acid conjugate is absorbed onto colloidal silver and is irradiated with laser light at a resonant frequency of the chromophore. See Graham et al., Anal. Chem., 69:4703-4707 (1997). The fluorescence correlation spectroscopy is based on the spatio-temporal correlations among fluctuating light signals and trapping single molecules in an electric field. See Eigen et al., Proc. Natl. Acad. Sci. USA, 91:5740-5747 (1994). In single-molecule electrophoresis, the electrophoretic velocity of a fluorescently tagged nucleic acid is determined by measuring the time required for the molecule to travel a predetermined distance between two laser beams. See Castro et al., Anal. Chem., 67:3181-3186 (1995).

[00229] In addition, the allele-specific oligonucleotides (ASO) can also be used in in situ hybridization using tissues or cells as samples. The oligonucleotide probes which can hybridize differentially with the wild-type gene sequence or the gene sequence harboring a mutation may be labeled with radioactive isotopes, fluorescence, or other detectable markers. In situ hybridization techniques are well known in the art and their adaptation to the present invention for detecting the presence or absence of a nucleotide variant in the one or more gene of a particular individual should be apparent to a skilled artisan apprised of this disclosure.

[00230] Accordingly, the presence or absence of one or more genes nucleotide variant or amino acid variant in an individual can be determined using any of the detection methods described above.

[00231] Typically, once the presence or absence of one or more gene nucleotide variants or amino acid variants is determined, physicians or genetic counselors or patients or other researchers may be informed of the result. Specifically the result can be cast in a transmittable form that can be communicated or transmitted to other researchers or physicians or genetic counselors or patients. Such a form can vary and can be tangible or intangible. The result with regard to the presence or absence of a nucleotide variant of the present invention in the individual tested can be embodied in descriptive statements, diagrams, photographs, charts, images or any other visual forms. For example, images of gel electrophoresis of PCR products can be used in explaining the results. Diagrams showing where a variant occurs in an individual's gene are also useful in indicating the testing results. The statements and visual forms can be recorded on a tangible media such as papers, computer readable media such as floppy disks, compact disks, etc., or on an intangible media, e.g., an electronic media in the form of email or website on internet or intranet. In addition, the result with regard to the presence or absence of a nucleotide variant or amino acid variant in

the individual tested can also be recorded in a sound form and transmitted through any suitable media, e.g., analog or digital cable lines, fiber optic cables, etc., via telephone, facsimile, wireless mobile phone, internet phone and the like.

[00232] Thus, the information and data on a test result can be produced anywhere in the world and transmitted to a different location. For example, when a genotyping assay is conducted offshore, the information and data on a test result may be generated and cast in a transmittable form as described above. The test result in a transmittable form thus can be imported into the U.S. Accordingly, the present invention also encompasses a method for producing a transmittable form of information on the genotype of the two or more suspected cancer samples from an individual. The method comprises the steps of (1) determining the genotype of the DNA from the samples according to methods of the present invention; and (2) embodying the result of the determining step in a transmittable form. The transmittable form is the product of the production method.

In Situ Hybridization

[00233] In situ hybridization assays are well known and are generally described in Angerer et al., Methods Enzymol. 152:649-660 (1987). In an in situ hybridization assay, cells, e.g., from a biopsy, are fixed to a solid support, typically a glass slide. If DNA is to be probed, the cells are denatured with heat or alkali. The cells are then contacted with a hybridization solution at a moderate temperature to permit annealing of specific probes that are labeled. The probes are preferably labeled, e.g., with radioisotopes or fluorescent reporters, or enzymatically. FISH (fluorescence in situ hybridization) uses fluorescent probes that bind to only those parts of a sequence with which they show a high degree of sequence similarity. CISH (chromogenic in situ hybridization) uses conventional peroxidase or alkaline phosphatase reactions visualized under a standard bright-field microscope.

[00234] In situ hybridization can be used to detect specific gene sequences in tissue sections or cell preparations by hybridizing the complementary strand of a nucleotide probe to the sequence of interest. Fluorescent in situ hybridization (FISH) uses a fluorescent probe to increase the sensitivity of in situ hybridization.

[00235] FISH is a cytogenetic technique used to detect and localize specific polynucleotide sequences in cells. For example, FISH can be used to detect DNA sequences on chromosomes. FISH can also be used to detect and localize specific RNAs, e.g., mRNAs, within tissue samples. In FISH uses fluorescent probes that bind to specific nucleotide sequences to which they show a high degree of sequence similarity. Fluorescence microscopy can be used to find out whether and where the fluorescent probes are bound. In addition to detecting specific nucleotide sequences, e.g., translocations, fusion, breaks, duplications and other chromosomal abnormalities, FISH can help define the spatial-temporal patterns of specific gene copy number and/or gene expression within cells and tissues.

[00236] Various types of FISH probes can be used to detect chromosome translocations. Dual color, single fusion probes can be useful in detecting cells possessing a specific chromosomal translocation. The DNA probe hybridization targets are located on one side of each of the two genetic breakpoints. “Extra signal” probes can reduce the frequency of normal cells exhibiting an abnormal FISH pattern due to the random

co-localization of probe signals in a normal nucleus. One large probe spans one breakpoint, while the other probe flanks the breakpoint on the other gene. Dual color, break apart probes are useful in cases where there may be multiple translocation partners associated with a known genetic breakpoint. This labeling scheme features two differently colored probes that hybridize to targets on opposite sides of a breakpoint in one gene. Dual color, dual fusion probes can reduce the number of normal nuclei exhibiting abnormal signal patterns. The probe offers advantages in detecting low levels of nuclei possessing a simple balanced translocation. Large probes span two breakpoints on different chromosomes. Such probes are available as Vysis probes from Abbott Laboratories, Abbott Park, IL.

[00237] CISH, or chromogenic in situ hybridization, is a process in which a labeled complementary DNA or RNA strand is used to localize a specific DNA or RNA sequence in a tissue specimen. CISH methodology can be used to evaluate gene amplification, gene deletion, chromosome translocation, and chromosome number. CISH can use conventional enzymatic detection methodology, e.g., horseradish peroxidase or alkaline phosphatase reactions, visualized under a standard bright-field microscope. In a common embodiment, a probe that recognizes the sequence of interest is contacted with a sample. An antibody or other binding agent that recognizes the probe, e.g., via a label carried by the probe, can be used to target an enzymatic detection system to the site of the probe. In some systems, the antibody can recognize the label of a FISH probe, thereby allowing a sample to be analyzed using both FISH and CISH detection. CISH can be used to evaluate nucleic acids in multiple settings, e.g., formalin-fixed, paraffin-embedded (FFPE) tissue, blood or bone marrow smear, metaphase chromosome spread, and/or fixed cells. In an embodiment, CISH is performed following the methodology in the SPoT-Light® HER2 CISH Kit available from Life Technologies (Carlsbad, CA) or similar CISH products available from Life Technologies. The SPoT-Light® HER2 CISH Kit itself is FDA approved for in vitro diagnostics and can be used for molecular profiling of HER2. CISH can be used in similar applications as FISH. Thus, one of skill will appreciate that reference to molecular profiling using FISH herein can be performed using CISH, unless otherwise specified.

[00238] Silver-enhanced in situ hybridization (SISH) is similar to CISH, but with SISH the signal appears as a black coloration due to silver precipitation instead of the chromogen precipitates of CISH.

[00239] Modifications of the in situ hybridization techniques can be used for molecular profiling according to the invention. Such modifications comprise simultaneous detection of multiple targets, e.g., Dual ISH, Dual color CISH, bright field double in situ hybridization (BDISH). *See e.g.,* the FDA approved INFORM HER2 Dual ISH DNA Probe Cocktail kit from Ventana Medical Systems, Inc. (Tucson, AZ); DuoCISH™, a dual color CISH kit developed by Dako Denmark A/S (Denmark).

[00240] Comparative Genomic Hybridization (CGH) comprises a molecular cytogenetic method of screening tumor samples for genetic changes showing characteristic patterns for copy number changes at chromosomal and subchromosomal levels. Alterations in patterns can be classified as DNA gains and losses. CGH employs the kinetics of in situ hybridization to compare the copy numbers of different DNA or RNA sequences from a sample, or the copy numbers of different DNA or RNA sequences in one sample to the copy numbers of the substantially identical sequences in another sample. In many useful

applications of CGH, the DNA or RNA is isolated from a subject cell or cell population. The comparisons can be qualitative or quantitative. Procedures are described that permit determination of the absolute copy numbers of DNA sequences throughout the genome of a cell or cell population if the absolute copy number is known or determined for one or several sequences. The different sequences are discriminated from each other by the different locations of their binding sites when hybridized to a reference genome, usually metaphase chromosomes but in certain cases interphase nuclei. The copy number information originates from comparisons of the intensities of the hybridization signals among the different locations on the reference genome. The methods, techniques and applications of CGH are known, such as described in U.S. Pat. No. 6,335,167, and in U.S. App. Ser. No. 60/804,818, the relevant parts of which are herein incorporated by reference.

[00241] In an embodiment, CGH used to compare nucleic acids between diseased and healthy tissues. The method comprises isolating DNA from disease tissues (e.g., tumors) and reference tissues (e.g., healthy tissue) and labeling each with a different "color" or fluor. The two samples are mixed and hybridized to normal metaphase chromosomes. In the case of array or matrix CGH, the hybridization mixing is done on a slide with thousands of DNA probes. A variety of detection system can be used that basically determine the color ratio along the chromosomes to determine DNA regions that might be gained or lost in the diseased samples as compared to the reference.

Molecular Profiling for Treatment Selection

[00242] The methods of the invention provide a candidate treatment selection for a subject in need thereof. Molecular profiling can be used to identify one or more candidate therapeutic agents for an individual suffering from a condition in which one or more of the biomarkers disclosed herein are targets for treatment. For example, the method can identify one or more chemotherapy treatments for a cancer. In an aspect, the invention provides a method comprising: performing at least one molecular profiling technique on at least one biomarker. Any relevant biomarker can be assessed using one or more of the molecular profiling techniques described herein or known in the art. The marker need only have some direct or indirect association with a treatment to be useful. Any relevant molecular profiling technique can be performed, such as those disclosed here. These can include without limitation, protein and nucleic acid analysis techniques. Protein analysis techniques include, by way of non-limiting examples, immunoassays, immunohistochemistry, and mass spectrometry. Nucleic acid analysis techniques include, by way of non-limiting examples, amplification, polymerase chain amplification, hybridization, microarrays, in situ hybridization, sequencing, dye-terminator sequencing, next generation sequencing, pyrosequencing, and restriction fragment analysis.

[00243] Molecular profiling may comprise the profiling of at least one gene (or gene product) for each assay technique that is performed. Different numbers of genes can be assayed with different techniques. Any marker disclosed herein that is associated directly or indirectly with a target therapeutic can be assessed. For example, any "druggable target" comprising a target that can be modulated with a therapeutic agent such as a small molecule or binding agent such as an antibody, is a candidate for inclusion in the molecular profiling methods of the invention. The target can also be indirectly drug

associated, such as a component of a biological pathway that is affected by the associated drug. The molecular profiling can be based on either the gene, e.g., DNA sequence, and/or gene product, e.g., mRNA or protein. Such nucleic acid and/or polypeptide can be profiled as applicable as to presence or absence, level or amount, activity, mutation, sequence, haplotype, rearrangement, copy number, or other measurable characteristic. In some embodiments, a single gene and/or one or more corresponding gene products is assayed by more than one molecular profiling technique. A gene or gene product (also referred to herein as "marker" or "biomarker"), e.g., an mRNA or protein, is assessed using applicable techniques (e.g., to assess DNA, RNA, protein), including without limitation ISH, gene expression, IHC, sequencing or immunoassay. Therefore, any of the markers disclosed herein can be assayed by a single molecular profiling technique or by multiple methods disclosed herein (e.g., a single marker is profiled by one or more of IHC, ISH, sequencing, microarray, etc.). In some embodiments, at least about 1, 2, 3, 4, 5, 6, 7, 8, 9, 10, 11, 12, 13, 14, 15, 16, 17, 18, 19, 20, 21, 22, 23, 24, 25, 26, 27, 28, 29, 30, 35, 40, 45, 50, 55, 60, 65, 70, 75, 80, 85, 90, 95 or at least about 100 genes or gene products are profiled by at least one technique, a plurality of techniques, or using a combination of ISH, gene expression, gene copy, IHC, and sequencing. In some embodiments, at least about 100, 200, 300, 400, 500, 600, 700, 800, 900, 1000, 2000, 3000, 4000, 5000, 6000, 7000, 8000, 9000, 10,000, 11,000, 12,000, 13,000, 14,000, 15,000, 16,000, 17,000, 18,000, 19,000, 20,000, 21,000, 22,000, 23,000, 24,000, 25,000, 26,000, 27,000, 28,000, 29,000, 30,000, 31,000, 32,000, 33,000, 34,000, 35,000, 36,000, 37,000, 38,000, 39,000, 40,000, 41,000, 42,000, 43,000, 44,000, 45,000, 46,000, 47,000, 48,000, 49,000, or at least 50,000 genes or gene products are profiled using various techniques. The number of markers assayed can depend on the technique used. For example, microarray and massively parallel sequencing lend themselves to high throughput analysis. Because molecular profiling queries molecular characteristics of the tumor itself, this approach provides information on therapies that might not otherwise be considered based on the lineage of the tumor.

[00244] In some embodiments, a sample from a subject in need thereof is profiled using methods which include but are not limited to IHC analysis, gene expression analysis, ISH analysis, and/or sequencing analysis (such as by PCR, RT-PCR, pyrosequencing) for one or more of the following: ABCC1, ABCG2, ACE2, ADA, ADH1C, ADH4, AGT, AR, AREG, ASNS, BCL2, BCRP, BDCA1, beta III tubulin, BIRC5, B-RAF, BRCA1, BRCA2, CA2, caveolin, CD20, CD25, CD33, CD52, CDA, CDKN2A, CDKN1A, CDKN1B, CDK2, CDW52, CES2, CK 14, CK 17, CK 5/6, c-KIT, c-Met, c-Myc, COX-2, Cyclin D1, DCK, DHFR, DNMT1, DNMT3A, DNMT3B, E-Cadherin, ECGF1, EGFR, EML4-ALK fusion, EPHA2, Epiregulin, ER, ERBR2, ERCC1, ERCC3, EREG, ESR1, FLT1, folate receptor, FOLR1, FOLR2, FSHB, FSHPRH1, FSHR, FYN, GART, GNA11, GNAQ, GNRH1, GNRHR1, GSTP1, HCK, HDAC1, hENT-1, Her2/Neu, HGF, HIF1A, HIG1, HSP90, HSP90AA1, HSPCA, IGF-1R, IGFRBP, IGFRBP3, IGFRBP4, IGFRBP5, IL13RA1, IL2RA, KDR, Ki67, KIT, K-RAS, LCK, LTB, Lymphotoxin Beta Receptor, LYN, MET, MGMT, MLH1, MMR, MRP1, MS4A1, MSH2, MSH5, Myc, NFKB1, NFKB2, NFKBIA, NRAS, ODC1, OGFR, p16, p21, p27, p53, p95, PARP-1, PDGFC, PDGFR, PDGFRA, PDGFRB, PGP, PGR, PI3K, POLA, POLA1, PPARG, PPARGC1, PR, PTEN, PTGS2, PTPN12, RAF1, RARA, ROS1, RRM1, RRM2, RRM2B, RXRB, RXRG, SIK2, SPARC, SRC, SSTR1,

SSTR2, SSTR3, SSTR4, SSTR5, Survivin, TK1, TLE3, TNF, TOP1, TOP2A, TOP2B, TS, TUBB3, TXN, TXNRD1, TYMS, VDR, VEGF, VEGFA, VEGFC, VHL, YES1, ZAP70.

[00245] Table 2 provides a listing of gene and corresponding protein symbols and names of many of the molecular profiling targets that are analyzed according to the methods of the invention. As understood by those of skill in the art, genes and proteins have developed a number of alternative names in the scientific literature. Thus, the listing in **Table 2** comprises an illustrative but not exhaustive compilation. A further listing of gene aliases and descriptions can be found using a variety of online databases, including GeneCards® (www.genecards.org), HUGO Gene Nomenclature (www.genenames.org), Entrez Gene (www.ncbi.nlm.nih.gov/entrez/query.fcgi?db=gene), UniProtKB/Swiss-Prot (www.uniprot.org), UniProtKB/TrEMBL (www.uniprot.org), OMIM (www.ncbi.nlm.nih.gov/entrez/query.fcgi?db=OMIM), GeneLoc (genecards.weizmann.ac.il/geneloc/), and Ensembl (www.ensembl.org). Generally, gene symbols and names below correspond to those approved by HUGO, and protein names are those recommended by UniProtKB/Swiss-Prot. Common alternatives are provided as well. Where a protein name indicates a precursor, the mature protein is also implied. Throughout the application, gene and protein symbols may be used interchangeably and the meaning can be derived from context, e.g., FISH is used to analyze nucleic acids whereas IHC is used to analyze protein.

Table 2: Gene and Protein Names

Gene Symbol	Gene Name	Protein Symbol	Protein Name
ABCB1, PGP	ATP-binding cassette, sub-family B (MDR/TAP), member 1	ABCB1, MDR1, PGP	Multidrug resistance protein 1; P-glycoprotein
ABCC1, MRP1	ATP-binding cassette, sub-family C (CFTR/MRP), member 1	MRP1, ABCC1	Multidrug resistance-associated protein 1
ABCG2, BCRP	ATP-binding cassette, sub-family G (WHITE), member 2	ABCG2	ATP-binding cassette sub-family G member 2
ACE2	angiotensin I converting enzyme (peptidyl-dipeptidase A) 2	ACE2	Angiotensin-converting enzyme 2 precursor
ADA	adenosine deaminase	ADA	Adenosine deaminase
ADH1C	alcohol dehydrogenase 1C (class I), gamma polypeptide	ADH1G	Alcohol dehydrogenase 1C
ADH4	alcohol dehydrogenase 4 (class II), pi polypeptide	ADH4	Alcohol dehydrogenase 4
AGT	angiotensinogen (serpin peptidase inhibitor, clade A, member 8)	ANGT, AGT	Angiotensinogen precursor
ALK	anaplastic lymphoma receptor tyrosine kinase	ALK	ALK tyrosine kinase receptor precursor
AR	androgen receptor	AR	Androgen receptor
AREG	amphiregulin	AREG	Amphiregulin precursor
ASNS	asparagine synthetase	ASNS	Asparagine synthetase [glutamine-hydrolyzing]
BCL2	B-cell CLL/lymphoma 2	BCL2	Apoptosis regulator Bcl-2
BDCA1, CD1C	CD1c molecule	CD1C	T-cell surface glycoprotein CD1c precursor
BIRC5	baculoviral IAP repeat-containing 5	BIRC5, Survivin	Baculoviral IAP repeat-containing protein 5; Survivin
BRAF	v-raf murine sarcoma viral oncogene homolog B1	B-RAF, BRAF	Serine/threonine-protein kinase B-raf
BRCA1	breast cancer 1, early onset	BRCA1	Breast cancer type 1 susceptibility

			protein
BRCA2	breast cancer 2, early onset	BRCA2	Breast cancer type 2 susceptibility protein
CA2	carbonic anhydrase II	CA2	Carbonic anhydrase 2
CAV1	caveolin 1, caveolae protein, 22kDa	CAV1	Caveolin-1
CCND1	cyclin D1	CCND1, Cyclin D1, BCL-1	G1/S-specific cyclin-D1
CD20, MS4A1	membrane-spanning 4-domains, subfamily A, member 1	CD20	B-lymphocyte antigen CD20
CD25, IL2RA	interleukin 2 receptor, alpha	CD25	Interleukin-2 receptor subunit alpha precursor
CD33	CD33 molecule	CD33	Myeloid cell surface antigen CD33 precursor
CD52, CDW52	CD52 molecule	CD52	CAMPATH-1 antigen precursor
CDA	cytidine deaminase	CDA	Cytidine deaminase
CDH1, ECAD	cadherin 1, type 1, E-cadherin (epithelial)	E-Cad	Cadherin-1 precursor (E-cadherin)
CDK2	cyclin-dependent kinase 2	CDK2	Cell division protein kinase 2
CDKN1A, P21	cyclin-dependent kinase inhibitor 1A (p21, Cip1)	CDKN1A, p21	Cyclin-dependent kinase inhibitor 1
CDKN1B	cyclin-dependent kinase inhibitor 1B (p27, Kip1)	CDKN1B, p27	Cyclin-dependent kinase inhibitor 1B
CDKN2A, P16	cyclin-dependent kinase inhibitor 2A (melanoma, p16, inhibits CDK4)	CD21A, p16	Cyclin-dependent kinase inhibitor 2A, isoforms 1/2/3
CES2	carboxylesterase 2 (intestine, liver)	CES2, EST2	Carboxylesterase 2 precursor
CK 5/6	cytokeratin 5 / cytokeratin 6	CK 5/6	Keratin, type II cytoskeletal 5; Keratin, type II cytoskeletal 6
CK14, KRT14	keratin 14	CK14	Keratin, type I cytoskeletal 14
CK17, KRT17	keratin 17	CK17	Keratin, type I cytoskeletal 17
COX2, PTGS2	prostaglandin-endoperoxide synthase 2 (prostaglandin G/H synthase and cyclooxygenase)	COX-2, PTGS2	Prostaglandin G/H synthase 2 precursor
DCK	deoxycytidine kinase	DCK	Deoxycytidine kinase
DHFR	dihydrofolate reductase	DHFR	Dihydrofolate reductase
DNMT1	DNA (cytosine-5-)-methyltransferase 1	DNMT1	DNA (cytosine-5)-methyltransferase 1
DNMT3A	DNA (cytosine-5-)-methyltransferase 3 alpha	DNMT3A	DNA (cytosine-5)-methyltransferase 3A
DNMT3B	DNA (cytosine-5-)-methyltransferase 3 beta	DNMT3B	DNA (cytosine-5)-methyltransferase 3B
ECGF1, TYMP	thymidine phosphorylase	TYMP, PD-ECGF, ECDF1	Thymidine phosphorylase precursor
EGFR, ERBB1, HER1	epidermal growth factor receptor (erythroblastic leukemia viral (v-erb-b) oncogene homolog, avian)	EGFR, ERBB1, HER1	Epidermal growth factor receptor precursor
EML4	echinoderm microtubule associated protein like 4	EML4	Echinoderm microtubule-associated protein-like 4
EPHA2	EPH receptor A2	EPHA2	Ephrin type-A receptor 2 precursor
ER, ESR1	estrogen receptor 1	ER, ESR1	Estrogen receptor
ERBB2, HER2/NEU	v-erb-b2 erythroblastic leukemia viral oncogene homolog 2,	ERBB2, HER2, HER-	Receptor tyrosine-protein kinase erbB-2 precursor

	neuro/glioblastoma derived oncogene homolog (avian)	2/neu	
ERCC1	excision repair cross-complementing rodent repair deficiency, complementation group 1 (includes overlapping antisense sequence)	ERCC1	DNA excision repair protein ERCC-1
ERCC3	excision repair cross-complementing rodent repair deficiency, complementation group 3 (xeroderma pigmentosum group B complementing)	ERCC3	TFIIH basal transcription factor complex helicase XPB subunit
EREG	Epiregulin	EREG	Proepiregulin precursor
FLT1	fms-related tyrosine kinase 1 (vascular endothelial growth factor/vascular permeability factor receptor)	FLT-1, VEGFR1	Vascular endothelial growth factor receptor 1 precursor
FOLR1	folate receptor 1 (adult)	FOLR1	Folate receptor alpha precursor
FOLR2	folate receptor 2 (fetal)	FOLR2	Folate receptor beta precursor
FSHB	follicle stimulating hormone, beta polypeptide	FSHB	Follitropin subunit beta precursor
FSHPRH1, CENP1	centromere protein I	FSHPRH1, CENP1	Centromere protein I
FSHR	follicle stimulating hormone receptor	FSHR	Follicle-stimulating hormone receptor precursor
FYN	FYN oncogene related to SRC, FGR, YES	FYN	Tyrosine-protein kinase Fyn
GART	phosphoribosylglycinamide formyltransferase, phosphoribosylglycinamide synthetase, phosphoribosylaminoimidazole synthetase	GART, PUR2	Trifunctional purine biosynthetic protein adenosine-3
GNA11, GA11	guanine nucleotide binding protein (G protein), alpha 11 (Gq class)	GNA11, G alpha-11, G-protein subunit alpha-11	Guanine nucleotide-binding protein subunit alpha-11
GNAQ, GAQ	guanine nucleotide binding protein (G protein), q polypeptide	GNAQ	Guanine nucleotide-binding protein G(q) subunit alpha
GNRH1	gonadotropin-releasing hormone 1 (luteinizing-releasing hormone)	GNRH1, GON1	Progonadoliberin-1 precursor
GNRHR1, GNRHR	gonadotropin-releasing hormone receptor	GNRHR1	Gonadotropin-releasing hormone receptor
GSTP1	glutathione S-transferase pi 1	GSTP1	Glutathione S-transferase P
HCK	hemopoietic cell kinase	HCK	Tyrosine-protein kinase HCK
HDAC1	histone deacetylase 1	HDAC1	Histone deacetylase 1
HGF	hepatocyte growth factor (hepapoietin A; scatter factor)	HGF	Hepatocyte growth factor precursor
HIF1A	hypoxia inducible factor 1, alpha subunit (basic helix-loop-helix transcription factor)	HIF1A	Hypoxia-inducible factor 1-alpha
HIG1, HIGD1A, HIG1A	HIG1 hypoxia inducible domain family, member 1A	HIG1, HIGD1A, HIG1A	HIG1 domain family member 1A
HSP90AA1, HSP90,	heat shock protein 90kDa alpha (cytosolic), class A member 1	HSP90, HSP90A	Heat shock protein HSP 90-alpha

HSPCA			
IGF1R	insulin-like growth factor 1 receptor	IGF-1R	Insulin-like growth factor 1 receptor precursor
IGFBP3, IGFRBP3	insulin-like growth factor binding protein 3	IGFBP-3, IBP-3	Insulin-like growth factor-binding protein 3 precursor
IGFBP4, IGFRBP4	insulin-like growth factor binding protein 4	IGFBP-4, IBP-4	Insulin-like growth factor-binding protein 4 precursor
IGFBP5, IGFRBP5	insulin-like growth factor binding protein 5	IGFBP-5, IBP-5	Insulin-like growth factor-binding protein 5 precursor
IL13RA1	interleukin 13 receptor, alpha 1	IL-13RA1	Interleukin-13 receptor subunit alpha-1 precursor
KDR	kinase insert domain receptor (a type III receptor tyrosine kinase)	KDR, VEGFR2	Vascular endothelial growth factor receptor 2 precursor
KIT, c-KIT	v-kit Hardy-Zuckerman 4 feline sarcoma viral oncogene homolog	KIT, c-KIT, CD117, SCFR	Mast/stem cell growth factor receptor precursor
KRAS	v-Ki-ras2 Kirsten rat sarcoma viral oncogene homolog	K-RAS	GTPase KRas precursor
LCK	lymphocyte-specific protein tyrosine kinase	LCK	Tyrosine-protein kinase Lck
LTB	lymphotoxin beta (TNF superfamily, member 3)	LTB, TNF3	Lymphotoxin-beta
LTBR	lymphotoxin beta receptor (TNFR superfamily, member 3)	LTBR, LTBR3, TNFR	Tumor necrosis factor receptor superfamily member 3 precursor
LYN	v-yes-1 Yamaguchi sarcoma viral related oncogene homolog	LYN	Tyrosine-protein kinase Lyn
MET, c-MET	met proto-oncogene (hepatocyte growth factor receptor)	MET, c-MET	Hepatocyte growth factor receptor precursor
MGMT	O-6-methylguanine-DNA methyltransferase	MGMT	Methylated-DNA--protein-cysteine methyltransferase
MKI67, KI67	antigen identified by monoclonal antibody Ki-67	Ki67, Ki-67	Antigen KI-67
MLH1	mutL homolog 1, colon cancer, nonpolyposis type 2 (E. coli)	MLH1	DNA mismatch repair protein Mlh1
MMR	mismatch repair (refers to MLH1, MSH2, MSH5)		
MSH2	mutS homolog 2, colon cancer, nonpolyposis type 1 (E. coli)	MSH2	DNA mismatch repair protein Msh2
MSH5	mutS homolog 5 (E. coli)	MSH5, hMSH5	MutS protein homolog 5
MYC, c-MYC	v-myc myelocytomatosis viral oncogene homolog (avian)	MYC, c-MYC	Myc proto-oncogene protein
NBN, P95	nibrin	NBN, p95	Nibrin
NDGR1	N-myc downstream regulated 1	NDGR1	Protein NDGR1
NFKB1	nuclear factor of kappa light polypeptide gene enhancer in B-cells 1	NFKB1	Nuclear factor NF-kappa-B p105 subunit
NFKB2	nuclear factor of kappa light polypeptide gene enhancer in B-cells 2 (p49/p100)	NFKB2	Nuclear factor NF-kappa-B p100 subunit
NFKBIA	nuclear factor of kappa light polypeptide gene enhancer in B-cells inhibitor, alpha	NFKBIA	NF-kappa-B inhibitor alpha
NRAS	neuroblastoma RAS viral (v-ras) oncogene homolog	NRAS	GTPase NRas, Transforming protein N-Ras

ODC1	ornithine decarboxylase 1	ODC	Ornithine decarboxylase
OGFR	opioid growth factor receptor	OGFR	Opioid growth factor receptor
PARP1	poly (ADP-ribose) polymerase 1	PARP-1	Poly [ADP-ribose] polymerase 1
PDGFC	platelet derived growth factor C	PDGF-C, VEGF-E	Platelet-derived growth factor C precursor
PDGFR	platelet-derived growth factor receptor	PDGFR	Platelet-derived growth factor receptor
PDGFRA	platelet-derived growth factor receptor, alpha polypeptide	PDGFRA, PDGFR2, CD140 A	Alpha-type platelet-derived growth factor receptor precursor
PDGFRB	platelet-derived growth factor receptor, beta polypeptide	PDGFRB, PDGFR, PDGFR1, CD140 B	Beta-type platelet-derived growth factor receptor precursor
PGR	progesterone receptor	PR	Progesterone receptor
PIK3CA	phosphoinositide-3-kinase, catalytic, alpha polypeptide	PI3K subunit p110α	phosphoinositide-3-kinase, catalytic, alpha polypeptide
POLA1	polymerase (DNA directed), alpha 1, catalytic subunit; polymerase (DNA directed), alpha, polymerase (DNA directed), alpha 1	POLA, POLA1, p180	DNA polymerase alpha catalytic subunit
PPARG, PPARG1, PPARG2, PPAR-gamma, NR1C3	peroxisome proliferator-activated receptor gamma	PPARG	Peroxisome proliferator-activated receptor gamma
PPARGC1 A, LEM6, PGC1, PGC1A, PPARGC1	peroxisome proliferator-activated receptor gamma, coactivator 1 alpha	PGC-1-alpha, PPARGC-1-alpha	Peroxisome proliferator-activated receptor gamma coactivator 1-alpha; PPAR-gamma coactivator 1-alpha
PSMD9, P27	proteasome (prosome, macropain) 26S subunit, non-ATPase, 9	p27	26S proteasome non-ATPase regulatory subunit 9
PTEN, MMAC1, TEP1	phosphatase and tensin homolog	PTEN	Phosphatidylinositol-3,4,5-trisphosphate 3-phosphatase and dual-specificity protein phosphatase; Mutated in multiple advanced cancers 1
PTPN12	protein tyrosine phosphatase, non-receptor type 12	PTPG1	Tyrosine-protein phosphatase non-receptor type 12; Protein-tyrosine phosphatase G1
RAF1	v-raf-1 murine leukemia viral oncogene homolog 1	RAF, RAF-1, c-RAF	RAF proto-oncogene serine/threonine-protein kinase
RARA	retinoic acid receptor, alpha	RAR, RAR-alpha, RARA	Retinoic acid receptor alpha
ROS1, ROS, MCF3	c-ros oncogene 1, receptor tyrosine kinase	ROS1, ROS	Proto-oncogene tyrosine-protein kinase ROS
RRM1	ribonucleotide reductase M1	RRM1, RR1	Ribonucleoside-diphosphate reductase large subunit
RRM2	ribonucleotide reductase M2	RRM2, RR2M, RR2	Ribonucleoside-diphosphate reductase subunit M2
RRM2B	ribonucleotide reductase M2 B (TP53 inducible)	RRM2B, P53R2	Ribonucleoside-diphosphate reductase subunit M2 B
RXRB	retinoid X receptor, beta	RXRB	Retinoic acid receptor RXR-beta

RXRG	retinoid X receptor, gamma	RXRG, RXRC	Retinoic acid receptor RXR-gamma
SIK2	salt-inducible kinase 2	SIK2, Q9H0K1	Salt-inducible protein kinase 2; Serine/threonine-protein kinase SIK2
SLC29A1	solute carrier family 29 (nucleoside transporters), member 1	ENT-1	Equilibrative nucleoside transporter 1
SPARC	secreted protein, acidic, cysteine-rich (osteonectin)	SPARC	SPARC precursor; Osteonectin
SRC	v-src sarcoma (Schmidt-Ruppin A-2) viral oncogene homolog (avian)	SRC	Proto-oncogene tyrosine-protein kinase Src
SSTR1	somatostatin receptor 1	SSTR1, SSR1, SS1R	Somatostatin receptor type 1
SSTR2	somatostatin receptor 2	SSTR2, SSR2, SS2R	Somatostatin receptor type 2
SSTR3	somatostatin receptor 3	SSTR3, SSR3, SS3R	Somatostatin receptor type 3
SSTR4	somatostatin receptor 4	SSTR4, SSR4, SS4R	Somatostatin receptor type 4
SSTR5	somatostatin receptor 5	SSTR5, SSR5, SS5R	Somatostatin receptor type 5
TK1	thymidine kinase 1, soluble	TK1, KITH	Thymidine kinase, cytosolic
TLE3	transducin-like enhancer of split 3 (E(sp1) homolog, Drosophila)	TLE3	Transducin-like enhancer protein 3
TNF	tumor necrosis factor (TNF superfamily, member 2)	TNF, TNF-alpha, TNF-a	Tumor necrosis factor precursor
TOP1, TOPO1	topoisomerase (DNA) I	TOP1, TOPO1	DNA topoisomerase 1
TOP2A, TOPO2A	topoisomerase (DNA) II alpha 170kDa	TOP2A, TOP2, TOPO2A	DNA topoisomerase 2-alpha; Topoisomerase II alpha
TOP2B, TOPO2B	topoisomerase (DNA) II beta 180kDa	TOP2B, TOPO2B	DNA topoisomerase 2-beta; Topoisomerase II beta
TP53	tumor protein p53	p53	Cellular tumor antigen p53
TUBB3	tubulin, beta 3	Beta III tubulin, TUBB3, TUBB4	Tubulin beta-3 chain
TXN	thioredoxin	TXN, TRX, TRX-1	Thioredoxin
TXNRD1	thioredoxin reductase 1	TXNRD1, TXNR	Thioredoxin reductase 1, cytoplasmic; Oxidoreductase
TYMS, TS	thymidylate synthetase	TYMS, TS	Thymidylate synthase
VDR	vitamin D (1,25- dihydroxyvitamin D3) receptor	VDR	Vitamin D3 receptor
VEGFA, VEGF	vascular endothelial growth factor A	VEGF-A, VEGF	Vascular endothelial growth factor A precursor
VEGFC	vascular endothelial growth factor C	VEGF-C	Vascular endothelial growth factor C precursor
VHL	von Hippel-Lindau tumor suppressor	VHL	Von Hippel-Lindau disease tumor suppressor
YES1	v-yes-1 Yamaguchi sarcoma viral oncogene homolog 1	YES1, Yes, p61-Yes	Proto-oncogene tyrosine-protein kinase Yes
ZAP70	zeta-chain (TCR) associated protein kinase 70kDa	ZAP-70	Tyrosine-protein kinase ZAP-70

[00246] In some embodiments, additional molecular profiling methods are performed. These can include without limitation PCR, RT-PCR, Q-PCR, SAGE, MPSS, immunoassays and other techniques to assess biological systems described herein or known to those of skill in the art. The choice of genes and gene products to be assayed can be updated over time as new treatments and new drug targets are identified. Once the expression or mutation of a biomarker is correlated with a treatment option, it can be assessed by molecular profiling. One of skill will appreciate that such molecular profiling is not limited to those techniques disclosed herein but comprises any methodology conventional for assessing nucleic acid or protein levels, sequence information, or both. The methods of the invention can also take advantage of any improvements to current methods or new molecular profiling techniques developed in the future. In some embodiments, a gene or gene product is assessed by a single molecular profiling technique. In other embodiments, a gene and/or gene product is assessed by multiple molecular profiling techniques. In a non-limiting example, a gene sequence can be assayed by one or more of FISH and pyrosequencing analysis, the mRNA gene product can be assayed by one or more of RT-PCR and microarray, and the protein gene product can be assayed by one or more of IHC and immunoassay. One of skill will appreciate that any combination of biomarkers and molecular profiling techniques that will benefit disease treatment are contemplated by the invention.

[00247] Genes and gene products that are known to play a role in cancer and can be assayed by any of the molecular profiling techniques of the invention include without limitation 2AR, A DISINTEGRIN, ACTIVATOR OF THYROID AND RETINOIC ACID RECEPTOR (ACTR), ADAM 11, ADIPOGENESIS INHIBITORY FACTOR (ADIF), ALPHA 6 INTEGRIN SUBUNIT, ALPHA V INTEGRIN SUBUNIT, ALPHA-CATENIN, AMPLIFIED IN BREAST CANCER 1 (AIB1), AMPLIFIED IN BREAST CANCER 3 (AIB3), AMPLIFIED IN BREAST CANCER 4 (AIB4), AMYLOID PRECURSOR PROTEIN SECRETASE (APPS), AP-2 GAMMA, APPS, ATP-BINDING CASSETTE TRANSPORTER (ABCT), PLACENTA-SPECIFIC (ABCP), ATP-BINDING CASSETTE SUBFAMILY C MEMBER (ABCC1), BAG-1, BASIGIN (BSG), BCEI, B-CELL DIFFERENTIATION FACTOR (BCDF), B-CELL LEUKEMIA 2 (BCL-2), B-CELL STIMULATORY FACTOR-2 (BSF-2), BCL-1, BCL-2-ASSOCIATED X PROTEIN (BAX), BCRP, BETA 1 INTEGRIN SUBUNIT, BETA 3 INTEGRIN SUBUNIT, BETA 5 INTEGRIN SUBUNIT, BETA-2 INTERFERON, BETA-CATENIN, BETA-CATENIN, BONE SIALOPROTEIN (BSP), BREAST CANCER ESTROGEN-INDUCIBLE SEQUENCE (BCEI), BREAST CANCER RESISTANCE PROTEIN (BCRP), BREAST CANCER TYPE 1 (BRCA1), BREAST CANCER TYPE 2 (BRCA2), BREAST CARCINOMA AMPLIFIED SEQUENCE 2 (BCAS2), CADHERIN, EPITHELIAL CADHERIN-11, CADHERIN-ASSOCIATED PROTEIN, CALCITONIN RECEPTOR (CTR), CALCIUM PLACENTAL PROTEIN (CAPL), CALCYCLIN, CALLA, CAM5, CAPL, CARCINOEMBRYONIC ANTIGEN (CEA), CATENIN, ALPHA 1, CATHEPSIN B, CATHEPSIN D, CATHEPSIN K, CATHEPSIN L2, CATHEPSIN O, CATHEPSIN O1, CATHEPSIN V, CD10, CD146, CD147, CD24, CD29, CD44, CD51, CD54, CD61, CD66e, CD82, CD87, CD9, CEA, CELLULAR RETINOL-BINDING PROTEIN 1 (CRBP1), c-ERBB-2, CK7, CK8, CK18, CK19, CK20, CLAUDIN-7, c-MET, COLLAGENASE, FIBROBLAST,

COLLAGENASE, INTERSTITIAL, COLLAGENASE-3, COMMON ACUTE LYMPHOCYTIC LEUKEMIA ANTIGEN (CALLA), CONNEXIN 26 (Cx26), CONNEXIN 43 (Cx43), CORTACTIN, COX-2, CTLA-8, CTR, CTSD, CYCLIN D1, CYCLOOXYGENASE-2, CYTOKERATIN 18, CYTOKERATIN 19, CYTOKERATIN 8, CYTOTOXIC T-LYMPHOCYTE-ASSOCIATED SERINE ESTERASE 8 (CTLA-8), DIFFERENTIATION-INHIBITING ACTIVITY (DIA), DNA AMPLIFIED IN MAMMARY CARCINOMA 1 (DAM1), DNA TOPOISOMERASE II ALPHA, DR-NM23, E-CADHERIN, EMMPRIN, EMS1, ENDOTHELIAL CELL GROWTH FACTOR (ECGR), PLATELET-DERIVED (PD-ECGF), ENKEPHALINASE, EPIDERMAL GROWTH FACTOR RECEPTOR (EGFR), EPISIALIN, EPITHELIAL MEMBRANE ANTIGEN (EMA), ER-ALPHA, ERBB2, ERBB4, ER-BETA, ERF-1, ERYTHROID-POTENTIATING ACTIVITY (EPA), ESR1, ESTROGEN RECEPTOR-ALPHA, ESTROGEN RECEPTOR-BETA, ETS-1, EXTRACELLULAR MATRIX METALLOPROTEINASE INDUCER (EMMPRIN), FIBRONECTIN RECEPTOR, BETA POLYPEPTIDE (FNRB), FIBRONECTIN RECEPTOR BETA SUBUNIT (FNRB), FLK-1, GA15.3, GA733.2, GALECTIN-3, GAMMA-CATENIN, GAP JUNCTION PROTEIN (26 kDa), GAP JUNCTION PROTEIN (43 kDa), GAP JUNCTION PROTEIN ALPHA-1 (GJA1), GAP JUNCTION PROTEIN BETA-2 (GJB2), GCP1, GELATINASE A, GELATINASE B, GELATINASE (72 kDa), GELATINASE (92 kDa), GLIOSTATIN, GLUCOCORTICOID RECEPTOR INTERACTING PROTEIN 1 (GRIP1), GLUTATHIONE S-TRANSFERASE p, GM-CSF, GRANULOCYTE CHEMOTACTIC PROTEIN 1 (GCP1), GRANULOCYTE-MACROPHAGE-COLONY STIMULATING FACTOR, GROWTH FACTOR RECEPTOR BOUND-7 (GRB-7), GSTp, HAP, HEAT-SHOCK COGNATE PROTEIN 70 (HSC70), HEAT-STABLE ANTIGEN, HEPATOCYTE GROWTH FACTOR (HGF), HEPATOCYTE GROWTH FACTOR RECEPTOR (HGFR), HEPATOCYTE-STIMULATING FACTOR III (HSF III), HER-2, HER2/NEU, HERMES ANTIGEN, HET, HHM, HUMORAL HYPERCALCEMIA OF MALIGNANCY (HHM), ICERE-1, INT-1, INTERCELLULAR ADHESION MOLECULE-1 (ICAM-1), INTERFERON-GAMMA-INDUCING FACTOR (IGIF), INTERLEUKIN-1 ALPHA (IL-1A), INTERLEUKIN-1 BETA (IL-1B), INTERLEUKIN-11 (IL-11), INTERLEUKIN-17 (IL-17), INTERLEUKIN-18 (IL-18), INTERLEUKIN-6 (IL-6), INTERLEUKIN-8 (IL-8), INVERSELY CORRELATED WITH ESTROGEN RECEPTOR EXPRESSION-1 (ICERE-1), KAI1, KDR, KERATIN 8, KERATIN 18, KERATIN 19, KISS-1, LEUKEMIA INHIBITORY FACTOR (LIF), LIF, LOST IN INFLAMMATORY BREAST CANCER (LIBC), LOT ("LOST ON TRANSFORMATION"), LYMPHOCYTE HOMING RECEPTOR, MACROPHAGE-COLONY STIMULATING FACTOR, MAGE-3, MAMMAGLOBIN, MASPIN, MC56, M-CSF, MDC, MDNCF, MDR, MELANOMA CELL ADHESION MOLECULE (MCAM), MEMBRANE METALLOENDOPEPTIDASE (MME), MEMBRANE-ASSOCIATED NEUTRAL ENDOPEPTIDASE (NEP), CYSTEINE-RICH PROTEIN (MDC), METASTASIN (MTS-1), MLN64, MMP1, MMP2, MMP3, MMP7, MMP9, MMP11, MMP13, MMP14, MMP15, MMP16, MMP17, MOESIN, MONOCYTE ARGININE-SERPIN, MONOCYTE-DERIVED NEUTROPHIL CHEMOTACTIC FACTOR, MONOCYTE-DERIVED PLASMINOGEN ACTIVATOR INHIBITOR, MTS-1, MUC-1, MUC18, MUCIN LIKE CANCER ASSOCIATED ANTIGEN (MCA), MUCIN, MUC-

1, MULTIDRUG RESISTANCE PROTEIN 1 (MDR, MDR1), MULTIDRUG RESISTANCE RELATED PROTEIN-1 (MRP, MRP-1), N-CADHERIN, NEP, NEU, NEUTRAL ENDOPEPTIDASE, NEUTROPHIL-ACTIVATING PEPTIDE 1 (NAP1), NM23-H1, NM23-H2, NME1, NME2, NUCLEAR RECEPTOR COACTIVATOR-1 (NCoA-1), NUCLEAR RECEPTOR COACTIVATOR-2 (NCoA-2), NUCLEAR RECEPTOR COACTIVATOR-3 (NCoA-3), NUCLEOSIDE DIPHOSPHATE KINASE A (NDPKA), NUCLEOSIDE DIPHOSPHATE KINASE B (NDPKB), ONCOSTATIN M (OSM), ORNITHINE DECARBOXYLASE (ODC), OSTEOCLAST DIFFERENTIATION FACTOR (ODF), OSTEOCLAST DIFFERENTIATION FACTOR RECEPTOR (ODFR), OSTEONECTIN (OSN, ON), OSTEOPONTIN (OPN), OXYTOCIN RECEPTOR (OXTR), p27/kip1, p300/CBP COINTEGRATOR ASSOCIATE PROTEIN (p/CIP), p53, p9Ka, PAI-1, PAI-2, PARATHYROID ADENOMATOSIS 1 (PRAD1), PARATHYROID HORMONE-LIKE HORMONE (PTHLH), PARATHYROID HORMONE-RELATED PEPTIDE (PTHrP), P-CADHERIN, PD-ECGF, PDGF, PEANUT-REACTIVE URINARY MUCIN (PUM), P-GLYCOPROTEIN (P-GP), PGP-1, PHGS-2, PHS-2, PIP, PLAKOGLOBIN, PLASMINOGEN ACTIVATOR INHIBITOR (TYPE 1), PLASMINOGEN ACTIVATOR INHIBITOR (TYPE 2), PLASMINOGEN ACTIVATOR (TISSUE-TYPE), PLASMINOGEN ACTIVATOR (UROKINASE-TYPE), PLATELET GLYCOPROTEIN IIIa (GP3A), PLAU, PLEOMORPHIC ADENOMA GENE-LIKE 1 (PLAGL1), POLYMORPHIC EPITHELIAL MUCIN (PEM), PRAD1, PROGESTERONE RECEPTOR (PgR), PROGESTERONE RESISTANCE, PROSTAGLANDIN ENDOPEROXIDE SYNTHASE-2, PROSTAGLANDIN G/H SYNTHASE-2, PROSTAGLANDIN H SYNTHASE-2, pS2, PS6K, PSORIASIN, PTHLH, PTHrP, RAD51, RAD52, RAD54, RAP46, RECEPTOR-ASSOCIATED COACTIVATOR 3 (RAC3), REPRESSOR OF ESTROGEN RECEPTOR ACTIVITY (REA), S100A4, S100A6, S100A7, S6K, SART-1, SCAFFOLD ATTACHMENT FACTOR B (SAF-B), SCATTER FACTOR (SF), SECRETED PHOSPHOPROTEIN-1 (SPP-1), SECRETED PROTEIN, ACIDIC AND RICH IN CYSTEINE (SPARC), STANNICALCIN, STEROID RECEPTOR COACTIVATOR-1 (SRC-1), STEROID RECEPTOR COACTIVATOR-2 (SRC-2), STEROID RECEPTOR COACTIVATOR-3 (SRC-3), STEROID RECEPTOR RNA ACTIVATOR (SRA), STROMELYSIN-1, STROMELYSIN-3, TENASCIN-C (TN-C), TESTES-SPECIFIC PROTEASE 50, THROMBOSPONDIN I, THROMBOSPONDIN II, THYMIDINE PHOSPHORYLASE (TP), THYROID HORMONE RECEPTOR ACTIVATOR MOLECULE 1 (TRAM-1), TIGHT JUNCTION PROTEIN 1 (TJP1), TIMP1, TIMP2, TIMP3, TIMP4, TISSUE-TYPE PLASMINOGEN ACTIVATOR, TN-C, TP53, tPA, TRANSCRIPTIONAL INTERMEDIARY FACTOR 2 (TIF2), TREFOIL FACTOR 1 (TFF1), TSG101, TSP-1, TSP1, TSP-2, TSP2, TSP50, TUMOR CELL COLLAGENASE STIMULATING FACTOR (TCSF), TUMOR-ASSOCIATED EPITHELIAL MUCIN, uPA, uPAR, UROKINASE, UROKINASE-TYPE PLASMINOGEN ACTIVATOR, UROKINASE-TYPE PLASMINOGEN ACTIVATOR RECEPTOR (uPAR), UVOMORULIN, VASCULAR ENDOTHELIAL GROWTH FACTOR, VASCULAR ENDOTHELIAL GROWTH FACTOR RECEPTOR-2 (VEGFR2), VASCULAR ENDOTHELIAL GROWTH FACTOR-A, VASCULAR PERMEABILITY FACTOR, VEGFR2, VERY LATE T-CELL ANTIGEN BETA (VLA-BETA), VIMENTIN, VITRONECTIN

RECEPTOR ALPHA POLYPEPTIDE (VNRA), VITRONECTIN RECEPTOR, VON WILLEBRAND FACTOR, VPF, VWF, WNT-1, ZAC, ZO-1, and ZONULA OCCLUDENS-1.

[00248] In some embodiments, IHC is used to detect on or more of the following proteins, including without limitation: ADA, AR, ASNA, BCL2, BRCA2, c-Met, CD33, CDW52, CES2, DNMT1, EGFR, EML4-ALK fusion, ERBB2, ERCC3, ESR1, FOLR2, GART, GSTP1, HDAC1, hENT-1, HIF1A, HSPCA, IGF-1R, IL2RA, KIT, MLH1, MMR, MS4A1, MASH2, NFKB2, NFKBIA, OGFR, p16, p21, p27, PARP-1, PI3K, PDGFC, PDGFRA, PDGFRB, PGR, POLA, PTEN, PTGS2, RAF1, RARA, RXRB, SPARC, SSTR1, TK1, TLE3, TNF, TOP1, TOP2A, TOP2B, TXNRD1, TYMS, VDR, VEGF, VHL, or ZAP70. The proteins can be detected by IHC using monoclonal or polyclonal antibodies. In some embodiments, both are used. As an illustrative example, SPARC can be detected by anti-SPARC monoclonal (SPARC mono, SPARC m) and/or anti-SPARC polyclonal (SPARC poly, SPARC p) antibodies. As described herein, the molecular characteristics of the tumor determined can be determined by IHC combined with one or more of gene copy number, gene expression, and mutation analysis. The genes and/or gene products used for IHC analysis can be at least 1, 2, 3, 4, 5, 6, 7, 8, 9, 10, 15, 20, 25, 30, 40, 50, 60, 70, 80, 90, 100 or all of the genes and/or gene products listed in **Table 2**.

[00249] In some embodiments, the genes used for gene expression profiling comprise one or more of: EGFR, SPARC, C-kit, ER, PR, Androgen receptor, PGP, RRM1, TOPO1, BRCP1, MRP1, MGMT, PDGFR, DCK, ERCC1, Thymidylate synthase, Her2/neu, TOPO2A, ADA, AR, ASNA, BCL2, BRCA2, CD33, CDW52, CES2, DNMT1, EGFR, ERBB2, ERCC3, ESR1, FOLR2, GART, GSTP1, HDAC1, HIF1A, HSPCA, IL2RA, KIT, MLH1, MS4A1, MASH2, NFKB2, NFKBIA, OGFR, PDGFC, PDGFRA, PDGFRB, PGR, POLA, PTEN, PTGS2, RAF1, RARA, RXRB, SPARC, SSTR1, TK1, TNF, TOP1, TOP2A, TOP2B, TXNRD1, TYMS, VDR, VEGF, VHL, and ZAP70. One or more of the following genes can also be assessed by gene expression profiling: ALK, EML4, hENT-1, IGF-1R, HSP90AA1, MMR, p16, p21, p27, PARP-1, PI3K and TLE3. The gene expression profiling can be performed using a low density microarray, an expression microarray, a comparative genomic hybridization (CGH) microarray, a single nucleotide polymorphism (SNP) microarray, a proteomic array an antibody array, or other array as disclosed herein or known to those of skill in the art. In some embodiments, high throughput expression arrays are used. Such systems include without limitation commercially available systems from Affymetrix, Agilent or Illumina, as described in more detail herein. Expression profiling can be performed using PCR, e.g., real-time PCR (qPCR or RT-PCR). Alternate gene expression techniques can be used as well. The genes and/or gene products examined gene expression profiling analysis can be at least 1, 2, 3, 4, 5, 6, 7, 8, 9, 10, 15, 20, 25, 30, 40, 50, 60, 70, 80, 90, 100 or all of the genes and/or gene products listed in **Table 2**.

[00250] ISH analysis can be used to profile one or more of HER2, CMET, PIK3CA, EGFR, TOP2A, CMYC and EML4-ALK fusion. ISH may include FISH, CISH or the like. In some embodiments, ISH is used to detect or test for one or more of the following genes, including without limitation: EGFR, SPARC, C-kit, ER, PR, AR, PGP, RRM1, TOPO1, BRCP1, MRP1, MGMT, PDGFR, DCK, ERCC1, TS, HER2, or TOPO2A. In some embodiments, ISH is used to detect or test for one or more of EML4-ALK fusion

and IGF-1R. In some embodiments, ISH is used to detect or test various biomarkers, including without limitation one or more of the following: ADA, AR, ASNA, BCL2, BRCA2, c-Met, CD33, CDW52, CES2, DNMT1, EGFR, EML4-ALK fusion, ERBB2, ERCC3, ESR1, FOLR2, GART, GSTP1, HDAC1, hENT-1, HIF1A, HSPCA, IGF-1R, IL2RA, KIT, MLH1, MMR, MS4A1, MASH2, NFKB2, NFKBIA, OGFR, p16, p21, p27, PARP-1, PI3K, PDGFC, PDGFRA, PDGFRB, PGR, POLA, PTEN, PTGS2, RAF1, RARA, RXRB, SPARC, SSTR1, TK1, TLE3, TNF, TOP1, TOP2A, TOP2B, TXNRD1, TYMS, VDR, VEGF, VHL, or ZAP70. The genes and/or gene products used for ISH analysis can be at least 1, 2, 3, 4, 5, 6, 7, 8, 9, 10, 15, 20, 25, 30, 40, 50, 60, 70, 80, 90, 100 or all of the genes and/or gene products listed in **Table 2**.

[00251] Mutation profiling can be determined by sequencing, including Sanger sequencing, array sequencing, pyrosequencing, NextGen sequencing, etc. Sequence analysis may reveal that genes harbor activating mutations so that drugs that inhibit activity are indicated for treatment. Alternately, sequence analysis may reveal that genes harbor mutations that inhibit or eliminate activity, thereby indicating treatment for compensating therapies. In embodiments, sequence analysis comprises that of exon 9 and 11 of c-KIT. Sequencing may also be performed on EGFR-kinase domain exons 18, 19, 20, and 21. Mutations, amplifications or misregulations of EGFR or its family members are implicated in about 30% of all epithelial cancers. Sequencing can also be performed on PI3K, encoded by the PIK3CA gene. This gene is a found mutated in many cancers. Sequencing analysis can also comprise assessing mutations in one or more ABCC1, ABCG2, ADA, AR, ASNS, BCL2, BIRC5, BRCA1, BRCA2, CD33, CD52, CDA, CES2, DCK, DHFR, DNMT1, DNMT3A, DNMT3B, ECGF1, EGFR, EPHA2, ERBB2, ERCC1, ERCC3, ESR1, FLT1, FOLR2, FYN, GART, GNRH1, GSTP1, HCK, HDAC1, HIF1A, HSP90AA1, IGFBP3, IGFBP4, IGFBP5, IL2RA, KDR, KIT, LCK, LYN, MET, MGMT, MLH1, MS4A1, MSH2, NFKB1, NFKB2, NFKBIA, NRAS, OGFR, PARP1, PDGFC, PDGFRA, PDGFRB, PGP, PGR, POLA1, PTEN, PTGS2, PTPN12, RAF1, RARA, RRM1, RRM2, RRM2B, RXRB, RXRG, SIK2, SPARC, SRC, SSTR1, SSTR2, SSTR3, SSTR4, SSTR5, TK1, TNF, TOP1, TOP2A, TOP2B, TXNRD1, TYMS, VDR, VEGFA, VHL, YES1, and ZAP70. One or more of the following genes can also be assessed by sequence analysis: ALK, EML4, hENT-1, IGF-1R, HSP90AA1, MMR, p16, p21, p27, PARP-1, PI3K and TLE3. The genes and/or gene products used for mutation or sequence analysis can be at least 1, 2, 3, 4, 5, 6, 7, 8, 9, 10, 15, 20, 25, 30, 40, 50, 60, 70, 80, 90, 100 or all of the genes and/or gene products listed in **Table 2**, **Table 6** or **Table 17**.

[00252] In embodiments, the methods of the invention are used detect gene fusions, such as those listed in U.S. Patent Application 12/658,770, filed February 12, 2010; International PCT Patent Application PCT/US2010/000407, filed February 11, 2010; and International PCT Patent Application PCT/US2010/54366, filed October 27, 2010; all of which applications are incorporated by reference herein in their entirety. A fusion gene is a hybrid gene created by the juxtaposition of two previously separate genes. This can occur by chromosomal translocation or inversion, deletion or via trans-splicing. The resulting fusion gene can cause abnormal temporal and spatial expression of genes, leading to abnormal expression of cell growth factors, angiogenesis factors, tumor promoters or other factors

contributing to the neoplastic transformation of the cell and the creation of a tumor. For example, such fusion genes can be oncogenic due to the juxtaposition of: 1) a strong promoter region of one gene next to the coding region of a cell growth factor, tumor promoter or other gene promoting oncogenesis leading to elevated gene expression, or 2) due to the fusion of coding regions of two different genes, giving rise to a chimeric gene and thus a chimeric protein with abnormal activity. Fusion genes are characteristic of many cancers. Once a therapeutic intervention is associated with a fusion, the presence of that fusion in any type of cancer identifies the therapeutic intervention as a candidate therapy for treating the cancer.

[00253] The presence of fusion genes, e.g., those described in U.S. Patent Application 12/658,770, filed February 12, 2010; International PCT Patent Application PCT/US2010/000407, filed February 11, 2010; and International PCT Patent Application PCT/US2010/54366, filed October 27, 2010 or elsewhere herein, can be used to guide therapeutic selection. For example, the BCR-ABL gene fusion is a characteristic molecular aberration in ~90% of chronic myelogenous leukemia (CML) and in a subset of acute leukemias (Kurzrock *et al., Annals of Internal Medicine* 2003; **138**:819-830). The BCR-ABL results from a translocation between chromosomes 9 and 22, commonly referred to as the Philadelphia chromosome or Philadelphia translocation. The translocation brings together the 5' region of the BCR gene and the 3' region of ABL1, generating a chimeric BCR-ABL1 gene, which encodes a protein with constitutively active tyrosine kinase activity (Mittleman et al., Nature *Reviews Cancer* 2007; 7:233-245). The aberrant tyrosine kinase activity leads to de-regulated cell signaling, cell growth and cell survival, apoptosis resistance and growth factor independence, all of which contribute to the pathophysiology of leukemia (Kurzrock et al., *Annals of Internal Medicine* 2003; **138**:819-830). Patients with the Philadelphia chromosome are treated with imatinib and other targeted therapies. Imatinib binds to the site of the constitutive tyrosine kinase activity of the fusion protein and prevents its activity. Imatinib treatment has led to molecular responses (disappearance of BCR-ABL+ blood cells) and improved progression-free survival in BCR-ABL+ CML patients (Kantarjian *et al., Clinical Cancer Research* 2007; **13**:1089-1097).

[00254] Another fusion gene, IGH-MYC, is a defining feature of ~80% of Burkitt's lymphoma (Ferry et al. *Oncologist* 2006; 11:375-83). The causal event for this is a translocation between chromosomes 8 and 14, bringing the c-Myc oncogene adjacent to the strong promoter of the immunoglobulin heavy chain gene, causing *c-myc* overexpression (Mittleman et al., *Nature Reviews Cancer* 2007; 7:233-245). The c-*myc* rearrangement is a pivotal event in lymphomagenesis as it results in a perpetually proliferative state. It has wide ranging effects on progression through the cell cycle, cellular differentiation, apoptosis, and cell adhesion (Ferry et al. *Oncologist* 2006; 11:375-83).

[00255] A number of recurrent fusion genes have been catalogued in the Mittleman database (cgap.nci.nih.gov/Chromosomes/Mitelman). The gene fusions can be used to characterize neoplasms and cancers and guide therapy using the subject methods described herein. For example, TMPRSS2-ERG, TMPRSS2-ETV and SLC45A3-ELK4 fusions can be detected to characterize prostate cancer; and ETV6-NTRK3 and ODZ4-NRG1 can be used to characterize breast cancer. The EML4-ALK, RLF-MYCL1, TGF-ALK, or CD74-ROS1 fusions can be used to characterize a lung cancer. The ACSL3-ETV1, C15ORF21-ETV1, FLJ35294-ETV1, HERV-ETV1, TMPRSS2-ERG, TMPRSS2-ETV1/4/5, TMPRSS2-

ETV4/5, SLC5A3-ERG, SLC5A3-ETV1, SLC5A3-ETV5 or KLK2-ETV4 fusions can be used to characterize a prostate cancer. The GOPC-ROS1 fusion can be used to characterize a brain cancer. The CHCHD7-PLAG1, CTNNB1-PLAG1, FHIT-HMGA2, HMGA2-NFIB, LIFR-PLAG1, or TCEA1-PLAG1 fusions can be used to characterize a head and neck cancer. The ALPHA-TFEB, NONO-TFE3, PRCC-TFE3, SFPQ-TFE3, CLTC-TFE3, or MALAT1-TFEB fusions can be used to characterize a renal cell carcinoma (RCC). The AKAP9-BRAF, CCDC6-RET, ERC1-RETM, GOLGA5-RET, HOOK3-RET, HRH4-RET, KTN1-RET, NCOA4-RET, PCM1-RET, PRKARA1A-RET, RFG-RET, RFG9-RET, Ria-RET, TGF-NTRK1, TPM3-NTRK1, TPM3-TPR, TPR-MET, TPR-NTRK1, TRIM24-RET, TRIM27-RET or TRIM33-RET fusions can be used to characterize a thyroid cancer and/or papillary thyroid carcinoma; and the PAX8-PPARy fusion can be analyzed to characterize a follicular thyroid cancer. Fusions that are associated with hematological malignancies include without limitation TTL-ETV6, CDK6-MLL, CDK6-TLX3, ETV6-FLT3, ETV6-RUNX1, ETV6-TTL, MLL-AFF1, MLL-AFF3, MLL-AFF4, MLL-GAS7, TCBA1-ETV6, TCF3-PBX1 or TCF3-TFPT, which are characteristic of acute lymphocytic leukemia (ALL); BCL11B-TLX3, IL2-TNFRFS17, NUP214-ABL1, NUP98-CCDC28A, TAL1-STIL, or ETV6-ABL2, which are characteristic of T-cell acute lymphocytic leukemia (T-ALL); ATIC-ALK, KIAA1618-ALK, MSN-ALK, MYH9-ALK, NPM1-ALK, TGF-ALK or TPM3-ALK, which are characteristic of anaplastic large cell lymphoma (ALCL); BCR-ABL1, BCR-JAK2, ETV6-EVI1, ETV6-MN1 or ETV6-TCBA1, characteristic of chronic myelogenous leukemia (CML); CBFB-MYH11, CHIC2-ETV6, ETV6-ABL1, ETV6-ABL2, ETV6-ARNT, ETV6-CDX2, ETV6-HLXB9, ETV6-PER1, MEF2D-DAZAP1, AML-AFF1, MLL-ARHGAP26, MLL-ARHGEF12, MLL-CASC5, MLL-CBL,MLL-CREBBP, MLL-DAB21P, MLL-ELL, MLL-EP300, MLL-EPS15, MLL-FNBP1, MLL-FOXO3A, MLL-GMPS, MLL-GPHN, MLL-MLLT1, MLL-MLLT11, MLL-MLLT3, MLL-MLLT6, MLL-MYO1F, MLL-PICALM, MLL-SEPT2, MLL-SEPT6, MLL-SORBS2, MYST3-SORBS2, MYST-CREBBP, NPM1-MLF1, NUP98-HOXA13, PRDM16-EVI1, RABEP1-PDGFRB, RUNX1-EVI1, RUNX1-MDS1, RUNX1-RPL22, RUNX1-RUNX1T1, RUNX1-SH3D19, RUNX1-USP42, RUNX1-YTHDF2, RUNX1-ZNF687, or TAF15-ZNF-384, which are characteristic of acute myeloid leukemia (AML); CCND1-FSTL3, which is characteristic of chronic lymphocytic leukemia (CLL); BCL3-MYC, MYC-BTG1, BCL7A-MYC, BRWD3-ARHGAP20 or BTG1-MYC, which are characteristic of B-cell chronic lymphocytic leukemia (B-CLL); CITTA-BCL6, CLTC-ALK, IL21R-BCL6, PIM1-BCL6, TFCR-BCL6, IKZF1-BCL6 or SEC31A-ALK, which are characteristic of diffuse large B-cell lymphomas (DLBCL); FLIP1-PDGFRA, FLT3-ETV6, KIAA1509-PDGFRA, PDE4DIP-PDGFRB, NIN-PDGFRB, TP53BP1-PDGFRB, or TPM3-PDGFRB, which are characteristic of hyper eosinophilia / chronic eosinophilia; and IGH-MYC or LCP1-BCL6, which are characteristic of Burkitt's lymphoma. One of skill will understand that additional fusions, including those yet to be identified to date, can be used to guide treatment once their presence is associated with a therapeutic intervention.

[00256] The fusion genes and gene products can be detected using one or more techniques described herein. In some embodiments, the sequence of the gene or corresponding mRNA is determined, e.g., using Sanger sequencing, NextGen sequencing, pyrosequencing, DNA microarrays, etc. Chromosomal

abnormalities can be assessed using FISH or PCR techniques, among others. For example, a break apart probe can be used for FISH detection of ALK fusions such as *EML4-ALK*, *KIF5B-ALK* and/or *TFG-ALK*. As an alternate, PCR can be used to amplify the fusion product, wherein amplification or lack thereof indicates the presence or absence of the fusion, respectively. In some embodiments, the fusion protein fusion is detected. Appropriate methods for protein analysis include without limitation mass spectroscopy, electrophoresis (e.g., 2D gel electrophoresis or SDS-PAGE) or antibody related techniques, including immunoassay, protein array or immunohistochemistry. The techniques can be combined. As a non-limiting example, indication of an ALK fusion by FISH can be confirmed for ALK expression using IHC, or vice versa.

Treatment Selection

[00257] The systems and methods allow identification of one or more therapeutic targets whose projected efficacy can be linked to therapeutic efficacy, ultimately based on the molecular profiling. Illustrative schemes for using molecular profiling to identify a treatment regime are shown in **FIGs. 2**, **49A-B** and **50**, each of which is described in further detail herein. The invention comprises use of molecular profiling results to suggest associations with treatment responses. In an embodiment, the appropriate biomarkers for molecular profiling are selected on the basis of the subject's tumor type. These suggested biomarkers can be used to modify a default list of biomarkers. In other embodiments, the molecular profiling is independent of the source material. In some embodiments, rules are used to provide the suggested chemotherapy treatments based on the molecular profiling test results. In an embodiment, the rules are generated from abstracts of the peer reviewed clinical oncology literature. Expert opinion rules can be used but are optional. In an embodiment, clinical citations are assessed for their relevance to the methods of the invention using a hierarchy derived from the evidence grading system used by the United States Preventive Services Taskforce. The "best evidence" can be used as the basis for a rule. The simplest rules are constructed in the format of "if biomarker positive then treatment option one, else treatment option two." Treatment options comprise no treatment with a specific drug, treatment with a specific drug or treatment with a combination of drugs. In some embodiments, more complex rules are constructed that involve the interaction of two or more biomarkers. In such cases, the more complex interactions are typically supported by clinical studies that analyze the interaction between the biomarkers included in the rule. Finally, a report can be generated that describes the association of the chemotherapy response and the biomarker and a summary statement of the best evidence supporting the treatments selected. Ultimately, the treating physician will decide on the best course of treatment.

[00258] As a non-limiting example, molecular profiling might reveal that the EGFR gene is amplified or overexpressed, thus indicating selection of a treatment that can block EGFR activity, such as the monoclonal antibody inhibitors cetuximab and panitumumab, or small molecule kinase inhibitors effective in patients with activating mutations in EGFR such as gefitinib, erlotinib, and lapatinib. Other anti-EGFR monoclonal antibodies in clinical development include zalutumumab, nimotuzumab, and matuzumab. The candidate treatment selected can depend on the setting revealed by molecular profiling. For example, kinase inhibitors are often prescribed with EGFR is found to have activating mutations.

Continuing with the illustrative embodiment, molecular profiling may also reveal that some or all of these treatments are likely to be less effective. For example, patients taking gefitinib or erlotinib eventually develop drug resistance mutations in EGFR. Accordingly, the presence of a drug resistance mutation would contraindicate selection of the small molecule kinase inhibitors. One of skill will appreciate that this example can be expanded to guide the selection of other candidate treatments that act against genes or gene products whose differential expression is revealed by molecular profiling. Similarly, candidate agents known to be effective against diseased cells carrying certain nucleic acid variants can be selected if molecular profiling reveals such variants.

[00259] As another example, consider the drug imatinib, currently marketed by Novartis as Gleevec in the US in the form of imatinib mesylate. Imatinib is a 2-phenylaminopyrimidine derivative that functions as a specific inhibitor of a number of tyrosine kinase enzymes. It occupies the tyrosine kinase active site, leading to a decrease in kinase activity. Imatinib has been shown to block the activity of Abelson cytoplasmic tyrosine kinase (ABL), c-Kit and the platelet-derived growth factor receptor (PDGFR). Thus, imatinib can be indicated as a candidate therapeutic for a cancer determined by molecular profiling to overexpress ABL, c-KIT or PDGFR. Imatinib can be indicated as a candidate therapeutic for a cancer determined by molecular profiling to have mutations in ABL, c-KIT or PDGFR that alter their activity, e.g., constitutive kinase activity of ABLs caused by the BCR-ABL mutation. As an inhibitor of PDGFR, imatinib mesylate appears to have utility in the treatment of a variety of dermatological diseases.

[00260] Cancer therapies that can be identified as candidate treatments by the methods of the invention include without limitation: 13-cis-Retinoic Acid, 2-CdA, 2-Chlorodeoxyadenosine, 5-Azacitidine, 5-Fluorouracil, 5-FU, 6-Mercaptopurine, 6-MP, 6-TG, 6-Thioguanine, Abraxane, Accutane®, Actinomycin-D, Adriamycin®, Adrucil®, Afinitor®, Agrylin®, Ala-Cort®, Aldesleukin, Alemtuzumab, ALIMTA, Alitretinoin, Alkaban-AQ®, Alkeran®, All-transretinoic Acid, Alpha Interferon, Altretamine, Amethopterin, Amifostine, Aminoglutethimide, Anagrelide, Anandron®, Anastrozole, Arabinosylcytosine, Ara-C, Aranesp®, Aredia®, Arimidex®, Aromasin®, Arranon®, Arsenic Trioxide, Asparaginase, ATRA, Avastin®, Azacitidine, BCG, BCNU, Bendamustine, Bevacizumab, Bexarotene, BEXXAR®, Bicalutamide, BiCNU, Blenoxane®, Bleomycin, Bortezomib, Busulfan, Busulfex®, C225, Calcium Leucovorin, Campath®, Camptosar®, Camptothecin-11, Capecitabine, Carac™, Carboplatin, Carmustine, Carmustine Wafer, Casodex®, CC-5013, CCI-779, CCNU, CDDP, CeeNU, Cerubidine®, Cetuximab, Chlorambucil, Cisplatin, Citrovorum Factor, Cladribine, Cortisone, Cosmegen®, CPT-11, Cyclophosphamide, Cytadren®, Cytarabine, Cytarabine Liposomal, Cytosar-U®, Cytoxan®, Dacarbazine, Dacogen, Dactinomycin, Darbepoetin Alfa, Dasatinib, Daunomycin Daunorubicin, Daunorubicin Hydrochloride, Daunorubicin Liposomal, DaunoXome®, Decadron, Decitabine, Delta-Cortef®, Deltasone®, Denileukin, Diftitox, DepoCyt™, Dexamethasone, Dexamethasone Acetate Dexamethasone Sodium Phosphate, Dexasone, Dexrazoxane, DHAD, DIC, Diodex Docetaxel, Doxil®, Doxorubicin, Doxorubicin Liposomal, Droxia™, DTIC, DTIC-Dome®, Duralone®, Efudex®, Eligard™, Ellence™, Eloxatin™, Elspar®, Emcyt®, Epirubicin, Epoetin Alfa, Erbitux, Erlotinib, Erwinia L-asparaginase, Estramustine, Ethyol Etopophos®, Etoposide, Etoposide Phosphate, Eulexin®, Everolimus,

Evista®, Exemestane, Fareston®, Faslodex®, Femara®, Filgrastim, Floxuridine, Fludara®, Fludarabine, Fluoroplex®, Fluorouracil, Fluorouracil (cream), Fluoxymesterone, Flutamide, Folinic Acid, FUDR®, Fulvestrant, G-CSF, Gefitinib, Gemcitabine, Gemtuzumab ozogamicin, Gemzar, Gleevec™, Gliadel® Wafer, GM-CSF, Goserelin, Granulocyte - Colony Stimulating Factor, Granulocyte Macrophage Colony Stimulating Factor, Halotestin®, Herceptin®, Hexadrol, Hexalen®, Hexamethylmelamine, HMM, Hycamtin®, Hydrea®, Hydrocort Acetate®, Hydrocortisone, Hydrocortisone Sodium Phosphate, Hydrocortisone Sodium Succinate, Hydrocortone Phosphate, Hydroxyurea, Ibritumomab, Ibritumomab, Tiuxetan, Idamycin®, Idarubicin, Ifex®, IFN-alpha, Ifosfamide, IL-11, IL-2, Imatinib mesylate, Imidazole Carboxamide, Interferon alfa, Interferon Alfa-2b (PEG Conjugate), Interleukin - 2, Interleukin-11, Intron A® (interferon alfa-2b), Iressa®, Irinotecan, Isotretinoin, Ixabepilone, Ixempra™, Kidrolase (t), Lanacort®, Lapatinib, L-asparaginase, LCR, Lenalidomide, Letrozole, Leucovorin, Leukeran, Leukine™, Leuprolide, Leurocristine, Leustatin™, Liposomal Ara-C Liquid Pred®, Lomustine, L-PAM, L-Sarcolysin, Lupron®, Lupron Depot®, Matulane®, Maxidex, Mechlorethamine, Mechlorethamine Hydrochloride, Medralone®, Medrol®, Megace®, Megestrol, Megestrol Acetate, Melphalan, Mercaptopurine, Mesna, Mesnex™, Methotrexate, Methotrexate Sodium, Methylprednisolone, Meticorten®, Mitomycin, Mitomycin-C, Mitoxantrone, M-Prednisol®, MTC, MTX, Mustargen®, Mustine, Mutamycin®, Myleran®, Mylocel™, Mylotarg®, Navelbine®, Nelarabine, Neosar®, Neulasta™, Neumega®, Neupogen®, Nexavar®, Nilandron®, Nilutamide, Nipent®, Nitrogen Mustard, Novaldex®, Novantrone®, Octreotide, Octreotide acetate, Oncospar®, Oncovin®, Ontak®, Onxal™, Oprevelkin, Orapred®, Orasone®, Oxaliplatin, Paclitaxel, Paclitaxel Protein-bound, Pamidronate, Panitumumab, Panretin®, Paraplatin®, Pediapred®, PEG Interferon, Pegaspargase, Pegfilgrastim, PEG-INTRON™, PEG-L-asparaginase, PEMETREXED, Pentostatin, Phenylalanine Mustard, Platinol®, Platinol-AQ®, Prednisolone, Prednisone, Prelone®, Procarbazine, PROCRIT®, Proleukin®, Prolifeprospan 20 with Carmustine Implant, Purinethol®, Raloxifene, Revlimid®, Rheumatrex®, Rituxan®, Rituximab, Roferon-A® (Interferon Alfa-2a), Rubex®, Rubidomycin hydrochloride, Sandostatin®, Sandostatin LAR®, Sargramostim, Solu-Cortef®, Solu-Medrol®, Sorafenib, SPRYCEL™, STI-571, Streptozocin, SU11248, Sunitinib, Sutent®, Tamoxifen, Tarceva®, Targretin®, Taxol®, Taxotere®, Temodar®, Temozolomide, Temsirolimus, Teniposide, TESPA, Thalidomide, Thalomid®, TheraCys®, Thioguanine, Thioguanine Tabloid®, Thiophosphoamide, Thioplex®, Thiotepa, TICE®, Toposar®, Topotecan, Toremifene, Torisel®, Tositumomab, Trastuzumab, Treanda®, Tretinoin, Trexall™, Trisenox®, TSPA, TYKERB®, VCR, Vectibix™, Velban®, Velcade®, VePesid®, Vesanoid®, Viadur™, Vidaza®, Vinblastine, Vinblastine Sulfate, Vincasar Pfs®, Vincristine, Vinorelbine, Vinorelbine tartrate, VLB, VM-26, Vorinostat, VP-16, Vumon®, Xeloda®, Zanosar®, Zevalin™, Zinecard®, Zoladex®, Zoledronic acid, Zolinza, Zometa®, and any appropriate combinations thereof.

[00261] The candidate treatments identified according to the subject methods can be chosen from the class of therapeutic agents identified as Anthracyclines and related substances, Anti-androgens, Anti-estrogens, Antigrowth hormones (e.g., Somatostatin analogs), Combination therapy (e.g., vincristine, bcnu,

melphalan, cyclophosphamide, prednisone (VBMCP)), DNA methyltransferase inhibitors, Endocrine therapy - Enzyme inhibitor, Endocrine therapy - other hormone antagonists and related agents, Folic acid analogs (e.g., methotrexate), Folic acid analogs (e.g., pemetrexed), Gonadotropin releasing hormone analogs, Gonadotropin-releasing hormones, Monoclonal antibodies (EGFR-Targeted - e.g., panitumumab, cetuximab), Monoclonal antibodies (Her2-Targeted - e.g., trastuzumab), Monoclonal antibodies (Multi-Targeted - e.g., alemtuzumab), Other alkylating agents, Other antineoplastic agents (e.g., asparaginase), Other antineoplastic agents (e.g., ATRA), Other antineoplastic agents (e.g., bexarotene), Other antineoplastic agents (e.g., celecoxib), Other antineoplastic agents (e.g., gemcitabine), Other antineoplastic agents (e.g., hydroxyurea), Other antineoplastic agents (e.g., irinotecan, topotecan), Other antineoplastic agents (e.g., pentostatin), Other cytotoxic antibiotics, Platinum compounds, Podophyllotoxin derivatives (e.g., etoposide), Progestogens, Protein kinase inhibitors (EGFR-Targeted), Protein kinase inhibitors (Her2 targeted therapy - e.g., lapatinib), Pyrimidine analogs (e.g., cytarabine), Pyrimidine analogs (e.g., fluoropyrimidines), Salicylic acid and derivatives (e.g., aspirin), Src-family protein tyrosine kinase inhibitors (e.g., dasatinib), Taxanes, Taxanes (e.g., nab-paclitaxel), Vinca Alkaloids and analogs, Vitamin D and analogs, Monoclonal antibodies (Multi-Targeted - e.g., bevacizumab), Protein kinase inhibitors (e.g., imatinib, sorafenib, sunitinib), Tyrosine Kinase inhibitors (TKI) (e.g., vemurafenib, sorafenib, imatinib, sunitinib, erlotinib, gefitinib, crizotinib, lapatinib).

[00262] In some embodiments, the candidate treatments identified according to the subject methods are chosen from at least the groups of treatments consisting of 5-fluorouracil, abarelix, alemtuzumab, aminoglutethimide, anastrozole, asparaginase, aspirin, ATRA, azacitidine, bevacizumab, bexarotene, bicalutamide, calcitriol, capecitabine, carboplatin, celecoxib, cetuximab, chemotherapy, cholecalciferol, cisplatin, cytarabine, dasatinib, daunorubicin, decitabine, doxorubicin, epirubicin, erlotinib, etoposide, exemestane, flutamide, fulvestrant, gefitinib, gemcitabine, gonadorelin, goserelin, hydroxyurea, imatinib, irinotecan, lapatinib, letrozole, leuprolide, liposomal-doxorubicin, medroxyprogesterone, megestrol, megestrol acetate, methotrexate, mitomycin, nab-paclitaxel, octreotide, oxaliplatin, paclitaxel, panitumumab, pegaspargase, pemetrexed, pentostatin, sorafenib, sunitinib, tamoxifen, Taxanes, temozolomide, toremifene, trastuzumab, VBMCP, and vincristine. The candidate treatments can be any of those in **Tables 3-5, 7-18, or 22** herein.

Rules Engine

[00263] In some embodiments, a database is created that maps treatments and molecular profiling results. The treatment information can include the projected efficacy of a therapeutic agent against cells having certain attributes that can be measured by molecular profiling. The molecular profiling can include differential expression or mutations in certain genes, proteins, or other biological molecules of interest. Through the mapping, the results of the molecular profiling can be compared against the database to select treatments. The database can include both positive and negative mappings between treatments and molecular profiling results. In some embodiments, the mapping is created by reviewing the literature for links between biological agents and therapeutic agents. For example, a journal article, patent publication or patent application publication, scientific presentation, etc can be reviewed for potential mappings. The

mapping can include results of in vivo, e.g., animal studies or clinical trials, or in vitro experiments, e.g., cell culture. Any mappings that are found can be entered into the database, e.g., cytotoxic effects of a therapeutic agent against cells expressing a gene or protein. In this manner, the database can be continuously updated. It will be appreciated that the methods of the invention are updated as well.

[00264] The rules can be generated by evidence-based literature review. Biomarker research continues to provide a better understanding of the clinical behavior and biology of cancer. This body of literature can be maintained in an up-to-date data repository incorporating recent clinical studies relevant to treatment options and potential clinical outcomes. The studies can be ranked so that only those with the strongest or most reliable evidence are selected for rules generation. For example, the rules generation can employ the grading system from the current methods of the U.S. Preventive Services Task Force. The literature evidence can be reviewed and evaluated based on the strength of clinical evidence supporting associations between biomarkers and treatments in the literature study. This process can be performed by a staff of scientists, physicians and other skilled reviewers. The process can also be automated in whole or in part by using language search and heuristics to identify relevant literature. The rules can be generated by a review of a plurality of literature references, e.g., tens, hundreds, thousands or more literature articles.

[00265] In another aspect, the invention provides a method of generating a set of evidence-based associations, comprising: (a) searching one or more literature database by a computer using an evidence-based medicine search filter to identify articles comprising a gene or gene product thereof, a disease, and one or more therapeutic agent; (b) filtering the articles identified in (a) to compile evidence-based associations comprising the expected benefit and/or the expected lack of benefit of the one or more therapeutic agent for treating the disease given the status of the gene or gene product; (c) adding the evidence-based associations compiled in (b) to the set of evidence-based associations; and (d) repeating steps (a)-(c) for an additional gene or gene product thereof. The status of the gene can include one or more assessments as described herein which relate to a biological state, e.g., one or more of an expression level, a copy number, and a mutation. The genes or gene products thereof can be one or more genes or gene products thereof selected from **Table 2**, **Table 6** or **Table 17**. For example, the method can be repeated for 1, 2, 3, 4, 5, 6, 7, 8, 9, 10, 15, 20, 25, 30, 40, 50 or more of the genes or gene products thereof in **Table 2**, **Table 6** or **Table 17**. The disease can be a disease described here, e.g., in embodiment the disease comprises a cancer. The one or more literature database can be selected from the group consisting of the National Library of Medicine's (NLM's) MEDLINE™ database of citations, a patent literature database, and a combination thereof.

[00266] Evidence-based medicine (EBM) or evidence-based practice (EBP) aims to apply the best available evidence gained from the scientific method to clinical decision making. This approach assesses the strength of evidence of the risks and benefits of treatments (including lack of treatment) and diagnostic tests. Evidence quality can be assessed based on the source type (from meta-analyses and systematic reviews of double-blind, placebo-controlled clinical trials at the top end, down to conventional wisdom at the bottom), as well as other factors including statistical validity, clinical relevance, currency, and peer-review acceptance. Evidence-based medicine filters are searches that have been developed to facilitate

searches in specific areas of clinical medicine related to evidence-based medicine (diagnosis, etiology, meta-analysis, prognosis and therapy). They are designed to retrieve high quality evidence from published studies appropriate to decision-making. The evidence-based medicine filter used in the invention can be selected from the group consisting of a generic evidence-based medicine filter, a McMaster University optimal search strategy evidence-based medicine filter, a University of York statistically developed search evidence-based medicine filter, and a University of California San Francisco systemic review evidence-based medicine filter. See e.g., US Patent Publication 20080215570; Shojania and Bero. Taking advantage of the explosion of systematic reviews: an efficient MEDLINE search strategy. Eff Clin Pract. 2001 Jul-Aug;4(4):157-62; Ingui and Rogers. Searching for clinical prediction rules in MEDLINE. J Am Med Inform Assoc. 2001 Jul-Aug;8(4):391-7; Haynes et al., Optimal search strategies for retrieving scientifically strong studies of treatment from Medline: analytical survey. BMJ. 2005 May 21;330(7501):1179; Wilczynski and Haynes. Consistency and accuracy of indexing systematic review articles and meta-analyses in medline. Health Info Libr J. 2009 Sep;26(3):203-10; which references are incorporated by reference herein in their entirety. A generic filter can be a customized filter based on an algorithm to identify the desired references from the one or more literature database. For example, the method can use one or more approach as described in US Patent 5168533 to Kato et al., US Patent 6886010 to Kostoff, or US Patent Application Publication No. 20040064438 to Kostoff; which references are incorporated by reference herein in their entirety.

[00267] The further filtering of articles identified by the evidence-based medicine filter can be performed using a computer, by one or more expert user, or combination thereof. The one or more expert can be a trained scientist or physician. In embodiments, the set of evidence-based associations comprise one or more of the rules in any of **Tables 3-4**, or **7-17**. For example, the set of evidence-based associations can include at least 5, 10, 25, 50 or 100 rules in **Tables 3-4**, or **7-17**. In some embodiments, the set of evidence-based associations comprises or consists of all of the rules in any of **Tables 3-4**, or **7-17**. In an aspect, the invention provides a computer readable medium comprising the set of evidence-based associations generated by the subject methods. The invention further provides a computer readable medium comprising one or more rules in any of **Tables 3-4**, or **7-17** herein. In an embodiment, the computer readable medium comprises at least 5, 10, 25, 50 or 100 rules in any of **Tables 3-4**, or **7-17**. For example, the computer readable medium can comprise all rules in any of **Tables 3-4**, or **7-17**, e.g., all rules in **Tables 3-4**, or **7-17**.

[00268] The rules for the mappings can contain a variety of supplemental information. In some embodiments, the database contains prioritization criteria. For example, a treatment with more projected efficacy in a given setting can be preferred over a treatment projected to have lesser efficacy. A mapping derived from a certain setting, e.g., a clinical trial, may be prioritized over a mapping derived from another setting, e.g., cell culture experiments. A treatment with strong literature support may be prioritized over a treatment supported by more preliminary results. A treatment generally applied to the type of disease in question, e.g., cancer of a certain tissue origin, may be prioritized over a treatment that is not indicated for that particular disease. Mappings can include both positive and negative correlations

between a treatment and a molecular profiling result. In a non-limiting example, one mapping might suggest use of a kinase inhibitor like erlotinib against a tumor having an activating mutation in EGFR, whereas another mapping might suggest against that treatment if the EGFR also has a drug resistance mutation. Similarly, a treatment might be indicated as effective in cells that overexpress a certain gene or protein but indicated as not effective if the gene or protein is underexpressed.

[00269] The selection of a candidate treatment for an individual can be based on molecular profiling results from any one or more of the methods described. Alternatively, selection of a candidate treatment for an individual can be based on molecular profiling results from more than one of the methods described. For example, selection of treatment for an individual can be based on molecular profiling results from FISH alone, IHC alone, or microarray analysis alone. In other embodiments, selection of treatment for an individual can be based on molecular profiling results from IHC, FISH, and microarray analysis; IHC and FISH; IHC and microarray analysis, or FISH and microarray analysis. Selection of treatment for an individual can also be based on molecular profiling results from sequencing or other methods of mutation detection. Molecular profiling results may include mutation analysis along with one or more methods, such as IHC, immunoassay, and/or microarray analysis. Different combinations and sequential results can be used. For example, treatment can be prioritized according the results obtained by molecular profiling. In an embodiment, the prioritization is based on the following algorithm: 1) IHC/FISH and microarray indicates same target as a first priority; 2) IHC positive result alone next priority; or 3) microarray positive result alone as last priority. Sequencing can also be used to guide selection. In some embodiments, sequencing reveals a drug resistance mutation so that the effected drug is not selected even if techniques including IHC, microarray and/or FISH indicate differential expression of the target molecule. Any such contraindication, e.g., differential expression or mutation of another gene or gene product may override selection of a treatment.

[00270] An illustrative listing of microarray expression results versus predicted treatments is presented in **Table 3**. As disclosed herein, molecular profiling is performed to determine whether a gene or gene product is differentially expressed in a sample as compared to a control. The expression status of the gene or gene product is used to select agents that are predicted to be efficacious or not. For example, **Table 3** shows that overexpression of the ADA gene or protein points to pentostatin as a possible treatment. On the other hand, underexpression of the ADA gene or protein implicates resistance to cytarabine, suggesting that cytarabine is not an optimal treatment.

Table 3: Molecular Profiling Results and Predicted Treatments

Gene Name	Expression Status	Candidate Agent(s)	Possible Resistance
ADA	Overexpressed	pentostatin	
ADA	Underexpressed		cytarabine
AR	Overexpressed	abarelix, bicalutamide, flutamide, gonadorelin, goserelin, leuprolide	
ASNS	Underexpressed	asparaginase, pegaspargase	
BCRP (ABCG2)	Overexpressed		cisplatin, carboplatin, irinotecan, topotecan
BRCA1	Underexpressed	mitomycin	

BRCA2	Underexpressed	mitomycin	
CD52	Overexpressed	alemtuzumab	
CDA	Overexpressed		cytarabine
CES2	Overexpressed	irinotecan	
c-kit	Overexpressed	sorafenib, sunitinib, imatinib	
COX-2	Overexpressed	celecoxib	
DCK	Overexpressed	gemcitabine	cytarabine
DHFR	Underexpressed	methotrexate, pemetrexed	
DHFR	Overexpressed		methotrexate
DNMT1	Overexpressed	azacitidine, decitabine	
DNMT3A	Overexpressed	azacitidine, decitabine	
DNMT3B	Overexpressed	azacitidine, decitabine	
EGFR	Overexpressed	erlotinib, gefitinib, cetuximab, panitumumab	
EML4-ALK	Overexpressed (present)	crizotinib	
EPHA2	Overexpressed	dasatinib	
ER	Overexpressed	anastrazole, exemestane, fulvestrant, letrozole, megestrol, tamoxifen, medroxyprogesterone, toremifene, aminoglutethimide	
ERCC1	Overexpressed		carboplatin, cisplatin
GART	Underexpressed	pemetrexed	
HER-2 (ERBB2)	Overexpressed	trastuzumab, lapatinib	
HIF-1α	Overexpressed	sorafenib, sunitinib, bevacizumab	
IκB-α	Overexpressed	bortezomib	
MGMT	Underexpressed	temozolomide	
MGMT	Overexpressed		temozolomide
MRP1 (ABCC1)	Overexpressed		etoposide, paclitaxel, docetaxel, vinblastine, vinorelbine, topotecan, teniposide
P-gp (ABCB1)	Overexpressed		doxorubicin, etoposide, epirubicin, paclitaxel, docetaxel, vinblastine, vinorelbine, topotecan, teniposide, liposomal doxorubicin
PDGFR-α	Overexpressed	sorafenib, sunitinib, imatinib	
PDGFR-β	Overexpressed	sorafenib, sunitinib, imatinib	
PR	Overexpressed	exemestane, fulvestrant, gonadorelin, goserelin, medroxyprogesterone, megestrol, tamoxifen, toremifene	
RARA	Overexpressed	ATRA	
RRM1	Underexpressed	gemcitabine,	

		hydroxyurea	
RRM2	Underexpressed	gemcitabine, hydroxyurea	
RRM2B	Underexpressed	gemcitabine, hydroxyurea	
RXR-α	Overexpressed	bexarotene	
RXR-β	Overexpressed	bexarotene	
SPARC	Overexpressed	nab-paclitaxel	
SRC	Overexpressed	dasatinib	
SSTR2	Overexpressed	octreotide	
SSTR5	Overexpressed	octreotide	
TOPO I	Overexpressed	irinotecan, topotecan	
TOPO IIα	Overexpressed	doxorubicin, epirubicin, liposomal- doxorubicin	
TOPO IIβ	Overexpressed	doxorubicin, epirubicin, liposomal- doxorubicin	
TS	Underexpressed	capecitabine, 5-fluorouracil, pemetrexed	
TS	Overexpressed		capecitabine, 5-fluorouracil
VDR	Overexpressed	calcitriol, cholecalciferol	
VEGFR1 (Flt1)	Overexpressed	sorafenib, sunitinib, bevacizumab	
VEGFR2	Overexpressed	sorafenib, sunitinib, bevacizumab	
VHL	Underexpressed	sorafenib, sunitinib	

[00271] Table 4 presents a selection of illustrative rules for treatment selection. The table is ordered by groups of related therapeutic agents. Each row describes a rule that maps the information derived from molecular profiling with an indication of benefit or lack of benefit for the therapeutic agent. Thus, the database contains a mapping of treatments whose biological activity is known against cancer cells that have alterations in certain genes or gene products, including gene copy alterations, chromosomal abnormalities, overexpression of or underexpression of one or more genes or gene products, or have various mutations. For each agent, a Lineage is presented as applicable which corresponds to a type of cancer associated with use of the agent. In this example, the agents can be used for all cancers. Agents with Benefit are listed along with a Benefit Summary Statement that describes molecular profiling information that relates to the predicted beneficial agent. Similarly, agents with Lack of Benefit are listed along with a Lack of Benefit Summary Statement that describes molecular profiling information that relates to the lack of benefit associated with the agent. Finally, the molecular profiling Criteria are shown. In the criteria, results from analysis using DNA microarray (DMA), IHC, FISH, and mutation analysis (MA) for one or more biomarkers is listed. For microarray analysis, expression can be reported as over (overexpressed) or under (underexpressed). When these criteria are met according to the application of the molecular profiling techniques to a sample, then the therapeutic agent or agents are predicted to have a benefit or lack of benefit as indicated in the corresponding row.

[00272] Further drug associations and rules that can be used in embodiments of the invention are found in U.S. Patent Application Publication 20100304989, filed February 12, 2010; International PCT Patent Application WO/2010/093465, filed February 11, 2010; and International PCT Patent Application

WO/2011/056688, filed October 27, 2010; all of which applications are incorporated by reference herein in their entirety. See e.g., "Table 4: Rules Summary for Treatment Selection" of WO/2011/056688.

Table 4: Exemplary Rules Summary for Treatment Selection

Therapeutic Agent	Lineage	Agents with Benefit	Benefit Summary Statement	Agents with Lack of Benefit	Lack of Benefit Summary Statement	Criteria
Protein kinase inhibitors (imatinib, sorafenib, sunitinib)	None	sunitinib, sorafenib	Presence of c-Kit mutation in exon 9 has been associated with benefit from sunitinib. In addition, over expression of HIF1A, VEGFR1, VEGFR2, c-Kit, PDGFRA and PDGFRB, and under expression of VHL have been associated with benefit from sunitinib and sorafenib.			DMA: VEGFR1 overexpressed. DMA: HIF1A overexpressed. DMA: VEGFR2 overexpressed. DMA: KIT overexpressed. DMA: PDGFRA overexpressed. DMA: PDGFRB overexpressed. DMA: VHL underexpressed. MA: c-kit mutated - Exon 9
Protein kinase inhibitors (imatinib, sorafenib, sunitinib)	None	sunitinib, sorafenib	Presence of c-Kit mutation in exon 9 has been associated with benefit from sunitinib. In addition, over expression of HIF1A, VEGFR1, VEGFR2, c-Kit, PDGFRA and PDGFRB have been associated with benefit from sunitinib and sorafenib.			DMA: VEGFR1 overexpressed. DMA: HIF1A overexpressed. DMA: VEGFR2 overexpressed. DMA: KIT overexpressed. DMA: PDGFRA overexpressed. DMA: PDGFRB overexpressed. DMA: VHL. MA: c-kit mutated - Exon 9
Protein kinase inhibitors (imatinib, sorafenib, sunitinib)	None	sunitinib, sorafenib	Presence of c-Kit mutation in exon 9 has been associated with benefit from sunitinib. In addition, over expression of			DMA: VEGFR1 overexpressed. DMA: HIF1A overexpressed. DMA: VEGFR2. DMA: KIT overexpressed. DMA: PDGFRA overexpressed.

			HIF1A, VEGFR1, c-Kit, PDGFRA and PDGFRB, and under expression of VHL have been associated with benefit from sunitinib and sorafenib.			DMA: PDGFRB overexpressed. DMA: VHL underexpressed. MA: c-kit mutated - Exon 9
Protein kinase inhibitors (imatinib, sorafenib, sunitinib)	None	sunitinib, sorafenib	Presence of c-Kit mutation in exon 9 has been associated with benefit from sunitinib. In addition, over expression of HIF1A, VEGFR1, c-Kit, PDGFRA and PDGFRB have been associated with benefit from sunitinib and sorafenib.			DMA: VEGFR1 overexpressed. DMA: HIF1A overexpressed. DMA: VEGFR2. DMA: KIT overexpressed. DMA: PDGFRA overexpressed. DMA: PDGFRB overexpressed. DMA: VHL. MA: c-kit mutated - Exon 9,
Protein kinase inhibitors (imatinib, sorafenib, sunitinib)	None	sunitinib, sorafenib	Presence of c-Kit mutation in exon 9 has been associated with benefit from sunitinib. In addition, over expression of HIF1A, VEGFR2, c-Kit, PDGFRA and PDGFRB, and under expression of VHL have been associated with benefit from sunitinib and sorafenib.			DMA: VEGFR1. DMA: HIF1A overexpressed. DMA: VEGFR2 overexpressed. DMA: KIT overexpressed. DMA: PDGFRA overexpressed. DMA: PDGFRB overexpressed. DMA: VHL underexpressed. MA: c-kit mutated - Exon 9
Protein kinase inhibitors (imatinib, sorafenib, sunitinib)	None	sunitinib, sorafenib	Presence of c-Kit mutation in exon 9 has been associated with benefit from sunitinib. In addition, over			DMA: VEGFR1. DMA: HIF1A overexpressed. DMA: VEGFR2 overexpressed. DMA: KIT overexpressed. DMA: PDGFRA

			expression of HIF1A, VEGFR2, c-Kit, PDGFRA and PDGFRB have been associated with benefit from sunitinib and sorafenib.			overexpressed. DMA: PDGFRB overexpressed. DMA: VHL. MA: c-kit mutated - Exon 9
Protein kinase inhibitors (imatinib, sorafenib, sunitinib)	None	sunitinib, sorafenib	Presence of c-Kit mutation in exon 9 has been associated with benefit from sunitinib. In addition, over expression of HIF1A, c-Kit, PDGFRA and PDGFRB, and under expression of VHL have been associated with benefit from sunitinib and sorafenib.			DMA: VEGFR1. DMA: HIF1A overexpressed. DMA: VEGFR2. DMA: KIT overexpressed. DMA: PDGFRA overexpressed. DMA: PDGFRB overexpressed. DMA: VHL underexpressed. MA: c-kit mutated - Exon 9
Protein kinase inhibitors (imatinib, sorafenib, sunitinib)	None	sunitinib, sorafenib	Presence of c-Kit mutation in exon 9 has been associated with benefit from sunitinib. In addition, over expression of HIF1A, c-Kit, PDGFRA and PDGFRB have been associated with benefit from sunitinib and sorafenib.			DMA: VEGFR1. DMA: HIF1A overexpressed. DMA: VEGFR2. DMA: KIT overexpressed. DMA: PDGFRA overexpressed. DMA: PDGFRB overexpressed. DMA: VHL. MA: c-kit mutated - Exon 9
Protein kinase inhibitors (imatinib, sorafenib, sunitinib)	None	sunitinib, sorafenib	Presence of c-Kit mutation in exon 9 has been associated with benefit from sunitinib. In addition, over expression of HIF1A, VEGFR1, VEGFR2, c-			DMA: VEGFR1 overexpressed. DMA: HIF1A overexpressed. DMA: VEGFR2 overexpressed. DMA: KIT overexpressed. DMA: PDGFRA overexpressed. DMA: PDGFRB. DMA: VHL

			Kit and PDGFRA, and under expression of VHL have been associated with benefit from sunitinib and sorafenib.			underexpressed. MA: c-kit mutated - Exon 9

[00273] The efficacy of various therapeutic agents given particular assay results, such as those in **Table 4** above, is derived from reviewing, analyzing and rendering conclusions on empirical evidence, such as that is available the medical literature or other medical knowledge base. The results are used to guide the selection of certain therapeutic agents in a prioritized list for use in treatment of an individual. When molecular profiling results are obtained, e.g., differential expression or mutation of a gene or gene product, the results can be compared against the database to guide treatment selection. The set of rules in the database can be updated as new treatments and new treatment data become available. In some embodiments, the rules database is updated continuously. In some embodiments, the rules database is updated on a periodic basis. Any relevant correlative or comparative approach can be used to compare the molecular profiling results to the rules database. In one embodiment, a gene or gene product is identified as differentially expressed by molecular profiling. The rules database is queried to select entries for that gene or gene product. Treatment selection information selected from the rules database is extracted and used to select a treatment. The information, e.g., to recommend or not recommend a particular treatment, can be dependent on whether the gene or gene product is over or underexpressed, or has other abnormalities at the genetic or protein levels as compared to a reference. In some cases, multiple rules and treatments may be pulled from a database comprising the comprehensive rules set depending on the results of the molecular profiling. In some embodiments, the treatment options are presented in a prioritized list. In some embodiments, the treatment options are presented without prioritization information. In either case, an individual, e.g., the treating physician or similar caregiver may choose from the available options.

[00274] The methods described herein are used to prolong survival of a subject by providing personalized treatment. In some embodiments, the subject has been previously treated with one or more therapeutic agents to treat the disease, e.g., a cancer. The cancer may be refractory to one of these agents, e.g., by acquiring drug resistance mutations. In some embodiments, the cancer is metastatic. In some embodiments, the subject has not previously been treated with one or more therapeutic agents identified by the method. Using molecular profiling, candidate treatments can be selected regardless of the stage, anatomical location, or anatomical origin of the cancer cells.

[00275] Progression-free survival (PFS) denotes the chances of staying free of disease progression for an individual or a group of individuals suffering from a disease, e.g., a cancer, after initiating a course of treatment. It can refer to the percentage of individuals in a group whose disease is likely to remain stable (e.g., not show signs of progression) after a specified duration of time. Progression-free survival rates are

an indication of the effectiveness of a particular treatment. Similarly, disease-free survival (DFS) denotes the chances of staying free of disease after initiating a particular treatment for an individual or a group of individuals suffering from a cancer. It can refer to the percentage of individuals in a group who are likely to be free of disease after a specified duration of time. Disease-free survival rates are an indication of the effectiveness of a particular treatment. Treatment strategies can be compared on the basis of the PFS or DFS that is achieved in similar groups of patients. Disease-free survival is often used with the term overall survival when cancer survival is described.

[00276] The candidate treatment selected by molecular profiling according to the invention can be compared to a non-molecular profiling selected treatment by comparing the progression free survival (PFS) using therapy selected by molecular profiling (period B) with PFS for the most recent therapy on which the patient has just progressed (period A). See **FIG. 30**. In one setting, a PFS(B)/PFS(A) ratio ≥ 1.3 was used to indicate that the molecular profiling selected therapy provides benefit for patient *(Robert Temple, Clinical measurement in drug evaluation. Edited by Wu Ningano and G.T. Thicker John Wiley and Sons Ltd. 1995; Von Hoff, D.D. Clin Can Res. 4: 1079, 1999: Dhani et al. Clin Cancer Res. 15: 118-123, 2009).* Other methods of comparing the treatment selected by molecular profiling to a non-molecular profiling selected treatment include determining response rate (RECIST) and percent of patients without progression or death at 4 months. The term "about" as used in the context of a numerical value for PFS means a variation of +/- ten percent (10%) relative to the numerical value. The PFS from a treatment selected by molecular profiling can be extended by at least 10%, 15%, 20%, 30%, 40%, 50%, 60%, 70%, 80%, or at least 90% as compared to a non-molecular profiling selected treatment. In some embodiments, the PFS from a treatment selected by molecular profiling can be extended by at least 100%, 150%, 200%, 300%, 400%, 500%, 600%, 700%, 800%, 900%, or at least about 1000% as compared to a non-molecular profiling selected treatment. In yet other embodiments, the PFS ratio (PFS on molecular profiling selected therapy or new treatment / PFS on prior therapy or treatment) is at least about 1.3. In yet other embodiments, the PFS ratio is at least about 1.1, 1.2, 1.3, 1.4, 1.5, 1.6, 1.7, 1.8, 1.9, or 2.0. In yet other embodiments, the PFS ratio is at least about 3, 4, 5, 6, 7, 8, 9 or 10.

[00277] Similarly, the DFS can be compared in patients whose treatment is selected with or without molecular profiling. In embodiments, DFS from a treatment selected by molecular profiling is extended by at least 10%, 15%, 20%, 30%, 40%, 50%, 60%, 70%, 80%, or at least 90% as compared to a non-molecular profiling selected treatment. In some embodiments, the DFS from a treatment selected by molecular profiling can be extended by at least 100%, 150%, 200%, 300%, 400%, 500%, 600%, 700%, 800%, 900%, or at least about 1000% as compared to a non-molecular profiling selected treatment. In yet other embodiments, the DFS ratio (DFS on molecular profiling selected therapy or new treatment / DFS on prior therapy or treatment) is at least about 1.3. In yet other embodiments, the DFS ratio is at least about 1.1, 1.2, 1.3, 1.4, 1.5, 1.6, 1.7, 1.8, 1.9, or 2.0. In yet other embodiments, the DFS ratio is at least about 3, 4, 5, 6, 7, 8, 9 or 10.

[00278] In some embodiments, the candidate treatment of the invention will not increase the PFS ratio or the DFS ratio in the patient, nevertheless molecular profiling provides invaluable patient benefit. For

example, in some instances no preferable treatment has been identified for the patient. In such cases, molecular profiling provides a method to identify a candidate treatment where none is currently identified. The molecular profiling may extend PFS, DFS or lifespan by at least 1 week, 2 weeks, 3 weeks, 4 weeks, 1 month, 5 weeks, 6 weeks, 7 weeks, 8 weeks, 2 months, 9 weeks, 10 weeks, 11 weeks, 12 weeks, 3 months, 4 months, 5 months, 6 months, 7 months, 8 months, 9 months, 10 months, 11 months, 12 months, 13 months, 14 months, 15 months, 16 months, 17 months, 18 months, 19 months, 20 months, 21 months, 22 months, 23 months, 24 months or 2 years. The molecular profiling may extend PFS, DFS or lifespan by at least 2 ½ years, 3 years, 4 years, 5 years, or more. In some embodiments, the methods of the invention improve outcome so that patient is in remission.

[00279] The effectiveness of a treatment can be monitored by other measures. A complete response (CR) comprises a complete disappearance of the disease: no disease is evident on examination, scans or other tests. A partial response (PR) refers to some disease remaining in the body, but there has been a decrease in size or number of the lesions by 30% or more. Stable disease (SD) refers to a disease that has remained relatively unchanged in size and number of lesions. Generally, less than a 50% decrease or a slight increase in size would be described as stable disease. Progressive disease (PD) means that the disease has increased in size or number on treatment. In some embodiments, molecular profiling according to the invention results in a complete response or partial response. In some embodiments, the methods of the invention result in stable disease. In some embodiments, the invention is able to achieve stable disease where non-molecular profiling results in progressive disease.

Computer Systems

[00280] The practice of the present invention may also employ conventional biology methods, software and systems. Computer software products of the invention typically include computer readable medium having computer-executable instructions for performing the logic steps of the method of the invention. Suitable computer readable medium include floppy disk, CD-ROM/DVD/DVD-ROM, hard-disk drive, flash memory, ROM/RAM, magnetic tapes and etc. The computer executable instructions may be written in a suitable computer language or combination of several languages. Basic computational biology methods are described in, for example Setubal and Meidanis et al., Introduction to Computational Biology Methods (PWS Publishing Company, Boston, 1997); Salzberg, Searles, Kasif, (Ed.), Computational Methods in Molecular Biology, (Elsevier, Amsterdam, 1998); Rashidi and Buehler, Bioinformatics Basics: Application in Biological Science and Medicine (CRC Press, London, 2000) and Ouelette and Bzevanis Bioinformatics: A Practical Guide for Analysis of Gene and Proteins (Wiley & Sons, Inc., 2.sup.nd ed., 2001). See U.S. Pat. No. 6,420,108.

[00281] The present invention may also make use of various computer program products and software for a variety of purposes, such as probe design, management of data, analysis, and instrument operation. See, U.S. Pat. Nos. 5,593,839, 5,795,716, 5,733,729, 5,974,164, 6,066,454, 6,090,555, 6,185,561, 6,188,783, 6,223,127, 6,229,911 and 6,308,170.

[00282] Additionally, the present invention relates to embodiments that include methods for providing genetic information over networks such as the Internet as shown in U.S. Ser. Nos. 10/197,621, 10/063,559

(U.S. Publication Number 20020183936), 10/065,856, 10/065,868, 10/328,818, 10/328,872, 10/423,403, and 60/482,389. For example, one or more molecular profiling techniques can be performed in one location, e.g., a city, state, country or continent, and the results can be transmitted to a different city, state, country or continent. Treatment selection can then be made in whole or in part in the second location. The methods of the invention comprise transmittal of information between different locations.

[00283] Conventional data networking, application development and other functional aspects of the systems (and components of the individual operating components of the systems) may not be described in detail herein but are part of the invention. Furthermore, the connecting lines shown in the various figures contained herein are intended to represent illustrative functional relationships and/or physical couplings between the various elements. It should be noted that many alternative or additional functional relationships or physical connections may be present in a practical system.

[00284] The various system components discussed herein may include one or more of the following: a host server or other computing systems including a processor for processing digital data; a memory coupled to the processor for storing digital data; an input digitizer coupled to the processor for inputting digital data; an application program stored in the memory and accessible by the processor for directing processing of digital data by the processor; a display device coupled to the processor and memory for displaying information derived from digital data processed by the processor; and a plurality of databases. Various databases used herein may include: patient data such as family history, demography and environmental data, biological sample data, prior treatment and protocol data, patient clinical data, molecular profiling data of biological samples, data on therapeutic drug agents and/or investigative drugs, a gene library, a disease library, a drug library, patient tracking data, file management data, financial management data, billing data and/or like data useful in the operation of the system. As those skilled in the art will appreciate, user computer may include an operating system (e.g., Windows NT, 95/98/2000, OS2, UNIX, Linux, Solaris, MacOS, etc.) as well as various conventional support software and drivers typically associated with computers. The computer may include any suitable personal computer, network computer, workstation, minicomputer, mainframe or the like. User computer can be in a home or medical/business environment with access to a network. In an illustrative embodiment, access is through a network or the Internet through a commercially-available web-browser software package.

[00285] As used herein, the term "network" shall include any electronic communications means which incorporates both hardware and software components of such. Communication among the parties may be accomplished through any suitable communication channels, such as, for example, a telephone network, an extranet, an intranet, Internet, point of interaction device, personal digital assistant (e.g., Palm Pilot®, Blackberry®), cellular phone, kiosk, etc.), online communications, satellite communications, off-line communications, wireless communications, transponder communications, local area network (LAN), wide area network (WAN), networked or linked devices, keyboard, mouse and/or any suitable communication or data input modality. Moreover, although the system is frequently described herein as being implemented with TCP/IP communications protocols, the system may also be implemented using IPX, Appletalk, IP-6, NetBIOS, OSI or any number of existing or future protocols. If the network is in the

nature of a public network, such as the Internet, it may be advantageous to presume the network to be insecure and open to eavesdroppers. Specific information related to the protocols, standards, and application software used in connection with the Internet is generally known to those skilled in the art and, as such, need not be detailed herein. See, for example, DILIP NAIK, INTERNET STANDARDS AND PROTOCOLS (1998); JAVA 2 COMPLETE, various authors, (Sybex 1999); DEBORAH RAY AND ERIC RAY, MASTERING HTML 4.0 (1997); and LOSHIN, TCP/IP CLEARLY EXPLAINED (1997) and DAVID GOURLEY AND BRIAN TOTTY, HTTP, THE DEFINITIVE GUIDE (2002), the contents of which are hereby incorporated by reference.

[00286] The various system components may be independently, separately or collectively suitably coupled to the network via data links which includes, for example, a connection to an Internet Service Provider (ISP) over the local loop as is typically used in connection with standard modem communication, cable modem, Dish networks, ISDN, Digital Subscriber Line (DSL), or various wireless communication methods, see, e.g., GILBERT HELD, UNDERSTANDING DATA COMMUNICATIONS (1996), which is hereby incorporated by reference. It is noted that the network may be implemented as other types of networks, such as an interactive television (ITV) network. Moreover, the system contemplates the use, sale or distribution of any goods, services or information over any network having similar functionality described herein.

[00287] As used herein, "transmit" may include sending electronic data from one system component to another over a network connection. Additionally, as used herein, "data" may include encompassing information such as commands, queries, files, data for storage, and the like in digital or any other form.

[00288] The system contemplates uses in association with web services, utility computing, pervasive and individualized computing, security and identity solutions, autonomic computing, commodity computing, mobility and wireless solutions, open source, biometrics, grid computing and/or mesh computing.

[00289] Any databases discussed herein may include relational, hierarchical, graphical, or object-oriented structure and/or any other database configurations. Common database products that may be used to implement the databases include DB2 by IBM (White Plains, NY), various database products available from Oracle Corporation (Redwood Shores, CA), Microsoft Access or Microsoft SQL Server by Microsoft Corporation (Redmond, Washington), or any other suitable database product. Moreover, the databases may be organized in any suitable manner, for example, as data tables or lookup tables. Each record may be a single file, a series of files, a linked series of data fields or any other data structure. Association of certain data may be accomplished through any desired data association technique such as those known or practiced in the art. For example, the association may be accomplished either manually or automatically. Automatic association techniques may include, for example, a database search, a database merge, GREP, AGREP, SQL, using a key field in the tables to speed searches, sequential searches through all the tables and files, sorting records in the file according to a known order to simplify lookup, and/or the like. The association step may be accomplished by a database merge function, for example, using a "key field" in pre-selected databases or data sectors.

[00290] More particularly, a "key field" partitions the database according to the high-level class of objects defined by the key field. For example, certain types of data may be designated as a key field in a plurality of related data tables and the data tables may then be linked on the basis of the type of data in the key field. The data corresponding to the key field in each of the linked data tables is preferably the same or of the same type. However, data tables having similar, though not identical, data in the key fields may also be linked by using AGREP, for example. In accordance with one embodiment, any suitable data storage technique may be used to store data without a standard format. Data sets may be stored using any suitable technique, including, for example, storing individual files using an ISO/IEC 7816-4 file structure; implementing a domain whereby a dedicated file is selected that exposes one or more elementary files containing one or more data sets; using data sets stored in individual files using a hierarchical filing system; data sets stored as records in a single file (including compression, SQL accessible, hashed vione or more keys, numeric, alphabetical by first tuple, etc.); Binary Large Object (BLOB); stored as ungrouped data elements encoded using ISO/IEC 7816-6 data elements; stored as ungrouped data elements encoded using ISO/IEC Abstract Syntax Notation (ASN.1) as in ISO/IEC 8824 and 8825; and/or other proprietary techniques that may include fractal compression methods, image compression methods, etc.

[00291] In one illustrative embodiment, the ability to store a wide variety of information in different formats is facilitated by storing the information as a BLOB. Thus, any binary information can be stored in a storage space associated with a data set. The BLOB method may store data sets as ungrouped data elements formatted as a block of binary via a fixed memory offset using either fixed storage allocation, circular queue techniques, or best practices with respect to memory management (*e.g.*, paged memory, least recently used, etc.). By using BLOB methods, the ability to store various data sets that have different formats facilitates the storage of data by multiple and unrelated owners of the data sets. For example, a first data set which may be stored may be provided by a first party, a second data set which may be stored may be provided by an unrelated second party, and yet a third data set which may be stored, may be provided by a third party unrelated to the first and second party. Each of these three illustrative data sets may contain different information that is stored using different data storage formats and/or techniques. Further, each data set may contain subsets of data that also may be distinct from other subsets.

[00292] As stated above, in various embodiments, the data can be stored without regard to a common format. However, in one illustrative embodiment, the data set (*e.g.*, BLOB) may be annotated in a standard manner when provided for manipulating the data. The annotation may comprise a short header, trailer, or other appropriate indicator related to each data set that is configured to convey information useful in managing the various data sets. For example, the annotation may be called a "condition header", "header", "trailer", or "status", herein, and may comprise an indication of the status of the data set or may include an identifier correlated to a specific issuer or owner of the data. Subsequent bytes of data may be used to indicate for example, the identity of the issuer or owner of the data, user, transaction/membership account identifier or the like. Each of these condition annotations are further discussed herein.

[00293] The data set annotation may also be used for other types of status information as well as various other purposes. For example, the data set annotation may include security information establishing access levels. The access levels may, for example, be configured to permit only certain individuals, levels of employees, companies, or other entities to access data sets, or to permit access to specific data sets based on the transaction, issuer or owner of data, user or the like. Furthermore, the security information may restrict/permit only certain actions such as accessing, modifying, and/or deleting data sets. In one example, the data set annotation indicates that only the data set owner or the user are permitted to delete a data set, various identified users may be permitted to access the data set for reading, and others are altogether excluded from accessing the data set. However, other access restriction parameters may also be used allowing various entities to access a data set with various permission levels as appropriate. The data, including the header or trailer may be received by a standalone interaction device configured to add, delete, modify, or augment the data in accordance with the header or trailer.

[00294] One skilled in the art will also appreciate that, for security reasons, any databases, systems, devices, servers or other components of the system may consist of any combination thereof at a single location or at multiple locations, wherein each database or system includes any of various suitable security features, such as firewalls, access codes, encryption, decryption, compression, decompression, and/or the like.

[00295] The computing unit of the web client may be further equipped with an Internet browser connected to the Internet or an intranet using standard dial-up, cable, DSL or any other Internet protocol known in the art. Transactions originating at a web client may pass through a firewall in order to prevent unauthorized access from users of other networks. Further, additional firewalls may be deployed between the varying components of CMS to further enhance security.

[00296] Firewall may include any hardware and/or software suitably configured to protect CMS components and/or enterprise computing resources from users of other networks. Further, a firewall may be configured to limit or restrict access to various systems and components behind the firewall for web clients connecting through a web server. Firewall may reside in varying configurations including Stateful Inspection, Proxy based and Packet Filtering among others. Firewall may be integrated within an web server or any other CMS components or may further reside as a separate entity.

[00297] The computers discussed herein may provide a suitable website or other Internet-based graphical user interface which is accessible by users. In one embodiment, the Microsoft Internet Information Server (IIS), Microsoft Transaction Server (MTS), and Microsoft SQL Server, are used in conjunction with the Microsoft operating system, Microsoft NT web server software, a Microsoft SQL Server database system, and a Microsoft Commerce Server. Additionally, components such as Access or Microsoft SQL Server, Oracle, Sybase, Informix MySQL, Interbase, etc., may be used to provide an Active Data Object (ADO) compliant database management system.

[00298] Any of the communications, inputs, storage, databases or displays discussed herein may be facilitated through a website having web pages. The term “web page” as it is used herein is not meant to limit the type of documents and applications that might be used to interact with the user. For example, a

typical website might include, in addition to standard HTML documents, various forms, Java applets, JavaScript, active server pages (ASP), common gateway interface scripts (CGI), extensible markup language (XML), dynamic HTML, cascading style sheets (CSS), helper applications, plug-ins, and the like. A server may include a web service that receives a request from a web server, the request including a URL (http://yahoo.com/stockquotes/ge) and an IP address (123.56.789.234). The web server retrieves the appropriate web pages and sends the data or applications for the web pages to the IP address. Web services are applications that are capable of interacting with other applications over a communications means, such as the internet. Web services are typically based on standards or protocols such as XML, XSLT, SOAP, WSDL and UDDI. Web services methods are well known in the art, and are covered in many standard texts. See, e.g., ALEX NGHIEM, IT WEB SERVICES: A ROADMAP FOR THE ENTERPRISE (2003), hereby incorporated by reference.

[00299] The web-based clinical database for the system and method of the present invention preferably has the ability to upload and store clinical data files in native formats and is searchable on any clinical parameter. The database is also scalable and may use an EAV data model (metadata) to enter clinical annotations from any study for easy integration with other studies. In addition, the web-based clinical database is flexible and may be XML and XSLT enabled to be able to add user customized questions dynamically. Further, the database includes exportability to CDISC ODM.

[00300] Practitioners will also appreciate that there are a number of methods for displaying data within a browser-based document. Data may be represented as standard text or within a fixed list, scrollable list, drop-down list, editable text field, fixed text field, pop-up window, and the like. Likewise, there are a number of methods available for modifying data in a web page such as, for example, free text entry using a keyboard, selection of menu items, check boxes, option boxes, and the like.

[00301] The system and method may be described herein in terms of functional block components, screen shots, optional selections and various processing steps. It should be appreciated that such functional blocks may be realized by any number of hardware and/or software components configured to perform the specified functions. For example, the system may employ various integrated circuit components, e.g., memory elements, processing elements, logic elements, look-up tables, and the like, which may carry out a variety of functions under the control of one or more microprocessors or other control devices. Similarly, the software elements of the system may be implemented with any programming or scripting language such as C, C++, Macromedia Cold Fusion, Microsoft Active Server Pages, Java, COBOL, assembler, PERL, Visual Basic, SQL Stored Procedures, extensible markup language (XML), with the various algorithms being implemented with any combination of data structures, objects, processes, routines or other programming elements. Further, it should be noted that the system may employ any number of conventional techniques for data transmission, signaling, data processing, network control, and the like. Still further, the system could be used to detect or prevent security issues with a client-side scripting language, such as JavaScript, VBScript or the like. For a basic introduction of cryptography and network security, see any of the following references: (1) "Applied Cryptography: Protocols, Algorithms, And Source Code In C," by Bruce Schneier, published by John Wiley & Sons (second edition, 1995); (2)

"Java Cryptography" by Jonathan Knudson, published by O'Reilly & Associates (1998); (3) "Cryptography & Network Security: Principles & Practice" by William Stallings, published by Prentice Hall; all of which are hereby incorporated by reference.

[00302] As used herein, the term "end user", "consumer", "customer", "client", "treating physician", "hospital", or "business" may be used interchangeably with each other, and each shall mean any person, entity, machine, hardware, software or business. Each participant is equipped with a computing device in order to interact with the system and facilitate online data access and data input. The customer has a computing unit in the form of a personal computer, although other types of computing units may be used including laptops, notebooks, hand held computers, set-top boxes, cellular telephones, touch-tone telephones and the like. The owner/operator of the system and method of the present invention has a computing unit implemented in the form of a computer-server, although other implementations are contemplated by the system including a computing center shown as a main frame computer, a mini-computer, a PC server, a network of computers located in the same of different geographic locations, or the like. Moreover, the system contemplates the use, sale or distribution of any goods, services or information over any network having similar functionality described herein.

[00303] In one illustrative embodiment, each client customer may be issued an "account" or "account number". As used herein, the account or account number may include any device, code, number, letter, symbol, digital certificate, smart chip, digital signal, analog signal, biometric or other identifier/indicia suitably configured to allow the consumer to access, interact with or communicate with the system (e.g., one or more of an authorization/access code, personal identification number (PIN), Internet code, other identification code, and/or the like). The account number may optionally be located on or associated with a charge card, credit card, debit card, prepaid card, embossed card, smart card, magnetic stripe card, bar code card, transponder, radio frequency card or an associated account. The system may include or interface with any of the foregoing cards or devices, or a fob having a transponder and RFID reader in RF communication with the fob. Although the system may include a fob embodiment, the invention is not to be so limited. Indeed, system may include any device having a transponder which is configured to communicate with RFID reader via RF communication. Typical devices may include, for example, a key ring, tag, card, cell phone, wristwatch or any such form capable of being presented for interrogation. Moreover, the system, computing unit or device discussed herein may include a "pervasive computing device," which may include a traditionally non-computerized device that is embedded with a computing unit. The account number may be distributed and stored in any form of plastic, electronic, magnetic, radio frequency, wireless, audio and/or optical device capable of transmitting or downloading data from itself to a second device.

[00304] As will be appreciated by one of ordinary skill in the art, the system may be embodied as a customization of an existing system, an add-on product, upgraded software, a standalone system, a distributed system, a method, a data processing system, a device for data processing, and/or a computer program product. Accordingly, the system may take the form of an entirely software embodiment, an entirely hardware embodiment, or an embodiment combining aspects of both software and hardware.

Furthermore, the system may take the form of a computer program product on a computer-readable storage medium having computer-readable program code means embodied in the storage medium. Any suitable computer-readable storage medium may be used, including hard disks, CD-ROM, optical storage devices, magnetic storage devices, and/or the like.

[00305] The system and method is described herein with reference to screen shots, block diagrams and flowchart illustrations of methods, apparatus (*e.g.*, systems), and computer program products according to various embodiments. It will be understood that each functional block of the block diagrams and the flowchart illustrations, and combinations of functional blocks in the block diagrams and flowchart illustrations, respectively, can be implemented by computer program instructions.

[00306] These computer program instructions may be loaded onto a general purpose computer, special purpose computer, or other programmable data processing apparatus to produce a machine, such that the instructions that execute on the computer or other programmable data processing apparatus create means for implementing the functions specified in the flowchart block or blocks. These computer program instructions may also be stored in a computer-readable memory that can direct a computer or other programmable data processing apparatus to function in a particular manner, such that the instructions stored in the computer-readable memory produce an article of manufacture including instruction means which implement the function specified in the flowchart block or blocks. The computer program instructions may also be loaded onto a computer or other programmable data processing apparatus to cause a series of operational steps to be performed on the computer or other programmable apparatus to produce a computer-implemented process such that the instructions which execute on the computer or other programmable apparatus provide steps for implementing the functions specified in the flowchart block or blocks.

[00307] Accordingly, functional blocks of the block diagrams and flowchart illustrations support combinations of means for performing the specified functions, combinations of steps for performing the specified functions, and program instruction means for performing the specified functions. It will also be understood that each functional block of the block diagrams and flowchart illustrations, and combinations of functional blocks in the block diagrams and flowchart illustrations, can be implemented by either special purpose hardware-based computer systems which perform the specified functions or steps, or suitable combinations of special purpose hardware and computer instructions. Further, illustrations of the process flows and the descriptions thereof may make reference to user windows, web pages, websites, web forms, prompts, etc. Practitioners will appreciate that the illustrated steps described herein may comprise in any number of configurations including the use of windows, web pages, web forms, popup windows, prompts and the like. It should be further appreciated that the multiple steps as illustrated and described may be combined into single web pages and/or windows but have been expanded for the sake of simplicity. In other cases, steps illustrated and described as single process steps may be separated into multiple web pages and/or windows but have been combined for simplicity.

Molecular Profiling Methods

[00308] **FIG. 1** illustrates a block diagram of an illustrative embodiment of a system **10** for determining individualized medical intervention for a particular disease state that uses molecular profiling of a patient's biological specimen. System **10** includes a user interface **12**, a host server **14** including a processor **16** for processing data, a memory **18** coupled to the processor, an application program **20** stored in the memory **18** and accessible by the processor **16** for directing processing of the data by the processor **16**, a plurality of internal databases **22** and external databases **24**, and an interface with a wired or wireless communications network **26** (such as the Internet, for example). System **10** may also include an input digitizer **28** coupled to the processor **16** for inputting digital data from data that is received from user interface **12**.

[00309] User interface **12** includes an input device **30** and a display **32** for inputting data into system **10** and for displaying information derived from the data processed by processor **16**. User interface **12** may also include a printer **34** for printing the information derived from the data processed by the processor **16** such as patient reports that may include test results for targets and proposed drug therapies based on the test results.

[00310] Internal databases **22** may include, but are not limited to, patient biological sample/specimen information and tracking, clinical data, patient data, patient tracking, file management, study protocols, patient test results from molecular profiling, and billing information and tracking. External databases **24** nay include, but are not limited to, drug libraries, gene libraries, disease libraries, and public and private databases such as UniGene, OMIM, GO, TIGR, GenBank, KEGG and Biocarta.

[00311] Various methods may be used in accordance with system **10**. **FIG. 2** shows a flowchart of an illustrative embodiment of a method **50** for determining individualized medical intervention for a particular disease state that uses molecular profiling of a patient's biological specimen that is non disease specific. In order to determine a medical intervention for a particular disease state using molecular profiling that is independent of disease lineage diagnosis (i.e. not single disease restricted), at least one test is performed for at least one target from a biological sample of a diseased patient in step **52**. A target is defined as any molecular finding that may be obtained from molecular testing. For example, a target may include one or more genes, one or more gene expressed proteins, one or more molecular mechanisms, and/or combinations of such. For example, the expression level of a target can be determined by the analysis of mRNA levels or the target or gene, or protein levels of the gene. Tests for finding such targets may include, but are not limited, fluorescent in-situ hybridization (FISH), in-situ hybridization (ISH), and other molecular tests known to those skilled in the art. PCR-based methods, such as real-time PCR or quantitative PCR can be used. Furthermore, microarray analysis, such as a comparative genomic hybridization (CGH) micro array, a single nucleotide polymorphism (SNP) microarray, a proteomic array, or antibody array analysis can also be used in the methods disclosed herein. In some embodiments, microarray analysis comprises identifying whether a gene is up-regulated or down-regulated relative to a reference with a significance of $p<0.001$. Tests or analyses of targets can also comprise

immunohistochemical (IHC) analysis. In some embodiments, IHC analysis comprises determining whether 30% or more of a sample is stained, if the staining intensity is +2 or greater, or both.

[00312] Furthermore, the methods disclosed herein also including profiling more than one target. For example, the expression of a plurality of genes can be identified. Furthermore, identification of a plurality of targets in a sample can be by one method or by various means. For example, the expression of a first gene can be determined by one method and the expression level of a second gene determined by a different method. Alternatively, the same method can be used to detect the expression level of the first and second gene. For example, the first method can be IHC and the second by microarray analysis, such as detecting the gene expression of a gene.

[00313] In some embodiments, molecular profiling can also including identifying a genetic variant, such as a mutation, polymorphism (such as a SNP), deletion, or insertion of a target. For example, identifying a SNP in a gene can be determined by microarray analysis, real-time PCR, or sequencing. Other methods disclosed herein can also be used to identify variants of one or more targets.

[00314] Accordingly, one or more of the following may be performed: an IHC analysis in step **54**, a microanalysis in step **56**, and other molecular tests know to those skilled in the art in step **58**.

[00315] Biological samples are obtained from diseased patients by taking a biopsy of a tumor, conducting minimally invasive surgery if no recent tumor is available, obtaining a sample of the patient's blood, or a sample of any other biological fluid including, but not limited to, cell extracts, nuclear extracts, cell lysates or biological products or substances of biological origin such as excretions, blood, sera, plasma, urine, sputum, tears, feces, saliva, membrane extracts, and the like.

[00316] In step **60**, a determination is made as to whether one or more of the targets that were tested for in step **52** exhibit a change in expression compared to a normal reference for that particular target. In one illustrative method of the invention, an IHC analysis may be performed in step **54** and a determination as to whether any targets from the IHC analysis exhibit a change in expression is made in step **64** by determining whether 30% or more of the biological sample cells were +2 or greater staining for the particular target. It will be understood by those skilled in the art that there will be instances where +1 or greater staining will indicate a change in expression in that staining results may vary depending on the technician performing the test and type of target being tested. In another illustrative embodiment of the invention, a micro array analysis may be performed in step **56** and a determination as to whether any targets from the micro array analysis exhibit a change in expression is made in step **66** by identifying which targets are up-regulated or down-regulated by determining whether the fold change in expression for a particular target relative to a normal tissue of origin reference is significant at $p < 0.001$. A change in expression may also be evidenced by an absence of one or more genes, gene expressed proteins, molecular mechanisms, or other molecular findings.

[00317] After determining which targets exhibit a change in expression in step **60**, at least one non-disease specific agent is identified that interacts with each target having a changed expression in step **70**. An agent may be any drug or compound having a therapeutic effect. A non-disease specific agent is a therapeutic drug or compound not previously associated with treating the patient's diagnosed disease that is capable

of interacting with the target from the patient's biological sample that has exhibited a change in expression. Some of the non-disease specific agents that have been found to interact with specific targets found in different cancer patients are shown in **Table 5** below.

Table 5: Illustrative target-drug associations

Patients	Target(s) Found	Treatment(s)
Advanced Pancreatic Cancer	HER 2/*neu*	Trastuzumab
Advanced Pancreatic Cancer	EGFR, HIF 1α	Cetuximab, Sirolimus
Advanced Ovarian Cancer	ERCC3	Irofulven
Advanced Adenoid Cystic Carcinoma	Vitamin D receptors, Androgen receptors	Calcitriol, Flutamide

[00318] Finally, in step **80**, a patient profile report may be provided which includes the patient's test results for various targets and any proposed therapies based on those results. An illustrative patient profile report **100** is shown in **FIGS. 3A-3D**. Patient profile report **100** shown in **FIG. 3A** identifies the targets tested **102**, those targets tested that exhibited significant changes in expression **104**, and proposed non-disease specific agents for interacting with the targets **106**. Patient profile report **100** shown in **FIG. 3B** identifies the results **108** of immunohistochemical analysis for certain gene expressed proteins **110** and whether a gene expressed protein is a molecular target **112** by determining whether 30% or more of the tumor cells were +2 or greater staining. Report **100** also identifies immunohistochemical tests that were not performed **114**. Patient profile report **100** shown in **FIG. 3C** identifies the genes analyzed **116** with a micro array analysis and whether the genes were under expressed or over expressed **118** compared to a reference. Finally, patient profile report **100** shown in **FIG. 3D** identifies the clinical history **120** of the patient and the specimens that were submitted **122** from the patient. Molecular profiling techniques can be performed anywhere, e.g., a foreign country, and the results sent by network to an appropriate party, e.g., the patient, a physician, lab or other party located remotely.

[00319] **FIG. 4** shows a flowchart of an illustrative embodiment of a method **200** for identifying a drug therapy/agent capable of interacting with a target. In step **202**, a molecular target is identified which exhibits a change in expression in a number of diseased individuals. Next, in step **204**, a drug therapy/agent is administered to the diseased individuals. After drug therapy/agent administration, any changes in the molecular target identified in step **202** are identified in step **206** in order to determine if the drug therapy/agent administered in step **204** interacts with the molecular targets identified in step **202**. If it is determined that the drug therapy/agent administered in step **204** interacts with a molecular target identified in step **202**, the drug therapy/agent may be approved for treating patients exhibiting a change in expression of the identified molecular target instead of approving the drug therapy/agent for a particular disease.

[00320] **FIGS. 5-14** are flowcharts and diagrams illustrating various parts of an information-based personalized medicine drug discovery system and method in accordance with the present invention. **FIG. 5** is a diagram showing an illustrative clinical decision support system of the information-based

personalized medicine drug discovery system and method of the present invention. Data obtained through clinical research and clinical care such as clinical trial data, biomedical/molecular imaging data, genomics/proteomics/chemical library/literature/expert curation, biospecimen tracking/LIMS, family history/environmental records, and clinical data are collected and stored as databases and datamarts within a data warehouse. **FIG. 6** is a diagram showing the flow of information through the clinical decision support system of the information-based personalized medicine drug discovery system and method of the present invention using web services. A user interacts with the system by entering data into the system via form-based entry/upload of data sets, formulating queries and executing data analysis jobs, and acquiring and evaluating representations of output data. The data warehouse in the web based system is where data is extracted, transformed, and loaded from various database systems. The data warehouse is also where common formats, mapping and transformation occurs. The web based system also includes datamarts which are created based on data views of interest.

[00321] A flow chart of an illustrative clinical decision support system of the information-based personalized medicine drug discovery system and method of the present invention is shown in **FIG. 7**. The clinical information management system includes the laboratory information management system and the medical information contained in the data warehouses and databases includes medical information libraries, such as drug libraries, gene libraries, and disease libraries, in addition to literature text mining. Both the information management systems relating to particular patients and the medical information databases and data warehouses come together at a data junction center where diagnostic information and therapeutic options can be obtained. A financial management system may also be incorporated in the clinical decision support system of the information-based personalized medicine drug discovery system and method of the present invention.

[00322] **FIG. 8** is a diagram showing an illustrative biospecimen tracking and management system which may be used as part of the information-based personalized medicine drug discovery system and method of the present invention. **FIG. 8** shows two host medical centers which forward specimens to a tissue/blood bank. The specimens may go through laboratory analysis prior to shipment. Research may also be conducted on the samples via micro array, genotyping, and proteomic analysis. This information can be redistributed to the tissue/blood bank. **FIG. 9** depicts a flow chart of an illustrative biospecimen tracking and management system which may be used with the information-based personalized medicine drug discovery system and method of the present invention. The host medical center obtains samples from patients and then ships the patient samples to a molecular profiling laboratory which may also perform RNA and DNA isolation and analysis.

[00323] A diagram showing a method for maintaining a clinical standardized vocabulary for use with the information-based personalized medicine drug discovery system and method of the present invention is shown in **FIG. 10**. **FIG. 10** illustrates how physician observations and patient information associated with one physician's patient may be made accessible to another physician to enable the other physician to use the data in making diagnostic and therapeutic decisions for their patients.

[00324] FIG. 11 shows a schematic of an illustrative microarray gene expression database which may be used as part of the information-based personalized medicine drug discovery system and method of the present invention. The micro array gene expression database includes both external databases and internal databases which can be accessed via the web based system. External databases may include, but are not limited to, UniGene, GO, TIGR, GenBank, KEGG. The internal databases may include, but are not limited to, tissue tracking, LIMS, clinical data, and patient tracking. **FIG. 12** shows a diagram of an illustrative micro array gene expression database data warehouse which may be used as part of the information-based personalized medicine drug discovery system and method of the present invention. Laboratory data, clinical data, and patient data may all be housed in the micro array gene expression database data warehouse and the data may in turn be accessed by public/private release and used by data analysis tools.

[00325] Another schematic showing the flow of information through an information-based personalized medicine drug discovery system and method of the present invention is shown in **FIG. 13**. Like **FIG. 7**, the schematic includes clinical information management, medical and literature information management, and financial management of the information-based personalized medicine drug discovery system and method of the present invention. **FIG. 14** is a schematic showing an illustrative network of the information-based personalized medicine drug discovery system and method of the present invention. Patients, medical practitioners, host medical centers, and labs all share and exchange a variety of information in order to provide a patient with a proposed therapy or agent based on various identified targets.

[00326] FIGS. 15-25 are computer screen print outs associated with various parts of the information-based personalized medicine drug discovery system and method shown in **FIGS. 5-14**. **FIGS. 15** and **16** show computer screens where physician information and insurance company information is entered on behalf of a client. **FIGS. 17-19** show computer screens in which information can be entered for ordering analysis and tests on patient samples.

[00327] FIG. 20 is a computer screen showing micro array analysis results of specific genes tested with patient samples. This information and computer screen is similar to the information detailed in the patient profile report shown in **FIG. 3C**. **FIG. 22** is a computer screen that shows immunohistochemistry test results for a particular patient for various genes. This information is similar to the information contained in the patient profile report shown in **FIG. 3B**.

[00328] FIG. 21 is a computer screen showing selection options for finding particular patients, ordering tests and/or results, issuing patient reports, and tracking current cases/patients.

[00329] FIG. 23 is a computer screen which outlines some of the steps for creating a patient profile report as shown in **FIGS. 3A** through **3D**. **FIG. 24** shows a computer screen for ordering an immunohistochemistry test on a patient sample and **FIG. 25** shows a computer screen for entering information regarding a primary tumor site for micro array analysis. It will be understood by those skilled in the art that any number and variety of computer screens may be used to enter the information necessary for using the information-based personalized medicine drug discovery system and method of the present

invention and to obtain information resulting from using the information-based personalized medicine drug discovery system and method of the present invention.

[00330] The systems of the invention can be used to automate the steps of identifying a molecular profile to assess a cancer. In an aspect, the invention provides a method of generating a report comprising a molecular profile. The method comprises: performing a search on an electronic medium to obtain a data set, wherein the data set comprises a plurality of scientific publications corresponding to plurality of cancer biomarkers; and analyzing the data set to identify a rule set linking a characteristic of each of the plurality of cancer biomarkers with an expected benefit of a plurality of treatment options, thereby identifying the cancer biomarkers included within a molecular profile. The method can further comprise performing molecular profiling on a sample from a subject to assess the characteristic of each of the plurality of cancer biomarkers, and compiling a report comprising the assessed characteristics into a list, thereby generating a report that identifies a molecular profile for the sample. The report can further comprise a list describing the expected benefit of the plurality of treatment options based on the assessed characteristics, thereby identifying candidate treatment options for the subject. The sample from the subject may comprise cancer cells. The cancer can be any cancer disclosed herein or known in the art.

[00331] The characteristic of each of the plurality of cancer biomarkers can be any useful characteristic for molecular profiling as disclosed herein or known in the art. Such characteristics include without limitation mutations (point mutations, insertions, deletions, rearrangements, etc), epigenetic modifications, copy number, nucleic acid or protein expression levels, post-translational modifications, and the like.

[00332] In an embodiment, the method further comprises identifying a priority list as amongst said plurality of cancer biomarkers. The priority list can be sorted according to any appropriate priority criteria. In an embodiment, the priority list is sorted according to strength of evidence in the plurality of scientific publications linking the cancer biomarkers to the expected benefit. In another embodiment, the priority list is sorted according to strength of the expected benefit. In still another embodiment, the priority list is sorted according to strength of the expected benefit. One of skill will appreciate that the priority list can be sorted according to a combination of these or other appropriate priority criteria. The candidate treatment options can be sorted according to the priority list, thereby identifying a ranked list of treatment options for the subject.

[00333] The candidate treatment options can be categorized by expected benefit to the subject. For example, the candidate treatment options can categorized as those that are expected to provide benefit, those that are not expected to provide benefit, or those whose expected benefit cannot be determined.

[00334] The candidate treatment options can include regulatory approved and/or on-compendium treatments for the cancer. The candidate treatment options can include regulatory approved but off-label treatments for the cancer, such as a treatment that has been approved for a cancer of another lineage. The candidate treatment options can include treatments that are under development, such as in ongoing clinical trials. The report may identify treatments as approved, on- or off-compendium, in clinical trials, and the like.

[00335] In some embodiments, the method further comprises analyzing the data set to select a laboratory technique to assess the characteristics of the biomarkers, thereby designating a technique that can be used to assess the characteristic for each of the plurality of biomarkers. In other embodiments, the laboratory technique is chosen based on its applicability to assess the characteristic of each of the biomarkers. The laboratory techniques can be those disclosed herein, including without limitation FISH for gene copy number or mutation analysis, IHC for protein expression levels, RT-PCR for mutation or expression analysis, sequencing or fragment analysis for mutation analysis. Sequencing includes any useful sequencing method disclosed herein or known in the art, including without limitation Sanger sequencing, pyrosequencing, or next generation sequencing methods.

[00336] In a related aspect, the invention provides a method comprising: performing a search on an electronic medium to obtain a data set comprising a plurality of scientific publications corresponding to plurality of cancer biomarkers; analyzing the data set to select a method to assess a characteristic of each of the cancer biomarkers, thereby designating a method for characterizing each of the biomarkers; further analyzing the data set to select a rule set that identifies a priority list as amongst the biomarkers; performing tumor profiling on a tumor sample from a subject comprising the selected methods to determine the status of the characteristic of each of the biomarkers; and compiling the status in a report according to said priority list; thereby generating a report that identifies a tumor profile.

Molecular Profiling Targets

[00337] The present invention provides methods and systems for analyzing diseased tissue using molecular profiling as previously described above. Because the methods rely on analysis of the characteristics of the tumor under analysis, the methods can be applied in for any tumor or any stage of disease, such an advanced stage of disease or a metastatic tumor of unknown origin. As described herein, a tumor or cancer sample is analyzed for molecular characteristics in order to predict or identify a candidate therapeutic treatment. The molecular characteristics can include the expression of genes or gene products, assessment of gene copy number, or mutational analysis. Any relevant determinable characteristic that can assist in prediction or identification of a candidate therapeutic can be included within the methods of the invention.

[00338] The biomarker patterns or biomarker signature sets can be determined for tumor types, diseased tissue types, or diseased cells including without limitation adipose, adrenal cortex, adrenal gland, adrenal gland – medulla, appendix, bladder, blood vessel, bone, bone cartilage, brain, breast, cartilage, cervix, colon, colon sigmoid, dendritic cells, skeletal muscle, endometrium, esophagus, fallopian tube, fibroblast, gallbladder, kidney, larynx, liver, lung, lymph node, melanocytes, mesothelial lining, myoepithelial cells, osteoblasts, ovary, pancreas, parotid, prostate, salivary gland, sinus tissue, skeletal muscle, skin, small intestine, smooth muscle, stomach, synovium, joint lining tissue, tendon, testis, thymus, thyroid, uterus, and uterus corpus.

[00339] The methods of the present invention can be used for selecting a treatment of any cancer or tumor type, including but not limited to breast cancer (including HER2+ breast cancer, HER2- breast cancer, ER/PR+, HER2- breast cancer, or triple negative breast cancer), pancreatic cancer, cancer of the colon

and/or rectum, leukemia, skin cancer, bone cancer, prostate cancer, liver cancer, lung cancer, brain cancer, cancer of the larynx, gallbladder, parathyroid, thyroid, adrenal, neural tissue, head and neck, stomach, bronchi, kidneys, basal cell carcinoma, squamous cell carcinoma of both ulcerating and papillary type, metastatic skin carcinoma, osteo sarcoma, Ewing's sarcoma, veticulum cell sarcoma, myeloma, giant cell tumor, small-cell lung tumor, islet cell carcinoma, primary brain tumor, acute and chronic lymphocytic and granulocytic tumors, hairy-cell tumor, adenoma, hyperplasia, medullary carcinoma, pheochromocytoma, mucosal neuroma, intestinal ganglioneuroma, hyperplastic corneal nerve tumor, marfanoid habitus tumor, Wilm's tumor, seminoma, ovarian tumor, leiomyoma, cervical dysplasia and in situ carcinoma, neuroblastoma, retinoblastoma, soft tissue sarcoma, malignant carcinoid, topical skin lesion, mycosis fungoides, rhabdomyosarcoma, Kaposi's sarcoma, osteogenic and other sarcoma, malignant hypercalcemia, renal cell tumor, polycythermia vera, adenocarcinoma, glioblastoma multiforma, leukemias, lymphomas, malignant melanomas, and epidermoid carcinomas. The cancer or tumor can comprise, without limitation, a carcinoma, a sarcoma, a lymphoma or leukemia, a germ cell tumor, a blastoma, or other cancers. Carcinomas that can be assessed using the subject methods include without limitation epithelial neoplasms, squamous cell neoplasms, squamous cell carcinoma, basal cell neoplasms basal cell carcinoma, transitional cell papillomas and carcinomas, adenomas and adenocarcinomas (glands), adenoma, adenocarcinoma, linitis plastica insulinoma, glucagonoma, gastrinoma, vipoma, cholangiocarcinoma, hepatocellular carcinoma, adenoid cystic carcinoma, carcinoid tumor of appendix, prolactinoma, oncocytoma, hurthle cell adenoma, renal cell carcinoma, grawitz tumor, multiple endocrine adenomas, endometrioid adenoma, adnexal and skin appendage neoplasms, mucoepidermoid neoplasms, cystic, mucinous and serous neoplasms, cystadenoma, pseudomyxoma peritonei, ductal, lobular and medullary neoplasms, acinar cell neoplasms, complex epithelial neoplasms, warthin's tumor, thymoma, specialized gonadal neoplasms, sex cord stromal tumor, thecoma, granulosa cell tumor, arrhenoblastoma, sertoli leydig cell tumor, glomus tumors, paraganglioma, pheochromocytoma, glomus tumor, nevi and melanomas, melanocytic nevus, malignant melanoma, melanoma, nodular melanoma, dysplastic nevus, lentigo maligna melanoma, superficial spreading melanoma, and malignant acral lentiginous melanoma. Sarcoma that can be assessed using the subject methods include without limitation Askin's tumor, botryodies, chondrosarcoma, Ewing's sarcoma, malignant hemangio endothelioma, malignant schwannoma, osteosarcoma, soft tissue sarcomas including: alveolar soft part sarcoma, angiosarcoma, cystosarcoma phyllodes, dermatofibrosarcoma, desmoid tumor, desmoplastic small round cell tumor, epithelioid sarcoma, extraskeletal chondrosarcoma, extraskeletal osteosarcoma, fibrosarcoma, hemangiopericytoma, hemangiosarcoma, kaposi's sarcoma, leiomyosarcoma, liposarcoma, lymphangiosarcoma, lymphosarcoma, malignant fibrous histiocytoma, neurofibrosarcoma, rhabdomyosarcoma, and synovialsarcoma. Lymphoma and leukemia that can be assessed using the subject methods include without limitation chronic lymphocytic leukemia/small lymphocytic lymphoma, B-cell prolymphocytic leukemia, lymphoplasmacytic lymphoma (such as waldenström macroglobulinemia), splenic marginal zone lymphoma, plasma cell myeloma, plasmacytoma, monoclonal immunoglobulin deposition diseases, heavy chain diseases, extranodal marginal zone B cell lymphoma,

also called malt lymphoma, nodal marginal zone B cell lymphoma (nmzl), follicular lymphoma, mantle cell lymphoma, diffuse large B cell lymphoma, mediastinal (thymic) large B cell lymphoma, intravascular large B cell lymphoma, primary effusion lymphoma, burkitt lymphoma/leukemia, T cell prolymphocytic leukemia, T cell large granular lymphocytic leukemia, aggressive NK cell leukemia, adult T cell leukemia/lymphoma, extranodal NK/T cell lymphoma, nasal type, enteropathy-type T cell lymphoma, hepatosplenic T cell lymphoma, blastic NK cell lymphoma, mycosis fungoides / sezary syndrome, primary cutaneous CD30-positive T cell lymphoproliferative disorders, primary cutaneous anaplastic large cell lymphoma, lymphomatoid papulosis, angioimmunoblastic T cell lymphoma, peripheral T cell lymphoma, unspecified, anaplastic large cell lymphoma, classical Hodgkin lymphomas (nodular sclerosis, mixed cellularity, lymphocyte-rich, lymphocyte depleted or not depleted), and nodular lymphocyte-predominant Hodgkin lymphoma. Germ cell tumors that can be assessed using the subject methods include without limitation germinoma, dysgerminoma, seminoma, nongerminomatous germ cell tumor, embryonal carcinoma, endodermal sinus turmor, choriocarcinoma, teratoma, polyembryoma, and gonadoblastoma. Blastoma includes without limitation nephroblastoma, medulloblastoma, and retinoblastoma. Other cancers include without limitation labial carcinoma, larynx carcinoma, hypopharynx carcinoma, tongue carcinoma, salivary gland carcinoma, gastric carcinoma, adenocarcinoma, thyroid cancer (medullary and papillary thyroid carcinoma), renal carcinoma, kidney parenchyma carcinoma, cervix carcinoma, uterine corpus carcinoma, endometrium carcinoma, chorion carcinoma, testis carcinoma, urinary carcinoma, melanoma, brain tumors such as glioblastoma, astrocytoma, meningioma, medulloblastoma and peripheral neuroectodermal tumors, gall bladder carcinoma, bronchial carcinoma, multiple myeloma, basalioma, teratoma, retinoblastoma, choroidea melanoma, seminoma, rhabdomyosarcoma, craniopharyngeoma, osteosarcoma, chondrosarcoma, myosarcoma, liposarcoma, fibrosarcoma, Ewing sarcoma, and plasmocytoma.

[00340] In an embodiment, the cancer may be a acute myeloid leukemia (AML), breast carcinoma, cholangiocarcinoma, colorectal adenocarcinoma, extrahepatic bile duct adenocarcinoma, female genital tract malignancy, gastric adenocarcinoma, gastroesophageal adenocarcinoma, gastrointestinal stromal tumors (GIST), glioblastoma, head and neck squamous carcinoma, leukemia, liver hepatocellular carcinoma, low grade glioma, lung bronchioloalveolar carcinoma (BAC), lung non-small cell lung cancer (NSCLC), lung small cell cancer (SCLC), lymphoma, male genital tract malignancy, malignant solitary fibrous tumor of the pleura (MSFT), melanoma, multiple myeloma, neuroendocrine tumor, nodal diffuse large B-cell lymphoma, non epithelial ovarian cancer (non-EOC), ovarian surface epithelial carcinoma, pancreatic adenocarcinoma, pituitary carcinomas, oligodendroglioma, prostatic adenocarcinoma, retroperitoneal or peritoneal carcinoma, retroperitoneal or peritoneal sarcoma, small intestinal malignancy, soft tissue tumor, thymic carcinoma, thyroid carcinoma, or uveal melanoma.

[00341] In a further embodiment, the cancer may be a lung cancer including non-small cell lung cancer and small cell lung cancer (including small cell carcinoma (oat cell cancer), mixed small cell/large cell carcinoma, and combined small cell carcinoma), colon cancer, breast cancer, prostate cancer, liver cancer, pancreas cancer, brain cancer, kidney cancer, ovarian cancer, stomach cancer, skin cancer, bone cancer,

gastric cancer, breast cancer, pancreatic cancer, glioma, glioblastoma, hepatocellular carcinoma, papillary renal carcinoma, head and neck squamous cell carcinoma, leukemia, lymphoma, myeloma, or a solid tumor.

[00342] In embodiments, the cancer comprises an acute lymphoblastic leukemia; acute myeloid leukemia; adrenocortical carcinoma; AIDS-related cancers; AIDS-related lymphoma; anal cancer; appendix cancer; astrocytomas; atypical teratoid/rhabdoid tumor; basal cell carcinoma; bladder cancer; brain stem glioma; brain tumor (including brain stem glioma, central nervous system atypical teratoid/rhabdoid tumor, central nervous system embryonal tumors, astrocytomas, craniopharyngioma, ependymoblastoma, ependymoma, medulloblastoma, medulloepithelioma, pineal parenchymal tumors of intermediate differentiation, supratentorial primitive neuroectodermal tumors and pineoblastoma); breast cancer; bronchial tumors; Burkitt lymphoma; cancer of unknown primary site; carcinoid tumor; carcinoma of unknown primary site; central nervous system atypical teratoid/rhabdoid tumor; central nervous system embryonal tumors; cervical cancer; childhood cancers; chordoma; chronic lymphocytic leukemia; chronic myelogenous leukemia; chronic myeloproliferative disorders; colon cancer; colorectal cancer; craniopharyngioma; cutaneous T-cell lymphoma; endocrine pancreas islet cell tumors; endometrial cancer; ependymoblastoma; ependymoma; esophageal cancer; esthesioneuroblastoma; Ewing sarcoma; extracranial germ cell tumor; extragonadal germ cell tumor; extrahepatic bile duct cancer; gallbladder cancer; gastric (stomach) cancer; gastrointestinal carcinoid tumor; gastrointestinal stromal cell tumor; gastrointestinal stromal tumor (GIST); gestational trophoblastic tumor; glioma; hairy cell leukemia; head and neck cancer; heart cancer; Hodgkin lymphoma; hypopharyngeal cancer; intraocular melanoma; islet cell tumors; Kaposi sarcoma; kidney cancer; Langerhans cell histiocytosis; laryngeal cancer; lip cancer; liver cancer; malignant fibrous histiocytoma bone cancer; medulloblastoma; medulloepithelioma; melanoma; Merkel cell carcinoma; Merkel cell skin carcinoma; mesothelioma; metastatic squamous neck cancer with occult primary; micropapillary urothelial carcinoma; mouth cancer; multiple endocrine neoplasia syndromes; multiple myeloma; multiple myeloma/plasma cell neoplasm; mycosis fungoides; myelodysplastic syndromes; myeloproliferative neoplasms; nasal cavity cancer; nasopharyngeal cancer; neuroblastoma; Non-Hodgkin lymphoma; nonmelanoma skin cancer; non-small cell lung cancer; oral cancer; oral cavity cancer; oropharyngeal cancer; osteosarcoma; other brain and spinal cord tumors; ovarian cancer; ovarian epithelial cancer; ovarian germ cell tumor; ovarian low malignant potential tumor; pancreatic cancer; papillomatosis; paranasal sinus cancer; parathyroid cancer; pelvic cancer; penile cancer; pharyngeal cancer; pineal parenchymal tumors of intermediate differentiation; pineoblastoma; pituitary tumor; plasma cell neoplasm/multiple myeloma; pleuropulmonary blastoma; primary central nervous system (CNS) lymphoma; primary hepatocellular liver cancer; prostate cancer; rectal cancer; renal cancer; renal cell (kidney) cancer; renal cell cancer; respiratory tract cancer; retinoblastoma; rhabdomyosarcoma; salivary gland cancer; Sézary syndrome; small cell lung cancer; small intestine cancer; soft tissue sarcoma; squamous cell carcinoma; squamous neck cancer; stomach (gastric) cancer; supratentorial primitive neuroectodermal tumors; T-cell lymphoma; testicular cancer; throat cancer; thymic carcinoma; thymoma; thyroid cancer; transitional cell cancer; transitional cell cancer of the renal

pelvis and ureter; trophoblastic tumor; ureter cancer; urethral cancer; uterine cancer; uterine sarcoma; vaginal cancer; vulvar cancer; Waldenström macroglobulinemia; or Wilm's tumor.

[00343] The methods of the invention can be used to determine biomarker patterns or biomarker signature sets in a number of tumor types, diseased tissue types, or diseased cells including accessory, sinuses, middle and inner ear, adrenal glands, appendix, hematopoietic system, bones and joints, spinal cord, breast, cerebellum, cervix uteri, connective and soft tissue, corpus uteri, esophagus, eye, nose, eyeball, fallopian tube, extrahepatic bile ducts, other mouth, intrahepatic bile ducts, kidney, appendix-colon, larynx, lip, liver, lung and bronchus, lymph nodes, cerebral, spinal, nasal cartilage, excl. retina, eye, nos, oropharynx, other endocrine glands, other female genital, ovary, pancreas, penis and scrotum, pituitary gland, pleura, prostate gland, rectum renal pelvis, ureter, peritonem, salivary gland, skin, small intestine, stomach, testis, thymus, thyroid gland, tongue, unknown, urinary bladder, uterus, nos, vagina & labia, and vulva,nos.

[00344] In some embodiments, the molecular profiling methods are used to identify a treatment for a cancer of unknown primary (CUP). Approximately 40,000 CUP cases are reported annually in the US. Most of these are metastatic and/or poorly differentiated tumors. Because molecular profiling can identify a candidate treatment depending only upon the diseased sample, the methods of the invention can be used in the CUP setting. Moreover, molecular profiling can be used to create signatures of known tumors, which can then be used to classify a CUP and identify its origin. In an aspect, the invention provides a method of identifying the origin of a CUP, the method comprising performing molecular profiling on a panel of diseased samples to determine a panel of molecular profiles that correlate with the origin of each diseased sample, performing molecular profiling on a CUP sample, and correlating the molecular profile of the CUP sample with the molecular profiling of the panel of diseased samples, thereby identifying the origin of the CUP sample. The identification of the origin of the CUP sample can be made by matching the molecular profile of the CUP sample with the molecular profiles that correlate most closely from the panel of disease samples. The molecular profiling can use any of the techniques described herein, e.g., IHC, FISH, microarray and sequencing. The diseased samples and CUP samples can be derived from a patient sample, e.g., a biopsy sample, including a fine needle biopsy. In one embodiment, DNA microarray and IHC profiling are performed on the panel of diseased samples, DNA microarray is performed on the CUP samples, and then IHC is performed on the CUP sample for a subset of the most informative genes as indicated by the DNA microarray analysis. This approach can identify the origin of the CUP sample while avoiding the expense of performing unnecessary IHC testing. The IHC can be used to confirm the microarray findings.

[00345] The biomarker patterns or biomarker signature sets of the cancer or tumor can be used to determine a therapeutic agent or therapeutic protocol that is capable of interacting with the biomarker pattern or signature set. For example, with advanced breast cancer, immunohistochemistry analysis can be used to determine one or more gene expressed proteins that are overexpressed. Accordingly, a biomarker pattern or biomarker signature set can be identified for advanced stage breast cancer and a therapeutic

agent or therapeutic protocol can be identified which is capable of interacting with the biomarker pattern or signature set.

[00346] The biomarker patterns and/or biomarker signature sets can comprise at least one biomarker. In yet other embodiments, the biomarker patterns or signature sets can comprise at least 2, 3, 4, 5, 6, 7, 8, 9, or 10 biomarkers. In some embodiments, the biomarker signature sets or biomarker patterns can comprise at least 15, 20, 30, 40, 50, or 60 biomarkers. In some embodiments, the biomarker signature sets or biomarker patterns can comprise at least 70, 80, 90, 100, 200, 300, 400, 500, 600, 700, 800, 900, 1000, 2000, 3000, 4000, 5000, 6000, 7000, 8000, 9000, 10,000, 15,000, 20,000, 25,000, 30,000, 35,000, 40,000, 45,000 or 50,000 biomarkers. Analysis of the one or more biomarkers can be by one or more methods. For example, analysis of 2 biomarkers can be performed using microarrays. Alternatively, one biomarker may be analyzed by IHC and another by microarray. Any such combinations of methods and biomarkers are contemplated herein.

[00347] The one or more biomarkers can be selected from the group consisting of, but not limited to: Her2/Neu, ER, PR, c-kit, EGFR, MLH1, MSH2, CD20, p53, Cyclin D1, bcl2, COX-2, Androgen receptor, CD52, PDGFR, AR, CD25, VEGF, HSP90, PTEN, RRM1, SPARC, Survivin, TOP2A, BCL2, HIF1A, AR, ESR1, PDGFRA, KIT, PDGFRB, CDW52, ZAP70, PGR, SPARC, GART, GSTP1, NFKBIA, MSH2, TXNRD1, HDAC1, PDGFC, PTEN, CD33, TYMS, RXRB, ADA, TNF, ERCC3, RAF1, VEGF, TOP1, TOP2A, BRCA2, TK1, FOLR2, TOP2B, MLH1, IL2RA, DNMT1, HSPCA, ERBR2, ERBB2, SSTR1, VHL, VDR, PTGS2, POLA, CES2, EGFR, OGFR, ASNS, NFKB2, RARA, MS4A1, DCK, DNMT3A, EREG, Epiregulin, FOLR1, GNRH1, GNRHR1, FSHB, FSHR, FSHPRH1, folate receptor, HGF, HIG1, IL13RA1, LTB, ODC1, PPARG, PPARGC1, Lymphotoxin Beta Receptor, Myc, Topoisomerase II, TOPO2B, TXN, VEGFC, ACE2, ADH1C, ADH4, AGT, AREG, CA2, CDK2, caveolin, NFKB1, ASNS, BDCA1, CD52, DHFR, DNMT3B, EPHA2, FLT1, HSP90AA1, KDR, LCK, MGMT, RRM1, RRM2, RRM2B, RXRG, SRC, SSTR2, SSTR3, SSTR4, SSTR5, VEGFA, or YES1.

[00348] For example, a biological sample from an individual can be analyzed to determine a biomarker pattern or biomarker signature set that comprises a biomarker such as HSP90, Survivin, RRM1, SSTRS3, DNMT3B, VEGFA, SSTR4, RRM2, SRC, RRM2B, HSP90AA1, STR2, FLT1, SSTR5, YES1, BRCA1, RRM1, DHFR, KDR, EPHA2, RXRG, or LCK. In other embodiments, the biomarker SPARC, HSP90, TOP2A, PTEN, Survivin, or RRM1 forms part of the biomarker pattern or biomarker signature set. In yet other embodiments, the biomarker MGMT, SSTRS3, DNMT3B, VEGFA, SSTR4, RRM2, SRC, RRM2B, HSP90AA1, STR2, FLT1, SSTR5, YES1, BRCA1, RRM1, DHFR, KDR, EPHA2, RXRG, CD52, or LCK is included in a biomarker pattern or biomarker signature set. In still other embodiments, the biomarker hENT1, cMet, P21, PARP-1, TLE3 or IGF1R is included in a biomarker pattern or biomarker signature set.

[00349] The expression level of HSP90, Survivin, RRM1, SSTRS3, DNMT3B, VEGFA, SSTR4, RRM2, SRC, RRM2B, HSP90AA1, STR2, FLT1, SSTR5, YES1, BRCA1, RRM1, DHFR, KDR, EPHA2, RXRG, or LCK can be determined and used to identify a therapeutic for an individual. The expression level of the biomarker can be used to form a biomarker pattern or biomarker signature set. Determining

the expression level can be by analyzing the levels of mRNA or protein, such as by microarray analysis or IHC. In some embodiments, the expression level of a biomarker is performed by IHC, such as for SPARC, TOP2A, or PTEN, and used to identify a therapeutic for an individual. The results of the IHC can be used to form a biomarker pattern or biomarker signature set. In yet other embodiments, a biological sample from an individual or subject is analyzed for the expression level of CD52, such as by determining the mRNA expression level by methods including, but not limited to, microarray analysis. The expression level of CD52 can be used to identify a therapeutic for the individual. The expression level of CD52 can be used to form a biomarker pattern or biomarker signature set. In still other embodiments, the biomarkers hENT1, cMet, P21, PARP-1, TLE3 and/or IGF1R are assessed to identify a therapeutic for the individual.

[00350] As described herein, the molecular profiling of one or more targets can be used to determine or identify a therapeutic for an individual. For example, the expression level of one or more biomarkers can be used to determine or identify a therapeutic for an individual. The one or more biomarkers, such as those disclosed herein, can be used to form a biomarker pattern or biomarker signature set, which is used to identify a therapeutic for an individual. In some embodiments, the therapeutic identified is one that the individual has not previously been treated with. For example, a reference biomarker pattern has been established for a particular therapeutic, such that individuals with the reference biomarker pattern will be responsive to that therapeutic. An individual with a biomarker pattern that differs from the reference, for example the expression of a gene in the biomarker pattern is changed or different from that of the reference, would not be administered that therapeutic. In another example, an individual exhibiting a biomarker pattern that is the same or substantially the same as the reference is advised to be treated with that therapeutic. In some embodiments, the individual has not previously been treated with that therapeutic and thus a new therapeutic has been identified for the individual.

[00351] Molecular profiling according to the invention can take on a biomarker-centric or a therapeutic-centric point of view. Although the approaches are not mutually exclusive, the biomarker-centric approach focuses on sets of biomarkers that are expected to be informative for a tumor of a given tumor lineage, whereas the therapeutic-centric point approach identifies candidate therapeutics using biomarker panels that are lineage independent. In a biomarker-centric view, panels of specific biomarkers are run on different tumor types. See **FIG. 26A**. This approach provides a method of identifying a candidate therapeutic by collecting a sample from a subject with a cancer of known origin, and performing molecular profiling on the cancer for specific biomarkers depending on the origin of the cancer. The molecular profiling can be performed using any of the various techniques disclosed herein. As an example, **FIG. 26A** shows biomarker panels for breast cancer, ovarian cancer, colorectal cancer, lung cancer, and a "complete" profile to run on any cancer. In the figure, markers shown in italics are assessed using mutational analysis (e.g., sequencing approaches), marker shown underlined are analyzed by FISH, and the remainder are analyzed using IHC. DNA microarray profiling can be performed on any sample. The candidate therapeutic is selected based on the molecular profiling results according to the subject methods. An advantage to the bio-marker centric approach is only performing assays that are most likely to yield informative results. Another advantage is that this approach can focus on identifying therapeutics

conventionally used to treat cancers of the specific lineage. In a therapeutic-centric approach, the biomarkers assessed are not dependent on the origin of the tumor. See **FIG. 26B**. This approach provides a method of identifying a candidate therapeutic by collecting a sample from a subject with a cancer, and performing molecular profiling on the cancer for a panel of biomarkers without regards to the origin of the cancer. The molecular profiling can be performed using any of the various techniques disclosed herein. As an example, in **FIG. 26B**, markers shown in italics are assessed using mutational analysis (e.g., sequencing approaches), marker shown underlined are analyzed by FISH, and the remainder are analyzed using IHC. DNA microarray profiling can be performed on any sample. The candidate therapeutic is selected based on the molecular profiling results according to the subject methods. An advantage to the therapeutic-marker centric approach is that the most promising therapeutics are identified only taking into account the molecular characteristics of the tumor itself. Another advantage is that the method can be preferred for a cancer of unidentified primary origin (CUP). In some embodiments, a hybrid of biomarker-centric and therapeutic-centric points of view is used to identify a candidate therapeutic. This method comprises identifying a candidate therapeutic by collecting a sample from a subject with a cancer of known origin, and performing molecular profiling on the cancer for a comprehensive panel of biomarkers, wherein a portion of the markers assessed depend on the origin of the cancer. For example, consider a breast cancer. A comprehensive biomarker panel is run on the breast cancer, e.g., the complete panel as shown in **FIG. 26B**, but additional sequencing analysis is performed on one or more additional markers, e.g., BRCA1 or any other marker with mutations informative for theranosis or prognosis of the breast cancer. Theranosis can be used to refer to the likely efficacy of a therapeutic treatment. Prognosis refers to the likely outcome of an illness. One of skill will appreciate that the hybrid approach can be used to identify a candidate therapeutic for any cancer having additional biomarkers that provide theranostic or prognostic information, including the cancers disclosed herein.

[00352] Methods for providing a theranosis of disease include selecting candidate therapeutics for various cancers by assessing a sample from a subject in need thereof (i.e., suffering from a particular cancer). The sample is assessed by performing an immunohistochemistry (IHC) to determine of the presence or level of: AR, BCRP, c-KIT, ER, ERCC1, HER2, IGF1R, MET (also referred to herein as cMet), MGMT, MRP1, PDGFR, PGP, PR, PTEN, RRM1, SPARC, TOPO1, TOP2A, TS, COX-2, CK5/6, CK14, CK17, Ki67, p53, CAV-1, CYCLIN D1, EGFR, E-cadherin, p95, TLE3 or a combination thereof; performing a microarray analysis on the sample to determine a microarray expression profile on one or more (such as at least five, 10, 15, 20, 25, 30, 40, 50, 60, 70 or all) of: ABCC1, ABCG2, ADA, AR, ASNS, BCL2, BIRC5, BRCA1, BRCA2, CD33, CD52, CDA, CES2, DCK, DHFR, DNMT1, DNMT3A, DNMT3B, ECGF1, EGFR, EPHA2, ERBB2, ERCC1, ERCC3, ESR1, FLT1, FOLR2, FYN, GART, GNRH1, GSTP1, HCK, HDAC1, HIF1A, HSP90AA1, IGFBP3, IGFBP4, IGFBP5, IL2RA, KDR, KIT, LCK, LYN, MET, MGMT, MLH1, MS4A1, MSH2, NFKB1, NFKB2, NFKBIA, OGFR, PARP1, PDGFC, PDGFRA, PDGFRB, PGP, PGR, POLA1, PTEN, PTGS2, PTPN12, RAF1, RARA, RRM1, RRM2, RRM2B, RXRB, RXRG, SIK2, SPARC, SRC, SSTR1, SSTR2, SSTR3, SSTR4, SSTR5, TK1, TNF, TOP1, TOP2A, TOP2B, TXNRD1, TYMS, VDR, VEGFA, VHL, YES1, and ZAP70; comparing the results

obtained from the IHC and microarray analysis against a rules database, wherein the rules database comprises a mapping of candidate treatments whose biological activity is known against a cancer cell that expresses one or more proteins included in the IHC expression profile and/or expresses one or more genes included in the microarray expression profile; and determining a candidate treatment if the comparison indicates that the candidate treatment has biological activity against the cancer.

[00353] Assessment can further comprise determining a fluorescent in-situ hybridization (FISH) profile of EGFR, HER2, cMYC, TOP2A, MET, or a combination thereof, comparing the FISH profile against a rules database comprising a mapping of candidate treatments predetermined as effective against a cancer cell having a mutation profile for EGFR, HER2, cMYC, TOP2A, MET, or a combination thereof, and determining a candidate treatment if the comparison of the FISH profile against the rules database indicates that the candidate treatment has biological activity against the cancer.

[00354] As explained further herein, the FISH analysis can be performed based on the origin of the sample. This can avoid unnecessary laboratory procedures and concomitant expenses by targeting analysis of genes that are known to play a role in a particular disorder, e.g., a particular type of cancer. In an embodiment, EGFR, HER2, cMYC, and TOP2A are assessed for breast cancer. In another embodiment, EGFR and MET are assessed for lung cancer. Alternately, FISH analysis of all of EGFR, HER2, cMYC, TOP2A, MET can be performed on a sample. The complete panel may be assessed, e.g., when a sample is of unknown or mixed origin, to provide a comprehensive view of an unusual sample, or when economies of scale dictate that it is more efficient to perform FISH on the entire panel than to make individual assessments.

[00355] In an additional embodiment, the sample is assessed by performing nucleic acid sequencing on the sample to determine a presence of a mutation of KRAS, BRAF, NRAS, PIK3CA (also referred to as PI3K), c-Kit, EGFR, or a combination thereof, comparing the results obtained from the sequencing against a rules database comprising a mapping of candidate treatments predetermined as effective against a cancer cell having a mutation profile for KRAS, BRAF, NRAS, PIK3CA, c-Kit, EGFR, or a combination thereof; and determining a candidate treatment if the comparison of the sequencing to the mutation profile indicates that the candidate treatment has biological activity against the cancer.

[00356] As explained further herein, the nucleic acid sequencing can be performed based on the origin of the sample. This can avoid unnecessary laboratory procedures and concomitant expenses by targeting analysis of genes that are known to play a role in a particular disorder, e.g., a particular type of cancer. In an embodiment, the sequences of PIK3CA and c-KIT are assessed for breast cancer. In another embodiment, the sequences of KRAS and BRAF are assessed for GI cancers such as colorectal cancer. In still another embodiment, the sequences of KRAS, BRAF and EGFR are assessed for lung cancer. Alternately, sequencing of all of KRAS, BRAF, NRAS, PIK3CA, c-Kit, EGFR can be performed on a sample. The complete panel may be sequenced, e.g., when a sample is of unknown or mixed origin, to provide a comprehensive view of an unusual sample, or when economies of scale dictate that it is more efficient to sequence the entire panel than to make individual assessments.

[00357] The genes and gene products used for molecular profiling, e.g., by microarray, IHC, FISH, sequencing, and/or PCR (e.g., qPCR), can be selected from those listed in **Table 2**, **Table 6** or **Table 17**. In an embodiment, IHC is performed for one or more, e.g., 2, 3, 4, 5, 6,7, 8, 9, 10, 15, 20 or more, of: AR, BCRP, CAV-1, CD20, CD52, CK 5/6, CK14, CK17, c-kit, CMET, COX-2, Cyclin D1, E-Cad, EGFR, ER, ERCC1, HER-2, IGF1R, Ki67, MGMT, MRP1, P53, p95, PDGFR, PGP, PR, PTEN, RRM1, SPARC, TLE3, TOPO1, TOPO2A, TS, TUBB3; expression analysis (e.g., microarray or RT-PCR) is performed on one or more, e.g. 2, 3, 4, 5, 6,7, 8, 9, 10, 15, 20, 25, 30, 40, 50 or more, of: ABCC1, ABCG2, ADA, AR, ASNS, BCL2, BIRC5, BRCA1, BRCA2, CD33, CD52, CDA, CES2, cKit, c-MYC, DCK, DHFR, DNMT1, DNMT3A, DNMT3B, ECGF1, EGFR, EPHA2, ERCC1, ERCC3, ESR1, FLT1, FOLR2, FYN, GART, GNRH1, GSTP1, HCK, HDAC1, HER2/ERBB2, HIF1A, HSP90, IGFBP3, IGFBP4, IGFBP5, IL2RA, KDR, LCK, LYN, MET, MGMT, MLH1, MS4A1, MSH2, NFKB1, NFKB2, NFKBIA, OGFR, PARP1, PDGFC, PDGFRa, PDGFRA, PDGFRB, PGP, PGR, POLA1, PTEN, PTGS2, RAF1, RARA, ROS1, RRM1, RRM2, RRM2B, RXRB, RXRG, SIK2, SRC, SSTR1, SSTR2, SSTR3, SSTR4, SSTR5, SPARC, TK1, TNF, TOP2B, TOP2A, TOPO1, TXNRD1, TYMS, VDR, VEGFA, VHL, YES1, and ZAP70; fluorescent in-situ hybridization (FISH) is performed on 1, 2, 3, 4, 5, 6 or 7 of ALK, cMET, c-MYC, EGFR, HER-2, PIK3CA, and TOPO2A; and DNA sequencing or PCR are performed on 1, 2, 3, 4, 5 or 6 of BRAF, c-kit, EGFR, KRAS, NRAS, and PIK3CA. In an embodiment, all of these genes and/or the gene products thereof are assessed.

[00358] Assessing one or more biomarkers disclosed herein can be used for characterizing any of the cancers disclosed herein. Characterizing includes the diagnosis of a disease or condition, the prognosis of a disease or condition, the determination of a disease stage or a condition stage, a drug efficacy, a physiological condition, organ distress or organ rejection, disease or condition progression, therapy-related association to a disease or condition, or a specific physiological or biological state.

[00359] A cancer in a subject can be characterized by obtaining a biological sample from a subject and analyzing one or more biomarkers from the sample. For example, characterizing a cancer for a subject or individual may include detecting a disease or condition (including pre-symptomatic early stage detecting), determining the prognosis, diagnosis, or theranosis of a disease or condition, or determining the stage or progression of a disease or condition. Characterizing a cancer can also include identifying appropriate treatments or treatment efficacy for specific diseases, conditions, disease stages and condition stages, predictions and likelihood analysis of disease progression, particularly disease recurrence, metastatic spread or disease relapse. Characterizing can also be identifying a distinct type or subtype of a cancer. The products and processes described herein allow assessment of a subject on an individual basis, which can provide benefits of more efficient and economical decisions in treatment.

[00360] In an aspect, characterizing a cancer includes predicting whether a subject is likely to respond to a treatment for the cancer. As used herein, a "responder" responds to or is predicted to respond to a treatment and a "non-responder" does not respond or is predicted to not respond to the treatment. Biomarkers can be analyzed in the subject and compared to biomarker profiles of previous subjects that were known to respond or not to a treatment. If the biomarker profile in a subject more closely aligns with

that of previous subjects that were known to respond to the treatment, the subject can be characterized, or predicted, as a responder to the treatment. Similarly, if the biomarker profile in the subject more closely aligns with that of previous subjects that did not respond to the treatment, the subject can be characterized, or predicted as a non-responder to the treatment.

[00361] The sample used for characterizing a cancer can be any disclosed herein, including without limitation a tissue sample, tumor sample, or a bodily fluid. Bodily fluids that can be used included without limitation peripheral blood, sera, plasma, ascites, urine, cerebrospinal fluid (CSF), sputum, saliva, bone marrow, synovial fluid, aqueous humor, amniotic fluid, cerumen, breast milk, broncheoalveolar lavage fluid, semen (including prostatic fluid), Cowper's fluid or pre-ejaculatory fluid, female ejaculate, sweat, fecal matter, hair, tears, cyst fluid, pleural and peritoneal fluid, pericardial fluid, malignant effusion, lymph, chyme, chyle, bile, interstitial fluid, menses, pus, sebum, vomit, vaginal secretions, mucosal secretion, stool water, pancreatic juice, lavage fluids from sinus cavities, bronchopulmonary aspirates or other lavage fluids. In an embodiment, the sample comprises vesicles. The biomarkers can be associated with the vesicles. In some embodiments, vesicles are isolated from the sample and the biomarkers associated with the vesicles are assessed.

Comprehensive and Standard-of-Care Molecular Profiling

[00362] Molecular profiling according to the invention can be used to guide treatment selection for cancers at any stage of disease or prior treatment. Molecular profiling comprises assessment of DNA mutations, gene rearrangements, gene copy number variation, RNA expression, protein expression, as well as assessment of other biological entities and phenomena that can inform clinical decision making. In some embodiments, the methods herein are used to guide selection of candidate treatments using the standard of care treatments for a particular type or lineage of cancer. Profiling of biomarkers that implicate standard-of-care treatments may be used to assist in treatment selection for a newly diagnosed cancer having multiple treatment options. Such profiling may be referred to herein as "select" profiling. Standard-of-care treatments may comprise NCCN on-compendium treatments or other standard treatments used for a cancer of a given lineage. One of skill will appreciate that such profiles can be updated as the standard of care and/or availability of experimental agents for a given disease lineage change. In other embodiments, molecular profiling is performed for additional biomarkers to identify treatments as beneficial or not beyond that go beyond the standard-of-care for a particular lineage or stage of the cancer. Such "comprehensive" profiling can be performed to assess a wide panel of druggable or drug-associated biomarker targets for any biological sample or specimen of interest. One of skill will appreciate that the select profiles generally comprise subsets of the comprehensive profile. The comprehensive profile can also be used to guide selection of candidate treatments for any cancer at any point of care. The comprehensive profile may also be preferable when standard-of-care treatments not expected to provide further benefit, such as in the salvage treatment setting for recurrent cancer or wherein all standard treatments have been exhausted. For example, the comprehensive profile may be used to assist in treatment selection when standard therapies are not an option for any reason including, without limitation, when standard treatments have been exhausted for the patient. The comprehensive

profile may be used to assist in treatment selection for highly aggressive or rare tumors with uncertain treatment regimens. For example, a comprehensive profile can be used to identify a candidate treatment for a newly diagnosed case or when the patient has exhausted standard of care therapies or has an aggressive disease. In practice, molecular profiling according to the invention has indeed identified beneficial therapies for a cancer patient when all standard-of-care treatments were exhausted the treating physician was unsure ofwhat treatment to select next. See the Examples herein. One of skill in the art will appreciate that by its very nature a comprehensive molecular profiling can be used to select a therapy for any appropriate indication independent of the nature of the indication (e.g., source, stage, prior treatment, etc). However, in some embodiments, a comprehensive molecular profile is tailored for a particular indication. For example, biomarkers associated with treatments that are known to be ineffective for a cancer from a particular lineage or anatomical origin may not be assessed as part of a comprehensive molecular profile for that particular cancer. Similarly, biomarkers associated with treatments that have been previously used and failed for a particular patient may not be assessed as part of a comprehensive molecular profile for that particular patient. In yet another non-limiting example, biomarkers associated with treatments that are only known to be effective for a cancer from a particular anatomical origin may only be assessed as part of a comprehensive molecular profile for that particular cancer. One of skill will further appreciate that the comprehensive molecular profile can be updated to reflect advancements, e.g., new treatments, new biomarker-drug associations, and the like, as available.

Molecular Intelligence Profiles

[00363] The invention provides molecular intelligence (MI) molecular profiles using a variety of techniques to assess panels of biomarkers in order to select or not select a candidate therapeutic for treating a cancer. Such techniques comprise IHC for expression profiling, CISH/FISH for DNA copy number, and Sanger, Pyrosequencing, PCR, RFLP, fragment analysis and Next Generation sequencing for mutational analysis. Such profiles are described in **FIGs. 27A-B**. The profiling can be performed using the biomarker – drug associations and related rules for the various cancer lineages as described for **FIGs. 27A-B** and **Tables 7-16**. MI profiles for all solid tumors or that have additional analyses based on tumor lineage include NextGen analysis of a panel of biomarkers linked to known therapies and clinical trials. The MI profiles can further be expanded to “MI PLUS” profiles that include sequencing of set of genes that are known to be involved in cancer and have alternative clinical utilities including predictive, prognostic or diagnostic uses.

[00364] The biomarkers which comprise the molecular intelligence molecular profiles can include genes or gene products that are known to be associated directly with a particular drug or class of drugs. The biomarkers can also be genes or gene products that interact with such drug associated targets, e.g., as members of a common pathway. The biomarkers can be selected from **Table 2**. In some embodiments, the genes and/or gene products included in the molecular intelligence (MI) molecular profiles are selected from **Table 6**.

Table 6: Exemplary Genes and Gene Products and Related Therapies

Biomarker	Description

ABL1	ABL1 Most CML patients have a chromosomal abnormality due to a fusion between Abelson (Abl) tyrosine kinase gene at chromosome 9 and break point cluster (Bcr) gene at chromosome 22 resulting in constitutive activation of the Bcr-Abl fusion gene. Imatinib is a Bcr-Abl tyrosine kinase inhibitor commonly used in treating CML patients. Mutations in the ABL1 gene are common in imatinib resistant CML patients which occur in 30-90% of patients. However, more than 50 different point mutations in the ABL1 kinase domain may be inhibited by the second generation kinase inhibitors, dasatinib, bosutinib and nilotinib. The gatekeeper mutation, T315I that causes resistance to all currently approved TKIs accounts for about 15% of the mutations found in patients with imatinib resistance. BCR-ABL1 mutation analysis is recommended to help facilitate selection of appropriate therapy for patients with CML after treatment with imatinib fails. Various clinical trials (on www.clinicaltrials.gov) investigating agents which target this gene may be available, which include the following: NCT01528085.
AKT1	AKT1 gene (v-akt murine thymoma viral oncogene homologue 1) encodes a serine/threonine kinase which is a pivotal mediator of the PI3K-related signaling pathway, affecting cell survival, proliferation and invasion. Dysregulated AKT activity is a frequent genetic defect implicated in tumorigenesis and has been indicated to be detrimental to hematopoiesis. Activating mutation E17K has been described in breast (2-4%), endometrial (2-4%), bladder cancers (3%), NSCLC (1%), squamous cell carcinoma of the lung (5%) and ovarian cancer (2%). This mutation in the pleckstrin homology domain facilitates the recruitment of AKT to the plasma membrane and subsequent activation by altering phosphoinositide binding. A mosaic activating mutation E17K has also been suggested to be the cause of Proteus syndrome. Mutation E49K has been found in bladder cancer, which enhances AKT activation and shows transforming activity in cell lines. Various clinical trials (on www.clinicaltrials.gov) investigating AKT inhibitor MK-2206 in patients carrying AKT mutations may be available, which include the following: NCT01277757, NCT01425879.
ALK	ALK rearrangements indicate the fusion of ALK (anaplastic lymphoma kinase) gene with the fusion partner, EML4. EML4-ALK fusion results in the pathologic expression of a fusion protein with constitutively active ALK kinase, resulting in aberrant activation of downstream signaling pathways including RAS-ERK, JAK3-STAT3 and PI3K-AKT. Patients with an EML4-ALK rearrangement are likely to respond to the ALK-targeted agent crizotinib and ceritinib.
AR	The androgen receptor (AR) is a member of the nuclear hormone receptor superfamily. Prostate tumor dependency on androgens / AR signaling is the basis for hormone withdrawal, or androgen ablation therapy, to treat men with prostate cancer. Androgen receptor antagonists as well as agents which block androgen production are indicated for the treatment of AR expressing prostate cancers.
APC	APC or adenomatous polyposis coli is a key tumor suppressor gene that encodes for a large multi-domain protein. This protein exerts its tumor suppressor function in the Wnt/b-catenin cascade mainly by controlling the degradation of b-catenin, the central activator of transcription in the Wnt signaling pathway. The Wnt signaling pathway mediates important cellular functions including intercellular adhesion, stabilization of the cytoskeleton, and cell cycle regulation and apoptosis, and it is important in embryonic development and oncogenesis. Mutation in APC results in a truncated protein product with abnormal function, lacking the domains involved in b-catenin degradation. Somatic mutation in the APC gene can be detected in the majority of colorectal tumors (80%) and it is an early event in colorectal tumorigenesis. APC wild type patients have shown better disease control rate in the metastatic setting when treated with oxaliplatin, while when treated with fluoropyrimidine regimens, APC wild type patients experience more hematological toxicities. APC mutation has also been identified in oral squamous cell carcinoma, gastric cancer as well as hepatoblastoma and may contribute to cancer formation. Various clinical trials (on www.clinicaltrials.gov) investigating agents which target this gene and/or its downstream or upstream effectors maybe available, which include the following: NCT01351103. Germline mutation in APC causes familial adenomatous polyposis, which is an autosomal dominant inherited disease that will inevitably develop to colorectal cancer if left untreated. COX-2 inhibitors including celecoxib may reduce the recurrence of adenomas and incidence of advanced adenomas in individuals with an increased risk of CRC. Turcot syndrome and Gardner's syndrome have also been associated with germline APC defects. Germline mutations

	of the APC have also been associated with an increased risk of developing desmoid disease, papillary thyroid carcinoma and hepatoblastoma.
AREG	AREG, also known as amphiregulin, is a ligand of the epidermal growth factor receptor. Overexpression of AREG in primary colorectal cancer patients has been associated with increased clinical benefit from cetuximab in KRAS wildtype patients.
ATM	ATM or ataxia telangiectasia mutated is activated by DNA double-strand breaks and DNA replication stress. It encodes a protein kinase that acts as a tumor suppressor and regulates various biomarkers involved in DNA repair, which include p53, BRCA1, CHK2, RAD17, RAD9, and NBS1. Although ATM is associated with hematologic malignancies, somatic mutations have been found in colon (18%), head and neck (14%), and prostate (12%) cancers. Patients with inactivating ATM mutations have been shown to respond poorly to DNA-damaging agents, as shown in a recent cohort of patients with leukemia, and therefore may potentially be more susceptible to PARP inhibitors. Various clinical trials (on www.clinicaltrials.gov) investigating agents which target this gene and/or its downstream or upstream effectors may be available, which include the following: NCT01434316. Germline mutations in ATM are associated with ataxia-telangiectasia (also known as Louis-Bar syndrome) and a predisposition to malignancy.
BRAF	BRAF encodes a protein belonging to the raf/mil family of serine/threonine protein kinases. This protein plays a role in regulating the MAPK signaling pathway initiated by EGFR activation, which affects cell division, differentiation, and secretion. Mutations in this gene, most frequently V600E, have been associated with various cancers, including colorectal cancer, malignant melanoma, thyroid carcinoma and non-small cell lung carcinoma. Recent publications have associated V600E mutations in BRAF with a reduced response to cetuximab and panitumumab in CRC, as well as sensitivity to vemurafenib, dabrafenib and trametinib in melanoma and other tumor types.
BRCA1	BRCA1 or breast cancer type 1 susceptibility gene encodes a protein involved in cell growth, cell division, and DNA-damage repair. It is a tumor suppressor gene which plays an important role in mediating double-strand DNA breaks by homologous recombination (HR). Tumors with BRCA1 mutation may be more sensitive to platinum agents and PARP inhibitors. Various clinical trials may be available (on clinicaltrials.gov) for patients with BRCA1 mutation.
BRCA2	BRCA2 or breast cancer type 2 susceptibility gene encodes a protein involved in cell growth, cell division, and DNA-damage repair. It is a tumor suppressor gene which plays an important role in mediating double-strand DNA breaks by homologous recombination (HR). Tumors with BRCA2 mutation may be more sensitive to platinum agents and PARP inhibitors. Various clinical trials may be available (on clinicaltrials.gov) for patients with BRCA2 mutation.
c-KIT	c-KIT is a receptor tyrosine kinase expressed by hematopoietic stem cells, interstitial cells of cajal (pacemaker cells of the gut) and other cell types. Upon binding of cKIT to stem cell factor (SCF), receptor dimerization initiates a phosphorylation cascade resulting in proliferation, apoptosis, chemotaxis and adhesion. Aberrations of cKIT, including protein overexpression and mutations, occur in a number of human malignancies, including gastrointestinal stromal tumors (GIST), seminoma, acral and mucosal melanomas and mastocytosis. c-Kit is inhibited by multi-targeted agents including imatinib and sunitinib.
CDH1	CDH1 (epithelial cadherin/E-cad) encodes a transmembrane calcium dependent cell adhesion glycoprotein that plays a major role in epithelial architecture, cell adhesion and cell invasion. Loss of function of CDH1 contributes to cancer progression by increasing proliferation, invasion, and/or metastasis. Various somatic mutations in CDH1 have been identified in diffuse gastric, lobular breast, endometrial and ovarian carcinomas; the resultant loss of function of E-cad may contribute to tumor growth and progression. Germline mutations in CDH1 cause hereditary diffuse gastric cancer and colorectal cancer; affected women are predisposed to lobular breast cancer with a risk of about 50%. CDH1 mutation carriers have an estimated cumulative risk of gastric cancer of 67% for men and 83% for women, by age of 80 years.
cMET	cMET is a tyrosine kinase receptor for hepatocyte growth factor (HGF) or scatter factor (SF) and is overexpressed and amplified in a wide range of tumors. cMET overexpression has been associated with a more aggressive biology and a worse prognosis in many human malignancies.

	Amplification of cMET has been implicated in the development of acquired resistance to erlotinib and gefitinib in NSCLC as well as response to cMET inhibitors available via clinical trials.
CSF1R	CSF1R or colony stimulating factor 1 receptor gene encodes a transmembrane tyrosine kinase, a member of the CSF1/PDGF receptor family. CSF1R mediates the cytokine (CSF-1) responsible for macrophage production, differentiation, and function. Although associated with hematologic malignancies, mutations of this gene are associated with cancers of the liver (21%), colon (13%), prostate (3%), endometrium (2%), and ovary (2%). Patients with CSF1R mutations may respond to imatinib. Various clinical trials (on www.clinicaltrials.gov) investigating agents which target this gene and/or its downstream or upstream effectors may be available, which include the following: NCT01346358, NCT01440959. Germline mutations in CSF1R are associated with diffuse leukoencephalopathy, a rapidly progressive neurodegenerative disorder.
CTNNB1	CTNNB1 or cadherin-associated protein, beta 1, encodes for ß-catenin, a central mediator of the Wnt signaling pathway which regulates cell growth, migration, differentiation and apoptosis. Mutations in CTNNB1 (often occurring in exon 3) prevent the breakdown of ß-catenin, which allows the protein to accumulate resulting in persistent transactivation of target genes, including c-myc and cyclin-D1. Somatic CTNNB1 mutations occur in 1-4% of colorectal cancers, 2-3% of melanomas, 25-38% of endometrioid ovarian cancers, 84-87% of sporadic desmoid tumors, as well as the pediatric cancers, hepatoblastoma, medulloblastoma and Wilms' tumors. A growing number of compounds that suppress the Wnt/ß-catenin pathway are available in clinical trials (on www.clinicaltrials.gov) including PRI-724 for advanced solid tumors (NCT01302405) and LGK974 for melanoma and lobular breast cancer (NCT01351103).
EGFR	EGFR (epidermal growth factor receptor) is a receptor tyrosine kinase and its abnormalities contribute to the growth and proliferation of many human cancers. Sensitizing mutations are commonly detected in NSCLC and patients harboring such mutations may respond to EGFR-targeted tyrosine kinase inhibitors including erlotinib, gefitinib and afatinib. Non-small cell lung cancer cancer patients overexpressing EGFR protein are known to respond to the EGFR monoclonal antibody, cetuximab. EGFR amplification may help enroll patients in various clinical trials with EGFR targeted agents.
EGFRvIII	EGFRvIII is a mutated form of EGFR with deletion of exon 2 to 7 on the extracellular ligand-binding domain. This genetic alteration has been found in about 30% of glioblastoma, 30% of head and neck squamous cell cancer, 30% of breast cancer and 15% of NSCLC, and has not been found in normal tissue. EGFRvIII can form homo-dimers or heterodimers with EGFR or ERBB2, resulting in constitutive activation in the absence of ligand binding, activating various downstream signaling pathways including the PI3K and MAPK pathways, leading to increased cell proliferation and motility as well as inhibition of apoptosis. Preliminary studies have shown that EGFRvIII expression may associate with higher sensitivity to erlotinib and gefitinib, as well as to pan-Her inhibitors including neratinib and dacomitinib, however, further studies are warranted to evaluate this association. EGFRvIII peptide vaccine rindopepimut (CDX-110) and monoclonal antibodies specific to EGFRvIII including ABT-806 and AMG595 are being actively investigated in clinical trials.
ER	The estrogen receptor (ER) is a member of the nuclear hormone family of intracellular receptors which is activated by the hormone estrogen. It functions as a DNA binding transcription factor to regulate estrogen-mediated gene expression. Estrogen receptors overexpressing breast cancers are referred to as "ER positive." Estrogen binding to ER on cancer cells leads to cancer cell proliferation. Breast tumors over-expressing ER are treated with hormone-based anti-estrogen therapy. Everolimus combined with exemestane significantly improves survival in ER positive Her2 negative breast cancer patients who are resistant to aromatase inhibitors.
ERBB2	ErbB2/Her2 encodes a member of the epidermal growth factor (EGF) receptor family of receptor tyrosine kinases. Her2 has no ligand-binding domain of its own and, therefore, cannot bind growth factors. It does, however, bind tightly to other ligand-bound EGF receptor family members to form a heterodimer and enhances kinase-mediated activation of downstream signaling pathways leading to cell proliferation. Her2 is overexpressed in 15-30% of newly diagnosed breast cancers. Clinically, Her2 is a target for the monoclonal antibodies trastuzumab, ado-trastuzumab emtansine and pertuzumab which bind to the receptor extracellularly; the kinase inhibitor lapatinib binds and blocks the receptor intracellularly. Other Her2-targeted agents under

	clinical investigation (on www.clinicaltrials.gov) may be available.
ERBB3	ERBB3 encodes for HER3, a member of the epidermal growth factor receptor (EGFR) family. This protein forms heterodimers with other EGF receptor family members which do have kinase activity. Amplification and/or overexpression of ERBB3 have been reported in numerous cancers, including breast cancer, and is a target for drug development.
ERBB4	ERBB4 is a member of the Erbb receptor family known to play a pivotal role in cell-cell signaling and signal transduction regulating cell growth and development. The most commonly affected signaling pathways are the PI3K-Akt and MAP kinase pathways. Erbb4 was found to be somatically mutated in 19% of melanomas and Erbb4 mutations may confer "oncogene addiction" on melanoma cells. Erbb4 mutations have also been observed in various other cancer types, including, gastric carcinomas (2%), colorectal carcinomas (1-3%), non-small cell lung cancer (2–5%) and breast carcinomas (1%), however, their biological impact is not uniform or consistent across these cancers. Based on activity of lapatinib in vitro, there is an active clinical trial (on www.clinicaltrials.gov) investigating lapatinib in stage IV melanoma patients with Erbb4 mutations, which includes NCT01264081.
ERCC1	ERCC1, or excision repair cross-complementation group 1, is a key component of the nucleotide excision repair (NER) pathway. NER is a DNA repair mechanism necessary for the repair of DNA damage from a variety of sources including platinum agents. Tumors with low expression of ERCC1 have impaired NER capacity and may be more sensitive to platinum agents.
EREG	EREG, also known as epiregulin, is a ligand of the epidermal growth factor receptor. Overexpression of EREG in primary colorectal cancer patients has been shown to significantly predict clinical outcome in KRAS wildtype patients treated with cetuximab indicating ligand driven autocrine oncogenic EGFR signaling.
FBXW7	FBXW7 or E3 ligase F-box and WD repeat domain containing 7, also known as Cdc4, encodes three protein isoforms which constitute a component of the ubiquitin-proteasome complex. Mutation of FBXW7 occurs in hotspots and disrupts the recognition of and binding with substrates which inhibits the proper targeting of proteins for degradation (e.g. Cyclin E, c-Myc, SREBP1, c-Jun, Notch-1, mTOR and MCL1). Mutation frequencies identified in cholangiocarcinomas, acute T-lymphoblastic leukemia/lymphoma, and carcinomas of endometrium, colon and stomach are 35%, 31%, 9%, 9%, and 6%, respectively. Targeting an oncoprotein downstream of FBXW7, such as mTOR or c-Myc, may provide a novel therapeutic strategy. Tumor cells with mutated FBXW7 are particularly sensitive to rapamycin treatment, suggesting FBXW7 loss (mutation) may be a predictive biomarker for treatment with inhibitors of the mTOR pathway. In addition, it has been proposed that loss of FBXW7 confers resistance to tubulin-targeting agents like paclitaxel or vinorelbine, by interfering with the degradation of MCL1, a regulator of apoptosis.
FGFR1	FGFR1 or fibroblast growth factor receptor 1, encodes for FGFR1 which is important for cell division, regulation of cell maturation, formation of blood vessels, wound healing and embryonic development. Somatic activating mutations are rare, but have been documented in melanoma, glioblastoma, and lung tumors. Other aberrations of FGFR1 including protein overexpression and gene amplification are common in breast cancer, squamous cell lung cancer, colorectal cancer, and, to a lesser extent in adenocarcinoma of the lung. Recently, it has been shown that osteosarcoma and advanced solid tumors that exhibit FGFR1 amplification are sensitive to the pan-FGFR inhibitor, NVP-BGJ398. Other FGFR1-targeted agents under clinical investigation (on www.clinicaltrials.gov) include dovitinib (NCT01440959). Germline, gain-of-function mutations in FGFR1 result in developmental disorders including Kallmann syndrome and Pfeiffer syndrome.
FGFR2	FGFR2 is a receptor for fibroblast growth factor. Activation of FGFR2 through mutation and amplification has been noted in a number of cancers. Somatic mutations of the fibroblast growth factor receptor 2 (FGFR2) tyrosine kinase are present in endometrial carcinoma, lung squamous cell carcinoma, cervical carcinoma, and melanoma. In the endometrioid histology of endometrial cancer, the frequency of FGFR2 mutation is 16% and the mutation is associated with shorter disease free survival in patients diagnosed with early stage disease. Loss of function FGFR2 mutations occur in about 8% melanomas and contribute to melanoma pathogenesis. Functional polymorphisms in the FGFR2 promoter are associated with breast cancer susceptibility. Various clinical trials (on www.clinicaltrials.gov) investigating agents which target this gene may be

	available, which include the following: NCT01379534. Germline mutations in FGFR2 are associated with numerous medical conditions that include congenital craniofacial malformation disorders, Apert syndrome and the related Pfeiffer and Crouzon syndromes.
FLT3	FLT3 or Fms-like tyrosine kinase 3 receptor is a member of class III receptor tyrosine kinase family, which includes PDGFRA/B and KIT. Signaling through FLT3 ligand-receptor complex regulates hematopoiesis, specifically lymphocyte development. The FLT3 internal tandem duplication (FLT3-ITD) is the most common genetic lesion in acute myeloid leukemia (AML), occurring in 25% of cases. FLT3 mutations are rare in solid tumors; however they have been documented in breast cancer. Several small molecule multikinase inhibitors targeting the RTK-III family are available (on www.clinicaltrials.gov) including phase II trials for crenolanib in AML (NCT01657682), famitinib for nasopharyngeal carcinoma (NCT01462474), dovitinib for GIST (NCT01440959), and phase I trial for PLX108-01 in solid tumors (NCT01004861).
GNA11	GNA11 is a proto-oncogene that belongs to the Gq family of the G alpha family of G protein coupled receptors. Known downstream signaling partners of GNA11 are phospholipase C beta and RhoA and activation of GNA11 induces MAPK activity. Over half of uveal melanoma patients lacking a mutation in GNAQ exhibit somatic mutations in GNA11. Activating mutations of GNA11 have not been found in other malignancies. Various clinical trials (on www.clinicaltrials.gov) investigating agents which target this gene may be available, which include the following: NCT01587352, NCT01390818, NCT01143402.
GNAQ	This gene encodes the Gq alpha subunit of G proteins. G proteins are a family of heterotrimeric proteins coupling seven-transmembrane domain receptors. Oncogenic mutations in GNAQ result in a loss of intrinsic GTPase activity, resulting in a constitutively active Galpha subunit. This results in increased signaling through the MAPK pathway. Somatic mutations in GNAQ have been found in 50% of primary uveal melanoma patients and up to 28% of uveal melanoma metastases. Various clinical trials (on www.clinicaltrials.gov) investigating agents which target this gene may be available, which include the following: NCT01587352, NCT01390818, NCT01143402.
GNAS	GNAS (or GNAS complex locus) encodes a stimulatory G protein alpha-subunit. These guanine nucleotide binding proteins (G proteins) are a family of heterotrimeric proteins which couple seven-transmembrane domain receptors to intracellular cascades. Stimulatory G-protein alpha-subunit transmits hormonal and growth factor signals to effector proteins and is involved in the activation of adenylate cyclases. Mutations of GNAS gene at codons 201 or 227 lead to constitutive cAMP signaling. GNAS somatic mutations have been found in pituitary (28%), pancreatic (20%), ovarian (11%), adrenal gland (6%), and colon (6%) cancers. SNPs in GNAS1 are a predictive marker for tumor response in cisplatin/fluorouracil-based radiochemotherapy in esophageal cancer. Patients with somatic GNAS mutations may derive benefit from MEK inhibitors. Germline mutations of GNAS have been shown to be the cause of McCune-Albright syndrome (MAS), a disorder marked by endocrine, dermatologic, and bone abnormalities. GNAS is usually found as a mosaic mutation in patients. Loss of function mutations are associated with pseudohypoparathyroidism and pseudopseudohypoparathyroidism.
HNF1A	HNF1A or hepatocyte nuclear factor 1 homeobox A encodes a transcription factor that is highly expressed in the liver, found on chromosome 12. It regulates a large number of genes, including those for albumin, alpha1-antitrypsin, and fibrinogen. HNF1A has been associated with an increased risk of pancreatic cancer. HNF1A somatic mutations are found in liver (30%), colon (15%), endometrium (11%), and ovarian (3%) cancers. Its prognostic and predictive value is under investigation. Germline mutations of HNF1A are associated with maturity-onset diabetes of the young type 3.
HRAS	HRAS (homologous to the oncogene of the Harvey rat sarcoma virus), together with KRAS and NRAS, belong to the superfamily of RAS GTPase. RAS protein activates RAS-MEK-ERK/MAPK kinase cascade and controls intracellular signaling pathways involved in fundamental cellular processes such as proliferation, differentiation, and apoptosis. Mutant Ras proteins are persistently GTP-bound and active, causing severe dysregulation of the effector

	signaling. HRAS mutations have been identified in cancers from the urinary tract (10%-40%), skin (6%) and thyroid (4%) and they account for 3% of all RAS mutations identified in cancer. RAS mutations (especially HRAS mutations) occur (5%) in cutaneous squamous cell carcinomas and keratoacanthomas that develop in patients treated with BRAF inhibitor vemurafenib, likely due to the paradoxical activation of the MAPK pathway. Various clinical trials (on www.clinicaltrials.gov) investigating agents which target this gene and/or its downstream or upstream effectors may be available, which include the following: NCT01306045. Germline mutation in HRAS has been associated with Costello syndrome, a genetic disorder that is characterized by delayed development and mental retardation and distinctive facial features and heart abnormalities.
IDH1	IDH1 encodes for isocitrate dehydrogenase in cytoplasm and is found to be mutated in 60-90% of secondary gliomas, 75% of cartilaginous tumors, 17% of thyroid tumors, 15% of cholangiocarcinoma, 12-18% of patients with acute myeloid leukemia, 5% of primary gliomas, 3% of prostate cancer, as well as in less than 2% in paragangliomas, colorectal cancer and melanoma. Mutated IDH1 results in impaired catalytic function of the enzyme, thus altering normal physiology of cellular respiration and metabolism. IDH1 mutation can also cause overproduction of onco-metabolite 2-hydroxy-glutarate, which can extensively alter the methylation profile in cancer. In gliomas, IDH1 mutations are associated with lower-grade astrocytomas and oligodendrogliomas (grade II/III), as well as secondary glioblastoma. IDH gene mutations are associated with markedly better survival in patients diagnosed with malignant astrocytoma; and clinical data support a more aggressive surgery for IDH1 mutated patients because these individuals may be able to achieve long-term survival. In contrast, IDH1 mutation is associated with a worse prognosis in AML. In glioblastoma, IDH1 mutation has been associated with significantly better response to alkylating agent temozolomide. Various clinical trials (on www.clinicaltrials.gov) investigating agents which target this gene and/or its downstream or upstream effectors may be available, which include the following: NCT01534845.
IDH2	IDH2 encodes for the mitochondrial form of isocitrate dehydrogenase, a key enzyme in the citric acid cycle, which is essential for cell respiration. Mutation in IDH2 not only results in impaired catalytic function of the enzyme, but also causes the overproduction of an onco-metabolite, 2-hydroxy-glutarate, which can extensively alter the methylation profile in cancer. IDH2 mutation is mutually exclusive of IDH1 mutation, and has been found in 2% of gliomas and 10% of AML, as well as in cartilaginous tumors and cholangiocarcinoma. In gliomas, IDH2 mutations are associated with lower grade astrocytomas, oligodendrogliomas (grade II/III), as well as secondary glioblastoma (transformed from a lower grade glioma), and are associated with a better prognosis. In secondary glioblastoma, preliminary evidence suggests that IDH2 mutation may associate with a better response to alkylating agent temozolomide, however, further studies are warranted to evaluate this association. IDH mutations have also been suggested to associate with a benefit from using hypomethylating agents in cancers including AML. Various clinical trials (on www.clinicaltrials.gov) investigating agents which target this gene and/or its downstream or upstream effectors may be available. Germline IDH2 mutation has been indicated to associate with a rare inherited neurometabolic disorder D-2-hydroxyglutaric aciduria.
JAK2	JAK2 or Janus kinase 2 is a part of the JAK/STAT pathway which mediates multiple cellular responses to cytokines and growth factors including proliferation and cell survival. It is also essential for numerous developmental and homeostatic processes, including hematopoiesis and immune cell development. Mutations in the JAK2 kinase domain result in constitutive activation of the kinase and the development of chronic myeloproliferative neoplasms such as polycythemia vera (95%), essential thrombocythemia (50%) and myelofibrosis (50%). JAK2 mutations were also found in BCR-ABL1-negative acute lymphoblastic leukemia patients and the mutated patients show a poor outcome. Various clinical trials (on www.clinicaltrials.gov) investigating agents which target this gene and/or its downstream or upstream effectors may be available for patients carrying JAK2 mutation, which include the following: NCT01038856. Germline mutations in JAK2 have been associated with myeloproliferative neoplasms and thrombocythemia.
JAK3	JAK3 or Janus activated kinase 3 is an intracellular tyrosine kinase involved in cytokine

	signaling, while interacting with members of the STAT family. Like JAK1, JAK2, and TYK2, JAK3 is a member of the JAK family of kinases. When activated, kinase enzymes phosphorylate one or more signal transducer and activator of transcription (STAT) factors, which translocate to the cell nucleus and regulate the expression of genes associated with survival and proliferation. JAK3 signaling is related to T cell development and proliferation. This biomarker is found in malignancies like head and neck (21%) colon (7%), prostate (5%), ovary (4%), breast (2%), lung (1%), and stomach (1%) cancer. Various clinical trials (on www.clinicaltrials.gov) investigating agents which target this gene and/or its downstream or upstream effectors may be available, which include the following: NCT01590459. Germline mutations of JAK3 are associated with severe, combined immunodeficiency disease (SCID).
KDR	KDR (VEGFR2) or Kinase insert domain receptor gene, also known as vascular endothelial growth factor receptor-2 (VEGFR2), is involved with angiogenesis and is expressed on almost all endothelial cells. VEGF ligands bind to KDR, which leads to receptor dimerization and signal transduction. Besides somatic mutations in angiosarcoma (10%), somatic KDR mutations have also been found in colon (13%), skin (13%), gastric (5%), lung (3%), renal (2%), and ovarian (2%) cancers. Several VEGFR antagonists are either FDA-approved or in clinical trials (i.e. bevacizumab, regorafenib, pazopanib, and vandetanib). Various clinical trials (on www.clinicaltrials.gov) investigating agents which target this gene and/or its downstream or upstream effectors may be available, which include the following: NCT01068587 and NCT01283945.
KRAS	Proto-oncogene of the Kirsten murine sarcoma virus (KRAS) is a signaling intermediate involved in many signaling cascades including the EGFR pathway. Mutations at activating hotspots are associated with resistance to EGFR tyrosine kinase inhibitors (erlotinib, gefitinib) in NSCLC and monoclonal antibodies (cetuximab, panitumumab) in CRC patients. Patients with KRAS G13D mutation have been shown to derive benefit from anti-EGFR monoclonal antibody therapy in CRC patients. Other targeted agents under clinical investigation (on www.clinicaltrials.gov) may be available for KRAS mutated patients.
MGMT	O-6-methylguanine-DNA methyltransferase (MGMT) encodes a DNA repair enzyme. MGMT expression is mainly regulated at the epigenetic level through CpG island promoter methylation which in turn causes functional silencing of the gene. MGMT methylation and/or low expression has been correlated with response to alkylating agents like temozolomide and dacarbazine.
MLH1	MLH1 - protein involved in mismatch repair system. MLH1 heterodimerizes with PMS2 to form the hMutL± complex which mediates excision of error-bearing strand and its resynthesis. Loss of protein expression is associated with deficiency in mismatch repair and results in microsatellite instability.
MPL	MPL or myeloproliferative leukemia gene encodes the thrombopoietin receptor, which is the main humoral regulator of thrombopoiesis in humans. MPL mutations cause constitutive activation of JAK-STAT signaling and have been detected in 5-7% of patients with primary myelofibrosis (PMF) and 1% of those with essential thrombocythemia (ET).
MSH2	MSH2 - protein involved in mismatch repair system. MSH2 heterodimerizes with MSH3 to form the hMutSß complex which functions to recognize large mismatches. Loss of protein expression is associated with deficiency is mismatch repair and results in microsatellite instability.
MSH6	MSH6 - protein involved in mismatch repair system. MSH6 heterodimerizes with MSH2 to form the hMutSa complex which functions to recognize single base insertion deletion loops/ small mismatches. Loss of protein expression is associated with deficiency in mismatch repair and results in microsatellite instability.
MSI	Microsatellites are short, tandem repeated DNA sequences from 1-6 base pairs in length. These repeats are distributed throughout the genome and often vary in length from one individual to another due to differences in the number of tandem repeats at each locus. They can be used to detect a form of genomic instability called microsatellite instability. MSI is a change in length of a microsatellite allele due to insertion or deletion of repeat units during DNA replication and failure of the DNA mis-match repair system to correct these errors. Therefore, the presence of MSI is indicative of a loss of mismatch repair (MMR) activity.
NOTCH1	NOTCH1 or notch homolog 1, translocation-associated, encodes a member of the Notch signaling network, an evolutionary conserved pathway that regulates developmental processes by regulating

	interactions between physically adjacent cells. Notch signaling modulates interplay between tumor cells, stromal matrix, endothelial cells and immune cells. Mutations in NOTCH1 play a central role in disruption of micro environmental communication, potentially leading to cancer progression. Due to the dual, bi-directional signaling of NOTCH1, activating mutations have been found in acute lymphoblastic leukemia and chronic lymphocytic leukemia, however loss of function mutations in NOTCH1 are prevalent in 11-15% of head and neck squamous cell carcinoma. NOTCH1 mutations have also been found in 2% of glioblastomas, 1% of ovarian cancers, 10% lung adenocarcinomas, 8% of squamous cell lung cancers and 5% of breast cancers. Notch pathway-directed therapy approaches differ depending on whether the tumor harbors gain or loss of function mutations, thus are classified as Notch pathway inhibitors or activators, respectively. Some Notch pathway modulators are being investigated (on www.clinicaltrials.gov) in phase I/II clinical trials, including MK0752 for advanced solid tumors (NCT01295632) and panobinostat (LBH589) for various refractory hematologic malignancies and many types of solid tumors including thyroid cancer (NCT01013597) and melanoma (NCT01065467).
NPM1	NPM1 or nucleophosmin is a nucleolar phosphoprotein belonging to a family of nuclear chaperones with proliferative and growth-suppressive roles. In several hematological malignancies, the NPM locus is lost or translocated, leading to expression of oncogenic proteins. NPM1 is mutated in one-third of patients with adult acute myeloid leukemia (AML) and leads to aberrant localization in the cytoplasm leading to activation of downstream pathways including JAK/STAT, RAS/ERK, and PI3K, leading to cell proliferation, survival and cytoskeletal rearrangements. In addition, the most common translocation in anaplastic large cell lymphoma (ALCL) is the NPM-ALK translocation which leads to expression of an oncogenic fusion protein with constitutive kinase activity. Although there are few NPM-directed therapies currently being investigated, research shows AML tumor cells with mutant NPM are more sensitive to chemotherapeutic agents, including daunorubicin and camptothecin. Further, ALK-targeted therapies like crizotinib are under clinical investigation (on www.clinicaltrials.gov) for ALK-NPM positive ALCL (NCT00939770).
NRAS	NRAS is an oncogene and a member of the (GTPase) ras family, which includes KRAS and HRAS. This biomarker has been detected in multiple cancers including melanoma, colorectal cancer, AML and bladder cancer. Evidence suggests that an acquired mutation in NRAS may be associated with resistance to vemurafenib in melanoma patients. In colorectal cancer patients NRAS mutation is associated with resistance to EGFR-targeted monoclonal antibodies.
PD-1	PD-1 - or programmed death 1 is a co-inhibitory receptor expressed on activated T, B and NK cells, and tumor infiltrating lymphocytes (TIL). PD-1 is a negative regulator of the immune system and inhibits the proliferation and effector function of the lymphocytes after binding with its ligands including PD-L1. PD-1/PD-L1 signaling pathway functions to attenuate or escape antitumor immunity by maintaining an immunosuppressive tumor microenvironment. Studies show that the presence of PD-1+ TIL is associated with a poor prognosis in various cancer types including lymphoma and breast cancer. Evidence suggests HER2 positive breast cancer patients with high levels of PD-1 respond well to trastuzumab. Anti PD-1 therapies may enhance endogenous antitumor immunity and is under investigation in multiple cancer types.
PD-L1	PD-L1 - or programmed cell death ligand 1, is a glycoprotein expressed in various tumor types and is associated with poor outcome. Upon binding to its receptor, PD-1, the PD-1/PD-L1 interaction functions to negatively regulate the immune system, attenuating antitumor immunity by maintaining an immunosuppressive tumor microenvironment. PD-L1 expression is upregulated in tumor cells through activation of common oncogenic pathways or exposure to inflammatory cytokines. Assessment of PD-L1 offers information on patient prognosis and also represents a target for immune manipulation in treatment of solid tumors. Clinical trials are currently recruiting patients with various tumor types testing immunomodulatory agents.
PDGFRA	PDGFRA is the alpha-type platelet-derived growth factor receptor, a surface tyrosine kinase receptor structurally homologous to c-KIT, which activates PIK3CA/AKT, RAS/MAPK and JAK/STAT signaling pathways. PDGFRA mutations are found in 5-8% of patients with gastrointestinal stromal tumors (GIST) and increases to 30% in KIT wildtype GIST. PDGFRA mutations in exons 12, 14 and 18 confer imatinib sensitivity, while the substitution mutation in exon 18 (D842V) shows resistance to imatinib. A novel PDGFRA mutation in the extracellular domain was shown to identify a subgroup of DIPG (diffuse intrinsic pontine glioma) patients with significantly worse outcome. Various clinical trials (on www.clinicaltrials.gov) investigating

	multikinase inhibitors which include PDGFRA as one of the targets for GIST, including dovitinib (NCT01478373), crenolanib for D842V-mutated GIST (NCT01243346) and pazopanib (NCT01524848). Germline mutations in PDGFRA have been associated with Familial gastrointestinal stromal tumors and Hypereosinophillic Syndrome (HES).
PGP	P-glycoprotein (MDR1, ABCB1) is an ATP-dependent, transmembrane drug efflux pump with broad substrate specificity, which pumps antitumor drugs out of cells. Its expression is often induced by chemotherapy drugs and is thought to be a major mechanism of chemotherapy resistance. Overexpression of p-gp is associated with resistance to anthracylines (doxorubicin, epirubicin). P-gp remains the most important and dominant representative of Multi-Drug Resistance phenotype and is correlated with disease state and resistant phenotype.
PIK3CA	The hot spot missense mutations in the gene PIK3CA are present in various malignancies including breast, colon and NSCLC resulting in activation of the PI3 kinase pathway. This pathway is an active target for drug development. PIK3CA exon 20 mutations have been associated with benefit from mTOR inhibitors (everolimus, temsirolimus). Evidence suggests that breast cancer patients with activation of the PI3K pathway due to PTEN loss or PIK3CA mutation/amplification have a significantly shorter survival following trastuzumab treatment. PIK3CA mutation causes reduced response to EGFR targeted therapies in colorectal cancer and NSCLC patients. Various clinical trials (on www.clinicaltrials.gov) investigating agents which target this gene may be available for PIK3CA mutated patients.
PMS2	PMS2 - protein involved in mismatch repair system. PMS2 heterodimerizes with MLH1 to form the hMutLa complex which mediates excision of error-bearing strand and its resynthesis. Loss of protein expression is associated with deficiency in mismatch repair and results in microsatellite instability.
PR	The progesterone receptor (PR or PGR) is an intracellular steroid receptor that specifically binds progesterone, an important hormone that fuels breast cancer growth. PR positivity in a tumor indicates that the tumor is more likely to be responsive to hormone therapy by anti-estrogens, aromatase inhibitors and progestogens.
PTEN	PTEN (phosphatase and tensin homolog) is a tumor suppressor gene that prevents cells from proliferating. Loss of PTEN protein is one of the most common occurrences in multiple advanced human cancers. PTEN is an important mediator in signaling downstream of EGFR, and its loss is associated with reduced benefit to trastuzumab in breast cancer and EGFR-targeted therapies in CRC and NSCLC.
PTPN11	PTPN11 or tyrosine-protein phosphatase non-receptor type 11 is a proto-oncogene that encodes a signaling molecule, Shp-2, which regulates various cell functions like mitogenic activation and transcription regulation. PTPN11 gain-of-function somatic mutations have been found to induce hyperactivation of the Akt and MAPK networks. Because of this hyperactivation, Ras effectors, such as Mek and PI3K, are potential targets for novel therapeutics in those with PTPN11 gain-of-function mutations. PTPN11 somatic mutations are found in hematologic and lymphoid malignancies (8%), gastric (2%), colon (2%), ovarian (2%), and soft tissue (2%) cancers. Germline mutations of PTPN11 are associated with Noonan syndrome, which itself is associated with juvenile myelomonocytic leukemia (JMML). PTPN11 is also associated with LEOPARD syndrome, which is associated with neuroblastoma and myeloid leukemia.
RB1	RB1 or retinoblastoma-1 is a tumor suppressor gene whose protein regulates the cell cycle by interacting with various transcription factors, including the E2F family (which controls the expression of genes involved in the transition of cell cycle checkpoints). Besides ocular cancer, RB1 mutations have also been detected in other malignancies, such as ovarian (10%), bladder (41%), prostate (8%), breast (6%), brain (6%), colon (5%), and renal (2%) cancers. RB1 status, along with other mitotic checkpoints, has been associated with the prognosis of GIST patients. Germline mutations of RB1 are associated with the pediatric tumor, retinoblastoma. Inherited retinoblastoma is usually bilateral. Studies indicate patients with a history of retinoblastoma are at increased risk for secondary malignancies.
RET	RET or rearranged during transfection gene, located on chromosome 10, activates cell signaling pathways involved in proliferation and cell survival. RET mutations are found in 23-69% of

	sporadic medullary thyroid cancers (MTC), but RET fusions are common in papillary thyroid cancer, and more recently have been found in 1-2% of lung adenocarcinoma. Amongst RET mutations in sporadic MTC, 85% involve the M918T mutation which is associated with a higher response rate to vandetanib in comparison to M918T negative patients. Further, a 10-year study notes that medullary thyroid cancer patients with somatic RET mutations have a poorer prognosis. Various clinical trials (on www.clinicaltrials.gov) investigating multikinase inhibitors which include RET as one of the targets may be available, including vandetanib for advanced cancers (NCT01582191) or cabozantinib for medullary thyroid cancer (NCT01683110). Germline activating mutations of RET are associated with multiple endocrine neoplasia type 2 (MEN2), which is characterized by the presence of medullary thyroid carcinoma, bilateral pheochromocytoma, and primary hyperparathyroidism. Germline inactivating mutations of RET are associated with Hirschsprung's disease.
ROS1	The proto-oncogene ROS1 is a receptor tyrosine kinase of the insulin receptor family. The ligand and function of ROS1 are unknown. Dimerization of ROS1-fused proteins results in constitutive activation of the receptor kinase, leading to cell proliferation and survival. Chromosomal rearrangements involving the ROS1 gene have been described in glioblastomas, NSCLC and cholangiocarcinoma. Clinical data show that ROS-rearranged NSCLC patients have increased sensitivity and improved response to the MET/ALK/ROS inhibitor, crizotinib.
RRM1	Ribonucleotide reductase subunit M1 (RRM1) is a component of the ribonucleotide reductase holoenzyme consisting of M1 and M2 subunits. The ribonucleotide reductase is a rate-limiting enzyme involved in the production of nucleotides required for DNA synthesis. Gemcitabine is a deoxycitidine analogue which inhibits ribonucleotide reductase activity. High RRM1 level is associated with resistance to gemcitabine.
SMAD4	SMAD4 or mothers against decapentaplegic homolog 4, is one of eight proteins in the SMAD family, involved in multiple signaling pathways and are key modulators of the transcriptional responses to the transforming growth factor-ß (TGFß) receptor kinase complex. SMAD4 resides on chromosome 18q21, one of the most frequently deleted chromosomal regions in colorectal cancer. Smad4 stabilizes Smad DNA-binding complexes and also recruits transcriptional coactivators such as histone acetyltransferases to regulatory elements. Dysregulation of SMAD4 occurs late in tumor development, and occurs through mutations of the MH1 domain which inhibits the DNA-binding function, thus dysregulating TGFßR signaling. Mutated (inactivated) SMAD4 is found in 50% of pancreatic cancers and 10-35% of colorectal cancers. Recent studies have shown that preservation of SMAD4 through retention of the 18q21 region, leads to clinical benefit from 5-fluorouracil-based therapy. In addition, various clinical trials investigating agents which target the TGFßR signaling axis are available (on www.clinicaltrials.gov), including PF-03446962 for advanced solid tumors including NCT00557856. Germline mutations in SMAD4 are associated with juvenile polyposis (JP) and combined syndrome of JP and hereditary hemorrhagic teleangiectasia (JP-HHT).
SMARCB1	SMARCB1 also known as SWI/SNF related, matrix associated, actin dependent regulator of chromatin, subfamily b, member 1, is a tumor suppressor gene implicated in cell growth and development. Loss of expression of SMARCB1 has been observed in tumors including epithelioid sarcoma, renal medullary carcinoma, undifferentiated pediatric sarcomas, and a subset of hepatoblastomas. Germline mutation in SMARCB1 causes about 20% of all rhabdoid tumors which makes it important for clinicians to facilitate genetic testing and refer families for genetic counseling. Germline SMARCB1 mutations have also been identified as the pathogenic cause of a subset of schwannomas and meningiomas.
SMO	SMO (smoothened) is a G protein-coupled receptor which plays an important role in the Hedgehog signaling pathway. It is a key regulator of cell growth and differentiation during development, and is important in epithelial and mesenchymal interaction in many tissues during embryogenesis. Dysregulation of the Hedgehog pathway is found in cancers including basal cell carcinomas (12%) and medulloblastoma (1%). A gain-of-function mutation in SMO results in constitutive activation of hedgehog pathway signaling, contributing to the genesis of basal cell carcinoma. SMO mutations have been associated with the resistance to SMO antagonist GDC-

	0449 in medulloblastoma patients by blocking the binding to SMO. SMO mutation may also contribute partially to resistance to SMO antagonist LDE225 in BCC. Various clinical trials (on www.clinicaltrials.gov) investigating SMO antagonists may be available, which include the following: NCT01529450.
SPARC	SPARC (secreted protein acidic and rich in cysteine) is a calcium-binding matricellular glycoprotein secreted by many types of cells. It has a normal role in wound repair, cell migration, and cell-matrix interactions. Its over-expression is thought to have a role in tumor invasion and angiogenesis. A few studies indicate that SPARC over-expression improves the response to the anti cancer drug, nab-paclitaxel. The improved response is thought to be related to SPARC's role in accumulating albumin and albumin targeted agents within tumor tissue.
STK11	STK11 also known as LKB1, is a serine/threonine kinase. It is thought to be a tumor suppressor gene which acts by interacting with p53 and CDC42. It modulates the activity of AMP-activated protein kinase, causes inhibition of mTOR, regulates cell polarity, inhibits the cell cycle, and activates p53. Somatic mutations in this gene are associated with a history of smoking and KRAS mutation in NSCLC patients. The frequency of STK11 mutation in lung adenocarcinomas ranges from 7%-30%. STK11 loss may play a role in development of metastatic disease in lung cancer patients. Mutations of this gene also drive progression of HPV-induced dysplasia to invasive, cervical cancer and hence STK11 status may be exploited clinically to predict the likelihood of disease recurrence. Various clinical trials (on www.clinicaltrials.gov) investigating agents which target this gene may be available, which include the following: NCT01578551. Germline mutations in STK11 are associated with Peutz-Jeghers syndrome which is characterized by early onset hamartomatous gastro-intestinal polyps and increased risk of breast, colon, gastric and ovarian cancer.
TLE3	TLE3 is a member of the transducin-like enhancer of split (TLE) family of proteins that have been implicated in tumorigenesis. It acts downstream of APC and beta-catenin to repress transcription of a number of oncogenes, which influence growth and microtubule stability. Studies indicate that TLE3 expression is associated with response to taxane therapy.
TOP2A	TOPOIIA is an enzyme that alters the supercoiling of double-stranded DNA and allows chromosomal segregation into daughter cells. Due to its essential role in DNA synthesis and repair, and frequent overexpression in tumors, TOPOIIA is an ideal target for antineoplastic agents. Amplification of TOPOIIA with or without HER2 co-amplification, as well as high protein expression of TOPOIIA, have been associated with benefit from anthracycline based therapy.
TOPO1	Topoisomerase I is an enzyme that alters the supercoiling of double-stranded DNA. TOPOI acts by transiently cutting one strand of the DNA to relax the coil and extend the DNA molecule. High expression of TOPOI has been associated with response to TOPOI inhibitors including irinotecan and topotecan.
TP53	TP53, or p53, plays a central role in modulating response to cellular stress through transcriptional regulation of genes involved in cell-cycle arrest, DNA repair, apoptosis, and senescence. Inactivation of the p53 pathway is essential for the formation of the majority of human tumors. Mutation in p53 (TP53) remains one of the most commonly described genetic events in human neoplasia, estimated to occur in 30-50% of all cancers with the highest mutation rates occurring in head and neck squamous cell carcinoma and colorectal cancer. Generally, presence of a disruptive p53 mutation is associated with a poor prognosis in all types of cancers, and diminished sensitivity to radiation and chemotherapy. In addition, various clinical trials (on www.clinicaltrials.gov) investigating agents which target p53's downstream or upstream effectors may have clinical utility depending on the p53 status. For p53 mutated patients, Chk1 inhibitors in advanced cancer (NCT01115790) and Wee1 inhibitors in ovarian cancer (NCT01164995, NCT01357161) are being investigated. For p53 wildtype patients with sarcoma, mdm2 inhibitors (NCT01605526) are being investigated. Germline p53 mutations are associated with the Li-Fraumeni syndrome (LFS) which may lead to early-onset of several forms of cancer currently known to occur in the syndrome, including sarcomas of the bone and soft tissues, carcinomas of the breast and adrenal cortex (hereditary adrenocortical carcinoma), brain tumors and acute leukemias.
TS	Thymidylate synthase (TS) is an enzyme involved in DNA synthesis that generates thymidine

	monophosphate (dTMP), which is subsequently phosphorylated to thymidine triphosphate for use in DNA synthesis and repair. Low levels of TS are predictive of response to fluoropyrimidines and other folate analogues.
TUBB3	Class III ß-Tubulin (TUBB3) is part of a class of proteins that provide the framework for microtubules, major structural components of the cytoskeleton. Due to their importance in maintaining structural integrity of the cell, microtubules are ideal targets for anti-cancer agents. Low expression of TUBB3 is associated with potential clinical benefit to taxane therapy.
VHL	VHL or von Hippel-Lindau gene encodes for tumor suppressor protein pVHL, which polyubiquitylates hypoxia-inducible factor in an oxygen dependent manner. Absence of pVHL causes stabilization of HIF and expression of its target genes, many of which are important in regulating angiogenesis, cell growth and cell survival. VHL somatic mutation has been seen in 20-70% of patients with sporadic clear cell renal cell carcinoma (ccRCC) and the mutation may imply a poor prognosis, adverse pathological features, and increased tumor grade or lymph-node involvement. Renal cell cancer patients with a 'loss of function' mutation in VHL show a higher response rate to therapy (bevacizumab or sorafenib) than is seen in patients with wild type VHL, however the mutation is not associated with improvement in progression free survival or overall survival. Various clinical trials (on www.clinicaltrials.gov) investigating angiogenesis inhibitors in various cancer types may be available, which include the following: NCT00693992. Germline mutations in VHL cause von Hippel-Lindau syndrome, associated with clear-cell renal-cell carcinomas, central nervous system hemangioblastomas, pheochromocytomas and pancreatic tumors.
VEGFR2	VEGFR2, vascular endothelial growth factor 2, is one of three main subtypes of VEGFR. This protein is an important signaling protein in angiogenesis. Evidence suggests that increased levels of VEGFR2 may be predictive of response to anti-angiogenic drugs.

[00365] **Table 7** presents a view of the information that can be gathered and reported for exemplary MI and MI Plus molecular profiles for a solid tumor. Lineage specific modications can be made as well. See, e.g., **Tables 11-16**. The columns headed "Agent/Biomarker Status Reported" provide either candidate agents (e.g., drugs) or biomarker status to be included in the report. Where agents are indicated, the association of the agent with the indicated biomarker is included in the report. Where a status is indicated (e.g., mutational status, protein expression status, gene copy number status), the biomarker status is indicated in the report instead of drug associations. The candidate agents may comprise those undergoing clinical trials, as indicated. Platform abbreviations are as used throughout the application, e.g., IHC: immunohistochemistry; FISH: fluorescent in situ hybridication; CISH: colorimetric in situ hybridization; NGS: next generation sequencing; PCR: polymerase chain reaction. The candidate agents may comprise those undergoing clinical trials, as indicated. As will be evident to one of skill, the same biomarkers in **Table 7** can be assessed using the indicated methodology for both MI and MI Plus molecular profiling.

[00366] The invention further provides a set of biomarker – treatment association rules, wherein the rules comprise a predicted likelihood of benefit or lack of benefit of a certain treatment for the cancer given an assessment of one or more biomarker. The associations/rules may comprise those presented in **Table 8**. In **Table 8**, the class of drug and illustrative drugs of the indicated class are indicated in the columns "Class of Drugs" and "Drugs," respectively. The columns headed "Biomarker Result" illustrate illustrative methods of profiling the indicated biomarkers, wherein the results are generally true ("T") or false ("F"), "Any," or "No Data." The data can also be labeled "Equivocal," "Equivocal Low," or "Equivocal High," e.g., for IHC where the observed expression level is near or at the threshold set to determine whether a protein is under-expressed, over-expressed, or expressed at normal levels. For mutations, in some cases a

particular mutation (e.g., BRAF V600E or V600K) or region / mutational hotspot is called out (e.g., c-KIT exon11 or exon13). In some cases, a particular mutation is called out from others in the "Biomarker Result." For example, in the case of cKIT, the V654A mutation or mutations in exon 14, exon 17, or exon 18 are called out in the rules for the tyrosine kinase inhibitor ("TKI") imatinib. Similarly, in the case of PDGFRA mutations, the PDGFRA D842V mutation may be called out in the tables apart from other PDGFRA mutations. One of skill will appreciate that alternative methods can be used to analyze the biomarkers as appropriate. For example, sequencing analysis performed by Next Generation methodology could also be performed by Sanger sequencing or other forms of sequence analysis method such as those described herein or known in the art that yield similar biological information (e.g., an expression or mutation status). The biomarker results combine to predict a benefit or lack of benefit from treatment with the indicated candidate drugs. Abbreviations used in **Table 8** include: tyrosine kinase inhibitor ("TKI"); Sequencing ("Seq."); Indeterminate ("Indet."); True ("T"); False ("F").

[00367] As an example in **Table 8**, consider that PIK3CA exon20 is mutated as determined by sequencing (PIK3CA Mutated | exon20 = T), then the mTOR inhibitor agents everolimus and/or temsirolimus are predicted to have treatment benefit (Overall Benefit = T). However, if PIK3CA exon20 mutation is determined to be false ("F") or is not determined ("No Data"), then the overall benefit of the mTOR inhibitors is indeterminate. As another example in **Table 8**, consider that the sample is determined to be ER positive by IHC. In such case, overall benefit from the hormonal agents leuprolide and/or megestrol acetate is expected to be likely (i.e., true or "T"). These results are independent of the status of PR as also determined by IHC. If ER is determined to not be overexpressed (i.e., false "F") or no data is available, and PR is determined to be positive by IHC, then overall benefit from the indicated hormonal agents such as leuprolide and megestrol acetate is also expected to be likely (i.e., true or "T"). If neither ER nor PR are expressed (i.e., ER Positive = false ("F") and PR Positive = false ("F")), then overall benefit from the hormonal agents leuprolide and/or megestrol acetate is expected to be not likely (i.e., false or "F"). The expected overall benefit from the hormonal agents is indeterminate (i.e., "Indet.") in either of the following situations: 1) ER is not expressed or data is unavailable (i.e., ER Positive = "No Data") and data is unavailable for PR (i.e., PR Positive = "No Data"); or 2) data is unavailable for ER (i.e., ER Positive = "No Data") and PR is not expressed (i.e., PR Positive = "F").

[00368] In an embodiment, the invention provides molecular intelligence (MI) profiles that can be used for any lineage of cancer, e.g., for any solid tumor. The MI molecular profiles can be based on assessing the biomarkers using the molecular profiling methods illustrated in **FIGs. 27A-B** or **Table 7**. In an embodiment, the invention provides a molecular intelligence molecular profile for a cancer comprises one or more, e.g., 1, 2, 3, 4, 5, 6, 7, 8, 9, 10, 11, 12, 13, 14, 15, 16, 17, 18, 19, 20, 21, 22, 23, 24, 25, 26, 27, 28, 29, 30, 31, 32, 33, 34, 35, 36, 37, 38, 39, 40, 41, 42, 43, 44, 45, 46, 47, 48, 49, 50, 51, 52, 53, 54, 55, 56 or 57, of: 1p19q, ABL1, AKT1, ALK, APC, AR, ATM, BRAF, BRCA1, BRCA2, cKIT, cMET, CSF1R, CTNNB1, EGFR, EGFRvIII, ER, ERBB2 (HER2), FGFR1, FGFR2, FLT3, GNA11, GNAQ, GNAS, HER2, HRAS, IDH1, IDH2, JAK2, KDR (VEGFR2), KRAS, MGMT, MGMT-Me, MLH1, MPL, NOTCH1, NRAS, PD-1, PDGFRA, PD-L1, Pgp, PIK3CA, PR, PTEN, RET, RRM1, SMO,

SPARC, TLE3, TOP2A, TOPO1, TP53, TS, TUBB3, VHL. The biomarker can be any single or group of biomarkers in **Table 6**. The invention further provides a method of selecting a candidate treatment for a cancer comprising assessment of one or more members of the cancer molecular profile using one or more molecular profiling technique presented herein, e.g., ISH (e.g., FISH, CISH), IHC, RT-PCR, expression array, mutation analysis (e.g., NextGen sequencing, Sanger sequencing, pyrosequencing, Fragment analysis (FA, e.g., RFLP), PCR), etc. In one embodiment, ISH is used to assess one or more, e.g., 1, 2 or 3 of: HER2, 1p19q and cMET. Any useful ISH technique can be used. For example, FISH can be used to assess cMET, 1p19q and/or HER2; or CISH can be used to assess cMET, 1p19q and/or HER2. In an embodiment, protein analysis such as IHC is used to assess one or more, e.g., 1, 2, 3, 4, 5, 6, 7, 8, 9, 10, 11, 12, 13, 14, 15, 16, 17, 18 or 19 of: AR, cMET, EGFR, ER, HER2, MGMT, PD-1, PD-L1, Pgp, PR, PTEN, RRM1, SPARC, TLE3, TOP2A, TOPO1, TS, TUBB3. In some embodiments, SPARC is assessed with IHC performed with both monoclonal ("m") or polyclonal ("p") primary antibodies. In some embodiments, sequence analysis is used to assess one or more, e.g., 1, 2, 3, 4, 5, 6, 7, 8, 9, 10, 11, 12, 13, 14, 15, 16, 17, 18, 19, 20, 21, 22, 23, 24, 25, 26, 27, 28, 29, 30, 31, 32, 33, 34, 35, 36, 37, 38, 39, 40, 41, 42, 43, 44, 45, 46 or 47 of: ABL1, AKT1, ALK, APC, ATM, BRAF, BRCA1, BRCA2, CDH1, cKIT, cMET, CSF1R, CTNNB1, EGFR, ERBB2, ERBB4, FBXW7, FGFR1, FGFR2, FLT3, GNA11, GNAQ, GNAS, HNF1A, HRAS, IDH1, JAK2, JAK3, KDR (VEGFR2), KRAS, MLH1, MPL, NOTCH1, NPM1, NRAS, PDGFRA, PIK3CA, PTEN, PTPN11, RB1, RET, SMAD4, SMARCB1, SMO, STK11, TP53, VHL. For example, the sequence analysis can be performed on one or more, e.g., 1, 2, 3, 4, 5, 6, 7, 8, 9, 10, 11, 12, 13 or 14 of ABL1, APC, BRAF, EGFR, FLT3, GNAQ, IDH1, JAK2, cKIT, KRAS, MPL, NRAS, PDGFRA, VHL. The sequence analysis can also be performed on one or more, e.g., 1, 2, 3, 4, 5, 6, 7, 8, 9, 10, 11, 12, 13, 14 or 15 of ABL1, APC, BRAF, EGFR, FLT3, GNAQ, IDH1, JAK2, cKIT, KRAS, MPL, NPM1, NRAS, PDGFRA, VHL. The sequencing may be performed using Next Generation sequencing technology or other technologies as described herein. For example, methylation of the MGMT promoter region can be assessed using pyrosequencing. The molecular profile can be based on assessing the biomarkers as illustrated in **FIGs. 27A-B** or **Table 7**.

[00369] In an embodiment, the invention provides a molecular intelligence molecular profile for a cancer comprising analysis of the biomarkers in **FIG. 27A**, which may be assessed as indicated in the paragraph above and/or as in **FIG. 27A** or **Table 7**. For example, the MI profile for a cancer such as a solid tumor may comprise: 1) ISH to assess one or more, e.g., 1, 2 or 3 of: HER2, 1p19q and cMET; 2) IHC to assess one or more, e.g., ., 1, 2, 3, 4, 5, 6, 7, 8, 9, 10, 11, 12, 13, 14, 15, 16, 17, 18 or 19 of: AR, cMET, EGFR, ER, HER2, MGMT, PD-1, PD-L1, Pgp, PR, PTEN, RRM1, SPARC, TLE3, TOP2A, TOPO1, TS, TUBB3; and/or 3) sequence analysis to assess one or more, e.g., 1, 2, 3, 4, 5, 6, 7, 8, 9, 10, 11, 12, 13, 14, 15, 16, 17, 18, 19, 20, 21, 22, 23, 24, 25, 26, 27, 28, 29, 30, 31, 32, 33, 34, 35 or 36 of: ABL1, AKT1, ALK, APC, ATM, BRAF, BRCA1, BRCA2, cKIT, cMET, CSF1R, CTNNB1, EGFR, ERBB2, FGFR1, FGFR2, FLT3, GNA11, GNAQ, GNAS, HRAS, IDH1, JAK2, KDR (VEGFR2), KRAS, MLH1, MPL, NOTCH1, NRAS, PDGFRA, PIK3CA, PTEN, RET, SMO, TP53, VHL. In another embodiment, the invention provides a molecular intelligence (MI) PLUS profile for a cancer comprising analysis of the

biomarkers in the molecular intelligence (MI) profile and the additional biomarker in **FIG. 27B**, i.e., 1, 2, 3, 4, 5, 6, 7, 8, 9, 10 or 11 of CDH1, ERBB4, FBXW7, HNF1A, JAK3, NPM1, PTPN11, RB1, SMAD4, SMARCB1 and STK11, which may be assessed as indicated this paragraph and/or as in **FIG. 27B** or **Table 7** below. The invention further provides a report comprising results of the molecular profiling and corresponding candidate treatments that are identified as likely beneficial or likely not beneficial, as further described herein.

[00370] Table 7 below presents a view of the information that is reported for a molecular intelligence molecular profile for a cancer, while the biomarker – treatment associations for the molecular profile for the cancer may comprise those associations in **Table 8**, which are generally interpreted as described above.

Table 7 – Molecular Profile and Report Parameters: Solid Tumor

Agents/Biomarker Status Reported	Biomarker	Platform
docetaxel, paclitaxel	TLE3	IHC
	TUBB3	IHC
	Pgp	IHC
nab-paclitaxel	SPARCm	IHC
	SPARCp	IHC
capecitabine, fluorouracil, pemetrexed	TS	IHC
doxorubicin, liposomal-doxorubicin, epirubicin	HER2	ISH
	TOP2A	IHC
	Pgp	IHC
irinotecan, topotecan	TOPO1	IHC
gemcitabine	RRM1	IHC
temozolomide, dacarbazine	MGMT	IHC[&&]
	MGMT-Me	Pyrosequencing[&]
	IDH1*	NGS
procarbazine, lomustine, vincristine	1p19q	ISH[&]
Hormone therapies†	ER	IHC
	PR	IHC
	AR	IHC
trastuzumab, lapatinib, pertuzumab, T-DM1, clinical trials	HER2	IHC, FISH/CISH
imatinib	cKIT	NGS
	PDGFRA	NGS
sunitinib *(GIST only)*	cKIT	NGS
everolimus, temsirolimus, clinical trials	PIK3CA	NGS
vandetanib	RET	NGS
vemurafenib, dabrafenib	BRAF	NGS
carboplatin, cisplatin, oxaliplatin	BRCA1	NGS
carboplatin, cisplatin, oxaliplatin	BRCA2	NGS
mutational status	EGFRvIII	Fragment Analysis (FA)[&]
Clinical Trials	IDH2	Sanger Sequencing[&]

Clinical Trials	PD-1	IHC
Clinical Trials	PD-L1	IHC
Clinical Trials	PTEN	IHC
Clinical Trials	cMET	IHC, ISH
Clinical Trials	ABL1	NGS
Clinical Trials	AKT1	NGS
Clinical Trials	ALK	NGS
Clinical Trials	APC	NGS
Clinical Trials	ATM	NGS
Clinical Trials	cMET	NGS
Clinical Trials	CSF1R	NGS
Clinical Trials	CTNNB1	NGS
Clinical Trials	EGFR	IHC, NGS
Clinical Trials	ERBB2 (HER2)	NGS
Clinical Trials	FGFR1	NGS
Clinical Trials	FGFR2	NGS
Clinical Trials	FLT3	NGS
Clinical Trials	GNA11	NGS
Clinical Trials	GNAQ	NGS
Clinical Trials	GNAS	NGS
Clinical Trials	HRAS	NGS
Clinical Trials	JAK2	NGS
Clinical Trials	KDR (VEGFR2)	NGS
Clinical Trials	KRAS	NGS
Clinical Trials	MLH1	NGS
Clinical Trials	MPL	NGS
Clinical Trials	NOTCH1	NGS
Clinical Trials	NRAS	NGS
Clinical Trials	PTEN	NGS
Clinical Trials	SMO	NGS
Clinical Trials	TP53	NGS
Clinical Trials	VHL	NGS

[00371] **IDH1 associate with temozolomide, dacarbazine in High Grade Glioma lineage.*

[00372] *† Hormone therapies may include: tamoxifen, toremifene, fulvestrant, letrozole, anastrozole, exemestane, megestrol acetate, leuprolide, goserelin, bicalutamide, flutamide, abiraterone, enzalutamide, triptorelin, abarelix, degarelix*

[00373] *† In prostate cancer, AR is associated with bicalutamide, flutamide, abiraterone, enzalutamide, goserelin, leuprolide, triptorelin, abarelix, and degarelix. In this same tumor type, ER and PR will be associated with protein expression only. The agents tamoxifen, toremifene, fulvestrant, letrozole, anastrozole, and exemestane, and megestrol acetate, then, may not be provided as recommendations for prostate cases.*

[00374] *& May only be recommended for glioma.*

[00375] *&& Only recommended for other than glioma.*

[00376] In addition to the columns in the tables above, **Table 8** provides a predicted benefit level and an evidence level, and list of references for each biomarker-drug association rule in the table. The benefit level is ranked from 1-5, wherein the levels indicate the predicted strength of the biomarker-drug association based on the indicated evidence. All relevant published studies were evaluated using the U.S. Preventive Services Task Force ("USPSTF") grading scheme for study design and validity. See, e.g., www.uspreventiveservicestaskforce.org/uspstf/grades.htm. The benefit level in the table ("Bene. Level") corresponds to the following:

[00377] 1: Expected benefit.

[00378] 2: Expected reduced benefit.

[00379] 3: Expected lack of benefit.

[00380] 4: No data is available.

[00381] 5: Data is available but no expected benefit or lack of benefit reported because the biomarker in this case is the not principal driver of that specific rule.

[00382] The evidence level in the table ("Evid. Level") corresponds to the following:

[00383] 1: Very high level of evidence. For example, the treatment comprises the standard of care.

[00384] 2: High level of evidence but perhaps insufficient to be considered for standard of care.

[00385] 3: Weaker evidence – fewer publications or clinical studies, or perhaps some controversial evidence.

[00386] Abbreviations used in **Table 8** include: Bene. (Benefit); Evid. (Evidence); Indet. (Indeterminate); Equiv. (Equivocal); Seq. (Sequencing). In the column "Drugs," under the section for Taxanes, the following abbreviations are used: PDN (paclitaxel, docetaxel, nab-paclitaxel) and N (nab-paclitaxel).

[00387] The column "Partial Report Overall Benefit" in **Table 8** is to make drug association in a preliminary molecular profiling report when all the biomarker assessment results may not be ready. For example, a preliminary report may be produced when requested by the treating physician. Interpretation of benefit of lack of benefit of the various drugs is more cautious in these scenarios to avoid potential change in drug association from benefit or lack of benefit or vice versa between the preliminary report and a final report that is produced when all biomarker results become available. Hence you will see some indeterminate scenarios.

Table 8 – Solid Tumor Drug – Biomarker Associations

Class of Drugs	Drugs	Biomarker Result	Bene. Level	Evid. Level	Ref. No.	Biomarker Result	Bene. Level	Evid. Level	Ref. No.	Biomarker Result	Bene. Level	Evid. Level	Ref. No.	Biomarker Result	Bene. Level	Evid. Level	Ref. No.	Biomarker Result	Bene. Level	Evid. Level	Ref. No.	Overall Bene.	Partial Report Overall Bene.
Antimetabolites	**gemcitabine**	**RRM1 Negative (IHC)**	**Bene. Level**	**Evid. Level**	**1**																	**Overall Bene.**	**Partial Report Overall Bene.**
		T	1	2																		T	T
		F	3	2																		F	F
		No Data	4																			Indet.	Indet.
Antimetabolites	**fluorouracil, capecitabine, pemetrexed**	**TS Negative (IHC)**	**Bene. Level**	**Evid. Level**	**2**																	**Overall Bene.**	**Partial Report Overall Bene.**
		T	1	2																		T	T
		F	3	2																		F	F
		No Data	4																			Indet.	Indet.
Topo1 inhibitors	**irinotecan, topotecan**	**TOPO1 Positive (IHC)**	**Bene. Level**	**Evid. Level**	**3**																	**Overall Bene.**	**Partial Report Overall Bene.**
		T	1	2																		T	T
		F	3	2																		F	F
		No Data	4																			Indet.	Indet.
Alkylating agents	**temozolomide, dacarbazine**	**MGMT Negative (IHC)**	**Bene. Level**	**Evid. Level**	**4**																	**Overall Bene.**	**Partial Report Overall Bene.**
		T	1	2																		T	T
		F	3	2																		F	F
		No Data	4																			Indet.	Indet.
Anti-androgens	**bicalutamide, flutamide, abiraterone, enzalutamide**	**AR Positive (IHC)**	**Bene. Level**	**Evid. Level**	**5**																	**Overall Bene.**	**Partial Report Overall Bene.**
		T	1	2																		T	T
		F	3	2																		F	F

		No Data	4																		Indet.	Indet.
Hormonal Agents	**tamoxifen, toremifene, fulvestrant, letrozole, anastrozole, exemestane, megestrol acetate**	**ER Positive (IHC)**	**Bene. Level**	**Evid. Level**	**6**	**PR Positive (IHC)**	**Bene. Level**	**Evid. Level**	**7**												**Overall Bene.**	**Partial Report Overall Bene.**
		T	1	1		T	1	1													T	T
		T	1	1		F	2	1													T	T
		T	1	1		No Data	4														T	T
		F	2	1		T	1	1													T	T
		F	3	1		F	3	1													F	F
		F	3	1		No Data	4														Indet.	Indet.
		No Data	4			T	1	1													T	T
		No Data	4			F	3	1													Indet.	Indet.
		No Data	4			No Data	4														Indet.	Indet.
TKI	**lapatinib**	**HER2 Positive (IHC)**	**Bene. Level**	**Evid. Level**	**8**	**HER2 Amplified (ISH)**	**Bene. Level**	**Evid. Level**	**9**												**Overall Bene.**	**Pending Report Overall Bene.**
		T	1	1		T	1	1													T	T
		T	1	1		F	5														T	T
		T	1	1		Equiv. High	1	1													T	T
		T	1	1		Equiv. Low	5														T	T
		T	1	1		No Data	4														T	T
		F	5			T	1	1													T	T
		F	3	1		F	3	1													F	F
		F	5			Equiv. High	1	1													T	T
		F	3	1		Equiv. Low	3	1													F	F
		F	3	1		No Data	4														Indet.	Indet.
		Equiv.	5			T	1	1													T	T
		Equiv.	5			F	3	1													F	F
		Equiv.	5			Equiv. High	1	1													T	T
		Equiv.	5			Equiv. Low	3	1													F	F

		Equiv.	5			No Data	4															Indet.	Indet.
		No Data	4			T	1	1														T	T
		No Data	4			F	3	1														Indet.	Indet.
		No Data	4			Equiv. High	1	1														T	T
		No Data	4			Equiv. Low	3	1														Indet.	Indet.
		No Data	4			No Data	4															Indet.	Indet.
Monoclonal antibodies (Her2-Targeted)	**trastuzumab, pertuzumab, ado-trastuzumab emtansine (T-DM1)**	**HER2 Positive (IHC)**	**Bene. Level**	**Evid. Level**	**10**	**HER2 Amplified (ISH)**	**Bene. Level**	**Evid. Level**	**11**													**Overall Bene.**	**Partial Report Overall Bene.**
		T	1	1		T	1	1														T	T
		T	1	1		F	5															T	T
		T	1	1		Equiv. low	5															T	T
		T	1	1		Equiv. high	1	1														T	T
		T	1	1		No Data	4															T	T
		F	5			T	1	1														T	T
		F	3	1		F	3	1														F	F
		F	3	1		Equiv. low	3	1														F	F
		F	5			Equiv. high	1	1														T	T
		F	3	1		No Data	4															Indet.	Indet.
		Equiv.	5			T	1	1														T	T
		Equiv.	5			F	3	1														F	F
		Equiv.	5			Equiv. low	3	1														F	F
		Equiv.	5			Equiv. high	1	1														T	T
		Equiv.	5			No Data	4															Indet.	Indet.
		No Data	4			T	1	1														T	T
		No Data	4			F	3	1														Indet.	Indet.
		No Data	4			Equiv. low	3	1														Indet.	Indet.
		No Data	4			Equiv. high	1	1														T	T
		No Data	4			No Data	4															Indet.	Indet.
Anthracyclines and related substances	**doxorubicin, liposomal-doxorubicin, epirubicin**	**TOP2A Amplified (ISH)**	**Bene. Level**	**Evid. Level**	**12**	**Her2 Amplified (ISH)**	**Bene. Level**	**Evid. Level**	**13**	**TOP2A Positive (IHC)**	**Bene. Level**	**Evid. Level**	**14**	**PGP Positive (IHC)**	**Bene. Level**	**Evid. Level**	**15**					**Overall Bene.**	**Partial Report Overall Bene.**
		T	1	1		T	1	1		T	1	2		T	2	2						T	T
		T	1	1		T	1	1		T	1	2		F	1	2						T	T
		T	1	1		T	1	1		T	1	2		No Data	4							T	T
		T	1	1		T	1	1		F	2	2		T	2	2						T	T

		T	1	1		T	1	1		F	2	2		F	1	2						T	T
		T	1	1		T	1	1		F	2	2		No Data	4							T	T
		T	1	1		T	1	1		No Data	4			T	2	2						T	T
		T	1	1		T	1	1		No Data	4			F	1	2						T	T
		T	1	1		T	1	1		No Data	4			No Data	4							T	T
		T	1	1		F	2	2		T	1	2		T	2	2						T	T
		T	1	1		F	2	2		T	1	2		F	1	2						T	T
		T	1	1		F	2	2		T	1	2		No Data	4							T	T
		T	1	1		F	2	1		F	2	2		T	2	2						T	T
		T	1	1		F	2	1		F	2	2		F	1	2						T	T
		T	1	1		F	2	1		F	2	2		No Data	4							T	T
		T	1	1		F	2	1		No Data	4			T	2	2						T	T
		T	1	1		F	2	1		No Data	4			F	1	2						T	T
		T	1	1		F	2	1		No Data	4			No Data	4							T	T
		T	1	1		No Data	4			T	1	2		T	2	2						T	T
		T	1	1		No Data	4			T	1	2		F	1	2						T	T
		T	1	1		No Data	4			T	1	2		No Data	4							T	T
		T	1	1		No Data	4			F	2	2		T	2	2						T	T
		T	1	1		No Data	4			F	2	2		F	1	2						T	T
		T	1	1		No Data	4			F	2	2		No Data	4							T	T
		T	1	1		No Data	4			No Data	4			T	2	2						T	T
		T	1	1		No Data	4			No Data	4			F	1	2						T	T
		T	1	1		No Data	4			No Data	4			No Data	4							T	T
		T	1	1		Equiv. high	1	1		T	1	2		T	2	2						T	T
		T	1	1		Equiv. high	1	1		T	1	2		F	1	2						T	T
		T	1	1		Equiv. high	1	1		T	1	2		No Data	4							T	T
		T	1	1		Equiv. high	1	1		F	2	2		T	2	2						T	T
		T	1	1		Equiv. high	1	1		F	2	2		F	1	2						T	T
		T	1	1		Equiv. high	1	1		F	2	2		No Data	4							T	T
		T	1	1		Equiv. high	1	1		No Data	4			T	2	2						T	T
		T	1	1		Equiv. high	1	1		No Data	4			F	1	2						T	T
		T	1	1		Equiv. high	1	1		No Data	4			No Data	4							T	T
		T	1	1		Equiv. low	2	2		T	1	2		T	2	2						T	T
		T	1	1		Equiv. low	2	2		T	1	2		F	1	2						T	T
		T	1	1		Equiv. low	2	2		T	1	2		No Data	4							T	T
		T	1	1		Equiv. low	2	1		F	2	2		T	2	2						T	T
		T	1	1		Equiv. low	2	1		F	2	2		F	1	2						T	T
		T	1	1		Equiv. low	2	1		F	2	2		No Data	4							T	T
		T	1	1		Equiv. low	2	1		No Data	4			T	2	2						T	T
		T	1	1		Equiv. low	2	1		No Data	4			F	1	2						T	T
		T	1	1		Equiv. low	2	1		No Data	4			No Data	4							T	T
		F	2	2		T	1	1		T	1	2		T	2	2						T	T
		F	2	2		T	1	1		T	1	2		F	1	2						T	T

		F	2	2		T	1	1		T	1	2		No Data	4							T	T
		F	2	1		T	1	1		F	2	2		T	2	2						T	T
		F	2	1		T	1	1		F	2	2		F	1	2						T	T
		F	2	1		T	1	1		F	2	2		No Data	4							T	T
		F	2	1		T	1	1		No Data	4			T	2	2						T	T
		F	2	1		T	1	1		No Data	4			F	1	2						T	T
		F	2	1		T	1	1		No Data	4			No Data	4							T	T
		F	2	2		F	2	2		T	1	2		T	2	2						T	T
		F	2	2		F	2	2		T	1	2		F	1	2						T	T
		F	2	2		F	2	2		T	1	2		No Data	4							T	T
		F	3	1		F	3	1		F	3	2		T	3	2						F	F
		F	3	1		F	3	1		F	3	2		F	2	2						F	F
		F	3	1		F	3	1		F	3	2		No Data	4							F	F
		F	3	1		F	3	1		No Data	4			T	3	2						F	Indet.
		F	3	1		F	3	1		No Data	4			F	2	2						F	Indet.
		F	3	1		F	3	1		No Data	4			No Data	4							F	Indet.
		F	2	2		No Data	4			T	1	2		T	2	2						T	T
		F	2	2		No Data	4			T	1	2		F	1	2						T	T
		F	2	2		No Data	4			T	1	2		No Data	4							T	T
		F	3	1		No Data	4			F	3	2		T	3	2						F	Indet.
		F	3	1		No Data	4			F	3	2		F	2	2						F	Indet.
		F	3	1		No Data	4			F	3	2		No Data	4							F	Indet.
		F	3	1		No Data	4			No Data	4			T	3	2						F	Indet.
		F	3	1		No Data	4			No Data	4			F	2	2						F	Indet.
		F	3	1		No Data	4			No Data	4			No Data	4							F	Indet.
		F	2	2		Equiv. high	1	1		T	1	2		T	2	2						T	T
		F	2	2		Equiv. high	1	1		T	1	2		F	1	2						T	T
		F	2	2		Equiv. high	1	1		T	1	2		No Data	4							T	T
		F	2	1		Equiv. high	1	1		F	2	2		T	2	2						T	T
		F	2	1		Equiv. high	1	1		F	2	2		F	1	2						T	T
		F	2	1		Equiv. high	1	1		F	2	2		No Data	4							T	T
		F	2	1		Equiv. high	1	1		No Data	4			T	2	2						T	T
		F	2	1		Equiv. high	1	1		No Data	4			F	1	2						T	T
		F	2	1		Equiv. high	1	1		No Data	4			No Data	4							T	T
		F	2	2		Equiv. low	2	2		T	1	2		T	2	2						T	T
		F	2	2		Equiv. low	2	2		T	1	2		F	1	2						T	T
		F	2	2		Equiv. low	2	2		T	1	2		No Data	4							T	T
		F	3	1		Equiv. low	3	1		F	3	2		T	3	2						F	F
		F	3	1		Equiv. low	3	1		F	3	2		F	2	2						F	F
		F	3	1		Equiv. low	3	1		F	3	2		No Data	4							F	F
		F	3	1		Equiv. low	3	1		No Data	4			T	3	2						F	Indet.
		F	3	1		Equiv. low	3	1		No Data	4			F	2	2						F	Indet.
		F	3	1		Equiv. low	3	1		No Data	4			No Data	4							F	Indet.

		No Data	4			T	1	1		T	1	2		T	2	2						T	T
		No Data	4			T	1	1		T	1	2		F	1	2						T	T
		No Data	4			T	1	1		T	1	2		No Data	4							T	T
		No Data	4			T	1	1		F	2	2		T	2	2						T	T
		No Data	4			T	1	1		F	2	2		F	1	2						T	T
		No Data	4			T	1	1		F	2	2		No Data	4							T	T
		No Data	4			T	1	1		No Data	4			T	2	2						T	T
		No Data	4			T	1	1		No Data	4			F	1	2						T	T
		No Data	4			T	1	1		No Data	4			No Data	4							T	T
		No Data	4			F	2	2		T	1	2		T	2	2						T	T
		No Data	4			F	2	2		T	1	2		F	1	2						T	T
		No Data	4			F	2	2		T	1	2		No Data	4							T	T
		No Data	4			F	3	1		F	3	2		T	3	2						F	Indet.
		No Data	4			F	3	1		F	3	2		F	2	2						F	Indet.
		No Data	4			F	3	1		F	3	2		No Data	4							F	Indet.
		No Data	4			F	3	1		No Data	4			T	3	2						F	Indet.
		No Data	4			F	3	1		No Data	4			F	2	2						F	Indet.
		No Data	4			F	3	1		No Data	4			No Data	4							F	Indet.
		No Data	4			No Data	4			T	1	2		T	2	2						T	T
		No Data	4			No Data	4			T	1	2		F	1	2						T	T
		No Data	4			No Data	4			T	1	2		No Data	4							T	T
		No Data	4			No Data	4			F	3	2		T	3	2						F	Indet.
		No Data	4			No Data	4			F	3	2		F	2	2						F	Indet.
		No Data	4			No Data	4			F	3	2		No Data	4							F	Indet.
		No Data	4			No Data	4			No Data	4			T	3	2						F	Indet.
		No Data	4			No Data	4			No Data	4			F	1	2						T	Indet.
		No Data	4			No Data	4			No Data	4			No Data	4							Indet.	Indet.
		No Data	4			Equiv. high	1	1		T	1	2		T	2	2						T	T
		No Data	4			Equiv. high	1	1		T	1	2		F	1	2						T	T
		No Data	4			Equiv. high	1	1		T	1	2		No Data	4							T	T
		No Data	4			Equiv. high	1	1		F	2	2		T	2	2						T	T
		No Data	4			Equiv. high	1	1		F	2	2		F	1	2						T	T
		No Data	4			Equiv. high	1	1		F	2	2		No Data	4							T	T
		No Data	4			Equiv. high	1	1		No Data	4			T	2	2						T	T
		No Data	4			Equiv. high	1	1		No Data	4			F	1	2						T	T
		No Data	4			Equiv. high	1	1		No Data	4			No Data	4							T	T
		No Data	4			Equiv. low	2	2		T	1	2		T	2	2						T	T
		No Data	4			Equiv. low	2	2		T	1	2		F	1	2						T	T
		No Data	4			Equiv. low	2	2		T	1	2		No Data	4							T	T
		No Data	4			Equiv. low	3	1		F	3	2		T	3	2						F	Indet.
		No Data	4			Equiv. low	3	1		F	3	2		F	2	2						F	Indet.
		No Data	4			Equiv. low	3	1		F	3	2		No Data	4							F	Indet.
		No Data	4			Equiv. low	3	1		No Data	4			T	3	2						F	Indet.

		No Data	4			Equiv. low	3	1		No Data	4			F	2	2						F	Indet.
		No Data	4			Equiv. low	3	1		No Data	4			No Data	4							F	Indet.
TKI	**imatinib**	**c-KIT exon11 | exon13 (Seq.)**	**Bene. Level**	**Evid. Level**	**16**	**PDGFRA exon 12 | exon 14 | exon 18 (Seq.)**	**Bene. Level**	**Evid. Level**	**17**													**Overall Bene.**	**Partial Report Overall Bene.**
		T	1	2		T	1	2														T	T
		T	1	2		F	5															T	T
		T	2	2		D842V	3	2														F	F
		T	1	2		No Data	4															T	Indet.
		F	2	2		T	1	2														T	T
		F	3	2		F	3	2														Indet.	Indet.
		F	3	2		D842V	3	2														F	F
		F	3	2		No Data	4															Indet.	Indet.
		V654A	3	2		T	2	2														F	F
		V654A	3	2		F	3	2														F	F
		V654A	3	2		D842V	3	2														F	F
		V654A	3	2		No Data	4															F	F
		exon 14	5			T	1	2														T	T
		exon 14	5			F	3	2														Indet.	Indet.
		exon 14	5			D842V	3	2														F	F
		exon 14	5			No Data	4															Indet.	Indet.
		exon 17 or 18	5			T	1	2														T	T
		exon 17 or 18	5			F	3	2														Indet.	Indet.
		exon 17 or 18	5			D842V	3	2														F	F
		exon 17 or 18	5			No Data	4															Indet.	Indet.
		No Data	4			T	1	2														T	Indet.
		No Data	4			F	3	2														Indet.	Indet.
		No Data	4			D842V	3	2														F	F
		No Data	4			No Data	4															Indet.	Indet.
TKI (crizotinib)	**crizotinib**	**ALK Positive (ISH)**	**Bene. Level**	**Evid. Level**	**18**	**ROS1 Positive (ISH)**	**Bene. Level**	**Evid. Level**	**19**													**Overall Bene.**	**Pending Report Overall Bene.**
		T	1	2		T	1	2														T	T
		F	5			T	1	2														T	T
		No Data	4			T	1	2														T	T

		T	1	2		F	5														T	T
		F	3	2		F	3	2													F	F
		No Data	4			F	3	2													Indet.	Indet.
		T	1	2		No Data	4														T	T
		F	3	2		No Data	4														F	Indet.
		No Data	4			No Data	4														Indet.	Indet.
mTOR inhibitors	**everolimus, temsirolimus**	**PIK3CA exon20 (Seq.)**	**Bene. Level**	**Evid. Level**	**20**																**Overall Bene.**	**Partial Report Overall Bene.**
		T	1	2																	T	T
		F	3	2																	Indet.	Indet.
		No Data	4																		Indet.	Indet.
TKI (RET-targeted)	**vandetanib**	**RET Mutated (Seq.)**	**Bene. Level**	**Evid. Level**	**21**																**Overall Bene.**	**Partial Report Overall Bene.**
		T	1	1																	T	T
		F	5																		Indet.	Indet.
		No Data	4																		Indet.	Indet.
Platinum compounds	**cisplatin, carboplatin, oxaliplatin**	**BRCA1 mutated (Seq.)**	**Bene. Level**	**Evid. Level**	**22**	**BRCA2 mutated (Seq.)**	**Bene. Level**	**Evid. Level**	**23**												**Overall Bene.**	**Partial Report Overall Benefft**
		T	1	2		T	1	2													T	T
		T	1	2		F	5														T	T
		T	1	2		No Data	4														T	T
		F	5			T	1	2													T	T
		F	3	2		F	3	2													Indet.	Indet.
		F	3	2		No Data	4														Indet.	Indet.
		No Data	4			T	1	2													T	T
		No Data	4			F	3	2													Indet.	Indet.
		No Data	4			No Data	4														Indet.	Indet.
GnRH agonists, antagonists	**goserelin, leuprolide, triptorelin, abarelix, degarelix**	**AR Positive (IHC)**	**Bene. Level**	**Evid. Level**	**24**	**ER Positive (IHC)**			**25**	**PR Positive**	**Bene. Level**	**Evid. Level**	**25**								**Overall Bene.**	**Partial Report Overall Benefft**
		T	1	2		T	1	2		T	1	2									T	T
		T	1	2		T	1	2		F	2	2									T	T

		T	1	2		T	1	2		No Data	4										T	T
		T	1	2		F	2	2		T	1	2									T	T
		T	1	2		F	2	2		F	2	2									T	T
		T	1	2		F	2	2		No Data	4										T	T
		T	1	2		No Data	4			T	1	2									T	T
		T	1	2		No Data	4			F	2	2									T	T
		T	1	2		No Data	4			No Data	4										T	T
		F	2	2		T	1	2		T	1	2									T	T
		F	2	2		T	1	2		F	2	2									T	T
		F	2	2		T	1	2		No Data	4										T	T
		F	2	2		F	2	2		T	1	2									T	T
		F	3	2		F	3	2		F	3	2									F	F
		F	3	2		F	3	2		No Data	4										F	Indet.
		F	2	2		No Data	4			T	1	2									T	T
		F	3	2		No Data	4			F	3	2									F	Indet.
		F	3	2		No Data	4			No Data	4										F	Indet.
		No Data	4			T	1	2		T	1	2									T	T
		No Data	4			T	1	2		F	2	2									T	Indet.
		No Data	4			T	1	2		No Data	4										T	Indet.
		No Data	4			F	2	2		T	1	2									T	T
		No Data	4			F	3	2		F	3	2									F	Indet.
		No Data	4			F	3	2		No Data	4										F	Indet.
		No Data	4			No Data	4			T	1	2									T	T
		No Data	4			No Data	4			F	3	2									F	Indet.
		No Data	4			No Data	4			No Data	4										Indet.	Indet.
Taxanes	**docetaxel, paclitaxel**	**TLE3 Positive (IHC)**	**Bene. Level**	**Evid. Level**	**26**	**TUBB3 Positive (IHC)**	**Bene. Level**	**Evid. Level**	**27**	**PGP Positive (IHC)**	**Bene. Level**	**Evid. Level**	**28**								**Overall Bene.**	**Partial Report Overall Benefft**
		T	1	2		T	2	2		T	2	3									T	T
		T	1	2		F	1	2		T	2	3									T	T
		T	1	2		No Data	4			T	2	3									T	T
		F	3	2		T	3	2		T	3	3									F	F
		F	2	2		F	1	2		T	2	3									T	T
		F	3	2		No Data	4			T	3	3									F	Indet.
		No Data	4			T	3	2		T	3	3									F	Indet.
		No Data	4			F	1	2		T	2	3									T	T
		No Data	4			No Data	4			T	3	3									Indet.	Indet.
		T	1	2		T	2	2		F	1	3									T	T
		T	1	2		F	1	2		F	1	3									T	T
		T	1	2		No Data	4			F	1	3									T	T
		F	3	2		T	3	2		F	1	3									F	F

		F	2	2		F	1	2		F	1	3										T	T
		F	3	2		No Data	4			F	1	3										F	Indet.
		No Data	4			T	3	2		F	1	3										F	Indet.
		No Data	4			F	1	2		F	1	3										T	T
		No Data	4			No Data	4			F	1	3										Indet.	Indet.
		T	1	2		T	2	2		No Data	4											T	T
		T	1	2		F	1	2		No Data	4											T	T
		T	1	2		No Data	4			No Data	4											T	T
		F	3	2		T	3	2		No Data	4											F	F
		F	2	2		F	1	2		No Data	4											T	T
		F	3	2		No Data	4			No Data	4											F	Indet.
		No Data	4			T	3	2		No Data	4											F	Indet.
		No Data	4			F	1	2		No Data	4											T	T
		No Data	4			No Data	4			No Data	4											Indet.	Indet.
Taxanes (nab-paclitaxel)	**nab-paclitaxel**	**SPARC IHC Mono Pos.**	**Bene. Level**	**Evid. Level**	**29**	**SPARC IHC Poly Pos.**	**Bene. Level**	**Evid. Level**	**29**													**Overall Bene.**	**Partial Report Overall Benefft**
		T	1	2		T	1	2														T	T
		T	1	2		F	2	2														T	T
		T	1	2		No Data	4															T	T
		F	2	2		T	1	2														T	T
		F	3	2		F	3	2														Indet.	Indet.
		F	3	2		No Data	4															Indet.	Indet.
		No Data	4			T	1	2														T	T
		No Data	4			F	3	2														Indet.	Indet.
		No Data	4			No Data	4															Indet.	Indet.
TKI	**vemurafenib, dabrafenib, trametinib**	**BRAF V600E (PCR or seq.)**	**Bene. Level**	**Evid. Level**	**30**																	**Overall Bene.**	**Partial Report Overall Benefft**
		T	1	2																		T	T
		F	3	2																		F	F
		No Data	4																			Indet.	Indet.
TKI	**ceritinib**	**ALK Positive**	**Bene. Level**	**Evid. Level**	**31**																	**Overall Bene.**	**Partial Report Overall Benefft**
		T	1	2																		T	T
		F	3	2																		F	F

| | | |

[00388] **Table 9** contains the references used to predict benefit level and provide an evidence level as shown in **Table 8** above. The "Ref. No." column in **Table 9** corresponds to the "Ref. No." columns in **Table 8**. Specifically, the reference numbers in **Table 8** include those references indicated in **Table 9**.

Table 9 – References for Comprehensive Cancer Molecular Profile

Ref. No.	References
1	Gong, W., J. Dong, et. al. (2012). "RRM1 expression and clinical outcome of gemcitabine-containing chemotherapy for advanced non-small-cell lung cancer: A meta-analysis." Lung Cancer. 75:374-380.
2	Qiu, L.X., M.H. Zheng, et. al. (2008). "Predictive value of thymidylate synthase expression in advanced colorectal cancer patients receiving fluoropyrimidine-based chemotherapy: Evidence from 24 studies." Int. J. Cancer: 123, 2384–2389. Chen, C.-Y., P.-C. Yang, et al. (2011). "Thymidylate synthase and dihydrofolate reductase expression in non-small cell lung carcinoma: The association with treatment efficacy of pemetrexed." Lung Cancer 74(1): 132-138. Lee, S.J., Y.H. Im, et. al. (2010). "Thymidylate synthase and thymidine phosphorylase as predictive markers of capecitabine monotherapy in patients with anthracycline- and taxane-pretreated metastatic breast cancer." Cancer Chemother. Pharmacol. DOI 10.1007/s00280-010-1545-0.
3	Braun, M.S., M.T. Seymour, et. al. (2008). "Predictive biomarkers of chemotherapy efficacy in colorectal cancer: results from the UK MRC FOCUS trial." J. Clin. Oncol. 26:2690-2698. Kostopoulos, I., G. Fountzilas, et. al. (2009). "Topoisomerase I but not thymidylate synthase is associated with improved outcome in patients with resected colorectal cancer treated with irinotecan containing adjuvant chemotherapy." BMC Cancer. 9:339. Ataka, M., K. Katano, et. al. (2007). "Topoisomerase I protein expression and prognosis of patients with colorectal cancer." Yonago Acta medica. 50:81–87.
4	Chinot, O. L., M. Barrie, et al. (2007). "Correlation between O6-methylguanine-DNA methyltransferase and survival in inoperable newly diagnosed glioblastoma patients treated with neoadjuvant temozolomide." J Clin Oncol 25(12): 1470-5. Kulke, M.H., M.S. Redston, et al. (2008). "06-Methylguanine DNA Methyltransferase Deficiency and Response to Temozolomide-Based Therapy in Patients with Neuroendocrine Tumors." Clin Cancer Res 15(1): 338-345.
5	El Sheikh, S. S., H. M. Romanska, et. al. (2008). "Predictive value of PTEN and AR coexpression of sustained responsiveness to hormonal therapy in prostate cancer--a pilot study." Neoplasia. 10(9): 949-53.
6	Lewis, J.D., M.J. Edwards, et al. (2010). "Excellent outcomes with adjuvant toremifene or tamoxifen in early stage breast cancer." Cancer116:2307-15. Bartlett, J.M.S., D. Rea, et al. (2011). "Estrogen receptor and progesterone receptor as predictive biomarkers of response to endocrine therapy: a prospectively powered pathology study in the Tamoxifen and Exemestane Adjuvant Multinational trial." J Clin Oncol 29 (12):1531-1538. Dowsett, M., C. Allred, et al. (2008). "Relationship between quantitative estrogen and progesterone receptor expression and human epidermal growth factor receptor 2 (HER-2) status with recurrence in the Arimidex, Tamoxifen, Alone or in Combination trial." J Clin Oncol 26(7): 1059-65. Viale, G., M. M. Regan, et al. (2008). "Chemoendocrine compared with endocrine adjuvant therapies for node-negative breast cancer: predictive value of centrally reviewed expression of estrogen and progesterone receptors--International Breast Cancer Study Group." J Clin Oncol 26(9): 1404-10. Anderson, H., M. Dowsett, et. al. (2011). "Relationship between estrogen receptor, progesterone receptor, HER-2 and Ki67 expression and efficacy of aromatase inhibitors in advanced breast cancer. Annals of Oncology. 22:1770-1776. Coombes, R.C., J.M. Bliss, et al. (2007). "Survival and safety of exemestane versus tamoxifen after 2-3 years' tamoxifen treatment (Intergroup Exemestane Study): a randomized controlled trial." The Lancet 369:559-570.

	Stuart, N.S.A., H. Earl, et. al. (1996). "A randomized phase III cross-over study of tamoxifen versus megestrol acetate in advanced and recurrent breast cancer." European Journal of Cancer. 32(11):1888-1892. Thurlimann, B., A. Goldhirsch, et al. (1997). "Formestane versus Megestrol Acetate in Postmenopausal Breast Cancer Patients After Failure of Tamoxifen: A Phase III Prospective Randomised Cross Over Trial of Second-line Hormonal Treatment (SAKK 20/90). E J Cancer 33 (7): 1017-1024. Cuzick J,LHRH-agonists in Early Breast Cancer Overview group. (2007). "Use of luteinising-hormone-releasing hormone agonists as adjuvant treatment in premenopausal patients with hormone-receptor-positive breast cancer: a meta-analysis of individual patient data from randomised adjuvant trials." The Lancet 369: 1711-1723.
7	Lewis, J.D., M.J. Edwards, et al. (2010). "Excellent outcomes with adjuvant toremifene or tamoxifen in early stage breast cancer." Cancer116:2307-15. Stendahl, M., L. Ryden, et al. (2006). "High progesterone receptor expression correlates to the effect of adjuvant tamoxifen in premenopausal breast cancer patients." Clin Cancer Res 12(15): 4614-8. Bartlett, J.M.S., D. Rea, et al. (2011). "Estrogen receptor and progesterone receptor as predictive biomarkers of response to endocrine therapy: a prospectively powered pathology study in the Tamoxifen and Exemestane Adjuvant Multinational trial." J Clin Oncol 29 (12):1531-1538. Dowsett, M., C. Allred, et al. (2008). "Relationship between quantitative estrogen and progesterone receptor expression and human epidermal growth factor receptor 2 (HER-2) status with recurrence in the Arimidex, Tamoxifen, Alone or in Combination trial." J Clin Oncol 26(7): 1059-65. Coombes, R.C., J.M. Bliss, et al. (2007). "Survival and safety of exemestane versus tamoxifen after 2-3 years' tamoxifen treatment (Intergroup Exemestane Study): a randomized controlled trial." The Lancet 369:559-570. Yamashita, H., Y. Yando, et al. (2006). "Immunohistochemical evaluation of hormone receptor status for predicting response to endocrine therapy in metastatic breast cancer." Breast Cancer 13(1): 74-83. Stuart, N.S.A., H. Earl, et. al. (1996). "A randomized phase III cross-over study of tamoxifen versus megestrol acetate in advanced and recurrent breast cancer." European Journal of Cancer. 32(11):1888-1892. Thurlimann, B., A. Goldhirsch, et al. (1997). "Formestane versus Megestrol Acetate in Postmenopausal Breast Cancer Patients After Failure of Tamoxifen: A Phase III Prospective Randomised Cross Over Trial of Second-line Hormonal Treatment (SAKK 20/90). E J Cancer 33 (7): 1017-1024. Cuzick J,LHRH-agonists in Early Breast Cancer Overview group. (2007). "Use of luteinising-hormone-releasing hormone agonists as adjuvant treatment in premenopausal patients with hormone-receptor-positive breast cancer: a meta-analysis of individual patient data from randomised adjuvant trials." The Lancet 369: 1711-1723.
8	Amir, E. et. al. (2010). "Lapatinib and HER2 status: results of a meta-analysis of randomized phase III trials in metastatic breast cancer." Cancer Treatment Reviews. 36:410-415. Johnston, S., Pegram M., et. al. (2009). "Lapatinib combined with letrozole versus letrozole and placebo as first-line therapy for postmenopausal hormone receptor-positive metastatic breast cancer. Journal of Clinical Oncology. Published ahead of print on September 28, 2009 as 10.1200/JCO.2009.23.3734. Press, M. F., R. S. Finn, et al. (2008). "HER-2 gene amplification, HER-2 and epidermal growth factor receptor mRNA and protein expression, and lapatinib efficacy in women with metastatic breast cancer." Clin Cancer Res 14(23): 7861-70.
9	Amir, E. et. al. (2010). "Lapatinib and HER2 status: results of a meta-analysis of randomized phase III trials in metastatic breast cancer." Cancer Treatment Reviews. 36:410-415. Johnston, S., Pegram M., et. al. (2009). "Lapatinib combined with letrozole versus letrozole and placebo as first-line therapy for postmenopausal hormone receptor-positive metastatic breast cancer. Journal of Clinical Oncology. Published ahead of print on September 28, 2009 as 10.1200/JCO.2009.23.3734. Press, M. F., R. S. Finn, et al. (2008). "HER-2 gene amplification, HER-2 and epidermal

	growth factor receptor mRNA and protein expression, and lapatinib efficacy in women with metastatic breast cancer." Clin Cancer Res 14(23): 7861-70. Bartlett, J.M.S., K. Miller, et. al. (2011). "A UK NEQAS ISH multicenter ring study using the Ventana HER2 dual-color ISH assay." Am. J. Clin. Pathol. 135:157-162.
10	Slamon, D., M. Buyse, et. al. (2011). "Adjuvant trastuzumab in HER2-positive breast cancer." N. Engl. J. Med. 365:1273-83. Yin, W., J. Lu, et. al. (2011). "Trastuzumab in adjuvant treatment HER2-positive early breast cancer patients: A meta-analysis of published randomized controlled trials." PLoS ONE 6(6): e21030. doi:10.1371/journal.pone.0021030. Cortes, J., J. Baselga, et. al. (2012). "Pertuzumab monotherapy after trastuzumab-based treatment and subsequent reintroduction of trastuzumab: activity and tolerability in patients with advanced human epidermal growth factor receptor-2-positive breast cancer." J. Clin. Oncol. 30. DOI: 10.1200/JCO.2011.37.4207. Bang, Y-J., Y-K. Kang, et. al. (2010). "Trastuzumab in combination with chemotherapy versus chemotherapy alone for treatment of HER2-positive advanced gastric or gastro-oesophageal junction cancer (ToGA): a phase 3, open-label, randomised controlled trial." Lancet. 376:687-97. Baselga, J., S.M. Swain, et. al. (2012). "Pertuzumab plus trastuzumab plus docetaxel for metastatic breast cancer". N. Engl. J. Med. 36:109-119. Verma, S., K. Blackwell, et. al. (2012) "Trastuzumab Emtansine for HER2-Positive Advanced Breast Cancer" N Engl J Med. 367(19):1783-91. Hurvitz, S.A., E.A. Perez, et. al. (2013) "Phase II randomized study of trastuzumab emtansine versus trastuzumab plus docetaxel in patients with human epidermal growth factor receptor 2-positive metastatic breast cancer." J Clin Oncol.31(9):1157-63
11	Slamon, D., M. Buyse, et. al. (2011). "Adjuvant trastuzumab in HER2-positive breast cancer." N. Engl. J. Med. 365:1273-83. Yin, W., J. Lu, et. al. (2011). "Trastuzumab in adjuvant treatment HER2-positive early breast cancer patients: A meta-analysis of published randomized controlled trials." PLoS ONE 6(6): e21030. doi:10.1371/journal.pone.0021030. Cortes, J., J. Baselga, et. al. (2012). "Pertuzumab monotherapy after trastuzumab-based treatment and subsequent reintroduction of trastuzumab: activity and tolerability in patients with advanced human epidermal growth factor receptor-2-positive breast cancer." J. Clin. Oncol. 30. DOI: 10.1200/JCO.2011.37.4207. Bang, Y-J., Y-K. Kang, et. al. (2010). "Trastuzumab in combination with chemotherapy versus chemotherapy alone for treatment of HER2-positive advanced gastric or gastro-oesophageal junction cancer (ToGA): a phase 3, open-label, randomised controlled trial." Lancet. 376:687-97. Bartlett, J.M.S., K. Miller, et. al. (2011). "A UK NEQAS ISH multicenter ring study using the Ventana HER2 dual-color ISH assay." Am. J. Clin. Pathol. 135:157-162. Baselga, J., S.M. Swain, et. al. (2012). "Pertuzumab plus trastuzumab plus docetaxel for metastatic breast cancer". N. Engl. J. Med. 36:109-119. Verma, S., K. Blackwell, et. al. (2012) "Trastuzumab Emtansine for HER2-Positive Advanced Breast Cancer" N Engl J Med. 367(19):1783-91. Hurvitz, S.A., E.A. Perez, et. al. (2013) "Phase II randomized study of trastuzumab emtansine versus trastuzumab plus docetaxel in patients with human epidermal growth factor receptor 2-positive metastatic breast cancer." J Clin Oncol.31(9):1157-63
12	Press, M.F., Slamon, D.J., et. al. (2011)."Alteration of topoisomerase II-alpha gene in human breast cancer: association with responsiveness to anthracycline based chemotherapy." J. Clin. Oncol, 29(7):859-67. Du, Y., J. Lu, et. al. (2011). "The role of topoisomerase II a in predicting sensitivity to anthracyclines in breast cancer patients: a meta-analysis of published literatures." Breast Can Res Treat. 129(3):839-848. O'Malley, F.P., K.I. Pritchard, et. al (2009) "Topoisomerase II alpha and responsiveness of breast cancer to adjuvant chemotherapy." J Natl Can Inst. 101: 644-650. Tanner, M., J. Bergh, et al. (2006). "Topoisomerase II-α Gene Amplification Predicts Favorable Treatment Response to Tailored and Dose-Escalated Anthracycline-Based Adjuvant Chemotherapy in HER-2/neu-Amplified Breast Cancer: Scandinavian Breast Group Trial

	9401." J Clin Oncol 24(16):2428-2436.
13	Press, M.F., Slamon, D.J., et. al. (2011)."Alteration of topoisomerase II-alpha gene in human breast cancer: association with responsiveness to anthracycline based chemotherapy." J. Clin. Oncol, 29(7):859-67. Gennari, A., P. Bruzzi, et. al (2008) "HER2 status and efficacy of adjuvant anthracyclines in early breast cancer: a pooled analysis of randomized trials." J Natl Can Inst. 100:14-20.
14	O'Malley, F.P., K.I. Pritchard, et al. (2011). "Topoisomerase II alpha protein and resposiveness of breast cancer to adjuvant chemotherapy with CEF compared to CMF in the NCIC CTG randomized MA.5 adjuvant trial." Breast Can Res Treat. 128, 401-409. Rodrigo, R.S., C. Axel le, et. al. (2011). "Topoisomerase II-alpha protein expression and histological response following doxorubicin-based induction chemotherapy predict survival of locally advanced soft tissues sarcomas." Eur J of Can. 47, 1319-1327.
15	Chintamani, J.P., Singh, et. al. (2005). "Role of p-glycoprotein expression in predicting response to neoadjuvant chemotherapy in breast cancer - a prospective clinical study." World J. Surg. Oncol. 3:61. Akimoto, M., H, Saisho, et al. (2006). "Relationship between therapeutic efficacy of arterial infusion chemotherapy and expression of P-glycoprotein and p53 protein in advanced hepatocellular carcinoma." World J of Gastroenterol, 12(6), 868-873.
16	Carvajal, R.D., G.K. Schwartz, et. al. (2011). "KIT as a therapeutic target in metastatic melanoma." JAMA. 305(22):2327-2334. Guo, Q.Z., Z.J. Wang, et. al. (2010). "High expression of ERCC1 is a poor prognostic factor in Chinese patients with non-small cell lung cancer receiving cisplatin-based therapy." Chin. J. Cancer Res. 22(4):296-302.
17	Cassier, P.A., P. Hohenberger, et al. (2012). "Outcome of Patients with Platelet-Derived Growth Factor Receptor Alpha-Mutated Gastrointestinal Stromal Tumors in the Tyrosine Kinase Inhibitor Era." Clin Cancer Res 18:4458-4464. Heinrich, M.C., J.A. Fletcher, et. al. (2008). "Correlation of kinase genotype and clinical outcome in North American Intergroup phase III trial of imatinib mesylate for treatment of advanced gastrointestinal stromal tumor: CALGB 150105 study by Cancer and Leukemia Group B and Southwest Oncology Group." J Clin Oncol. 26(33): 5360–5367. Debiec-Rychter, M., I. Judson, et al. (2006). "KIT mutations and dose selection for imatinib in patients with advanced gastrointestinal stromal tumours." Eur J Cancer 42:1093-1103.
18	Kwak, E.L., A.J. Iafrate, et. al. (2010). "Anaplastic lymphoma kinase inhibition in non-small cell lung cancer." N. Engl. J. Med. 363:1693-703. Lin, E., Modrusan, Z., (2009). Exon array profiling detects EML4-ALK fusion in breast, colorectal and non-small lung cancers, Mol. Cancer Res. 7(9):1466-76.
19	Bergethon, K., A.J. Iafrate, et. al. (2012) "ROS1 Rearrangements Define a Unique Molecular Class of Lung Cancers." J. Clin. Oncol. 30(8):863-70. Davies, K.D., R.C. Deobele, et. al. (2012) "Identifying and Targeting ROS1 Gene Fusions in Non–Small Cell Lung Cancer." Clin. Cancer Res. 18(17): 4570-9. Shaw, A.T., S.I. Ou, et. al. (2012) "Clinical activity of crizotinib in advanced non-small cell lung cancer (NSCLC) harboring ROS1 gene rearrangement." J Clin Oncol 30 (suppl; abstr 7508). National Comprehensive Cancer Network. NCCN Clinical Practice Guidelines in Oncology. Non-Small Cell Lung Cancer 2.2013. 2013; National Comprehensive Cancer Network.
20	Janku, F., R. Kurzrock, et. al. (2011). "PIK3CA mutations in patients with advanced cancers treated with PI3K/AKT/mTOR axis inhibitors." Molecular Cancer Therapeutics. 10(3):558–65. Janku, F., R. Kurzrock, et. al. (2012). "PI3K/Akt/mTOR inhibitors in patients with breast and gynecologic malignancies harboring PIK3CA mutations." Journal of Clinical Oncology. DOI: 10.1200/JCO.2011.36.1196. Moroney, J.W., R. Kurzrock, et. al. (2011). "A phase I trial of liposomal doxorubicin, bevacizumab, and temsirolimus in patients with advanced gynecologic and breast malignancies." Clin. Cancer Res. 17:6840-6846.
21	Wells, S.A., M.J. Schlumberger, et al. (2012). "Vandetanib in Patients with Locally Advanced or Metastatic Medullary Thyroid Cancer: A Randomized, Double-Blind Phase III Trial." J Clin Oncol 30: 134-141.

22	Lowery, M.A., et al. (2011) "An emerging entity: pancreatic adenocarcinoma associated with a known BRCA mutation: clinical descriptors, treatment implications, and future directions." Oncologist. 16(10):1397-402. Tan, D.S.P., et al. (2008) ""BRCAness" syndrome in ovarian cancer: a case-control study describing the clinical features and outcome of patients with epithelial ovarian cancer associated with BRCA1 and BRCA2 mutations." J Clin Oncol. 26(34):5530-6 Hennessy, B.T., et al. (2010) "Somatic mutations in BRCA1 and BRCA2 could expand the number of patients that benefit from poly (ADP ribose) polymerase inhibitors in ovarian cancer" J Clin Oncol. 28(22):3570-6 Byrski, T., S. Narod, et al. (2009) "Pathologic complete response rates in young women with BRCA1-positive breast cancers after neoadjuvant chemotherapy." J Clin Oncol. 28(3):275-9
23	Lowery, M.A., et al. (2011) "An emerging entity: pancreatic adenocarcinoma associated with a known BRCA mutation: clinical descriptors, treatment implications, and future directions." Oncologist. 16(10):1397-402. Tan, D.S.P., et al. (2008) ""BRCAness" syndrome in ovarian cancer: a case-control study describing the clinical features and outcome of patients with epithelial ovarian cancer associated with BRCA1 and BRCA2 mutations." J Clin Oncol. 26(34):5530-6 Hennessy, B.T., et al. (2010) "Somatic mutations in BRCA1 and BRCA2 could expand the number of patients that benefit from poly (ADP ribose) polymerase inhibitors in ovarian cancer" J Clin Oncol. 28(22):3570-6
24	El Sheikh, S.S. et al. (2008). "Predictive value of PTEN and AR coexpression of sustained responsiveness to hormonal therapy in prostate cancer--a pilot study." Neoplasia. 10(9): 949-53
25	Cuzick J, LHRH-agonists in Early Breast Cancer Overview group. (2007). "Use of luteinising-hormone-releasing hormone agonists as adjuvant treatment in premenopausal patients with hormone-receptor-positive breast cancer: a meta-analysis of individual patient data from randomised adjuvant trials." The Lancet 369: 1711-1723.
26	Kulkarni, S.A., D.T. Ross, et. al. (2009). "TLE3 as a candidate biomarker of response to taxane therapy". Breast Cancer Research. 11:R17 (doi:10.1186/bcr2241).
27	Zhang, H.-L., X.-W. Zhou, et al. (2012). "Association between class III β-tubulin expression and response to paclitaxel/vinorelbine-based chemotherapy for non-small cell lung cancer: A meta-analysis." Lung Cancer 77: 9-15. Seve, P., C. Dumontet, et al. (2005). "Class III β-tubulin expression in tumor cells predicts response and outcome in patients with non-small cell lung cancer receiving paclitaxel." Mol Cancer Ther 4(12): 2001-2007. Gao, S., J. Gao, et al. (2012). "Clinical implications of REST and TUBB3 in ovarian cancer and its relationship to paclitaxel resistance." Tumor Biol 33:1759-1765. Ploussard, G., A. de la Taille, et al. (2010). "Class III β-Tubulin Expression Predicts Prostate Tumor Aggressiveness and Patient Response to Docetaxel-Based Chemotherapy." Clin Cancer Res 70(22): 9253-9264.
28	Penson, R.T., M.V. Seiden, et al. (2004). "Expression of multidrug resistance-1 protein inversely correlates with paclitaxel response and survival in ovarian cancer patients: a study in serial samples." Gynecologic Oncology 93:98-106. Yeh, J.J., A. Kao, et al. (2003). "Predicting Chemotherapy Response to Paclitaxel-Based Therapy in Advanced Non-Small-Cell Lung Cancer with P-Glycoprotein Expression." Respiration 70:32-35.
29	Desai, N., Soon-Shiong, P., et al. (2009). "SPARC Expression Correlates with Tumor Response to Albumin-Bound Paclitaxel in Head and Neck Cancer Patients." Translational Oncology 2(2): 59-64. Von Hoff, D.D., M. Hidalgo, et. al. (2011). "Gemcitabine plus nab-paclitaxel is an active regimen in patients with advanced pancreatic cancer: a phase I/II trial." J. Clin. Oncol. DOI: 10.1200/JCO.2011.36.5742.
30	Chapman, P.B., et al. (2011). "Improved survival with vemurafenib in melanoma with BRAF V600E mutation." N. Engl. J. Med. This article (10.1056/NEJMoa1103782) was published on June 5, 2011, at nejm.org. Hauschild, A., et al. (2012). "Dabrafenib in BRAF-mutated metastatic melanoma: a multicentre, open-label, phase 3 randomised controlled trial." Lancet 358-365 Falchook, G.S., et al. (2012). "Dabrafenib in patients with melanoma, untreated brain

	metastases, and other solid tumours: a phase I dose-escalation trial." Lancet 379:1893-901. Flaherty, K.T., et al. (2010). "Inhibition of Mutated, Activated BRAF in Metastatic Melanoma." N Engl J Med 363:809-819.
31	Shaw, A.T., et al. (2014). "Ceritinib in ALK-Rearranged Non-small-Cell Lung Cancer". N Engl J Med. 370:1189-1197.

[00389] The PLUS profiles described above and shown in the appropriate panels in **FIGs. 27A-B** include next generation sequencing as in **Table 10**.

Table 10 – PLUS Sequencing panel

ABL1	CTNNB1	GNAS	MLH1	SMARCB1
AKT1	EGFR	HNF1A	MPL	SMO
ALK	ERBB2 (Her2)	HRAS	NOTCH1	STK11
APC	ERBB4	IDH1	NPM1	TP53
ATM	FBXW7	JAK2	NRAS	VHL
BRAF	FGFR1	JAK3	PDGFRA	RET
BRCA1	FGFR2	KDR (VGFR2)	PIK3CA	SMAD4
BRCA2	FLT3	cKIT	PTEN	
CDH1	GNA11	KRAS	PTPN11	
CSF1R	GNAQ	cMET	RB1	

[00390] Any of the biomarker assays herein, e.g., as shown in **FIGs. 27A-B** or **Tables 7-18** can be performed individually as desired. One of skill will appreciate that any combination of the individual biomarker assays could be performed. For example, a treating physician may choose to order one or more of the following to profile a particular patient's tumor: IHC for 1, 2, 3, 4, 5, 6, 7, 8, 9, 10, 11, 12, 13, 14, 15, 16, 17, 18 or 19 of AR, cMET, EGFR (including H-score for lung cancer such as NSCLC), ER, HER2, MGMT, Pgp, PR, PTEN, PD-1, PD-L1, RRM1, SPARCm, SPARCp, TLE3, TOPO1, TOP2A, TS, TUBB3; FISH or CISH for 1, 2, 3, 4, or 5 of ALK, cMET, HER2, ROS1, TOP2A; Mutational Analysis of 1, 2, 3 or 4 of BRAF (e.g., cobas® PCR), IDH2 (e.g., Sanger Sequencing), MGMT-Me (e.g., by PyroSequencing); EGFR (e.g., fragment analysis to detect EGFRvIII); and/or Mutational Analysis (e.g., by Next-Generation Sequencing) of 1, 2, 3, 4, 5, 6, 7, 8, 9, 10, 11, 12, 13, 14, 15, 16, 17, 18, 19, 20, 21, 22, 23, 24, 25, 26, 27, 28, 29, 30, 31, 32, 33, 34, 35, 36, 37, 38, 39, 40, 41, 42, 43, 44, 45, 46 or 47 of ABL1, AKT1, ALK, APC, ATM, BRAF, BRCA1, BRCA2, CDH1, CSF1R, CTNNB1, EGFR, ERBB2 (HER2), ERBB4, FBXW7, FGFR1, FGFR2, FLT3, GNA11, GNAQ, GNAS, HNF1A, HRAS, IDH1, JAK2, JAK3, KDR (VEGFR2), KIT, KRAS, MET, MLH1, MPL, NOTCH1, NPM1, NRAS, PDGFRA, PIK3CA, PTEN, PTPN11, RB1, RET, SMAD4, SMARCB1, SMO, STK11, TP53, VHL. In some embodiments, a selection of individual tests is made when insufficient tumor sample is available for performing all molecular profiling tests in **FIGs. 27A-B** or **Tables 7-18**.

[00391] **FIGs. 28A-C** illustrate biomarkers assessed using a molecular profiling approach as outlined in **FIGs. 27A-B** or **Tables 7-18**, and accompanying text herein. **FIG. 28A** illustrates biomarkers that are assessed. The biomarkers that are assessed according to the Next Generation sequencing panel are shown in **FIG. 28B**. **FIG. 28C** illustrates sample requirements that can be used to perform molecular profiling on

a patient tumor sample according to the panels in **FIGs. 28A-B**. **FIG. 28D** and **FIG. 28E** detail the biomarkers assessed, technology platforms utilized and associated therapies or clinical trials.

[00392] In certain embodiments, ERCC1 is assessed according to the profiles of the invention, such as described in any of **FIGs. 27A-B** or **Tables 7-16**. Lack of ERCC1 expression, e.g., as determined by IHC, can indicate positive benefit for platinum compounds (cisplatin, carboplatin, oxaliplatin), and conversely positive expression of ERCC1 can indicate lack of benefit of these drugs. Additional biomarkers that can be assessed according to the molecular profiles include EGFRvIII, IDH2, PD1, PD-L1, PD-L2, BAP1, SETD2, PBRM1, MLH1, MSH2, MSH6, PMS2, BRCA1, BRCA2, and microsatillite instability (MSI). The presence of EGFRvIII may be assessed using expression analysis at the protein or mRNA level, e.g., by either IHC or PCR, respectively. Expression of EGFRvIII can suggest treatment with EGFR inhibitors. Mutational analysis can be performed for IDH2, e.g., by Sanger sequencing, pyrosequencing or by next generation sequencing approaches. IDH2 mutations suggest the same therapy indications as IDH1 mutations, e.g., for decarbazine and temozolomide as described herein.

[00393] The biomarkers assessed according to the invention may depend on lineage as desired. See **Tables 11-16** for exemplary lineage-specialized profiles. For example, **Table 12** shows a profile for colorectal cancer that contain various biomarkers involved in mismatch repair, such as MLH1, MSH2, MSH6, PMS2, which may all be assessed using IHC or similar technique. The profiles for colorectal cancer can also include assessment of microsatellite instability (MSI) assessed by fragment analysis or similar technique. The profiles for ovarian cancer may include analysis of ERCC1 by IHC or similar technique. See **Table 16**. The profiles for NSCLC may include analysis of ALK and ROS1 by ISH or similar technique. See **Table 15**. The analysis can also depend on lineage as desired. For example, TOP2A may be assessed by ISH but not IHC for breast cancer profiles. See **Table 11**. In still other cases, the analysis performed for each biomarker can depend on the lineage as desired. For example, EGFR IHC results may be assessed using H-SCORE for NSCLC but not other lineages.

[00394] **Table 11** presents a view of the information that is reported for breast cancer molecular intelligence molecular profiles, which can be interpreted as described for **Table 7** above.

Table 11 – Molecular Profile and Report Parameters: Breast Cancer

Agents/Biomarker Status Reported	Biomarker	Platform
Hormone therapies†	ER	IHC
	PR	IHC
trastuzumab	HER2	IHC; CISH
	PTEN	IHC
	PIK3CA	NGS
lapatinib, pertuzumab, T-DM1, Clinical Trials	HER2	IHC; CISH
doxorubicin, liposomal-doxorubicin, epirubicin	TOP2A	CISH
	HER2	CISH
fluorouracil, capecitabine, pemetrexed	TS	IHC
docetaxel, paclitaxel, nab-paclitaxel	TLE3	IHC
	Pgp	IHC
nab-paclitaxel	SPARCm	IHC
	SPARCp	IHC

gemcitabine	RRM1	IHC
irinotecan	TOPO1	IHC
everolimus, temsirolimus, Clinical Trials	ER	IHC
	PIK3CA	NGS
carboplatin, cisplatin, oxaliplatin	BRCA1	NGS
	BRCA2	NGS
protein expression status	TUBB3	IHC
imatinib	cKIT	NGS
	PDGFRA	NGS
vandetanib	RET	NGS
dabrafenib, vemurafenib	BRAF	NGS
Clinical Trials	AR	IHC
Clinical Trials	cMET	IHC, CISH
Clinical Trials	PD-1	IHC
Clinical Trials	PD-L1	IHC
Clinical Trials	EGFR	IHC
Clinical Trials	ABL1	NGS
Clinical Trials	AKT1	NGS
Clinical Trials	ALK	NGS
Clinical Trials	APC	NGS
Clinical Trials	ATM	NGS
Clinical Trials	cMET	NGS
Clinical Trials	CSF1R	NGS
Clinical Trials	CTNNB1	NGS
Clinical Trials	EGFR	NGS
Clinical Trials	ERBB2 (HER2)	NGS
Clinical Trials	FGFR1	NGS
Clinical Trials	FGFR2	NGS
Clinical Trials	FLT3	NGS
Clinical Trials	GNA11	NGS
Clinical Trials	GNAQ	NGS
Clinical Trials	GNAS	NGS
Clinical Trials	HRAS	NGS
Clinical Trials	IDH1	NGS
Clinical Trials	JAK2	NGS
Clinical Trials	KDR (VEGFR2)	NGS
Clinical Trials	KRAS	NGS
Clinical Trials	MPL	NGS
Clinical Trials	NOTCH1	NGS
Clinical Trials	NRAS	NGS
Clinical Trials	PTEN	NGS
Clinical Trials	SMO	NGS
Clinical Trials	TP53	NGS
Clinical Trials	VHL	NGS

† Hormone therapies may include: tamoxifen, toremifene, fulvestrant, letrozole, anastrozole, exemestane, megestrol acetate, leuprolide, goserelin, bicalutamide, flutamide, abiraterone, enzalutamide, triptorelin, abarelix, degarelix

[00395] Table 12 presents a view of the information that is reported for colorectal cancer (CRC) molecular intelligence molecular profiles, which can be interpreted as described for **Table 7** above.

Table 12 – Molecular Profile and Report Parameters: Colorectal Cancer

Agents/Biomarker Status Reported	Biomarker	Platform
cetuximab, panitumumab	KRAS	NGS
	BRAF	NGS
	NRAS	NGS
	PIK3CA	NGS
	PTEN	IHC
fluorouracil, capecitabine, pemetrexed	TS	IHC
irinotecan	TOPO1	IHC
protein expression status	AR	IHC
	ER	IHC
	PR	IHC
imatinib	cKIT	NGS
	PDGFRA	NGS
doxorubicin, liposomal-doxorubicin, epirubicin	HER2	CISH
	TOP2A	IHC
	Pgp	IHC
trastuzumab, lapatinib, pertuzumab, T-DM1	HER2	IHC, CISH
gemcitabine	RRM1	IHC
temozolomide, dacarbazine	MGMT	IHC
docetaxel, paclitaxel	TLE3	IHC
	TUBB3	IHC
	Pgp	IHC
nab-paclitaxel	SPARCm	IHC
	SPARCp	IHC
vandetanib	RET	NGS
dabrafenib, vemurafenib	BRAF	NGS
carboplatin, cisplatin, oxaliplatin	BRCA1	NGS
	BRCA2	NGS
Clinical Trials	cMET	IHC, CISH
Clinical Trials	PD-1	IHC
Clinical Trials	PD-L1	IHC
Clinical Trials	EGFR	IHC
Clinical Trials	MLH1	IHC
Clinical Trials	MSH2	IHC
Clinical Trials	MSH6	IHC
Clinical Trials	PMS2	IHC
Clinical Trials	MSI	Fragment analysis
Clinical Trials	ABL1	NGS
Clinical Trials	AKT1	NGS
Clinical Trials	ALK	NGS
Clinical Trials	APC	NGS

Clinical Trials	ATM	NGS
Clinical Trials	cMET	NGS
Clinical Trials	CSF1R	NGS
Clinical Trials	CTNNB1	NGS
Clinical Trials	EGFR	NGS
Clinical Trials	ERBB2 (HER2)	NGS
Clinical Trials	FGFR1	NGS
Clinical Trials	FGFR2	NGS
Clinical Trials	FLT3	NGS
Clinical Trials	GNA11	NGS
Clinical Trials	GNAQ	NGS
Clinical Trials	GNAS	NGS
Clinical Trials	HRAS	NGS
Clinical Trials	IDH1	NGS
Clinical Trials	JAK2	NGS
Clinical Trials	KDR (VEGFR2)	NGS
Clinical Trials	MPL	NGS
Clinical Trials	NOTCH1	NGS
Clinical Trials	PTEN	NGS
Clinical Trials	SMO	NGS
Clinical Trials	TP53	NGS
Clinical Trials	VHL	NGS

[00396] Table 13 presents a view of the information that is reported for melanoma molecular intelligence molecular profiles, which can be interpreted as described for **Table 7** above.

Table 13 – Molecular Profile and Report Parameters: Melanoma

Agents/Biomarker Status Reported	Biomarker	Platform
dabrafenib, vemurafenib, trametinib	BRAF	cobas PCR
		NGS
temozolomide, dacarbazine	MGMT	IHC
imatinib	cKIT	NGS
	PDGFRA	NGS
everolimus, temsirolimus, Clinical Trials	PIK3CA	NGS
protein expression status	AR	IHC
	ER	IHC
	PR	IHC
paclitaxel, docetaxel	TLE3	IHC
	TUBB3	IHC
	Pgp	IHC
nab-paclitaxel	SPARCm	IHC
	SPARCp	IHC
doxorubicin, liposomal-doxorubicin, epirubicin	HER2	CISH
	TOP2A	IHC
	Pgp	IHC
trastuzumab, lapatinib, pertuzumab, T-DM1	HER2	IHC, CISH
gemcitabine	RRM1	IHC

irinotecan	TOPO1	IHC
fluorouracil, capecitabine, pemetrexed	TS	IHC
vandetanib	RET	NGS
carboplatin, cisplatin, oxaliplatin	BRCA1	NGS
	BRCA2	NGS
Clinical Trials	cMET	IHC, CISH
Clinical Trials	PD-1	IHC
Clinical Trials	PD-L1	IHC
Clinical Trials	EGFR	IHC
Clinical Trials	PTEN	IHC
Clinical Trials	ABL1	NGS
Clinical Trials	AKT1	NGS
Clinical Trials	ALK	NGS
Clinical Trials	APC	NGS
Clinical Trials	ATM	NGS
Clinical Trials	BRAF	NGS
Clinical Trials	cMET	NGS
Clinical Trials	CSF1R	NGS
Clinical Trials	CTNNB1	NGS
Clinical Trials	EGFR	NGS
Clinical Trials	ERBB2 (HER2)	NGS
Clinical Trials	FGFR1	NGS
Clinical Trials	FGFR2	NGS
Clinical Trials	FLT3	NGS
Clinical Trials	GNA11	NGS
Clinical Trials	GNAQ	NGS
Clinical Trials	GNAS	NGS
Clinical Trials	HRAS	NGS
Clinical Trials	IDH1	NGS
Clinical Trials	JAK2	NGS
Clinical Trials	KDR (VEGFR2)	NGS
Clinical Trials	KRAS	NGS
Clinical Trials	MPL	NGS
Clinical Trials	NOTCH1	NGS
Clinical Trials	NRAS	NGS
Clinical Trials	PTEN	NGS
Clinical Trials	SMO	NGS
Clinical Trials	TP53	NGS
Clinical Trials	VHL	NGS

[00397] Table 14 presents a view of the information that is reported for uveal melanoma molecular intelligence molecular profiles, which can be interpreted as described for **Table 7** above.

Table 14 – Molecular Profile and Report Parameters: Uveal Melanoma

Agents/Biomarker Status Reported	Biomarker	Platform
vemurafenib, dabrafenib	BRAF	NGS
temozolomide, dacarbazine	MGMT	IHC

imatinib	cKIT	NGS
	PDGFRA	NGS
everolimus, temsirolimus, Clinical Trials	PIK3CA	NGS
protein expression status	AR	IHC
	ER	IHC
	PR	IHC
paclitaxel, docetaxel	TLE3	IHC
	TUBB3	IHC
	Pgp	IHC
nab-paclitaxel	SPARCm	IHC
	SPARCp	IHC
doxorubicin, liposomal-doxorubicin, epirubicin	HER2	CISH
	TOP2A	IHC
	Pgp	IHC
trastuzumab, lapatinib, pertuzumab, T-DM1	HER2	IHC, CISH
gemcitabine	RRM1	IHC
irinotecan	TOPO1	IHC
fluorouracil, capecitabine, pemetrexed	TS	IHC
vandetanib	RET	NGS
carboplatin, cisplatin, oxaliplatin	BRCA1	NGS
	BRCA2	NGS
Clinical Trials	cMET	IHC, CISH
Clinical Trials	PD-1	IHC
Clinical Trials	PD-L1	IHC
Clinical Trials	EGFR	IHC
Clinical Trials	PTEN	IHC
Clinical Trials	ABL1	NGS
Clinical Trials	AKT1	NGS
Clinical Trials	ALK	NGS
Clinical Trials	APC	NGS
Clinical Trials	ATM	NGS
Clinical Trials	BRAF	cobas PCR
Clinical Trials	cMET	NGS
Clinical Trials	CSF1R	NGS
Clinical Trials	CTNNB1	NGS
Clinical Trials	EGFR	NGS
Clinical Trials	ERBB2 (HER2)	NGS
Clinical Trials	FGFR1	NGS
Clinical Trials	FGFR2	NGS
Clinical Trials	FLT3	NGS
Clinical Trials	GNA11	NGS
Clinical Trials	GNAQ	NGS
Clinical Trials	GNAS	NGS
Clinical Trials	HRAS	NGS
Clinical Trials	IDH1	NGS
Clinical Trials	JAK2	NGS
Clinical Trials	KDR (VEGFR2)	NGS

Clinical Trials	KRAS	NGS
Clinical Trials	MPL	NGS
Clinical Trials	NOTCH1	NGS
Clinical Trials	NRAS	NGS
Clinical Trials	PTEN	NGS
Clinical Trials	SMO	NGS
Clinical Trials	TP53	NGS
Clinical Trials	VHL	NGS

[00398] Table 15 presents a view of the information that is reported for lung cancer (including NSCLC) molecular intelligence molecular profiles, which can be interpreted as described for **Table 7** above.

Table 15 – Molecular Profile and Report Parameters: Lung Cancer

Agents/Biomarker Status Reported	Biomarker	Platform
erlotinib, gefitinib	EGFR	NGS
	KRAS	NGS
	cMET	CISH
	PIK3CA	NGS
	PTEN	IHC
afatinib	EGFR	NGS
crizotinib	ALK	FISH
	ROS1	FISH
ceritinib	ALK	FISH
pemetrexed, fluorouracil, capecitabine	TS	IHC
gemcitabine	RRM1	IHC
docetaxel, paclitaxel	TLE3	IHC
	TUBB3	IHC
	Pgp	IHC
nab-paclitaxel	SPARCm	IHC
	SPARCp	IHC
cetuximab	EGFR	IHC (H-Score)
everolimus, temsirolimus, Clinical Trials	PIK3CA	NGS
protein expression status	AR	IHC
	ER	IHC
	PR	IHC
imatinib	cKIT	NGS
	PDGFRA	NGS
doxorubicin, liposomal-doxorubicin, epirubicin	HER2	CISH
	TOP2A	IHC
	Pgp	IHC
irinotecan	TOPO1	IHC
temozolomide, dacarbazine	MGMT	IHC
vandetanib	RET	NGS
Clinical Trials	cMET	IHC
lapatinib, pertuzumab, T-DM1, Clinical Trials	HER2	IHC, CISH
trastuzumab	ERBB2 (HER2)	IHC, CISH, NGS
dabrafenib, vemurafenib	BRAF	NGS

carboplatin, cisplatin, oxaliplatin	BRCA1	NGS
	BRCA2	NGS
Clinical Trials	PD-1	IHC
Clinical Trials	PD-L1	IHC
Clinical Trials	ABL1	NGS
Clinical Trials	AKT1	NGS
Clinical Trials	ALK	NGS
Clinical Trials	APC	NGS
Clinical Trials	ATM	NGS
Clinical Trials	BRAF	NGS
Clinical Trials	cMET	NGS
Clinical Trials	CSF1R	NGS
Clinical Trials	CTNNB1	NGS
Clinical Trials	FGFR1	NGS
Clinical Trials	FGFR2	NGS
Clinical Trials	FLT3	NGS
Clinical Trials	GNA11	NGS
Clinical Trials	GNAQ	NGS
Clinical Trials	GNAS	NGS
Clinical Trials	HRAS	NGS
Clinical Trials	IDH1	NGS
Clinical Trials	JAK2	NGS
Clinical Trials	KDR (VEGFR2)	NGS
Clinical Trials	KRAS	NGS
Clinical Trials	MPL	NGS
Clinical Trials	NOTCH1	NGS
Clinical Trials	NRAS	NGS
Clinical Trials	PTEN	NGS, IHC
Clinical Trials	SMO	NGS
Clinical Trials	TP53	NGS
Clinical Trials	VHL	NGS

[00399] Table 16 presents a view of the information that is reported for ovarian cancer molecular intelligence molecular profiles, which can be interpreted as described for **Table 7** above.

Table 16 – Molecular Profile and Report Parameters: Ovarian Cancer

Agents/Biomarker Status Reported	Biomarker	Platform
carboplatin, cisplatin, oxaliplatin	ERCC1	IHC
	BRCA1	NGS
	BRCA2	NGS
docetaxel, paclitaxel	TUBB3	IHC
	Pgp	IHC
nab-paclitaxel	SPARCm	IHC
	SPARCp	IHC
irinotecan, topotecan	TOPO1	IHC
gemcitabine	RRM1	IHC
doxorubicin, liposomal-doxorubicin, epirubicin	HER2	CISH

	TOP2A	IHC
	Pgp	IHC
megestrol acetate, leuprolide, goserelin, tamoxifen, fulvestrant, toremifene, letrozole, anastrozole, exemestane	ER	IHC
	PR	IHC
pemetrexed, capecitabine, fluorouracil	TS	IHC
trastuzumab, pertuzumab, T-DM1, Clinical Trials	HER2	IHC, CISH
everolimus, temsirolimus, Clinical Trials	PIK3CA	NGS
protein expression status	AR	IHC
	TLE3	IHC
imatinib	cKIT	NGS
	PDGFRA	NGS
temozolomide, dacarbazine	MGMT	IHC
vandetanib	RET	NGS
vemurafenib, dabrafenib	BRAF	NGS
Clinical Trials	cMET	IHC, CISH
Clinical Trials	PD-1	IHC
Clinical Trials	PD-L1	IHC
Clinical Trials	EGFR	IHC
Clinical Trials	PTEN	IHC
Clinical Trials	ABL1	NGS
Clinical Trials	AKT1	NGS
Clinical Trials	ALK	NGS
Clinical Trials	APC	NGS
Clinical Trials	ATM	NGS
Clinical Trials	BRAF	NGS
Clinical Trials	cMET	NGS
Clinical Trials	CSF1R	NGS
Clinical Trials	CTNNB1	NGS
Clinical Trials	EGFR	NGS
Clinical Trials	ERBB2 (HER2)	NGS
Clinical Trials	FGFR1	NGS
Clinical Trials	FGFR2	NGS
Clinical Trials	FLT3	NGS
Clinical Trials	GNA11	NGS
Clinical Trials	GNAQ	NGS
Clinical Trials	GNAS	NGS
Clinical Trials	HRAS	NGS
Clinical Trials	IDH1	NGS
Clinical Trials	JAK2	NGS
Clinical Trials	KDR (VEGFR2)	NGS
Clinical Trials	KRAS	NGS
Clinical Trials	MPL	NGS
Clinical Trials	NOTCH1	NGS
Clinical Trials	NRAS	NGS
Clinical Trials	PTEN	NGS
Clinical Trials	SMO	NGS

Clinical Trials	TP53	NGS
Clinical Trials	VHL	NGS

[00400] Additional biomarkers that may be assessed according to the molecular profiling of the invention include BAP1 (BRCA1 Associated Protein-1 (Ubiquitin Carboxy-Terminal Hydrolase)), SETD2 (SET Domain Containing 2), PBRM1 (Polybromo 1), MLH1 (mutL homolog 1), MSH2 (mutS homolog 2), MSH6 (mutS homolog 6) and/or PMS2 (PMS2 Postmeiotic Segregation Increased 2 (S. Cerevisiae)). In some embodiments of the invention, their expression is assessed at the protein and/or mRNA level. For example, IHC can be used to assess the protein expression of one or more of these biomarkers.

[00401] Molecular profiling of the invention can include at least one of TOP2A by CISH, Chromosome 17 by CISH, PBRM1 (PB1/BAF180) by IHC, BAP1 by IHC, SETD2 (ANTI-HISTONE H3) by IHC, MDM2 by CISH, Chromosome 12 by CISH, ALK by IHC, CTLA4 by IHC, CD3 by IHC, NY-ESO-1 by IHC, MAGE-A by IHC, TP by IHC, and EGFR by CISH.

Lab Technique Substitution

[00402] One of skill will appreciate that the laboratory techniques of the molecular profiles herein can be substituted by alternative techniques if appropriate, including alternative techniques as disclosed herein or known in the art. For example, FISH and CISH are generally interchangeable methods so that one can often be used in place of the other. Similarly, Dual ISH methods such as described herein can be substituted for conventional ISH methods. In an embodiment, the FDA approved INFORM HER2 Dual ISH DNA Probe Cocktail kit from Ventana Medical Systems, Inc. (Tucson, AZ) is used for FISH/CISH analysis of HER2. This kit allows the determination of the HER2 gene status by enumeration of the ratio of the HER2 gene to Chromosome 17. The HER2 and Chromosome 17 probes are detected using two color chromogenic in situ hybridization (CISH) reactions. A number of methods can be used to assess nucleic acid sequences, and any alterations thereof, including without limitation point mutations, insertions, deletions, translocations, rearrangements. Nucleic acid analysis methods include Sanger sequencing, next generation sequencing, polymerase chain reaction (PCR), real-time PCR (qPCR; RT-PCR), a low density microarray, a DNA microarray, a comparative genomic hybridization (CGH) microarray, a single nucleotide polymorphism (SNP) microarray, fragment analysis, RFLP, pyrosequencing, methylation specific PCR, mass spec, Southern blotting, hybridization, and related methods such as described herein. Similarly, a number of methods can be used to assess gene expression, including without limitation next generation sequencing, polymerase chain reaction (PCR), real-time PCR (qPCR; RT-PCR), a low density microarray, a DNA microarray, a comparative genomic hybridization (CGH) microarray, a single nucleotide polymorphism (SNP) microarray, proteomic arrays, antibody arrays or mass spec. The presence or level of a protein can also be assessed using multiple methods as appropriate, including without limitation IHC, immunocapture, immunoblotting, Western analysis, ELISA, immunoprecipitation, flow cytometry, and the like. The desired laboratory technique can be chosen based of multiple criteria, including without limitation accuracy, precision, reproduceability, cost,

amount of sample available, type of sample available, time to perform the technique, regulatory approval status of the technique platform, regulatory approval status of the particular test, and the like.

[00403] In some embodiments, more than one technique is used to assess a same biomarker. For example, results of profiling both gene expression and protein expression can provide confirmatory results. In other cases, a certain method may provide optimal results depending on the available sample. In some embodiments, sequencing is used to assess EGFR if the sample is more than 50% tumor. Fragment analysis (FA) can also be used to assess EGFR. In some embodiments, FA, e.g., RFLP, is used to assess EGFR if the sample is less than 50% tumor. In still other cases, one technique may indicate a desire to perform another technique, e.g., a less expensive technique or one that requires lesser sample quantity may indicate a desire to perform a more expensive technique or one that consumes more sample. In an embodiment, FA of ALK is performed first, and then FISH or PCR is performed if the FA indicates the presence of a particular ALK alteration such as an ALK fusion. The FISH and/or PCR assay can be designed such that only certain fusion products are detected, e.g., EML4-ALK. The alternate methods may also provide different information about the biomarker. For example, sequence analysis may reveal the presence of a mutant protein, whereas IHC of the protein may reveal its level and/or cellular location. As another example, gene copy number or gene expression at the RNA level may be elevated, but the presence of interfering RNAs may still downregulate protein expression. As still another example, a biomarker can be assessed using a same technique but with different reagents that provide actionable results. As an example, SPARC can be assessed by IHC using either a polyclonal or a monoclonal antibody. This context is identified herein, e.g., as SPARCp, SPARC poly, or variants thereof for SPARC detected using a polyclonal antibody), and as SPARCm, SPARC mono, or variants thereof, for SPARC detected using a monoclonal antibody). SPARC (m/p) and similar derivations can be used to refer to IHC performed using both polyclonal and monoclonal antibodies.

[00404] One of skill will appreciate that molecular profiles of the invention can be updated as new evidence becomes available. For example, new evidence may appear in the literature describing an association between a treatment and potential benefit for cancer or a certain lineage of cancer. This information can be incorporated into an appropriate molecular profile. As another example, new evidence may be presented for a biomarker that is already assessed according to the invention. Consider the BRAF V600E mutation that is currently FDA approved for directed treatment with vemurafenib for melanoma. If the treatment is determined to be effective in another setting, e.g., for another lineage of cancer, BRAF V600E can be added to an appropriate molecular profile for that setting.

Mutational Analysis

[00405] Mutational or sequence analysis can be performed using any number of techniques described herein or known in the art, including without limitation sequencing (e.g., Sanger, Next Generation, pyrosequencing), PCR, variants of PCR such as RT-PCR, fragment analysis, and the like. **Table 17** describes a number of genes bearing mutations that have been identified in various cancer lineages. In an aspect, the invention provides a molecular profile comprising one or more genes in **Table 17**. In one embodiment, the genes are assessed using Next Generation sequencing methods, e.g., using a

TruSeq/MiSeq/HiSeq/NexSeq system offered by Illumina Corporation or an Ion Torrent system from Life Technologies. One of skill will appreciate that the profiling may be used to identify candidate treatments for cancer lineages other than those described in **Table 17**. Clinical trials in the table can be found at www.clinicaltrials.gov using the indicated identifiers.

Table 17: Exemplary Mutated Genes and Gene Products and Related Therapies

Biomarker	Description
ABL1	ABL1 also known as Abelson murine leukemia homolog 1. Most CML patients have a chromosomal abnormality due to a fusion between Abelson (Abl) tyrosine kinase gene at chromosome 9 and break point cluster (Bcr) gene at chromosome 22 resulting in constitutive activation of the Bcr-Abl fusion gene. Imatinib is a Bcr-Abl tyrosine kinase inhibitor commonly used in treating CML patients. Mutations in the ABL1 gene are common in imatinib resistant CML patients which occur in 30-90% of patients. However, more than 50 different point mutations in the ABL1 kinase domain may be inhibited by the second generation kinase inhibitors, dasatinib, bosutinib and nilotinib. The gatekeeper mutation, T315I that causes resistance to all currently approved TKIs accounts for about 15% of the mutations found in patients with imatinib resistance. BCR-ABL1 mutation analysis is recommended to help facilitate selection of appropriate therapy for patients with CML after treatment with imatinib fails. Various clinical trials (on www.clinicaltrials.gov) investigating agents which target this gene may be available for ABL1 mutated patients.
AKT1	AKT1 gene (v-akt murine thymoma viral oncogene homologue 1) encodes a serine/threonine kinase which is a pivotal mediator of the PI3K-related signaling pathway, affecting cell survival, proliferation and invasion. Dysregulated AKT activity is a frequent genetic defect implicated in tumorigenesis and has been indicated to be detrimental to hematopoiesis. Activating mutation E17K has been described in breast (2-4%), endometrial (2-4%), bladder cancers (3%), NSCLC (1%), squamous cell carcinoma of the lung (5%) and ovarian cancer (2%). This mutation in the pleckstrin homology domain facilitates the recruitment of AKT to the plasma membrane and subsequent activation by altering phosphoinositide binding. A mosaic activating mutation E17K has also been suggested to be the cause of Proteus syndrome. Mutation E49K has been found in bladder cancer, which enhances AKT activation and shows transforming activity in cell lines. Various clinical trials (on www.clinicaltrials.gov) investigating AKT inhibitor in patients carrying AKT mutations may be available.
ALK	ALK or anaplastic lymphoma receptor tyrosine kinase belongs to the insulin receptor superfamily. It has been found to be rearranged or mutated in tumors including anaplastic large cell lymphomas, neuroblastoma, anaplastic thyroid cancer and non-small cell lung cancer. EML4-ALK fusion or point mutations of ALK result in the constitutively active ALK kinase, causing aberrant activation of downstream signaling pathways including RAS-ERK, JAK3-STAT3 and PI3K-AKT. Patients with an EML4-ALK rearrangement are likely to respond to the ALK-targeted agent crizotinib and ceritinib. ALK secondary mutations found in NSCLC have been associated with acquired resistance to ALK inhibitor, crizotinib and ceritinib. Various clinical trials (on www.clinicaltrials.gov) investigating agents may be available for patients with ALK mutation.
APC	APC or adenomatous polyposis coli is a key tumor suppressor gene that encodes for a large multi-domain protein. This protein exerts its tumor suppressor function in the Wnt/ß-catenin cascade mainly by controlling the degradation of ß-catenin, the central activator of transcription in the Wnt signaling pathway. The Wnt signaling pathway mediates important cellular functions including intercellular adhesion, stabilization of the cytoskeleton, and cell cycle regulation and apoptosis, and it is important in embryonic development and oncogenesis. Mutation in APC results in a truncated protein product with abnormal function, lacking the domains involved in ß-catenin degradation. Somatic mutation in the APC gene can be detected in the majority of colorectal tumors (80%) and it is an early event in colorectal tumorigenesis. APC wild type patients have shown better disease control rate in the metastatic setting when treated with oxaliplatin, while when treated with fluoropyrimidine regimens, APC wild type patients experience more hematological

	toxicities. APC mutation has also been identified in oral squamous cell carcinoma, gastric cancer as well as hepatoblastoma and may contribute to cancer formation. Various clinical trials (on www.clinicaltrials.gov) investigating agents which target this gene and/or its downstream or upstream effectors maybe available for APC mutated patients. Germline mutation in APC causes familial adenomatous polyposis, which is an autosomal dominant inherited disease that will inevitably develop to colorectal cancer if left untreated. COX-2 inhibitors including celecoxib may reduce the recurrence of adenomas and incidence of advanced adenomas in individuals with an increased risk of CRC. Turcot syndrome and Gardner's syndrome have also been associated with germline APC defects. Germline mutations of the APC have also been associated with an increased risk of developing desmoid disease, papillary thyroid carcinoma and hepatoblastoma.
ATM	ATM or ataxia telangiectasia mutated is activated by DNA double-strand breaks and DNA replication stress. It encodes a protein kinase that acts as a tumor suppressor and regulates various biomarkers involved in DNA repair, which include p53, BRCA1, CHK2, RAD17, RAD9, and NBS1. Although ATM is associated with hematologic malignancies, somatic mutations have been found in colon (18%), head and neck (14%), and prostate (12%) cancers. Inactivating ATM mutations make patients potentially more susceptible to PARP inhibitors. Various clinical trials (on www.clinicaltrials.gov) investigating agents which target this gene and/or its downstream or upstream effectors may be available for ATM mutated patients. Germline mutations in ATM are associated with ataxia-telangiectasia (also known as Louis-Bar syndrome) and a predisposition to malignancy.
BRAF	BRAF encodes a protein belonging to the raf/mil family of serine/threonine protein kinases. This protein plays a role in regulating the MAP kinase/ERK signaling pathway initiated by EGFR activation, which affects cell division, differentiation, and secretion. BRAF somatic mutations have been found in melanoma (43%), thyroid (39%), biliary tree (14%), colon (12%), and ovarian tumors (12%). Patients with V600E BRAF mutation have a reduced likelihood of response to EGFR targeted monoclonal antibodies in colorectal cancer and sensitivity to BRAF inhibitors, vemurafenib and dabrafenib, and MEK1/2 inhibitor, trametinib in various solid tumors. Various clinical trials (on www.clinicaltrials.gov) investigating agents which target this gene may be available for BRAF mutated patients. BRAF inherited mutations are associated with Noonan/Cardio-Facio-Cutaneous (CFC) syndrome, syndromes associated with short stature, distinct facial features, and potential heart/skeletal abnormalities.
c-KIT	c-KIT is a receptor tyrosine kinase expressed by hematopoietic stem cells, interstitial cells of cajal (pacemaker cells of the gut) and other cell types. Upon binding of c-KIT to stem cell factor (SCF), receptor dimerization initiates a phosphorylation cascade resulting in proliferation, apoptosis, chemotaxis and adhesion. C-KIT mutation has been identified in various cancer types including gastrointestinal stromal tumors (GIST) (up to 85%) and melanoma (chronic sun damage type, acral or mucosal) (20-40%). C-KIT is inhibited by multi-targeted agents including imatinib and sunitinib. Various clinical trials (on www.clinicaltrials.gov) investigating agents which target this gene and/or its downstream or upstream effectors may be available for c-KIT mutated patients. Germline mutations in c-KIT have been associated with multiple gastrointestinal stromal tumors (GIST) and Piebald trait.
CDH1	CDH1 (epithelial cadherin/E-cad) encodes a transmembrane calcium dependent cell adhesion glycoprotein that plays a major role in epithelial architecture, cell adhesion and cell invasion. Loss of function of CDH1 contributes to cancer progression by increasing proliferation, invasion, and/or metastasis. Various somatic mutations in CDH1 have been identified in diffuse gastric, lobular breast, endometrial and ovarian carcinomas; the resultant loss of function of E-cad may contribute to tumor growth and progression. Germline mutations in CDH1 cause hereditary diffuse gastric cancer and colorectal cancer;

	affected women are predisposed to lobular breast cancer with a risk of about 50%. CDH1 mutation carriers have an estimated cumulative risk of gastric cancer of 67% for men and 83% for women, by age of 80 years.
cMET	C-Met is a proto-oncogene that encodes the tyrosine kinase receptor of hepatocyte growth factor (HGF) or scatter factor (SF). c-Met mutation causes aberrant MET signaling in various cancer types including renal papillary, hepatocellular, head and neck squamous, gastric carcinomas and non-small cell lung cancer. Mutations in the juxtamembrane domain (exon 14, 15) results in the constitutive activation and show enhanced tumorigenicity. Various clinical trials (on www.clinicaltrials.gov) investigating c-MET inhibitors in patients carrying MET mutations may be available. Germline mutations in c-MET have been associated with hereditary papillary renal cell carcinoma.
CSF1R	CSF1R or colony stimulating factor 1 receptor gene encodes a transmembrane tyrosine kinase, a member of the CSF1/PDGF receptor family. CSF1R mediates the cytokine (CSF-1) responsible for macrophage production, differentiation, and function. Although associated with hematologic malignancies, mutations of this gene are associated with cancers of the liver (21%), colon (13%), prostate (3%), endometrium (2%), and ovary (2%). It is suggested that patients with CSF1R mutations could respond to imatinib. Various clinical trials (on www.clinicaltrials.gov) investigating agents may be available for CSF1R mutated patients. Germline mutations in CSF1R are associated with diffuse leukoencephalopathy, a rapidly progressive neurodegenerative disorder.
CTNNB1	CTNNB1 or cadherin-associated protein, beta 1, encodes for ß-catenin, a central mediator of the Wnt signaling pathway which regulates cell growth, migration, differentiation and apoptosis. Mutations in CTNNB1 (often occurring in exon 3) prevent the breakdown of ß-catenin, which allows the protein to accumulate resulting in persistent transactivation of target genes, including c-myc and cyclin-D1. Somatic CTNNB1 mutations occur in 1-4% of colorectal cancers, 2-3% of melanomas, 25-38% of endometrioid ovarian cancers, 84-87% of sporadic desmoid tumors, as well as the pediatric cancers, hepatoblastoma, medulloblastoma and Wilms' tumors. A growing number of compounds that suppress the Wnt/ß-catenin pathway are available in clinical trials for CTNNB1 mutated patients.
EGFR	EGFR, or epidermal growth factor receptor, is a transmembrane receptor tyrosine kinase belonging to the ErbB family of receptors. Upon ligand binding, the activated receptor triggers a series of intracellular pathways (Ras/MAPK, PI3K/Akt, JAK-STAT) that result in cell proliferation, migration and adhesion. EGFR mutations have been observed in 20-25% of non-small cell lung cancer (NSCLC), 10% of endometrial and peritoneal cancers. Somatic gain-of-function EGFR mutations, including in-frame deletions in exon 19 or point mutations in exon 21, confer sensitivity to first- and second-generation tyrosine kinase inhibitors (TKIs), whereas the secondary mutation, T790M in exon 20, confers reduced response. New agents and novel combination therapies are being explored (www.clinicaltrials.gov) for EGFR mutated patients. Germline mutations and polymorphisms of EGFR have been associated with familial lung adenocarcinomas.
ERBB2	ERBB2 (HER2) or v-erb-b2 erythroblastic leukemia viral oncogene homolog 2, encodes a member of the epidermal growth factor (EGF) receptor family of receptor tyrosine kinases. This gene binds to other ligand-bound EGF receptor family members to form a heterodimer and enhances kinase-mediated activation of downstream signaling pathways, leading to cell proliferation. Most common mechanism for activation of HER2 are gene amplification and over-expression with somatic mutations being rare. NCCN NSCLC guidelines recommend trastuzumab for activity against HER2 mutations in patients with NSCLC. Various clinical trials (on www.clinicaltrials.gov) investigating agents which target this gene may be available for patients with ERBB2 mutation.
ERBB4	ERBB4 is a member of the Erbb receptor family known to play a pivotal role in cell-cell signaling and signal transduction regulating cell growth and development. The most commonly affected signaling pathways are the PI3K-Akt and MAP kinase pathways. Erbb4

	was found to be somatically mutated in 19% of melanomas and Erbb4 mutations may confer "oncogene addiction" on melanoma cells. Erbb4 mutations have also been observed in various other cancer types, including, gastric carcinomas (2%), colorectal carcinomas (1-3%), non-small cell lung cancer (2–5%) and breast carcinomas (1%), however, their biological impact is not uniform or consistent across these cancers.
FBXW7	FBXW7 or E3 ligase F-box and WD repeat domain containing 7, also known as Cdc4, encodes three protein isoforms which constitute a component of the ubiquitin-proteasome complex. Mutation of FBXW7 occurs in hotspots and disrupts the recognition of and binding with substrates which inhibits the proper targeting of proteins for degradation (e.g. Cyclin E, c-Myc, SREBP1, c-Jun, Notch-1, mTOR and MCL1). Mutation frequencies identified in cholangiocarcinomas, acute T-lymphoblastic leukemia/lymphoma, and carcinomas of endometrium, colon and stomach are 35%, 31%, 9%, 9%, and 6%, respectively. Targeting an oncoprotein downstream of FBXW7, such as mTOR or c-Myc, may provide a novel therapeutic strategy.
FGFR1	FGFR1 or fibroblast growth factor receptor 1, encodes for FGFR1 which is important for cell division, regulation of cell maturation, formation of blood vessels, wound healing and embryonic development. Somatic activating mutations are rare, but have been documented in melanoma, glioblastoma, and lung tumors. FGFR1-targeted agents under clinical investigation (on www.clinicaltrials.gov) may be available for FGFR1 mutated patients. Germline, gain-of-function mutations in FGFR1 result in developmental disorders including Kallmann syndrome and Pfeiffer syndrome.
FGFR2	FGFR2 is a receptor for fibroblast growth factor. Activation of FGFR2 through mutation and amplification has been noted in a number of cancers. Somatic mutations of the fibroblast growth factor receptor 2 (FGFR2) tyrosine kinase are present in endometrial carcinoma, lung squamous cell carcinoma, cervical carcinoma, and melanoma. In the endometrioid histology of endometrial cancer, the frequency of FGFR2 mutation is 16% and the mutation is associated with shorter disease free survival in patients diagnosed with early stage disease. Loss of function FGFR2 mutations occur in about 8% melanomas and contribute to melanoma pathogenesis. Various clinical trials (on www.clinicaltrials.gov) investigating agents which target this gene may be available for FGFR2 mutated patients. Germline mutations in FGFR2 are associated with numerous medical conditions that include congenital craniofacial malformation disorders, Apert syndrome and the related Pfeiffer and Crouzon syndromes.
FLT3	FLT3 or Fms-like tyrosine kinase 3 receptor is a member of class III receptor tyrosine kinase family, which includes PDGFRA/B and KIT. Signaling through FLT3 ligand-receptor complex regulates hematopoiesis, specifically lymphocyte development. The FLT3 internal tandem duplication (FLT3-ITD) is the most common genetic lesion in acute myeloid leukemia (AML), occurring in 25% of cases. FLT3 mutations are rare in solid tumors; however they have been documented in breast cancer. Several small molecule multikinase inhibitors targeting the RTK-III family are available (on www.clinicaltrials.gov) for FLT3 mutated patients.
GNA11	GNA11 is a proto-oncogene that belongs to the Gq family of the G alpha family of G protein coupled receptors. Known downstream signaling partners of GNA11 are phospholipase C beta and RhoA and activation of GNA11 induces MAPK activity. Over half of uveal melanoma patients lacking a mutation in GNAQ exhibit somatic mutations in GNA11. Activating mutations of GNA11 have not been found in other malignancies. Various clinical trials (on www.clinicaltrials.gov) investigating agents which target this gene may be available for GNA11 mutated patients.
GNAQ	This gene encodes the Gq alpha subunit of G proteins. G proteins are a family of heterotrimeric proteins coupling seven-transmembrane domain receptors. Oncogenic mutations in GNAQ result in a loss of intrinsic GTPase activity, resulting in a constitutively active Galpha subunit. This results in increased signaling through the MAPK pathway. Somatic mutations in GNAQ have been found in 50% of primary uveal melanoma patients and up to 28% of uveal melanoma metastases. Various clinical trials (on www.clinicaltrials.gov) investigating agents which target this gene may be available for

	GNAQ mutated patients.
GNAS	GNAS (or GNAS complex locus) encodes a stimulatory G protein alpha-subunit. These guanine nucleotide binding proteins (G proteins) are a family of heterotrimeric proteins which couple seven-transmembrane domain receptors to intracellular cascades. Stimulatory G-protein alpha-subunit transmits hormonal and growth factor signals to effector proteins and is involved in the activation of adenylate cyclases. Mutations of GNAS gene at codons 201 or 227 lead to constitutive cAMP signaling. GNAS somatic mutations have been found in pituitary (28%), pancreatic (20%), ovarian (11%), adrenal gland (6%), and colon (6%) cancers. Patients with somatic GNAS mutations may derive benefit from clinical trials with MEK inhibitors. Germline mutations of GNAS have been shown to be the cause of McCune-Albright syndrome (MAS), a disorder marked by endocrine, dermatologic, and bone abnormalities. GNAS is usually found as a mosaic mutation in patients. Loss of function mutations are associated with pseudohypoparathyroidism and pseudopseudohypoparathyroidism.
HNF1A	HNF1A or hepatocyte nuclear factor 1 homeobox A encodes a transcription factor that is highly expressed in the liver, found on chromosome 12. It regulates a large number of genes, including those for albumin, alpha1-antitrypsin, and fibrinogen. HNF1A has been associated with an increased risk of pancreatic cancer. HNF1A somatic mutations are found in liver (30%), colon (15%), endometrium (11%), and ovarian (3%) cancers. Its prognostic and predictive value is under investigation. Germline mutations of HNF1A are associated with maturity-onset diabetes of the young type 3.
HRAS	HRAS (homologous to the oncogene of the Harvey rat sarcoma virus), together with KRAS and NRAS, belong to the superfamily of RAS GTPase. RAS protein activates RAS-MEK-ERK/MAPK kinase cascade and controls intracellular signaling pathways involved in fundamental cellular processes such as proliferation, differentiation, and apoptosis. Mutant Ras proteins are persistently GTP-bound and active, causing severe dysregulation of the effector signaling. HRAS mutations have been identified in cancers from the urinary tract (10%-40%), skin (6%) and thyroid (4%) and they account for 3% of all RAS mutations identified in cancer. RAS mutations (especially HRAS mutations) occur (5%) in cutaneous squamous cell carcinomas and keratoacanthomas that develop in patients treated with BRAF inhibitor vemurafenib, likely due to the paradoxical activation of the MAPK pathway. Various clinical trials (on www.clinicaltrials.gov) investigating agents which target this gene and/or its downstream or upstream effectors may be available for HRAS mutated patients. Germline mutation in HRAS has been associated with Costello syndrome, a genetic disorder that is characterized by delayed development and mental retardation and distinctive facial features and heart abnormalities.
IDH1	IDH1 encodes for isocitrate dehydrogenase in cytoplasm and is found to be mutated in 60-90% of secondary gliomas, 75% of cartilaginous tumors, 17% of thyroid tumors, 15% of cholangiocarcinoma, 12-18% of patients with acute myeloid leukemia, 5% of primary gliomas, 3% of prostate cancer, as well as in less than 2% in paragangliomas, colorectal cancer and melanoma. Mutated IDH1 results in impaired catalytic function of the enzyme, thus altering normal physiology of cellular respiration and metabolism. IDH1 mutation can also cause overproduction of onco-metabolite 2-hydroxy-glutarate, which can extensively alter the methylation profile in cancer. In gliomas, IDH1 mutations are associated with lower-grade astrocytomas and oligodendrogliomas (grade II/III), as well as secondary glioblastoma. IDH gene mutations are associated with markedly better survival in patients diagnosed with malignant astrocytoma; and clinical data support a more aggressive surgery for IDH1 mutated patients because these individuals may be able to achieve long-term survival. In contrast, IDH1 mutation is associated with a worse prognosis in AML. In glioblastoma, IDH1 mutation has been associated with significantly better response to alkylating agent temozolomide. Various clinical trials (on www.clinicaltrials.gov) investigating agents which target this gene and/or its downstream or upstream effectors may be available for IDH1 mutated patients.

JAK2	JAK2 or Janus kinase 2 is a part of the JAK/STAT pathway which mediates multiple cellular responses to cytokines and growth factors including proliferation and cell survival. It is also essential for numerous developmental and homeostatic processes, including hematopoiesis and immune cell development. Mutations in the JAK2 kinase domain result in constitutive activation of the kinase and the development of chronic myeloproliferative neoplasms such as polycythemia vera (95%), essential thrombocythemia (50%) and myelofibrosis (50%). JAK2 mutations were also found in BCR-ABL1-negative acute lymphoblastic leukemia patients and the mutated patients show a poor outcome. Various clinical trials (on www.clinicaltrials.gov) investigating agents which target this gene and/or its downstream or upstream effectors may be available for patients carrying JAK2 mutation. Germline mutations in JAK2 have been associated with myeloproliferative neoplasms and thrombocythemia.
JAK3	JAK3 or Janus activated kinase 3 is an intracellular tyrosine kinase involved in cytokine signaling, while interacting with members of the STAT family. Like JAK1, JAK2, and TYK2, JAK3 is a member of the JAK family of kinases. When activated, kinase enzymes phosphorylate one or more signal transducer and activator of transcription (STAT) factors, which translocate to the cell nucleus and regulate the expression of genes associated with survival and proliferation. JAK3 signaling is related to T cell development and proliferation. This biomarker is found in malignancies like head and neck (21%) colon (7%), prostate (5%), ovary (4%), breast (2%), lung (1%), and stomach (1%) cancer. Its prognostic and predictive utility is under investigation. Germline mutations of JAK3 are associated with severe, combined immunodeficiency disease (SCID).
KDR	KDR (VEGFR2) or Kinase insert domain receptor gene, also known as vascular endothelial growth factor receptor-2 (VEGFR2), is involved with angiogenesis and is expressed on almost all endothelial cells. VEGF ligands bind to KDR, which leads to receptor dimerization and signal transduction. Besides somatic mutations in angiosarcoma (10%), somatic KDR mutations have also been found in colon (13%), skin (13%), gastric (5%), lung (3%), renal (2%), and ovarian (2%) cancers. Several VEGFR antagonists are either FDA-approved or in clinical trials (i.e. bevacizumab, cabozantinib, regorafenib, pazopanib, and vandetanib). Various clinical trials (on www.clinicaltrials.gov) investigating agents which target this gene and/or its downstream or upstream effectors may be available for KDR mutated patients.
KRAS	KRAS or V-Ki-ras2 Kirsten rat sarcoma viral oncogene homolog encodes a signaling intermediate involved in many signaling cascades including the EGFR pathway. KRAS somatic mutations have been found in pancreatic (57%), colon (35%), lung (16%), biliary tract (28%), and endometrial (15%) cancers. Mutations at activating hotspots are associated with resistance to EGFR tyrosine kinase inhibitors (erlotinib, gefitinib) in NSCLC and monoclonal antibodies (cetuximab, panitumumab) in CRC patients. Patients with KRAS G13D mutation have been shown to derive benefit from anti-EGFR monoclonal antibody therapy in CRC patients. Various clinical trials (on www.clinicaltrials.gov) investigating agents which target this gene may be available for KRAS mutated patients. Several germline mutations of KRAS (V14I, T58I, and D153V amino acid substitutions) are associated with Noonan syndrome.
MLH1	MLH1 or mutL homolog 1, colon cancer, nonpolyposis type 2 (E. coli) gene encodes a mismatch repair (MMR) protein which repairs DNA mismatches that occur during replication. Although the frequency is higher in colon cancer (10%), MLH1 somatic mutations have been found in esophageal (6%), ovarian (5%), urinary tract (5%), pancreatic (5%), and prostate (5%) cancers. Its prognostic and predictive utility is under investigation. Germline mutations of MLH1 are associated with Lynch syndrome, also known as hereditary non-polyposis colorectal cancer (HNPCC). Patients with Lynch syndrome are at increased risk for various malignancies, including intestinal, gynecologic, and upper urinary tract cancers and in its variant, Muir-Torre syndrome, with sebaceous tumors.
MPL	MPL or myeloproliferative leukemia gene encodes the thrombopoietin receptor, which is the

	main humoral regulator of thrombopoiesis in humans. MPL mutations cause constitutive activation of JAK-STAT signaling and have been detected in 5-7% of patients with primary myelofibrosis (PMF) and 1% of those with essential thrombocythemia (ET).
NOTCH1	NOTCH1 or notch homolog 1, translocation-associated, encodes a member of the Notch signaling network, an evolutionary conserved pathway that regulates developmental processes by regulating interactions between physically adjacent cells. Mutations in NOTCH1 play a central role in disruption of micro environmental communication, potentially leading to cancer progression. Due to the dual, bi-directional signaling of NOTCH1, activating mutations have been found in acute lymphoblastic leukemia and chronic lymphocytic leukemia, however loss of function mutations in NOTCH1 are prevalent in 11-15% of head and neck squamous cell carcinoma. NOTCH1 mutations have also been found in 2% of glioblastomas, 1% of ovarian cancers, 10% lung adenocarcinomas, 8% of squamous cell lung cancers and 5% of breast cancers. Notch pathway-directed therapy approaches differ depending on whether the tumor harbors gain or loss of function mutations, thus are classified as Notch pathway inhibitors or activators, respectively. Some Notch pathway modulators are being investigated (on www.clinicaltrials.gov) for NOTCH1 mutated patients.
NPM1	NPM1 or nucleophosmin is a nucleolar phosphoprotein belonging to a family of nuclear chaperones with proliferative and growth-suppressive roles. In several hematological malignancies, the NPM locus is lost or translocated, leading to expression of oncogenic proteins. NPM1 is mutated in one-third of patients with adult acute myeloid leukemia (AML) leading to activation of downstream pathways including JAK/STAT, RAS/ERK, and PI3K. Although there are few NPM-directed therapies currently being investigated, research shows AML tumor cells with mutant NPM are more sensitive to chemotherapeutic agents, including daunorubicin and camptothecin.
NRAS	NRAS is an oncogene and a member of the (GTPase) ras family, which includes KRAS and HRAS. This biomarker has been detected in multiple cancers including melanoma (15%), colorectal cancer (4%), AML (10%) and bladder cancer (2%). Evidence suggests that an acquired mutation in NRAS may be associated with resistance to vemurafenib in melanoma patients. In colorectal cancer patients NRAS mutation is associated with resistance to EGFR-targeted monoclonal antibodies. Various clinical trials (on www.clinicaltrials.gov) investigating agents which target this gene and/or its downstream or upstream effectors may be available for NRAS mutated patients. Germline mutations in NRAS have been associated with Noonan syndrome, autoimmune lymphoproliferative syndrome and juvenile myelomonocytic leukemia.
PDGFRA	PDGFRA is the alpha-type platelet-derived growth factor receptor, a surface tyrosine kinase receptor structurally homologous to c-KIT, which activates PIK3CA/AKT, RAS/MAPK and JAK/STAT signaling pathways. PDGFRA mutations are found in 5-8% of patients with gastrointestinal stromal tumors (GIST) and increases to 30% in KIT wildtype GIST. PDGFRA mutations in exons 12, 14 and 18 confer imatinib sensitivity, while the substitution mutation in exon 18 (D842V) shows resistance to imatinib. Various clinical trials (on www.clinicaltrials.gov) investigating multikinase inhibitors may be available for PDGFRA mutated patients. Germline mutations in PDGFRA have been associated with Familial gastrointestinal stromal tumors and Hypereosinophillic Syndrome (HES).
PIK3CA	PIK3CA or phosphoinositide-3-kinase catalytic alpha polypeptide encodes a protein in the PI3 kinase pathway. This pathway is an active target for drug development. PIK3CA somatic mutations have been found in breast (26%), endometrial (23%), urinary tract (19%), colon (13%), and ovarian (11%) cancers. Somatic mosaic activating mutations in PIK3CA are said to cause CLOVES syndrome. PIK3CA exon 20 mutations have been associated with benefit from mTOR inhibitors (everolimus, temsirolimus). Evidence suggests that breast cancer patients with PIK3CA mutation have a significantly shorter survival following trastuzumab treatment. PIK3CA mutated colorectal cancer patients are less likely to respond to EGFR targeted monoclonal antibody therapy. Various clinical trials (on www.clinicaltrials.gov) investigating agents which target this gene may be available for PIK3CA mutated patients.

PTEN	PTEN or phosphatase and tensin homolog is a tumor suppressor gene that prevents cells from proliferating. PTEN is an important mediator in signaling downstream of EGFR, and loss of PTEN gene function/expression due to gene mutations or allele loss is associated with reduced benefit to EGFR-targeted monoclonal antibodies. Mutation in PTEN is found in 5-14% of colorectal cancer and 7% of breast cancer. PTEN mutation leads to loss of function of the encoded phosphatase, and an upregulation of the PIK3CA/AKT pathway. Various clinical trials (on www.clinicaltrials.gov) investigating agents which target this gene and/or its downstream or upstream effectors may be available for patients with PTEN alteration. Germline PTEN mutations associate with Cowden disease and Bannayan-Riley-Ruvalcaba syndrome. These dominantly inherited disorders belong to a family of hamartomatous polyposis syndromes which feature multiple tumor-like growths (hamartomas) accompanied by an increased risk of breast carcinoma, follicular carcinoma of the thyroid, glioma, prostate and endometrial cancer. Trichilemmoma, a benign, multifocal neoplasm of the skin is also associated with PTEN germline mutations.
PTPN11	PTPN11 or tyrosine-protein phosphatase non-receptor type 11 is a proto-oncogene that encodes a signaling molecule, Shp-2, which regulates various cell functions like mitogenic activation and transcription regulation. PTPN11 gain-of-function somatic mutations have been found to induce hyperactivation of the Akt and MAPK networks. Because of this hyperactivation, Ras effectors, such as Mek and PI3K, are potential targets for novel therapeutics in those with PTPN11 gain-of-function mutations. PTPN11 somatic mutations are found in hematologic and lymphoid malignancies (8%), gastric (2%), colon (2%), ovarian (2%), and soft tissue (2%) cancers. Germline mutations of PTPN11 are associated with Noonan syndrome, which itself is associated with juvenile myelomonocytic leukemia (JMML). PTPN11 is also associated with LEOPARD syndrome, which is associated with neuroblastoma and myeloid leukemia.
RB1	RB1 or retinoblastoma-1 is a tumor suppressor gene whose protein regulates the cell cycle by interacting with various transcription factors, including the E2F family (which controls the expression of genes involved in the transition of cell cycle checkpoints). Besides ocular cancer, RB1 mutations have also been detected in other malignancies, such as ovarian (10%), bladder (41%), prostate (8%), breast (6%), brain (6%), colon (5%), and renal (2%) cancers. RB1 status, along with other mitotic checkpoints, has been associated with the prognosis of GIST patients. Germline mutations of RB1 are associated with the pediatric tumor, retinoblastoma. Inherited retinoblastoma is usually bilateral. Studies indicate patients with a history of retinoblastoma are at increased risk for secondary malignancies.
RET	RET or rearranged during transfection gene, located on chromosome 10, activates cell signaling pathways involved in proliferation and cell survival. RET mutations are found in 23-69% of sporadic medullary thyroid cancers (MTC), but RET fusions are common in papillary thyroid cancer, and more recently have been found in 1-2% of lung adenocarcinoma. Amongst RET mutations in sporadic MTC, 85% involve the M918T mutation which is associated with a higher response rate to vandetanib in comparison to M918T negative patients. Further, a 10-year study notes that medullary thyroid cancer patients with somatic RET mutations have a poorer prognosis. Various clinical trials (on www.clinicaltrials.gov) investigating multikinase inhibitors which include RET as one of the targets may be available for RET mutated patients. Germline activating mutations of RET are associated with multiple endocrine neoplasia type 2 (MEN2), which is characterized by the presence of medullary thyroid carcinoma, bilateral pheochromocytoma, and primary hyperparathyroidism. Germline inactivating mutations of RET are associated with Hirschsprung's disease.
SMAD4	SMAD4 or mothers against decapentaplegic homolog 4, is one of eight proteins in the SMAD family, involved in multiple signaling pathways and are key modulators of the transcriptional responses to the transforming growth factor-ß (TGFß) receptor kinase complex. SMAD4 resides on chromosome 18q21, one of the most frequently deleted

	chromosomal regions in colorectal cancer. Smad4 stabilizes Smad DNA-binding complexes and also recruits transcriptional coactivators such as histone acetyltransferases to regulatory elements. Dysregulation of SMAD4 occurs late in tumor development, and occurs through mutations of the MH1 domain which inhibits the DNA-binding function, thus dysregulating TGFßR signaling. Mutated (inactivated) SMAD4 is found in 50% of pancreatic cancers and 10-35% of colorectal cancers. Germline mutations in SMAD4 are associated with juvenile polyposis (JP) and combined syndrome of JP and hereditary hemorrhagic teleangiectasia (JP-HHT).
SMARCB1	SMARCB1 also known as SWI/SNF related, matrix associated, actin dependent regulator of chromatin, subfamily b, member 1, is a tumor suppressor gene implicated in cell growth and development. Loss of expression of SMARCB1 has been observed in tumors including epithelioid sarcoma, renal medullary carcinoma, undifferentiated pediatric sarcomas, and a subset of hepatoblastomas. Germline mutation in SMARCB1 causes about 20% of all rhabdoid tumors which makes it important for clinicians to facilitate genetic testing and refer families for genetic counseling. Germline SMARCB1 mutations have also been identified as the pathogenic cause of a subset of schwannomas and meningiomas.
SMO	SMO (smoothened) is a G protein-coupled receptor which plays an important role in the Hedgehog signaling pathway. It is a key regulator of cell growth and differentiation during development, and is important in epithelial and mesenchymal interaction in many tissues during embryogenesis. Dysregulation of the Hedgehog pathway is found in cancers including basal cell carcinomas (12%) and medulloblastoma (1%). A gain-of-function mutation in SMO results in constitutive activation of hedgehog pathway signaling, contributing to the genesis of basal cell carcinoma. SMO mutations have been associated with the resistance to SMO antagonist GDC-0449 in medulloblastoma patients by blocking the binding to SMO. SMO mutation may also contribute partially to resistance to SMO antagonist LDE225 in BCC. Various clinical trials (on www.clinicaltrials.gov) investigating SMO antagonists may be available for SMO mutated patients.
STK11	STK11 also known as LKB1, is a serine/threonine kinase. It is thought to be a tumor suppressor gene which acts by interacting with p53 and CDC42. It modulates the activity of AMP-activated protein kinase, causes inhibition of mTOR, regulates cell polarity, inhibits the cell cycle, and activates p53. Somatic mutations in this gene are associated with a history of smoking and KRAS mutation in NSCLC patients. The frequency of STK11 mutation in lung adenocarcinomas ranges from 7%-30%. STK11 loss may play a role in development of metastatic disease in lung cancer patients. Mutations of this gene also drive progression of HPV-induced dysplasia to invasive, cervical cancer and hence STK11 status may be exploited clinically to predict the likelihood of disease recurrence. Germline mutations in STK11 are associated with Peutz-Jeghers syndrome which is characterized by early onset hamartomatous gastro-intestinal polyps and increased risk of breast, colon, gastric and ovarian cancer.
TP53	TP53, or p53, plays a central role in modulating response to cellular stress through transcriptional regulation of genes involved in cell-cycle arrest, DNA repair, apoptosis, and senescence. Inactivation of the p53 pathway is essential for the formation of the majority of human tumors. Mutation in p53 (TP53) remains one of the most commonly described genetic events in human neoplasia, estimated to occur in 30-50% of all cancers. Generally, presence of a disruptive p53 mutation is associated with a poor prognosis in all types of cancers, and diminished sensitivity to radiation and chemotherapy. In addition, various clinical trials (on www.clinicaltrials.gov) investigating agents which target p53's downstream or upstream effectors may have clinical utility depending on the p53 status. Germline p53 mutations are associated with the Li-Fraumeni syndrome (LFS) which may lead to early-onset of several forms of cancer currently known to occur in the syndrome, including sarcomas of the bone and soft tissues, carcinomas of the breast and adrenal cortex (hereditary adrenocortical carcinoma), brain tumors and acute leukemias.

VHL	VHL or von Hippel-Lindau gene encodes for tumor suppressor protein pVHL, which polyubiquitylates hypoxia-inducible factor. Absence of pVHL causes stabilization of HIF and expression of its target genes, many of which are important in regulating angiogenesis, cell growth and cell survival. VHL somatic mutation has been seen in 20-70% of patients with sporadic clear cell renal cell carcinoma (ccRCC) and the mutation may imply a poor prognosis, adverse pathological features, and increased tumor grade or lymph-node involvement. Renal cell cancer patients with a 'loss of function' mutation in VHL show a higher response rate to therapy (bevacizumab or sorafenib) than is seen in patients with wild type VHL, however the mutation is not associated with improvement in progression free survival or overall survival. Various clinical trials (on www.clinicaltrials.gov) investigating angiogenesis inhibitors in various cancer types may be available for VHL mutated patients. Germline mutations in VHL cause von Hippel-Lindau syndrome, associated with clear-cell renal-cell carcinomas, central nervous system hemangioblastomas, pheochromocytomas and pancreatic tumors.

[00406] In an aspect, the invention provides a molecular profile for a cancer which comprises mutational analysis of a panel of genes, e.g., at least 2, 3, 4, 5, 6, 7, 8, 9, 10, 12, 15, 20, 25, 30, 35, 40, 45 or at least 50 genes. As described herein, the molecular profile can be used to identify a candidate agent that is likely to benefit the cancer patient. The molecular profile can also be used to identify a candidate agent that is not likely to benefit the cancer patient. Further as described, a report can be generated that describes results of the molecular profile. The report may include a summary of the mutational analysis for the genes assessed. The report may also provide a linkage of the mutational analysis with the predicted efficacy of various treatments based on the mutational analysis. Such rules for mutation – drug association are provided herein, e.g., in **Table 17** or any of **Tables 7-16**. The report may also comprise one or more clinical trials associated with one or more identified mutation in the patient. Mutational analysis can also be used to detect mutations of genes that are known to affect a prognosis or provide other characterization of a cancer.

[00407] The molecular profile may comprise mutational analysis of one or more gene in **Table 17**. For example, the molecular profile may include the mutational analysis of at least 1, 2, 3, 4, 5, 6, 7, 8, 9, 10, 11, 12, 13, 14, 15, 16, 17, 18, 19, 20, 21, 22, 23, 24, 25, 26, 27, 28, 29, 30, 31, 32, 33, 34, 35, 36, 37, 38, 39, 40, 41, 42, 43, 44, 45, 46, 47, 48, 49, or at least 50 genes in **Table 17**. The molecular profile may include the mutational analysis of at least 1, 2, 3, 4, 5, 6, 7, 8, 9, 10, 11, 12, 13, 14, 15, 16, 17, 18, 19, 20, 21, 22, 23, 24, 25, 26, 27, 28, 29, 30, 31, 32, 33, 34, 35, 36, 37, 38, 39, 40, 41, 42, 43, 44, 45, 46, 47, 48, 49, or 50 or ABL1, AKT1, ALK, APC, ATM, BRAF, BRCA1, BRCA2, CDH1, CDKN2A, c-Kit, C-Met, CSF1R, CTNNB1, EGFR, ERBB2, ERBB4, FBXW7, FGFR1, FGFR2, FGFR3, FLT3, GNA11, GNAQ, GNAS, HNF1A, HRAS, IDH1, JAK2, JAK3, KDR, KRAS, MLH1, MPL, NOTCH1, NPM1, NRAS, PDGFRA, PIK3CA, PTEN, PTPN11, RB1, RET, SMAD4, SMARCB1, SMO, SRC, STK11, TP53, VHL. In an embodiment, the molecular profile comprises mutational analysis of at least 1, 2, 3, 4, 5, 6, 7, 8, 9, 10, 11, 12, 13, 14, 15, 16, 17, 18, 19, 20, 21, 22, 23, 24, 25, 26, 27, 28, 29, 30, 31, 32, 33, 34, 35, 36, 37, 38, 39, 40, 41, 42, 43, 44, 45, 46 or 47 of ABL1, AKT1, ALK, APC, ATM, BRAF, BRCA1, BRCA2, CDH1, CSF1R, CTNNB1, EGFR, ERBB2 (HER2), ERBB4, FBXW7, FGFR1, FGFR2, FLT3, GNA11, GNAQ, GNAS, HNF1A, HRAS, IDH1, JAK2, JAK3, KDR (VEGFR2), KIT, KRAS, MET, MLH1,

MPL, NOTCH1, NPM1, NRAS, PDGFRA, PIK3CA, PTEN, PTPN11, RB1, RET, SMAD4, SMARCB1, SMO, STK11, TP53, VHL. For example, the molecular profile may comprise mutational analysis of ABL1, AKT1, ALK, APC, ATM, BRAF, BRCA1, BRCA2, CDH1, CSF1R, CTNNB1, EGFR, ERBB2 (HER2), ERBB4, FBXW7, FGFR1, FGFR2, FLT3, GNA11, GNAS, HNF1A, HRAS, IDH1, JAK2, JAK3, KDR (VEGFR2), KIT, KRAS, MET, MLH1, MPL, NOTCH1, NPM1, NRAS, PDGFRA, PIK3CA, PTEN, PTPN11, RB1, RET, SMAD4, SMARCB1, SMO, STK11, TP53, and VHL. In an embodiment, the mutational analysis molecular profile is performed in concert with another molecular profile provided herein. For example, the analysis of at least 1, 2, 3, 4, 5, 6, 7, 8, 9, 10, 11, 12, 13, 14, 15, 16, 17, 18, 19, 20, 21, 22, 23, 24, 25, 26, 27, 28, 29, 30, 31, 32, 33, 34, 35, 36, 37, 38, 39, 40, 41, 42, 43, 44, 45, 46 or 47 of ABL1, AKT1, ALK, APC, ATM, BRAF, BRCA1, BRCA2, CDH1, CSF1R, CTNNB1, EGFR, ERBB2 (HER2), ERBB4, FBXW7, FGFR1, FGFR2, FLT3, GNA11, GNAS, HNF1A, HRAS, IDH1, JAK2, JAK3, KDR (VEGFR2), KIT, KRAS, MET, MLH1, MPL, NOTCH1, NPM1, NRAS, PDGFRA, PIK3CA, PTEN, PTPN11, RB1, RET, SMAD4, SMARCB1, SMO, STK11, TP53 and VHL can be reported together with the molecular profiling described in any of **FIGs. 27A-B**, and/or **Tables 7-17**. In an embodiment, the mutational analysis of ABL1, AKT1, ALK, APC, ATM, BRAF, BRCA1, BRCA2, CDH1, CSF1R, CTNNB1, EGFR, ERBB2 (HER2), ERBB4, FBXW7, FGFR1, FGFR2, FLT3, GNA11, GNAS, HNF1A, HRAS, IDH1, JAK2, JAK3, KDR (VEGFR2), KIT, KRAS, MET, MLH1, MPL, NOTCH1, NPM1, NRAS, PDGFRA, PIK3CA, PTEN, PTPN11, RB1, RET, SMAD4, SMARCB1, SMO, STK11, TP53 and VHL genes is reported together with the molecular profiling described in any of **FIGs. 27A-B**, and/or **Tables 7-17**.

[00408] In an embodiment, the molecular profile comprises mutational analysis of at least 1, 2, 3, 4, 5, 6, 7, 8, 9, 10, 11, 12, 13, 14, 15, 16, 17, 18, 19, 20, 21, 22, 23, 24, 25, 26, 27, 28, 29, 30, 31, 32, 33, 34, 35 or 36 of ABL1, AKT1, ALK, APC, ATM, BRAF, BRCA1, BRCA2, cKIT, cMET, CSF1R, CTNNB1, EGFR, ERBB2, FGFR1, FGFR2, FLT3, GNA11, GNAQ, GNAS, HRAS, IDH1, JAK2, KDR (VEGFR2), KRAS, MLH1, MPL, NOTCH1, NRAS, PDGFRA, PIK3CA, PTEN, RET, SMO, TP53, VHL. For example, ABL1, AKT1, ALK, APC, ATM, BRAF, cKIT, cMET, CSF1R, CTNNB1, EGFR, ERBB2, FGFR1, FGFR2, FLT3, GNA11, GNAQ, GNAS, HRAS, IDH1, JAK2, KDR (VEGFR2), KRAS, MLH1, MPL, NOTCH1, NRAS, PDGFRA, PIK3CA, PTEN, RET, SMO, TP53, VHL may be assessed. As desired, additional biomarkers may be assessed for mutational analysis including at least 1, 2, 3, 4, 5, 6, 7, 8, 9, 10 or 11 of CDH1, ERBB4, FBXW7, HNF1A, JAK3, NPM1, PTPN11, RB1, SMAD4, SMARCB1, STK11. For example, CDH1, ERBB4, FBXW7, HNF1A, JAK3, NPM1, PTPN11, RB1, SMAD4, SMARCB1, STK11 may be assessed in addition to the biomarkers above. In an embodiment, the molecular profile comprises mutational analysis of at least 1, 2, 3, 4, 5, 6, 7, 8, 9, 10, 11, 12, 13, 14, 15, 16, 17, 18, 19, 20, 21, 22, 23, 24, 25, 26, 27, 28, 29, 30, 31, 32, 33, 34, 35, 36, 37, 38, 39, 40, 41, 42, 43, 44, 45, 46 or 47 of ABL1, AKT1, ALK, APC, ATM, BRAF, BRCA1, BRCA2, CDH1, cKIT, cMET, CSF1R, CTNNB1, EGFR, ERBB2, ERBB4, FBXW7, FGFR1, FGFR2, FLT3, GNA11, GNAQ, GNAS, HNF1A, HRAS, IDH1, JAK2, JAK3, KDR (VEGFR2), KRAS, MLH1, MPL, NOTCH1, NPM1, NRAS, PDGFRA, PIK3CA, PTEN, PTPN11, RB1, RET, SMAD4, SMARCB1, SMO, STK11, TP53, VHL. For

example, the molecular profile may comprise or consist of mutational analysis of ABL1, AKT1, ALK, APC, ATM, BRAF, BRCA1, BRCA2, CDH1, cKIT, cMET, CSF1R, CTNNB1, EGFR, ERBB2, ERBB4, FBXW7, FGFR1, FGFR2, FLT3, GNA11, GNAQ, GNAS, HNF1A, HRAS, IDH1, JAK2, JAK3, KDR (VEGFR2), KRAS, MLH1, MPL, NOTCH1, NPM1, NRAS, PDGFRA, PIK3CA, PTEN, PTPN11, RB1, RET, SMAD4, SMARCB1, SMO, STK11, TP53, VHL.

[00409] In still other embodiments, the molecular profile comprises mutational analysis of 1, 2, 3, 4, 5, 6, 7, 8, 9, 10, 11, 12, 13, 14, 15, 16, 17, 18, 19 or 20 of ALK, BRAF, BRCA1, BRCA2, EGFR, ERRB2, GNA11, GNAQ, IDH1, IDH2, KIT, KRAS, MET, NRAS, PDGFRA, PIK3CA, PTEN, RET, SRC, TP53. The molecular profile may comprise mutational analysis of 1, 2, 3, 4, 5, 6, 7, 8, 9, 10, 11, 12, 13, 14, 15, 16, 17, 18, 19, 20, 21, 22, 23, 24, 25, 26, 27 or 28 of AKT1, HRAS, GNAS, MEK1, MEK2, ERK1, ERK2, ERBB3, CDKN2A, PDGFRB, IFG1R, FGFR1, FGFR2, FGFR3, ERBB4, SMO, DDR2, GRB1, PTCH, SHH, PD1, UGT1A1, BIM, ESR1, MLL, AR, CDK4, SMAD4. The molecular profile may also comprise mutational analysis of 1, 2, 3, 4, 5, 6, 7, 8, 9, 10, 11, 12, 13, 14, 15, 16, 17, 18, 19, 20 or 21 of ABL, APC, ATM, CDH1, CSFR1, CTNNB1, FBXW7, FLT3, HNF1A, JAK2, JAK3, KDR, MLH1, MPL, NOTCH1, NPM1, PTPN11, RB1, SMARCB1, STK11, VHL. The genes assessed by mutational analysis may comprise at least 1, 2, 3, 4, 5, 6, 7, 8, 9, 10, 11, 12, 13, 14, 15, 16, 17, 18, 19, 20, 21, 22, 23, 24, 25, 26, 27, 28, 29, 30, 31, 32, 33, 34, 35, 36, 37, 38, 39, 40, 41, 42, 43, 44, 45, 46, 47, 48, 49, 50, 60, 70, 80, 90, 100, 110, 120, 130, 140, 150, 160, 170, 180, 190, 200, 250, 300, or all genes, selected from the group consisting of ABL1, ABL2, ACVR1B, AKT1, AKT2, AKT3, ALK, AMER1 (FAM123B), APC, AR, ARAF, ARFRP1, ARID1A, ARID1B, ARID2, ASXL1, ATM, ATR, ATRX, AURKA, AURKB, AXIN1, AXL, BAP1, BARD1, BCL2, BCL2L1, BCL2L2, BCL6, BCOR, BCORL1, BCR, BLM, BRAF, BRCA1, BRCA2, BRD4, BRIP1, BTG1, BTK, C11orf30 (EMSY), CARD11, CBFB, CBL, CCND1, CCND2, CCND3, CCNE1, CD274, CD79A, CD79B, CDC73, CDH1, CDK12, CDK4, CDK6, CDK8, CDKN1A, CDKN1B, CDKN2A, CDKN2B, CDKN2C, CEBPA, CHD2, CHD4, CHEK1, CHEK2, CIC, CREBBP, CRKL, CRLF2, CSF1R, CTCF, CTNNA1, CTNNB1, CUL3, CYLD, DAXX, DDR2, DICER1, DNMT3A, DOT1L, EGFR, EP300, EPHA3, EPHA5, EPHA7, EPHB1, ERBB2, ERBB3, ERBB4, ERG, ERRFI1, ESR1, ETV1, ETV4, ETV5, ETV6, EZH2, FAM46C, FANCA, FANCC, FANCD2, FANCE, FANCF, FANCG, FANCL, FAS, FAT1, FBXW7, FGF10, FGF14, FGF19, FGF23, FGF3, FGF4, FGF6, FGFR1, FGFR2, FGFR3, FGFR4, FH, FLCN, FLT1, FLT3, FLT4, FOXL2, FOXP1, FRS2, FUBP1, GABRA6, GATA1, GATA2, GATA3, GATA4, GATA6, GID4 (C17orf39), GLI1, GNA11, GNA13, GNAQ, GNAS, GPR124, GRIN2A, GRM3, GSK3B, H3F3A, HGF, HNF1A, HRAS, HSD3B1, HSP90AA1, IDH1, IDH2, IGF1R, IGF2, IKBKE, IKZF1, IL7R, INHBA, INPP4B, IRF2, IRF4, IRS2, JAK1, JAK2, JAK3, JUN, KAT6A (MYST3), KDM5A, KDM5C, KDM6A, KDR, KEAP1, KEL, KIT, KLHL6, KMT2A (MLL), KMT2C (MLL3), KMT2D (MLL2), KRAS, LMO1, LRP1B, LYN, LZTR1, MAGI2, MAP2K1, MAP2K2, MAP2K4, MAP3K1, MCL1, MDM2, MDM4, MED12, MEF2B, MEN1, MET, MITF, MLH1, MPL, MRE11A, MSH2, MSH6, MTOR, MUTYH, MYB, MYC, MYCL (MYCL1), MYCN, MYD88, NF1, NF2, NFE2L2, NFKBIA, NKX2-1, NOTCH1, NOTCH2, NOTCH3, NPM1, NRAS, NSD1, NTRK1, NTRK2, NTRK3, NUP93, PAK3, PALB2, PARK2, PAX5, PBRM1,

PDCD1LG2, PDGFRA, PDGFRB, PDK1, PIK3C2B, PIK3CA, PIK3CB, PIK3CG, PIK3R1, PIK3R2, PLCG2, PMS2, POLD1, POLE, PPP2R1A, PRDM1, PREX2, PRKAR1A, PRKCI, PRKDC, PRSS8, PTCH1, PTEN, PTPN11, QKI, RAC1, RAD50, RAD51, RAF1, RANBP2, RARA, RB1, RBM10, RET, RICTOR, RNF43, ROS1, RPTOR, RUNX1, RUNX1T1, SDHA, SDHB, SDHC, SDHD, SETD2, SF3B1, SLIT2, SMAD2, SMAD3, SMAD4, SMARCA4, SMARCB1, SMO, SNCAIP, SOCS1, SOX10, SOX2, SOX9, SPEN, SPOP, SPTA1, SRC, STAG2, STAT3, STAT4, STK11, SUFU, SYK, TAF1, TBX3, TERC, TERT (promoter only), TET2, TGFBR2, TMPRSS2, TNFAIP3, TNFRSF14, TOP1, TOP2A, TP53, TSC1, TSC2, TSHR, U2AF1, VEGFA, VHL, WISP3, WT1, XPO1, ZBTB2, ZNF217, ZNF703. The mutational analysis may be performed to detect a gene rearrangement, e.g., a rearrangement in at least 1, 2, 3, 4, 5, 6, 7, 8, 9, 10, 11, 12, 13, 14, 15, 16, 17, 18, 19, 20, 21, 22, 23, 24, 25, 26, 27, 28, 29 or 30, of ALK, BCR, BCL2, BRAF, BRCA1, BRCA2, BRD4, EGFR, ETV1, ETV4, ETV5, ETV6, EWSR1, FGFR1, FGFR2, FGFR3, KIT, MSH2, MLL, MYB, MYC, NTRK1, NTRK2, PDGFRA, RAF1, RARA, RET, ROS1, TMPRSS2.

[00410] Various cancer genes disclosed in the COSMIC (Catalogue Of Somatic Mutations In Cancer) database (available at cancer.sanger.ac.uk/cancergenome/projects/cosmic/) can be assessed as well.

Clinical Trial Connector

[00411] Thousands of clinical trials for therapies are underway in the United States, with several hundred of these tied to biomarker status. In an embodiment, the molecular intelligence molecular profiles of the invention include molecular profiling of markers that are associated with ongoing clinical trials. Thus, the molecular profile can be linked to clinical trials of therapies that are correlated to a subject's biomarker profile. The method can further comprise identifying trial location(s) to facilitate patient enrollment. The database of ongoing clinical trials can be obtained from www.clinicaltrials.gov in the United States, or similar source in other locations. The molecular profiles generated by the methods of the invention can be linked to ongoing clinical trials and updated on a regular basis, e.g., daily, bi-weekly, weekly, monthly, or other appropriate time period.

[00412] Although significant advances in cancer treatment have been made in recent years, not all patients can be effectively treated within the standard of care paradigm. Many patients are eligible for clinical trials participation, yet less than 3 percent are actually enrolled in a trial, according to recent National Cancer Institute (NCI) statistics. The Clinical Trials Connector allows caregivers such as physicians to quickly identify and review global clinical trial opportunities in real-time that are molecularly targeted to each patient. In embodiments, the Clinical Trials Connector has one or more of the following features: Examines thousands of open and enrolling clinical trials; Individualizes clinical trials based on molecular profiling as described herein; Includes interactive and customizable trial search filters by: Biomarker, Mechanism of action, Therapy, Phase of study, and other clinical factors (age, sex, etc.). The Clinical Trials Connector can be a computer database that is accessed once molecular profiling results are available. In some embodiments, the database comprises the EmergingMed database (EmergingMed, New York, NY).

[00413] **Tables 7** and **Tables 11-16** herein indicates an association of certain biomarkers in the molecular profiles of the invention with ongoing clinical trials. Profiling of the specified markers can provide an indication that a subject is a candidate for a clinical trial, e.g., by suggesting that an agent in a clinical trial may benefit the subject. For example, **Table 16** indicates that molecular profiling of HER2, PIK3CA, PTEN, cMET and the other indicated gene mutations (i.e., as profiled using NGS) can associate ovarian cancer with ongoing clinical trials. **Table 11** indicates that molecular profiling of HER2, ER/HER2/PIK3CA, AR, cMET and the other indicated gene mutations (i.e., as profiled using NGS) can associate breast cancer with ongoing clinical trials. **Table 13** indicates that molecular profiling of PIK3CA, PTEN, cMET and the other indicated gene mutations (i.e., as profiled using NGS) can associate melanoma with ongoing clinical trials. **Table 14** indicates that molecular profiling of PIK3CA, PTEN, cMET and the other indicated gene mutations (i.e., as profiled using NGS) can associate uveal melanoma with ongoing clinical trials. **Table 12** indicates that molecular profiling of cMET and the other indicated gene mutations (i.e., as profiled using NGS) can associate colorectal cancer with ongoing clinical trials. **Table 15** indicates that molecular profiling of HER2, PIK3CA, cMET and the indicated gene mutations (i.e., as profiled using NGS) can associate lung cancer, e.g., NSCLC, with ongoing clinical trials. **Table 7** indicates that molecular profiling of HER2, PIK3CA, PTEN, cMET, EGFRvIII, IDH2 and the indicated gene mutations (i.e., as profiled using NGS) can associate various solid tumors with ongoing clinical trials. An illustrative listing of such clinical trials is found in **Table 18** below.

[00414] In an aspect, the invention provides a set of rules for matching of clinical trials to biomarker status as determined by the molecular profiling described herein. In some embodiments, the matching of clinical trials to biomarker status is performed using one or more pre-specified criteria: 1) Trials are matched based on the OFF NCCN Compendia drug/drug class associated with potential benefit by the molecular profiling rules; 2) Trials are matched based on biomarker driven eligibility requirement of the trial; and 3) Trials are matched based on the molecular profile of the patient, the biology of the disease and the associated signaling pathways. In the latter case, i.e. item 3, clinical trial matching may comprise further criteria as follows. First, for directly targetable markers, match trials with agents directly targeting the gene (e.g., FGFR results map to anti-FGFR therapy trials; ERBB2 results map to anti-HER2 agents, etc). In addition, for directly targetable markers, trial matching considers downstream markers under the following scenarios: a) a known resistance mechanism is available (e.g., cMET inhibitors for EGFR gene); b) clinical evidence associates the (mutated) biomarker with drugs targeting downstream pathways (e.g., mTOR inhibitors when PIK3CA is mutated); and c) active clinical trials are enrolling patients (with the biomarker aberration in the inclusion criteria) with drugs targeting the downstream pathways (e.g., SMO inhibitors for BCR-ABL mutation T315I). In the case of markers that are not directly targetable by a known therapeutic agent, trial matching may consider alternative, downstream markers (e.g., platinum agents for ATM gene; MEK inhibitors for GNAS/GNAQ/GNA11 mutation). The clinical trials that are matched may be identified based on results of "pathogenic," "presumed pathogenic," or variant of uncertain (or unknown) significance ("VUS"). In some embodiments, the decision to incorporate/associate a drug class with a biomarker mutation can further depend on one or more of the

following: 1) Clinical evidence; 2) Preclinical evidence; 3) Understanding of the biological pathway affected by the biomarker; and 4) expert analysis. In some embodiments, the mutation of biomarkers in the above section "Mutational Analysis" is linked to clinical trials using one or more of these criteria.

[00415] The guiding principle above can be used to identify classes of drugs that are linked to certain biomarkers. The biomarkers can be linked to various clinical trials that are studying these biomarkers, including without limitation requiring a certain biomarker status for clinical trial inclusion. **Table 18** presents an illustrative overview of biomarker statuses that are matched to classes of drugs. In the table, the column headed "Biomarker" identifies that biomarker that is assessed according to the molecular profiling technique specified in the column headed "Technique." It will be appreciated that equivalent methods can be used as desired. For example, Next Generation Sequencing (NGS; Next Gen SEQ) is used to identify mutations, but alternate nucleic acid sequencing and analysis techniques (Sanger sequencing, PCR, RFLP, etc) can be used in the alternative or in the conjunction. Results that indicate a potential match (e.g., a potential benefit) to a class of drugs are indicated in the column "Result." For sequencing methods, "Pathogenic/Presumed Pathogenic/Variant of Unknown Significance" refer to mutations that are detected and are known, presumed, or potentially pathogenic. As appropriate, particular mutations or other alterations in the biomarker that are potentially matched to the class of drugs are identified in the column headed "Mutation Type/Alteration." The matched drug classes are identified in the column headed "Drug Class (Associated Agents)." Associated agents are illustrative drugs that are members of the class. Clinical trials studying the drug classes and/or specific agents listed can be matched to the biomarker. In an aspect, the invention provides a method of selecting a clinical trial for enrollment of a patient, comprising performing molecular profiling of one or more biomarker on a sample from the patient using the methods described herein. For example, the profiling can be performed for one on more biomarker in **Table 18** using the technique indicated in the table. The results of the profiling are matched to classes of drugs using the above criteria. Clinical trials studying members of the classes of drugs are identified. The patient is a potential candidate for the so-identified clinical trials.

Table 18 – Illustrative Biomarker – Drug Associations for Drugs in Matched Clinical Trials

Biomarker	Technique	Result	Mutation Type / Alteration	Drug Class (Associated Agents) matched by clinical trials
NGS tests				
ATM	Next Gen SEQ	Pathogenic/Presumed Pathogenic/Variant of Unknown Significance		**PARP inhibitors** (ABT-767, CEP9722, E7016, iniparib, MK4827, olaparib, rucaparib, veliparib), **HDAC inhibitors** (abexinostat, ACY-1215, AR-42, belinostat, CUDC-907, entinostat, FK228, givinostat, JNJ26481585, mocetinostat, panobinostat, SHP-141, valproic acid, vorinostat, 4SC-202) **Platinum compounds** (carboplatin, cisplatin, oxaliplatin)
CSF1R	Next Gen SEQ	Pathogenic/Presumed Pathogenic/Variant of		**FGFR TKI** (dovitinib), **anti-CSF1R monoclonal**

		Unknown Significance		**antibody** (IMC-CS4)
ERBB2	Next Gen SEQ	Pathogenic/Presumed Pathogenic/Variant of Unknown Significance		**anti-HER2 monoclonal antibody** (pertuzumab, trastuzumab) **HER2-targeted tyrosine kinase inhibitors** (afatinib, dacomitinib, lapatinib, neratinib) **anti-HER2 monoclonal antibody - drug conjugate** (ado-trastuzumab emtansine (T-DM1))
GNAS	Next Gen SEQ	Pathogenic/Presumed Pathogenic/Variant of Unknown Significance		**MEK inhibitors** (AZD8330, BAY86-9766, CI-1040, GDC-0623, GDC-0973, MEK162, MSC1936369B, MSC2015103B, PD0325901, pimasertib (AS-703026), selumetinib, TAK-733, trametinib, XL518)
GNAQ	Next Gen SEQ	Pathogenic/Presumed Pathogenic/Variant of Unknown Significance		**MEK inhibitors** (AZD8330, BAY86-9766, CI-1040, GDC-0623, GDC-0973, MEK162, MSC1936369B, MSC2015103B, PD0325901, pimasertib (AS-703026), selumetinib, TAK-733, trametinib, XL518)
GNA11	Next Gen SEQ	Pathogenic/Presumed Pathogenic/Variant of Unknown Significance		**MEK inhibitors** (AZD8330, BAY86-9766, CI-1040, GDC-0623, GDC-0973, MEK162, MSC1936369B, MSC2015103B, PD0325901, pimasertib (AS-703026), selumetinib, TAK-733, trametinib, XL518)
KDR	Next Gen SEQ	Pathogenic/Presumed Pathogenic/Variant of Unknown Significance		**VEGFR2-targeted tyrosine kinase inhibitors** (apatinib, axitinib, cabozantinib, famitinib, fruquintinib, lenvatinib, motesanib, ninedanib, pazopanib, regorafenib, sorafenib, sunitinib, tivozanib, vandetanib, vatalanib) **anti-VEGFR2-targeted monoclonal antibody** (ramucirumab, tanibirumab)
MLH1	Next Gen SEQ	Pathogenic/Presumed Pathogenic/Variant of Unknown Significance		**PARP inhibitors** (ABT-767, CEP9722, E7016, iniparib, MK4827, olaparib, rucaparib, veliparib)
VHL	Next Gen SEQ	Pathogenic/Presumed Pathogenic/Variant of Unknown Significance		**VEGF, VEGFR targeted therapies:** Aflibercept, Axitinib, Bevacizumab, Cabozantinib, Pazopanib, Regorafenib, Sorafenib, Sunitinib, Tivozanib, Apatinib, Famitinib, Fruquintinib, Lenvatinib, Motesanib, Ninedanib, Vandetanib, Vatalanib, Ramucirumab, Tanibirumab, IMC-3C5, IMC-18F1

				PI3K/Akt/mTor inhibitors: Temsirolimus, Everolimus, CC-223, Ridaforolimus, sirolimus, MLN0128, GDC0941, Deforolimus, BEZ235, DS-7423, GDC-0980, PF-04691502, PF-05212384, SAR245409, BKM120, BYL719, PX-866, GDC-0068, MK2206, GSK2131795, GSK2110183, GSK2141795, XL147 (SAR245408), INK1117, AZD5363, Perifosine, ARQ092, AZD8055, OSI-027, BAY80-6946
c-KIT	Next Gen SEQ	Pathogenic/Presumed Pathogenic/Variant of Unknown Significance	all mutations except V654A, T670I, D820A, D820E, D820G, D820Y, N822H, N822K, Y823D, D816A, D816G, D816H, D816V, A829P	**KIT inhibitiors:** Sorafenib, Dasatinib, Sunitinib, Nilotinib, Imatinib, Regorafenib, Vatalanib, Masitinib, Pazopanib
c-KIT	Next Gen SEQ	Pathogenic/Presumed Pathogenic/Variant of Unknown Significance	V654A, T670I, D820A, D820E, D820G, D820Y, N822H, N822K, Y823D, D816A, D816G, D816H, D816V, A829P	**KIT inhibitiors:** Sorafenib, Dasatinib, Sunitinib, Nilotinib, Regorafenib, Vatalanib, Masitinib, Pazopanib
PDGFRA	Next Gen SEQ	Pathogenic/Presumed Pathogenic/Variant of Unknown Significance	all mutations except D842V	**PDGFRA inhibitors**: Sorafenib, Dasatinib, Sunitinib, Nilotinib, Imatinib, Crenolanib (CP 868-956), Masitinib, Pazopanib
PDGFRA	Next Gen SEQ	Pathogenic/Presumed Pathogenic/Variant of Unknown Significance	D842V	**PDGFRA inhibitors**: Sorafenib, Dasatinib, Sunitinib, Nilotinib, Crenolanib (CP 868-956), Masitinib, Pazopanib
ABL1	Next Gen SEQ	Pathogenic/Presumed Pathogenic/Variant of Unknown Significance	T315I	**PI3K/Akt/mTor inhibitors:**Temsirolimus, Everolimus, CC-223, Ridaforolimus, sirolimus, MLN0128, GDC0941, Deforolimus, BEZ235, DS-7423, GDC-0980, PF-04691502, PF-05212384, SAR245409, BKM120, BYL719, PX-866, GDC-0068, MK2206, GSK2131795, GSK2110183, GSK2141795, XL147(SAR245408), INK1117, AZD5363, Perifosine, ARQ092, AZD8055, OSI-027, BAY80-6946 **SMO antagonists:** GDC-0449, LDE225, BMS833923
ABL1	Next Gen SEQ	Pathogenic/Presumed Pathogenic/Variant of Unknown Significance	all mutations except T315I	**PI3K/Akt/mTor inhibitors:**Temsirolimus, Everolimus, CC-223, Ridaforolimus, sirolimus, MLN0128, GDC0941, Deforolimus, BEZ235, DS-7423, GDC-0980, PF-04691502, PF-05212384, SAR245409, BKM120,

				BYL719, PX-866, GDC-0068, MK2206, GSK2131795, GSK2110183, GSK2141795, XL147 (SAR245408), INK1117, AZD5363, Perifosine, ARQ092, AZD8055, OSI-027, BAY80-6946 **SMO antagonists:** GDC-0449, LDE225, BMS833923 **BCR-ABL inhibitors:** nilotinib, dasatinib, ponatinib, bosutinib
cMET	Next Gen SEQ	Pathogenic/Presumed Pathogenic/Variant of Unknown Significance		**anti-HGF monoclonal antibody** (Ficlatuzumab, Rilotumumab, TAK-701) **cMET-targeted inhibitors** (AMG-208, BMS-777607, Compound 1 (Amgen), EMD 1214063/EMD 1204831, INC280, JNJ38877605, Onartuzumab (MetMAb), MK-2461, MK-8033, NK4, PF4217903, PHA665752, SGX126, Tivantinib (ARQ 197), cabozantinib, crizotinib, foretenib, MGCD265)
FGFR1	Next Gen SEQ	Pathogenic/Presumed Pathogenic/Variant of Unknown Significance		**Small molecule tyrosine kinase inhibitors** (TKI258, BIBF1120, BMS-582,664(Brivanib), E7080, TSU-68, AZD4547, Dovitinib, E-3810, BGJ398, TKI258, FP-1039, Ponatinib, JNJ-42756493) **FGFR antibodies and FGF ligand traps:** (1A6, FP-1039)
FGFR2	Next Gen SEQ	Pathogenic/Presumed Pathogenic/Variant of Unknown Significance		**Small molecule tyrosine kinase inhibitors** (TKI258, BIBF1120, BMS-582,664(Brivanib), E7080, TSU-68, AZD4547, Dovitinib, E-3810, BGJ398, TKI258, FP-1039, Ponatinib, JNJ-42756493) **FGFR antibodies and FGF ligand traps:** (1A6, FP-1039)
RET	Next Gen SEQ	Pathogenic/Presumed Pathogenic/Variant of Unknown Significance		**RET inhibitors** (Sorafenib, sunitinib, motesanib, cabozantinib, vandetanib, lenvatinib)
PIK3CA	Next Gen SEQ	Pathogenic/Presumed Pathogenic/Variant of Unknown Significance		**PI3K/Akt/mTor inhibitors:**Temsirolimus, Everolimus, CC-223, Ridaforolimus, sirolimus, MLN0128, GDC0941, Deforolimus, BEZ235, DS-7423, GDC-0980, PF-04691502, PF-05212384, SAR245409, BKM120, BYL719, PX-866, GDC-0068, MK2206, GSK2131795, GSK2110183, GSK2141795, XL147 (SAR245408), INK1117, AZD5363, Perifosine, ARQ092, AZD8055, OSI-027, BAY80-6946

				Aspirin: aspirin
PTEN	Next Gen SEQ	Pathogenic/Presumed Pathogenic/Variant of Unknown Significance		**PI3K/Akt/mTor inhibitors:**Temsirolimus, Everolimus, CC-223, Ridaforolimus, sirolimus, MLN0128, GDC0941, Deforolimus, BEZ235, DS-7423, GDC-0980, PF-04691502, PF-05212384, SAR245409, BKM120, BYL719, PX-866, GDC-0068, MK2206, GSK2131795, GSK2110183, GSK2141795, XL147 (SAR245408), INK1117, AZD5363, Perifosine, ARQ092, AZD8055, OSI-027, BAY80-6946 **Parp inhibitors:** ABT-767, CEP9722, E7016, iniparib, MK4827, olaparib, rucaparib, veliparib, ABT-888
AKT1	Next Gen SEQ	Pathogenic/Presumed Pathogenic/Variant of Unknown Significance		**Akt inhibitors:** AZD5363, GDC-0068, MK2206, Perifosine, ARQ092
ALK	Next Gen SEQ	Pathogenic/Presumed Pathogenic/Variant of Unknown Significance		**ALK inhibitors:** crizotinib, AP26113, X-396, CH5424802(AF-802), ASP3026, CEP-28122, CEP-37440, LDK378
SMO	Next Gen SEQ	Pathogenic/Presumed Pathogenic/Variant of Unknown Significance		**SMO inhibitors:** Vismodegib, Erismodegib (LDE255), IPI-926, BMS-838923, PF-04449913, LEQ506, TAK441, LY2940680.
KRAS	Next Gen SEQ	Pathogenic/Presumed Pathogenic/Variant of Unknown Significance		**MEK inhibitors:** AZD8330, BAY86-9766, CI-1040, GDC-0623, GDC-0973, MEK162, MSC1936369B, MSC2015103B, PD0325901, pimasertib (AS-703026), selumetinib, TAK-733, trametinib, XL518, ARRY-438162 **ERK inhibitors:** LY2228820, LY3007113, BVD-523, BAY86-9766, ARRY-614 **Regorafenib:** regorafenib
NRAS	Next Gen SEQ	Pathogenic/Presumed Pathogenic/Variant of Unknown Significance		**MEK inhibitors:** AZD8330, BAY86-9766, CI-1040, GDC-0623, GDC-0973, MEK162, MSC1936369B, MSC2015103B, PD0325901, pimasertib (AS-703026), selumetinib, TAK-733, trametinib, XL518, ARRY-438162 **ERK inhibitors:** LY2228820, LY3007113, BVD-523, BAY86-9766, ARRY-614
HRAS	Next Gen SEQ	Pathogenic/Presumed Pathogenic/Variant of Unknown Significance		**MEK inhibitors:** AZD8330, BAY86-9766, CI-1040, GDC-0623, GDC-0973, MEK162, MSC1936369B,

				MSC2015103B, PD0325901, pimasertib (AS-703026), selumetinib, TAK-733, trametinib, XL518, ARRY-438162 **ERK inhibitors:** LY2228820, LY3007113, BVD-523, BAY86-9766, ARRY-614
IDH1	Next Gen SEQ	Pathogenic/Presumed Pathogenic/Variant of Unknown Significance		**Alkylating agents:** temozolomide, dacarbazine **Hypomethylating agents:** azacitidine, decitabine
JAK2	Next Gen SEQ	Pathogenic/Presumed Pathogenic/Variant of Unknown Significance		**JAK2 inhibitors:** ruxolitinib, tg101348 (panolosetron), CEP-701 (lestaurtinib), NS-018, LY278544
MPL	Next Gen SEQ	Pathogenic/Presumed Pathogenic/Variant of Unknown Significance		**JAK2 inhibitors**: ruxolitinib, tg101348 (panolosetron), CEP-701 (lestaurtinib), NS-018, LY278544
FLT3	Next Gen SEQ	Pathogenic/Presumed Pathogenic/Variant of Unknown Significance		**FLT3 inhibitors**: CEP-701 (lestaurtinib), sunitinib, MLN518 (tandutinib), PKC412 (midostaurin)
APC	Next Gen SEQ	Pathogenic/Presumed Pathogenic/Variant of Unknown Significance		**Wnt pathway inhibitors**: PRI-724
CTNNB1	Next Gen SEQ	Pathogenic/Presumed Pathogenic/Variant of Unknown Significance		**Wnt pathway inhibitors**: PRI-724
BRAF	Next Gen SEQ	Pathogenic/Presumed Pathogenic/Variant of Unknown Significance		**BRAF inhibitors:** sorafenib, vemurafenib, RAF-265, XL281, LGX818, GSK2118436 (dabrafenib), ARQ736, RO5212054 **MEK inhibitors:** AZD8330, BAY86-9766, CI-1040, GDC-0623, GDC-0973, MEK162, MSC1936369B, MSC2015103B, PD0325901, pimasertib (AS-703026), selumetinib, TAK-733, trametinib, XL518, ARRY-438162 **ERK inhibitors:** LY2228820, LY3007113, BVD-523, BAY86-9766, ARRY-614
EGFR	Next Gen SEQ	Pathogenic/Presumed Pathogenic/Variant of Unknown Significance	T790M; exon20 insert (A763_Y764insFQEA, A767_D770dup, A767_V769dup, D770delinsGY, D770dup, D770_N771insG, D770_N771insGF, D770_N771insGT, D770_N771insGY, D770_N771insNPH, D770_P772delinsKG, H770dup, H773dup, H773_V774dup, H773_V774insAH, H773_V774insY, N771delinsGF,	**Pan HER inhibitors:** (afatinib, dacomitinib, CO-1686, XL647, neratinib, BMS-690514, Icotinib, poziotinib)

			N771delinsGY, N771delinsKG, N771delinsRY, N771_H773delinsTGG, N771_H773dup, N771_P772insH, P772_H773insGNP, S768_D770dup, V769_D770dup, V769_D770insDNP, V769_D770insGG, V769_D770insVTW, Y764_V765insHH)	
EGFR	Next Gen SEQ	Pathogenic/Presumed Pathogenic/Variant of Unknown Significance	all mutations except T790M and exon20 insert (A763_Y764insFQEA, A767_D770dup, A767_V769dup, D770delinsGY, D770dup, D770_N771insG, D770_N771insGF, D770_N771insGT, D770_N771insGY, D770_N771insNPH, D770_P772delinsKG, H770dup, H773dup, H773_V774dup, H773_V774insAH, H773_V774insY, N771delinsGF, N771delinsGY, N771delinsKG, N771delinsRY, N771_H773delinsTGG, N771_H773dup, N771_P772insH, P772_H773insGNP, S768_D770dup, V769_D770dup, V769_D770insDNP, V769_D770insGG, V769_D770insVTW, Y764_V765insHH)	**Pan HER inhibitors:** (afatinib, dacomitinib, CO-1686, XL647, neratinib, BMS-690514, Icotinib, poziotinib) **EGFR TKIs:** (erlotinib, gefitinib)
EGFR T790M	Next Gen SEQ	Present		**Pan HER inhibitors:** (afatinib, dacomitinib, CO-1686, XL647, neratinib, BMS-690514, Icotinib, poziotinib)
NOTCH1	Next Gen SEQ	Pathogenic/Presumed Pathogenic/Variant of Unknown Significance		**HDAC inhibitors**: HDAC inhibitors (abexinostat, ACY-1215, AR-42, belinostat, CUDC-907, entinostat, FK228, givinostat, JNJ26481585, mocetinostat, panobinostat, SHP-141, valproic acid, vorinostat, 4SC-202) **GSI**: (MK0752, RO4929097, R4733, BMS-906024, PF-03084014, MEDI0639)
TP53	Next Gen SEQ	Pathogenic/Presumed Pathogenic/Variant of Unknown Significance		**WEE1 inhibitors**: MK-1775 **CHK1 inhibitors**: LY2606368, SCH 900776 **Biologicals (gene therapy, vaccines)**: rAd-p53, P53-SLP,

				Ad5CMV-p53, adenovirus-p53 transduced dendritic cell vaccine (Ad.p53-DC vaccines), modified vaccinia virus ankara vaccine expressing p53, ALT-801 **p53 activators**: PRIMA
TP53	Next Gen SEQ	Wild Type		**P53-MDM2 interaction inhibitors**: CGM097, RO5503781, RO5045337, Kevetrin (thioureidobutyronitrile), DS-3032
Sanger SEQ				
PIK3CA	Sanger SEQ	Exon 20; Exon 9; Mutated - Other		**PI3K/Akt/mTor inhibitors:**Temsirolimus, Everolimus, CC-223, Ridaforolimus, sirolimus, MLN0128, GDC0941, Deforolimus, BEZ235, DS-7423, GDC-0980, PF-04691502, PF-05212384, SAR245409, BKM120, BYL719, PX-866, GDC-0068, MK2206, GSK2131795, GSK2110183, GSK2141795, XL147 (SAR245408), INK1117, AZD5363, Perifosine, ARQ092, AZD8055, OSI-027, BAY80-6946 **Aspirin:** aspirin
KRAS	Sanger SEQ	G12, G13; G13D; Q61; Mutated - Other		**MEK inhibitors:** AZD8330, BAY86-9766, CI-1040, GDC-0623, GDC-0973, MEK162, MSC1936369B, MSC2015103B, PD0325901, pimasertib (AS-703026), selumetinib, TAK-733, trametinib, XL518, ARRY-438162 **ERK inhibitors:** LY2228820, LY3007113, BVD-523, BAY86-9766, ARRY-614 **Regorafenib:** regorafenib
KRAS G13D	Sanger SEQ	Present		**MEK inhibitors:** AZD8330, BAY86-9766, CI-1040, GDC-0623, GDC-0973, MEK162, MSC1936369B, MSC2015103B, PD0325901, pimasertib (AS-703026), selumetinib, TAK-733, trametinib, XL518, ARRY-438162 **ERK inhibitors:** LY2228820, LY3007113, BVD-523, BAY86-9766, ARRY-614 **Regorafenib:** regorafenib
NRAS	Sanger SEQ	G12, G13; Q61; Mutated - Other		**MEK inhibitors:** AZD8330, BAY86-9766, CI-1040, GDC-0623, GDC-0973, MEK162, MSC1936369B, MSC2015103B, PD0325901, pimasertib (AS-703026),

				selumetinib, TAK-733, trametinib, XL518, ARRY-438162 **ERK inhibitors:** LY2228820, LY3007113, BVD-523, BAY86-9766, ARRY-614
BRAF	Sanger SEQ	V600D; V600E; V600K; V600R; Exon11; Mutated - Other; SEQ-MUT/PCR-WT; SEQ-WT/PCR-MUT;		**BRAF inhibitors:** sorafenib, vemurafenib, RAF-265, XL281, LGX818, GSK2118436 (dabrafenib), ARQ736, RO5212054 **MEK inhibitors:** AZD8330, BAY86-9766, CI-1040, GDC-0623, GDC-0973, MEK162, MSC1936369B, MSC2015103B, PD0325901, pimasertib (AS-703026), selumetinib, TAK-733, trametinib, XL518, ARRY-438162 **ERK inhibitors:** LY2228820, LY3007113, BVD-523, BAY86-9766, ARRY-614
EGFR	Sanger SEQ and RFLP	Exon 18 G719A; Exon 19 del; Exon 20 R776; Exon 21 L858R; Exon 21 L861;		**EGFR TKIs:** (erlotinib, gefitinib) **Pan HER inhibitors:** (afatinib, dacomitinib, CO-1686, XL647, neratinib, BMS-690514, Icotinib, poziotinib)
EGFR T790M	Sanger SEQ and RFLP	Present		**Pan HER inhibitors:** (afatinib, dacomitinib, CO-1686, XL647, neratinib, BMS-690514, Icotinib, poziotinib)
EGFR Exon 20 ins	Sanger SEQ and RFLP	Present		**Pan HER inhibitors:** (afatinib, dacomitinib, CO-1686, XL647, neratinib, BMS-690514, Icotinib, poziotinib)
IDH2	Sanger SEQ	Mutated-Other, **R140, R172**		**Alkylating agents:** temozolomide, dacarbazine **Hypomethylating agents:** azacitidine, decitabine
IHC Tests				
Her2/Neu	IHC	Positive		**anti-HER2 monoclonal antibody** (pertuzumab, trastuzumab) **HER2-targeted tyrosine kinase inhibitors** (afatinib, dacomitinib, lapatinib, neratinib) **anti-HER2 monoclonal antibody - drug conjugate** (ado-trastuzumab emtansine (T-DM1))
cMET	IHC	Positive		**anti-HGF monoclonal antibody** (Ficlatuzumab, Rilotumumab, TAK-701) **cMET-targeted inhibitors** (AMG-208, BMS-777607, Compound 1 (Amgen), EMD 1214063/EMD 1204831, INC280, JNJ38877605, Onartuzumab (MetMAb), MK-2461, MK-8033, NK4,

				PF4217903, PHA665752, SGX126, Tivantinib (ARQ 197), cabozantinib, crizotinib, foretenib, MGCD265) **cMET antibody:** ABT-700
PTEN	IHC	Negative		**PI3K/Akt/mTor inhibitors:**Temsirolimus, Everolimus, CC-223, Ridaforolimus, sirolimus, MLN0128, GDC0941, Deforolimus, BEZ235, DS-7423, GDC-0980, PF-04691502, PF-05212384, SAR245409, BKM120, BYL719, PX-866, GDC-0068, MK2206, GSK2131795, GSK2110183, GSK2141795, XL147 (SAR245408), INK1117, AZD5363, Perifosine, ARQ092, AZD8055, OSI-027, BAY80-6946 **Parp inhibitors:** ABT-767, CEP9722, E7016, iniparib, MK4827, olaparib, rucaparib, veliparib, ABT-888
Androgen Receptor	IHC	positive		**Anti androgens:** (Bicalutamide, flutamide, abiraterone, enzalutamide, TAK-700, ARN-509) **GnRH agonists/antagonists:** (goserelin, leuprolide, degarelix, abarelix);
EGFR	IHC	Positive		**EGFR monoclonal antibody:** cetuximab, nimotuzumab
CISH/FISH Tests				
Her2/Neu	CISH/FISH	Amplified		**anti-HER2 monoclonal antibody** (pertuzumab, trastuzumab) **HER2-targeted tyrosine kinase inhibitors** (afatinib, dacomitinib, lapatinib, neratinib) **anti-HER2 monoclonal antibody - drug conjugate** (ado-trastuzumab emtansine (T-DM1))
cMET	CISH/FISH	Amplified		**anti-HGF monoclonal antibody** (Ficlatuzumab, Rilotumumab, TAK-701) **cMET-targeted inhibitors** (AMG-208, BMS-777607, Compound 1 (Amgen), EMD 1214063/EMD 1204831, INC280, JNJ38877605, Onartuzumab (MetMAb), MK-2461, MK-8033, NK4, PF4217903, PHA665752, SGX126, Tivantinib (ARQ 197), cabozantinib, crizotinib, foretenib, MGCD265) **cMET antibody:** ABT-700
ALK	FISH	Positive		**ALK inhibitors:** crizotinib, AP26113, X-396,

				CH5424802(AF-802), ASP3026, CEP-28122, CEP-37440, LDK378 **HSP90 inhibitors:** AUY922, Ganetespib, 17-AGG
Cobas PCR				
BRAF	Cobas PCR (qPCR)	V600E		**BRAF inhibitors:** sorafenib, vemurafenib, RAF-265, XL281, LGX818, GSK2118436 (dabrafenib), ARQ736, RO5212054 **MEK inhibitors:** AZD8330, BAY86-9766, CI-1040, GDC-0623, GDC-0973, MEK162, MSC1936369B, MSC2015103B, PD0325901, pimasertib (AS-703026), selumetinib, TAK-733, trametinib, XL518, ARRY-438162 **ERK inhibitors:** LY2228820, LY3007113, BVD-523, BAY86-9766, ARRY-614
Fragment Analysis				
EGFRvIII	Fragment Analysis	present		**EGFRvIII targeted peptide vaccine:** rindopepimut (CDX-110; PEP-3-KLH) **EGFRvIII targeted antibodies and antibody conjugates:** ABT-806 (mAb806), ABT-414, AMG 595 **EGFR TKIs**:erlotinib, gefitinib **Pan HER inhibitors:** afatinib, dacomitinib, CO-1686, XL647, neratinib, BMS-690514, Icotinib, poziotinib

Report

[00416] In an embodiment, the methods of the invention comprise generating a molecular profile report. The report can be delivered to the treating physician or other caregiver of the subject whose cancer has been profiled. The report can comprise multiple sections of relevant information, including without limitation: 1) a list of the genes and/or gene products in the molecular profile; 2) a description of the molecular profile of the genes and/or gene products as determined for the subject; 3) a treatment associated with one or more of the genes and/or gene products in the molecular profile; and 4) and an indication whether each treatment is likely to benefit the patient, not benefit the patient, or has indeterminate benefit. The list of the genes and/or gene products in the molecular profile can be those presented herein for the molecular intelligence profiles of the invention. The description of the molecular profile of the genes and/or gene products as determined for the subject may include such information as the laboratory technique used to assess each biomarker (e.g., RT-PCR, FISH/CISH, IHC, PCR, FA/RFLP, sequencing, etc) as well as the result and criteria used to score each technique. By way of example, the criteria for scoring a protein as positive or negative for IHC may comprise the amount of staining and/or percentage of positive cells, the criteria for scoring a nucleic acid RT-PCR may be a cycle number

indicating whether the level of the appropriate nucleic acid is differentially regulated as compared to a control sample, or criteria for scoring a mutation may be a presence or absence. The treatment associated with one or more of the genes and/or gene products in the molecular profile can be determined using a rule set as described herein, e.g., in any of **Tables 7-17**. The indication whether each treatment is likely to benefit the patient, not benefit the patient, or has indeterminate benefit may be weighted. For example, a potential benefit may be a strong potential benefit or a lesser potential benefit. Such weighting can be based on any appropriate criteria, e.g., the strength of the evidence of the biomarker-treatment association, or the results of the profiling, e.g., a degree of over- or underexpression.

[00417] Various additional components can be added to the report as desired. In an embodiment, the report comprises a list having an indication of whether one or more of the genes and/or gene products in the molecular profile are associated with an ongoing clinical trial. The report may include identifiers for any such trials, e.g., to facilitate the treating physician's investigation of potential enrollment of the subject in the trial. In some embodiments, the report provides a list of evidence supporting the association of the genes and/or gene products in the molecular profile with the reported treatment. The list can contain citations to the evidentiary literature and/or an indication of the strength of the evidence for the particular biomarker-treatment association. In still another embodiment, the report comprises a description of the genes and/or gene products in the molecular profile. The description of the genes and/or gene products in the molecular profile may comprise without limitation the biological function and/or various treatment associations.

[00418] FIGs. 29A-X herein presents an illustrative patient report according to the invention. The illustrative report was derived from molecular profiling of a metastatic pancreatic adenocarcinoma with mutational analysis using Next Generation sequencing as described above (see, e.g., **Tables 7** and **10**).

[00419] As noted herein, the same biomarker may be assessed by one or more technique. In such cases, the results of the different analysis may be prioritized in case of inconsistent results. For example, the different methods may detect different aspects of a single biomarker (e.g., expression level versus mutation), or one method may be more sensitive than another. In the profiles presented above in **Tables 13-14**, BRAF mutations for melanoma and uveal melanoma samples are assessed by both PCR and Next Generation sequencing. Results obtained using the FDA approved cobas PCR (Roche Diagnostics) may be prioritized over the Next Generation results. However, if the sequencing detects a mutation, e.g., V600E, V600E2 or V600K, when PCR either detects wild type or is not determinable, the report may contain a note describing both sets of results including any therapy that may be implicated. In the case of melanoma, when the result of BRAF cobas PCR is "Wild type" or "no data" whereas BRAF sequencing is "V600E" or "V600E2", the report may comprise a note that BRAF mutation was not detected by the FDA-approved Cobas PCR test, however, a V600E/E2 mutation was detected by alternative methods (next generation/ Sanger sequencing) and that evidence suggests that the presence of a V600E mutation associates with potential clinical benefit from vemurafenib, dabrafenib or trametinib therapy. Similarly, when the result of BRAF cobas PCR is "Wild type" or "no data" and BRAF sequencing is "V600K", the report may comprise a note that BRAF mutation was not detected by the FDA-approved Cobas PCR test,

however, a V600K mutation was detected by alternative methods (next generation/ Sanger sequencing) and that evidence suggests that the presence of a V600K mutation associates with potential clinical benefit from trametinib therapy. In the case of uveal melanoma, when the result of BRAF cobas PCR is "Wild type" or "no data" and BRAF sequencing is "V600E", or "V600E2" or "V600K", the report may comprise a note that BRAF mutation was not detected by the FDA-approved Cobas PCR test, however, a V600E/E2 or a V600K mutation was detected by alternative methods (next generation/ Sanger sequencing) and that evidence suggests that the presence of a V600E or V600K mutation associates with potential clinical benefit from vemurafenib.

[00420] The molecular profiling report can be delivered to the caregiver for the subject, e.g., the oncologist or other treating physician. The caregiver can use the results of the report to guide a treatment regimen for the subject. For example, the caregiver may use one or more treatments indicated as likely benefit in the report to treat the patient. Similarly, the caregiver may avoid treating the patient with one or more treatments indicated as likely lack of benefit in the report.

Immune Modulators

[00421] PD1 (programmed death-1, PD-1) is a transmembrane glycoprotein receptor that is expressed on CD4-/CD8-thymocytes in transition to CD4+/CD8+ stage and on mature T and B cells upon activation. It is also present on activated myeloid lineage cells such as monocytes, dendritic cells and NK cells. In normal tissues, PD-1 signaling in T cells regulates immune responses to diminish damage, and counteracts the development of autoimmunity by promoting tolerance to self-antigens. PD-L1 (programmed cell death 1 ligand 1, PDL1, cluster of differentiation 274, CD274, B7 homolog 1, B7-H1, B7H1) and PD-L2 (programmed cell death 1 ligand 2, PDL2, B7-DC, B7DC, CD273, cluster of differentiation 273) are PD1 ligands. PD-L1 is constitutively expressed in many human cancers including without limitation melanoma, ovarian cancer, lung cancer, clear cell renal cell carcinoma (CRCC), urothelial carcinoma, HNSCC, and esophageal cancer. Blockade of PD-1 which is expressed in tumor-infiltrating T cells (TILs) has created an important rationale for development to monoclonal antibody therapy to target blockade of PD1/PDL-1 pathway. Tumor cell expression of PD-L1 is used as a mechanism to evade recognition/destruction by the immune system as in normal cells the PD1/PDL1 interplay is an immune checkpoint. Monoclonal antibodies targeting PD-1/PD-L1 that boost the immune system are being developed for the treatment of cancer. *See, e.g.,* Flies *et al*, Blockade of the B7-H1/PD-1 pathway for cancer immunotherapy. Yale J Biol Med. 2011 Dec;84(4):409-21; Sznol and Chen, Antagonist Antibodies to PD-1 and B7-H1 (PD-L1) in the Treatment of Advanced Human Cancer, Clin Cancer Res; 19(5) March 1, 2013; Momtaz and Postow, Immunologic checkpoints in cancer therapy: focus on the programmed death-1 (PD-1) receptor pathway. Pharmgenomics Pers Med. 2014 Nov 15;7:357-65; Shin and Ribas, The evolution of checkpoint blockade as a cancer therapy: what's here, what's next?, Curr Opin Immunol. 2015 Jan 23;33C:23-35; which references are incorporated by reference herein in their entirety. Several drugs are in clinical development that affect the PDL1/PD1 pathway include: 1) Nivolumab (BMS936558/MDX-1106), an anti-PD1 drug from Bristol Myers Squib drug which was approved by the U.S. FDA in late 2014 under the brand name OPDIVO for the treatment of patients with unresectable or

metastatic melanoma and disease progression following ipilimumab and, if BRAF V600 mutation positive, a BRAF inhibitor; 2) Pembrolizumab (formerly lambrolizumab, MK-3475, trade name Keytruda), an anti-PD1 drug from Merck approved in late 2014 for use following treatment with ipilimumab, or after treatment with ipilimumab and a BRAF inhibitor in patients who carry a BRAF mutation; 3) BMS-936559/MDX-1105, an anti-PDL1 drug from Bristol Myers Squib with initial evidence in advanced solid tumors; and 4) MPDL3280A, an anti-PDL1 drug from Roche with initial evidence in NSCLC.

[00422] Expression of PD1, PD-L1 and/or PD-L2 expression can be assessed at the protein and/or mRNA level according to the methods of the invention. For example, IHC can be used to assess their protein expression. Expression may indicate likely benefit of inhibitors of the B7-H1/PD-1 pathway, whereas lack of expression may indicate lack of benefit thereof. In some embodiments, expression of both PD-1 and PD-L1 is assessed and likely benefit of inhibitors of the B7-H1/PD-1 pathway is determined only upon co-expression of both of these immunosuppressive components. Certain cells express PD-L1 mRNA, but not the protein, due to translational suppression by microRNA miR-513. Therefore, analysis of PD-L1 protein may be desirable for molecular profiling. Molecular profiling may also include that of miR-513. Expression of miR-513 above a certain threshold may indicate lack of benefit of immune modulation therapy.

[00423] In an aspect, the invention provides a method of identifying at least one treatment associated with a cancer in a subject, comprising: a) determining a molecular profile for at least one sample from the subject by assessing a plurality of gene or gene products, wherein the plurality of gene or gene products comprises at least one of PD-1 and PD-L1; and b) identifying, based on the molecular profile, at least one of: i) at least one treatment that is associated with benefit for treatment of the cancer; ii) at least one treatment that is associated with lack of benefit for treatment of the cancer; and iii) at least one treatment associated with a clinical trial. Expression of PD-1 and/or PD-L1 may be performed along with that of additional biomarkers that guide treatment selection according to the invention. Such additional biomarkers can be additional immune modulators including without limitation CTL4A, IDO1, COX2, CD80, CD86, CD8A, Granzyme A, Granzyme B, CD19, CCR7, CD276, LAG-3, TIM-3, and a combination thereof. The additional biomarkers could also comprise other useful biomarkers disclosed herein, such any of **Tables 2**, **6**, **7** or **10-17**. For example, the additional biomarkers may comprise at least one of 1p19q, ABL1, AKT1, ALK, APC, AR, ATM, BRAF, BRCA1, BRCA2, cKIT, cMET, CSF1R, CTNNB1, EGFR, EGFRvIII, ER, ERBB2 (HER2), FGFR1, FGFR2, FLT3, GNA11, GNAQ, GNAS, HER2, HRAS, IDH1, IDH2, JAK2, KDR (VEGFR2), KRAS, MGMT, MGMT-Me, MLH1, MPL, NOTCH1, NRAS, PDGFRA, Pgp, PIK3CA, PR, PTEN, RET, RRM1, SMO, SPARC, TLE3, TOP2A, TOPO1, TP53, TS, TUBB3, VHL, CDH1, ERBB4, FBXW7, HNF1A, JAK3, NPM1, PTPN11, RB1, SMAD4, SMARCB1, STK1, MLH1, MSH2, MSH6, PMS2, microsatellite instability (MSI), ROS1 and ERCC1. These additional analyses may suggest combinations of therapies likely to benefit the patient, such as a PD-1/PD-L1 pathway inhibitor and another therapy suggested by the molecular profiling. See, e.g., additional biomarker-drug associations in any of **Tables 3-7**, **Table 8**, **Tables 11-17**, **Table 19**,

Tables 24-26 and **FIGs. 28D-E**. In some embodiments, anti-CTLA-4 therapy, including without limitation ipilimumab, is administered with PD-1/PD-L1 pathway therapy.

[00424] The invention further provides association of immune modulation therapy, including without limitation PD-1/PD-L1 pathway inhibitor treatments, with molecular profiling of biomarkers in addition to PD-1/PD-L1 themselves. In an embodiment of the invention, beneficial treatment of the cancer with immunotherapy targeting at least one of PD-1, PD-L1, CTLA-4, IDO-1, and CD276, is associated with a molecular profile indicating that the cancer is AR-/HER2-/ER-/PR- (quadruple negative) and/or carries a mutation in BRCA1. See, e.g., Example 7 herein. In some embodiments, the invention provides associating beneficial treatment of the cancer with immunotherapy targeting immune modulating therapy wherein the molecular profile indicates that the cancer carries a mutation in at least one cancer-related gene. The cancer-related gene can include at least one, e.g., at least 1, 2, 3, 4, 5, 6, 7, 8, 9, 10, 11, 12, 13, 14, 15, 16, 17, 18, 19, 20, 21, 22, 23, 24, 25, 26, 27, 28, 29, 30, 31, 32, 33, 34, 35, 36, 37, 38, 39, 40, 41, 42, 43, 44, 45, 46 or 47, of ABL1, AKT1, ALK, APC, ATM, BRAF, BRCA1, BRCA2, cKIT, cMET, CSF1R, CTNNB1, EGFR, ERBB2, FGFR1, FGFR2, FLT3, GNA11, GNAQ, GNAS, HRAS, IDH1, JAK2, KDR (VEGFR2), KRAS, MLH1, MPL, NOTCH1, NRAS, PDGFRA, PIK3CA, PTEN, RET, SMO, TP53, VHL, CDH1, ERBB4, FBXW7, HNF1A, JAK3, NPM1, PTPN11, RB1, SMAD4, SMARCB1 and STK1. Other cancer related genes, such as those disclosed herein or in the COSMIC (Catalogue Of Somatic Mutations In Cancer) database (available at cancer.sanger.ac.uk/cancergenome/projects/cosmic/), can be assessed as well. See Example 11 herein. It will be apparent to one of skill that such profiling may be performed independently of direct assessment of immune modulators themselves. As an illustrative example, a tumor determined to carry a mutation in BRCA1 may be a candidate for anti-PD-1 and/or anti-PD-L1 therapy. Thus, in a related aspect, the invention provides a method of identifying at least one treatment associated with a cancer in a subject, comprising: a) determining a molecular profile for at least one sample from the subject by assessing a plurality of gene or gene products other than PD-1 and/or PD-L1; and b) identifying, based on the molecular profile, that the cancer is likely to benefit from anti-PD-1 or anti-PD-L1 therapy.

[00425] Expression of PD-1 is generally assessed in tumor infiltrating lymphocytes (TILs). PD-L1 may be expressed in various cells in the tumor microenvironment. In addition to tumor cells, PD-L1 can be expressed by T cells, natural killer (NK) cells, macrophages, myeloid dendritic cells (DCs), B cells, epithelial cells, and vascular endothelial cells. In some cases, the response to anti-PD-1/PD-L1 therapy may be dependent on which cells in the tumor microenvironment express PD-L1. Thus, in some embodiments of the invention, the tumor microenvionment is assessed to determine the expression patterns of PD-L1 and the likely benefit or lack thereof is dependent on the cells determined to express PD-L1. Such PD-L1 expression can be determined in various cells, including without limitation one or more of T cells, natural killer (NK) cells, macrophages, myeloid dendritic cells (DCs), B cells, epithelial cells, and endothelial cells.

[00426] Certain tumor cells may also more susceptible to immune modulating therapy and thus more likely associated with likely treatment benefit. An “immune modulating therapy” can include antagonists

such as antibodies to PD-1, PD-L1, PD-L2, CTL4A, IDO1, COX2, CD80, CD86, CD8A, Granzyme A, Granzyme B, CD19, CCR7, CD276, LAG-3 or TIM-3. The antagonist could also be a soluble ligand or small molecule inhibitor. As a non-limiting example, a soluble PD-L1 construct may bind PD-1 and thus block its immunosuppressive activity. In an embodiment, the invention provides for determining the apoptotic or necrotic environment of the tumor. Apoptotic or necrotic cells may be associated with likely treatment benefit from immune modulating therapy. Thus, the invention provides a method of identifying at least one treatment associated with a cancer in a subject, comprising: a) determining a molecular profile for at least one sample from the subject by assessing tumor necrosis or apoptosis; and b) associating the cancer with likely to benefit from immune modulating therapy, including without limitation anti-PD-1 or anti-PD-L1 therapy, if apoptotic or necrotic tumor cells are identified.

EXAMPLES

Example 1: Molecular profiling to find targets and select treatments for refractory cancers

[00427] The primary objective was to compare progression free survival (PFS) using a treatment regimen selected by molecular profiling with the PFS for the most recent regimen the patient progressed on (e.g. patients are their own control) (**FIG. 30**). The molecular profiling approach was deemed of clinical benefit for the individual patient who had a PFS ratio (PFS on molecular profiling selected therapy/PFS on prior therapy) of $\geq$1.3.

[00428] The study was also performed to determine the frequency with which molecular profiling by IHC, FISH and microarray yielded a target against which there is a commercially available therapeutic agent and to determine response rate (RECIST) and percent of patients without progression or death at 4 months.

[00429] The study was conducted in 9 centers throughout the United States. An overview of the method is depicted in **FIG. 31.** As can be seen in **FIG. 31**, the patient was screened and consented for the study. Patient eligibility was verified by one of two physician monitors. The same physicians confirmed whether the patients had progressed on their prior therapy and how long that PFS (TTP) was. A tumor biopsy was then performed, as discussed below. The tumor was assayed using IHC, FISH (on paraffin-embedded material) and microarray (on fresh frozen tissue) analyses.

[00430] The results of the IHC/FISH and microarray were given to two study physicians who in general used the following algorithm in suggesting therapy to the physician caring for the patient: 1) IHC/FISH and microarray indicated same target was first priority; 2) IHC positive result alone next priority; and 3) microarray positive result alone the last priority.

[00431] The patient's physician was informed of the suggested treatment and the patient was treated with the suggested agent(s) (package insert recommendations). The patient's disease status was assessed every 8 weeks and adverse effects were assessed by the NCI CTCAE version 3.0.

[00432] To be eligible for the study, the patient was required to: 1) provide informed consent and HIPAA authorization; 2) have any histologic type of metastatic cancer; 3) have progressed by RECIST criteria on at least 2 prior regimens for advanced disease; 4) be able to undergo a biopsy or surgical procedure to

obtain tumor samples; 5) be ≥18 years, have a life expectancy > 3 months, and an Eastern Cooperative Oncology Group (ECOG) Performance Status or 0-1; 6) have measurable or evaluable disease; 7) be refractory to last line of therapy (documented disease progression under last treatment; received ≥6 weeks of last treatment; discontinued last treatment for progression); 8) have adequate organ and bone marrow function; 9) have adequate methods of birth control; and 10) if CNS metastases then adequately controlled. The ECOG performance scale is described in Oken, M.M., Creech, R.H., Tormey, D.C., Horton, J., Davis, T.E., McFadden, E.T., Carbone, P.P.: Toxicity And Response Criteria Of The Eastern Cooperative Oncology Group. Am J Clin Oncol 5:649-655, 1982, which is incorporated by reference in its entirety. Before molecular profiling was performed, the principal investigator at the site caring for the patient must designate what they would treat the patient with if no molecular profiling results were available.

[00433] ***Methods***

[00434] All biopsies were performed at local investigators' sites. For needle biopsies, 2-3 18 gauge needle core biopsies were performed. For DNA microarray (MA) analysis, tissue was immediately frozen and shipped on dry ice via FedEx to a central CLIA certified laboratory, Caris MPI in Phoenix, Arizona. For IHC, paraffin blocks were shipped on cold packs. IHC was considered positive for target if 2+ in ≥ 30% of cells. The MA was considered positive for a target if the difference in expression for a gene between tumor and control organ tissue was at a significance level of $p \leq 0.001$.

[00435] Ascertainment of the Time to Progression to Document the Progression-Free Survival Ratio

[00436] Time to progression under the last line of treatment was documented by imaging in 58 patients (88%). Among these 58 patients, documentation by imaging alone occurred in 49 patients (74%), and documentation by imaging with tumor markers occurred in nine patients (14%; ovarian cancer, n 3; colorectal, n 1; pancreas, n 1; prostate, n 3; breast, n 1). Patients with clinical proof of progression were accepted when the investigator reported the assessment of palpable and measurable lesions (i.e., inflammatory breast cancer, skin/subcutaneous nodules, or lymph nodes), which occurred in six patients (9%). One patient (2%) with prostate cancer was included with progression by tumor marker. In one patient (2%) with breast cancer, the progression was documented by increase of tumor marker and worsening of bone pain. The time to progression achieved with a treatment based on molecular profiling was documented by imaging in 44 patients (67%) and by clinical events detected between two scheduled tumor assessments in 20 patients. These clinical events were reported as serious adverse events related to disease progression (e.g., death, bleeding, bowel obstruction, hospitalization), and the dates of reporting were censored as progression of disease. The remaining two patients were censored at the date of last follow-up.

[00437] IHC/FISH

[00438] For IHC studies, the formalin fixed, paraffin embedded tumor samples had slices from these blocks submitted for IHC testing for the following proteins: EGFR, SPARC, C-kit, ER, PR, Androgen receptor, PGP, RRM1, TOPO1, BRCP1, MRP1, MGMT, PDGFR, DCK, ERCC1, Thymidylate synthase, Her2/neu and TOPO2A. IHCs for all proteins were not carried out on all patients' tumors.

[00439] Formalin-fixed paraffin-embedded patient tissue blocks were sectioned (4μm thick) and mounted onto glass slides. After deparaffination and rehydration through a series of graded alcohols, pretreatment was performed as required to expose the targeted antigen.

[00440] Human epidermal growth factor receptor 2 (HER2) and epidermal growth factor receptor (EGFR) were stained as specified by the vendor (DAKO, Denmark). All other antibodies were purchased from commercial sources and visualized with a DAB biotin-free polymer detection kit. Appropriate positive control tissue was used for each antibody. Negative control slides were stained by replacing the primary antibody with an appropriately matched isotype negative control reagent. All slides were counterstained with hematoxylin as the final step and cover slipped. Tissue microarray sections were analyzed by FISH for EGFR and HER-2/neu copy number per the manufacturer's instructions. FISH for HER-2/neu (was done with the PathVysion HER2 DNA Probe Kit (Abbott Molecular, Abbott Park, IL). FISH for EGFR was done with the LSI EGFR/CEP 7 Probe (Abbott Molecular).

[00441] All slides were evaluated semi-quantitatively by a first pathologist, who confirmed the original diagnosis as well as read each of the immunohistochemical stains using a light microscope. Some lineage immunohistochemical stains were performed to confirm the original diagnosis, as necessary. Staining intensity and extent of staining were determined; both positive, tumor-specific staining of tumor cells and highly positive (≥2+), pervasive (≥30%) tumor specific staining results were recorded. IHC was considered positive for target if staining was ≥ 2+ in ≥ 30% of cells. Rather than look for a positive signal without qualification, this approach raises the stringency of the cut point such that it would be a significant or more demonstrative positive. A higher positive is more likely to be associated with a therapy that would affect the time to progression. The cut point used (i.e., staining was ≥ 2+ in ≥ 30% of cells) is similar to some cut points used in breast cancer for HER2/neu. When IHC cut points were compared with evidence from the tissue of origin of the cancer, the cut points were equal to or higher (more stringent) than the evidence cut points. A standard 10% quality control was performed by a second pathologist.

[00442] Microarray

[00443] Tumor samples obtained for microarray were snap frozen within 30 minutes of resection and transmitted to Caris-MPI on dry ice. The frozen tumor fragments were placed on a 0.5mL aliquot of frozen 0.5M guanidine isothiocyanate solution in a glass tube, and simultaneously thawed and homogenized with a Covaris S2 focused acoustic wave homogenizer (Covaris, Woburn, MA). A 0.5mL aliquot of TriZol was added, mixed and the solution was heated to 65˚C for 5 minutes then cooled on ice and phase separated by the addition of chloroform followed by centrifugation. An equal volume of 70% ethanol was added to the aqueous phase and the mixture was chromatographed on a Qiagen RNeasy column (Qiagen, Germantown, MD). RNA was specifically bound and then eluted. The RNA was tested for integrity by assessing the ratio of 28S to 18S ribosomal RNA on an Agilent BioAnalyzer (Agilent, Santa Clara, CA). Two to five micrograms of tumor RNA and two to five micrograms of RNA from a sample of a normal tissue representative of the tumor's tissue of origin were separately converted to cDNA and then labeled during T7 polymerase amplification with contrasting fluor tagged (Cy3, Cy5)

cytidine triphosphate. The labeled tumor and its tissue of origin reference were hybridized to an Agilent H1Av2 60-mer olio array chip with 17,085 unique probes.

[00444] The arrays contain probes for 50 genes for which there is a possible therapeutic agent that would potentially interact with that gene (with either high expression or low expression). Those 50 genes included: ADA, AR, ASNA, BCL2, BRCA2, CD33, CDW52, CES2, DNMT1, EGFR, ERBB2, ERCC3, ESR1, FOLR2, GART, GSTP1, HDAC1, HIF1A, HSPCA, IL2RA, KIT, MLH1, MS4A1, MASH2, NFKB2, NFKBIA, OGFR, PDGFC, PDGFRA, PDGFRB, PGR, POLA, PTEN, PTGS2, RAF1, RARA, RXRB, SPARC, SSTR1, TK1, TNF, TOP1, TOP2A, TOP2B, TXNRD1, TYMS, VDR, VEGF, VHL, and ZAP70.

[00445] The chips were hybridized from 16 to 18 hours at 60°C and then washed to remove non-stringently hybridized probe and scanned on an Agilent Microarray Scanner. Fluorescent intensity data were extracted, normalized, and analyzed using Agilent Feature Extraction Software. Gene expression was judged to be different from its reference based on an estimate of the significance of the extent of change, which was estimated using an error model that takes into account the levels of signal to noise for each channel, and uses a large number of positive and negative controls replicated on the chip to condition the estimate. Expression changes at the level of $p \leq 0.001$ were considered as significantly different.

[00446] Statistical Considerations

[00447] The protocol called for a planned 92 patients to be enrolled of which an estimated 64 patients would be treated with therapy assigned by molecular profiling. The other 28 patients were projected to not have molecular profiling results available because of (a) inability to biopsy the patient; (b) no target identified by the molecular profiling; or (c) deteriorating performance status. Sixty four patients were required to receive molecular profiling treatment in order to reject the null hypothesis (Ho) that: ≤15% of patients would have a PFS ratio of ≥1.3 (e.g. a non-promising outcome).

[00448] Treatment Selection

[00449] Treatment for the patients based on molecular profiling results was selected using the following algorithm: 1) IHC/FISH and microarray indicates same target; 2) IHC positive result alone; 3) microarray positive result alone. The patient's physician was informed of suggested treatment and the patient was treated based on package insert recommendations. Disease status was assessed every 8 weeks. Adverse effects were assessed by NCI CTCAE version 3.0.

[00450] The targets and associated drugs are listed in **Table 19**.

Table 19: Pairings of Targets and Drugs

Potential Target	Agents Suggested as Interacting With the Target
IHC	
EGFR	Cetuximab, erlotinib, gefitinib
SPARC	Nanoparticle albumin-bound paclitaxel
c-KIT	Imatinib, sunitinib, sorafenib
ER	Tamoxifen, aromatase inhibitors, toremifene, progestational agent
PR	Progestational agents, tamoxifen, aromatase inhibitor, goserelin
Androgen receptor	Flutamide, abarelix, bicalutamide, leuprolide, goserelin
PGP	Avoid natural products, doxorubicin, etoposide, docetaxel, vinorelbine
HER2/NEU	Trastuzumab

PDGFR	Sunitinib, imatinib, sorafenib
CD52	Alemtuzumab
CD25	Denileukin diftitox
HSP90	Geldanamycin, CNF2024
TOP2A	Doxorubicin, epirubicin, etoposide
Microarray	
ADA	Pentostatin, cytarabine
AR	Flutamide, abarelix, bicalutamide, leuprolide, goserelin
ASNA	Asparaginase
BCL2	Oblimersen sodium†
BRCA2	Mitomycin
CD33	Gemtuzumab ozogamicin
CDW52	Alemtuzumab
CES-2	Irinotecan
DCK	Gemcitabine
DNMT1	Azacitidine, decitabine
EGFR	Cetuximab, erlotinib, gefitinib
ERBB2	Trastuzumab
ERCC1	Cisplatin, carboplatin, oxaliplatin
ESR1	Tamoxifen, aromatase inhibitors, toremifene, progestational agent
FOLR2	Methotrexate, pemetrexed
GART	Pemetrexed
GSTP1	Platinum
HDAC1	Vorinostat
HIF1α	Bevacizumab, sunitinib, sorafenib
HSPCA	Geldanamycin, CNF2024
IL2RA	Aldesleukin
KIT	Imatinib, sunitinib, sorafenib
MLH-1	Gemcitabine, oxaliplatin
MSH1	Gemcitabine
MSH2	Gemcitabine, oxaliplatin
NFKB2	Bortezomib
NFKB1	Bortezomib
OGFR	Opioid growth factor
PDGFC	Sunitinib, imatinib, sorafenib
PDGFRA	Sunitinib, imatinib, sorafenib
PDGFRB	Sunitinib, imatinib, sorafenib
PGR	Progestational agents, tamoxifen, aromatase inhibitors, goserelin
POLA	Cytarabine
PTEN	Rapamycin (if low)
PTGS2	Celecoxib
RAF1	Sorafenib
RARA	Bexarotene, all-trans-retinoic acid
RXRB	Bexarotene
SPARC	Nanoparticle albumin-bound paclitaxel
SSTR1	Octreotide
TK1	Capecitabine
TNF	Infliximab
TOP1	Irinotecan, topotecan
TOP2A	Doxorubicin, etoposide, mitoxantrone
TOP2B	Doxorubicin, etoposide, mitoxantrone
TXNRD1	Px12
TYMS	Fluorouracil, capecitabine
VDR	Calcitriol
VEGF	Bevacizumab, sunitinib, sorafenib

VHL	Bevacizumab, sunitinib, sorafenib
ZAP70	Geldanamycin, CNF2024

[00451] ***Results***

[00452] The distribution of the patients is diagrammed in **FIG. 32** and the characteristics of the patients shown in **Tables 20** and **21**. As can be seen in **FIG. 32**, 106 patients were consented and evaluated. There were 20 patients who did not proceed with molecular profiling for the reasons outlined in **FIG. 32** (mainly worsening condition or withdrawing their consent or they did not want any additional therapy). There were 18 patients who were not treated following molecular profiling (mainly due to worsening condition or withdrawing consent because they did not want additional therapy). There were 68 patients treated, with 66 of them treated according to molecular profiling results and 2 not treated according to molecular profiling results. One of the two was treated with another agent because the clinician caring for the patient felt a sense of urgency to treat and the other was treated with another agent because the insurance company would not cover the molecular profiling suggested treatment.

[00453] The median time for molecular profiling results being made accessible to a clinician was 16 days from biopsy (range 8 to 30 days) and a median of 8 days (range 0 to 23 days) from receipt of the tissue sample for analysis. Some modest delays were caused by the local teams not sending the patients' blocks immediately (due to their need for a pathology workup of the specimen). Patient tumors were sent from 9 sites throughout the United States including: Greenville, SC; Tyler, TX; Beverly Hills, CA; Huntsville, AL; Indianapolis, IN; San Antonio, TX; Scottsdale, AZ and Los Angeles, CA.

[00454] **Table 20** details the characteristics of the 66 patients who had molecular profiling performed on their tumors and who had treatment according to the molecular profiling results. As seen in **Table 21**, of the 66 patients the majority were female, with a median age of 60 (range 27-75). The number of prior treatment regimens was 2-4 in 53% of patients and 5-13 in 38% of patients. There were 6 patients (9%), who had only 1 prior therapy because no approved active 2nd line therapy was available. Twenty patients had progressed on prior phase I therapies. The majority of patients had an ECOG performance status of 1.

Table 20: Patient Characteristics (n=66)

Characteristic	**n**	**%**
Gender		
Female	43	65
Male	23	35
Age		
Median (range)	60	(27-75)
Number of Prior Treatments		
2-4*	35	53
5-13	25	38
ECOG		
0	18	27
1	48	73

*Note: 6 patients (9%) had 1 prior

[00455] As seen in **Table 21**, tumor types in the 66 patients included breast cancer 18 (27%), colorectal 11 (17%), ovarian 5 (8%), and 32 patients (48%) were in the miscellaneous categories. Many patients had the more rare types of cancers.

Table 21: Patient Tumor Types (n=66)

Tumor Type	n	%
Breast	***18***	***27***
Colorectal	***11***	***17***
Ovarian	***5***	***8***
Miscellaneous	***32***	***48***
Prostate	4	6
Lung	3	5
Melanoma	2	3
Small cell (esopha/retroperit)	2	3
Cholangiocarcinoma	2	3
Mesothelioma	2	3
H&N (SCC)	2	3
Pancreas	2	3
Pancreas neuroendocrine	1	1.5
Unknown (SCC)	1	1.5
Gastric	1	1.5
Peritoneal pseudomyxoma	1	1.5
Anal Canal (SCC)	1	1.5
Vagina (SCC)	1	1.5
Cervis	1	1.5
Renal	1	1.5
Eccrine seat adenocarinoma	1	1.5
Salivary gland adenocarinoma	1	1.5
Soft tissue sarcoma (uterine)	1	1.5
GIST (Gastric)	1	1.5
Thyroid-Anaplastic	1	1.5

[00456] *Primary Endpoint: PFS Ratio ≥ 1.3*

[00457] As far as the primary endpoint for the study is concerned (PFS ratio of ≥1.3), in the 66 patients treated according to molecular profiling results, the number of patients with PFS ratio greater or equal to 1.3 was 18 out of the 66 or 27%, 95% CI 17-38% one-sided, one-sample non parametric test p=0.007. The null hypothesis was that ≤15% of this patient population would have a PFS ratio of ≥1.3. Therefore, the null hypothesis is rejected and our conclusion is that this molecular profiling approach is beneficial. **FIG. 33** details the comparison of PFS on molecular profiling therapy (the bar) versus PFS (TTP) on the patient's last prior therapy (the boxes) for the 18 patients. The median PFS ratio is 2.9 (range 1.3-8.15).

[00458] If the primary endpoint is examined, as shown in **Table 22**, a PFS ratio ≥1.3 was achieved in 8/18 (44%) of patients with breast cancer, 4/11 (36%) patients with colorectal cancer, 1/5 (20%) of patients with ovarian cancer and 5/32 (16%) patients in the miscellaneous tumor types (note that miscellaneous tumor types with PFS ratio ≥1.3 included: lung 1/3, cholangiocarcinoma 1/3, mesothelioma 1/2, eccrine sweat gland tumor 1/1, and GIST (gastric) 1/1).

Table 22: Primary Endpoint – PFS Ratio ≥ 1.3 By Tumor Type

Tumor Type	Total Treated	Number with PFS Ratio ≥ 1.3	%

Breast	18	8	44
Colorectal	11	4	36
Ovarian	5	1	20
Miscellaneous*	32	5	16
Total	**66**	**18**	**27**

*lung 1/3, cholangiocarcinoma ½, mesothelioma ½, eccrine sweat 1/1, GIST (gastric) 1/1

[00459] The treatment that the 18 patients with the PFS ≥1.3 received based on profiling is detailed in **Table 23**. As can be seen in that table for breast cancer patients, the treatment ranged from diethylstibesterol to nab paclitaxel + gemcitabine to doxorubicin. Treatments for patients with other tumor types are also detailed in **Table 23**. The table further shows a comparison of the drugs that the responding patients received versus the drugs that would have been suggested without molecular profiling and indicates which targets were used to suggest the therapies. Overall, 14 were treated with combinations and 4 were treated with single agents.

Table 23: Targets Noted in Patients' Tumors, Treatment Suggested on the Basis of These Results, and Treatment Investigator Would Use if No Target Was Identified (in patients with PFS ratio ≥ 1.3)

Location of Primary Tumor	Targets Used to Suggest Treatment and Method Used	Treatment Suggested on Basis of Patient's Tumor Molecular Profiling	Treatment the Investigator Would Have Used if No Results From Molecular Profiling
Breast	ESR1: I; *ESR1*: M	DES 5 mg TID	Investigational
Cholangiocarcinoma	EGFR: I; *TOP1*: M	CPT-11 350 mg/m^2 every 3 weeks; cetuximab 400 mg/m^2 day 1, 250 mg/m^2 every week	Investigational
Breast	SPARC: I; *SPARC*, *ERBB2*: M	NAB paclitaxel 260 mg/m^2 every 3 weeks; trastuzumab 6 mg/kg every 3 weeks	Docetaxel, trastuzumab
Eccrine sweat gland (right forearm)	c-KIT: I; *c-KIT*:M	Sunitinib 50 mg/d, 4 weeks on/2 weeks off	Best supportive care
Ovary	HER2/NEU, ER: I; *HER2/NEU*: M	Lapatinib 1,250 mg PO days 1-21; tamoxifen 20 mg PO	Bevacizumab
Colon/rectum	PDGFR, c-KIT: I I; *PDGFR, TOP1*: M	CPT-11 70 mg/m^2 weekly for 4 weeks on/2 weeks off; sorafenib 400 mg BID	Cetuximab
Breast	SPARC: I; *DCK*: M	NAB paclitaxel 90 mg/m^2 every 3 weeks; gemcitabine 750 mg/m^2 days 1, 8, 15, every 3 weeks	Mitomycin
Breast	ER: I; *ER, TYMS*: M	Letrozole 2.5 mg daily; capecitabine 1,250 mg/m^2 BID, 2 weeks on/1 week off	Capecitabine
Malignant mesothelioma	MLH1, MLH2: I;	Gemcitabine 1,000	Gemcitabine

	RRM2B, *RRM1*, *RRM2*, *TOP2B*: M	mg/m2 days 1 and 8, every 3 weeks; etoposide 50 mg/m^2 3 days every 3 weeks	
Breast	MSH2	Oxaliplatin 85 mg/m^2 every 2 weeks; fluorouracil (5FU) 1,200 mg/m^2 days 1 and 2, every 2 weeks; trastuzumab 4 mg/kg day 1, 2 mg/kg every week	Investigational
Non–small-cell lung cancer	EGFR: I; *EGFR*	Cetuximab 400 mg/m^2 day 1, 250 mg/m^2 every week; CPT-11 125 mg/m^2 weekly for 4 weeks on/2 weeks off	Vinorelbine
Colon/rectum	*MGMT*	Temozolomide 150 mg/m^2 for 5 days every 4 weeks; bevacizumab 5 mg/kg every 2 weeks	Capecitabine
Colon/rectum	PDGFR, c-KIT: I; *PDGFR*: *KDR*, *HIF1A*, *BRCA2*: M	Mitomycin 10 mg once every 4-6 weeks; sunitinib 37.5 mg/d, 4 weeks on/2 weeks off	Capecitabine
Breast	*DCK*, *DHFR*: M	Gemcitabine 1,000 mg/m^2 days 1 and 8 every 3 weeks; pemetrexed 500 mg/m^2 days 1 and 8, every 3 weeks	Best supportive care
Breast	TOP2A: I; *TOP2A*: M	Doxorubicin 50 mg/m^2 every 3 weeks	Vinorelbine
Colon/rectum	*MGMT*, *VEGFA*, *HIF1A*: M	Temozolomide 150 mg/m^2 for 5 days every 4 weeks; sorafenib 400 mg BID	Panitumumab
Breast	ESR1, PR: I; *ESR1, PR*: M	Exemestane 25 mg every day	Doxorubicin liposomal
GIST (stomach)	EGFR: I; *EGFR*, *RRM2*: M	Gemcitabine 1,000 mg/m^2 days 1, 8, and 15 every 4 weeks; cetuximab 400 mg/m^2 day 1, 250 mg/m^2 every week	None

* Abbreviations used in **Table 23**: I, immunohistochemistry; M, microarray; DES, diethylstilbestrol; CPT-11, irinotecan; TID, three times a day; NAB, nanoparticle albumin bound; PO, orally; BID, twice a day; GIST, GI stromal tumor.

[00460] *Secondary Endpoints*

[00461] The results for the secondary endpoint for this study are as follows. The frequency with which molecular profiling of a patients' tumor yielded a target in the 86 patients where molecular profiling was attempted was 84/86 (98%). Broken down by methodology, 83/86 (97%) yielded a target by IHC/FISH and 81/86 (94%) yielding a target by microarray. RNA was tested for integrity by assessing the ratio of

28S to 18S ribosomal RNA on an Agilent BioAnalyzer. 83/86 (97%) specimens had ratios of 1 or greater and gave high intra-chip reproducibility ratios. This demonstrates that very good collection and shipment of patients' specimens throughout the United States and excellent technical results can be obtained.

[00462] By RECIST criteria in 66 patients, there was 1 complete response and 5 partial responses for an overall response rate of 10% (one CR in a patient with breast cancer and PRs in breast, ovarian, colorectal and NSCL cancer patients). Patients without progression at 4 months included 14 out of 66 or 21%.

[00463] In an exploratory analysis, a waterfall plot for all patients for maximum % change of the summed diameters of target lesions with respect to baseline diameters was generated. The patients who had progression and the patients who had some shrinkage of their tumor sometime during their course along with those partial responses by RECIST criteria is demonstrated in **FIG. 34**. There is some shrinkage of patient's tumors in over 47% of the patients (where 2 or more evaluations were completed).

[00464] *Other Analyses – Safety*

[00465] As far as safety analyses there were no treatment related deaths. There were nine treatment related serious adverse events including anemia (2 patients), neutropenia (2 patients), dehydration (1 patient), pancreatitis (1 patient), nausea (1 patient), vomiting (1 patient), and febrile neutropenia (1 patient). Only one patient (1.5%) was discontinued due to a treatment related adverse event of grade 2 fatigue.

[00466] *Other Analyses – Relationship between What the Clinician Caring for the Patient Would Have Selected versus What the Molecular Profiling Selected*

[00467] The relationship between what the clinician selected to treat the patient before knowing what molecular profiling results suggested for treatment was also examined. As detailed in **FIG. 35**, there is no pattern between the two. More specifically, no matches for the 18 patients with PFS ratio ≥1.3 were noted.

[00468] The overall survival for the 18 patients with a PFS ratio of ≥1.3 versus all 66 patients is shown in **FIG. 36**. This exploratory analysis was done to help determine if the PFS ratio had some clinical relevance. The overall survival for the 18 patients with the PFS ratio of ≥1.3 is 9.7 months versus 5 months for the whole population – log rank 0.026. This exploratory analysis indicates that the PFS ratio is correlated with the clinical parameter of survival.

[00469] ***Conclusions***

[00470] This prospective multi-center pilot study demonstrates: (a) the feasibility of measuring molecular targets in patients' tumors from 9 different centers across the US with good quality and sufficient tumor collection – and treat patients based on those results; (b) this molecular profiling approach gave a longer PFS for patients on a molecular profiling suggested regimen than on the regimen they had just progressed on for 27% of the patients (confidence interval 17-38%) p = 0.007; and (c) this is a promising result demonstrating use and benefits of molecular profiling.

[00471] The results also demonstrate that patients with refractory cancer can commonly have simple targets (such as ER) for which therapies are available and can be beneficial to them. Molecular profiling for patients who have exhausted other therapies and who are perhaps candidates for phase I or II trials could have this molecular profiling performed.

Example 2: Molecular Profiling System

[00472] Molecular profiling is performed to determine a treatment for a disease, typically a cancer. Using a molecular profiling approach, molecular characteristics of the disease itself are assessed to determine a candidate treatment. Thus, this approach provides the ability to select treatments without regard to the anatomical origin of the diseased tissue, or other "one-size-fits-all" approaches that do not take into account personalized characteristics of a particular patient's affliction. The profiling comprises determining gene and gene product expression levels, gene copy number and mutation analysis. Treatments are identified that are indicated to be effective against diseased cells that overexpress certain genes or gene products, underexpress certain genes or gene products, carry certain chromosomal aberrations or mutations in certain genes, or any other measureable cellular alterations as compared to non-diseased cells. Because molecular profiling is not limited to choosing amongst therapeutics intended to treat specific diseases, the system has the power to take advantage of any useful technique to measure any biological characteristic that can be linked to a therapeutic efficacy. The end result allows caregivers to expand the range of therapies available to treat patients, thereby providing the potential for longer life span and/or quality of life than traditional "one-size-fits-all" approaches to selecting treatment regimens.

[00473] **FIG. 37** illustrates a molecular profiling system that performs analysis of a cancer sample using a variety of components that measure expression levels, chromosomal aberrations and mutations. The molecular "blueprint" of the cancer is used to generate a prioritized ranking of druggable targets and/or drug associated targets in tumor and their associated therapies.

[00474] A system for carrying out molecular profiling according to the invention comprises the components used to perform molecular profiling on a patient sample, identify potentially beneficial and non-beneficial treatment options based on the molecular profiling, and return a report comprising the results of the analysis to the treating physician or other appropriate caregiver.

[00475] Formalin-fixed paraffin-embedded (FFPE) are reviewed by a pathologist for quality control before subsequent analysis. Nucleic acids (DNA and RNA) are extracted from FFPE tissues after microdissection of the fixed slides. Nucleic acids are extracted using phenol-chlorform extraction or a kit such as the QIAamp DNA FFPE Tissue kit according to the manufacturer's instructions (QIAGEN Inc., Valencia, CA).

[00476] Gene expression analysis is performed using an expression microarray or qPCR (RT-PCR). The qPCR can be performed using a low density microarray. In addition to gene expression analysis, the system can perform a set of immunohistochemistry assays on the input sample. Gene copy number is determined for a number of genes via FISH (fluorescence in situ hybridization) and mutation analysis is done by DNA sequencing (including sequence sensitive PCR assays and fragment analysis such as RFLP, as desired) for a several specific mutations. All of this data is stored for each patient case. Data is reported from the expression, IHC, FISH and DNA sequencing analysis. All laboratory experiments are performed according to Standard Operating Procedures (SOPs).

[00477] Expression can be measured using real-time PCR (qPCR, RT-PCR). The analysis can employ a low density microarray. The low density microarray can be a PCR-based microarray, such as a Taqman™ Low Density Microarray (Applied Biosystems, Foster City, CA).

[00478] Expression can be measured using a microarray. The expression microarray can be an Agilent 44K chip (Agilent Technologies, Inc., Santa Clara, CA). This system is capable of determining the relative expression level of roughly 44,000 different sequences through RT-PCR from RNA extracted from fresh frozen tissue. Alternately, the system uses the Illumina Whole Genome DASL assay (Illumina Inc., San Diego, CA), which offers a method to simultaneously profile over 24,000 transcripts from minimal RNA input, from both fresh frozen (FF) and formalin-fixed paraffin embedded (FFPE) tissue sources, in a high throughput fashion. The analysis makes use of the Whole-Genome DASL Assay with UDG (Illumina, cat#DA-903-1024/DA-903-1096), the Illumina Hybridization Oven, and the Illumina iScan System according to the manufacturer's protocols. **FIG. 38** shows results obtained from microarray profiling of an FFPE sample. Total RNA was extracted from tumor tissue and was converted to cDNA. The cDNA sample was then subjected to a whole genome (24K) microarray analysis using the Illumina Whole Genome DASL process. The expression of a subset of 80 genes was then compared to a tissue specific normal control and the relative expression ratios of these 80 target genes indicated in the figure was determined as well as the statistical significance of the differential expression.

[00479] Polymerase chain reaction (PCR) amplification is performed using the ABI Veriti Thermal Cycler (Applied Biosystems, cat#9902). PCR is performed using the Platinum Taq Polymerase High Fidelity Kit (Invitrogen, cat#11304-029). Amplified products can be purified prior to further analysis with Sanger sequencing, pyrosequencing or the like. Purification is performed using CleanSEQ reagent, (Beckman Coulter, cat#000121), AMPure XP reagent (Beckman Coulter, cat#A63881) or similar. Sequencing of amplified DNA is performed using Applied Biosystem's ABI Prism 3730xl DNA Analyzer and BigDye® Terminator V1.1 chemistry (Life Technologies Corporation, Carlsbad, CA). The BRAF V600E mutation is assessed using the FDA approved cobas® 4800 BRAF V600 Mutation Test from Roche Molecular Diagnostics (Roche Diagnostics, Indianapolis, IN). NextGeneration sequencing is performed using the MiSeq platform from Illumina Corporation (San Diego, California, USA) according to the manufacturer's recommended protocols.

[00480] For RFLP, ALK fragment analysis is performed on reverse transcribed mRNA isolated from a formalin-fixed paraffin-embedded tumor sample using FAM-linked primers designed to flank and amplify EML4-ALK fusion products. The assay is designed to detect variants v1, v2, v3a, v3b, 4, 5a, 5b, 6, 7, 8a and 8b. Other rare translocations may be detected by this assay; however, detection is dependent on the specific rearrangement. This test does not detect ALK fusions to genes other than EML4.

[00481] IHC is performed according to standard protocols. IHC detection systems vary by marker and include Dako's Autostainer Plus (Dako North America, Inc., Carpinteria, CA), Ventana Medical Systems Benchmark® XT (Ventana Medical Systems, Tucson, AZ), and the Leica/Vision Biosystems Bond System (Leica Microsystems Inc., Bannockburn, IL). All systems are operated according to the manufacturers' instructions.

[00482] FISH is performed on formalin-fixed paraffin-embedded (FFPE) tissue. FFPE tissue slides for FISH must be Hematoxylin and Eosion (H & E) stained and given to a pathologist for evaluation. Pathologists will mark areas of tumor to be FISHed for analysis. The pathologist report must show tumor is present and sufficient enough to perform a complete analysis. FISH is performed using the Abbott Molecular VP2000 according to the manufacturer's instructions (Abbott Laboratories, Des Plaines, IA). ALK is assessed using the Vysis ALK Break Apart FISH Probe Kit from Abbott Molecular, Inc. (Des Plaines, IL). HER2 is assessed using the INFORM HER2 Dual ISH DNA Probe Cocktail kit from Ventana Medical Systems, Inc. (Tucson, AZ) and/or SPoT-Light® HER2 CISH Kit available from Life Technologies (Carlsbad, CA).

[00483] DNA for mutation analysis is extracted from formalin-fixed paraffin-embedded (FFPE) tissues after macrodissection of the fixed slides in an area that % tumor nuclei $\geq$ 10% as determined by a pathologist. Extracted DNA is only used for mutation analysis if % tumor nuclei $\geq$ 10%. DNA is extracted using the QIAamp DNA FFPE Tissue kit according to the manufacturer's instructions (QIAGEN Inc., Valencia, CA). DNA can also be extracted using the QuickExtract™ FFPE DNA Extraction Kit according to the manufacturer's instructions (Epicentre Biotechnologies, Madison, WI). The BRAF Mutector I BRAF Kit (TrimGen, cat#MH1001-04) is used to detect BRAF mutations (TrimGen Corporation, Sparks, MD). Roche's Cobas PCR kit can be used to assess the BRAF V600E mutation. The DxS KRAS Mutation Test Kit (DxS, #KR-03) is used to detect KRAS mutations (QIAGEN Inc., Valencia, CA). BRAF and KRAS sequencing of amplified DNA is performed using Applied Biosystems' BigDye® Terminator V1.1 chemistry (Life Technologies Corporation, Carlsbad, CA).

[00484] Next generation sequencing is performed using a TruSeq/MiSeq/HiSeq/NexSeq system offered by Illumina Corporation (San Diego, CA) or an Ion Torrent system from Life Technologies (Carlsbad, CA, a division of Thermo Fisher Scientific Inc.) according to the manufacturer's instructions.

Example 3: Molecular Profiling Reports

[00485] An exemplary report generated by the molecular profiling systems and methods of the invention is shown in **FIGs. 29A-X**. The figures illustrate an exemplary patient report based on molecular profiling for a metastatic pancreatic adenocarinoma. **FIG. 29A** illustrates a cover page of a report indicating patient and specimen information for the patient. **FIG. 29A** also displays a summary of agents associated with potential benefit, potential lack of benefit, or intermediate benefit wherein the molecular profiling results do not impact the potential benefit or lack of potential benefit. Agents associated with potential benefit are further annotated as on NCCN Compendium™ (i.e., recommended by NCCN guidelines for the particular tumor lineage) or off NCCN Compendium™ (i.e., not part of the NCCN guidelines for the particular tumor lineage). **FIG. 29A** also lists clinical trials which may be available given the molecular profiling results, here no trials were matched. **FIG. 29B** reports further summary of biomarker results including biomarkers with notable results (e.g., impacting potential benefit or lack thereof for one or more drug) and those without notable results. **FIG. 29C** illustrates more detailed information for biomarker profiling used to associate agents with potential benefit. **FIGs. 29D-E** illustrate more detailed information for biomarker profiling used to associate agents with lack of potential benefit. **FIG. 29F** illustrates more detailed

information for biomarker profiling used to associate agents with indeterminate benefit. **FIG. 29G** illustrates more detailed information for biomarker profiling used to associate clinical trials. **FIG. 29H**, **FIG. 29I**, **FIG. 29J** and **FIG. 29K** provide a listing of published references used to provide evidence of the biomarker – agent association rules used to construct the report. **FIG. 29L** presents a description of the specimen/s received and a disclaimer, e.g., that ultimate treatment decisions reside solely within the discretion of the treating physician. **FIG. 29M** provides a cover page for an Appendix to the report. **FIG. 29N**, **FIG. 29O**, and **FIG. 29P** provide more information about the mutational analysis performed by Next Generation sequencing and other sequencing tests performed, which can depend on the tumor lineage. **FIG. 29Q** provides more information about the IHC analysis performed on the patient sample, e.g., the staining threshold and results for each marker. **FIG. 29R** provides more information about the ISH analysis performed on the patient sample, which comprised CISH for this tumor. **FIG. 29S**, **FIG. 29T**, **FIG. 29U**, **FIG. 29V**, and **FIG. 29W**, provide a description of the biomarkers assessed per the molecular profiling. **FIG. 29X** provides the framework used for the literature level of evidence as included in the report.

Example 4: Molecular Profiling Panels

[00486] **FIGs. 28A-C** illustrate biomarkers assessed using a molecular profiling approach as outlined in **FIGs. 27A-B**, **Tables 7-17**, and accompanying text herein. **FIG. 28A** illustrates biomarkers that are assessed. The row labeled MI Profile™ does not include the Next Generation sequencing panel. The row labeled MI Profile™ Plus includes the Next Generation sequencing panel. The biomarkers that are assessed according to the Next Generation sequencing panel are shown in **FIG. 28B**. **FIG. 28C** illustrates sample requirements that can be used to perform molecular profiling on a patient tumor sample according to the panels in **FIGs. 28A-B**. **FIG. 28D** and **FIG. 28E** detail the biomarkers assessed, technology platforms utilized and associated therapies or clinical trials.

Example 5: Biomarker- Drug Associations

[00487] Molecular profiling according to the invention leverages multiple technologies to provide evidence-based, clinically actionable information FDA approved cancer drugs. At present, such information is reported for 48 different FDA approved cancer drugs. This Example summarizes biomarker – drug associations available with Level 1 or Level 2 evidence. As described above, Level 1 evidence comprises very high level of evidence. For example, the treatment comprises the standard of care. Level 2 evidence comprises high level of evidence but perhaps insufficient to be considered for standard of care. **Table 24** lists 32 drugs whose biomarker – drug associations are based on IHC or IHC/ISH combination. **Table 25** lists 9 drugs whose biomarker – drug associations are based on sequencing/IHC combination. **Table 26** lists 7 drugs whose biomarker – drug associations are based on sequencing alone. The sequencing can comprise Next Generation Sequencing (NGS), Sanger sequencing, qPCR, or a combination thereof.

[00488] For each row in **Tables 24-26**, the markers and technologies are listed in respective order. For example, in the fourth row in **Table 24**, drug name "ado-trastuzumab emtansine (T-DM1)", the markers

"Her2/Neu, Her2/Neu" are assessed by "FISH" and "IHC," respectively. As another example, in the eighth row in **Table 24**, drug name "crizotinib", the markers "ALK, ROS1" are assessed by "FISH" and "FISH," respectively.

Table 24: Drugs Associations supported by Evidence by IHC and ISH

Drug Name	Markers	Illustrative Technologies
abarelix	Androgen Receptor	IHC
abiraterone	Androgen Receptor	IHC
ado-trastuzumab emtansine (T-DM1)	Her2/Neu, Her2/Neu	FISH, IHC
anastrozole	ER, PR	IHC, IHC
bicalutamide	Androgen Receptor	IHC
capecitabine	TS	IHC
crizotinib	ALK, ROS1	FISH, FISH
degarelix	Androgen Receptor	IHC
docetaxel	SPARC Polyclonal, TUBB3, SPARC Monoclonal, PGP, TLE3	IHC, IHC, IHC, IHC, IHC
doxorubicin	TOP2A, TOP2A, Her2/Neu, PGP	FISH, IHC, FISH, IHC
enzalutamide	Androgen Receptor	IHC
epirubicin	TOP2A, PGP, TOP2A, Her2/Neu	FISH, IHC, IHC, FISH
exemestane	ER, PR	IHC, IHC
fluorouracil	TS	IHC
flutamide	Androgen Receptor	IHC
fulvestrant	ER, PR	IHC, IHC
gemcitabine	RRM1	IHC
goserelin	PR, ER, AR	IHC, IHC, IHC
irinotecan	TOPO1	IHC
lapatinib	Her2/Neu, Her2/Neu	FISH, IHC
letrozole	ER, PR	IHC, IHC
leuprolide	ER, PR	IHC, IHC
liposomal-doxorubicin	TOP2A, TOP2A, PGP, Her2/Neu	FISH, IHC, IHC, FISH
megestrol acetate	PR, ER	IHC, IHC
nab-paclitaxel	SPARC Monoclonal, SPARC Polyclonal, TLE3, PGP, TUBB3	IHC, IHC, IHC, IHC, IHC
paclitaxel	TUBB3, SPARC Polyclonal, TLE3, SPARC Monoclonal, PGP	IHC, IHC, IHC, IHC, IHC
pemetrexed	TS	IHC
pertuzumab	Her2/Neu, Her2/Neu	IHC, FISH
tamoxifen	PR, ER	IHC, IHC
topotecan	TOPO1	IHC
toremifene	PR, ER	IHC, IHC
triptorelin	Androgen Receptor	IHC

Table 25: Drugs Associations supported by evidence by IHC, ISH and Sequencing

Drug Name	Markers	Illustrative Technologies
cetuximab	PTEN, EGFR, BRAF, KRAS, NRAS, PIK3CA	IHC, IHC, Sanger SEQ/NGS, Sanger SEQ/NGS, Sanger SEQ/NGS, Sanger SEQ/NGS
dacarbazine	MGMT, MGMT, MGMT, IDH1	MGMT Methylation, Pyro SEQ, IHC, NGS
erlotinib	PTEN, KRAS, cMET, PIK3CA,	IHC, Sanger SEQ/NGS, FISH, Sanger

	EGFR	SEQ/NGS, Sanger SEQ/NGS
everolimus	Her2/Neu, PIK3CA, Her2/Neu, ER	IHC, Sanger SEQ/NGS, Sanger SEQ/NGS, FISH, Sanger SEQ/NGS, IHC, IHC
gefitinib	PTEN, EGFR, PIK3CA, cMET, KRAS, cMET	IHC, Sanger SEQ/NGS, Sanger SEQ/NGS, IHC, Sanger SEQ/NGS, FISH
panitumumab	KRAS, BRAF, NRAS, PTEN, PIK3CA	Sanger SEQ/NGS, Sanger SEQ/NGS, Sanger SEQ/NGS, IHC, Sanger SEQ/NGS
temozolomide	MGMT, MGMT, IDH1, MGMT	MGMT Methylation, Pyro SEQ, NGS, IHC
temsirolimus	PIK3CA, Her2/Neu, Her2/Neu, ER	Sanger SEQ/NGS, Sanger SEQ/NGS, Sanger SEQ/NGS, FISH, IHC, IHC, IHC
trastuzumab	Her2/Neu, PTEN, PIK3CA, Her2/Neu	FISH, IHC, Sanger SEQ/NGS, IHC

Table 26: Drugs Associations supported by evidence by Sequencing

Drug Name	Markers	Illustrative Technologies
afatinib	EGFR	Sanger SEQ/NGS
dabrafenib	BRAF, BRAF	Sanger SEQ/NGS, qPCR
imatinib	c-KIT, PDGFRA	NGS, NGS
sunitinib	c-KIT	NGS
trametinib	BRAF, BRAF	Sanger SEQ/NGS, qPCR
vandetanib	RET	NGS
vemurafenib	BRAF, BRAF	Sanger SEQ/NGS, qPCR

Example 6: Molecular Profiling Reagents

[00489] Molecular profiling according to the invention is performed using various analysis methods as described herein. The analysis includes sequence variant analysis (e.g., Sanger sequencing, Next Generation Sequencing (NGS) or pyrosequencing), immunohistochemistry (protein expression), CISH or FISH (gene amplification), and/or RNA fragment analysis (FA). Various reagents used for IHC and ISH analysis as described herein are shown in **Table 27**.

Table 27: Reagents used for molecular profiling

Product Name	Vendor	Catalog Number	Clone
AR antibody	LEICA	NCL-AR-318	AR27
Chr7 CISH probe	VENTANA	760-1219	(PROBE)
cMet antibody	VENTANA	790-4430	SP44
cMet CISH probe	VENTANA	760-1228	(PROBE)
EGFR antibody	DAKO	K1494	2-18C9
ER antibody	VENTANA	790-4325	SP1
ERCC1 antibody	ABCAM	AB2356	8F1
HER2 CISH probe	VENTANA	780-4422	(PROBE)
HER2/neu antibody	VENTANA	790-2991	4B5
MGMT antibody	INVITROGEN	18-7337	MT23.2
NEGATIVE MOUSE	VENTANA	760-2014	MOPC21
NEGATIVE MOUSE	DAKO	IR750	
NEGATIVE RABBIT	VENTANA	760-1029	(POLY)
NEGATIVE RABBIT	DAKO	IR600	
PGP (MDR1) antibody	INVITROGEN	18-7243	C494
PR antibody	VENTANA	790-4296	IE2
PTEN antibody	DAKO	M 3627	6H2.1
RRM1 antibody	PROTEINTECH	10526-1-AP	(POLY)

SPARC-MONO antibody	R&D SYSTEMS	MAB941	122511
SPARC-POLY antibody	EXALPHA	X1867P	(POLY)
TLE3 antibody	SANTA CRUZ	SC-9124	(POLY)
TOPO2A antibody	LEICA	NCL-TOPO11A	3F6
TOPO1 antibody	LEICA	NCL-TOPO1	1D6
TS antibody	INVITROGEN	18-0405	TS106/4H4B1
TUBB3 antibody	COVANCE	PRB-435P	(POLY)
MLH-1 antibody	VENTANA	790-4535	M1
MSH-2 antibody	VENTANA (CELL MARQUE)	760-4265	G219-1129
MSH-6 antibody	VENTANA	790-4455	44
PMS-2 antibody	VENTANA (CELL MARQUE)	760-4531	EPR3947
PD-1 antibody	BD PHARMINGEN	562138	EH12.1
PD-L1 antibody	R&D SYSTEMS	MAB1561	130021
PBRM1 (PB1/BAF180) antibody	BETHYL LABORATORIES	A301-591A	(POLY)
BAP1 antibody	SANTA CRUZ	SC-28383	C-4
SETD2 (ANTI-HISTONE H3) antibody	ABCAM	AB9050	(POLY)

Example 7: Distribution of immune markers in Breast cancer

[00490] This Example studied the expression of PDL1 and eight other immune markers in breast cancer cohorts defined by ER, PR and Her2 status as determined by IHC analysis. This Example further considers the expression of immune markers in a triple negative breast cancer (TNBC) population. Triple negative breast cancer (TNBC) is an aggressive form of breast cancer that lack expression of ER, PR, HER2. There is no optimal standard of care of the management of these patients. TNBC constitutes about 20% of all breast cancers and have a poorer prognosis than women with other breast cancers. Targeted treatment options for TNBC are limited.

[00491] Programmed cell death 1 ligand 1 (PD-L1; PDL1), also known as cluster of differentiation 274 (CD274) or B7 homolog 1 (B7-H1; B7H1), is a protein encoded by the CD274 gene. PD-L1 is expressed in hematopoietic cells and can be found in various tissues, such as pancreatic islets, heart, endothelium, and small intestine. Programmed cell death protein 1 (PD-1; PD1) is a 288 amino acid cell surface protein molecule encoded by the PDCD1 gene. PDCD1 has also been designated as CD279 (cluster of differentiation 279). PD-L1 binds to its receptor, PD-1, found on activated T cells, B cells, and myeloid cells, to modulate activation or inhibition. The formation of PD-1 receptor / PD-L1 ligand complex transmits an inhibitory signal which reduces the proliferation of these CD8+ T cells at the lymph nodes and supplementary to that PD-1 is also able to control the accumulation of foreign antigen specific T cells in the lymph nodes through apoptosis which is further mediated by a lower regulation of the gene Bcl-2. Tumor cell expression of PD-L1 is used as a mechanism to evade recognition/destruction by the immune system as the PD1/PDL1 interplay is an immune checkpoint in normal cells. Overexpression of PD-L1 expression on malignant cells may occur through various mechanisms, such as: 1) activation of common oncogenic pathways; and 2) exposure to inflammatory cytokine produced by infiltrating immune cells.

Such overexpression of PDL1 results in deregulation of the immune checkpoint resulting in immune resistance.

[00492] Several drugs are in clinical development that affect the PDL1/PD1 pathway, including: 1) Nivolumab (BMS936558/MDX-1106), an anti-PD1 drug from Bristol Myers Squib drug which was approved in late 2014 under the brand name OPDIVO for the treatment of patients with unresectable or metastatic melanoma and disease progression following ipilimumab and, if BRAF V600 mutation positive, a BRAF inhibitor; 2) Lambrolizumab (MK-3475), an anti-PD1 drug from Merck shown to shrink tumors in a cohort of patients with advanced melanoma; 3) BMS-936559/MDX-1105, an anti-PDL1 drug from Bristol Myers Squib with initial evidence in advanced solid tumors; and 4) MPDL3280A, an anti-PDL1 drug from Roche with initial evidence in NSCLC.

[00493] Cytotoxic T-lymphocyte Antigen-4 (CTLA-4) functions as an "off" switch to T-cell activity in the priming phase. Anti-CTLA-4 targeted therapies include ipilumunab (Yervoy, Bristol-Myers Squibb) and tremelimumab (Pfizer). IDO1 indoleamine 2,3-dioxygenase 1 (IDO-1) catalyzes the first and rate-limiting step in tryptophan catabolism. It can play an important to immune tolerance and immunosuppression. Current trials are now underway for IDO-1 inhibitors.

[00494] In this Example, expression of PDL1 (CD274) mRNA was examined by microarray (HumanHT-12 v4 beadChip Illumina Inc., San Diego, CA) in a cohort of 3993 breast cancer patients that had molecular profiling performed as described herein. The cases represent a portion of those in the Example above. All cases were analyzed for the expression of ER, PR, AR and Her2 by immunohistochemistry. Results for ER/PR/HER2 status are shown in **Table 28**. 511 cases were TNBC. Additionally, certain cases were analyzed using immunohistochemical analysis and next generation sequencing as described herein using Illumina's Miseq platform. For IHC, slides were stained using an automated system (Ventana Medical Systems, Tucson, AZ) as per manufacturer's protocol with proprietary reagents. IHC stained slides were scored by pathologists. Tumor staining was scored for all markers except for PD1 which was scored in the tumor infiltrating lymphocytes (TILs).The Comprehensive R Archive Network program ("R") was used for statistical computing and graphics. The study was IRB approved.

Table 28: Receptor Status

ER Status	Her2 Status	PR Status	Identifier	Number
Positive	Positive	Positive	ER+PR+Her+	133
		Negative	ER+PR-Her+	125
	Negative	Positive	ER+PR+Her-	1867
		Negative	ER+PR-Her-	924
Negative	Positive	Positive	ER-PR+Her+	33
		Negative	ER-PR-Her+	271
	Negative	Positive	ER-PR+Her-	125
		Negative	ER-PR-Her-	515

[00495] First, the expression of CD274 (which encodes PDL1 protein), CTLA4 (which encodes CTLA-4 (Cytotoxic T-Lymphocyte Antigen 4), also known as CD153 (Cluster of differentiation 153)), IDO1 (which encodes Indoleamine-pyrrole 2,3-dioxygenase protein (IDO or INDO)), and PTGS2 (which encodes COX2 protein) mRNAs were examined by microarray in the breast cancer cohorts defined by

receptor status as shown in **Table 28**. These markers comprise negative immune markers by playing roles in downregulating the immune response. Results of the microarray analysis are shown in **FIGs. 39A-D** (**FIG. 39A**: PDL1; **FIG. 39B**: CTL4A; **FIG. 39C**: IDO1; **FIG. 39D**: COX2). In the figures, positive receptor status is indicated on the Y-axis and receptors not shown are negative, except that triple negative samples are represented by "TNBC." The figures show box plots of expression of the indicated mRNAs over control levels in the patient cohorts identified along the X-axis. All four of the assessed genes are expressed at higher levels versus the controls in all BCa cohorts. COX2 is expressed at particularly high levels, suggesting a general inflammatory response in the cohorts. The over expression of CTLA4, COX2 and PDL1 indicates potential benefit of targeted therapies. For example, CTLA4, COX2 and PDL1 can be therapeutically targeted by ipilumumab, anti-COX2 and anti-PD1/PDL1 antibodies, respectively.

[00496] Expression of PDL1 was then compared with other immune related biomarkers. **FIGs. 39E-H** show plots of correlation of PDL1 mRNA expression levels in TNBC patients with the indicated markers as determined by microarray (**FIG. 39E**: CD86; **FIG. 39F**: CTL4A; **FIG. 39G**: CD8A; **FIG. 39H**: IDO1). **FIG. 39I** shows a table of Spearman correlation coefficients amongt a larger group of immune markers that were found to be overexpressed in the TNBC group, ordered as PDL1/CD274, PTGS2/COX2, CTLA4, CD86, Granzyme A (GZMA), CD8A, CD19, Granzyme B (GZMB), CCR7, CD276 and IDO1. There is a general correlation between several of the various immune markers tested (e.g., coefficient > ~0.5). However, none of the markers are very highly correlated or negatively correlated with each other (e.g., coefficient > +/- 0.8), indicating that each of the markers can provide independent biological information. COX2 showed little correlation with other immune markers other than perhaps a slight negative correlation with CD8A.

[00497] TNBC patient cohort showed a variable expression of immune genes including PDL1, CTLA4, IDO1 and B7-H3 with some patients expressing relatively high levels of the immune markers (in this context, 'high' refers to expression levels 2 standard deviation above the sample mean). Furthermore, analysis of AR protein levels showed overexpression of AR in 17.5% of TNBC cases. Stratification based on AR expression revealed that AR negative TNBC patients were more likely to express PDL-1 (p =.05); CTLA 4 gene (p =.001); and IDO1 (p = 2.8e-05). Spearman correlation test showed a positive correlation of PDL-1 with CTLA4 (correlation coefficient 0.53), IDO1 (correlation coefficient 0.48), and differential correlation with members of the Phosphatidylinositol 3-kinase (PI3-kinase) Pathway: PIK3CA (correlation coefficient 0.39) and PTEN (correlation coefficient 0.11). Differential expression analysis between high and low PDL1 expressing patients identified 144 genes. Pathway Analysis of the 144 genes indicated significant enrichment of the DNA repair genes including BRCA1 which was negatively correlated with PDL1, and HUS1 and FANCA which were positively correlated with PDL1.

[00498] Expression of immune markers above was compared by AR status (i.e. AR+ vs AR-) in the TNBC population. **FIG. 39J** suggests that there is a relationship between AR expression and PDL1 expression, wherein TNBCs with low AR expression are more likely to express PDL1. **FIG. 39K** suggests that TNBCs with low AR expression are more likely to express IDO1. Similar results were observed for CTL4A (**FIG. 39L**) and CD276 (**FIG. 39M**).

[00499] Although all mRNAs were overexpressed in the TNBC population, there was no difference observed between patients stratified according to AR status. Similarly, there was no difference in mRNA expression patterns among the 8 cohorts identified by ER, PR and Her2 staining as described above (see **Table 28**).

[00500] The incidence of TP53 mutations as assessed by Sanger Sequencing was then compared in the TNBC patients according to AR status. Results are shown in **Table 29**. The different percentages in TP53 mutations were not statistically significant between the AR+ and AR- groups (t-test p-value = 0.14).

Table 29: AR, TP53 mutation association in TNBC patients

AR Status	p53 MT	Total Tested	Percentage
AR+	16	35	46
AR-	144	240	60

[00501] Cluster analysis was performed for the genes most differentially expressed between high and low PDL1 expressors as determined by t-test. Results are shown in **FIG. 39N**. The figure show heat map analysis of 144 genes selected based on T test in patients with top 20% high and lowest 20% PDL1 expressers. For each class we identified genes that exhibit a distinct pattern of expression as compared to the other group. In the heatmap, rows represent genes and columns represent samples. For each class, "upregulated" (depicted in red) is defined as a gene with median transcript level that is >1.5 fold relative to control for a given group and <1.2 fold in the other groups, and down regulated (depicted in blue) is defined as a gene with median transcript level that is <0.66 fold relative to control for a given group and >0.8 fold in the other group. WebGestalt, a "WEB-based GEne SeT AnaLysis Toolkit," was used to do enrichment analysis of the heatmap data. Pathways and interactions that were overrepresented included PDGFR-beta signaling pathway, ErbB1 downstream signaling, EGFR-dependent Endothelin signaling events, Canonical Wnt signaling pathway, E-cadherin signaling in the nascent adherens junction, Regulation of nuclear beta catenin signaling and target gene transcription, mTOR signaling pathway, Class I PI3K signaling events mediated by Akt, VEGF and VEGFR signaling network, Integrin family cell surface interactions, Signaling events mediated by Hepatocyte Growth Factor Receptor (c-Met), Insulin Pathway, Gene expression pathway, Fanconi anemia pathway, ATM pathway and DNA Repair. DNA repair genes were significant (adjusted p value=0.02), including BRCA1, Fanconi anemia, complementation goup A, and HUS1 checkpoint homolog (S. Pombe).

[00502] Protein expression and mutational studies were performed on 36 TNBC tumor samples. The tumors were profiled for PD1, PD-L1, AR, and BRCA1 mutation. Slides were stained using a Ventana Discovery XT automated system (Ventana Medical Systems, Tucson, AZ) as per manufacturer's protocol. Stained slides were scored by pathologists. BRCA1 somatic mutation testing was performed by Next Gen Sequencing (Illumina MiSeq). The data suggest that expression of PD-L1 is correlated with BRCA1 mutation and AR levels, as shown in **Table 30**:

Table 30: Expression of PD-L1 is correlated with BRCA1 mutation and AR levels

BRCA1 mutated/PD-L1 high= 3 additional 4 samples showed similar trends
BRCA1 mutated/PD-L1 low = 0

BRCA1 not mutated/PD-L1 low= 19
BRCA1 not mutated/PD-L1 high = 4
AR high/PD-L1 high= 1
AR high /PD-L1 low = 8
AR low/PD-L1 low= 16
AR low/PD-L1 high =8

[00503] PD-L1 expression was present in 10 (28%) of TNBC patients. PD-1 expression was present in 22 (61%) of TNBC patients. There was a correlation between PD-1 and PD-L1 expression (7 out of 10 or 70% of tumors co-expressed PD-1 and PD-L1). The IHC data suggest that PD-L1 expression may be correlated with BRCA1 mutation status. Without being bound by theory, it may be that DNA damaging effects of chemotherapy and radiation therapy are potentiated in heavily pretreated TNBC patients with BRCA1 mutation, leading to increased apoptosis which leads to inflammatory conditions and increased PD-L1 expression. AR expression found in 9 (25%) patients, of which 1 patient (11%) was PD-L1+. 33% of AR- TNBC were PD-L1+, and 90% PD-L1+ were AR-. All (3/3) BRCA1 mutated patients were PD-L1+. Out of the 8 PD-L1 positive cases, 3 harbored a BRCA1 mutation, 4 harbored a P53 mutation. Mutation testing was not successful in one of the PD-L1 positive samples. PD-L1 expression was more likely to be found in AR negative TNBC cohort.

[00504] The relationship of PD-L1 and the PI-3 kinase pathway was further investigated. Loss of PTEN expression was present in 19 patients (54%), of which 4 of these patients were PD-L1+ (21%). PI3K mutation was present in 5 patients (14%) and 1 of these patients (20%) was PD-L1+.

[00505] The expression of immune regulatory targets in the TNBC population supports the evaluation of immune targeted therapies in this cohort. Androgen receptor negative TNBC population (quadruple negative) may be potential candidates for immunotherapy targeting PD-1, PD-L1, CTLA-4, IDO-1, and CD276. Inverse correlation of BRCA1 with PDL1 (i.e., BRCA mutation or loss is correlated with high PDL1 levels) indicates potential for platinum salts and/or PARP inhibitors and anti PD/PDL1 combination therapy. Positive correlation of PIK3CA and PDL1 gene indicate potential for therapeutic strategies targeting the PI3K pathway and the PD1/PDL1 pathway.

[00506] These data show a correlation between BRCA mutation and PDL1 expression. Out of 3 TNBC patients who harbored a true pathogenic mutation in BRCA1, all had high PD-L1 status and out of the 17 that did not have BRCA1 mutated 15 patients had PD-L1 negative expression. This observation suggests benefit from platinum-based therapy in combination with anti-PD-L1 antibody therapy.

Example 8: Molecular Profiling of Immune checkpoint related genes

[00507] Clinical response to immune checkpoint inhibitor therapy ranges from 18% to 28% by tumor type. There is unmet clinical need for laboratory tests that can identify patients likely to respond to such therapy. Reports indicate that 36% of transgenic tumors with PD-1 expression responded to anti-PD1 therapy while no PD-1 negative cases responded. Estimated objective responses for tumors expressing FoxP3 and IDO by IHC were 10.38 and 8.72 respectively. This Example used microarray expression data to characterize the presence of immune response modulators in human tumors and possibly identify a subset of cases as the candidates for immune checkpoint inhibitor therapy.

[00508] A retrospective analysis of gene expression microarray data for immune related genes was performed on 9,025 qualifying paraffin embedded human tumor specimens (HumanHT-12 v4 beadChip Illumina Inc., San Diego, CA). Samples from LN metastases were excluded from analysis. Immune checkpoint-related genes examined included CTLA4, its binding partners CD80 and CD86, PD-L1, CD276 (B7-H3), Granzymes A and B, CD8a, CD19 and the chemokine receptor CCR7. The normalized expression values for these genes were plotted by tumor types to compare relative expression levels and Principal Component Analysis was performed.

[00509] The results of this analysis showed that PD-L1 expression was above the 90th percentile of normal control tissue in 4% of breast cancers, 3% of renal cancers, 7% of NSCLC, 3% ovarian cancer and 5% of colon cancer tumors. Principal component analysis of the immune checkpoint-related genes showed the greatest percentage of "distinct" cases within ovarian, melanoma, colon, gastric and pancreatic cancers.

[00510] Microarray analysis can identify tumors with unique immune components that are more likely to respond to immune checkpoint therapy.

Example 9: Genomic and Protein Alterations in Triple Negative (TN) Metaplastic Breast Cancer

[00511] Metaplastic breast cancer ("MpBC") is a rare subtype (less than 1% of all breast cancers), is generally ER, PR and HER2-negative (triple negative, "TN"), demonstrates a claudin-low gene expression profile, and is poorly responsive to cytotoxic therapy. Little is known about the genomic alterations (GA) in MpBC nor about overexpressed proteins that may be amenable to targeted therapy.

[00512] Molecular profiling according as described herein was used to assess 126 cases of TN MpBCs. Specific testing was performed per physician request and included sequencing (Sanger or next generation sequencing [NGS]), protein expression (immunohistochemistry [IHC]), and/or gene amplification (CISH or FISH) as described herein.

[00513] The 126 member patient cohort had a median age of 60 years old, range 21-94 (6 patients < 50 years old). 81% of patients had documented metastatic disease. Sites of metastasis included 12 in the chest wall/skin/soft tissue, eight in the lung, four in the lymph nodes, one in the bone, and 61 unreported. By ICD-O code, 55 patients had metaplastic carcinoma, NOS, 23 patients had an adenocarcinoma with spindle cell metaplasia, 20 had an adenocarcinoma with squamous features and 8 had an adenocarcinoma with cartilage elements.

[00514] **Table 31** shows the percentage of gene mutations, amplifications, and IHC findings for biomarkers that were different between TNBC and MpBCs, as a percentage of total patients tested.

Table 31: Molecular Profiling differences between TNBC and MpBCs

	Gene Mutation, %				ISH, % Positive	IHC, % Positive				
	TP53	PIK3CA	HRAS	cMET	EGFR	PTEN loss	AR	cMET	Ki67	TOPO1
TNBC	64	13	0	0	22	66	17	13	85	70
Metaplastic	32	39	21	4	17	44	8	3	95	49

P value	0.101	0.002	0.002	0.430	0.801	0.001	0.046	0.250	0.650	0.147

[00515] The above analysis revealed that the biomarker profile of MpBC was more similar to non-TNBC than to TNBC (data not shown). mTOR pathway involvement (PIK3CA mutated and PTEN loss) was significantly different between TNBC and MpBC. In the MpBC cohort, 2 of 14 cases had PIK3CA and TP53 co-mutated (14%), whereas in the TNBC cohort, 26 of 55 cases had PIK3CA and TP53 co-mutated (47%).

[00516] **Table 32** shows the results of IHC profiling of the MpBCs in more detail. In the table, a "$" symbol next to the biomarker name indicates that expression of the biomarker below the threshold is considered predictive of response to therapy. In all other cases, expression above the threshold is considered predictive of response to therapy. Thresholds are set for each biomarker based on staining intensity and/or percentage of positive cells.

Table 32: IHC profiling of MpBCs

	Total Positive	Total Cases Evaluated	% Positive
AR	8	97	8.2
BCRP	7	11	63.6
cKit	5	57	8.8
cMET	1	37	2.7
EGFR	7	9	77.8
ER	0	98	0
ERCC1	19	40	47.5
HER2	0	99	0
MGMT$	39	69	56.5
MRP1	46	54	85.2
p53	20	42	48.6
PDGFR	5	22	22.7
PGP	8	82	9.8
PR	2	98	2
PTEN$	55	100	55
RRM1$	20	63	31.7
SPARC	40	92	43.5
TLE3	32	87	36.8
TOP2A	37	58	63.8
TOPO1	28	56	50
TS$	42	81	51.9
TUBB3$	17	25	68

[00517] **FIG. 40A** shows selected results of mutational analysis deteted by Sanger sequencing or NGS along with suggested therapy. Mutations were not detected in this cohort in the following genes: ABL1, AKT1, ALK, APC, ATM, CDH1, cKIT, CSF1R, CTNNB1, EGFR, ERBB2, ERBB4, FBXW7, FGFR1, FGFR2, FLT3, GNA11, GNAQ, GNAS, HNF1A, IDH1, JAK2, KDR, KRAS, MPL, NOTCH1, NPM1, NRAS, PDGFRA, RET, SMAD4, SMARCB1, SMO, STK11, VHL. A breakdown of specific mutations in the genes indicated in **FIG. 40A** is shown in **Table 33**:

Table 33: Mutations observed in MpBCs

Gene	Mutation	# Observed	Exon
APC	L1129S	1	16

BRAF	N581I	1	15
cMET	T1010I	1	14
HRAS	G12D	1	2
	G13V	1	2
	Q61L	1	3
MLH1	S406N	1	12
PIK3CA	E545K	1	9
	G106R	1	1
	H1047L	2	20
	H1047R	10	20
	N345K	1	4
PTEN	R233X	1	7
JAK3	V722I	1	16
TP53	S106R	1	4
	Y163C	1	5
	R213X	1	6
	G244S	1	7
	Y236S	1	7
	D281E	1	8
	R273H	1	8
	R333fs	1	10

[00518] FIG. 40B and **Table 34** present comparison of p53 Mutated, PIK3CA Mutated, and EGFR amplified MpBC patients. **Table 34** shows patient characteristics of those harboring mutations in PIK3CA and p53/TP53, and amplification of EGFR. **FIG. 40B** shows a selection of molecular alterations detected in these tumors as indicated.

Table 34: Patient Characteristics

	PIK3CA MT, n=14	**TP53 MT, n=8**	**EGFR Amp, n=4**
Median Age	65	51	63
Percent Metastatic	85.7%	87.5%	75.0%
Histology, Metaplastic	Sarcomatoid=2 Squamous=6 NOS=5	Sarcomatoid=0 Squamous=2 NOS=6	Sarcomatoid=2 Squamous=1 NOS=1

[00519] FIGs. 40C-D present further omparison of PIK3CA mutant vs. TP53 mutant vs. EGFR amplified MpBC for individual patients. **FIG. 40C** presents data for fourteen PIK3CA mutant patients. Under "Demographics," "Met?" indicates whether the cancer is metastatic. Under the different analysis techniques (*i.e.*, IHC, ISH and DNA Sequencing), the biomarker is indicated, "n/t" means non-tested, and a check mark indicates an actionable finding (*i.e.*, suggesting potential drug therapy). Under Ki67%, the percentage Ki67 positivity is indicated and under PIK3CA, the specific mutation is indicated. **FIG. 40D** is similar to **FIG. 40C** except that data for TP53 mutant MpBCs and EGFR amplified MpBCs is shown. These data suggest that subgroups within MpBC may have different pathways of origin and therapy oppportunities. For example, EGFR Amplified MpBCs may have lower incidence of MGMT underexpression but higher incidence of SPARC expression as compared to PIK3CA and TP53 mutants.

[00520] FIG. 40E presents further Ki67 analysis, a proliferative marker, for 64 patients. The median Ki67 percent positive cells was 46.7%. Proliferation of MpBC is highly variable, reflective of the indolent to

highly proliferative spectrum of progression seen in MpBC, compared to TNBC, which tends to be more proliferative. Six cases were both AR positive/Ki67>20% (median Ki67 for AR+ MpBC=24).

[00521] **FIG. 40F** indicates potential therapeutic strategies suggested by molecular evaluation of MpBC by IHC (immunohistochemistry) and/or ISH (in situ hybridization). The figure shows results for a selection of biomarkers assessed by IHC and EGFR by ISH (bar labeled "EGFR amp"). The x-axis indicates the biomarker and whether it was detected as high or low (depending on actionability) in the indicated number of patients. Potential targeted drug therapies are shown above each bar. The Ki67 spectrum reflects variable history and spread between indolent and aggressive progression. PD-1 (71%) and PD-L1 (100%) were expressed at high levels in the samples tested.

[00522] Comparison of the genomic and protein expression profiles highlights some differences between the two cancers. Although poorly responsive to cytotoxic therapies, molecular alterations identified in 97% of cases in this large series by multiplatform profiling points to many potential therapeutic strategies for MpBCs, including: mTOR pathways inhibitors suggested by gene alterations in the PI3K pathway (52% of cases had PTEN/PIK3CA mutations or PTEN loss); immunomodulatory agents, approved or currently in clinical trials, suggested by the presence of PD-1/PD-L1; gemcitabine treatment suggested by low RRM1 expression in 68% of MpBCs; imitinab or anti-androgen therapies suggested by cKIT (9%) and AR protein overexpression (8%); MEK inhibitors suggested by HRAS mutations (21%) and BRAF mutations (2%). Other potential therapeutically targetable gene alterations were present at low incidence, thus indicating a benefit of comprehensive molecular profiling in these patients. These results highlight the benefit of comprehensive molecular profiling of the invention to identify both common and potentially rare tumor characteristics that can guide therapeutic strategy.

[00523] **References**:

1. Song, Y, et al. Unique clinicopathological features of metaplastic breast carcinoma compared with invasive ductal carcinoma and poor prognostic indicators. World Journal of Surgical Oncology 2013, 11:129.
2. Cooper et al. Molecular alterations in metaplastic breast carcinoma. J Clin Pathol. 2013 Jun;66(6):522-8.
3. Hu et al. Current progress in the treatment of metaplastic breast carcinoma. Asian Pac J Cancer Prev. 2013;14(11):6221-5.

Example 10: PD1 and PDL1 in HPV+ and HPV-/TP53 mutated head and neck squamous cell carcinomas

[00524] This Example investigated the role of the programmed death 1 (PD1) and programmed death ligand 1 (PDL1) immunomodulatory axis in head and neck squamous cell carcinoma (HNSCC), a cancer with viral and non-viral etiologies. Determination of the impact of this testing in human papilloma virus (HPV)-positive and HPV–negative/TP53-mutated HNSCC carries great importance due to the development of new immunomodulatory agents.

[00525] Thirty-four HNSCC cases, including 16 HPV+ and 18 HPV-/TP53 mutant, were analyzed for the PD1/PDL1 immunomodulatory axis by immunohistochemical methods. HNSCC arising in the following

anatomic sites were assessed: pharynx, larynx, mouth, parotid gland, paranasal sinuses, tongue and metastatic SCC consistent with head and neck primary.

[00526] Results are summarized in **FIG. 41**. 8/34 (24%) HNSCC were positive for cancer cells expression of PDL1, and 13/34 (38%) HNSCC were positive for PD1+ tumor infiltrating lymphocytes (TILs). 3/34 (8.8%) were positive for both components of the PD1/PDL1 axis. Comparison of PD1 and PDL1 expression in HPV + and HPV-/TP53mutant HNSCC showed PD1 + TILs were more frequent in HPV+ vs. HPV– HNSCC (56% vs. 22%; p=0.07), whereas PDL1+ tumor cells more frequent in HPV– vs. HPV+ HNSCC (38% vs. 13%; p=0.14). PD1 and PDL1 were expressed in both oropharyngeal and non-oropharyngeal HNSCC: 33% vs. 39% for PD1 + TILs, respectively, and 11% and 33% for PDL-1, respectively. To examine the role of PD1 and PDL1 in progression of disease, expression was compared between metastatic and non-metastatic HNSCC. PD1 + TILs were detected in 45% of metastatic vs. 25% non-metastatic HNSCC (p=0.29), and PDL1 was detected in 27% vs. 17% of metastatic vs. non-metastatic HNSCC. Interestingly, the three cases that were positive for both PD1 and PDL1 were metastatic HNSCC, including a tumor of the mandible which had metastasized to the bone of the arm, and two unknown primary consistent with head and neck primary, one metastatasized to the lymph nodes and the other metastasized to the lung.

[00527] Immune evasion through the PD1/PDL1 axis is relevant to both viral (HPV) and non-viral (TP53) etiologies of HNSCC. Expression of both axis components was less frequently observed across HNSCC tumor sites, and elevated expression of both PD1 and PLD1 was seen at a higher frequency in metastatic HNSCC. In summary, we observed that: 1) PDL1 + TILs were more frequent (56%) in HPV + HNSCC; 2) PD1 expression was more frequent (38%) in HPV-/TP53 mutated HNSCC; 3) elevation of both components of the axis (PD1 and PDL1), occurs at low frequency (8%); 4) expression of PDL1 and PD1 occurs in head and neck cancers that occur in oropharyngeal and non-oropharyngeal sites; and 5) the PD1/PDL1 pathway is more frequently expressed in metastatic cases vs. non-metastatic HNSCC.

Example 11: Programmed cell death 1 (PD-1) and its ligand (PD-L1) in common cancers and their correlation with molecular cancer type

[00528] Programmed death-1 (PD-1, CD279) is an immune suppressive molecule that is upregulated on activated T cells and other immune cells. It is activated by binding to its ligand PD-L1 (B7-H1, CD274), which results in intracellular responses that reduce T-cell activation. Aberrant PD-L1 expression had been observed on cancer cells, leading to the development of PD-1/PD-L1-directed cancer therapies, which have shown promising results in late phase clinical trials. Blockade of the PD-1 and PD-L1 interaction led to good clinical responses in several, but not all cancer types, and the heterogeneous cellular expression of PD-1/PD-L1 may underlie these selective responses (1-6).

[00529] PD-1/PD-L1 expression has been studied by various methods in different cancer subtypes (7). Most of the published papers focused on prognostic relevance of PD-1/PD-L1 and less is known about their predictive value as well as their relationship to molecular genetic alterations in solid tumors (1). In this Example, we analyzed distribution of PD-1+ tumor-infiltrating lymphocytes (TIL) and PD-L1

expression in the most common solid cancers and further correlated these biomarkers with genotypic and phenotypic characteristics of tumors.

[00530] Material and Methods

[00531] *Tumor samples*

[00532] The study cohort consisted of 437 tumor samples (both primary and metastatic) representing both major and some rare solid cancer types: 380 carcinomas (breast, colon, lung, pancreas, prostate, Merkel cell, ovary, liver, endometrial, bladder, kidney and cancers of unknown primary [CUP]), 33 soft tissue sarcomas (liposarcomas, chondrosarcomas, extraskeletal myxoid chondrosarcomas and uterine sarcomas) and 24 malignant cutaneous melanomas.

[00533] *Molecular methods*

[00534] Tumor samples were evaluated using a commercial multiplatform approach consisting of protein analysis (immunohistochemistry), gene copy number analysis (in-situ hybridization) and gene sequencing (Next-Generation Sequencing with the Illumina MiSeq platform) as described herein. See also reference 8.

[00535] The presence of PD-1+ lymphocytes was evaluated with monoclonal antibody NAT105 (Cell Marque) while the expression of PD-L1 was analyzed with B7-H1 antibody (R&D Systems), using automated immunohistochemical methods.

[00536] Due to the biopsy size-related dependence on the detection of PD-1 TILs (9, 10), we evaluated their density using a hot-spot approach, analogous to the previously described method for measuring neoangiogenesis (11). The whole tumor sample was reviewed at a low power (4x objective) and the area of highest density of TILs in direct contact with malignant cells of the tumor at 400x visual field (40x objective x 10x ocular) was enumerated (number of PD-1+ TIL/ high power fields (hpf)). The intensity of the cancer cells expression of PD-L1 was recorded on a semiquantitative scale (0-3+): 0 for no staining, 1+ for weak cytoplasmic staining, 2+ moderate membranous and cytoplasmic staining and 3+ strong membranous and cytoplasmic staining. Percent of tumor cells expressing PD-L1 at the highest intensity was recorded.

[00537] Statistical methods

[00538] The 2-tail Fisher's exact test and Chi-square test were applied for the correlation between the variables ($p \leq 0.05$).

[00539] Results

[00540] PD-1 and PD-L1 expression in solid tumors

[00541] PD-1 and PD-L1 expression in solid tumors and their subtypes are summarized in **Tables 35-38**.

Table 35: Overview over PD-1 and PD-L1 expression in various types of solid tumors

Tumor types (n=437 total)	PD-1 expression (% and range)	PD-L1 (tumor cells) (%)	Concurrent PD-1 and PD-L1 expression (%)
Carcinomas (n=380 total):			
a) Breast (**n=116**)	51% (1-20)	45%	29%
b) Colon (**n=87**)	50% (1->20)	21%	12%
c) Non-small cell lung cancer (**n=44**)	75% (1-20)	50%	43%
d) Pancreas (**n=23**)	43% (1-16)	23%	9%

e) Prostate (**n=20**)	35% (1-6)	25%	5%
f) Merkel cell carcinoma (**n=19**)	17% (1-4)	0%	0%
g) Endometrium (**n=16**)	86% (1-13)	88%	79%
h) Ovary (**n=14**)	93% (1-16)	43%	36%
i) Liver (**n=13**)	38% (1-5)	8%	0%
j) Bladder (**n=11**)	73% (1-10)	55%	55%
k) Kidney (**n=11**)	36% (1-3)	67%	33%
l) CUP (**n=6**)	50% (1-4)	33%	33%
Sarcomas (n=33 total)	30% (1->10)	97%	31%
Melanoma (n=24 total)	58% (1-15)	92%	58%

Table 36: PD-1 and PD-L1 expression in breast cancer according to the molecular subtype

Breast cancer subtypes (n=116)	PD-1 expression/HPF (TILs) (% and range)	PD-L1 (tumor cells) (%)	Concurrent PD-1 and PD-L1 expression (%)
Luminal tumors (n=58)			
a) Luminal A (n=33)	25% (1->10)	33%	13%
b) Luminal B (n=25)	44% (1-20)	33%	17%
HER2 positive (n=5)	60% (1-9)	20%	20%
Triple-negative (n=53)	70% (1-20)*	59%*	45%*

*Significantly higher than in luminal tumors.

[00542] Table 37 shows that PD-1 and PD-L1 exhibited higher expression in tumors with high microsatellite instability ("MSI-H") versus microsatellite stable tumors ("MSS"). The MSI-H cases here comprised Lynch syndrome and sporadic colon cancers.

Table 37: PD-1 and PD-L1 expression in colorectal carcinomas in relationship to the microsatellite instability status

Colon cancer subtypes (n=87)	PD-1 expression/HPF (TILs) (% and range)	PD-L1 (tumor cells) (%)	Concurrent PD-1/ PD-L1 expression (%)
MSS colon cancers (n=60)	39% (1-11)	13%	4%
MSI-H colon cancers (n=27)	77% (1->20)*	38%*	32%*

*Significantly higher ($p<0.05$)

Table 38: Overview over PD-1 and PD-L1 expression in sarcoma subtypes

Sarcoma subtypes (n=33)	PD-1 expression/HPF (TILs) (% and range)	PD-L1 (tumor cells) (%)	Concurrent PD-1 and PD-L1 expression (%)
Liposarcoma (n=20)	45% (1->10)	100%	45%
Chondrosarcoma (n=8)	12% (1-)	100%	12%
Extraskeletal myxoid chondrosarcoma (n=3)	0%	67%	0%
Uterine sarcoma (n=2)	0%	100%	0%

[00543] PD-1+ lymphocytes were consistently identified in reactive, peri-tumoral lymphoid follicles which served as an internal positive control.

[00544] PD-1+ TILs in direct contact with cancer cells were uncommon in some cancer types (e.g. 0% observed in extraskeletal myxoid chondrosarcoma in this cohort), although triple-negative breast cancer (TNBC), bladder cancer, microsatellite instability high (MSI-H) colon cancer, non-small cell lung cancer (NSCLC), endometrial and ovarian cancer were frequently (70-100%) infiltrated with PD-1+ TILs. When present, PD-1+ TILs density varied from 1 to >20/hpf. See **Table 35**.

[00545] PD-L1 was consistently expressed in the tumor microenvironment including endothelial cells, macrophages and dendritic cells, at strong (2+/3+) intensity and was used as internal positive control. In contrast, the cancer cells expressed PD-L1 at widely varying levels and proportions. Consistent, strong membranous staining was a feature of only a few, specific cancer types including endometrial carcinomas (see **FIGs. 42A-D**) and malignant melanomas (88% and 92%, respectively), metaplastic breast carcinomas, chondrosarcomas and liposarcomas (both 100%). See **Tables 35** and **38**.

[00546] Simultaneous expression of PD-L1 in tumor cells and presence of PD-1+ TILs was frequently observed in kidney cancer (33%), ovarian cancer (36%), NSCLC (43%), TNBC (45%), dedifferentiated liposarcomas (50%), bladder cancer (55%), malignant melanomas (58%), endometrial cancer (79%), but was infrequent in other cancer types in our cohort, e.g., 0% in liver cancer and Merkel cell carcinoma, 4% microsatellite-stable (MSS) colon cancer, 5% prostate cancer, 8% liver cancer, 9% pancreatic cancer, and 13% in luminal A breast cancer. See **Table 35**.

[00547] Association of PD-1 and PD-L1 expression with genotypic and phenotypic characteristics of the tumors

[00548] In the sample set used in this Example, expression of PD-1+ TILs was associated with an increasing number of mutations in tumor cells ($p=0.029$, Fisher's exact test) whereas PD-L1 status showed the opposite association ($p=0.004$, Fisher's exact test). Consequently, co-presence of PD-1+ TILs and cancer cells expressing PD-L1 showed no association with overall mutational status ($p=0.67$, Fisher's exact test).

[00549] In breast cancer PD-1+ TILs were significantly more common in TNBC than in luminal-type tumors (70% vs. 25-44%, $p<0.001$, Chi-square test). See **Table 36**. Similarly, PD-L1 expression was the highest in TNBC as compared to other subtypes (59% vs. 33% in luminal tumors, $p=0.017$). Among TNBC, 9 cases were metaplastic breast carcinomas and all were positive for PD-L1. Consequently, co-expression of PD-1+TIL/cancer cells PD-L1+ was the highest in the TNBC subgroup (45% vs. 13-17% non-TNBC, $p=0.001$, Chi-Square test). Similarly, TP53 mutated breast cancers exhibited significantly higher PD-1 TIL positivity compared with breast cancers that harbored other mutations (e.g. PIK3CA mutations) or breast tumors without mutations (42% vs. 10%, $p=0.002$, Chi-square test). In contrast, PD-L1+ did not correlate with any of the detected mutations in breast cancer.

[00550] In the colon cancer cohort, MSI-H tumors exhibited a significantly higher rate of positivity for PD-1+ TILs than MSS colon cancers (77% vs. 39%, $p=0.002$, Fisher's exact test). See **Table 37**. Also, the proportion of PD-L1+ cancers was significantly higher in MSI-H than in the MSS colon cancers (38% vs.

13%, p=0.02, Fisher's exact test). Of note, MSI-H cases were predominantly stage I and II (75%) whereas the majority of the MSS cases were at advanced stage (III and IV, 93%) ($p<0.001$). Both PD-1 and PD-L1 positivity significantly decreased with the tumor stage in CRC ($p=0.021$ and 0.031, respectively).

[00551] In NSCLC, PD-1+TILs and PD-L1 expressing tumor cells were seen in 18/42 cases (43%) of which 8 cases lacked other biological targets (such as activating EGFR mutations, HER2, cMET, ALK or ROS1 rearrangements).

[00552] Discussion

[00553] Recent clinical trials have demonstrated that blocking of the PD-1/PD-L1 pathway induces an objective and durable remission in patients with advanced solid tumors (2-6). The efficacy of these agents has been primarily linked to the expression of PD-L1 in the tumor cells and PD-1 on activated T lymphocytes (12-14). Expression of both markers has already been explored in several human malignancies, particularly in renal cell carcinomas, malignant melanoma and NSCLC (13-15). Our PD-L1 results for these three cancer types are comparable with the data provided by Taube et al (13). Consistent with a previous report by Vanderstraeten et al. (16), endometrial cancer appears to be abundantly enriched with both PD-1 and PD-L1.

[00554] The broad array of tumors screened for this study also allowed the assessment of PD-1/PD-L1 expression in several less common cancer types. Our study revealed a low expression of both PD-1 and PD-L1 in several highly aggressive tumors including Merkel cell carcinoma, hepatocellular and pancreatic carcinoma. In contrast, PD-L1 expression was particularly high in dedifferentiated liposarcomas, which is in line with a recent report by Kim et al. (17). We also found PD-L1 positivity in chondrosarcomas and extraskeletal myxoid chondrosarcomas. Furthermore, PD-1 and PD-L1 positivity was observed in cancers of unknown primary, a group of cancers with particularly difficult treatment decisions.

[00555] Marked variations in PD-1/PD-L1 positivity have also been observed within general histologic types, but subtype analysis revealed significant correlations. For example, PD-1/PD-L1 were differently expressed in molecular subtypes of breast (TNBC vs. non-TNBC) and colon cancer (MSI-H vs. MSS cases) providing an indication for potential benefit of targeted immunotherapy in aggressive subtypes of breast and colon cancers for which no targeted therapy is currently available. We found PD-L1 expression to be the highest in TNBC (59%) whereas a recent study that reported the highest frequency (34%) in HER2-positive breast cancers (18). The difference may be due to the cohorts analyzed. Our cohort was enriched (8%) for rare metaplastic TNBC, which were all PD-L1 positive whereas we analyzed only 5 HER2-positive breast cases. Of note, TP53 mutated breast carcinomas exhibited significantly higher PD-1 expression in comparison with breast carcinomas harboring other types of mutations. High PD-1+TILs had been recently associated with a more aggressive phenotype and poorer outcome in operable breast cancers (19).

[00556] Upon interferon-gamma (IFN-γ) stimulation, PD-L1 is expressed on T-cells, NK-cells, macrophages and vascular endothelial cells, all present in tumor microenvironment and detected in nearly all of our cases. Some immunogenic tumors (e.g. MSI-H CRC) attract TILs which produce IFN-γ and could upregulate PD-L1 on tumor epithelial cells. IFN-γ receptor (IFN-γRα) expressed on tumor epithelial

cells plays a critical role in tumor immunoediting (20), including acquisition of stem cell-like phenotype (21) and resistance to granule-mediated cytotoxic T-lymphocyte killing (22).

[00557] Our data for colon cancer also appear to differ from those reported by Droeser et al. who reported more frequent expression of PD-L1 in the MSS than in MSI-H colon cancers (23). The discrepancy may be caused by the fact that tested MSI-H and MSS cases differed significantly in regards to the tumor stage as the majority of MSI-H was at stage I and II while MSS tumors were predominantly stage III and IV. Overall, the expression of both PD-1 and PD-L1 in colon cancer inversely correlated with the tumor stage.

[00558] Another relevant finding in our study is that a substantial proportion of NSCLCs with PD-1/PD-L1 positivity were devoid of the most common and targetable alterations (e.g. EGFR, HER2, cMET, ALK, ROS1). In contrast to previous studies, we did not find any association between PD-1/PD-L1 expression and EGFR alterations in lung cancer (24, 25).

[00559] Without being bound by theory, low percentage of intra-tumoral PD-1+ lymphocytes and PD-L1 cancer cells in certain solid tumors (see **Tables 35-38**) may explain—in whole or in part—the observed lack of a benefit from therapies targeting this pathway. Also without being bound by theory, these data are consistent with the idea that PD-1 lymphocytes that are in direct contact with (PD-L1 expressing cancer cell) are most relevant for response to PD-1 / PD-L1 targeted therapies. Thus, cell-to-cell contact (PD-1 lymphocytes with PD-L1 cancer cell) may be used as a potential biomarker of response. Such interactions in a tumor may indicate the efficacy of PD-1 / PD-L1 pathway modulators. Dual IHC and/or flow cytometry may provide such a signal. *See, e.g.,* Segal and Stephany, The Measurement of Specific Cell1:Cell Interactions by Dual-Parameter Flow Cytometry, Cytometry **5**:169-181 (1984).

[00560] In summary, our survey demonstrated expression of two potentially targetable immune checkpoint proteins (PD-1/PD-L1) in a substantial proportion of solid tumors including some aggressive subtypes that lack targeted treatment modalities. In some other tumor types, expression of the immune checkpoint proteins was rare. Taken together, these data indicate that molecular profiling can be used to assess likely benefit of PD-1 and PD-L1 therapies across a broad variety of tumor types.

[00561] Literature

1. Sznol M, Chen L. Antagonist antibodies to PD-1 and B7-H1 (PD-L1) in the treatment of advanced human cancer. Clin Cancer Res 2013;19:1021-34.

2. Hodi FS, O'Day SJ, McDermott DF, Weber RW, Sosman JA, Haanen JB, et al. Improved survival with ipilimumab in patients with metastatic melanoma. N Engl J Med 2010; 363:711-23.

3. Hamid O, Robert C, Daud A, Hodi FS, Hwu WJ, Kefford J, et al. Safety and tumor responses with lambrolizumab (anti-PD-1) in melanoma. N Engl J Med 2013;369:134-44.

4. Brahmer JR, Tykodi SS, Chow LQ, Hwu WJ, Topalian SL, Hwu P, et al. Safety and activity of anti-PD-L1 antibody in patients with advanced cancer. N Engl J Med 2012; 366:2455-65.

5. Topalian SL, Hodi FS, Brahmer JR, Gettinger SN, Smith DC, McDermott DF, et al. Safety, activity, and immune correlates of anti-PD-1 antibody in cancer. N Engl J Med 2012;366:2443-54.

6. Topalian SL, Sznol M, McDermott DF, Kluger HM, Carvajal RD, et al. Survival, durable tumor remission, and long-term safety in patients with advanced melanoma receiving nivolumab. J Clin Oncol 2014;32:1020-30.

7. Velcheti V, Schalper KA, Carvajal DE, Anagnostou VK, Syrigos KN, Sznol M, et al. Programmed death ligand-1 expression in non-small cell lung cancer. Lab Invest 2014; 94:107-16.

8. Millis S, Bryant D, Basu G, Bender R, Vranic S, Gatalica Z, Vogelzang N. Molecular profiling of infiltrating urothelial carcinomaof the bladder. Clin Genitourin Cancer 2014 Aug.1 DOI:10.1016/j.clgc2014.07.010.

9. Ghebeh H, Mohammed S, Al-Omair A, Qattan A, Lehe C, Al-Quadihi G, et al. The B7–H1 (PDL1) T lymphocyte-inhibitory molecule is expressed in breast cancer patients with infiltrating ductal carcinoma: correlation with important high-risk prognostic factors. Neoplasia 2006;8:190–8.

10. Muenst S, Soysal SD, Gao F, Obermann EC, Oertli, Gillanders WE. The presence of programmed death 1 (PD-1)-positive tumor-infiltrating lymphocytes is associated with poor prognosis in human breast cancer. Breast Cancer Res Treat 2013;139:667-76.

11. Weidner N. Measuring intratumoral microvessel density. Methods Enzymol 2008; 444:305-23.

12. Lipson EJ, Vicent JG, Loyo M, Kagohara LT, Luber BS, Wang H, et al. PD-L1 expression in the Merkel cell carcinoma microenvironment: Association with inflammation, Merkel cell polyomavirus and overall survival. Cancer Immunol Res 2013;1:54-63.

13. Taube JM, Klein AP, Brahmer JR, Xu H, Pan X, Kim JH, et al. Association of PD-1, PD-1 ligands, and other features of the tumor immune microenvironment with response to anti-PD-1 therapy. Clin Cancer Res 2014 Apr 8. [Epub ahead of print]

14. Langer CJ. Emerging Immunotherapies in the Treatment of Non-Small Cell Lung Cancer (NSCLC): The Role of Immune Checkpoint Inhibitors. Am J Clin Oncol 2014 Mar 28. [Epub ahead of print]

15. Jilaveanu LB, Shuch B, Zito CR, Parisi F, Barr M, Kluger Y, et al. PD-L1 Expression in Clear Cell Renal Cell Carcinoma: An Analysis of Nephrectomy and Sites of Metastases. J Cancer 2014;5:166-72.

16. Vanderstraeten A, Luyten C, Verbist G, Tuyaerts S, Amant F. Mapping the immunosuppressive environment in uterine tumors: implications for immunotherapy. Cancer Immunol Immunother 2014;63:545-57

17. Kim JR, Moon YJ, Kwon KS, Bae JS, Wagle S, Kim KM, et al. Tumor infiltrating PD1-positive lymphocytes and the expression of PD-L1 predict poor prognosis of soft tissue sarcomas. PLoS One 2013;8:e82870.

18. Muenst S, Schaerli AR, Gao F, Däster S, Trella E, Droeser RA, et al. Expression of programmed death ligand 1 (PD-L1) is associated with poor prognosis in human breast cancer. Breast Cancer Res Treat 2014;146:15-24.

19. Sun S, Fei X, Mao Y, Wang X, Garfield DH, Huang O, et al. PD-1(+) immune cell infiltration inversely correlates with survival of operable breast cancer patients. Cancer Immunol Immunother 2014;63:395-406.

20. Schreiber RD, Old LJ and Smyth MJ. Cancer Immunoediting: Integrating Immunity's role in Cancer Suppression and Promotion. Science 2011;331:1565-1570

21. Kmieciak M, Payne KK, Wang X-Y, Manjili MH. IFN-γ Rα is a key determinant of CD8+ T cell-mediated tumor elimination of tumor escape and relapse in FVB mouse. PLoS One 2013;8:e82544

22. Hallermalm K, Seki K, De Geer A, Motyka B, Bleackley RC, Jager MJ, Froelich CJ, Kiessling R, Levitsky V and Levitskaya J. Modulation of the tumor cell phenotype by IFN-γ results in resistance of uveal melanoma cells to granule-mediated lysis by cytotoxic lymphocytes. J Immunol 2008;180:3766-74.

23. Droeser RA, Hirt C, Viehl CT, Frey DM, Nebiker C, Huber X, et al. Clinical impact of programmed cell death ligant 1 expression in colorectal cancer. Eur J Cancer 2013;49:2233-42

24. Yang CY, Lin MW, Chang YL, Wu CT, Yang PC. Programmed cell death-ligand 1 expression in surgically resected stage I pulmonary adenocarcinoma and its correlation with driver mutations and clinical outcomes. Eur J Cancer 2014;50:1361-9.

25. Akbay EA, Koyama S, Carretero J, Altabef A, Tchaicha JH, Christensen CL, et al. Activation of the PD-1 pathway contributes to immune escape in EGFR-driven lung tumors. Cancer Discov 2013;3:1355-63.

Example 12: Predicative biomarker profiling of 2000 sarcomas

[00562] Sarcomas are a heterogeneous group of tumors with more than 50 subtypes. First line chemotherapy such as doxorubicin and ifosfamide yield limited survival benefit. In this Example, molecular profiling of the invention was used to guide new therapeutic options for sarcoma.

[00563] A total of 2047 sarcomas (including soft tissue sarcomas - leiomyosarcoma, fibrosarcoma, rhabdomyosarcoma, liposarcoma, angiosarcoma, synovial sarcoma, malignant peripheral nerve sheath tumor; organ specific sarcomas - angiosarcoma of the breast, osteosarcoma and chondrosarcoma, Ewing sarcoma of bone) were profiled using comprehensive molecular profiling as described herein, including biomarker assessment using IHC, Sanger sequencing, Next Generation sequencing, and FISH/CISH. IHC data was available for 1968 samples, FISH/CISH data for 1048 samples, and sequencing data for 261 samples. Data for all platforms was available for 256 samples. 713 samples were known to be from a metastatic site, the median patient age was 55 (range: 1-92), and 62% of the patients were female. Tumor types are displayed in **Table 39**.

Table 39: Histological types

Alveolar soft part sarcoma (ASPS)	14
Angiosarcoma (11=breast)	65
Chondrosarcoma	47
Chordoma	12
Clear cell sarcoma	14
Desmoplastic small round cell tumor (DSRCT)	31

Epithelioid hemangioendothelioma (EHE)	12
Epithelioid sarcoma	14
Endometrial stromal sarcoma (ESS)	72
Ewing sarcoma	66
Fibromatosis	36
Fibrosarcoma	61
Giant cell tumour	11
Leiomyosarcoma (LMS; 355 are uterine)	630
Liposarcoma	168
Malignant fibrous histiocytoma (MFH/UPS)	145
Malignant peripheral nerve sheath tumor (MPNST)	31
Osteosarcoma	84
Perivascular epithelioid cell tumor (PEComa)	16
Rhabdomyosarcoma	67
Solitary fibrous tumor (SFT)	36
Synovial sarcoma	61
fibromyxoid sarcoma	9
Fibrous hamartoma of infancy	1
hereditary leiomyomatosis	1
angiomyolipoma	1
angiomyxoma	1
Atypical spindle cell lesion (with fibrohistiocytic differentiation)	1
chondroblastoma	1
dendritic cell sarcoma	3
Granular cell tumor	6
High grade myxoid sarcoma	1
high-grade myoepithelial carcinoma	1
Hyalinizing fibroblastic sarcoma	1
Inflammatory myofibroblastic sarcoma	1
Interdigitating Dendritic Cell Tumor	1
Intimal sarcoma	3
leiomyoma	2
lymphangitic sarcomatosis	1
malignant glomus tumor	1
Malignant myoepithelioma	1
melanocytic neoplasm	1
mesenchymal neoplasm	3
Mesenteric glomangioma	1
Metastatic histocytoid neoplasm	1
myoepithelioma	1
myxoid sarcoma	4
myxoid stromal	1
neurilemmoma	1
phyllodes	12
rhabdoid	4
Round cell	2

Sarcoma, NOS	204
sarcomatous mesothelioma	1
schwannoma	4
Spindle and round cell sarcoma	1
Spindle cell	75
Spinocellular mesenchymal tumor	1

[00564] Overall IHC results are displayed in **Table 40**. About 50% or the sarcomas overexpressed TOPO2a, and there was PTEN loss in 43%. EGFR was overexpressed in 36% in a wide variety of sarcomas (liposarcoma, UPS, LMS). CKIT, HER2, and cMet were overexpressed at low levels overall.

Table 40: Overall Immunohistochemistry Results

Protein	Below Threshold	Above Threshold	Total	% Above
AR	1653	256	1909	13.4
BCRP	228	224	452	49.6
cKIT	1333	60	1393	4.3
cMET	528	33	561	5.9
EGFR	125	70	195	35.9
ER	1583	337	1920	17.6
ERCC1	881	589	1470	40.1
Her2	1949	1	1950	0.1
MGMT	1221	641	1862	34.4
MRP1	412	867	1279	67.8
PDGFR	443	128	571	22.4
PGP	1414	223	1637	13.6
PR	1508	404	1912	21.1
PTEN	816	1091	1907	57.2
RRM1	1161	548	1709	32.1
SPARC	1264	704	1968	35.8
TLE3	441	142	583	24.4
TOP2A	822	844	1666	50.7
TOPO1	884	767	1651	46.5
TS	1321	380	1701	22.3
TUBB3	186	106	292	36.3

[00565] Table 41 shows a selection of IHC results by histology. For MGMT and RRM1, underexpression potentially confers sensitivity to an agent. Therefore, low MGMT expression in the majority of fibromatosis and LMS potentially confers sensitivity to alkylating agents such as temozolomide. Low RRM1 expression the ASPS tumors and the majority of fibromatosis and liposarcoma potentially confers sensitivity to gemcitiabine. On the other hand, overexpression of SPARC, as observed in 50-70% of angiosarcoma, chondrosarcoma and EHE and osteosarcoma, indicates likely benefit of nab-paclitaxel. TOPO2A overexpression, which indicates likely benefit of anthracyclines, was seen in approximately 60% of angiosarcoma, LMS and UPS.

Table 41: Immunohistochemistry Results by Histology (% overexpressing)

Histology	N	MGMT*	RRM1*	SPARC	TOPO2A
ASPS	14	21.4	0.0	14.3	9.1
Angiosarcoma	64	48.4	39.0	53.1	63.8
Chondrosarcoma	47	70.2	20.5	51.1	14.3
EHE	12	16.7	27.3	66.7	0.0
Epithelioid sarcoma	14	46.2	38.5	30.8	15.4
Fibromatosis	34	3.2	10.7	48.5	0.0
LMS	610	22.8	33.3	30.7	62.0
Liposarcoma	158	40.0	15.8	35.4	31.4
MFH/UPS	140	24.8	25.6	36.6	63.9
Osteosarcoma	80	29.1	38.2	47.6	48.6

*Expression of the biomarker below the threshold is considered predictive of a positive response to therapy

[00566] Table 42 shows another selection of IHC results by histology. AR overexpression was noted in 20-40% of chondrosarcoma, DSRCT, ESS and LMS. cKIT overexpression was noted in 29% angiosarcoma, 37% Ewing sarcoma. cMET overexpression 25% Ewing sarcoma. ER alpah overexpression as expected was seen in 20-45% of ESS and LMS. 60% in uterine and 21% in extrauterine. There was also expression above average in PEComa. PTEN loss was seen in 60-80% of epithelioid sarcoma, osteosarcoma and rhabdomyosarcoma. 41% of LMS had PTEN loss the same regardless of anatomical site.

Table 42: Immunohistochemistry Results by Histology (% overexpressing)

Histology	N	AR	cKIT	cMET	ERα	PDGFRA	PTEN*
Angiosarcoma	64	0.0	28.6	9.5	0.0	46.7	50.8
Clear cell sarcoma	12	0.0	0.0	50.0	0.0	50.0	63.6
Chondrosarcoma	47	23.9	4.3	9.1	0.0	40.0	63.8
DSRCT	30	40.0	19.0	11.1	0.0	7.7	50.0
EHE	12	8.3	0.0	0.0	0.0	33.3	75.0
Epithelioid sarcoma	14	0.0	0.0	0.0	0.0	25.0	23.1
ESS	71	28.2	1.8	5.9	46.5	40.0	78.9
Ewing sarcoma	63	3.6	37.3	25.0	5.4	31.8	41.7
LMS	610	22.4	1.1	3.9	43.2	15.4	59.2
Osteosarcoma	80	2.6	0.0	0.0	0.0	27.8	29.6
PEComa	16	12.5	0.0	0.0	25.0	0.0	81.3
Rhabdomyosarcoma	64	8.5	9.3	15.0	3.3	17.4	41.0

*Expression of the biomarker below the threshold is considered predictive of a positive response to therapy

[00567] 33 additional patients had tumor submitted for PD1 and PDL1 analysis. As shown in **Table 43**, all of the 20 liposarcomas and 9 chondrosarcomas expressed PDL1 in at least 5% of cells with at least a staining intensity of 2.

Table 43: Immunohistochemistry Results for PD-1/PD-L1 expression

Sarcoma subtype	N (33)	PD-1 expression/hpf (TILs)	PD-L1 (tumor cells)	Concurrent PD-1 and PD-L1 expression
Liposarcoma	20	45%	100%	45%

Chondrosarcoma	9	11%	100%	11%
Extraskeletal myxoid chondrosarcoma	3	0%	67%	0%
Uterine sarcoma	1	0%	100%	0%

[00568] Overall FISH/CISH results are displayed in **Table 44**. There was a low level of TOPO2a amplification, but amplification of EGFR was observed in 17% of the cases. Although the level of HER2 amplification was low overall, this was amplification was concentrated in 3-8% of ESS and LMS, the latter more commonly found in the extrauterine LMS: 1.5% in uterine LMS and 4% in extrauterine LMS. EGFR amplification was observed in greater than 5% of Chondrosarcoma, ESS and Ewing sarcoma, greater than 10% of Fibrosarcoma, Liposarcoma and Rhabdomyosarcoma, and greater than 20% of LMS, MPNST, Osteosarcoma and UPS.

Table 44: Overall In situ Hybridization (ISH) Results

Assay	Total	Normal	Amplified	% Amplified
cMET	431	414	17	3.9
cMYC	18	17	1	5.6
EGFR	1048	872	176	16.8
HER2	573	565	8	1.4
TOP2A	107	105	2	1.9

[00569] PTEN loss was observed in up to 80% in several different histopathology types including angiosarcoma, Kaposi's sarcoma, LMS, liposarcoma, rhabdomyosarcoma, Ewing's sarcoma, Osteosarcoma, chondrosarcoma and others. Overexpression of TOPO2 and TOPO1 proteins were observed in more than 50% of angiosarcomas, fibrosarcomas, leiomyosarcomas, rhabdomyosarcomas, malignant fibrous histiocytomas, malignant peripheral nerve sheath tumors, desmoplastic small round cell tumors, synovial sarcomas, and hemangiopericytoma. Low MGMT expression was observed in 75% of osteosarcoma. Absence or low TS expression was seen in Kaposi sarcoma, leiomyosarcomas, hemangiopericytomas and liposarcomas. Steroid hormone receptor overexpression was observed in Ewing sarcomas (52%) and desmoplastic small round cell tumors (44%), followed by rhabdomyosarcomas(36%) and leiomyosarcomas(25%). cMET by FISH showed amplification in 17 % of leiomyosarcoma tested, while EGFR FISH showed > 4 copies in more than 30% of malignant fibrous histiocytomas and malignant peripheral nerve sheath tumors tested.

[00570] Of 261 patients tested using NGS with the panel in **Table 10**, 156 had no mutations (60%) and the rest had 123 gene aberrations detected in 25 genes. Some of the most common mutations in the overall population are shown in **Table 45**. Most of the mutations were at low levels in the entire population, 22.4% had p53 mutations. In this population, only 1 mutant was found each of ABL1, AKT1, AKT1, FGFR2, FLT3, GNA11, KDR, MLH1, SMARCB1 and SMO. And no mutations were detected in ALK, CDH1, CSF1R, EGFR, ERBB2, ERBB4, FBXW7, FGFR1, GNAQ, GNAS, HRAS, JAK2, MPL, NOTCH1, NPM1, PDGFRA, PTPN11, SMAD4 and VHL.

Table 45: Next Generation Sequencing

Gene	Total Tested	WildType	Mutated	% Mutated
APC	261	254	7	2.7

ATM	258	252	6	2.3
BRAF	542	534	8	1.5
cKIT	394	389	5	1.3
cMET	260	254	6	2.3
CTNNB1	261	255	6	2.3
IDH1	261	257	4	1.5
JAK3	260	257	3	1.2
KRAS	1473	1454	19	1.3
NRAS	365	362	3	0.8
PIK3CA	333	323	10	3
PTEN	249	241	8	3.2
RB1	258	252	6	2.3
STK11	247	243	4	1.6
TP53	254	197	57	22.4

[00571] Some of the mutations occuring at higher frequencies in various histologies are shown in **Table 46**, including some known mutation such as IDH1 in chondrosaroma or cKIT in synovial sarcoma, and others such as BRAF in angio, PIK3CA in a variety of sarcomas. The data include both Sanger and NGS results. **Table 47** displays similar data for rare sarcomas. The data revealed known mutations such as CTNNB1 in fibromatosis, and also BRAF in MPSNT and PIK3CA and PTEN in fibrosarcoma. Other histologies had either no mutations detected other than TP53.

Table 46: % mutated by histology

Histology	Angio (all)	Chondro	LMS (all)	Liposarcoma	UPS	Synovial sarcoma
APC	13.3	0	2.3	0	0	0
ATM	6.7	0	0	3.3	0	10
BRAF	10	0	0	2.1	2.4	0
cKIT	0	0	0	0	3.1	11.8
cMET	6.7	0	4.5	3.3	0	0
CTNNB1	0	0	0	0	0	0
IDH1	0	25	0	0	4.2	0
JAK3	0	0	0	3.3	0	0
KRAS	5.8	0	0	0	2.7	0
NRAS	13.3	0	0	0	0	0
PIK3CA	0	0	1.6	5.6	3.8	0
PTEN	6.7	16.7	7.1	3.6	0	0
RB1	0	0	7	0	4.2	0
STK11	0	0	2.5	3.7	0	0
TP53	26.7	25	41.5	13.3	34.8	0

Table 47: % mutated by histology, rare sarcomas

Histology	ESS	Fibromatosis	Fibrosarcoma	MPNST	Giant cell tumor	Rhabdo
APC	NT	14.3	0	0	33.3	0
ATM	NT	0	14.3	0	0	0
BRAF	0	0	0	7.1	0	4.2
cKIT	0	0	0	0	0	0
cMET	NT	0	0	0	0	0
CTNNB1	NT	85.7	0	0	0	0
IDH1	NT	0	0	0	0	0
JAK3	NT	0	0	0	0	0
KRAS	6.1	0	6.1	3.8	20	2.4
NRAS	0	0	0	0	0	0

PIK3CA	0	0	6.7	0	0	10
PTEN	NT	0	16.7	0	0	0
RB1	NT	0	0	0	0	0
STK11	NT	14.3	0	0	0	0
TP53	NT	0	28.6	11.1	0	11.1

[00572] Some specific mutations observed are shown in **Table 48**. These mutations were observed most frequently excluding p53. BRAF v600E was the most common BRAF mutation. PTEN alterations were mostly frame shift mutations with some missence mutations.

Table 48: Specific mutations

Gene	Alteration	Exon	Frequency	Histology (N)
BRAF	V600E	15	5	Liposarcoma(1), angiosarcoma (1), MPNST (1), other (2)
cKIT	T67S	2	2	UPS(1), synovial sarcoma (1)
cMET	T1010I	14	3	Angiosarcoma (1), LMS (1), liposarcoma (1)
IDH1	R132C	3	4	Chondrosarcoma (3), UPS (1)
KRAS	G12C	5	2	UPS(1), angiosarcoma (2), giant cell tumor (2)
KRAS	G12V	2	4	Fibrosarcoma (1), UPS (1), MPNST (1), other (2)
PIK3CA	H1047R	20	3	Liposarcoma (1), fibrosarcoma (1), other (1)
PIK3CA	E545K	9	3	LMS (1), liposarcoma (1), other (1)

[00573] In sum, the following mutations other than TP53 were detected with frequency ≥ 5%: 1) Synovial sarcoma and ATM, cKIT; 2) Angiosarcoma and BRAF, APC, NRAS, ATM, cMET, KRAS, PTEN; 3) Chondrosarcoma and IDH1, PTEN; 4) Liposarcoma and PIK3CA; and 5) LMS and PTEN, RB1. These data suggest targeted therapy such as against cKit in synovial and against BRAF in angiosarcoma.

[00574] Association of p53 mutations with other alterations and PIK3CA mutations and other alterations were investigated. See **Tables 49-50**. 82% of samples were both TOPO2 positive by IHC and p53 mutated. These data suggest that P53 mutations may serve as a biomarker of sensitivity to anthracyclines. One patient had PTEN loss and PIK3CA mutation, which is not previously described in the literature. PIK3CA and PTEN mutations were mutually exclusive in the tumors tested.

Table 49: Coincidence of alterations with TP53 mutation

	PTEN Loss IHC	TOPO2A IHC+	PTEN MT	cMET MT	IDH MT	CTNNB1 MT	APC MT	KRAS MT
TP53wt	24/197 (12.2%)	98/182 (53.8%)	5/192 (2.6%)	2/201 (1.0%)	1/202 (0.5%)	6/202 (3.0%)	5/202 (2.5%)	6/201 (3.0%)
TP53 mutated	10/51 (19.6%)	41/50 (82%)	3/52 (2.6%)	4/52 (7.7%)	3/52 (5.8%)	0/52 (0)	2/52 (3.8%)	0/52 (0)
P value	0.17	0.0003	0.37	0.03	0.03	0.35	0.63	0.35

Table 50: Coincidence of alterations with TP53 mutation

	TP53 MT	PTEN Loss IHC	TOPO2A IHC+	PTEN MT
PIK3CA mutated	3/7 (42.9%; 1LMS, 1 lipo)	1/10 (10.0%)	7/8 (87.5%)	0/6 (0)
PIK3CA WT	54/243 (22.2%)	39/316 (12.3%)	136/229 (59.4%)	2/240 (0.8%)
P value	0.40	1.0	0.15	1.0

26. Distinct biomarker expression and molecular phenotypes identified therapeutic strategies not otherwise considered in the treatment of sarcoma. Alterations with therapeutic implications were found in

99% of sarcomas. For example, PTEN protein expression and EGFR polysomy/amplification have been associated with potential benefit to EGFR pathway targeted therapy. Overexpression of TOPO2 and Topo1 can fine tune the use of anthracyclines and irinotecan in significant numbers of patients. The overexpression of TOPO2 was observed in approximately 50% of sarcomas, without concomitant amplification. This was most commonly observed in angiosarcoma, LMS, and UPS. These data suggest sensitivity to anthracyclines, especially in relation to TP53 status in a tumor. SPARC is overexpressed in angiosarcoma, chondrosarcoma, EHE and osteosarcoma, which suggests sensitiivty to nab-paclitaxel in addition to the current taxane therapy. PTEN loss was seen in 80% of sarcomas without associated mutations and the use of PI3kinase inhibitors in this subset of patients may be beneficial. PDL1 was expressed in all of the liposarcomas (mostly dedifferentiated) and chondrosarcomas, this indicating potential benefit from the use of the new immune checkpoint inhibitors (anti-PD-1 or anti-PD-L1 therapy). High level of steroid hormone receptor expression uncovers the potential to use anti-steroid hormone therapy in some rare sarcomas. Low MGMT expression suggests potential benefit from radiation therapy and temozolomide, while tumors with low TS expression may benefit from the use of fluorouracil based therapies. The presence of activating mutations BRAF V600E and PIK3CA E545K or H1047L provide for highly specific targeted inhibitors. Trials of agents like mTOR and PI3K inhibitors could benefit from designs in which patient selection is based on PTEN loss or PIK3CA mutations instead of sarcoma histology. Overall, molecular profiling through protein expression, gene copy variations and mutations identified alterations in 99% of sarcoma samples which may guide the most beneficial treatment options.

Example 13: Identification of actionable targets in rare cancers using a multiplatform molecular analysis

[00575] Chordomas are a rare cancer. Limited biomarker data exists to prognosticate outcome or predict response to therapy. This Example explores the utility of multiplatform tumor profiling, which uses immunohistochemistry (IHC) and next generation sequencing (NGS) as described herein to identify druggable targets in patients with chordoma.

[00576] All tissues were internally reviewed by a pathologist. Immunohistochemistry (IHC) was performed on AR, BCRP, cKIT, cMET, EGFR, ER, ERCC1, HER2, MGMT, MRP1, PD-1, PD-L1, PDGFR, PGP, PR, PTEN, RRM1 SPARC, TLE3, TOP2A, TOPO1, TS and TUBB3. In situ hybridization (fluorescence or chromogenic) was performed on EGFR, HER2, cMET and TOP2A. Sequencing (Sanger or NGS) was performed on 47 genes (see **Table 10**).

[00577] 31 chordoma patients were profiled, of which 12 had metastatic disease. The median age was 58 years old; 58% of patients were male. Overexpression of EGFR and TOPO1 were identified in 50% and 54% of cases, while Phosphatase and tensin homolog (PTEN), thymidylate synthase (TS), ribonucleotide reductase M1 (RRM1), and O-6-methylguanine-DNA methyltransferase (MGMT) expression was absent in 15/25, 22/26, 21/26 and 8/23 tumors, respectively. A pathogenic point mutation in PIK3CA (Q546R) was detected in 1 of 12 tumors tested whereas no mutations were identified in the other 46 genes tested by

sequence analysis. No changes in copy number were identified using ISH. Notably, PD-1 tumor infiltrating lymphocytes (TILs) and PD-L1 were seen in 20% and 60% of cases tested, respectively.

[00578] Biomarker analysis indicates that chordomas might be amenable to chemotherapy with 5-fluorouracil, gemcitabine, or temozolamide due to absence of TS, RRM1, and MGMT expression, respectively. Targeting the PI3 kinase pathway is supported by the high loss of PTEN and the PIK3CA mutation. Additionally, immunotherapies, e.g., anti-PD1 therapy, might be of utility in this rare cancer based on the 60% of cases in which tumor infiltrating lymphocytes were identified.

[00579] Similar analysis as above was performed for a cohort of rare adrenal tumors, including 142 tumors of the adrenal cortex, 33 of the adrenal medulla, 2 paraganglia and 7 soft tissues of the abdomen (specifically, neuroblastoma/ganglioneuroblastoma of the periadrenal soft tissue). 49% of the tumors were recurrent and 8.6% were metastatic. The average age was 48 (range=20-86) and 59% were female. Of the 142 adrenal cortical tumors, 137 were adrenal cortical carcinoma, one was a large cell carcinoma, one was a carcinosarcoma, one was a neuroendocrine tumor, one was a malignant neoplasm, and one was unspecified.

[00580] Results of protein expression analysis are shown in **Table 51**. Results of amplification/ rearrangements analysis are shown in **Table 52**. Mutations by detected by next generation sequencing are shown in **Table 53**. No mutations were observed in this cohort in ABL1, ALK, BRAF, BRCA1, CDH1, CSF1R, ERBB2, FBXW7, FGFR1, FGFR2, FLT3, GNA11, GNAQ, GNAS, HNF1A, HRAS, IDH1, JAK2, KRAS, MLH1, MPL, NOTCH1, NPM1, NRAS, PDGFRA, PIK3CA, PTEN, PTPN11, RB1, RET, SMAD4, SMARCB1, SMO, STK11 or VHL.

Table 51: Immunohistochemistry Results for Adrenal Cortical tumors

	Expression observed	Total tested	%
AR	10	135	7.4
BCRP	26	34	76.5
c-kit	6	82	7.3
cMET	1	56	1.8
EGFR	10	30	33.3
ER	3	135	2.2
ERCC1[$]	33	76	43.4
HER2	0	132	0
MGMT[$]	38	118	32.2
MRP1	57	59	96.6
PD-1	4	11	36.4
PD-L1	4	11	36.4
PDGFR	4	56	7.1
PGP[$]	62	111	55.9
PR	79	134	59
PTEN[$]	53	124	42.7
RRM1[$]	47	115	40.9
SPARC	65	139	46.8
TLE3	5	58	8.6
TOP2A	51	115	44.3
TOPO1	50	108	46.3
TS[$]	42	115	36.5
TUBB3[$]	5	43	11.6

[$]*Expression of the biomarker below the threshold is considered predictive of response to therapy.*

Table 52: ISH Results for Adrenal Cortical tumors

	Alterations	Total tested	%
cMET	1	42	2.4
EGFR	5	46	10.9
HER2	1	66	1.5

Table 53: NGS Results for Adrenal Cortical tumors

	Mutation Detected	Total tested	%
AKT1	1	32	3.1
APC	1	33	3
ATM	1	32	3.1
BRCA2	1	7	14.3
c-KIT	1	32	3.1
cMET	1	33	3
CTNNB1	11	33	33.3
EGFR	1	35	2.9
ERBB4	1	33	3
JAK3	1	32	3.1
KDR	1	32	3.1
TP53	15	32	46.9

[00581] Of note, over a third of the tumors tested expressed both PD-1 and PD-L1 (see **Table 51**). These data suggest utility of targeted immunotherapies, e.g., anti-PD1 therapy or anti-PD-L1 therapy, to treat these rare cancers.

[00582] Although preferred embodiments of the present invention have been shown and described herein, it will be obvious to those skilled in the art that such embodiments are provided by way of example only. Numerous variations, changes, and substitutions will now occur to those skilled in the art without departing from the invention. It should be understood that various alternatives to the embodiments of the invention described herein may be employed in practicing the invention. It is intended that the following claims define the scope of the invention and that methods and structures within the scope of these claims and their equivalents be covered thereby.

CLAIMS

WHAT IS CLAIMED IS:

1. A method of identifying at least one treatment associated with a cancer in a subject, comprising:

(a) determining a molecular profile for at least one sample from the subject by assessing a plurality of gene or gene products, wherein the plurality of gene or gene products comprises at least one of PD-1 and PD-L1; and

(b) identifying, based on the molecular profile, at least one of: i) at least one treatment that is associated with benefit for treatment of the cancer; ii) at least one treatment that is associated with lack of benefit for treatment of the cancer; and iii) at least one treatment associated with a clinical trial.

2. The method of claim **1**, wherein the plurality of gene or gene products further comprises at least one gene or gene product selected from the group consisting of CTL4A, IDO1, COX2, CD80, CD86, CD8A, Granzyme A, Granzyme B, CD19, CCR7, CD276, LAG-3, TIM-3 and a combination thereof.

3. The method of claim **1**, wherein the plurality of gene or gene products further comprises at least one gene or gene product selected from any of **Tables 2**, **6**, **7** or **10-17**.

4. The method of claim **1**, wherein the plurality of gene or gene products further comprises at least one of 1p19q, ABL1, AKT1, ALK, APC, AR, ATM, BRAF, BRCA1, BRCA2, cKIT, cMET, CSF1R, CTNNB1, EGFR, EGFRvIII, ER, ERBB2 (HER2), FGFR1, FGFR2, FLT3, GNA11, GNAQ, GNAS, HER2, HRAS, IDH1, IDH2, JAK2, KDR (VEGFR2), KRAS, MGMT, MGMT-Me, MLH1, MPL, NOTCH1, NRAS, PDGFRA, Pgp, PIK3CA, PR, PTEN, RET, RRM1, SMO, SPARC, TLE3, TOP2A, TOPO1, TP53, TS, TUBB3 and VHL.

5. The method of claim **1**, wherein assessing the plurality of gene or gene products comprises using ISH to assess at least one of HER2, 1p19q and cMET.

6. The method of claim **1**, wherein assessing the plurality of gene or gene products comprises using IHC to assess at least one of AR, cMET, EGFR, ER, HER2, MGMT, PD-1, PD-L1, Pgp, PR, PTEN, RRM1, SPARC, TLE3, TOP2A, TOPO1, TS and TUBB3.

7. The method of claim **1**, wherein assessing the plurality of gene or gene products comprises using sequence analysis to assess at least one of ABL1, AKT1, ALK, APC, ATM, BRAF, BRCA1, BRCA2, cKIT, cMET, CSF1R, CTNNB1, EGFR, ERBB2, FGFR1, FGFR2, FLT3, GNA11, GNAQ, GNAS, HRAS, IDH1, JAK2, KDR (VEGFR2), KRAS, MLH1, MPL, NOTCH1, NRAS, PDGFRA, PIK3CA, PTEN, RET, SMO, TP53 and VHL.

8. The method of claim **1**, wherein assessing the plurality of gene or gene products comprises using ISH to assess at least one of HER2, 1p19q and cMET; using IHC to assess at least one of AR, cMET, EGFR, ER, HER2, MGMT, PD-1, PD-L1, Pgp, PR, PTEN, RRM1, SPARC, TLE3, TOP2A, TOPO1, TS and TUBB3; and/or using sequence analysis to assess at least one of ABL1, AKT1, ALK, APC, ATM, BRAF, BRCA1, BRCA2, cKIT, cMET, CSF1R, CTNNB1, EGFR, ERBB2, FGFR1, FGFR2,

FLT3, GNA11, GNAQ, GNAS, HRAS, IDH1, JAK2, KDR (VEGFR2), KRAS, MLH1, MPL, NOTCH1, NRAS, PDGFRA, PIK3CA, PTEN, RET, SMO, TP53 and VHL.

9. The method of claim **7** or **8**, wherein assessing the plurality of gene or gene products comprises using sequence analysis to assess at least one of CDH1, ERBB4, FBXW7, HNF1A, JAK3, NPM1, PTPN11, RB1, SMAD4, SMARCB1 and STK11.

10. The method of any of claims **7-9**, wherein the sequence analysis comprises Next Generation Sequencing.

11. The method of any preceding claim, wherein the plurality of gene or gene products further comprises at least one of MLH1, MSH2, MSH6, PMS2, microsatellite instability (MSI), ROS1 and ERCC1.

12. The method of claim **11**, wherein at least one of MLH1, MSH2, MSH4, PMS2 are assessed by IHC.

13. The method of claim **11**, wherein microsatellite instability is assessed by fragment analysis.

14. The method of claim **11**, wherein ROS1 and/or ERCC1 are assessed by ISH.

15. The method of any preceding claim, wherein the plurality of gene or gene products is according to any of **Tables 7** or **10-16**.

16. The method of any preceding claim, wherein the step of correlating the molecular profile with treatments comprises associating beneficial treatment of the cancer with immune modulating therapy targeting at least one of PD-1, PD-L1, PD-L2, CTL4A, IDO1, COX2, CD80, CD86, CD8A, Granzyme A, Granzyme B, CD19, CCR7, CD276, LAG-3 or TIM-3, wherein the cancer is apoptotic or necrotic.

17. The method of any preceding claim, wherein the step of identifying based on the molecular profile comprises correlating the molecular profile with treatments whose benefit has been assessed for cancers characterized by presence or level, overexpression, underexpression, copy number, mutation, deletion, insertion, translocation, amplification, rearrangement, or other molecular alteration in at least one member of the plurality of gene or gene products.

18. The method of claim **17**, wherein the step of correlating the molecular profile with treatments is according to at least one biomarker-drug association in any of **Tables 3-7**, **Table 8**, **Tables 11-17**, **Table 19**, **Tables 24-26** and **FIGs. 28D-E**.

19. The method of claim **17**, wherein the step of correlating the molecular profile with treatments is according to at least one biomarker-drug association rule selected from:

(a) performing IHC on PD1 to determine likely benefit or lack of benefit from a PD-1 modulating therapy, PD-1 inhibitor, anti-PD-1 immunotherapy, anti-PD-1 monoclonal antibody, nivolumab, pidilizumab (CT-011, CureTech, LTD), pembrolizumab (lambrolizumab, MK-3475, Merck), a PD-1 antagonist, a PD-1 ligand soluble construct, and/or AMP-224 (Amplimmune);

(b) performing IHC on PD-L1 to determine likely benefit or lack of benefit from a PD-L1 modulating therapy, PD-L1 inhibitor, anti-PD-L1 immunotherapy, anti-PD-L1 monoclonal antibody, BMS-936559, MPDL3280A/RG7446, and/or MEDI4736 (MedImmune);

(c) performing IHC on RRM1 to determine likely benefit or lack of benefit from an antimetabolite and/or gemcitabine;

(d) performing IHC on TS to determine likely benefit or lack of benefit from a antimetabolite, fluorouracil, capecitabine, and/or pemetrexed;

(e) performing IHC on TOPO1 to determine likely benefit or lack of benefit from a TOPO1 inhibitor, irinotecan and/or topotecan;

(f) performing at least one of IHC on MGMT, pyrosequencing for MGMT promoter methylation, and sequencing on IDH1 to determine likely benefit or lack of benefit from an alkylating agent, temozolomide, and/or dacarbazine;

(g) performing IHC on AR to determine likely benefit or lack of benefit from an anti-androgen, bicalutamide, flutamide, abiraterone and/or enzalutamide;

(h) performing IHC on ER to determine likely benefit or lack of benefit from a hormonal agent, tamoxifen, fulvestrant, letrozole, and/or anastrozole;

(i) performing IHC on at least one of ER, PR and AR to determine likely benefit or lack of benefit from a hormonal agent, tamoxifen, toremifene, fulvestrant, letrozole, anastrozole, exemestane, megestrol acetate, leuprolide, goserelin, bicalutamide, flutamide, abiraterone, enzalutamide, triptorelin, abarelix, and/or degarelix;

(j) performing at least one of IHC on HER2 and ISH on HER2 to determine likely benefit or lack of benefit from a tyrosine kinase inhibitor and/or lapatinib, pertuzumab, and/or ado-trastuzumab emtansine (T-DM1);

(k) performing at least one of IHC on HER2, ISH on HER2, IHC on PTEN and sequencing on PIK3CA to determine likely benefit or lack of benefit from HER2 targeted therapy, and/or trastuzumab;

(l) performing at least one of ISH on TOP2A, ISH on HER2, IHC on TOP2A and IHC on PGP to determine likely benefit or lack of benefit from an anthracycline, doxorubicin, liposomal-doxorubicin, and/or epirubicin;

(m) performing sequencing on at least one of cKIT and PDGFRA to determine likely benefit or lack of benefit from a tyrosine kinase inhibitor and/or imatinib;

(n) performing at least one of ISH on ALK and ISH on ROS1 to determine likely benefit or lack of benefit from a tyrosine kinase inhibitor and/or crizotinib;

(o) performing at least one of IHC on ER or sequencing on PIK3CA to determine likely benefit or lack of benefit from an mTOR inhibitor, everolimus, and/or temsirolimus;

(p) performing sequencing on RET to determine likely benefit or lack of benefit from a tyrosine kinase inhibitor, and/or vandetanib;

(q) performing IHC on at least one of TLE3, TUBB3 and PGP to determine likely benefit or lack of benefit from a taxane, paclitaxel, and/or docetaxel;

(r) performing IHC on SPARC to determine likely benefit or lack of benefit from a taxane, and/or nab-paclitaxel;

(s) performing at least one of PCR and sequencing on BRAF to determine likely benefit or lack of benefit from a tyrosine kinase inhibitor, vemurafenib, dabrafenib, and/or trametinib;

(t) performing at least one of sequencing on KRAS, sequencing on BRAF, sequencing on NRAS, sequencing on PIK3CA and IHC on PTEN to determine likely benefit or lack of benefit from an EGFR-targeted antibody, cetuximab, and/or panitumumab;

(u) performing sequencing on EGFR to determine likely benefit or lack of benefit from an EGFR-targeted antibody, and/or cetuximab;

(v) performing at least one of sequencing on EGFR, sequencing on KRAS, ISH on cMET, sequencing on PIK3CA and IHC on PTEN to determine likely benefit or lack of benefit from a tyrosine kinase inhibitor, erlotinib, and/or gefitinib;

(w) performing sequencing on EGFR to determine likely benefit or lack of benefit from a tyrosine kinase inhibitor, and/or afatinib;

(x) performing sequencing on cKIT to determine likely benefit or lack of benefit from a tyrosine kinase inhibitor, and/or sunitinib;

(y) performing sequencing on at least one of BRCA1, BRCA2 and/or IHC on ERCC1 to determine likely benefit or lack of benefit from carboplatin, cisplatin, and/or oxaliplatin;

(z) performing ISH on ALK to determine likely benefit or lack of benefit from ceritinib; and

(aa) performing ISH to detect 1p19q codeletion to determine likely benefit or lack of benefit from procarbazine, lomustine, and/or vincristine (PCV).

20. The method of claim **17**, wherein the step of correlating the molecular profile with treatments comprises associating beneficial treatment of the cancer with immune modulating therapy targeting at least one of PD-1, PD-L1, PD-L2, CTL4A, IDO1, COX2, CD80, CD86, CD8A, Granzyme A, Granzyme B, CD19, CCR7, CD276, LAG-3 or TIM-3, wherein determining the molecular profile indicates that the cancer is AR-/HER2-/ER-/PR- (quadruple negative) and/or carries a mutation in BRCA1.

21. The method of claim **17**, wherein the step of correlating the molecular profile with treatments comprises associating beneficial treatment of the cancer with immune modulating therapy targeting at least one of PD-1, PD-L1, PD-L2, CTL4A, IDO1, COX2, CD80, CD86, CD8A, Granzyme A, Granzyme B, CD19, CCR7, CD276, LAG-3 or TIM-3, wherein determining the molecular profile indicates that the cancer carries a mutation in at least one cancer related gene.

22. The method of claim **21**, wherein the at least one cancer related gene comprises at least one of ABL1, AKT1, ALK, APC, ATM, BRAF, BRCA1, BRCA2, cKIT, cMET, CSF1R, CTNNB1, EGFR, ERBB2, FGFR1, FGFR2, FLT3, GNA11, GNAQ, GNAS, HRAS, IDH1, JAK2, KDR (VEGFR2), KRAS, MLH1, MPL, NOTCH1, NRAS, PDGFRA, PIK3CA, PTEN, RET, SMO, TP53, VHL, CDH1, ERBB4, FBXW7, HNF1A, JAK3, NPM1, PTPN11, RB1, SMAD4, SMARCB1 and STK11.

23. The method of claim **17**, wherein the step of correlating the molecular profile with treatments comprises associating beneficial treatment of the cancer with immune modulating therapy targeting at least one of PD-1, PD-L1, PD-L2, CTL4A, IDO1, COX2, CD80, CD86, CD8A, Granzyme A,

Granzyme B, CD19, CCR7, CD276, LAG-3 or TIM-3, wherein determining the molecular profile indicates that the cancer microenvironment expresses PD-L1.

24. The method of claim **23**, wherein the expression of PD-L1 in the cancer microenvironment comprises determining expression of PD-L1 in at least one of tumor cells, T cells, natural killer (NK) cells, macrophages, dendritic cells (DCs), B cells, epithelial cells, and vascular endothelial cells.

25. The method of any preceding claim, further comprising identifying at least one candidate clinical trial for the subject based on the molecular profiling.

26. The method of any preceding claim, wherein the step of identifying based on the molecular profile comprises correlating the molecular profile with treatments whose benefit has been assessed for cancers characterized by presence or level, overexpression, underexpression, copy number, mutation, deletion, insertion, translocation, amplification, rearrangement, or other molecular alteration in PD-1 and/or PD-L1.

27. The method of claim **26**, wherein the at least one treatment comprises a modulator of PD-1 and/or PD-L1.

28. The method of claim **27**, wherein the modulator of PD-1 is selected from the group consisting of a PD-1 inhibitor, anti-PD-1 immunotherapy, anti-PD-1 monoclonal antibody, nivolumab, pidilizumab (CT-011, CureTech, LTD), pembrolizumab (lambrolizumab, MK-3475, Merck), a PD-1 antagonist, a PD-1 ligand soluble construct, AMP-224 (Amplimmune), and a combination thereof.

29. The method of claim **27**, wherein the modulator of PD-L1 is selected from the group consisting of a PD-L1 inhibitor, anti-PD-L1 immunotherapy, anti-PD-L1 monoclonal antibody, BMS-936559, MPDL3280A/RG7446, MEDI4736 (MedImmune), and a combination thereof.

30. The method of any of claims **26-29**, wherein the inhibitor of PD-1 and/or PD-L1 is associated with benefit for treatment of the cancer if the sample expresses both PD-1 and PD-L1.

31. The method of any preceding claim, wherein the presence or level of PD-1 is determined in tumor infiltrating lymphocytes (TILs).

32. The method of any preceding claim, wherein the presence or level of PD-L1 is determined in at least one of tumor cells, T cells, natural killer (NK) cells, macrophages, dendritic cells (DCs), B cells, epithelial cells, and vascular endothelial cells.

33. The method of any preceding claim, wherein the at least one sample comprises formalin-fixed paraffin-embedded (FFPE) tissue, fixed tissue, core needle biopsy, fine needle aspirate, unstained slides, fresh frozen (FF) tissue, formalin samples, tissue comprised in a solution that preserves nucleic acid or protein molecules, and/or a bodily fluid sample.

34. The method of any preceding claim, wherein the sample comprises cells from a solid tumor.

35. The method of any of claims **1-31**, wherein the at least one sample comprises a bodily fluid.

36. The method of claim **35**, wherein the bodily fluid comprises a malignant fluid.

37. The method of claim **35**, wherein the bodily fluid comprises a pleural fluid or peritoneal fluid.

38. The method of any of claims **35-37**, wherein the bodily fluid comprises peripheral blood, sera, plasma, ascites, urine, cerebrospinal fluid (CSF), sputum, saliva, bone marrow, synovial fluid, aqueous humor, amniotic fluid, cerumen, breast milk, broncheoalveolar lavage fluid, semen, prostatic fluid, cowper's fluid, pre-ejaculatory fluid, female ejaculate, sweat, fecal matter, tears, cyst fluid, pleural fluid, peritoneal fluid, pericardial fluid, lymph, chyme, chyle, bile, interstitial fluid, menses, pus, sebum, vomit, vaginal secretions, mucosal secretion, stool water, pancreatic juice, lavage fluids from sinus cavities, bronchopulmonary aspirates, blastocyst cavity fluid, or umbilical cord blood.

39. The method of any preceding claim, wherein the at least one sample comprises a microvesicle population.

40. The method of claim **39**, wherein at least one member of the plurality of gene or gene products is associated with the microvesicle population.

41. The method of any preceding claim, wherein the subject has not previously been treated with the at least one treatment that is associated with benefit for treatment of the cancer.

42. The method of any preceding claim, wherein the cancer comprises a metastatic and/or recurrent cancer.

43. The method of any preceding claim, wherein the cancer is refractory to a prior treatment.

44. The method of claim **43**, wherein the prior treatment comprises the standard of care for the cancer.

45. The method of claim **43**, wherein the cancer is refractory to all known standard of care treatments.

46. The method of any of claims **1-42**, wherein the subject has not previously been treated for the cancer.

47. The method of any preceding claim, wherein progression free survival (PFS), disease free survival (DFS), or lifespan is extended by administration of the at least one treatment that is associated with benefit for treatment of the cancer to the subject.

48. The method of any preceding claim, wherein the cancer comprises an acute lymphoblastic leukemia; acute myeloid leukemia; adrenocortical carcinoma; AIDS-related cancer; AIDS-related lymphoma; anal cancer; appendix cancer; astrocytomas; atypical teratoid/rhabdoid tumor; basal cell carcinoma; bladder cancer; brain stem glioma; brain tumor, brain stem glioma, central nervous system atypical teratoid/rhabdoid tumor, central nervous system embryonal tumors, astrocytomas, craniopharyngioma, ependymoblastoma, ependymoma, medulloblastoma, medulloepithelioma, pineal parenchymal tumors of intermediate differentiation, supratentorial primitive neuroectodermal tumors and pineoblastoma; breast cancer; bronchial tumors; Burkitt lymphoma; cancer of unknown primary site (CUP); carcinoid tumor; carcinoma of unknown primary site; central nervous system atypical teratoid/rhabdoid tumor; central nervous system embryonal tumors; cervical cancer; childhood cancers; chordoma; chronic lymphocytic leukemia; chronic myelogenous leukemia; chronic myeloproliferative

disorders; colon cancer; colorectal cancer; craniopharyngioma; cutaneous T-cell lymphoma; endocrine pancreas islet cell tumors; endometrial cancer; ependymoblastoma; ependymoma; esophageal cancer; esthesioneuroblastoma; Ewing sarcoma; extracranial germ cell tumor; extragonadal germ cell tumor; extrahepatic bile duct cancer; gallbladder cancer; gastric (stomach) cancer; gastrointestinal carcinoid tumor; gastrointestinal stromal cell tumor; gastrointestinal stromal tumor (GIST); gestational trophoblastic tumor; glioma; hairy cell leukemia; head and neck cancer; heart cancer; Hodgkin lymphoma; hypopharyngeal cancer; intraocular melanoma; islet cell tumors; Kaposi sarcoma; kidney cancer; Langerhans cell histiocytosis; laryngeal cancer; lip cancer; liver cancer; malignant fibrous histiocytoma bone cancer; medulloblastoma; medulloepithelioma; melanoma; Merkel cell carcinoma; Merkel cell skin carcinoma; mesothelioma; metastatic squamous neck cancer with occult primary; mouth cancer; multiple endocrine neoplasia syndromes; multiple myeloma; multiple myeloma/plasma cell neoplasm; mycosis fungoides; myelodysplastic syndromes; myeloproliferative neoplasms; nasal cavity cancer; nasopharyngeal cancer; neuroblastoma; Non-Hodgkin lymphoma; nonmelanoma skin cancer; non-small cell lung cancer; oral cancer; oral cavity cancer; oropharyngeal cancer; osteosarcoma; other brain and spinal cord tumors; ovarian cancer; ovarian epithelial cancer; ovarian germ cell tumor; ovarian low malignant potential tumor; pancreatic cancer; papillomatosis; paranasal sinus cancer; parathyroid cancer; pelvic cancer; penile cancer; pharyngeal cancer; pineal parenchymal tumors of intermediate differentiation; pineoblastoma; pituitary tumor; plasma cell neoplasm/multiple myeloma; pleuropulmonary blastoma; primary central nervous system (CNS) lymphoma; primary hepatocellular liver cancer; prostate cancer; rectal cancer; renal cancer; renal cell (kidney) cancer; renal cell cancer; respiratory tract cancer; retinoblastoma; rhabdomyosarcoma; salivary gland cancer; Sézary syndrome; small cell lung cancer; small intestine cancer; soft tissue sarcoma; squamous cell carcinoma; squamous neck cancer; stomach (gastric) cancer; supratentorial primitive neuroectodermal tumors; T-cell lymphoma; testicular cancer; throat cancer; thymic carcinoma; thymoma; thyroid cancer; transitional cell cancer; transitional cell cancer of the renal pelvis and ureter; trophoblastic tumor; ureter cancer; urethral cancer; uterine cancer; uterine sarcoma; vaginal cancer; vulvar cancer; Waldenström macroglobulinemia; or Wilm's tumor.

49. The method of any preceding claim, wherein the cancer comprises an acute myeloid leukemia (AML), breast carcinoma, cholangiocarcinoma, colorectal adenocarcinoma, extrahepatic bile duct adenocarcinoma, female genital tract malignancy, gastric adenocarcinoma, gastroesophageal adenocarcinoma, gastrointestinal stromal tumor (GIST), glioblastoma, head and neck squamous carcinoma, leukemia, liver hepatocellular carcinoma, low grade glioma, lung bronchioloalveolar carcinoma (BAC), non-small cell lung cancer (NSCLC), lung small cell cancer (SCLC), lymphoma, male genital tract malignancy, malignant solitary fibrous tumor of the pleura (MSFT), melanoma, multiple myeloma, neuroendocrine tumor, nodal diffuse large B-cell lymphoma, non epithelial ovarian cancer (non-EOC), ovarian surface epithelial carcinoma, pancreatic adenocarcinoma, pituitary carcinomas, oligodendroglioma, prostatic adenocarcinoma, retroperitoneal or peritoneal carcinoma, retroperitoneal or

peritoneal sarcoma, small intestinal malignancy, soft tissue tumor, thymic carcinoma, thyroid carcinoma, or uveal melanoma.

50. The method of any preceding claim, wherein the cancer comprises a breast cancer, triple negative breast cancer, metaplastic breast cancer (MpBC), head and neck squamous cell carcinoma (HNSCC), human papilloma virus (HPV)-positive HNSCC, HPV–negative/TP53-mutated HNSCC, metastatic HNSCC, oropharyngeal HNSCC, non-oropharyngeal HNSCC, a carcinoma, a sarcoma, a melanoma, a luminal A breast cancer, a luminal B breast cancer, HER2+ breast cancer, a high microsatellite instability (MSI-H) colorectal cancer, a microsatellite stable colorectal cancer (MSS), non-small cell lung cancer (NSCLC), chordoma, or adrenal cortical carcinoma.

51. The method of claim **50**, wherein the carcinoma comprises a carcinoma of the breast, colon, lung, pancreas, prostate, Merkel cell, ovary, liver, endometrial, bladder, kidney or cancer of unknown primary (CUP).

52. The method of claim **50**, wherein the sarcoma comprises a liposarcoma, chondrosarcoma, extraskeletal myxoid chondrosarcoma or uterine sarcoma.

53. The method of claim **50**, wherein the sarcoma comprises an alveolar soft part sarcoma (ASPS), angiosarcoma, breast angiosarcoma, chondrosarcoma, chordoma, clear cell sarcoma, desmoplastic small round cell tumor (DSRCT), epithelioid hemangioendothelioma (EHE), epithelioid sarcoma, endometrial stromal sarcoma (ESS), ewing sarcoma, fibromatosis, fibrosarcoma, giant cell tumour, leiomyosarcoma (LMS), uterine LMS, liposarcoma, malignant fibrous histiocytoma (MFH/UPS), malignant peripheral nerve sheath tumor (MPNST), osteosarcoma, perivascular epithelioid cell tumor (PEComa), rhabdomyosarcoma, solitary fibrous tumor (SFT), synovial sarcoma, fibromyxoid sarcoma, fibrous hamartoma of infancy, hereditary leiomyomatosis, angiomyolipoma, angiomyxoma, atypical spindle cell lesion (with fibrohistiocytic differentiation), chondroblastoma, dendritic cell sarcoma, granular cell tumor, high grade myxoid sarcoma, high-grade myoepithelial carcinoma, hyalinizing fibroblastic sarcoma, inflammatory myofibroblastic sarcoma, interdigitating dendritic cell tumor, intimal sarcoma, leiomyoma, lymphangitic sarcomatosis, malignant glomus tumor, malignant myoepithelioma, melanocytic neoplasm, mesenchymal neoplasm, mesenteric glomangioma, metastatic histocytoid neoplasm, myoepithelioma, myxoid sarcoma, myxoid stromal, neurilemmoma, phyllodes, rhabdoid, round cell, sarcoma not otherwise specified (NOS), sarcomatous mesothelioma, schwannoma, spindle and round cell sarcoma, spindle cell or spinocellular mesenchymal tumor.

54. A method of generating a molecular profiling report comprising preparing a report comprising results of the determining and identifying steps according to any preceding claim.

55. The method of claim **54**, wherein the report further comprises a list of the at least one treatment that is associated with benefit for treatment of the cancer.

56. The method of claim **55**, wherein the report further comprises a list of the at least one treatment that is associated with lack of benefit for treatment of the cancer.

57. The method of claim **55**, wherein the report further comprises a list of at least one treatment that is associated with indeterminate benefit for treating the cancer.

58. The method of claim **55**, wherein the report further comprises identification of the at least one treatment as standard of care or not for the cancer lineage.

59. The method of claim **54**, wherein the report further comprises a listing of at least one member of the plurality of genes or gene products assessed with description of the at least one member.

60. The method of claim **54**, wherein the report further comprises a listing of members of the plurality of genes or gene products assessed by at least one of ISH, IHC, Next Generation sequencing, Sanger sequencing, PCR, pyrosequencing and fragment analysis.

61. The method of claim **54**, wherein the report further comprises a list of clinical trials for which the subject is indicated and/or eligible based on the molecular profile.

62. The method of claim **54**, wherein the report further comprises a list of evidence supporting the identification of certain treatments as likely to benefit the patient, not benefit the patient, or having indeterminate benefit.

63. The method of claim **54**, wherein the report further comprises: 1) a list of the genes and/or gene products in the molecular profile; 2) a description of the molecular profile of the genes and/or gene products as determined for the subject; 3) a treatment associated with at least one of the genes and/or gene products in the molecular profile; and 4) and an indication whether each treatment is likely to benefit the patient, not benefit the patient, or has indeterminate benefit.

64. The method of claim **63**, wherein the description of the molecular profile of the genes and/or gene products as determined for the subject comprises the technique used to assess the gene and/or gene products and the results of the assessment.

65. The method of any of claims **54-64**, wherein the report is computer generated.

66. The method of claim **65**, wherein the report is a printed report or a computer file.

67. The method of claim **65**, wherein the report is accessible via a web portal.

68. Use of a reagent in carrying out the method of any of claims **1-53**.

69. Use of a reagent in the manufacture of a reagent or kit for carrying out the method of any of claims **1-53**.

70. A kit comprising a reagent for carrying out the method of any of claims **1-53**.

71. The use of of any of claims **68-69** or kit of claim **70**, wherein the reagent comprises at least one of a reagent for extracting nucleic acid from a sample, a reagent for performing ISH, a reagent for performing IHC, a reagent for performing PCR, a reagent for performing Sanger sequencing, a reagent for performing next generation sequencing, a reagent for a DNA microarray, a reagent for performing pyrosequencing, a nucleic acid probe, a nucleic acid primer, an antibody, a reagent for performing bisulfite treatment of nucleic acid, and a combination thereof.

72. A report generated by the method of any of claims **54-67**.

73. A computer system for generating the report of claim **72**.

74. A system for identifying at least one treatment associated with a cancer in a subject, comprising:

(a) a host server;

(b) a user interface for accessing the host server to access and input data;

(c) a processor for processing the inputted data;

(d) a memory coupled to the processor for storing the processed data and instructions for:

i. accessing a molecular profile generated by the method of any of claims **1-53**;

ii. identifying, based on the molecular profile, at least one of: A) at least one treatment that is associated with benefit for treatment of the cancer; B) at least one treatment that is associated with lack of benefit for treatment of the cancer; and C) at least one treatment associated with a clinical trial; and

(e) a display for displaying the identified at least one of: A) at least one treatment that is associated with benefit for treatment of the cancer; B) at least one treatment that is associated with lack of benefit for treatment of the cancer; and C) at least one treatment associated with a clinical trial.

75. The system of claim **74**, wherein the display comprises a report of claim **72**.

76. A computer medium comprising at least one rule from **Table 8**.

77. A computer medium comprising at least one at least one rule, e.g., 1, 2, 3, 4, 5, 6, 7, 8, 9, 10, 11, 12, 13, 14, 15, 16, 17, 18, 19, 20, 21,22, 23, 24, 25, 26 or 27 rules, selected from claim 19.

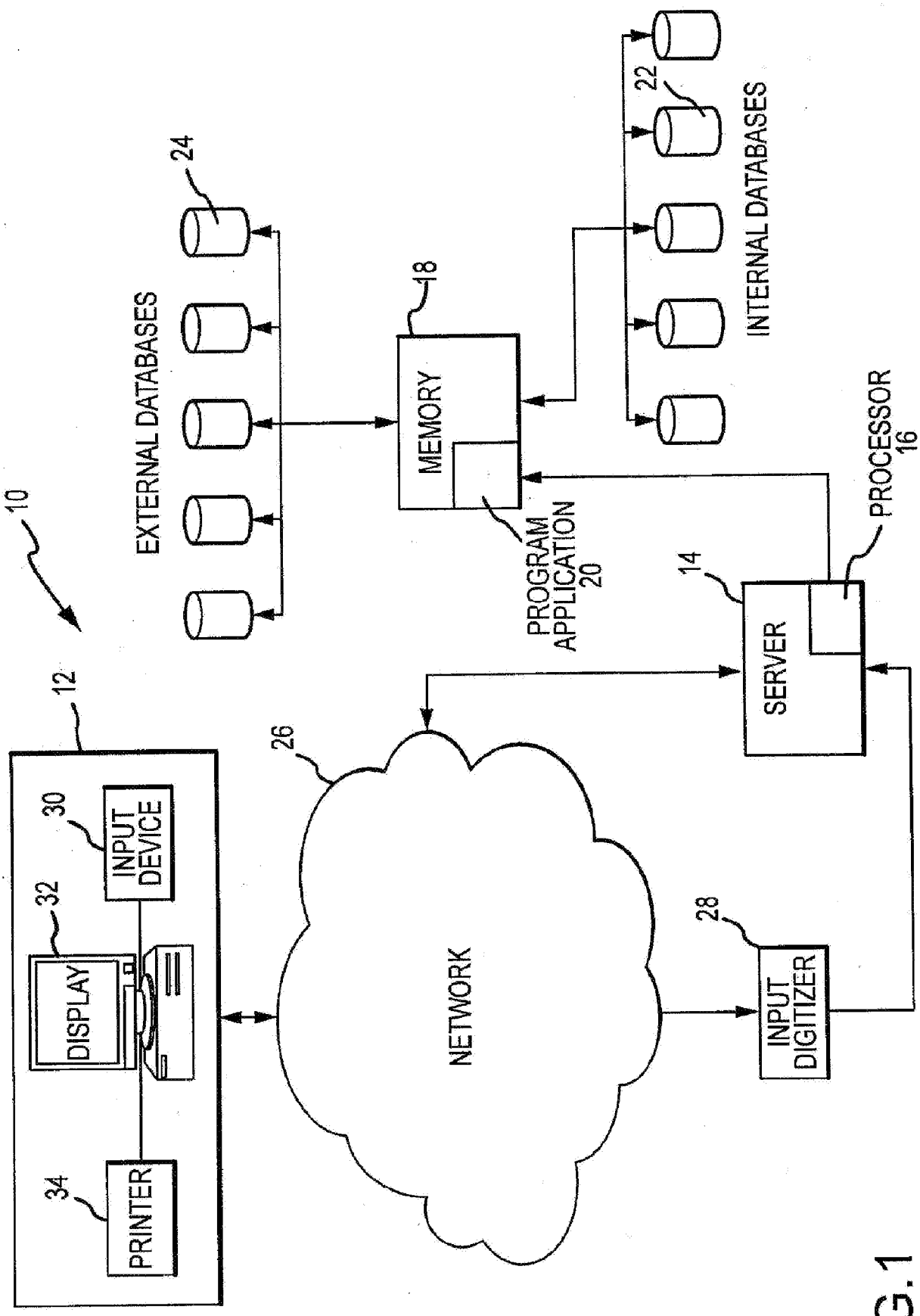

10
12
30
INPUT DEVICE
32
DISPLAY
34
PRINTER
26
NETWORK
28
INPUT DIGITIZER
14
SERVER
PROCESSOR 16
PROGRAM APPLICATION 20
18
MEMORY
EXTERNAL DATABASES
24
INTERNAL DATABASES
22

FIG. 1

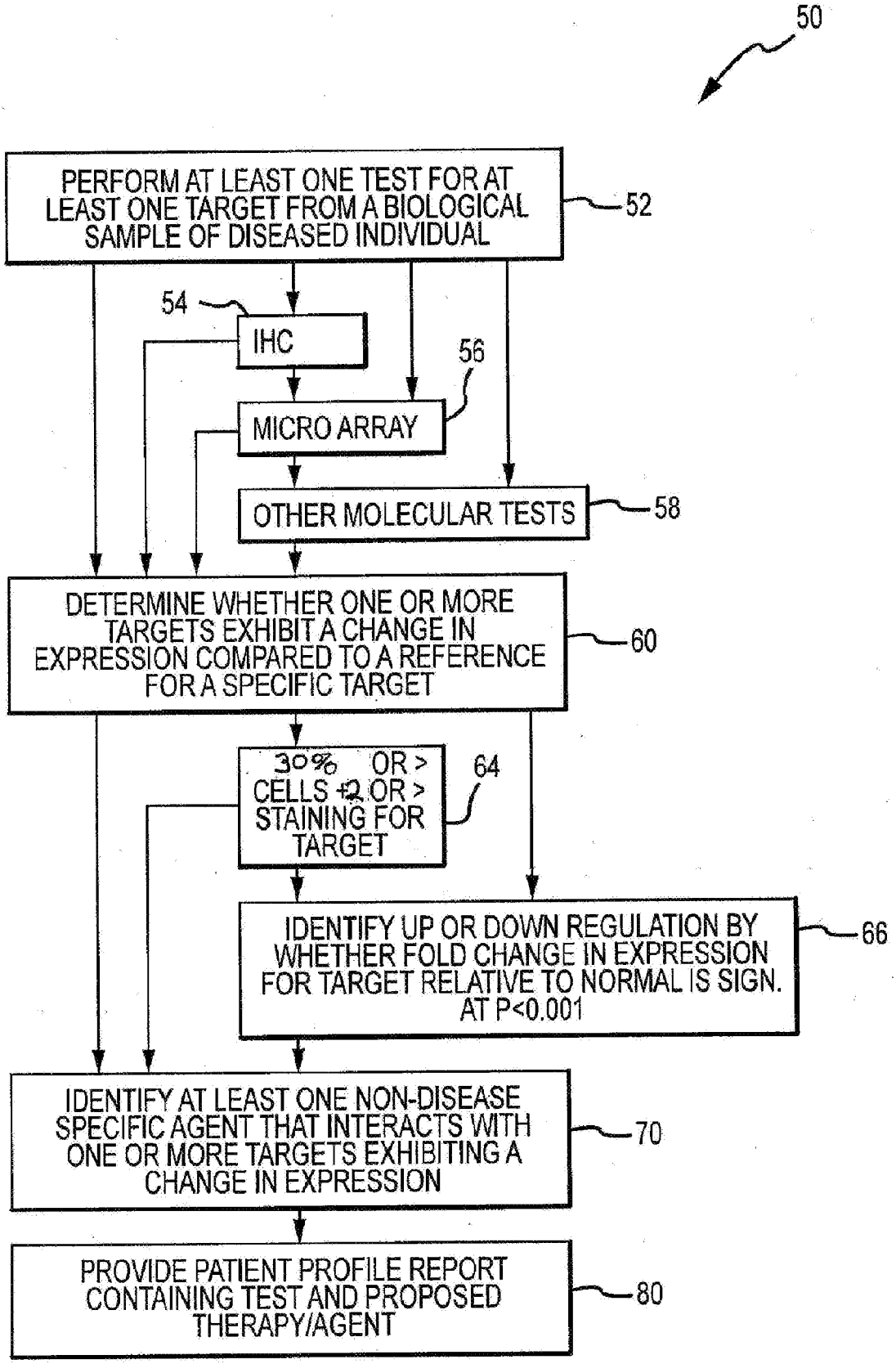

50
PERFORM AT LEAST ONE TEST FOR AT LEAST ONE TARGET FROM A BIOLOGICAL SAMPLE OF DISEASED INDIVIDUAL
52
54
IHC
56
MICRO ARRAY
OTHER MOLECULAR TESTS
58
DETERMINE WHETHER ONE OR MORE TARGETS EXHIBIT A CHANGE IN EXPRESSION COMPARED TO A REFERENCE FOR A SPECIFIC TARGET
60
30% OR > CELLS +2 OR > STAINING FOR TARGET
64
IDENTIFY UP OR DOWN REGULATION BY WHETHER FOLD CHANGE IN EXPRESSION FOR TARGET RELATIVE TO NORMAL IS SIGN. AT P<0.001
66
IDENTIFY AT LEAST ONE NON-DISEASE SPECIFIC AGENT THAT INTERACTS WITH ONE OR MORE TARGETS EXHIBITING A CHANGE IN EXPRESSION
70
PROVIDE PATIENT PROFILE REPORT CONTAINING TEST AND PROPOSED THERAPY/AGENT
80

FIG.2

MOLECULAR PROFILING INSTITUTE	PATIENT INFORMATION	PHYSICIAN INFORMATION
	NAME: SAMPLE PATIENT SEX: FEMALE DOB: 6/1/1974 SSN#: 123-45-6789	SOME DOCTOR, M.D. 1234 E. SOUTH ST. TUCSON, AX 12345 480-123-4567
	REPORT INFORMATION VER 1.6.2:4-25-06	
	DATE SPECIMEN RECEIVED: 02/01/2006 DATE REPORTED: 02/09/2006 CASE NO. MP-TN06-05040 DATE SPECIMEN COLLECTED AT HOST MEDICAL CENTER: 01/24/2006	

SPECIAL STUDIES
RESULTS AND INTERPRETATION

INTERPRETATION:

REVIEW OF PATHOLOGY SLIDES: (RECEIVED FROM MAIN HOSPITAL, TUCSON, AZ, ONE PARAFFIN BLOCK LABELED M01-123 AND FROZEN TISSUE).

PELVIC AND RETROPERITONEAL TUMOR: INFLAMMATORY MYOFIBROLASTIC TUMOR.

100

102 104 106

POSSIBLE AGENTS THAT MIGHT INTERACT WITH CANDIDATE GENE TARGETS:

ASSAY*	CANDIDATE TARGET	SIGNIFICANT RESULT	POSSIBLE AGENT(S)
MICROARRAY	NFKBIA	(INCREASED 1.78)**	VELCADE
IHC	C-KIT	(INCREASED +2, 90%)	GLEEVEC, SUTENT
MICROARRAY	PDGFRA	(INCREASED 4.74)**	GLEEVEC, SORAFENIB, SUTENT
MICROARRAY	GART	(INCREASED 1.90)**	ALIMTA
MICROARRAY	VDR	(INCREASED 37.30)**	CALCITRIOL
MICROARRAY	ADA	(INCREASED 5.26)**	PENTOSTATIN
MICROARRAY	TOP1	(INCREASED 2.78)**	TOPOTECAN, CAMPTOSAR (CPT11)
MICROARRAY	HIF1A	(INCREASED 4.03)**	AVASTIN, SCRAFENIB. SUTENT
MICROARRAY	DNMT1	(INCREASED 1.51)**	VIDAZA (5-AZACYTIDINE)

*IHC = IMMUNOHISTOCHEMISTRY
** INCREASED OR DECREASED ARE RELATIVE TO NORMAL CONTRLS.

FIG.3A

MOLECULAR PROFILING INSTITUTE	PATIENT INFORMATION	PHYSICIAN INFORMATION
	NAME: SAMPLE PATIENT SEX: FEMALE DOB: 6/1/1974 SSN#: 123-45-6789	SOME DOCTOR, M.D. 1234 E. SOUTH ST. TUCSON, AX 12345 480-123-4567

REPORT INFORMATION	VER 1.6.2:4-25-06
DATE SPECIMEN RECEIVED: 02/01/2006 DATE REPORTED: 02/09/2006 CASE NO. MP-TN06-05040	
DATE SPECIMEN COLLECTED AT HOST MEDICAL CENTER: 01/24/2006	

SPECIAL STUDIES

RESULTS AND INTERPRETATION

ADVANCED IMMUNOHISTOCHEMICAL ANALYSIS:

112

GENE EXPRESSED PROTEIN	CONCLUSION	SPECIFICITY	INTENSITY	%	TARGET STATUS*
HER2/NEU	NEGATIVE				
ER	NEGATIVE				
PR	NEGATIVE				
C-KIT	POSITIVE	SPECIFIC	2	90	TARGET
EGFR	NEGATIVE				
COX-2	NEGATIVE				
ANDROGEN RECEPTOR	NEGATIVE				
CD52	NEGATIVE				
PDGFR	NEGATIVE	NON-SPECIFIC			
CD25	NEGATIVE				

100

110 108

*2+ IHC IN GREATER THAN 30% OF THE TUMOR CELLS HAS BEEN CHOSEN AS A CONSERVATIVE DIVIDING POINT TO REPORT A POTENTIAL TARGET AS POSITIVE TO HELP INCREASE PHARMACOLOGIC EFFECTIVENESS.

114

IMMUNOHISTOCHEMICAL TESTS NOT PERFORMED

IL-2	TOPOISOMERASE I	MLH1
NF-KAPPA BETA	TOPOISOMERASE II	MSH2
THYMIDYLATE SYNTHASE	RETINOIC ACID RECEPTOR	CD20
ERCC3 (HELICASE)	R X R	P53
THYMIDINE PHOSPHORYLASE	ORNITHINE DECARBOXYLASE	CYCLIN D1
NGF	SOMATOSTATIN	BCL-2
MTAP	RAS (MUTATED)	VEGF
MAP KINASE PROTEIN	ASPARAGINE SYNTHETASE	
XANTHINE OXIDASE		

FIG.3B

MOLECULAR PROFILING INSTITUTE

PATIENT INFORMATION	PHYSICIAN INFORMATION
NAME: SAMPLE PATIENT SEX: FEMALE DOB: 6/1/1974 SSN#: 123-45-6789	SOME DOCTOR, M.D. 1234 E. SOUTH ST. TUCSON, AX 12345 480-123-4567

REPORT INFORMATION VER 1.6.2:4-25-06

DATE SPECIMEN RECEIVED: 02/01/2006 DATE REPORTED: 02/09/2006 CASE NO. MP-TN06-05040

DATE SPECIMEN COLLECTED AT HOST MEDICAL CENTER: 01/24/2006

SPECIAL STUDIES

MICROARRAY RESULTS

MICROARRAY ANALYSIS: (116, 118, 100)

GENE	RATIO	EXPRESSION*	ANALYSIS
AR	0.02	UNDER EXPRESSED	
ESR1	0.09	UNDER EXPRESSED	
PGR	0.10	UNDER EXPRESSED	
VEGF	0.33	UNDER EXPRESSED	
KIT	0.51	UNDER EXPRESSED	
PDGFC	0.53	UNDER EXPRESSED	
RXRB	0.62	NO CHANGE	
TOP2B	0.62	UNDER EXPRESSED	
RAF1	0.68	NO CHANGE	
ERBB2	0.69	NO CHANGE	
ERCC3	0.71	NO CHANGE	
BCL2	0.71	NO CHANGE	
PDGFRB	0.78	NO CHANGE	
BCL2	0.80	NO CHANGE	
GSTP1	0.85	NO CHANGE	
SPARC	0.92	NO CHANGE	
HDAC1	0.95	NO CHANGE	
POLA	0.98	NO CHANGE	
MSH2	0.98	NO CHANGE	
CES2	1.05	NO CHANGE	
VEGF	1.09	NO CHANGE	
SSTR1	1.11	NO CHANGE	
PTEN	1.11	NO CHANGE	

GENE	RATIO	EXPRESSION*	ANALYSIS
EGFR	1.16	NO CHANGE	
OGFR	1.17	NO CHANGE	
MLH1	1.19	NO CHANGE	
VHL	1.22	NO CHANGE	
TNF	1.29	NO CHANGE	
RARA	1.38	NO CHANGE	
HSPCA	1.42	NO CHANGE	
TXNRD1	1.42	NO CHANGE	
ASNS	1.44	NO CHANGE	
DNMT1	1.51	OVER EXPRESSED	
NFKB2	1.74	NO CHANGE	
NFKBIA	1.78	OVER EXPRESSED	
PTGS2	1.81	NO CHANGE	
BRCA2	1.83	NO CHANGE	
GART	1.90	OVER EXPRESSED	
CDW52	2.15	OVER EXPRESSED	
ZAP70	2.18	NO CHANGE	
FOLR2	2.21	OVER EXPRESSED	
ZAP70	2.76	NO CHANGE	
TOP1	2.78	OVER EXPRESSED	
MS4A1	2.86	NO CHANGE	
ZAP70	2.86	NO CHANGE	
ZAP70	2.92	NO CHANGE	

GENE	RATIO	EXPRESSION*	ANALYSIS
ZAP70	3.00	NO CHANGE	
ZAP70	3.02	NO CHANGE	
CD33	3.05	OVER EXPRESSED	
ZAP70	3.06	NO CHANGE	
ZAP70	3.13	NO CHANGE	
ZAP70	3.18	NO CHANGE	
ZAP70	3.40	NO CHANGE	
CD33	3.52	OVER EXPRESSED	
HIF1A	3.84	OVER EXPRESSED	
HIF1A	3.85	OVER EXPRESSED	
HIF1A	3.88	OVER EXPRESSED	
HIF1A	3.90	OVER EXPRESSED	
HIF1A	3.90	OVER EXPRESSED	
HIF1A	3.91	OVER EXPRESSED	
HIF1A	3.94	OVER EXPRESSED	
HIF1A	3.97	OVER EXPRESSED	
HIF1A	4.01	OVER EXPRESSED	
HIF1A	4.03	OVER EXPRESSED	
PDGFRA	4.74	OVER EXPRESSED	
TK1	4.94	OVER EXPRESSED	
IL2RA	5.07	NO CHANGE	
ADA	5.26	OVER EXPRESSED	
TOP2A	9.34	NO CHANGE	
TYMS	22.95	OVER EXPRESSED	
VDR	37.30	OVER EXPRESSED	

*"NO CHANGE" INDICATES THAT THERE IS NO DIFFERENCE IN EXPRESSION FOR THIS GENE BETWEEN THE TUMOR AND CONTROL TISSUES AT A SIGNIFICANCE LEVEL OF P<=0.001. A SIGNIFICANCE LEVEL OF P<=0.001 HAS BEEN CHOSEN SINCE GENES PASSING THIS THRESHOLD CAN BE VALIDATED AS DIFFERENTIALLY EXPRESSED BY ALTERNATIVE METHODS APPROXIMATELY 95% OF THE TIME.

FIG.3C

100

PATIENT: SAMPLE PATIENT CASE NO. MP-TN06-05040 DATE REPORTED: 2/9/2006

CLINICAL INFORMATION

CLINICAL HISTORY

120 THE PATIENT WAS DIAGNOSED WITH INFLAMATORY MYOFIBROBLASTIC TUMOR IN FEB, 2004. AT THAT TIME A LARGE MASS WAS REMOVED FROM HER ABDOMEN. THE PATIENT NOW HAS RECURRENT MASSES ON HER LEFT UPPER QUADRANT AND IN THE PELVIS. PER THE PATIENT CHART, DR SOME REVIEWED THIS CASE WITH DR VON HOFF AND IT WAS AGREED THAT PERFORMING DNA MICROARRAY AND IHC TESTING ON THIS PATIENT MAY PROVIDE INSIGHT INTO FURTHER TREATMENT OPTIONS.

SPECIMENS SUBMITTED

122 RECEIVED FROM MAIN HOSPITAL, TUCSON, AZ, ONE PARAFFIN BLOCK LABELED M01-123 AND FROZEN TISSUE WITH THE ACCOMPANYING SURGICAL PATHOLOGY REPORT.

DISCLAIMER

THESE TESTS WERE DEVELOPED BY MOLECULAR PROFILING AND THEIR PERFORMANCE CHARACTERISTICS DETERMINED BY MOLECULAR PROFILING. IT HAS NOT BEEN CLEARED OR APPROVED BY THE U.S. FOOD AND DRUG ADMINISTRATION (FDA). THESE TESTS ARE PERMITTED FOR CLINICAL PURPOSES AND SHOULD NOT BE REGARDED AS PURELY INVESTIGATIONAL OR FOR RESEARCH. MOLECULAR PROFILING IS CERTIFIED UNDER THE CLINICAL LABORATORY IMPROVEMENT AMENDMENTS OF 1988 (CLIA) AS QUALIFIED TO PERFORM HIGH-COMPLEXITY CLINICAL TESTING.

DECISIONS REGARDING CARE AND TREATMENT SHOULD NOT BE BASED ON A SINGLE TEST SUCH AS THIS TEST. RATHER DECISIONS ON CARE AND TREATMENT SHOULD BE BASED ON THE INDEPENDENT MEDICAL JUDGMENT OF THE TREATING PHYSICIAN TAKING INTO CONSIDERATION ALL AVAILABLE INFORMATION CONCERNING THE PATIENT'S CONDITION, INCLUDING OTHER LABORATORY TESTS, IN ACCORDANCE WITH THE STANDARD OF CARE IN A GIVEN COMMUNITY.

THE FINDING OF A TARGET DOES NOT NECESSARILY INDICATE PHARMACOLOGIC EFFECTIVENESS.

ROBERT J. PENNY, MD, PHD, PATHOLOGIST AND MEDICAL DIRECTOR — 2/9/2006 DATE

FIG.3D

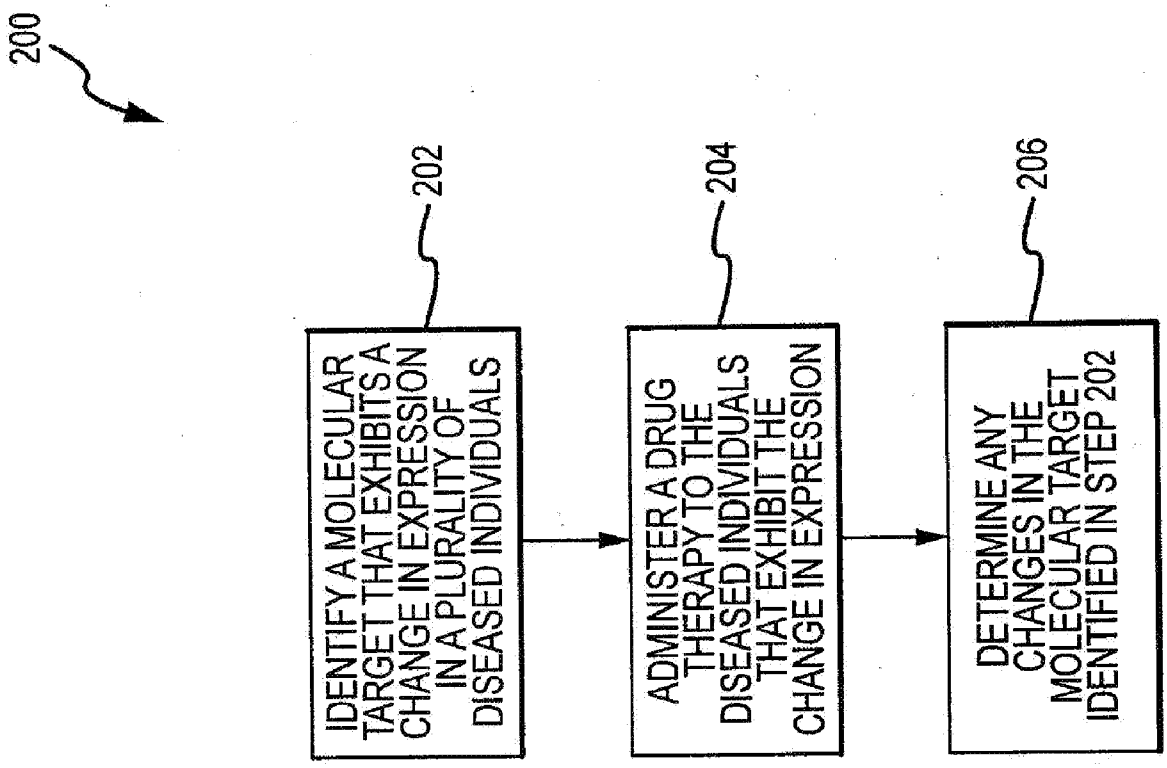

200
202
IDENTIFY A MOLECULAR TARGET THAT EXHIBITS A CHANGE IN EXPRESSION IN A PLURALITY OF DISEASED INDIVIDUALS
204
ADMINISTER A DRUG THERAPY TO THE DISEASED INDIVIDUALS THAT EXHIBIT THE CHANGE IN EXPRESSION
206
DETERMINE ANY CHANGES IN THE MOLECULAR TARGET IDENTIFIED IN STEP 202

FIG.4

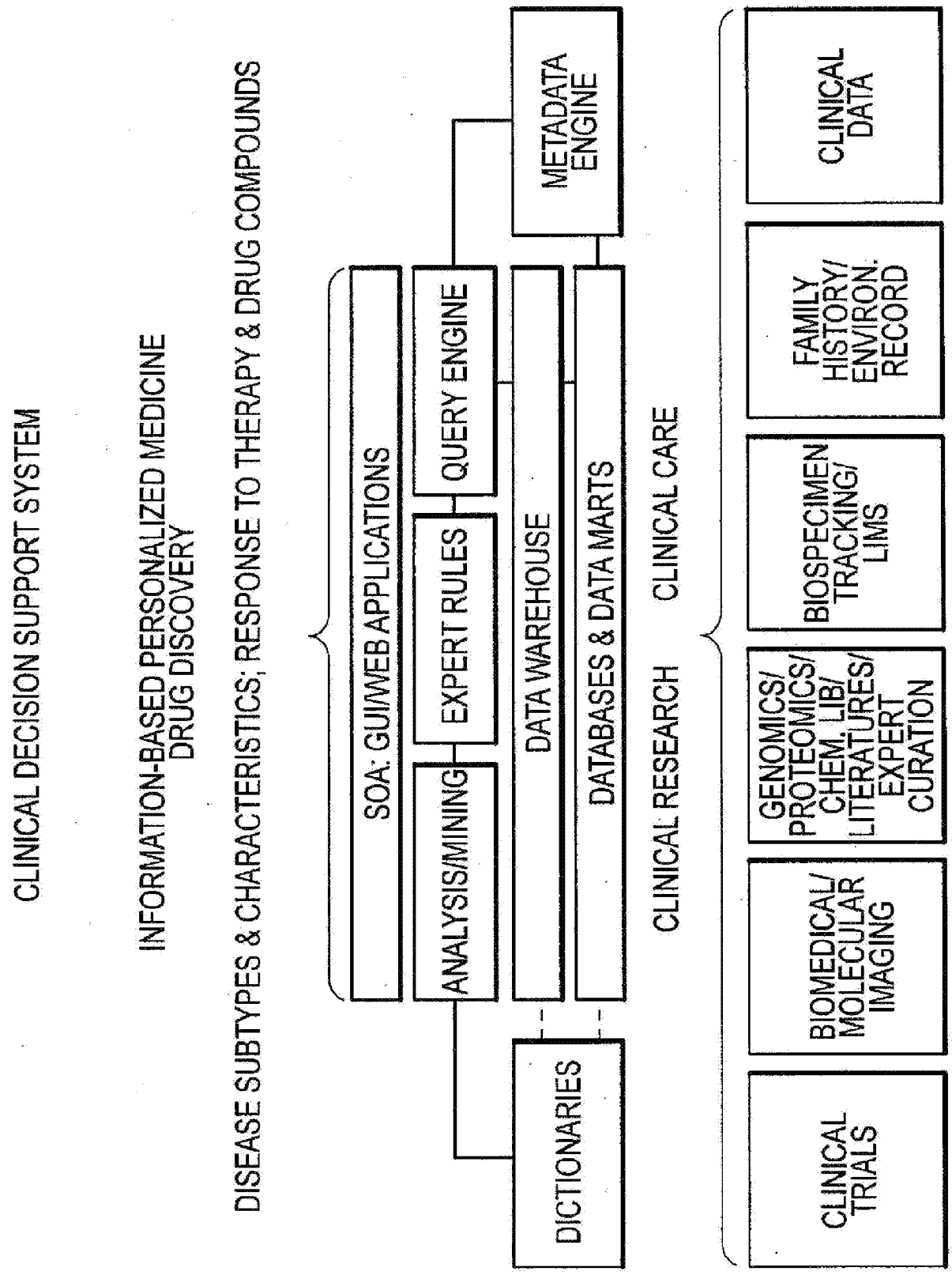

CLINICAL DECISION SUPPORT SYSTEM
INFORMATION-BASED PERSONALIZED MEDICINE
DRUG DISCOVERY
DISEASE SUBTYPES & CHARACTERISTICS; RESPONSE TO THERAPY & DRUG COMPOUNDS
SOA: GUI/WEB APPLICATIONS
ANALYSIS/MINING
EXPERT RULES
QUERY ENGINE
METADATA ENGINE
DATA WAREHOUSE
DICTIONARIES
DATABASES & DATA MARTS
CLINICAL RESEARCH
CLINICAL CARE
CLINICAL TRIALS
BIOMEDICAL/ MOLECULAR IMAGING
GENOMICS/ PROTEOMICS/ CHEM. LIB/ LITERATURES/ EXPERT CURATION
BIOSPECIMEN TRACKING/ LIMS
FAMILY HISTORY/ ENVIRON. RECORD
CLINICAL DATA

FIG.5

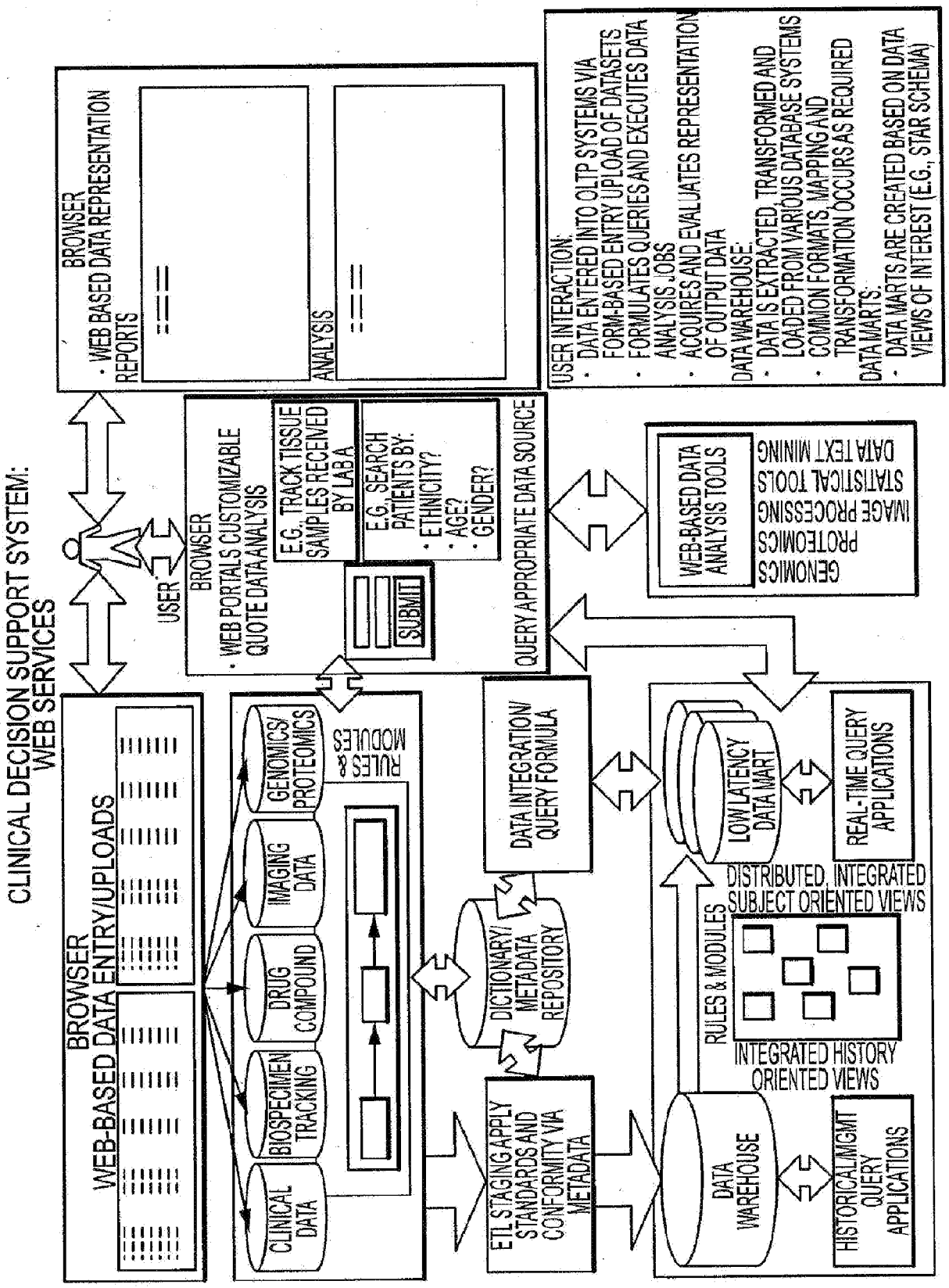

CLINICAL DECISION SUPPORT SYSTEM:
WEB SERVICES
BROWSER
WEB-BASED DATA ENTRY/UPLOADS
USER
BROWSER
· WEB PORTALS CUSTOMIZABLE QUOTE DATA ANALYSIS
E.G., TRACK TISSUE SAMPLES RECEIVED BY LAB A
E.G., SEARCH PATIENTS BY:
· ETHNICITY?
· AGE?
· GENDER?
SUBMIT
QUERY APPROPRIATE DATA SOURCE
BROWSER
· WEB BASED DATA REPRESENTATION REPORTS
ANALYSIS
USER INTERACTION:
· DATA ENTERED INTO OLTP SYSTEMS VIA FORM-BASED ENTRY UPLOAD OF DATASETS
· FORMULATES QUERIES AND EXECUTES DATA ANALYSIS JOBS
· ACQUIRES AND EVALUATES REPRESENTATION OF OUTPUT DATA
DATA WAREHOUSE:
· DATA IS EXTRACTED, TRANSFORMED AND LOADED FROM VARIOUS DATABASE SYSTEMS
· COMMON FORMATS, MAPPING AND TRANSFORMATION OCCURS AS REQUIRED
DATA MARTS:
· DATA MARTS ARE CREATED BASED ON DATA VIEWS OF INTEREST (E.G., STAR SCHEMA)
WEB-BASED DATA ANALYSIS TOOLS
GENOMICS
PROTEOMICS
IMAGE PROCESSING
STATISTICAL TOOLS
DATA TEXT MINING
RULES & MODULES
GENOMICS/ PROTEOMICS
IMAGING DATA
DRUG COMPOUND
BIOSPECIMEN TRACKING
CLINICAL DATA
DATA INTEGRATION/ QUERY FORMULA
DICTIONARY/ METADATA REPOSITORY
ETL STAGING APPLY STANDARDS AND CONFORMITY VIA METADATA
DISTRIBUTED, INTEGRATED SUBJECT ORIENTED VIEWS
LOW LATENCY DATA MART
REAL-TIME QUERY APPLICATIONS
RULES & MODULES
INTEGRATED HISTORY ORIENTED VIEWS
DATA WAREHOUSE
HISTORICAL/MGMT QUERY APPLICATIONS

FIG.6

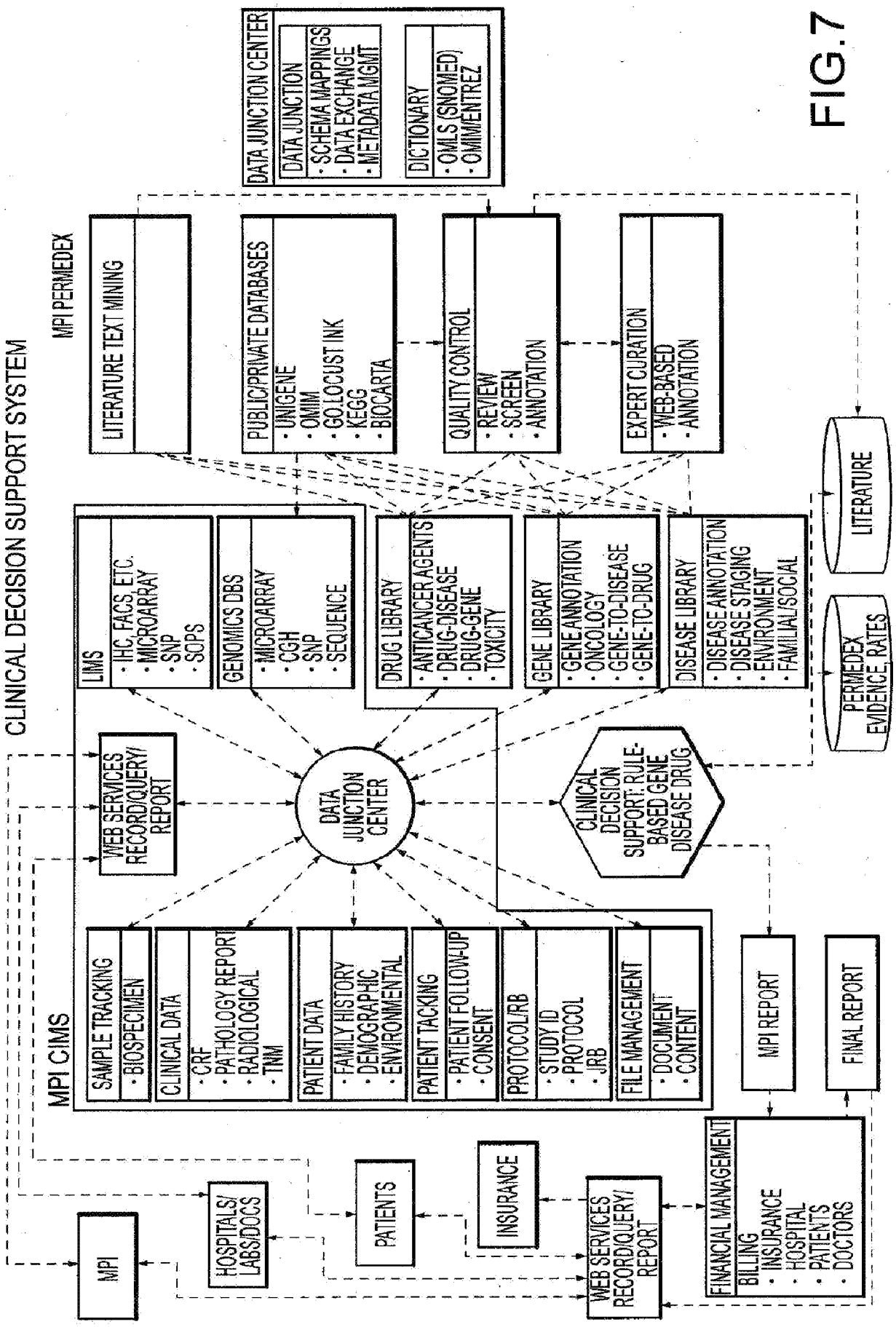

CLINICAL DECISION SUPPORT SYSTEM
MPI PERMEDEX
MPI CIMS
DATA JUNCTION CENTER
DATA JUNCTION
· SCHEMA MAPPINGS
· DATA EXCHANGE
· METADATA MGMT
DICTIONARY
· OMLS (SNOMED)
· OMIM/ENTREZ
LITERATURE TEXT MINING
PUBLIC/PRIVATE DATABASES
· UNIGENE
· OMIM
· GO,LOCUST INK
· KEGG
· BIOCARTA
QUALITY CONTROL
· REVIEW
· SCREEN
· ANNOTATION
EXPERT CURATION
· WEB-BASED
· ANNOTATION
LITERATURE
PERMEDEX EVIDENCE, RATES
LIMS
· IHC, FACS, ETC.
· MICROARRAY
· SNP
· SOPS
GENOMICS DBS
· MICROARRAY
· CGH
· SNP
· SEQUENCE
DRUG LIBRARY
· ANTICANCER AGENTS
· DRUG-DISEASE
· DRUG-GENE
· TOXICITY
GENE LIBRARY
· GENE ANNOTATION
· ONCOLOGY
· GENE-TO-DISEASE
· GENE-TO-DRUG
DISEASE LIBRARY
· DISEASE ANNOTATION
· DISEASE STAGING
· ENVIRONMENT
· FAMILIAL/SOCIAL
WEB SERVICES RECORD/QUERY/ REPORT
DATA JUNCTION CENTER
CLINICAL DECISION SUPPORT: RULE-BASED GENE DISEASE DRUG
SAMPLE TRACKING
· BIOSPECIMEN
CLINICAL DATA
· CRF
· PATHOLOGY REPORT
· RADIOLOGICAL
· TNM
PATIENT DATA
· FAMILY HISTORY
· DEMOGRAPHIC
· ENVIRONMENTAL
PATIENT TACKING
· PATIENT FOLLOW-UP
· CONSENT
PROTOCOL/RB
· STUDY ID
· PROTOCOL
· JRB
FILE MANAGEMENT
· DOCUMENT
· CONTENT
MPI REPORT
FINAL REPORT
FINANCIAL MANAGEMENT
BILLING
· INSURANCE
· HOSPITAL
· PATIENTS
· DOCTORS
MPI
HOSPITALS/ LABS/DOCS
PATIENTS
INSURANCE
WEB SERVICES RECORD/QUERY/ REPORT

FIG.7

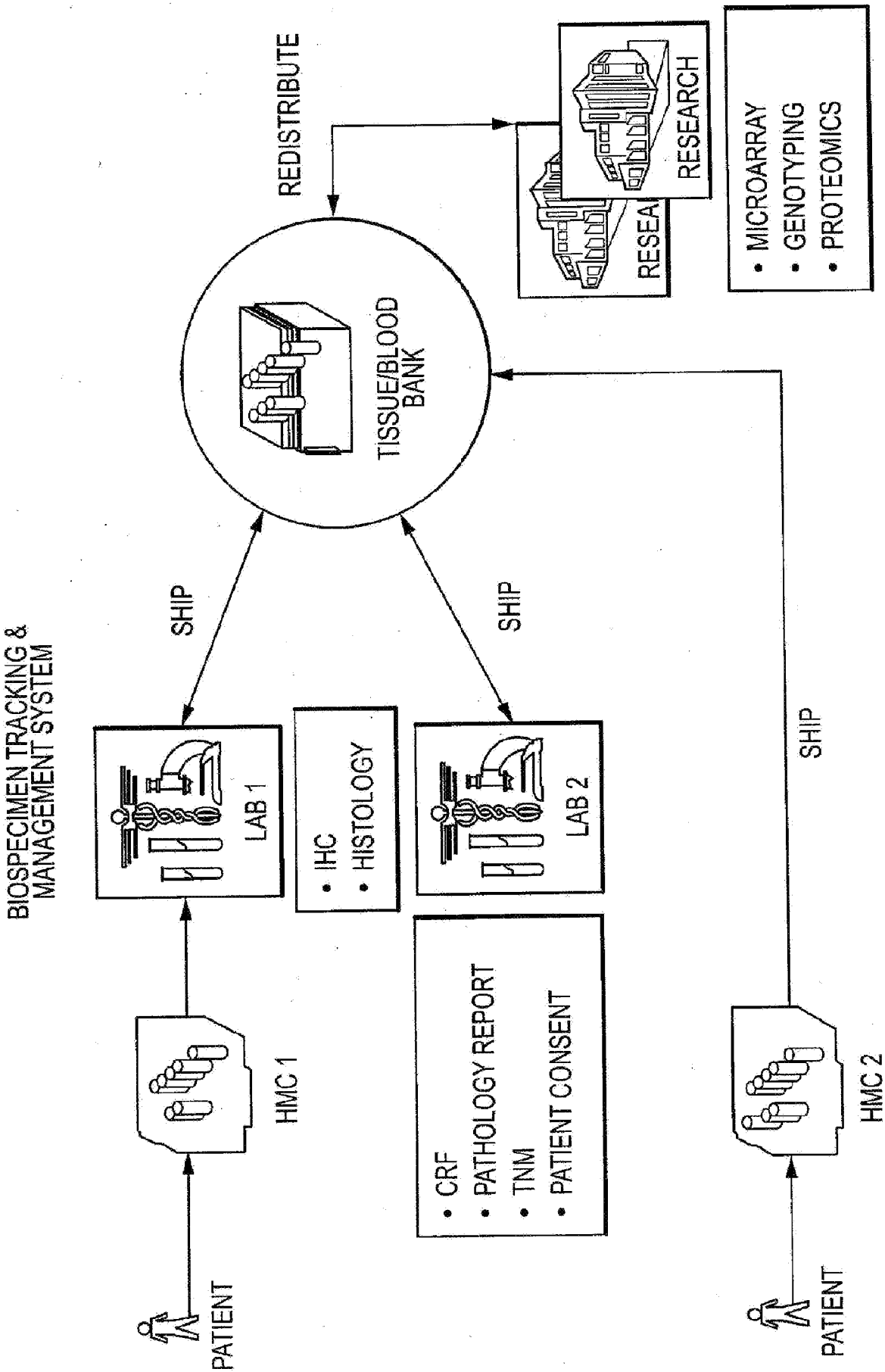

BIOSPECIMEN TRACKING & MANAGEMENT SYSTEM
PATIENT
HMC 1
LAB 1
• IHC
• HISTOLOGY
• CRF
• PATHOLOGY REPORT
• TNM
• PATIENT CONSENT
LAB 2
SHIP
SHIP
TISSUE/BLOOD BANK
REDISTRIBUTE
RESEARCH
RESEA
• MICROARRAY
• GENOTYPING
• PROTEOMICS
SHIP
HMC 2
PATIENT

FIG.8

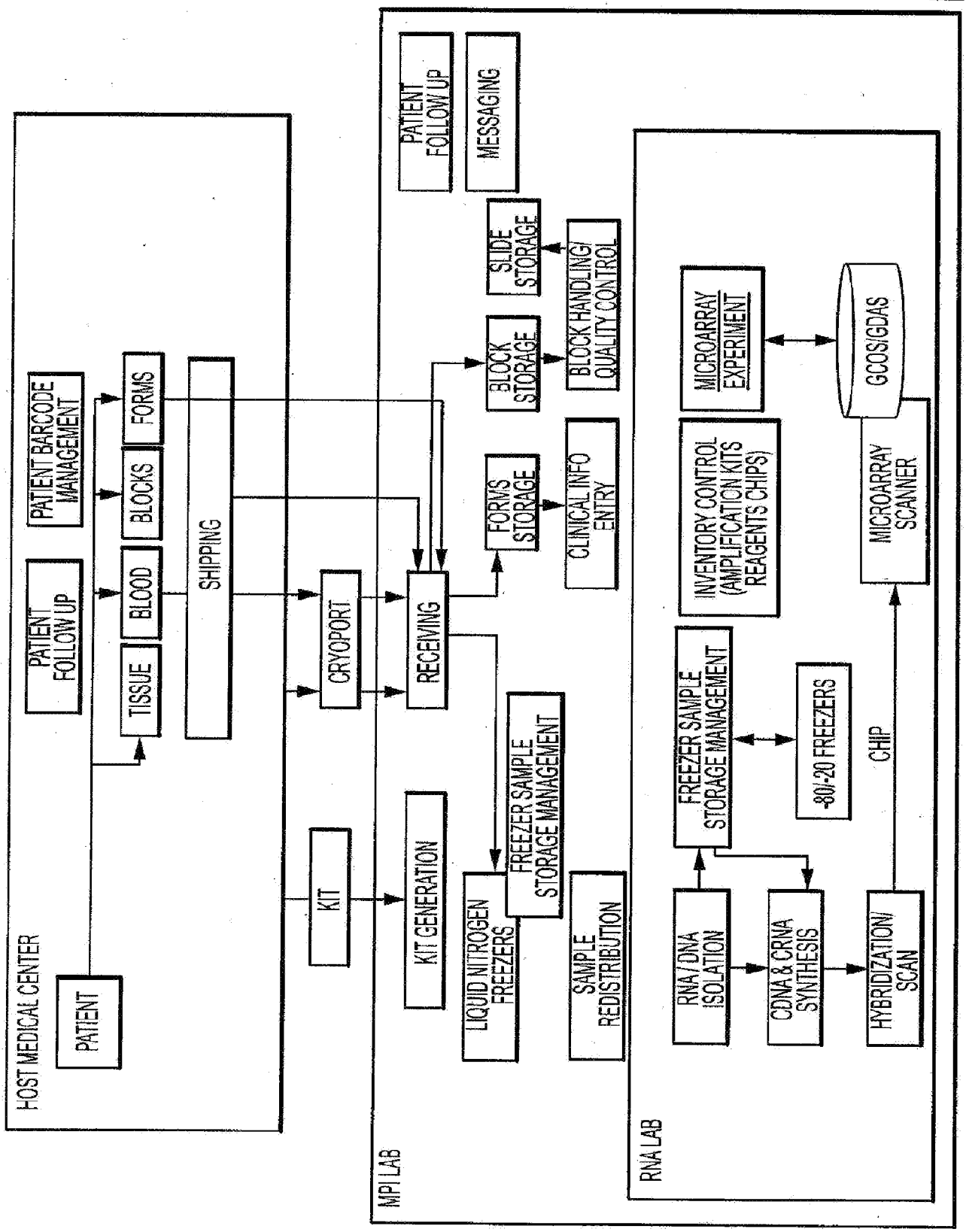

HOST MEDICAL CENTER
PATIENT
PATIENT FOLLOW UP
PATIENT BARCODE MANAGEMENT
TISSUE
BLOOD
BLOCKS
FORMS
SHIPPING
KIT
CRYOPORT
KIT GENERATION
RECEIVING
LIQUID NITROGEN FREEZERS
FREEZER SAMPLE STORAGE MANAGEMENT
SAMPLE REDISTRIBUTION
FORMS STORAGE
CLINICAL INFO ENTRY
BLOCK STORAGE
BLOCK HANDLING/ QUALITY CONTROL
SLIDE STORAGE
PATIENT FOLLOW UP
MESSAGING
MPI LAB
RNA LAB
RNA/DNA ISOLATION
CDNA & CRNA SYNTHESIS
HYBRIDIZATION/ SCAN
FREEZER SAMPLE STORAGE MANAGEMENT
-80/-20 FREEZERS
CHIP
INVENTORY CONTROL (AMPLIFICATION KITS REAGENTS CHIPS)
MICROARRAY EXPERIMENT
MICROARRAY SCANNER
GCOS/GDAS

FIG.9

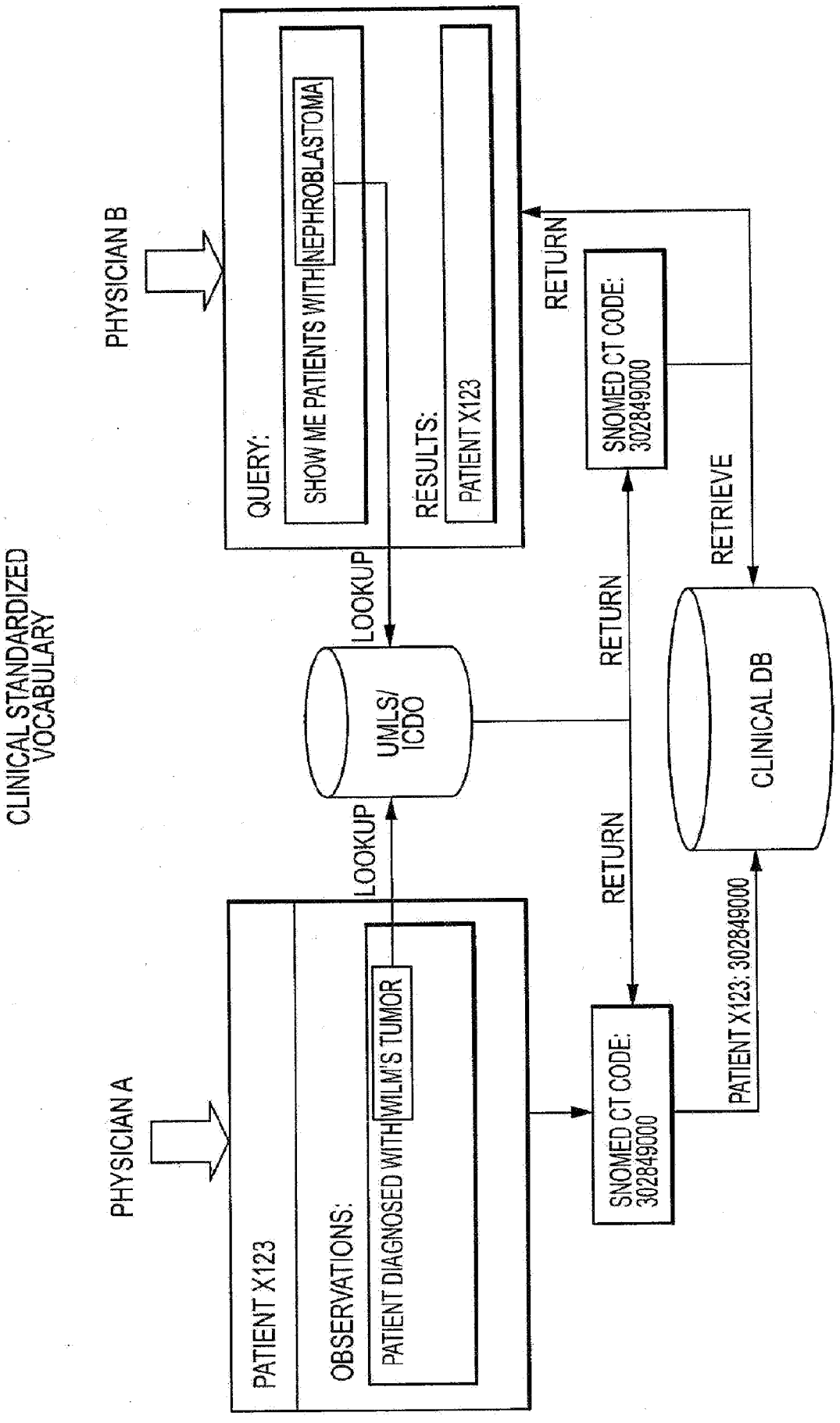

CLINICAL STANDARDIZED VOCABULARY
PHYSICIAN A
PHYSICIAN B
PATIENT X123
OBSERVATIONS:
PATIENT DIAGNOSED WITH WILM'S TUMOR
QUERY:
SHOW ME PATIENTS WITH NEPHROBLASTOMA
RESULTS:
PATIENT X123
LOOKUP
LOOKUP
UMLS/ ICDO
RETURN
RETURN
SNOMED CT CODE: 302849000
SNOMED CT CODE: 302849000
RETURN
RETRIEVE
CLINICAL DB
PATIENT X123: 302849000

FIG.10

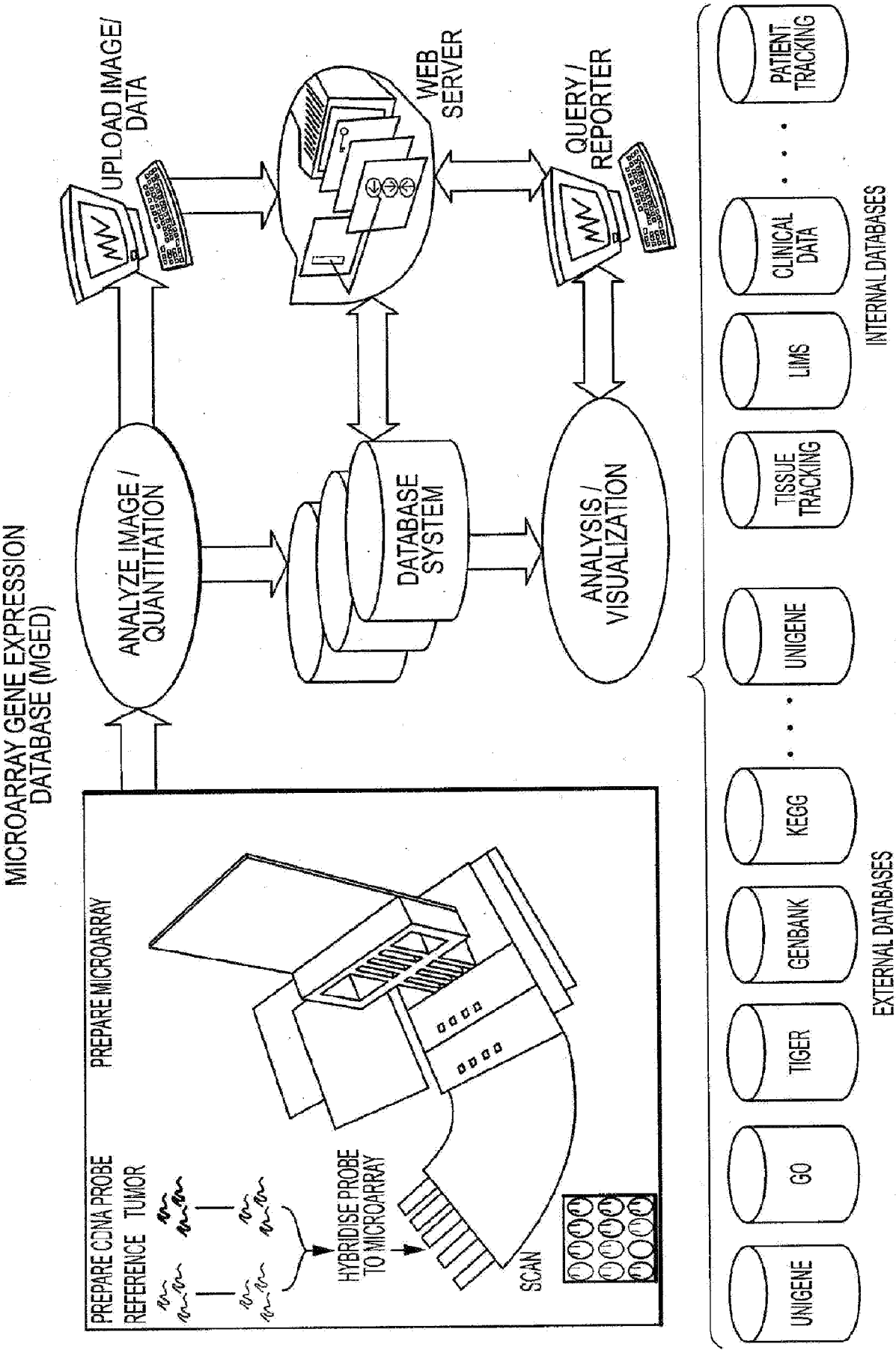

MICROARRAY GENE EXPRESSION DATABASE (MGED)
UPLOAD IMAGE/ DATA
WEB SERVER
QUERY / REPORTER
ANALYZE IMAGE / QUANTITATION
DATABASE SYSTEM
ANALYSIS / VISUALIZATION
PREPARE MICROARRAY
PREPARE CDNA PROBE
REFERENCE TUMOR
HYBRIDISE PROBE TO MICROARRAY
SCAN
UNIGENE
GO
TIGER
GENBANK
KEGG
UNIGENE
TISSUE TRACKING
LIMS
CLINICAL DATA
PATIENT TRACKING
EXTERNAL DATABASES
INTERNAL DATABASES

FIG.11

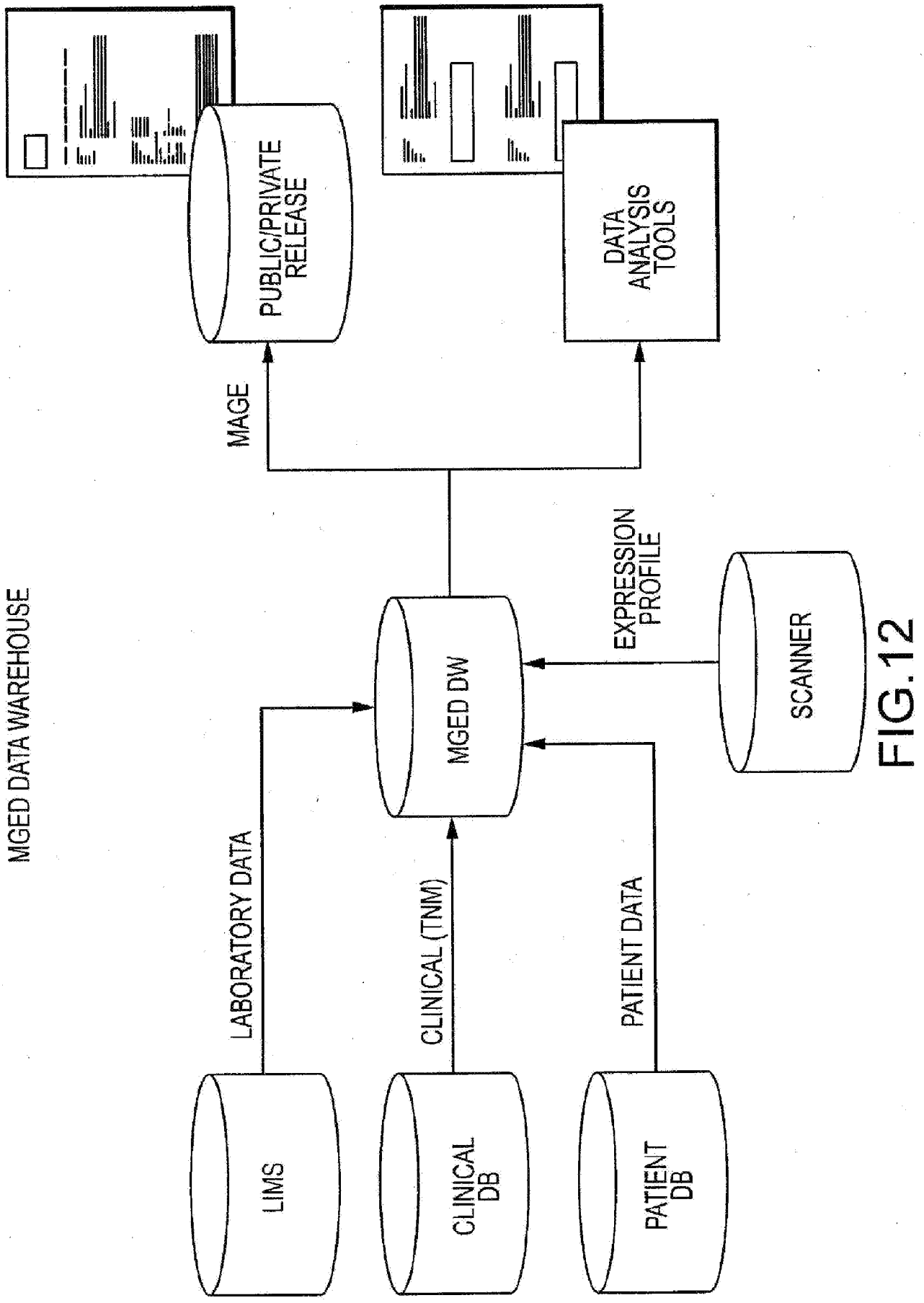

MGED DATA WAREHOUSE
LABORATORY DATA
CLINICAL (TNM)
PATIENT DATA
LIMS
CLINICAL DB
PATIENT DB
MGED DW
EXPRESSION PROFILE
SCANNER
MAGE
PUBLIC/PRIVATE RELEASE
DATA ANALYSIS TOOLS

FIG.12

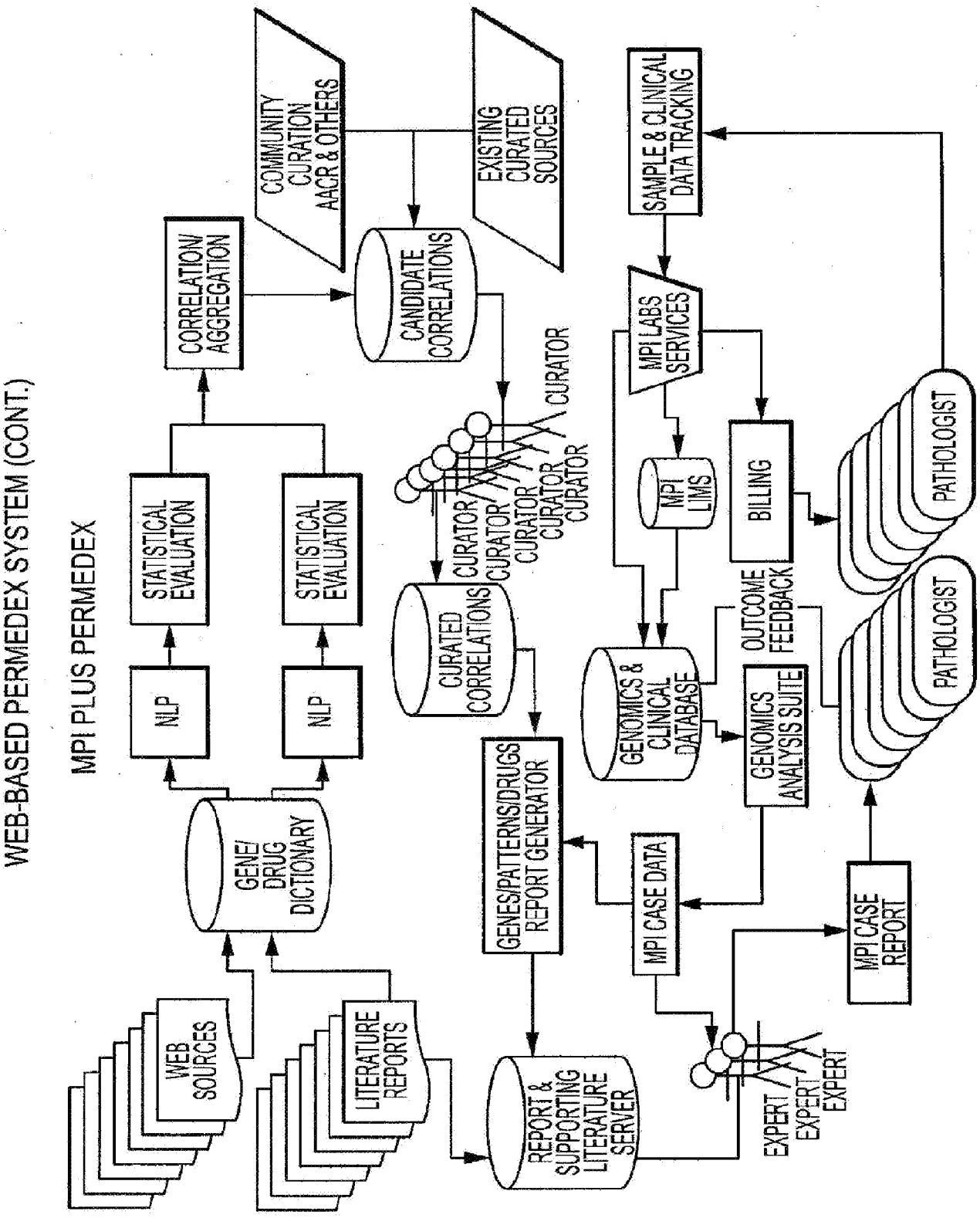

WEB-BASED PERMEDEX SYSTEM (CONT.)
MPI PLUS PERMEDEX
WEB SOURCES
LITERATURE REPORTS
GENE/ DRUG DICTIONARY
NLP
NLP
STATISTICAL EVALUATION
STATISTICAL EVALUATION
CORRELATION/ AGGREGATION
COMMUNITY CURATION AACR & OTHERS
EXISTING CURATED SOURCES
CANDIDATE CORRELATIONS
CURATOR
CURATED CORRELATIONS
GENES/PATTERNS/DRUGS REPORT GENERATOR
REPORT & SUPPORTING LITERATURE SERVER
SAMPLE & CLINICAL DATA TRACKING
MPI LABS SERVICES
MPI LIMS
BILLING
GENOMICS & CLINICAL DATABASE
GENOMICS ANALYSIS SUITE
OUTCOME FEEDBACK
MPI CASE DATA
MPI CASE REPORT
EXPERT
PATHOLOGIST

FIG. 13

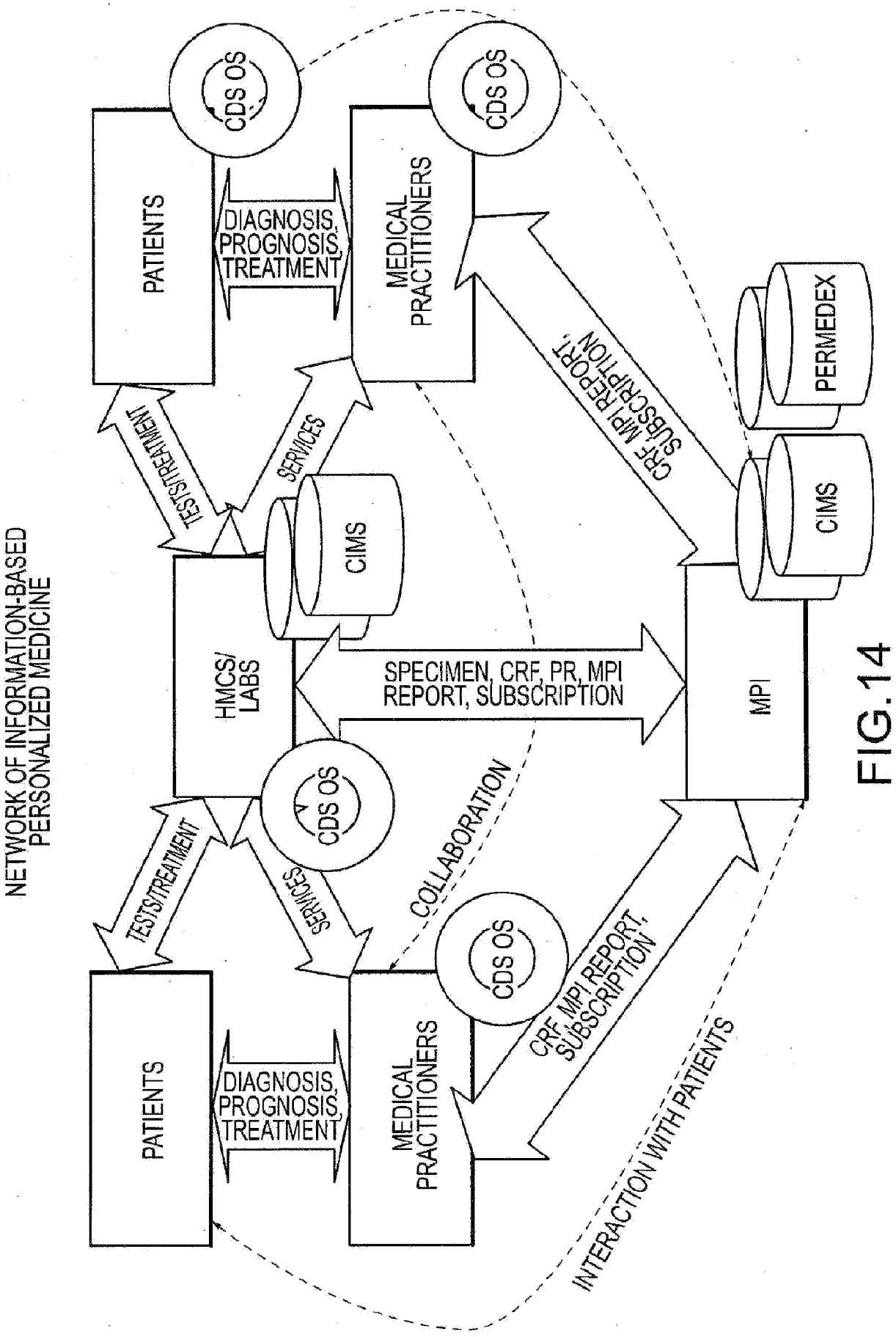

NETWORK OF INFORMATION-BASED PERSONALIZED MEDICINE
PATIENTS
DIAGNOSIS, PROGNOSIS, TREATMENT
MEDICAL PRACTITIONERS
CDS OS
CDS OS
TESTS/TREATMENT
SERVICES
CRF, MPI REPORT, SUBSCRIPTION
PERMEDEX
CIMS
CIMS
HMCS/ LABS
SPECIMEN, CRF, PR, MPI REPORT, SUBSCRIPTION
MPI
CDS OS
TESTS/TREATMENT
SERVICES
COLLABORATION
CDS OS
CRF, MPI REPORT, SUBSCRIPTION
PATIENTS
DIAGNOSIS, PROGNOSIS, TREATMENT
MEDICAL PRACTITIONERS
INTERACTION WITH PATIENTS

FIG. 14

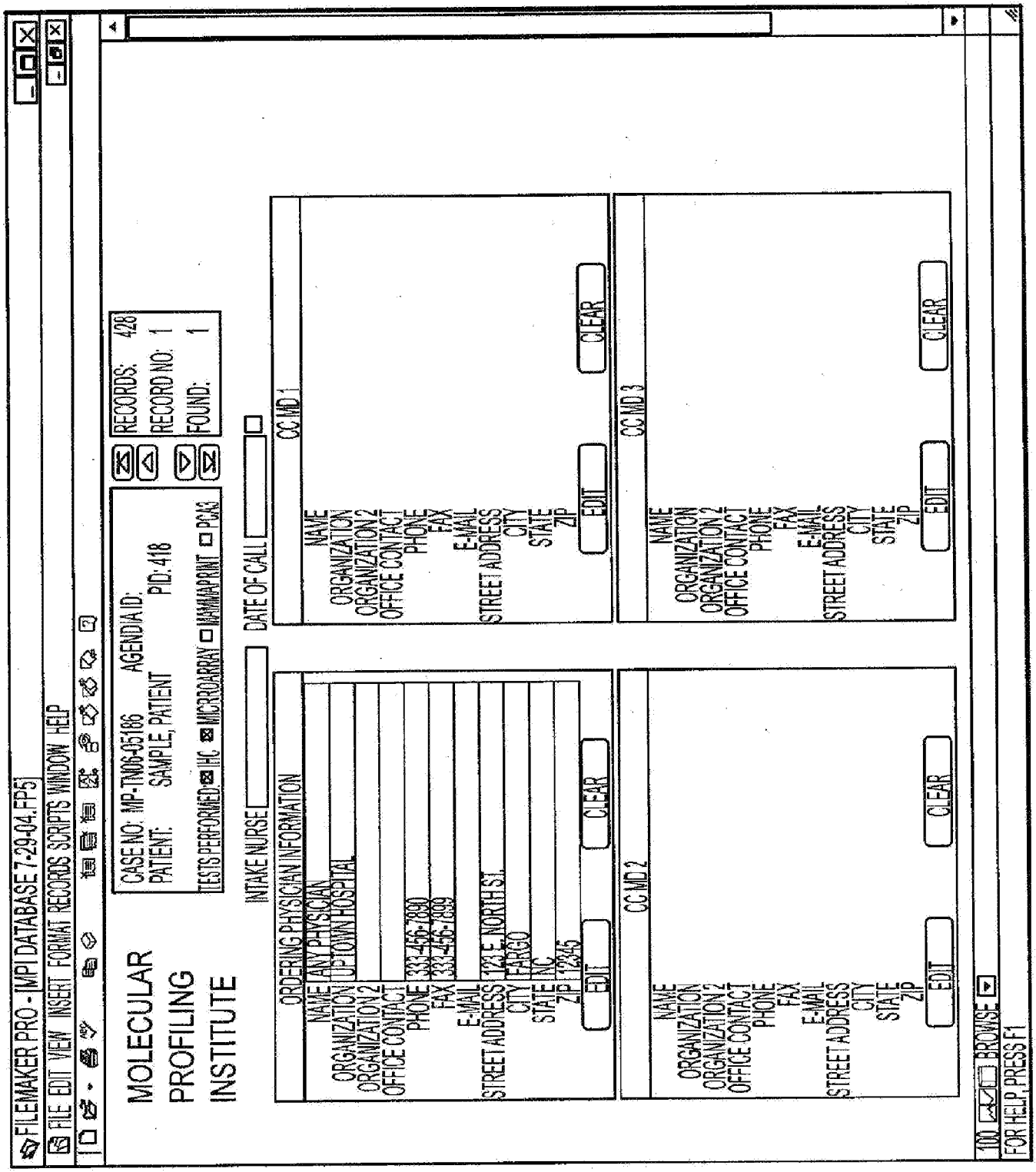

FILEMAKER PRO - IMPI DATABASE 7-29-04.FP5
FILE EDIT VIEW INSERT FORMAT RECORDS SCRIPTS WINDOW HELP
MOLECULAR PROFILING INSTITUTE
CASE NO: IMP-TN06-05186
PATIENT: SAMPLE, PATIENT
AGENDA ID:
PID: 418
TESTS PERFORMED: IHC MICROARRAY MAMMAPRINT PCR
RECORDS: 428
RECORD NO: 1
FOUND: 1
INTAKE NURSE
DATE OF CALL
ORDERING PHYSICIAN INFORMATION
NAME ANY PHYSICIAN
ORGANIZATION UPTOWN HOSPITAL
ORGANIZATION 2
OFFICE CONTACT
PHONE 333-456-7890
FAX 333-456-7899
E-MAIL
STREET ADDRESS 123 E. NORTH ST.
CITY FARGO
STATE ND
ZIP 12345
EDIT
CLEAR
CC MD 1
NAME
ORGANIZATION
ORGANIZATION 2
OFFICE CONTACT
PHONE
FAX
E-MAIL
STREET ADDRESS
CITY
STATE
ZIP
EDIT
CLEAR
CC MD 2
NAME
ORGANIZATION
ORGANIZATION 2
OFFICE CONTACT
PHONE
FAX
E-MAIL
STREET ADDRESS
CITY
STATE
ZIP
EDIT
CLEAR
CC MD 3
NAME
ORGANIZATION
ORGANIZATION 2
OFFICE CONTACT
PHONE
FAX
E-MAIL
STREET ADDRESS
CITY
STATE
ZIP
EDIT
CLEAR
100 BROWSE
FOR HELP, PRESS F1

FIG.15

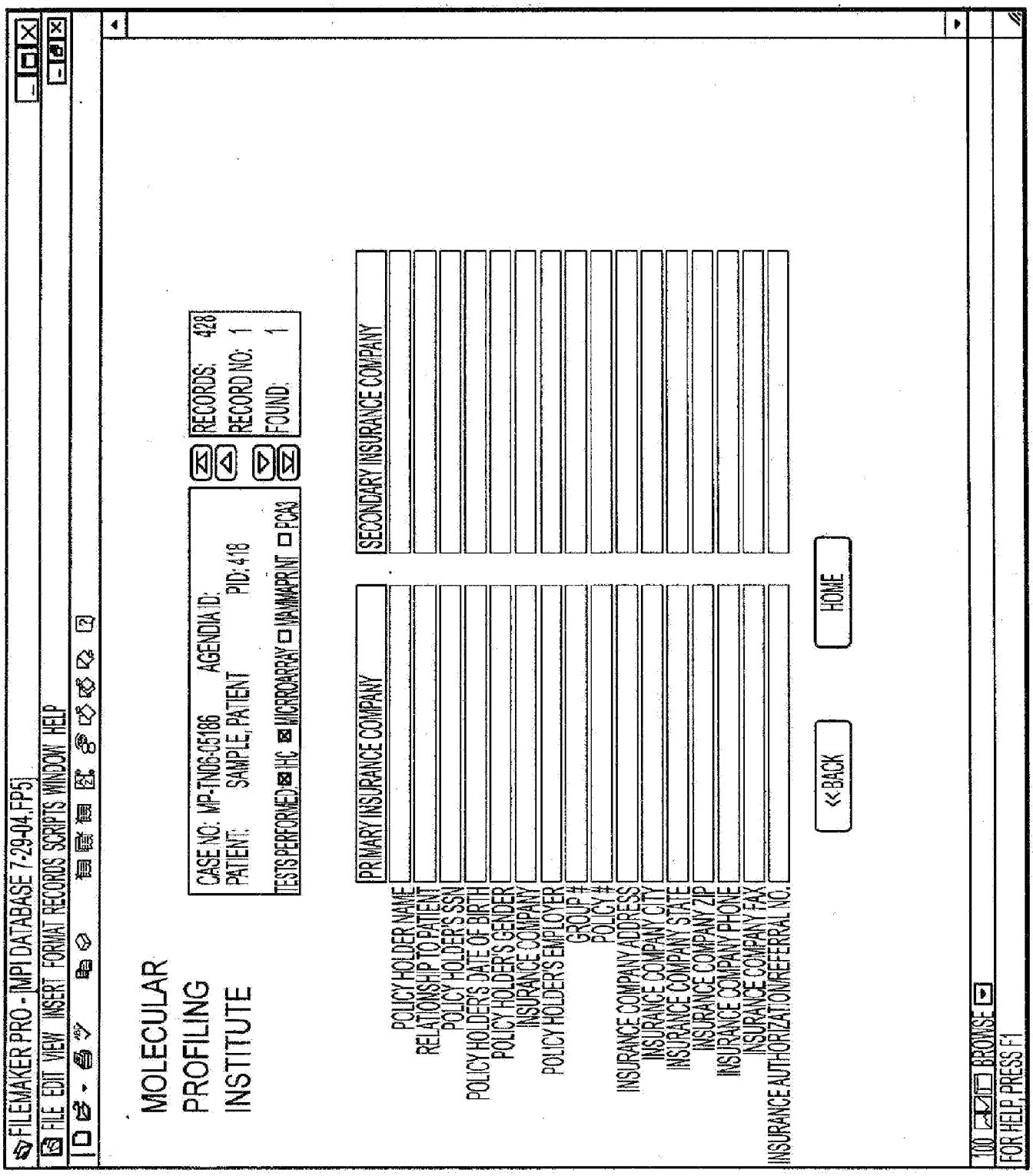

FILEMAKER PRO - IMPI DATABASE 7-29-04.FP5
FILE EDIT VIEW INSERT FORMAT RECORDS SCRIPTS WINDOW HELP
MOLECULAR
PROFILING
INSTITUTE
CASE NO: MP-TN06-05186 AGENDA ID:
PATIENT: SAMPLE, PATIENT PID: 418
TESTS PERFORMED: IHC MICRORARRAY MAMMAPRINT PCR
RECORDS: 428
RECORD NO: 1
FOUND: 1
PRIMARY INSURANCE COMPANY
SECONDARY INSURANCE COMPANY
POLICY HOLDER NAME
RELATIONSHIP TO PATIENT
POLICY HOLDER'S SSN
POLICY HOLDER'S DATE OF BIRTH
POLICY HOLDER'S GENDER
INSURANCE COMPANY
POLICY HOLDER'S EMPLOYER
GROUP #
POLICY #
INSURANCE COMPANY ADDRESS
INSURANCE COMPANY CITY
INSURANCE COMPANY STATE
INSURANCE COMPANY ZIP
INSURANCE COMPANY PHONE
INSURANCE COMPANY FAX
INSURANCE AUTHORIZATION/REFERRAL NO.
<<BACK
HOME
100 BROWSE
FOR HELP, PRESS F1

FIG.16

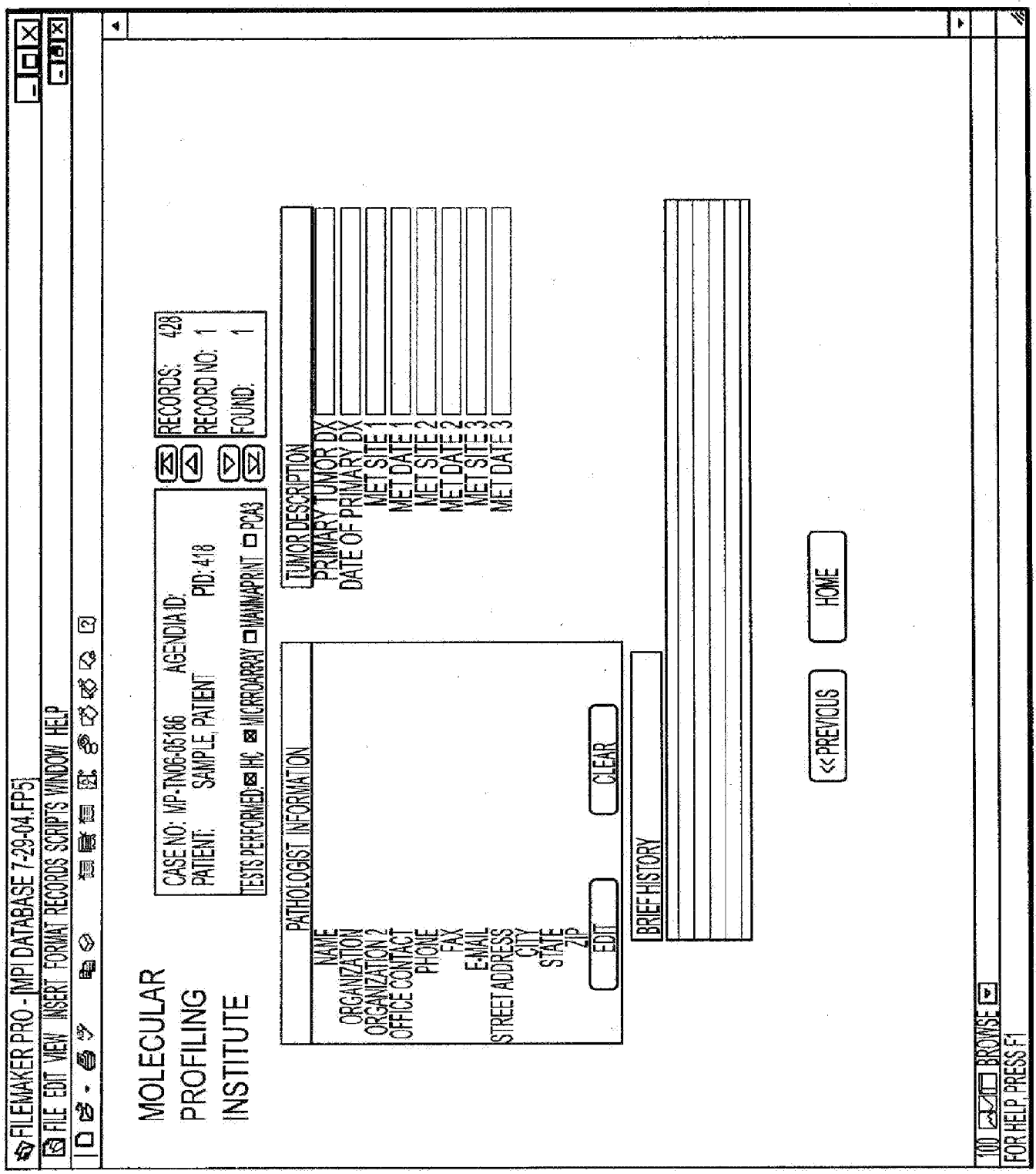

FILEMAKER PRO - [MPI DATABASE 7-29-04.FP5]
FILE EDIT VIEW INSERT FORMAT RECORDS SCRIPTS WINDOW HELP
MOLECULAR PROFILING INSTITUTE
CASE NO: MP-TN06-05186 AGENDA ID:
PATIENT: SAMPLE, PATIENT PID: 418
TESTS PERFORMED: HIC MICROARRAY MAMMAPRINT PCA3
RECORDS: 428
RECORD NO: 1
FOUND: 1
PATHOLOGIST INFORMATION
NAME
ORGANIZATION
ORGANIZATION 2
OFFICE CONTACT
PHONE
FAX
E-MAIL
STREET ADDRESS
CITY
STATE
ZIP
EDIT
CLEAR
TUMOR DESCRIPTION
PRIMARY TUMOR DX
DATE OF PRIMARY DX
MET SITE 1
MET DATE 1
MET SITE 2
MET DATE 2
MET SITE 3
MET DATE 3
BRIEF HISTORY
<<PREVIOUS
HOME
100
BROWSE
FOR HELP, PRESS F1

FIG.17

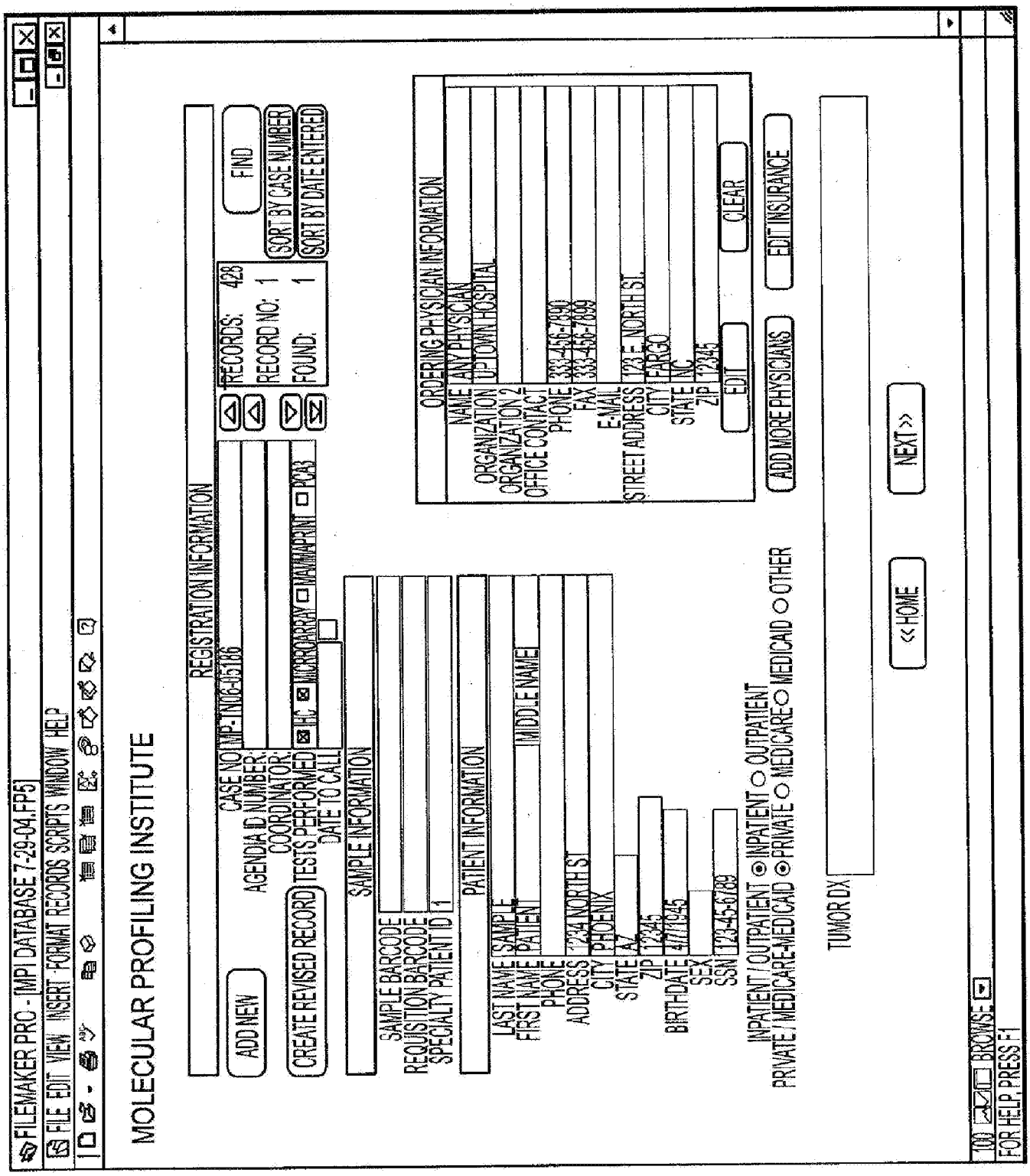

FILEMAKER PRO - IMPI DATABASE 7-29-04.FP5
FILE EDIT VIEW INSERT FORMAT RECORDS SCRIPTS WINDOW HELP
MOLECULAR PROFILING INSTITUTE
REGISTRATION INFORMATION
ADD NEW
CASE NO: IMP-TN06-06186
AGENDIA ID NUMBER:
COORDINATOR:
CREATE REVISED RECORD
TESTS PERFORMED
HC
MICROARRAY
MAM/APRINT
PCR3
DATE TO CALL
RECORDS: 428
RECORD NO: 1
FOUND: 1
FIND
SORT BY CASE NUMBER
SORT BY DATE ENTERED
SAMPLE INFORMATION
SAMPLE BARCODE
REQUISITION BARCODE
SPECIALTY PATIENT ID 1
PATIENT INFORMATION
LAST NAME SAMPLE
FIRST NAME PATIENT
MIDDLE NAME
PHONE
ADDRESS 1234 NORTH ST
CITY PHOENIX
STATE AZ
ZIP 12345
BIRTHDATE 1/1/1945
SEX
SSN 123-45-6789
ORDERING PHYSICIAN INFORMATION
NAME ANY PHYSICIAN
ORGANIZATION UPTOWN HOSPITAL
ORGANIZATION 2
OFFICE CONTACT
PHONE 333-456-7890
FAX 333-456-7899
E-MAIL
STREET ADDRESS 123 E. NORTH ST.
CITY FARGO
STATE NC
ZIP 12345
EDIT
CLEAR
ADD MORE PHYSICIANS
EDIT INSURANCE
INPATIENT / OUTPATIENT ⊙ INPATIENT ○ OUTPATIENT
PRIVATE / MEDICARE-MEDICAID ⊙ PRIVATE ○ MEDICARE ○ MEDICAID ○ OTHER
TUMOR DX
« HOME
NEXT »
100
BROWSE
FOR HELP, PRESS F1

FIG.18

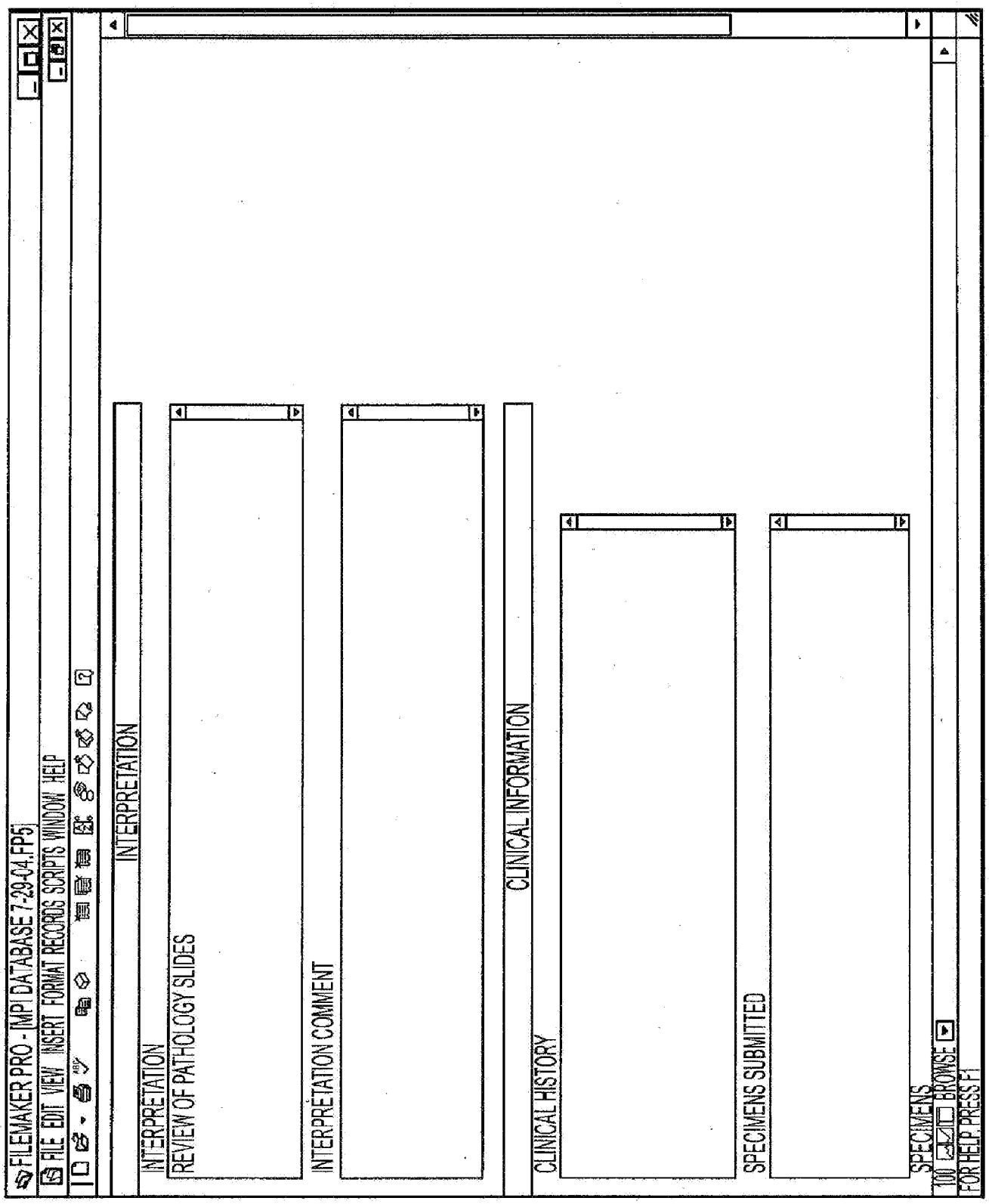
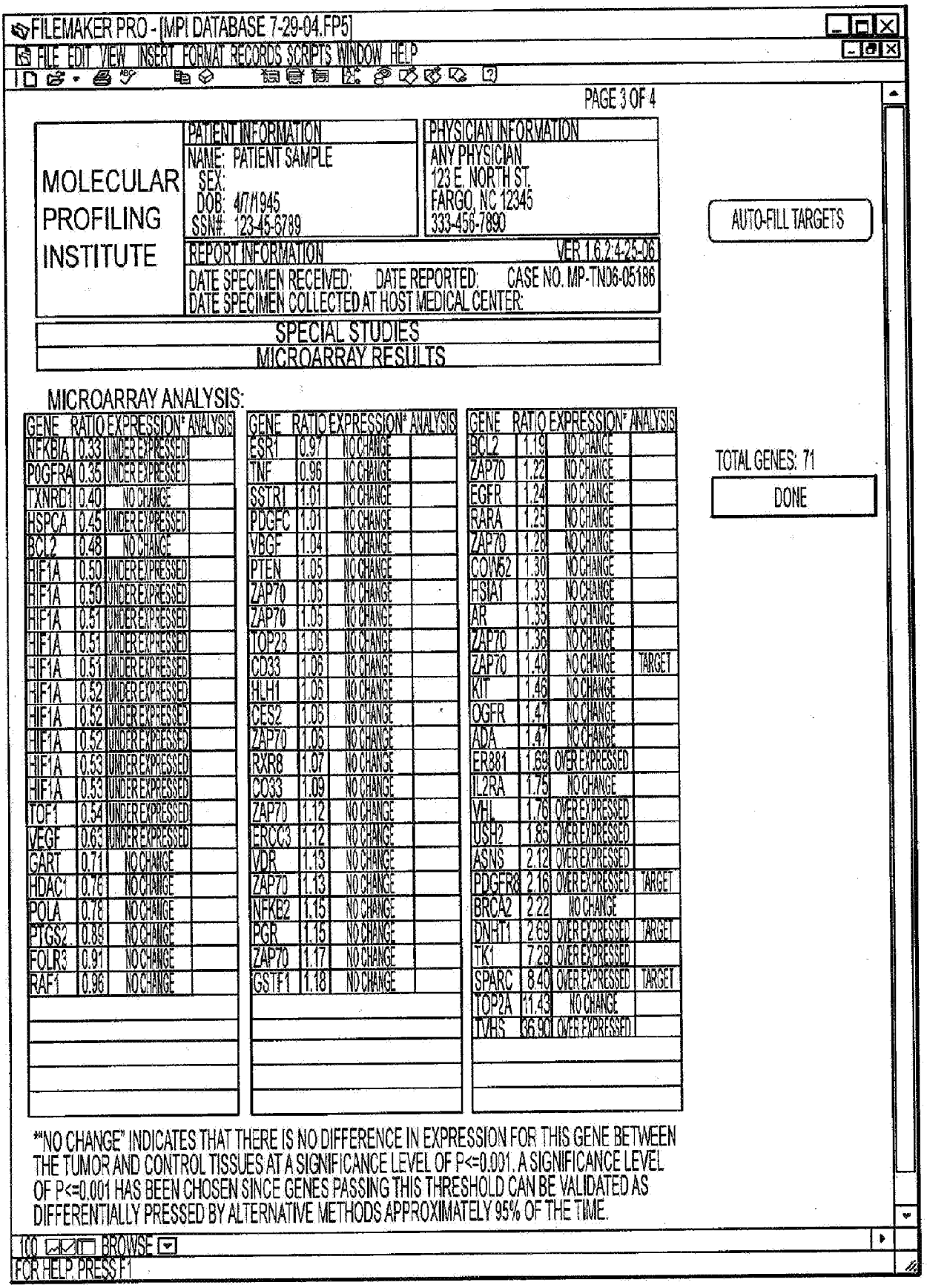

FILEMAKER PRO - IMPI DATABASE 7-29-04.FP5
FILE EDIT VIEW INSERT FORMAT RECORDS SCRIPTS WINDOW HELP
INTERPRETATION
INTERPRETATION
REVIEW OF PATHOLOGY SLIDES
INTERPRETATION COMMENT
CLINICAL INFORMATION
CLINICAL HISTORY
SPECIMENS SUBMITTED
SPECIMENS
100
BROWSE
FOR HELP, PRESS F1

FIG.19



GENE	RATIO	EXPRESSION*	ANALYSIS
NFKBIA	0.33	UNDER EXPRESSED	
PDGFRA	0.35	UNDER EXPRESSED	
TXNRD1	0.40	NO CHANGE	
HSPCA	0.45	UNDER EXPRESSED	
BCL2	0.48	NO CHANGE	
HIF1A	0.50	UNDER EXPRESSED	
HIF1A	0.50	UNDER EXPRESSED	
HIF1A	0.51	UNDER EXPRESSED	
HIF1A	0.51	UNDER EXPRESSED	
HIF1A	0.51	UNDER EXPRESSED	
HIF1A	0.52	UNDER EXPRESSED	
HIF1A	0.52	UNDER EXPRESSED	
HIF1A	0.52	UNDER EXPRESSED	
HIF1A	0.53	UNDER EXPRESSED	
HIF1A	0.53	UNDER EXPRESSED	
TOP1	0.54	UNDER EXPRESSED	
VEGF	0.63	UNDER EXPRESSED	
GART	0.71	NO CHANGE	
HDAC1	0.76	NO CHANGE	
POLA	0.78	NO CHANGE	
PTGS2	0.89	NO CHANGE	
FOLR3	0.91	NO CHANGE	
RAF1	0.96	NO CHANGE	

GENE	RATIO	EXPRESSION*	ANALYSIS
ESR1	0.97	NO CHANGE	
TNF	0.96	NO CHANGE	
SSTR1	1.01	NO CHANGE	
PDGFC	1.01	NO CHANGE	
VBGF	1.04	NO CHANGE	
PTEN	1.05	NO CHANGE	
ZAP70	1.05	NO CHANGE	
ZAP70	1.05	NO CHANGE	
TOP2B	1.05	NO CHANGE	
CD33	1.05	NO CHANGE	
HLH1	1.06	NO CHANGE	
CES2	1.06	NO CHANGE	
ZAP70	1.06	NO CHANGE	
RXR8	1.07	NO CHANGE	
CO33	1.09	NO CHANGE	
ZAP70	1.12	NO CHANGE	
ERCC3	1.12	NO CHANGE	
VDR	1.13	NO CHANGE	
ZAP70	1.13	NO CHANGE	
NFKB2	1.15	NO CHANGE	
PGR	1.15	NO CHANGE	
ZAP70	1.17	NO CHANGE	
GSTF1	1.18	NO CHANGE	

GENE	RATIO	EXPRESSION*	ANALYSIS
BCL2	1.19	NO CHANGE	
ZAP70	1.22	NO CHANGE	
EGFR	1.24	NO CHANGE	
RARA	1.25	NO CHANGE	
ZAP70	1.28	NO CHANGE	
COX52	1.30	NO CHANGE	
HSIAT	1.33	NO CHANGE	
AR	1.35	NO CHANGE	
ZAP70	1.36	NO CHANGE	
ZAP70	1.40	NO CHANGE	TARGET
KIT	1.46	NO CHANGE	
OGFR	1.47	NO CHANGE	
ADA	1.47	NO CHANGE	
ER881	1.69	OVER EXPRESSED	
IL2RA	1.75	NO CHANGE	
VHL	1.76	OVER EXPRESSED	
USH2	1.85	OVER EXPRESSED	
ASNS	2.12	OVER EXPRESSED	
PDGFRB	2.16	OVER EXPRESSED	TARGET
BRCA2	2.22	NO CHANGE	
DNHT1	2.69	OVER EXPRESSED	TARGET
TK1	7.28	OVER EXPRESSED	
SPARC	8.40	OVER EXPRESSED	TARGET
TOP2A	11.43	NO CHANGE	
TVHS	36.90	OVER EXPRESSED	

FIG.20

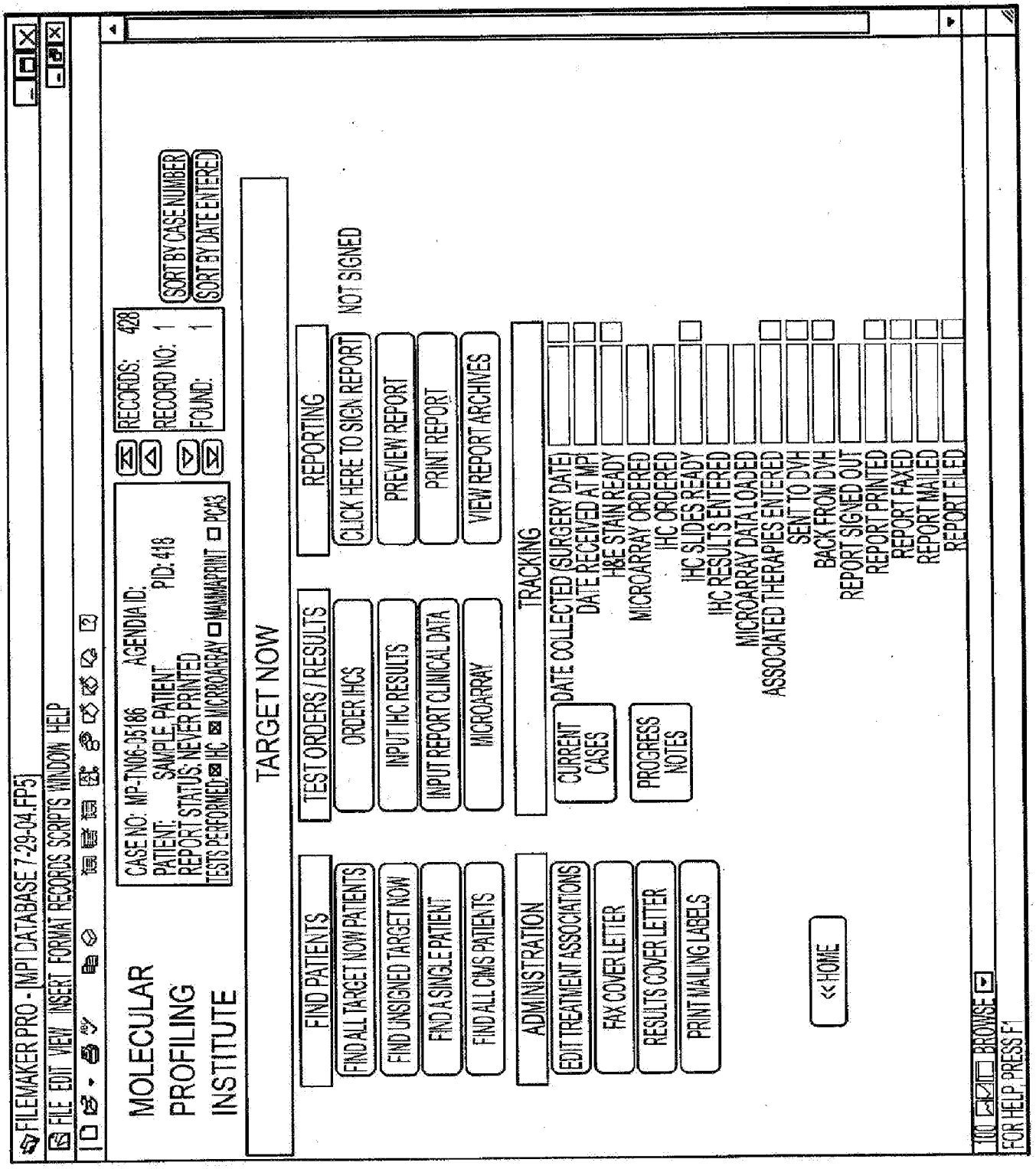

FILEMAKER PRO - [MPI DATABASE 7-29-04.FP5]
FILE EDIT VIEW INSERT FORMAT RECORDS SCRIPTS WINDOW HELP
MOLECULAR PROFILING INSTITUTE
CASE NO: MP-TN06-05186 AGENDIA ID:
PATIENT: SAMPLE, PATIENT PID: 418
REPORT STATUS: NEVER PRINTED
TESTS PERFORMED: IHC MICROARRAY MAMMAPRINT PIK3
RECORDS: 428
RECORD NO: 1
FOUND: 1
SORT BY CASE NUMBER
SORT BY DATE ENTERED
TARGET NOW
FIND PATIENTS
FIND ALL TARGET NOW PATIENTS
FIND UNSIGNED TARGET NOW
FIND A SINGLE PATIENT
FIND ALL CIMS PATIENTS
TEST ORDERS / RESULTS
ORDER IHCS
INPUT IHC RESULTS
INPUT REPORT CLINICAL DATA
MICROARRAY
REPORTING
CLICK HERE TO SIGN REPORT
NOT SIGNED
PREVIEW REPORT
PRINT REPORT
VIEW REPORT ARCHIVES
ADMINISTRATION
EDIT TREATMENT ASSOCIATIONS
FAX COVER LETTER
RESULTS COVER LETTER
PRINT MAILING LABELS
<<HOME
TRACKING
CURRENT CASES
PROGRESS NOTES
DATE COLLECTED (SURGERY DATE)
DATE RECEIVED AT MPI
H&E STAIN READY
MICROARRAY ORDERED
IHC ORDERED
IHC SLIDES READY
IHC RESULTS ENTERED
MICROARRAY DATA LOADED
ASSOCIATED THERAPIES ENTERED
SENT TO DVH
BACK FROM DVH
REPORT SIGNED OUT
REPORT PRINTED
REPORT FAXED
REPORT MAILED
REPORT FILED
100 BROWSE
FOR HELP, PRESS F1

FIG.21

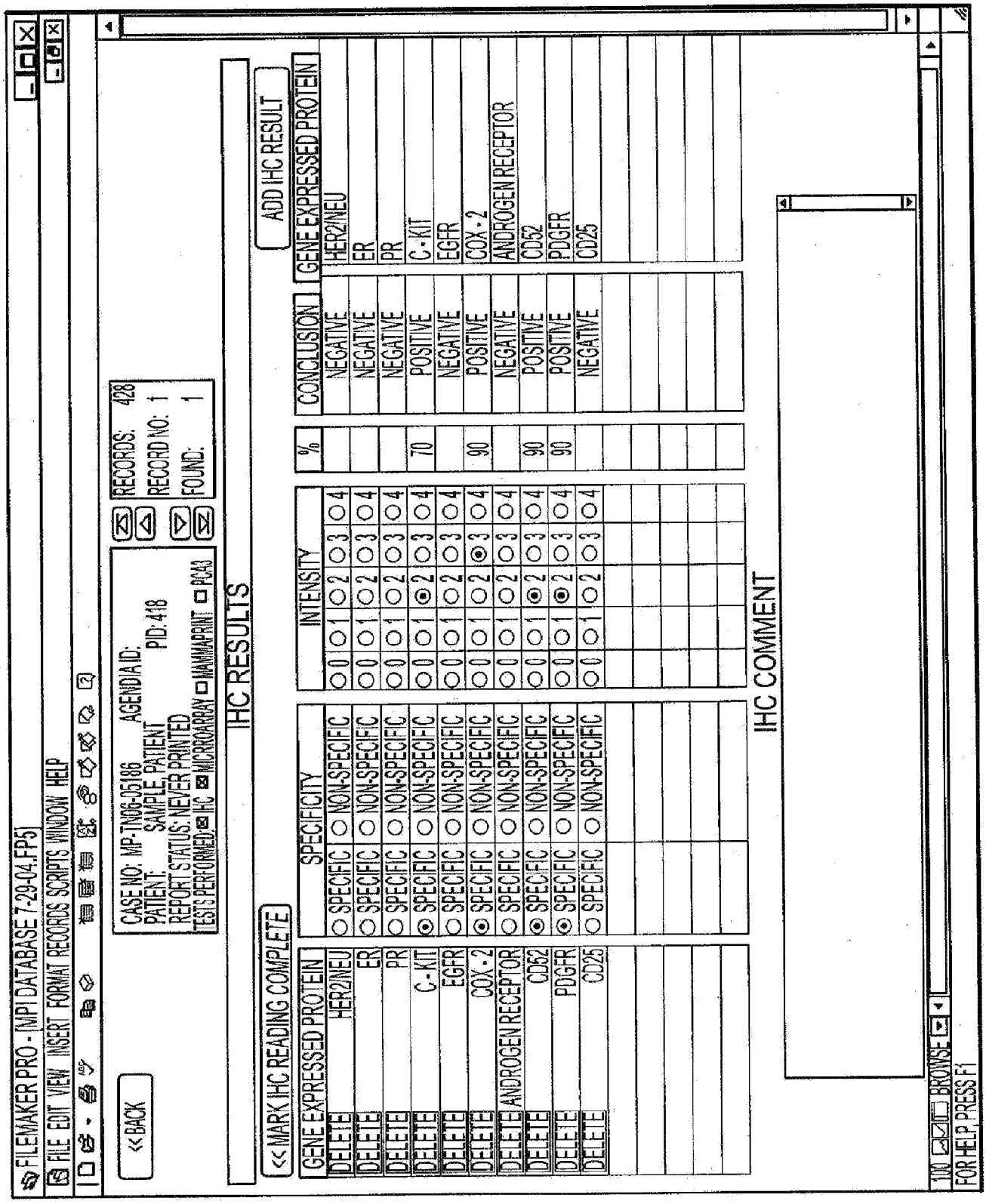

FILEMAKER PRO - [MPI DATABASE 7-29-04.FP5]
FILE EDIT VIEW INSERT FORMAT RECORDS SCRIPTS WINDOW HELP
<<BACK
CASE NO: MP-TNG6-05186 AGENDA ID:
PATIENT: SAMPLE, PATIENT PID: 418
REPORT STATUS: NEVER PRINTED
TESTS PERFORMED: IHC MICROARRAY MAMMAPRINT PCR3
RECORDS: 428
RECORD NO: 1
FOUND: 1
IHC RESULTS
<<MARK IHC READING COMPLETE
ADD IHC RESULT
GENE EXPRESSED PROTEIN
SPECIFICITY
INTENSITY
%
CONCLUSION
GENE EXPRESSED PROTEIN
DELETE HER2/NEU SPECIFIC NON-SPECIFIC 0 1 2 3 4 NEGATIVE HER2/NEU
DELETE ER SPECIFIC NON-SPECIFIC 0 1 2 3 4 NEGATIVE ER
DELETE PR SPECIFIC NON-SPECIFIC 0 1 2 3 4 NEGATIVE PR
DELETE C-KIT SPECIFIC NON-SPECIFIC 0 1 2 3 4 70 POSITIVE C-KIT
DELETE EGFR SPECIFIC NON-SPECIFIC 0 1 2 3 4 NEGATIVE EGFR
DELETE COX-2 SPECIFIC NON-SPECIFIC 0 1 2 3 4 90 POSITIVE COX-2
DELETE ANDROGEN RECEPTOR SPECIFIC NON-SPECIFIC 0 1 2 3 4 NEGATIVE ANDROGEN RECEPTOR
DELETE CD52 SPECIFIC NON-SPECIFIC 0 1 2 3 4 90 POSITIVE CD52
DELETE PDGFR SPECIFIC NON-SPECIFIC 0 1 2 3 4 90 POSITIVE PDGFR
DELETE CD25 SPECIFIC NON-SPECIFIC 0 1 2 3 4 NEGATIVE CD25
IHC COMMENT
100 BROWSE
FOR HELP, PRESS F1

FIG.22

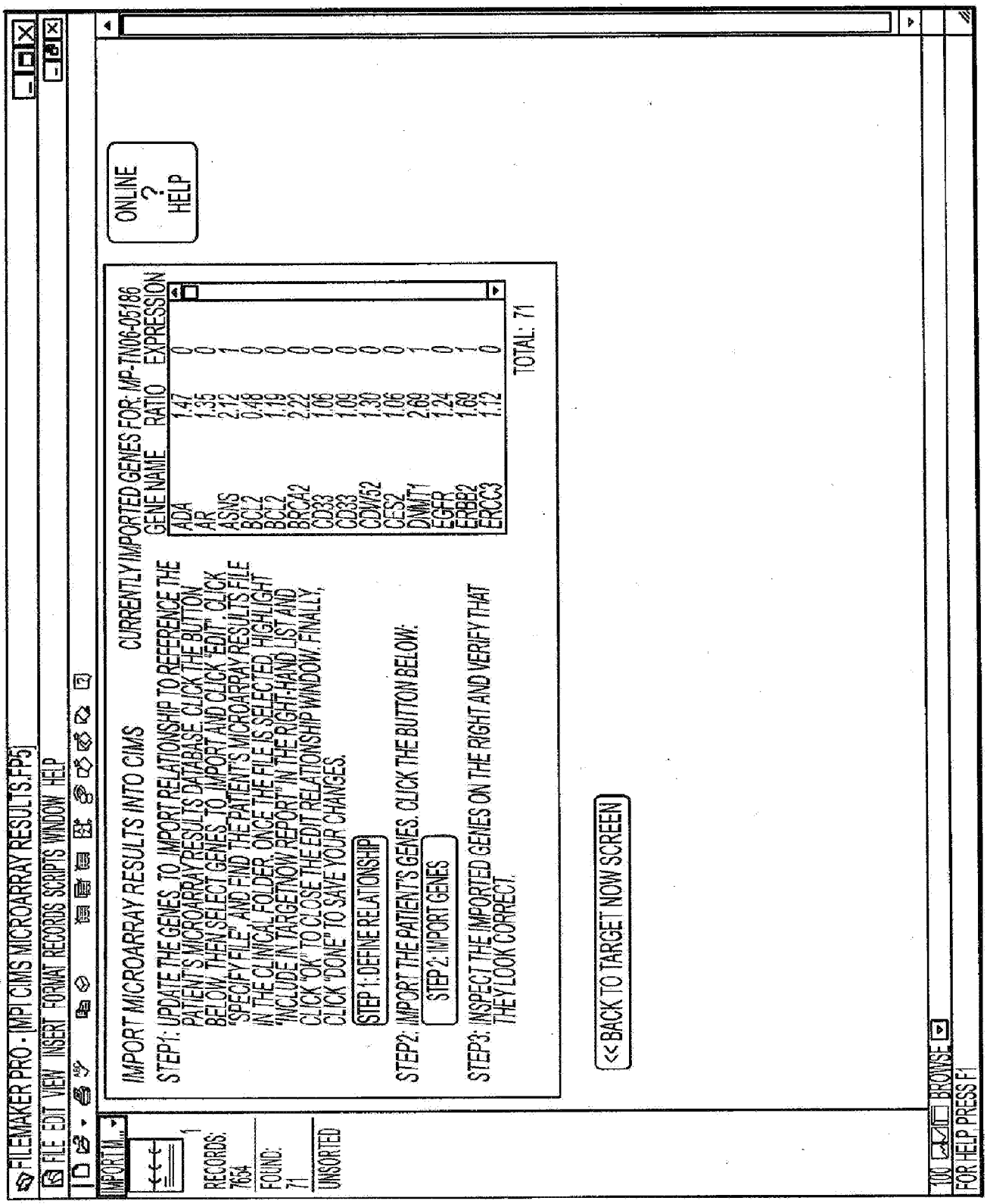

FILEMAKER PRO - [MPI CIMS MICROARRAY RESULTS.FP5]
FILE EDIT VIEW INSERT FORMAT RECORDS SCRIPTS WINDOW HELP
IMPORT M...
RECORDS: 7654
FOUND: 71
UNSORTED
IMPORT MICROARRAY RESULTS INTO CIMS
CURRENTLY IMPORTED GENES FOR: MP-TN06-05186
GENE NAME RATIO EXPRESSION
ADA 1.47 0
AR 1.35 0
ASNS 2.12 1
BCL2 0.48 0
BCL2 1.19 0
BRCA2 2.22 0
CD33 1.06 0
CD33 1.09 0
CDW52 1.30 0
CES2 1.06 0
DIMT1 2.69 1
EGFR 1.24 0
ERBB2 1.69 1
ERCC3 1.12 0
TOTAL: 71
STEP1: UPDATE THE GENES_TO_IMPORT RELATIONSHIP TO REFERENCE THE PATIENT'S MICROARRAY RESULTS DATABASE. CLICK THE BUTTON BELOW, THEN SELECT GENES_TO_IMPORT AND CLICK "EDIT". CLICK "SPECIFY FILE", AND FIND THE PATIENT'S MICROARRAY RESULTS FILE IN THE CLINICAL FOLDER. ONCE THE FILE IS SELECTED, HIGHLIGHT "INCLUDE IN TARGETNOW REPORT" IN THE RIGHT-HAND LIST AND CLICK "OK" TO CLOSE THE EDIT RELATIONSHIP WINDOW. FINALLY, CLICK "DONE" TO SAVE YOUR CHANGES.
STEP 1: DEFINE RELATIONSHIP
STEP2: IMPORT THE PATIENT'S GENES. CLICK THE BUTTON BELOW:
STEP 2: IMPORT GENES
STEP3: INSPECT THE IMPORTED GENES ON THE RIGHT AND VERIFY THAT THEY LOOK CORRECT.
ONLINE ? HELP
<< BACK TO TARGET NOW SCREEN
100 BROWSE
FOR HELP, PRESS F1

FIG.23

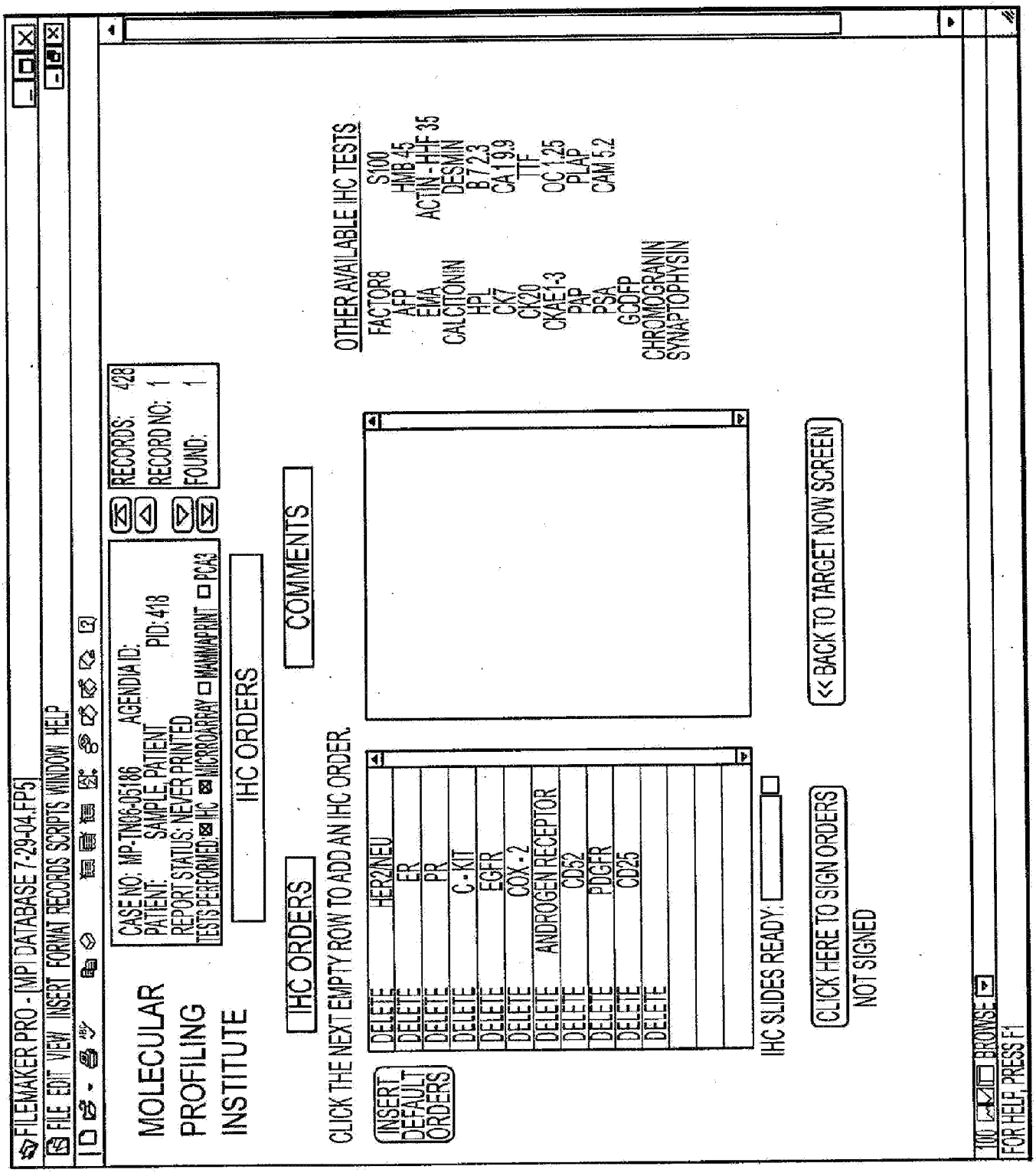

FILEMAKER PRO - IMPI DATABASE 7-29-04.FP5
FILE EDIT VIEW INSERT FORMAT RECORDS SCRIPTS WINDOW HELP
MOLECULAR PROFILING INSTITUTE
CASE NO: MP-TN06-05186 AGENDIA ID:
PATIENT: SAMPLE, PATIENT PID: 418
REPORT STATUS: NEVER PRINTED
TESTS PERFORMED: IHC MICROARRAY MAMMAPRINT PDA3
RECORDS: 428
RECORD NO: 1
FOUND: 1
IHC ORDERS
COMMENTS
CLICK THE NEXT EMPTY ROW TO ADD AN IHC ORDER.
INSERT DEFAULT ORDERS
DELETE HER2/NEU
DELETE ER
DELETE PR
DELETE C-KIT
DELETE EGFR
DELETE COX-2
DELETE ANDROGEN RECEPTOR
DELETE CD52
DELETE PDGFR
DELETE CD25
DELETE
IHC SLIDES READY:
CLICK HERE TO SIGN ORDERS
NOT SIGNED
OTHER AVAILABLE IHC TESTS
FACTOR8
AFP
EMA
CALCITONIN
HPL
CK7
CK20
CKAE1-3
PAP
PSA
GCDFP
CHROMOGRANIN
SYNAPTOPHYSIN
S100
HMB 45
ACTIN - HHF 35
DESMIN
B72.3
CA19.9
TTF
CC1.25
PLAP
CAM 5.2
<< BACK TO TARGET NOW SCREEN
100 BROWSE
FOR HELP, PRESS F1

FIG.24

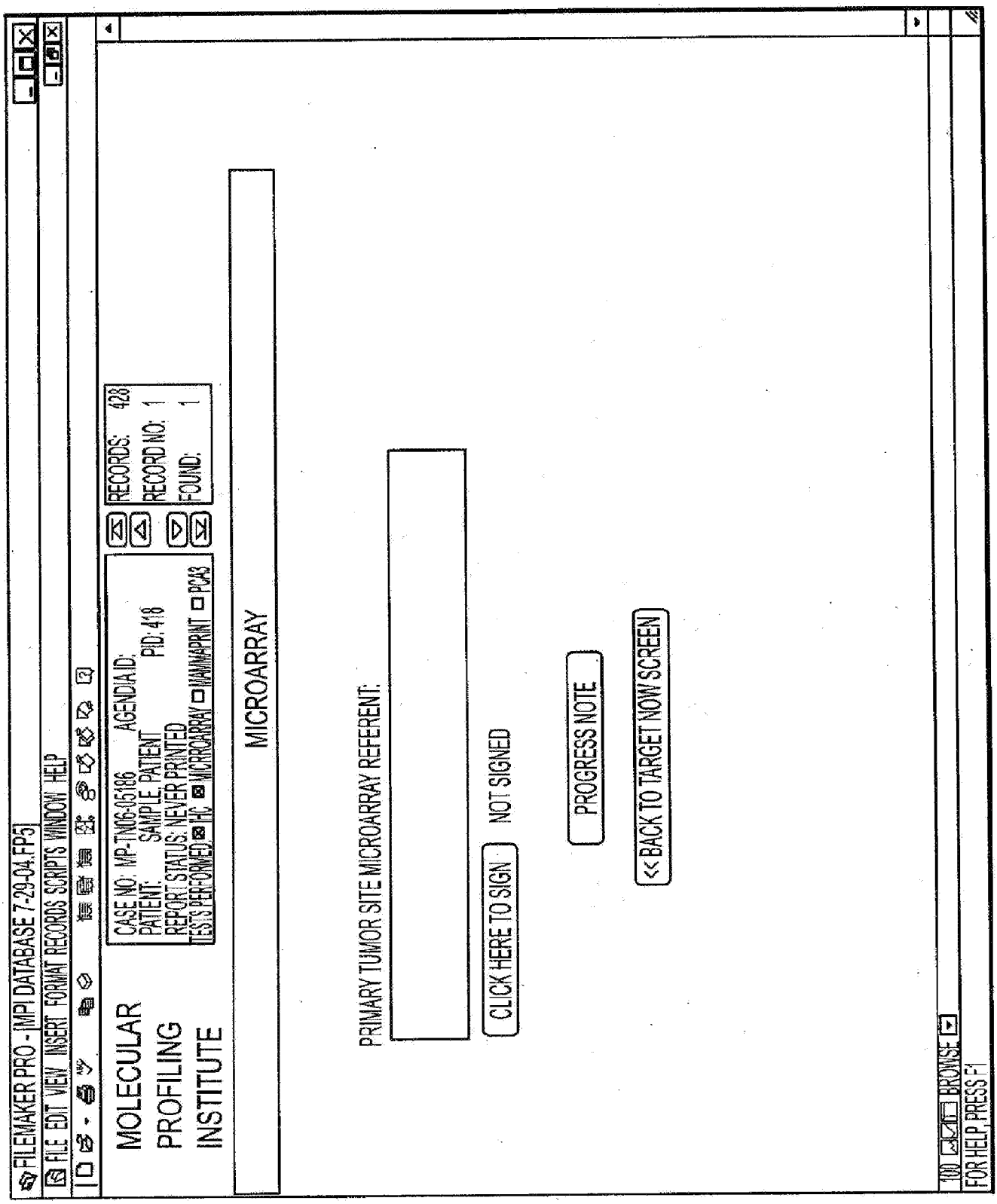

FILEMAKER PRO - [MPI DATABASE 7-29-04.FP5]
FILE EDIT VIEW INSERT FORMAT RECORDS SCRIPTS WINDOW HELP
MOLECULAR PROFILING INSTITUTE
CASE NO: MP-TN06-05186 AGENDA ID:
PATIENT: SAMPLE, PATIENT PID: 418
REPORT STATUS: NEVER PRINTED
TESTS PERFORMED: HC MICROARRAY MAMMAPRINT PQA3
RECORDS: 428
RECORD NO: 1
FOUND: 1
MICROARRAY
PRIMARY TUMOR SITE MICROARRAY REFERENT:
CLICK HERE TO SIGN
NOT SIGNED
PROGRESS NOTE
<< BACK TO TARGET NOW SCREEN
100
BROWSE
FOR HELP, PRESS F1

FIG.25

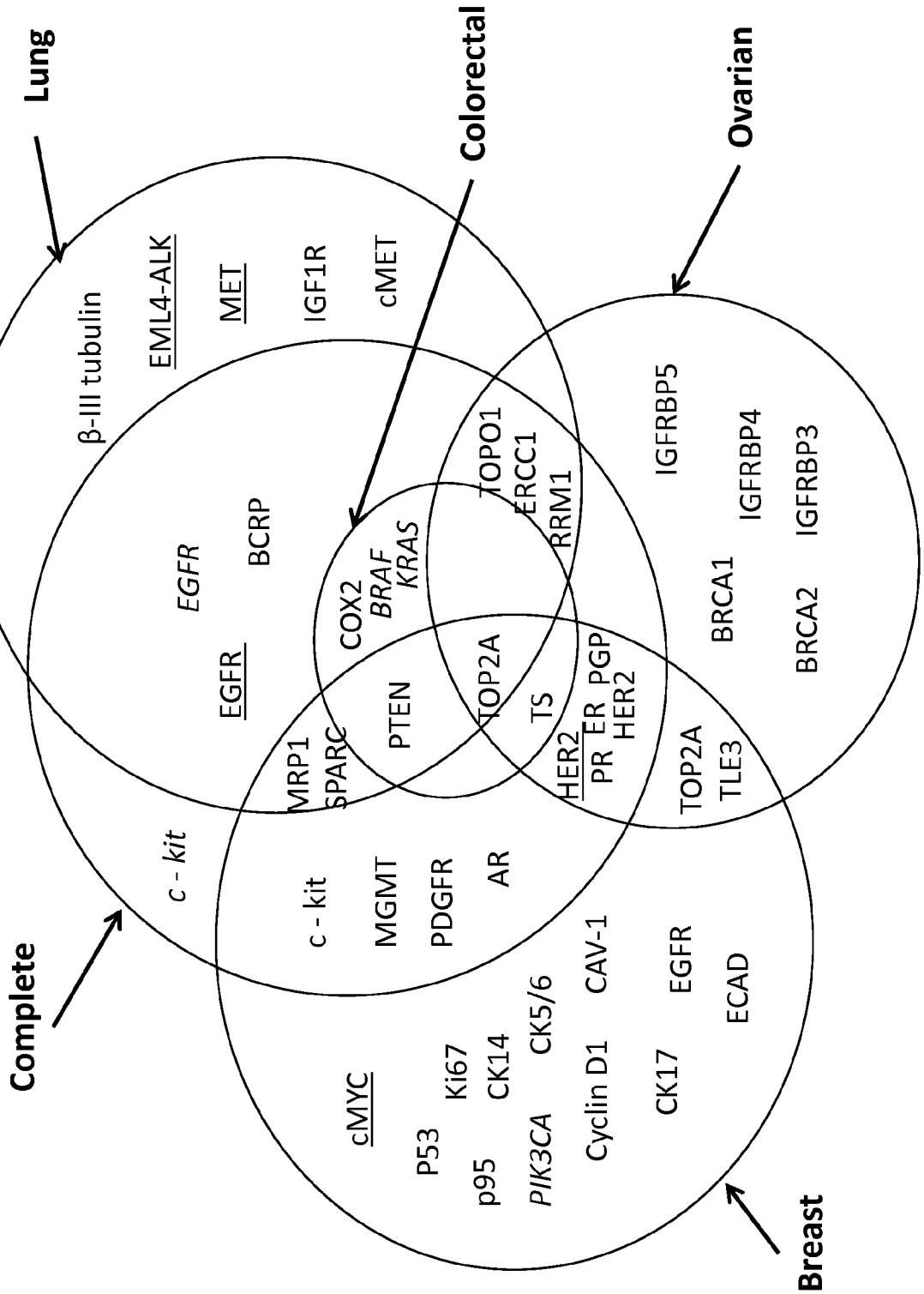

Lung
Colorectal
Ovarian
Complete
Breast
β-III tubulin
EML4-ALK
MET
IGF1R
cMET
EGFR
BCRP
COX2
BRAF
KRAS
TOPO1
ERCC1
RRM1
IGFRBP5
IGFRBP4
IGFRBP3
BRCA1
BRCA2
MRP1
SPARC
PTEN
TOP2A
TS
HER2
PR
ER
PGP
HER2
TOP2A
TLE3
c - kit
MGMT
PDGFR
AR
cMYC
P53
Ki67
p95
CK14
PIK3CA
CK5/6
Cyclin D1
CAV-1
CK17
EGFR
ECAD

FIG. 26A

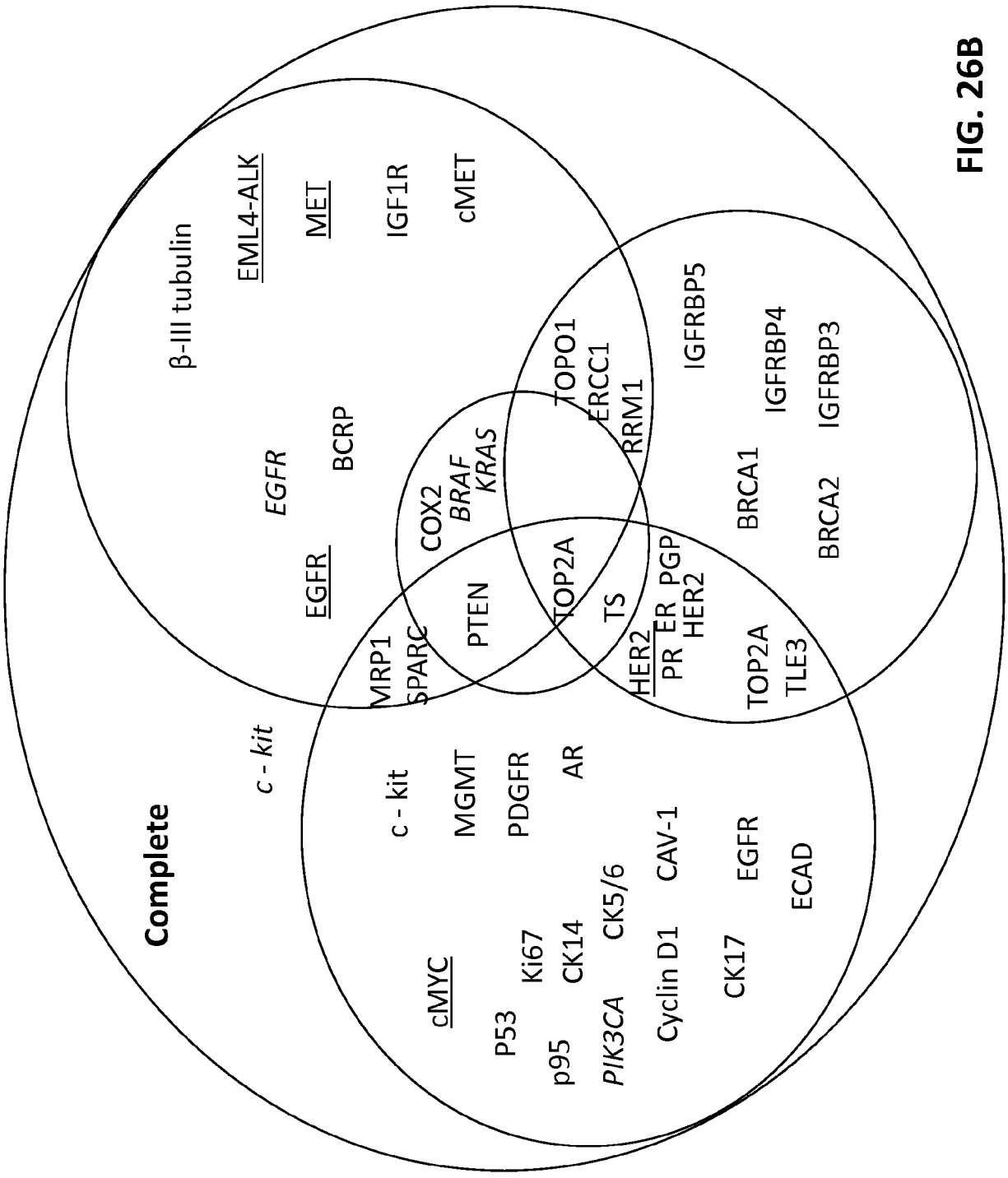

Complete
c - kit
β-III tubulin
EML4-ALK
MET
IGF1R
cMET
EGFR
BCRP
EGFR
COX2
BRAF
KRAS
TOPO1
ERCC1
RRM1
IGFRBP5
IGFRBP4
IGFRBP3
MRP1
SPARC
PTEN
TOP2A
TS
HER2
PR
ER
PGP
HER2
BRCA1
BRCA2
TOP2A
TLE3
c - kit
MGMT
PDGFR
AR
cMYC
Ki67
CK14
CK5/6
P53
p95
PIK3CA
Cyclin D1
CAV-1
CK17
EGFR
ECAD

FIG. 26B

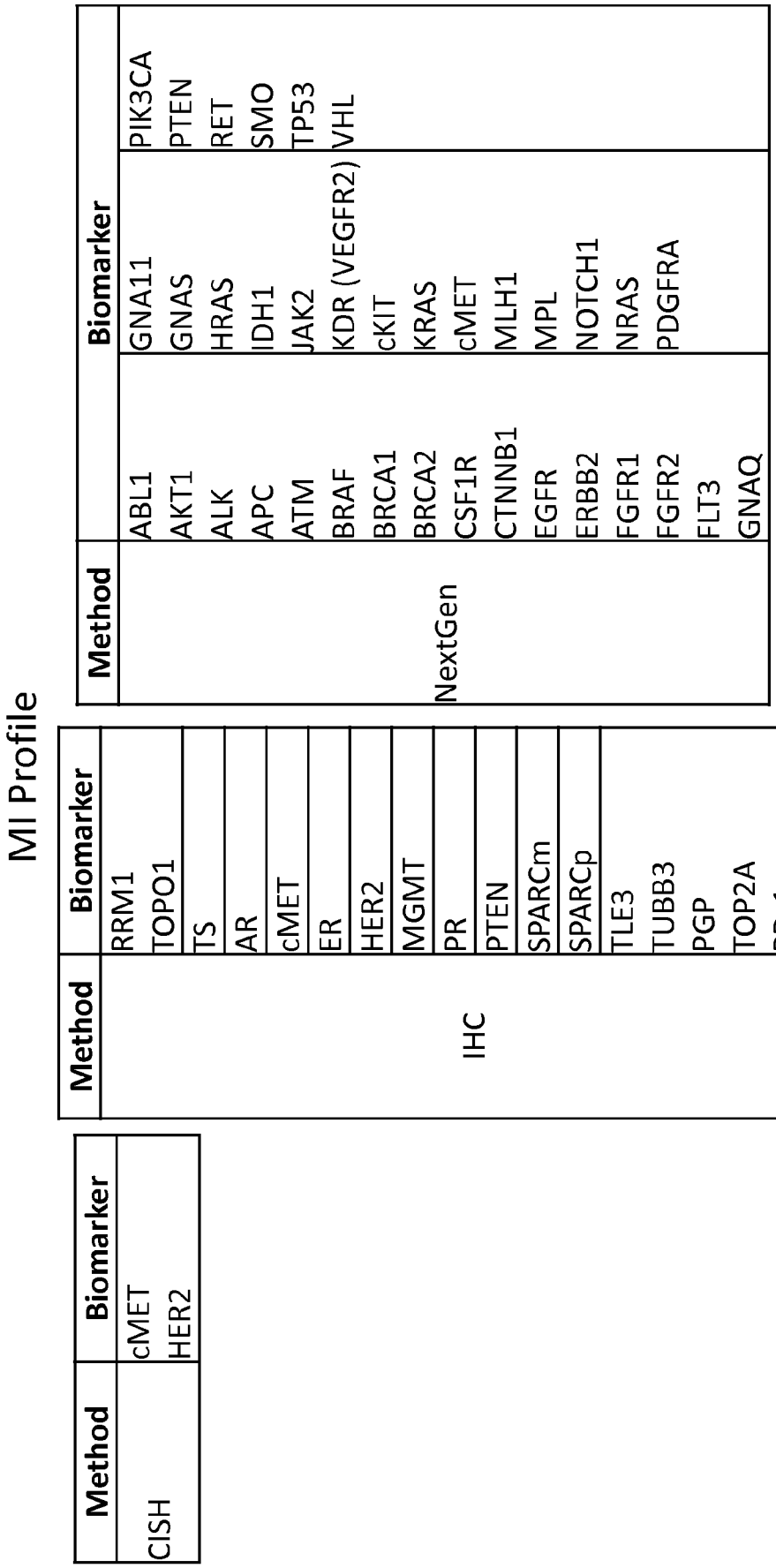

MI Profile
Method
Biomarker
CISH
cMET
HER2
Method
Biomarker
IHC
RRM1
TOPO1
TS
AR
cMET
ER
HER2
MGMT
PR
PTEN
SPARCm
SPARCp
TLE3
TUBB3
PGP
TOP2A
PD-1
PD-L1
Method
Biomarker
NextGen
ABL1
AKT1
ALK
APC
ATM
BRAF
BRCA1
BRCA2
CSF1R
CTNNB1
EGFR
ERBB2
FGFR1
FGFR2
FLT3
GNAQ
GNA11
GNAS
HRAS
IDH1
JAK2
KDR (VEGFR2)
cKIT
KRAS
cMET
MLH1
MPL
NOTCH1
NRAS
PDGFRA
PIK3CA
PTEN
RET
SMO
TP53
VHL

FIG. 27A

MI Profile PLUS

Method	Biomarker
CISH	cMET
	HER2

Method	Biomarker
IHC	RRM1
	TOPO1
	TS
	AR
	cMET
	ER
	HER2
	MGMT
	PR
	PTEN
	SPARCm
	SPARCp
	TLE3
	TUBB3
	PGP
	TOP2A

Method	Biomarker		
NextGen	ABL1	FLT3	NPM1
	AKT1	GNAQ	NRAS
	ALK	GNA11	PDGFRA
	APC	GNAS	PIK3CA
	ATM	HNF1A	PTEN
	BRAF	HRAS	PTPN11
	BRCA1	IDH1	RB1
	BRCA2	JAK2	RET
	CDH1	JAK3	SMAD4
	CSF1R	KDR (VEGFR2)	SMARCB1
	CTNNB1	cKIT	SMO
	EGFR	KRAS	STK11
	ERBB2	cMET	TP53
	ERBB4	MLH1	VHL
	FBXW7	MPL	
	FGFR1	NOTCH1	
	FGFR2		

FIG. 27B

MI Profile™

IHC	FISH / CISH	Mutational Analysis	
		Next-Generation Sequencing	Other
AR, cMET, EGFR *(H-score; NSCLC only)*, EGFR *(excluding NSCLC)*, ER, ERCC1 *(ovarian only)*, HER2, MGMT *(excluding glioma)*, MLH1 *(CRC only)*, MSH2 *(CRC only)*, MSH6 *(CRC only)*, PD-1, PD-L1, Pgp, PMS2 *(CRC only)*, PR, PTEN, RRM1, SPARCm, SPARCp, TLE3, TOP2A *(excluding breast)*, TOPO1, TS, TUBB3	1p19q# *(glioma only)*; cMET#, HER2#, TOP2A# *(breast only)*, ALK# *(NSCLC only)*, ROS1# *(NSCLC only)*	*See MI Profile™ Next-Generation Sequencing Genes below.*	*EGFRvIII (Frag. Analysis; glioma only)*, IDH2 *(SangerSeq; glioma only)*, MGMT Methylation Analysis *(PyroSeq; glioma only)* MSI *(Frag. Analysis; CRC only)*

FIG. 28A

Next-Generation Sequencing Cancer Service											
Mutations, Amplifications, Deletions, Translocations, Fusions								Additional Non-Cancerous Genetic Variants, Carrier Status, Ancestry			
ABL1	ESCAT*	EGFR	GNA11	JAK2	MPL	PTEN		CDH1	MPA1	STK11	
AKT1	BRCA2*	ERBB2 (HER2)	GNAQ	KDR (VEGFR2)	NOTCH1	RET		ERBB4	PTPN11		
ALK	BRAF	FGFR1	GNAS	KIT	NRAS	SMO		FBXW7	RB1		
APC	CSF1R	FGFR2	HRAS	KRAS	PDGFRA	TP53		FNFTA	SMAD4		
ATM	CTNNB1	FLT3	IDH1	MET	PIK3CA	VHL		JAK3	SMARCB1		

FIG. 28B

Formalin Fixed Paraffin Embedded (FFPE) Samples

Sufficient tumor must be present to complete all analysis. If you have any questions, please contact Client Services at (888) 979-8669.

SPECIMEN TYPE	SPECIMEN REQUIREMENTS
Fixed Tissue	One (1) tumor-containing formalin fixed paraffin embedded block (FFPE) from most recent surgery or biopsy. Successive four (4) micron sections will be created from the block until sufficient material for the testing orders is obtained. For the molecular analysis, tumor cells will be excised by microdissection until a total area of at least 50mm² is obtained.
Core Needle Biopsy	Four to six (4-6) biopsies formalin fixed paraffin embedded • 18 gauge needle preferred
Fine Needle Aspirate (FNA)	One (1) formalin fixed paraffin embedded block containing sufficient tumor
Unstained Slides	Unstained, positively charged, unbaked slides from one single, tumor-containing formalin fixed paraffin embedded block; 4 micron sections • MI Profile™ - 55 slides • Next-Generation Sequencing only - 15 slides Note: At least a 5mm x 5mm section of tissue per slide is required. For small biopsies (tissue area < 5 mm x 5 mm) please cut two sections per slide for at least one half of the slides to ensure sufficient material for molecular assays.
Malignant Fluid	One (1) formalin fixed paraffin embedded cell block containing sufficient tumor.

FIG. 28C

Agent	Biomarker	Platform
docetaxel, paclitaxel	Pgp	IHC
	TLE3 (excluding Ovarian)	IHC
	TUBB3 (excluding Breast)	IHC
nab-paclitaxel	SPARCm	IHC
	SPARCp	IHC
capecitabine, fluorouracil, pemetrexed	TS	IHC
doxorubicin, liposomal-doxorubicin, epirubicin	HER2	FISH/CISH
	TOP2A	IHC (excluding Breast)
		FISH/CISH (Breast only)
	Pgp	IHC
carboplatin, cisplatin, oxaliplatin	BRCA1	NGS
	BRCA2	NGS
	ERCC1 (assoc. in Ovarian only)	IHC
irinotecan topotecan (excluding Breast, CRC, Melanoma, Uveal Melanoma, NSCLC)	TOPO1	IHC
gemcitabine	RRM1	IHC
imatinib	cKIT	NextGen Seq
	PDGFRA	NextGen Seq
temozolomide, dacarbazine	MGMT (excluding Glioma)	IHC (excluding Glioma)
	MGMT-Methylation (Glioma only)	Pyrosequencing (Glioma only)
	IDH1 (assoc. in High Grade Glioma only)	NextGen Seq
procarbazine, lomustine, vincristine	1p19q (Glioma only)	FISH/CISH (Glioma only)
vandetanib	RET	NextGen Seq
abiraterone, bicalutamide	AR	IHC
hormone therapies[1]	AR	IHC
	ER	IHC
	PR	IHC
trastuzumab	HER2	IHC; FISH/CISH
	PTEN (assoc. in Breast only)	IHC
	PIK3CA (assoc. in Breast only)	NextGen Seq
lapatinib, pertuzumab, T-DM1	HER2	IHC; FISH/CISH
everolimus, temsirolimus (assoc. excludes CRC)	ER (assoc. in Breast only)	IHC
	PIK3CA	NextGen Seq

[1] *Hormone therapies may include: tamoxifen, toremifene, fulvestrant, letrozole, anastrozole, exemestane, megestrol acetate, leuprolide, goserelin, bicalutamide, flutamide, abiraterone, enzalutamide, triptorelin, abarelix, degarelix.*

FIG. 28D

Agent	Biomarker	Platform
cetuximab, panitumumab (assoc. in CRC only)	BRAF	NextGen Seq
	KRAS	NextGen Seq
	NRAS	NextGen Seq
	PIK3CA	NextGen Seq
	PTEN	IHC
cetuximab (assoc. in NSCLC only)	EGFR (NSCLC only)	IHC (H-score) (NSCLC only)
erlotinib, gefitinib (assoc. in NSCLC only)	EGFR (NSCLC only)	NextGen Seq (NSCLC only)
	KRAS	NextGen Seq
	PIK3CA	NextGen Seq
	cMET	FISH/CISH
	PTEN	IHC
crizotinib	ALK (assoc. in NSCLC only)	FISH (NSCLC only)
	ROS1 (assoc. in NSCLC only)	
ceritinib	ALK	FISH
afatinib (assoc. in NSCLC only)	EGFR	NextGen Seq
sunitinib (assoc. in GIST only)	cKIT	NextGen Seq
dabrafenib, vemurafenib	BRAF	NextGen Seq
dabrafenib, vemurafenib, trametinib (assoc. in Melanoma only)	BRAF	NextGen Seq
clinical trials	EGFRvIII (assoc. in Glioma only)	Fragment Analysis (Glioma only)
clinical trials	IDH2 (assoc. in Glioma only)	Sanger Seq (Glioma only)
clinical trials	EGFR, PD-1, PD-L1, PTEN	IHC
clinical trials	cMET	IHC; FISH/CISH; NGS
clinical trials (CRC only)	MLH1, MSH2, MSH6, PMS2	IHC
	MSI	Fragment Analysis
clinical trials	BRAF	NGS
clinical trials	ABL1, AKT1, ALK, APC, ATM, CSF1R, CTNNB1, EGFR, ERBB2 (HER2), FGFR1, FGFR2, FLT3, GNA11, GNAQ, GNAS, HRAS, IDH1, JAK2, KDR (VEGFR2), KRAS, MPL, NOTCH1, NRAS, PTEN, SMO, TP53, VHL	NGS

** Biomarkers listed above may also be assessed for clinical trial opportunities and may include clinical trials associations.*

FIG. 28E

PATIENT	SPECIMEN INFORMATION	ORDERED BY
Test Patient Date Of Birth: XX/XX/1947 Sex: Male Case Number: TN14-111111	Primary Tumor Site: Pancreas, NOS Specimen Site: Lower lobe, lung Specimen Collected: XX/XX/2014 Specimen Received: XX/XX/2014 Initiation of Testing: XX/XX/2014 Completion of Testing: XX/XX/2014	Ordering Physician, MD Springfield Medical Center 123 Main Street Springfield, XY 12345 1 (234) 567-8910

Clinical History: Per the submitted documents, the patient is a 67 year-old male with metastatic pancreatic adenocarcinoma.
Pathologic Diagnosis: Lung, left upper lobe, wedge biopsy: Moderately differentiated adenocarcinoma, consistent with metastatic pancreatic adenocarcinoma.

Caris Molecular Intelligence™ – Final Report

Agents Associated with Potential BENEFIT

On NCCN Compendium™

capecitabine, fluorouracil

docetaxel

gemcitabine

Off NCCN Compendium™

pemetrexed

paclitaxel

Current Agents in CLINICAL TRIALS Associated by Biomarker Results

Chemotherapies (3)

Targeted Therapies (8)

For a detailed list of clinical trial opportunities, please see the Clinical Trials Connector™ results page or visit MI Portal.

Agents Associated With Potential LACK OF BENEFIT

abarelix, degarelix, goserelin, leuprolide, triptorelin

abiraterone, bicalutamide, enzalutamide, flutamide

ado-trastuzumab emtansine (T-DM1), pertuzumab, trastuzumab

anastrozole, exemestane, fulvestrant, letrozole, megestrol acetate, tamoxifen, toremifene

dabrafenib, vemurafenib

dacarbazine, temozolomide

doxorubicin, epirubicin, liposomal doxorubicin

irinotecan, topotecan

lapatinib

Agents With Indeterminate Benefit (Biomarker Results Do Not Impact Potential Benefit or Lack of Potential Benefit)

carboplatin	cisplatin	everolimus	imatinib
nab-paclitaxel	oxaliplatin	temsirolimus	vandetanib

SAMPLE REPORT. ILLUSTRATIVE PURPOSES ONLY. NOT FOR CLINICAL USE.

Agents associated with potential benefit or lack of benefit, as indicated above, are based on biomarker results provided in this report and are based on published medical evidence. This evidence may have been obtained from studies performed in the cancer type present in the tested patient's sample or derived from another tumor type. The selection of any, all, or none of the matched agents resides solely with the discretion of the treating physician. Decisions on patient care and treatment must be based on the independent medical judgment of the treating physician, taking into consideration all available information in addition to this report concerning the patient's condition in accordance with the applicable standard of care.

FIG. 29A

SUMMARY OF BIOMARKER RESULTS (see appendix for full results)

Biomarkers With Notable Results

Biomarker	Method	Result
EGFR	IHC	Positive
KRAS	NGS	Mutated (G12R
PD-1 IHC	IHC	Positive
PGP	IHC	Positive
PTEN	IHC	Negative

Biomarker	Method	Result
RRM1	IHC	Negative
TP53	NGS	Mutated (P278S
TS	IHC	Negative
TUBB3	IHC	Negative

Biomarkers Without Notable Results

Biomarker	Method	Result
ABL1	NGS	Wild Type
AKT1	NGS	Wild Type
ALK	NGS	Wild Type
Androgen Receptor	IHC	Negative
APC	NGS	Wild Type
ATM	NGS	Wild Type
BRAF	NGS	Wild Type
BRCA1	NGS	Wild Type
BRCA2	NGS	Wild Type
c-KIT	NGS	Wild Type
cMET	IHC	Negative
cMET	CISH	Not Amplified
cMET	NGS	Wild Type
CSF1R	NGS	Wild Type
CTNNB1	NGS	Wild Type
EGFR	NGS	Wild Type
ER	IHC	Negative
FGFR1	NGS	Wild Type
FGFR2	NGS	Wild Type
FLT3	NGS	Wild Type
GNA11	NGS	Wild Type
GNAQ	NGS	Wild Type
GNAS	NGS	Wild Type
Her2/Neu	IHC	Equivocal

Biomarker	Method	Result
Her2/Neu	CISH	Not Amplified
Her2/Neu (ERBB2)	NGS	Wild Type
HRAS	NGS	Wild Type
IDH1	NGS	Wild Type
JAK2	NGS	Wild Type
KDR (VEGFR2)	NGS	Wild Type
MGMT	IHC	Positive
MPL	NGS	Wild Type
NOTCH1	NGS	Wild Type
NRAS	NGS	Wild Type
PDGFRA	NGS	Wild Type
PD-L1 IHC	IHC	Negative
PIK3CA	NGS	Wild Type
PR	IHC	Negative
PTEN	NGS	Wild Type
RET	NGS	Wild Type
SMO	NGS	Wild Type
SPARC Monoclonal	IHC	Negative
SPARC Polyclonal	IHC	Negative
TLE3	IHC	Negative
TOP2A	IHC	Negative
TOPO1	IHC	Negative
VHL	NGS	Wild Type

IHC: Immunohistochemistry CISH: Chromogenic In situ Hybridization NGS: Next-Generation Sequencing

See the Appendix section for a detailed overview of the biomarker test results for each technology.

SAMPLE REPORT - NOT FOR CLINICAL USE

FIG. 29B

Agents Associated with Potential BENEFIT

Agents	Test	Method	Result	Value†	Clinical Association			Literature Assessment*	
					Potential Benefit	Decreased Potential Benefit	Lack of Potential Benefit	Highest Level of Evidence	References
capecitabine, fluorouracil, pemetrexed	TS	IHC	Negative	1+ 6%	✓			II / Good	24, 25, 26
docetaxel, paclitaxel	PGP	IHC	Positive	2+ 12%		✓		II-3 / Fair	41, 42
	TLE3	IHC	Negative	2+ 25%		✓		II-2 / Good	43
	TUBB3	IHC	Negative	2+ 15%	✓			II / Good	37, 38, 39, 40
gemcitabine	RRM1	IHC	Negative	2+ 5%	✓			II / Good	53

*The level of evidence for all references is assigned according to the Literature Level of Evidence Framework consistent with the US Preventive Services Task Force described further in the Appendix of this report. The level of evidence reported is the highest level of evidence based on the body of evidence, overall clinical utility, competing biomarker interactions and tumor type from which the evidence was gathered.

† Refer to Appendix for detailed Result and Value information for each biomarker, including appropriate cutoffs, unit of measure, etc.

SAMPLE REPORT - ILLUSTRATIVE PURPOSES ONLY - NOT A REAL CASE

FIG. 29C

Agents Associated with Potential LACK OF BENEFIT

Agents	Test	Method	Result	Value[7]	Clinical Association			Literature Assessment	
					Potential Benefit	Decreased Potential Benefit	Lack of Potential Benefit	Highest Level of Evidence[8]	Reference
abarelix, degarelix, goserelin, leuprolide, triptorelin	Androgen Receptor	IHC	Negative	0+ 100%			✓	II-3 / Good	2
	ER	IHC	Negative	0+ 100%			✓	I / Good	1
	PR	IHC	Negative	0+ 100%			✓	I / Good	1
abiraterone, bicalutamide, enzalutamide, flutamide	Androgen Receptor	IHC	Negative	0+ 100%			✓	I / Good	2, 3, 4, 5
ado-trastuzumab emtansine (T-DM1), pertuzumab, trastuzumab	Her2/Neu	CISH	Not Amplified	1.19			✓	I / Good	6, 7, 8, 9, 10, 11, 12, 13
	Her2/Neu	IHC	Equivocal	2+ 20%				I / Good	6, 7, 8, 9, 10, 11, 12
anastrozole, exemestane, fulvestrant, letrozole, megestrol acetate, tamoxifen, toremifene	ER	IHC	Negative	0+ 100%			✓	I / Good	14, 15, 16, 17, 18, 19, 20, 21
	PR	IHC	Negative	0+ 100%			✓	I / Good	15, 16, 17, 18, 19, 20, 22, 23
dabrafenib, vemurafenib	BRAF	Next Gen SEQ	Wild Type				✓	I / Good	31, 32, 33, 34
dacarbazine, temozolomide	MGMT	IHC	Positive	2+ 90%			✓	II-2 / Good	35, 36
doxorubicin, epirubicin, liposomal doxorubicin	Her2/Neu	CISH	Not Amplified	1.19			✓	I / Good	44, 45
	PGP	IHC	Positive	2+ 12%			✓	II-1 / Fair	48, 49
	TOP2A	IHC	Negative	2+ 1%			✓	I / Good	46, 47
irinotecan, topotecan	TOPO1	IHC	Negative	1+ 2%			✓	II-1 / Good	59, 60, 61

FOR REFERENCE PURPOSES ONLY. MAY NOT CONFORM

FIG. 29D

Agents Associated with Potential LACK OF BENEFIT

Agents	Test	Method	Result	Value†	Clinical Association			Literature Assessment	
					Potential Benefit	Decreased Potential Benefit	Lack of Potential Benefit	Highest Level of Evidence*	Reference
lapatinib	Her2/Neu	CISH	Not Amplified	1.19			✓	I / Good	13, 62, 63, 64
	Her2/Neu	IHC	Equivocal	2+ 20%				I / Good	62, 63, 64

*The level of evidence for all references is assigned according to the Literature Level of Evidence Framework consistent with the US Preventive Services Task Force described further in the Appendix of this report. The level of evidence reported is the highest level of evidence based on the body of evidence, overall clinical utility, competing biomarker interactions and tumor type from which the evidence was gathered.

† Refer to Appendix for detailed Result and Value information for each biomarker, including appropriate cutoffs, unit of measure, etc.

SAMPLE REPORT. ILLUSTRATIVE PURPOSES ONLY. NOT FOR COMMERCIAL USE.

FIG. 29E

Agents with Indeterminate Benefit (Biomarker Results Do Not Impact Potential Benefit or Lack of Potential Benefit)

Agents	Test	Method	Result	Value†	Clinical Association			Literature Assessment	
					Potential Benefit	Decreased Potential Benefit	Lack of Potential Benefit	Highest Level of Evidence*	Reference
carboplatin, cisplatin, oxaliplatin	BRCA1	Next Gen SEQ	Mutation Not Detected				✓	II-2 / Good	27, 28, 29, 30
	BRCA2	Next Gen SEQ	Mutation Not Detected				✓	II-2 / Good	27, 28, 29
everolimus, temsirolimus	PIK3CA	Next Gen SEQ	Wild Type			✓		II-2 / Good	50, 51, 52
imatinib	c-KIT	Next Gen SEQ	Wild Type				✓	II-2 / Good	54, 55
	PDGFRA	Next Gen SEQ	Wild Type				✓	II-3 / Good	56, 57, 58
nab-paclitaxel	SPARC Monoclonal	IHC	Negative	2+ 10%			✓	II-2 / Good	65, 66
	SPARC Polyclonal	IHC	Negative	2+ 5%			✓	II-2 / Good	65, 66
vandetanib	RET	Next Gen SEQ	Wild Type					I / Good	67

*The level of evidence for all references is assigned according to the Literature Level of Evidence Framework consistent with the US Preventive Services Task Force described further in the Appendix of this report. The level of evidence reported is the highest level of evidence based on the body of evidence, overall clinical utility, competing biomarker interactions and tumor type from which the evidence was gathered.

† Refer to Appendix for detailed Result and Value information for each biomarker, including appropriate cutoffs, unit of measure, etc.

SAMPLE REPORT. ILLUSTRATIVE PURPOSES ONLY. NOT FOR CLINICAL USE.

FIG. 29F

Clinical Trials Connector™ Results Summary

For a complete list of open, enrolling clinical trials visit MI Portal to access the Clinical Trials Connector. This highly personalized, real-time web-based service provides additional clinical trial information and enhanced searching capabilities, including, but not limited to:

- Location: filter by geographic area
- Biomarker(s): identify specific biomarkers associated with open clinical trials to choose from
- Drug(s): search for specific therapies
- Trial Sponsor: locate trials based on the organization supporting the trial(s)

Visit www.CarisMolecularIntelligence.com to view all matched trials.

Chemotherapies		
Drug Class	Biomarker	Investigational Agent(s)
Antifolates	TS	pemetrexed, methotrexate
Nucleoside analog	RRM1	gemcitabine
Pyrimidine analog	TS	fluorouracil, capecitabine

Targeted Therapies		
Drug Class	Biomarker	Investigational Agent(s)
ERK inhibitors	KRAS	BVD-523
Cell cycle inhibitors	TP53	MK-1775
EGFR monoclonal antibody	EGFR	cetuximab
Immunomodulatory agents	PD-1	nivolumab, MK-3475, MPDL3280A
PI3K/Akt/mTor inhibitors	PTEN	MLN0128, temsirolimus, LY2780301, BEZ235, ZSTK474, CC-223, PF-04691502, GSK2110183, BAY80-6946, XL147(SAR245408), AZD5363, INK1117, PF-05212384, ARQ092, BYL719, MLN1117, AZD2014, sirolimus, everolimus, BKM120, GDC-0068, MK2206
Multikinase inhibitors	KRAS	regorafenib
MEK inhibitors	KRAS	XL518, trametinib, selumetinib, GDC-0973, PD0325901
PARP inhibitors	PTEN	BMN-673, veliparib, rucaparib, olaparib

SAMPLE REPORT, ILLUSTRATIVE PURPOSES ONLY. NOT FOR CLINICAL USE

FIG. 29G

References	Level of Evidence
[1] Cuzick J,LHRH-agonists in Early Breast Cancer Overview group. (2007). "Use of luteinising-hormone-releasing hormone agonists as adjuvant treatment in premenopausal patients with hormone-receptor-positive breast cancer: a meta-analysis of individual patient data from randomised adjuvant trials." The Lancet 369: 1711-1723. View Citation Online	I / Good
[2] El Sheikh, S. S., H. M. Romanska, et al. (2008). "Predictive value of PTEN and AR coexpression of sustained responsiveness to hormonal therapy in prostate cancer--a pilot study." Neoplasia. 10(9): 949-53. View Citation Online	II-3 / Good
[3] Jaspers, H.C., C.M. van Herpen, et. al. (2011). "Androgen-receptor-positive salivary duct carcinoma: a disease entity with promising new treatment options". J Clin Oncol. 29(16):e473-476. View Citation Online	II-3 / Fair
[4] Scher, H.I., J.S. de Bono, et al. (2012). "Increased Survival with Enzalutamide in Prostate Cancer after Chemotherapy". N Engl J Med. 367:1187-1197. View Citation Online	I / Good
[5] de Bono, J.S., H.I. Scher, et al. (2011). "Abiraterone and Increased Survival in Metastatic Prostate Cancer". N Engl J Med. 364:1995-2005. View Citation Online	I / Good
[6] Bang, Y-J., Y-K. Kang, et. al. (2010). "Trastuzumab in combination with chemotherapy versus chemotherapy alone for treatment of HER2-positive advanced gastric or gastro-oesophageal junction cancer (ToGA): a phase 3, open-label, randomised controlled trial." Lancet. 376:687-97. View Citation Online	I / Good
[7] Baselga, J., S.M. Swain, et. al. (2012). "Pertuzumab plus trastuzumab plus docetaxel for metastatic breast cancer". N. Engl. J. Med. 36:109-119. View Citation Online	I / Good
[8] Yin, W., J. Lu, et. al. (2011). "Trastuzumab in adjuvant treatment HER2-positive early breast cancer patients: A meta-analysis of published randomized controlled trials." PLoS ONE 6(6): e21030. doi:10.1371/journal.pone.0021030. View Citation Online	I / Good
[9] Cortes, J., J. Baselga, et. al. (2012). "Pertuzumab monotherapy after trastuzumab-based treatment and subsequent reintroduction of trastuzumab: activity and tolerability in patients with advanced human epidermal growth factor receptor-2-positive breast cancer." J. Clin. Oncol. 30. DOI: 10.1200/JCO.2011.37.4207. View Citation Online	II-1 / Good
[10] Hurvitz, S.A., E.A. Perez, et. al. (2013) "Phase III randomized study of trastuzumab emtansine versus trastuzumab plus docetaxel in patients with human epidermal growth factor receptor 2-positive metastatic breast cancer." J Clin Oncol.31(9):1157-63. View Citation Online	I / Good
[11] Slamon, D., M. Buyse, et. al. (2011). "Adjuvant trastuzumab in HER2-positive breast cancer." N. Engl. J. Med. 365:1273-83. View Citation Online	I / Good
[12] Verma, S., K. Blackwell, et. al. (2012). "Trastuzumab Emtansine for HER2-Positive Advanced Breast Cancer" N Engl J Med. 367(19):1783-91. View Citation Online	I / Good
[13] Bartlett, J.M.S., K. Miller, et. al. (2011). "A UK NEQAS ISH multicenter ring study using the Ventana HER2 dual-color ISH assay." Am. J. Clin. Pathol. 135:157-162. View Citation Online	II-3 / Good
[14] Anderson, H., M. Dowsett, et. al. (2011). "Relationship between estrogen receptor, progesterone receptor, HER-2 and KI67 expression and efficacy of aromatase inhibitors in advanced breast cancer. Annals of Oncology. 22:1770-1776. View Citation Online	II-3 / Good
[15] Bartlett, J.M.S., O. Rea, et al. (2011). "Estrogen receptor and progesterone receptor as predictive biomarkers of response to endocrine therapy: a prospectively powered pathology study in the Tamoxifen and Exemestane Adjuvant Multinational trial." J Clin Oncol 29 (12):1631-1638. View Citation Online	I / Good
[16] Bajetta, N.S.A., H. Earl, et. al. (1996). "A randomized phase III cross-over study of tamoxifen versus megestrol acetate in advanced and recurrent breast cancer." European Journal of Cancer. 32(11):1886-1892. View Citation Online	II-2 / Fair
[17] Coombes, R.C., J.M. Bliss, et al. (2007). "Survival and safety of exemestane versus tamoxifen after 2-3 years' tamoxifen treatment (Intergroup Exemestane Study): a randomized controlled trial." The Lancet 369:559-570. View Citation Online	I / Good
[18] Thurmann, B., A. Gochitzki, et al. (1997). "Formestane versus Megestrol Acetate in Postmenopausal Breast Cancer Patients After Failure of Tamoxifen: A Phase III Prospective Randomised Cross Over Trial of Second-line Hormonal Treatment (SAKK 20/90). E J Cancer 33 (7): 1017-1024. View Citation Online	II-2 / Fair

SUBMITTED FROM IBUS FRAME ONLY - NOT FOR COMMERCIAL USE

FIG. 29H

[19] Lewis, J.D., M.J. Edwards, et al. (2010). "Excellent outcomes with adjuvant toremifene or tamoxifen in early stage breast cancer." Cancer116:2307-15. View Citation Online I / Good

[20] Dowsett, M., C. Allred, et al. (2008). "Relationship between quantitative estrogen and progesterone receptor expression and human epidermal growth factor receptor 2 (HER-2) status with recurrence in the Arimidex, Tamoxifen, Alone or in Combination trial." J Clin Oncol 26(7): 1059-65. View Citation Online II-2 / Fair

[21] Viale, G., M. M. Regan, et al. (2008). "Chemoendocrine compared with endocrine adjuvant therapies for node-negative breast cancer: predictive value of centrally reviewed expression of estrogen and progesterone receptors--International Breast Cancer Study Group." J Clin Oncol 26(9): 1484-10. View Citation Online II-2 / Good

[22] Stendahl, M., L. Ryden, et al. (2006). "High progesterone receptor expression correlates to the effect of adjuvant tamoxifen in premenopausal breast cancer patients." Clin Cancer Res 12(15): 4614-9. View Citation Online I / Good

[23] Yamashita, H., Y. Yando, et al. (2006). "Immunohistochemical evaluation of hormone receptor status for predicting response to endocrine therapy in metastatic breast cancer." Breast Cancer 13(1): 74-83. View Citation Online II-3 / Good

[24] Chen, C.-Y., P.-C. Yang, et al. (2011). "Thymidylate synthase and dihydrofolate reductase expression in non-small cell lung carcinoma: The association with treatment efficacy of pemetrexed." Lung Cancer 74(1): 132-138. View Citation Online II-1 / Good

[25] Qiu, L.X., M.H. Zheng, et al. (2008). "Predictive value of thymidylate synthase expression in advanced colorectal cancer patients receiving fluoropyrimidine-based chemotherapy: Evidence from 24 studies." Int. J. Cancer 123: 2364-2369. View Citation Online I / Good

[26] Lee, S.J., Y.H. Im, et al. (2010). "Thymidylate synthase and thymidine phosphorylase as predictive markers of capecitabine monotherapy in patients with anthracycline- and taxane-pretreated metastatic breast cancer." Cancer Chemother. Pharmacol. DOI 10.1007/s00280-010-1545-0. View Citation Online II-3 / Good

[27] Tan, D.S.P., M.E. Gore, et. Al. (2008) "'BRCAness' syndrome in ovarian cancer: a case-control study describing the clinical features and outcome of patients with epithelial ovarian cancer associated with BRCA1 and BRCA2 mutations." J Clin Oncol. 26(34):5530-6 View Citation Online II-2 / Good

[28] Hennessy, B.T., G.B. Mills, et al. (2010) "Somatic mutations in BRCA1 and BRCA2 could expand the number of patients that benefit from poly (ADP ribose) polymerase inhibitors in ovarian cancer" J Clin Oncol. 28(23): 3570-6 View Citation Online II-3 / Good

[29] Lowery, M.A., E.M. O'Reilly, et.al. (2011) "An emerging entity: pancreatic adenocarcinoma associated with a known BRCA mutation: clinical descriptors, treatment implications, and future directions." Oncologist. 16(10):1397-402. View Citation Online II-3 / Fair

[30] Byrski, T., S. Narod, et. Al. (2009) "Pathologic complete response rates in young women with BRCA1-positive breast cancers after neoadjuvant chemotherapy." J Clin Oncol. 28(3):375-9. View Citation Online II-3 / Good

[31] Flaherty, K.T., P.B. Chapman, et al. (2010). "Inhibition of Mutated, Activated BRAF in Metastatic Melanoma." N Engl J Med 363:809-819. View Citation Online II-2 / Good

[32] Hauschild, A., P.B. Chapman, et al. (2012). "Dabrafenib in BRAF-mutated metastatic melanoma: a multicentre, open-label, phase 3 randomised controlled trial." Lancet 368-365. View Citation Online I / Good

[33] Chapman, P.B., G.A. McArthur, et. al. (2011). "Improved survival with vemurafenib in melanoma with BRAF V600E mutation." N. Engl. J. Med. This article (10.1056/NEJMoa1103782) was published on June 5, 2011, at nejm.org. View Citation Online I / Good

[34] Falchook, G.S., R. F. Kefford, et al. (2012). "Dabrafenib in patients with melanoma, untreated brain metastases, and other solid tumours: a phase I dose-escalation trial." Lancet 379:1893-901. View Citation Online II-2 / Good

[35] Kulke, M.H., M.S. Ramdon, et al. (2006). "O6-Methylguanine DNA Methyltransferase Deficiency and Response to Temozolomide-Based Therapy in Patients with Neuroendocrine Tumors." Clin Cancer Res 15(1): 338-345. View Citation Online II-2 / Good

[36] Ohlmot, G. L., M. Sante, et al. (2007). "Correlation between O6-methylguanine-DNA methyltransferase and survival in inoperable newly diagnosed glioblastoma patients treated with neoadjuvant temozolomide." J Clin Oncol 25(12): 1470-5. View Citation Online II-3 / Good

[37] Ploussard, G., A. de la Taille, et al. (2010). "Class III β-Tubulin Expression Predicts Prostate Tumor Aggressiveness and Patient Response to Docetaxel-Based Chemotherapy." Clin Cancer Res 70(22): 9253-9264. View Citation Online II-3 / Good

[38] Gao, S., J. Gao, et al. (2012). "Clinical implications of REST and TUBB3 in ovarian cancer and its relationship to paclitaxel resistance." Tumor Biol 33:1759-1765. View Citation Online II-3 / Good

SAMPLE PROTOCOL FOR PROPOSED COMMERCIAL USE

FIG. 29I

[39]	Zhang, H.-L., X.-W. Zhou, et al. (2012). "Association between class III β-tubulin expression and response to paclitaxel/vinorelbine-based chemotherapy for non-small cell lung cancer: A meta-analysis." Lung Cancer 77: 9-15. View Citation Online	I / Good
[40]	Seve, P., C. Dumontet, et al. (2005). "Class III β-tubulin expression in tumor cells predicts response and outcome in patients with non-small cell lung cancer receiving paclitaxel." Mol Cancer Ther 4(12): 2001-2007. View Citation Online	II-3 / Good
[41]	Penson, R.T., M.V. Seiden, et al. (2004). "Expression of multidrug resistance-1 protein inversely correlates with paclitaxel response and survival in ovarian cancer patients: a study in serial samples." Gynecologic Oncology 93:98-106. View Citation Online	II-3 / Fair
[42]	Yeh, J.J., A. Kao, et al. (2003). "Predicting Chemotherapy Response to Paclitaxel-Based Therapy in Advanced Non-Small-Cell Lung Cancer with P-Glycoprotein Expression." Respiration 70:32-38. View Citation Online	II-3 / Fair
[43]	Kulkarni, S.A., D.T. Ross, et al. (2009). "TLE3 as a candidate biomarker of response to taxane therapy". Breast Cancer Research. 11:R17 (doi:10.1186/bcr2241). View Citation Online	II-2 / Good
[44]	Gennari, A., P. Bruzzi, et al. (2008) "HER2 status and efficacy of adjuvant anthracyclines in early breast cancer: a pooled analysis of randomized trials." J Natl Can Inst. 100:14-20. View Citation Online	I / Good
[45]	Press, M.F., Slamon, D.J., et al. (2011)."Alteration of topoisomerase II-alpha gene in human breast cancer: association with responsiveness to anthracycline based chemotherapy." J. Clin. Oncol. 29(7):859-67. View Citation Online	I / Good
[46]	O'Malley, F.P., K.I. Pritchard, et al. (2011). "Topoisomerase II alpha protein and responsiveness of breast cancer to adjuvant chemotherapy with CEF compared to CMF in the NCIC CTG randomized MA.8 adjuvant trial." Breast Can Res Treat. 126, 401-409. View Citation Online	I / Good
[47]	Rodrigo, R.S., C. Axel III, et. al. (2011). "Topoisomerase II-alpha protein expression and histological response following doxorubicin-based induction chemotherapy predict survival of locally advanced soft tissues sarcomas." Eur J of Can. 47, 1319-1327. View Citation Online	II-3 / Good
[48]	Chintamani, J.P., Singh, et. al. (2005). "Role of p-glycoprotein expression in predicting response to neoadjuvant chemotherapy in breast cancer - a prospective clinical study." World J. Surg. Oncol. 3:61. View Citation Online	II-3 / Good
[49]	Akimoto, M., H. Saisho, et al. (2006). "Relationship between therapeutic efficacy of arterial infusion chemotherapy and expression of P-glycoprotein and p53 protein in advanced hepatocellular carcinoma." World J of Gastroenterol. 12(6). 868-873. View Citation Online	II-1 / Fair
[50]	Moroney, J.W., R. Kurzrock, et. al. (2011). "A phase I trial of liposomal doxorubicin, bevacizumab, and temsirolimus in patients with advanced gynecologic and breast malignancies." Clin. Cancer Res. 17:6840-6846. View Citation Online	II-3 / Fair
[51]	Janku, F., R. Kurzrock, et. al. (2012) "PIK3CA Mutation H1047R is Associated with Response to PI3K/AKT/mTOR Signaling Pathway Inhibitors in Early-Phase Clinical Trials", Cancer Res; 73(1); 276-84. View Citation Online	II-2 / Good
[52]	Janku, F., R. Kurzrock, et. al. (2012). "PI3K/AktmTOR Inhibitors in patients with breast and gynecologic malignancies harboring PIK3CA mutations." Journal of Clinical Oncology. DOI: 10.1200/JCO.2011.36.1196. View Citation Online	II-3 / Good
[53]	Gong, W., J. Dong, et. al. (2012). "RRM1 expression and clinical outcome of gemcitabine-containing chemotherapy for advanced non-small-cell lung cancer: A meta-analysis." Lung Cancer. 75:374-380. View Citation Online	I / Good
[54]	Guo, J., S. Qin, et. al. (2011). "Phase II, open-label, single-arm trial of imatinib mesylate in patients with metastatic melanoma harboring c-Kit mutation or amplification." J. Clin. Oncol. 29:2904-2909. View Citation Online	II-2 / Good
[55]	Carvajal, R.D., G.K. Schwartz, et. al. (2011). "KIT as a therapeutic target in metastatic melanoma." JAMA. 305(22):2327-2334. View Citation Online	II-2 / Good
[56]	Cassier, P.A., P. Hohenberger, et al. (2012). "Outcome of Patients with Platelet-Derived Growth Factor Receptor Alpha-Mutated Gastrointestinal Stromal Tumors in the Tyrosine Kinase Inhibitor Era." Clin Cancer Res 18:4458-4464. View Citation Online	II-3 / Good
[57]	Debiec-Rychter, M., I. Judson, et al. (2006). "KIT mutations and dose selection for imatinib in patients with advanced gastrointestinal stromal tumours." Eur J Cancer 42:1093-1103. View Citation Online	II-3 / Good
[58]	Heinrich, M.C., J.A. Fletcher, et. al. (2008). "Correlation of kinase genotype and clinical outcome in North American intergroup phase III trial of imatinib mesylate for treatment of advanced gastrointestinal stromal tumor: CALGB 150105 study by Cancer and Leukemia Group B and Southwest Oncology Group." J Clin Oncol 26(33):5360-5367. View Citation Online	II-3 / Good

SAMPLE REPORT ONLY - NOT FOR COMMERCIAL USE

FIG. 29J

[59] Koutopoulos, I., G. Fountzilas, et al. (2009). "Topoisomerase I but not thymidylate synthase is associated with improved outcome in patients with resected colorectal cancer treated with irinotecan containing adjuvant chemotherapy." BMC Cancer. 9:339 View Citation Online II-2 / Fair

[60] Ataka, M., K. Katano, et al. (2007). "Topoisomerase I protein expression and prognosis of patients with colorectal cancer." Yonago Acta medica. 50:61-67 View Citation Online II-3 / Fair

[61] Braun, M.S., M.T. Seymour, et al. (2005). "Predictive biomarkers of chemotherapy efficacy in colorectal cancer: results from the UK MRC FOCUS trial." J. Clin. Oncol. 23:3590-3596. View Citation Online II-1 / Good

[62] Amir, E. et al. (2010). "Lapatinib and HER2 status: results of a meta-analysis of randomized phase III trials in metastatic breast cancer." Cancer Treatment Reviews. 36:410-415. View Citation Online 1 / Good

[63] Johnston, S., Pegram M., et al. (2009). "Lapatinib combined with letrozole versus letrozole and placebo as first-line therapy for postmenopausal hormone receptor-positive metastatic breast cancer. Journal of Clinical Oncology. Published ahead of print on September 28, 2009 as 10.1200/JCO.2009.23.3734. View Citation Online 1 / Good

[64] Press, M. F., R. S. Finn, et al. (2008). "HER-2 gene amplification, HER-2 and epidermal growth factor receptor mRNA and protein expression, and lapatinib efficacy in women with metastatic breast cancer." Clin Cancer Res 14(23): 7861-70. View Citation Online 1 / Good

[65] Desai, N., Soon-Shiong, P., et al. (2008). "SPARC Expression Correlates with Tumor Response to Albumin-Bound Paclitaxel in Head and Neck Cancer Patients." Translational Oncology 2(2): 59-64. View Citation Online II-3 / Good

[66] Von Hoff, D.D., M. Hidalgo, et al. (2011). "Gemcitabine plus nab-paclitaxel is an active regimen in patients with advanced pancreatic cancer: a phase I/II trial." J. Clin. Oncol. DOI: 10.1200/JCO.2011.36.5742. View Citation Online II-2 / Good

[67] Wells, S.A., M.J. Schlumberger, et al. (2012). "Vandetanib in Patients with Locally Advanced or Metastatic Medullary Thyroid Cancer: A Randomized, Double-Blind Phase III Trial." J Clin Oncol 30: 134-141. View Citation Online 1 / Good

SAMPLE REPORT. ILLUSTRATIVE PURPOSES ONLY. NOT FOR COMMERCIAL USE

FIG. 29K

Specimens Received (Gross Description)

The specimens consist of:

1 (A) Paraffin Block - Client ID(XYZ-1234-5678-AB) from Springfield Medical Center, Springfield, XY, with the corresponding surgical pathology report labeled "XYZ-1234-5678".

Specimen Id: XYZ-1234-5678-AB

Disclaimer

All of the individual assays that are available through Caris Life Sciences® Molecular Intelligence™ Services (Caris Molecular Intelligence) were developed and validated by Caris MPI, Inc. d/b/a Caris Life Sciences and their test performance characteristics were determined and validated by Caris Life Sciences pursuant to the Clinical Laboratory Improvements Amendments and accompanying regulations ("CLIA"). Some of the assays that are part of Caris Molecular Intelligence have been cleared or approved by the U.S. Food and Drug Administration (FDA). The clinical reference laboratory of Caris MPI, Inc. is certified under CLIA to perform high complexity testing, including all of the assays that are part of the Caris Molecular Intelligence.

The CLIA certification number of each Caris MPI, Inc. laboratory performing testing in connection with Caris Molecular Intelligence can be found at the bottom of each page. This Report includes information about therapeutic agents that appear to be associated with clinical benefit based on NCCN Compendium guidelines, relevance of tumor lineage, level of published evidence and strength of biomarker expression, as available, reviewed and assessed by Caris Life Sciences. The agents are not ranked in order of potential or predicted efficacy. The finding of a biomarker expression does not necessarily indicate pharmacologic effectiveness or lack thereof. The agents identified may or may not be suitable for use with a particular patient and the report does not guarantee or suggest that any particular agent will be effective with the treatment of any particular condition. Caris Life Sciences expressly disclaims and makes no representation or warranty whatsoever relating, directly or indirectly, to this review of evidence or identified scientific literature, the conclusions drawn from it or any of the information set forth in this Report that is derived from such review, including information and conclusions relating to therapeutic agents that are included or omitted from this Report.

The decision to select any, all or none of the matched agents resides solely with the discretion of the treating physician. Decisions on patient care and treatment must be based on the independent medical judgment of the treating physician, taking into consideration all applicable information concerning the patient's condition, such as patient and family history, physical examinations, information from other diagnostic tests, and patient preferences, in accordance with the applicable standard of care. Decisions regarding care and treatment should not be based on a single test such as this test or the information contained in this report.

The information presented in the Clinical Trials Connector™ section of the Report is compiled from sources believed to be reliable and current. We have used our best efforts to make this information as accurate as possible. However, the accuracy and completeness of this information cannot be guaranteed. The contents are to be used for clinical trial guidance and may not include all relevant trials. Current enrollment status for these trials is unknown. The clinical trials information present in the biomarker description was compiled from www.clinicaltrials.gov. The contents are to be used only as a guide, and health care providers should employ clinical judgment in interpreting this information for individual patients. Specific entrance criteria for each clinical trial should be reviewed as additional inclusion criteria may apply. Caris Life Sciences makes no promises or guarantees that a healthcare provider, insurer or other third party payor, whether private or governmental, will provide reimbursement (instead of coverage) for any of the tests performed.

The next generation sequencing assay performed by Caris Life Sciences examines tumor tissue only and does not examine normal tissues such as tumor adjacent tissue or whole peripheral blood. As such, the origin of any mutation detected by our assay may either be a somatic (not inherited) or a germline mutation (inherited) and will not be distinguishable by this assay. It is recommended that results be considered within the clinical context and history of the patient. If a germline inheritance pattern is suspected then counseling by a board certified genetic counselor is recommended.

Electronic Signature

CE EC REP

SAMPLE REPORT - ILLUSTRATIVE PURPOSES ONLY - NOT FOR CLINICAL USE.

FIG. 29L

Appendix

MI-2014-10-280

Note: The initial pages of this Appendix contain patient specific Result and Value information for each biomarker, including appropriate cutoffs, unit of measure, etc.

SAMPLE REPORT - ILLUSTRATIVE PURPOSES ONLY - NOT FOR CLINICAL USE.

FIG. 29M

Mutational Analysis by Next Generation Sequencing

Genes Tested With Alterations

Gene	Alteration	Frequency (%)	Exon	Result
KRAS	G12R	16	2	Mutated, Pathogenic

Interpretation: A pathogenic mutation was detected in KRAS

KRAS or V-Ki-ras2 Kirsten rat sarcoma viral oncogene homolog encodes a signaling intermediate involved in many signaling cascades including the EGFR pathway. KRAS somatic mutations have been found in pancreatic (57%), colon (35%), lung (16%), biliary tract (28%), and endometrial (16%) cancers. Mutations at activating hotspots are associated with resistance to EGFR tyrosine kinase inhibitors (erlotinib, gefitinib) in NSCLC and monoclonal antibodies (cetuximab, panitumumab) in CRC patients. Retrospective clinical studies raised the possibility that KRAS G13D mutations may not be absolutely predictive of non-response; however, this finding is not supported by published analysis of 3 randomized controlled phase III trials. Various clinical trials (on www.clinicaltrials.gov) investigating agents which target this gene may be available for KRAS mutated patients.
Several germline mutations of KRAS (V14I, T58I, and D153V amino acid substitutions) are associated with Noonan syndrome.

TP53	P278S	17	8	Mutated, Presumed Pathogenic

Interpretation: A TP53 mutation was detected in this sample. This mutation has been reported previously in numerous publications and various tumor types. In addition, biochemical studies have shown that this mutation causes TP53 to be inactive at physiological temperatures (Dearth et al, 2007, Carcinogenesis). As such, it is very likely that this mutation is pathogenic.

TP53, or p53, plays a central role in modulating response to cellular stress through transcriptional regulation of genes involved in cell-cycle arrest, DNA repair, apoptosis, and senescence. Inactivation of the p53 pathway is essential for the formation of the majority of human tumors. Mutation in p53 (TP53) remains one of the most commonly described genetic events in human neoplasia, estimated to occur in 30-50% of all cancers. Generally, presence of a disruptive p53 mutation is associated with a poor prognosis in all types of cancers, and diminished sensitivity to radiation and chemotherapy. In addition, various clinical trials (on www.clinicaltrials.gov) investigating agents which target p53's downstream or upstream effectors may have clinical utility depending on the p53 status. Germline p53 mutations are associated with the Li-Fraumeni syndrome (LFS) which may lead to early-onset of several forms of cancer currently known to occur in the syndrome, including sarcomas of the bone and soft tissues, carcinomas of the breast and adrenal cortex (hereditary adrenocortical carcinoma), brain tumors and acute leukemias.

Genes Tested Without Alterations

ABL1	AKT1	ALK	APC	ATM	BRAF
c-KIT	cMET	CSF1R	CTNNB1	EGFR	ERBB2
FGFR1	FGFR2	FLT3	GNA11	GNAQ	GNAS
HRAS	IDH1	JAK2	KDR	MPL	NOTCH1
NRAS	PDGFRA	PIK3CA	PTEN	RET	SMO
VHL					

Comments on Next Gen Profile Analysis

Molecular testing of this specimen was performed after harvesting of targeted tissues with an approved manual microdissection technique. Candidate slides were examined and areas containing tumor cells (and separately normal cells, when necessary for testing) were circled. A laboratory technician harvested targeted tissues for extraction from the marked areas. The areas marked and extracted were examined on post-microdissected slides and adequacy of microdissection was verified by a board certified Pathologist.

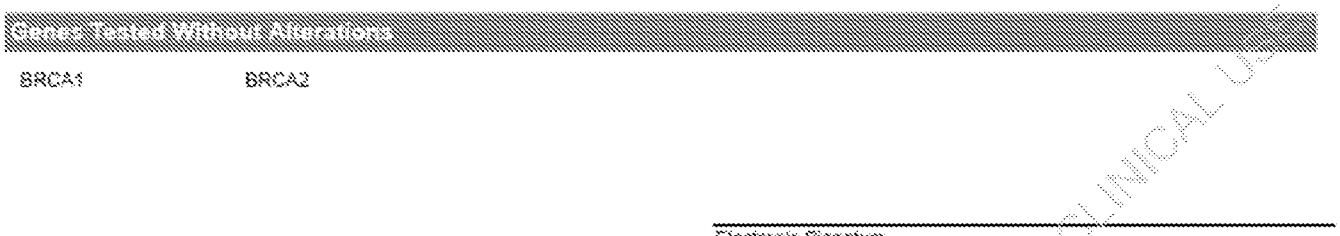
SAMPLE REPORT. ILLUMINA CONFIDENTIAL

FIG. 29N

Electronic Signature

SAMPLE REPORT, ILLUSTRATIVE PURPOSES ONLY. NOT FOR CLINICAL USE.

FIG. 29O



SAMPLE REPORT. ILLUSTRATIVE PURPOSES ONLY. NOT FOR CLINICAL USE

FIG. 29P

IHC Biomarker Detail

Biomarker	Patient Tumor			Threshold
	Staining Intensity	Percent Staining	Result	Biomarker Intensity/Percentage
MGMT	2	90	Positive	=0+ or ≤35% or ≥1+ and >35%
TLE3	2	25	Negative	<30% or <2+ or ≥2+ and ≥30%
cMET	2	25	Negative	<50% or <2+ or ≥2+ and ≥50%
Her2/Neu	2	20	Equivocal	≤1+ or =2+ and ≤10% or ≥3+ and >10%
TUBB3	2	15	Negative	<30% or <2+ or ≥2+ and ≥30%
PGP	2	12	Positive	=0+ or <10% or ≥1+ and ≥10%
SPARC Monoclonal	2	10	Negative	<30% or <2+ or ≥2+ and ≥30%
RRM1	2	5	Negative	=0+ or <50% or <2+ or ≥2+ and ≥50%
SPARC Polyclonal	2	5	Negative	<30% or <2+ or ≥2+ and ≥30%
TOP2A	2	1	Negative	=0+ or <10% or ≥1+ and ≥10%
EGFR	1	75	Positive	=0+ or <10% or ≥1+ and ≥10%
PTEN	1	20	Negative	=0+ or ≤50% or ≥1+ and >50%
TS	1	6	Negative	=0+ or ≤3+ and <10% or ≥1+ and ≥10%
TOPO1	1	2	Negative	=0+ or <30% or <2+ or ≥2+ and ≥30%
Androgen Receptor	0	100	Negative	=0+ or <10% or ≥1+ and ≥10%
ER	0	100	Negative	=0+ or <10% or ≥1+ and ≥10%
PD-L1	0	100	Negative	<5% or <2+ or ≥2+ and ≥5%
PR	0	100	Negative	=0+ or <10% or ≥1+ and ≥10%

These tests were developed and their performance characteristics determined by Caris Life Sciences, Inc.
* Caris Life Sciences has defined threshold levels of reactivity of IHC to establish cutoff points based on published evidence. Polymer detection systems are used for each IHC.
Clones used: MGMT(MT23.2), TLE3(Polyclonal), cMET(SP44), Her2/Neu(4B5), TUBB3(Polyclonal), PGP(C464), SPARC Monoclonal(122511), RRM1(Polyclonal), SPARC Polyclonal(Polyclonal), TOP2A(3F6), EGFR(H11), PTEN(6H2.1), TS(TS106/4H4B1), TOPO1(1D6), Androgen Receptor(AR27), ER(SP1), PD-L1(130021), PR(1E2).

Electronic Signature

Biomarker	TIL Count/HPF w/40X Objective	Result	Threshold
PD-1	2-5/HPF	Positive	=0+ or ≥1+

These tests were developed and their performance characteristics determined by Caris Life Sciences, Inc.
* Please note that PD1 staining is read from the tumor infiltrating lymphocytes (TIL).
Clones used: PD-1(MRQ-22).

Electronic Signature

SAMPLE REPORT ILLUSTRATION ONLY

FIG. 29Q

ANALYSIS BY CISH FOR AMPLIFICATION

Gene / ISCN	Cells Counted	Result	Avg Gene Copy Number	Avg Control Copy Number	% Cells with 2-4 Copies	% Cells with 2+5 Copies	Ratio Calculation	Ratio
Her2/Neu nuc ish (D17Z1x1-2,HER2x1-2)[/30]	20	Not Amplified	2.50	2.10	N/A	N/A	Her2/neu/ Chromosome 17	1.19
	Reference Range: Her2/Neu:CEP 17 signal ratio of >= 2.0; and non-amplification as <2.0 per Ventana INFORM HER2 CISH Package insert.							
cMET nuc ish (D7Z1x1-2,cMETx1-2)[100/100]	20	Not Amplified	2.00	1.95	N/A	N/A		1.03
	Reference Range: Positivity for increased gene copy number for cMET CISH has been defined as >= 5 copies of mean MET gene copy number per cell in NSCLC based on cMET FISH evidence (Cappuzzo et al 2009). The gene copy number threshold for other tumor types has not been determined.							

HER2 CISH test was carried out using the INFORM DUAL HER2 ISH Assay (Ventana Medical Systems, Inc.), which has been cleared by the US Food and Drug Administration (FDA) for enumerating the ratio of HER2/Chr 17 in Breast Cancer samples.

cMET CISH was carried out using a probe specific for cMET and a probe for the pericentromeric region of chromosome 7 (Ventana).

TOP2A CISH was carried out using a probe specific for TOP2 and a probe for the pericentromeric region of chromosome 17 (Ventana).

MDM2 CISH was carried out using a probe specific for MDM2 and a probe for the pericentromeric region of chromosome 12 (Ventana).

EGFR CISH was carried out using a probe specific for EGFR and a probe for the pericentromeric region of chromosome 7 (Ventana).

All CISH testing has been developed and its performance characteristics determined by Caris MPI, Inc. (d/b/a Caris Life Sciences), and has not been cleared or approved by the FDA. The FDA has determined that such clearance or approval is not currently necessary. These tests should not be regarded as investigational or research as they are used for clinical purpose and determined to be medically necessary by the ordering physician, who is not employed by Caris MPI, Inc. or its affiliates. This laboratory is certified under Clinical Laboratory Improvement Amendment of 1988 (CLIA-88) and is qualified to perform high complexity testing. CLIA 03D1019490

Electronic Signature

SAMPLE REPORT. ILLUSTRATIVE PURPOSES ONLY. NOT A REAL PATIENT REPORT.

FIG. 29R

BIOMARKER DESCRIPTION

Target	Biomarker Description
ABL1	ABL1 also known as Abelson murine leukemia homolog 1. Most CML patients have a chromosomal abnormality due to a fusion between Abelson (Abl) tyrosine kinase gene at chromosome 9 and break point cluster (Bcr) gene at chromosome 22 resulting in constitutive activation of the Bcr-Abl fusion gene. Imatinib is a Bcr-Abl tyrosine kinase inhibitor commonly used in treating CML patients. Mutations in the ABL1 gene are common in imatinib-resistant CML patients which occur in 30-90% of patients. However, more than 50 different point mutations in the ABL1 kinase domain may be inhibited by the second generation kinase inhibitors, dasatinib, bosutinib and nilotinib. The gatekeeper mutation, T315I that causes resistance to all currently approved TKIs accounts for about 15% of the mutations found in patients with imatinib resistance. BCR-ABL1 mutation analysis is recommended to help facilitate selection of appropriate therapy for patients with CML after treatment with imatinib fails. Various clinical trials (on www.clinicaltrials.gov) investigating agents which target this gene may be available for ABL1 mutated patients.
AKT1	AKT1 gene (v-akt murine thymoma viral oncogene homologue 1) encodes a serine/threonine kinase which is a pivotal mediator of the PI3K-related signaling pathway, affecting cell survival, proliferation and invasion. Dysregulated AKT activity is a frequent genetic defect implicated in tumorigenesis and has been indicated to be detrimental to hematopoiesis. Activating mutation E17K has been described in breast (2-4%), endometrial (2-4%), bladder cancers (3%), NSCLC (1%), squamous cell carcinoma of the lung (5%) and ovarian cancer (2%). This mutation in the pleckstrin homology domain facilitates the recruitment of AKT to the plasma membrane and subsequent activation by altering phosphoinositide binding. A mosaic activating mutation E17K has also been suggested to be the cause of Proteus syndrome. Mutation E49K has been found in bladder cancer, which enhances AKT activation and shows transforming activity in cell lines. Various clinical trials (on www.clinicaltrials.gov) investigating AKT inhibitor in patients carrying AKT mutations may be available.
ALK	ALK rearrangements indicates the fusion of ALK (anaplastic lymphoma kinase) gene with the fusion partner, EML4. EML4-ALK fusion results in the pathologic expression of a fusion protein with constitutively active ALK kinase, resulting in aberrant activation of downstream signaling pathways including RAS-ERK, JAK3-STAT3 and PI3K-AKT. Patients with an EML4-ALK rearrangement are likely to respond to the ALK-targeted agent crizotinib and ceritinib.
Androgen Receptor	The androgen receptor (AR) is a member of the nuclear hormone receptor superfamily. Prostate tumor dependency on androgens / AR signaling is the basis for hormone withdrawal, or androgen ablation therapy, to treat men with prostate cancer. Androgen receptor antagonists as well as agents which block androgen production are indicated for the treatment of AR expressing prostate cancers.
APC	APC or adenomatous polyposis coli is a key tumor suppressor gene that encodes for a large multi-domain protein. This protein exerts its tumor suppressor function in the Wnt/β-catenin cascade mainly by controlling the degradation of β-catenin, the central activator of transcription in the Wnt signaling pathway. The Wnt signaling pathway mediates important cellular functions including intercellular adhesion, stabilization of the cytoskeleton, and cell cycle regulation and apoptosis, and it is important in embryonic development and oncogenesis. Mutation in APC results in a truncated protein product with abnormal function, lacking the domains involved in β-catenin degradation. Somatic mutation in the APC gene can be detected in the majority of colorectal tumors (80%) and it is an early event in colorectal tumorigenesis. APC wild type patients have shown better disease control rate in the metastatic setting when treated with oxaliplatin, while when treated with fluoropyrimidine regimens, APC wild type patients experience more hematological toxicities. APC mutation has also been identified in oral squamous cell carcinoma, gastric cancer as well as hepatoblastoma and may contribute to cancer formation. Various clinical trials (on www.clinicaltrials.gov) investigating agents which target this gene and/or its downstream or upstream effectors maybe available for APC mutated patients. Germline mutation in APC causes familial adenomatous polyposis, which is an autosomal dominant inherited disease that will inevitably develop to colorectal cancer if left untreated. COX-2 inhibitors including celecoxib may reduce the recurrence of adenomas and incidence of advanced adenomas in individuals with an increased risk of CRC. Turcot syndrome and Gardner's syndrome have also been associated with germline APC defects. Germline mutations of the APC have also been associated with an increased risk of developing desmoid disease, papillary thyroid carcinoma and hepatoblastoma.
ATM	ATM or ataxia telangiectasia mutated is activated by DNA double-strand breaks and DNA replication stress. It encodes a protein kinase that acts as a tumor suppressor and regulates various biomarkers involved in DNA repair, which include p53, BRCA1, CHK2, RAD17, RAD9, and NBS1. Although ATM is associated with hematologic malignancies, somatic mutations have been found in colon (18%), breast and neck (14%), and prostate (12%) cancers. Inactivating ATM mutations make patients potentially more susceptible to PARP inhibitors. Various clinical trials (on www.clinicaltrials.gov) investigating agents which target this gene and/or its downstream or upstream effectors may be available for ATM mutated patients. Germline mutations in ATM are associated with ataxia-telangiectasia (also known as Louis-Bar syndrome) and a predisposition to malignancy.
BRAF	BRAF encodes a protein belonging to the raf/mil family of serine/threonine protein kinases. This protein plays a role in regulating the MAPK signaling pathway initiated by EGFR activation, which affects cell division, differentiation, and secretion. Mutations in this gene, most frequently V600E, have been associated with various cancers, including colorectal cancer, malignant melanoma, thyroid carcinoma and non-small cell lung carcinoma. Recent publications have associated V600E mutations in BRAF with a reduced response to cetuximab and panitumumab in CRC, as well as sensitivity to vemurafenib, dabrafenib and trametinib in melanoma and other tumor types.
BRCA1	BRCA1 or breast cancer type 1 susceptibility gene encodes a protein involved in cell growth, cell division, and DNA-damage repair. It is a tumor suppressor gene which plays an important role in mediating double-strand DNA breaks by homologous recombination (HR). Tumors with BRCA1 mutation may be more sensitive to platinum agents and PARP inhibitors. Various clinical trials may be available (on clinicaltrials.gov) for patients with BRCA1 mutation.
BRCA2	BRCA2 or breast cancer type 2 susceptibility gene encodes a protein involved in cell growth, cell division, and DNA-damage repair. It is a tumor suppressor gene which plays an important role in mediating double-strand DNA breaks by homologous recombination (HR). Tumors with BRCA2 mutation may be more sensitive to platinum agents and PARP inhibitors. Various clinical trials may be available (on clinicaltrials.gov) for patients with BRCA2 mutation.
c-KIT	c-KIT is a receptor tyrosine kinase expressed by hematopoietic stem cells, interstitial cells of cajal (pacemaker cells of the gut) and other cell types. Upon binding of cKIT to stem cell factor (SCF), receptor dimerization initiates a phosphorylation cascade resulting in proliferation, apoptosis, chemotaxis and adhesion. Alterations of cKIT, including protein overexpression and mutations, occur in a number of human malignancies, including gastrointestinal stromal tumors (GIST), seminoma, acral and mucosal melanomas and mastocytosis. c-Kit is inhibited by multi-targeted agents including imatinib and sunitinib.

SAMPLE REPORT - NOT FOR CLINICAL USE

FIG. 29S

BIOMARKER DESCRIPTION

Target	Biomarker Description
cMET	cMET is a tyrosine kinase receptor for hepatocyte growth factor (HGF) or scatter factor (SF) and is overexpressed and amplified in a wide range of tumors. cMET overexpression has been associated with a more aggressive biology and a worse prognosis in many human malignancies. Amplification of cMET has been implicated in the development of acquired resistance to erlotinib and gefitinib in NSCLC as well as response to cMET inhibitors available via clinical trials.
CSF1R	CSF1R or colony stimulating factor 1 receptor gene encodes a transmembrane tyrosine kinase, a member of the CSF1/PDGF receptor family. CSF1R mediates the cytokine (CSF-1) responsible for macrophage production, differentiation, and function. Although associated with hematologic malignancies, mutations of this gene are associated with cancers of the liver (21%), colon (13%), prostate (3%), endometrium (2%), and ovary (2%). It is suggested that patients with CSF1R mutations could respond to imatinib. Various clinical trials (on www.clinicaltrials.gov) investigating agents may be available for CSF1R mutated patients. Germline mutations in CSF1R are associated with diffuse leukoencephalopathy, a rapidly progressive neurodegenerative disorder.
CTNNB1	CTNNB1 or cadherin-associated protein, beta 1, encodes for β-catenin, a central mediator of the Wnt signaling pathway which regulates cell growth, migration, differentiation and apoptosis. Mutations in CTNNB1 (often occurring in exon 3) prevent the breakdown of β-catenin, which allows the protein to accumulate resulting in persistent transactivation of target genes, including c-myc and cyclin-D1. Somatic CTNNB1 mutations occur in 1-4% of colorectal cancers, 2-3% of melanomas, 25-38% of endometrioid ovarian cancers, 64-87% of sporadic desmoid tumors, as well as the pediatric cancers, hepatoblastoma, medulloblastoma and Wilms' tumors. A growing number of compounds that suppress the Wnt/β-catenin pathway are available in clinical trials for CTNNB1 mutated patients.
EGFR	EGFR (epidermal growth factor receptor) is a receptor tyrosine kinase and its abnormalities contribute to the growth and proliferation of many human cancers. Sensitizing mutations are commonly detected in NSCLC and patients harboring such mutations may respond to EGFR-targeted tyrosine kinase inhibitors including erlotinib, gefitinib and afatinib. Non-small cell lung cancer cancer patients overexpressing EGFR protein are known to respond to the EGFR monoclonal antibody, cetuximab. EGFR amplification may help enroll patients in various clinical trials with EGFR targeted agents.
ER	The estrogen receptor (ER) is a member of the nuclear hormone family of intracellular receptors which is activated by the hormone estrogen. It functions as a DNA binding transcription factor to regulate estrogen-mediated gene expression. Estrogen receptors overexpressing breast cancers are referred to as "ER positive." Estrogen binding to ER on cancer cells leads to cancer cell proliferation. Breast tumors over-expressing ER are treated with hormone-based anti-estrogen therapy. Everolimus combined with exemestane significantly improves survival in ER positive Her2 negative breast cancer patients who are resistant to aromatase inhibitors.
ERBB2	ErbB2/Her2 encodes a member of the epidermal growth factor (EGF) receptor family of receptor tyrosine kinases. Her2 has no ligand-binding domain of its own and, therefore, cannot bind growth factors. It does, however, bind tightly to other ligand-bound EGF receptor family members to form a heterodimer and enhances kinase-mediated activation of downstream signaling pathways leading to cell proliferation. Her2 is overexpressed in 15-30% of newly diagnosed breast cancers. Clinically, Her2 is a target for the monoclonal antibodies trastuzumab, ado-trastuzumab emtansine and pertuzumab which bind to the receptor extracellularly; the kinase inhibitor lapatinib binds and blocks the receptor intracellularly. Other Her2-targeted agents under clinical investigation (on www.clinicaltrials.gov) may be available.
FGFR1	FGFR1 or fibroblast growth factor receptor 1, encodes for FGFR1 which is important for cell division, regulation of cell maturation, formation of blood vessels, wound healing and embryonic development. Somatic activating mutations are rare, but have been documented in melanoma, glioblastoma, and lung tumors. FGFR1-targeted agents under clinical investigation (on www.clinicaltrials.gov) may be available for FGFR1 mutated patients. Germline, gain-of-function mutations in FGFR1 result in developmental disorders including Kallmann syndrome and Pfeiffer syndrome.
FGFR2	FGFR2 is a receptor for fibroblast growth factor. Activation of FGFR2 through mutation and amplification has been noted in a number of cancers. Somatic mutations of the fibroblast growth factor receptor 2 (FGFR2) tyrosine kinase are present in endometrial carcinoma, lung squamous cell carcinoma, cervical carcinoma, and melanoma. In the endometrioid histology of endometrial cancer, the frequency of FGFR2 mutation is 16% and the mutation is associated with shorter disease free survival in patients diagnosed with early stage disease. Loss of function FGFR2 mutations occur in about 9% melanomas and contribute to melanoma pathogenesis. Various clinical trials (on www.clinicaltrials.gov) investigating agents which target this gene may be available for FGFR2 mutated patients. Germline mutations in FGFR2 are associated with numerous medical conditions that include congenital craniofacial malformation disorders, Apert syndrome and the related Pfeiffer and Crouzon syndromes.
FLT3	FLT3 or Fms-like tyrosine kinase 3 receptor is a member of class III receptor tyrosine kinase family, which includes PDGFRA/B and KIT. Signaling through FLT3 ligand-receptor complex regulates hematopoiesis, specifically lymphocyte development. The FLT3 internal tandem duplication (FLT3-ITD) is the most common genetic lesion in acute myeloid leukemia (AML), occurring in 25% of cases. FLT3 mutations are rare in solid tumors, however they have been documented in breast cancer. Several small molecule multikinase inhibitors targeting the RTK-III family are available (on www.clinicaltrials.gov) for FLT3 mutated patients.
GNA11	GNA11 is a proto-oncogene that belongs to the Gq family of the G alpha family of G protein coupled receptors. Known downstream signaling partners of GNA11 are phospholipase C beta and RhoA and activation of GNA11 induces MAPK activity. Over half of uveal melanoma patients lacking a mutation in GNAQ exhibit somatic mutations in GNA11. Activating mutations of GNA11 have not been found in other malignancies. Various clinical trials (on www.clinicaltrials.gov) investigating agents which target this gene may be available for GNA11 mutated patients.
GNAQ	This gene encodes the Gq alpha subunit of G proteins. G proteins are a family of heterotrimeric proteins coupling seven-transmembrane domain receptors. Oncogenic mutations in GNAQ result in a loss of intrinsic GTPase activity, resulting in a constitutively active Galpha subunit. This results in increased signaling through the MAPK pathway. Somatic mutations in GNAQ have been found in 50% of primary uveal melanoma patients and up to 28% of uveal melanoma metastases. Various clinical trials (on www.clinicaltrials.gov) investigating agents which target this gene may be available for GNAQ mutated patients.

FIG. 29T

BIOMARKER DESCRIPTION

Target	Biomarker Description
GNAS	GNAS (or GNAS complex locus) encodes a stimulatory G protein alpha-subunit. These guanine nucleotide binding proteins (G proteins) are a family of heterotrimeric proteins which couple seven-transmembrane domain receptors to intracellular cascades. Stimulatory G-protein alpha-subunit transmits hormonal and growth factor signals to effector proteins and is involved in the activation of adenylate cyclases. Mutations of GNAS gene at codons 201 or 227 lead to constitutive cAMP signaling. GNAS somatic mutations have been found in pituitary (36%), pancreatic (20%), ovarian (11%), adrenal gland (6%), and colon (6%) cancers. Patients with somatic GNAS mutations may derive benefit from clinical trials with MEK inhibitors. Germline mutations of GNAS have been shown to be the cause of McCune-Albright syndrome (MAS), a disorder marked by endocrine, dermatologic, and bone abnormalities. GNAS is usually found as a mosaic mutation in patients. Loss of function mutations are associated with pseudohypoparathyroidism and pseudopseudohypoparathyroidism.
Her2/Neu	ErbB2/Her2 encodes a member of the epidermal growth factor (EGF) receptor family of receptor tyrosine kinases. Her2 has no ligand-binding domain of its own and, therefore, cannot bind growth factors. It does, however, bind tightly to other ligand-bound EGF receptor family members to form a heterodimer and enhances kinase-mediated activation of downstream signaling pathways leading to cell proliferation. Her2 is overexpressed in 15-30% of newly diagnosed breast cancers. Clinically, Her2 is a target for the monoclonal antibodies trastuzumab and pertuzumab which bind to the receptor extracellularly; the kinase inhibitor lapatinib binds and blocks the receptor intracellularly.
HRAS	HRAS (homologous to the oncogene of the Harvey rat sarcoma virus), together with KRAS and NRAS, belong to the superfamily of RAS GTPase. RAS protein activates RAS-MEK-ERK/MAPK kinase cascade and controls intracellular signaling pathways involved in fundamental cellular processes such as proliferation, differentiation, and apoptosis. Mutant Ras proteins are persistently GTP-bound and active, causing severe dysregulation of the effector signaling. HRAS mutations have been identified in cancers from the urinary tract (10%-40%), skin (6%) and thyroid (4%) and they account for 3% of all RAS mutations identified in cancer. RAS mutations (especially HRAS mutations) occur (5%) in cutaneous squamous cell carcinomas and keratoacanthomas that develop in patients treated with BRAF inhibitor vemurafenib, likely due to the paradoxical activation of the MAPK pathway. Various clinical trials (on www.clinicaltrials.gov) investigating agents which target this gene and/or its downstream or upstream effectors may be available for HRAS mutated patients. Germline mutation in HRAS has been associated with Costello syndrome, a genetic disorder that is characterized by delayed development and mental retardation and distinctive facial features and heart abnormalities.
IDH1	IDH1 encodes for isocitrate dehydrogenase in cytoplasm and is found to be mutated in 60-90% of secondary gliomas, 75% of cartilaginous tumors, 17% of thyroid tumors, 15% of cholangiocarcinoma, 12-18% of patients with acute myeloid leukemia, 5% of primary gliomas, 3% of prostate cancer, as well as in less than 2% in paragangliomas, colorectal cancer and melanoma. Mutated IDH1 results in impaired catalytic function of the enzyme, thus altering normal physiology of cellular respiration and metabolism. IDH1 mutation can also cause overproduction of onco-metabolite 2-hydroxy-glutarate, which can extensively alter the methylation profile in cancer. In gliomas, IDH1 mutations are associated with lower-grade astrocytomas and oligodendrogliomas (grade II/III), as well as secondary glioblastoma. IDH gene mutations are associated with markedly better survival in patients diagnosed with malignant astrocytoma, and clinical data support a more aggressive surgery for IDH1 mutated patients because these individuals may be able to achieve long-term survival. In contrast, IDH1 mutation is associated with a worse prognosis in AML. In glioblastoma, IDH1 mutation has been associated with significantly better response to alkylating agent temozolomide. Various clinical trials (on www.clinicaltrials.gov) investigating agents which target this gene and/or its downstream or upstream effectors may be available for IDH1 mutated patients.
JAK2	JAK2 or Janus kinase 2 is a part of the JAK/STAT pathway which mediates multiple cellular responses to cytokines and growth factors including proliferation and cell survival. It is also essential for numerous developmental and homeostatic processes, including hematopoiesis and immune cell development. Mutations in the JAK2 kinase domain result in constitutive activation of the kinase and the development of chronic myeloproliferative neoplasms such as polycythemia vera (95%), essential thrombocythemia (50%) and myelofibrosis (50%). JAK2 mutations were also found in BCR-ABL1-negative acute lymphoblastic leukemia patients and the mutated patients show a poor outcome. Various clinical trials (on www.clinicaltrials.gov) investigating agents which target this gene and/or its downstream or upstream effectors may be available for patients carrying JAK2 mutation. Germline mutations in JAK2 have been associated with myeloproliferative neoplasms and thrombocythemia.
KDR	KDR (VEGFR2) or Kinase insert domain receptor gene, also known as vascular endothelial growth factor receptor-2 (VEGFR2), is involved with angiogenesis and is expressed on almost all endothelial cells. VEGF ligands bind to KDR, which leads to receptor dimerization and signal transduction. Besides somatic mutations in angiosarcoma (10%), somatic KDR mutations have also been found in colon (13%), skin (13%), gastric (5%), lung (3%), renal (2%), and ovarian (2%) cancers. Several VEGFR antagonists are either FDA-approved or in clinical trials (i.e. bevacizumab, cabozantinib, regorafenib, pazopanib, and vandetanib). Various clinical trials (on www.clinicaltrials.gov) investigating agents which target this gene and/or its downstream or upstream effectors may be available for KDR mutated patients.
KRAS	Proto-oncogene of the Kirsten murine sarcoma virus (KRAS) is a signaling intermediate involved in many signaling cascades including the EGFR pathway. Mutations at activating hotspots are associated with resistance to EGFR tyrosine kinase inhibitors (erlotinib, gefitinib) in NSCLC and monoclonal antibodies (cetuximab, panitumumab) in CRC patients. Retrospective clinical studies raised the possibility that KRAS G13D mutations may not be absolutely predictive of non-response; however, this finding is not supported by published analysis of 3 randomized controlled phase III trials. Other targeted agents under clinical investigation (on www.clinicaltrials.gov) may be available for KRAS mutated patients.
MGMT	O-6-methylguanine-DNA methyltransferase (MGMT) encodes a DNA repair enzyme. MGMT expression is mainly regulated at the epigenetic level through CpG island promoter methylation which in turn causes functional silencing of the gene. MGMT methylation and/or low expression has been correlated with response to alkylating agents like temozolomide and dacarbazine.
MPL	MPL or myeloproliferative leukemia gene encodes the thrombopoietin receptor, which is the main humoral regulator of thrombopoiesis in humans. MPL mutations cause constitutive activation of JAK-STAT signaling and have been detected in 5-7% of patients with primary myelofibrosis (PMF) and 1% of those with essential thrombocythemia (ET).

SAMPLE REPORT

FIG. 29U

BIOMARKER DESCRIPTION

Target	Biomarker Description
NOTCH1	NOTCH1 or notch homolog 1, translocation-associated, encodes a member of the Notch signaling network, an evolutionary conserved pathway that regulates developmental processes by regulating interactions between physically adjacent cells. Mutations in NOTCH1 play a central role in disruption of micro environmental communication, potentially leading to cancer progression. Due to the dual, bi-directional signaling of NOTCH1, activating mutations have been found in acute lymphoblastic leukemia and chronic lymphocytic leukemia, however loss of function mutations in NOTCH1 are prevalent in 11-15% of head and neck squamous cell carcinoma. NOTCH1 mutations have also been found in 2% of glioblastomas, 1% of ovarian cancers, 10% lung adenocarcinomas, 8% of squamous cell lung cancers and 5% of breast cancers. Notch pathway-directed therapy approaches differ depending on whether the tumor harbors gain or loss of function mutations, thus are classified as Notch pathway inhibitors or activators, respectively. Some Notch pathway modulators are being investigated (on www.clinicaltrials.gov) for NOTCH1 mutated patients.
NRAS	NRAS is an oncogene and a member of the (GTPase) ras family, which includes KRAS and HRAS. This biomarker has been detected in multiple cancers including melanoma, colorectal cancer, AML and bladder cancer. Evidence suggests that an acquired mutation in NRAS may be associated with resistance to vemurafenib in melanoma patients. In colorectal cancer patients NRAS mutation is associated with resistance to EGFR-targeted monoclonal antibodies.
PD-1	PD-1 - or programmed death 1 is a co-inhibitory receptor expressed on activated T, B and NK cells, and tumor infiltrating lymphocytes (TIL). PD-1 is a negative regulator of the immune system and inhibits the proliferation and effector function of the lymphocytes after binding with its ligands including PD-L1. PD-1/PD-L1 signaling pathway functions to attenuate or escape antitumor immunity by maintaining an immunosuppressive tumor microenvironment. Studies show that the presence of PD-1+ TIL is associated with a poor prognosis in various cancer types including lymphoma and breast cancer. Evidence suggests HER2 positive breast cancer patients with high levels of PD-1 respond well to trastuzumab. Anti PD-1 therapies may enhance endogenous antitumor immunity and is under investigation in multiple cancer types.
PDGFRA	PDGFRA is the alpha-type platelet-derived growth factor receptor, a surface tyrosine kinase receptor structurally homologous to c-KIT, which activates PIK3CA/AKT, RAS/MAPK and JAK/STAT signaling pathways. PDGFRA mutations are found in 5-8% of patients with gastrointestinal stromal tumors (GIST) and increases to 35% in KIT wildtype GIST. PDGFRA mutations in exons 12, 14 and 18 confer imatinib sensitivity, while the substitution mutation in exon 18 (D842V) shows resistance to imatinib. Various clinical trials (on www.clinicaltrials.gov) investigating multikinase inhibitors may be available for PDGFRA mutated patients. Germline mutations in PDGFRA have been associated with Familial gastrointestinal stromal tumors and Hypereosinophilic Syndrome (HES).
PD-L1	PD-L1 - or programmed cell death ligand 1, is a glycoprotein expressed in various tumor types and is associated with poor outcome. Upon binding to its receptor, PD-1, the PD-1/PD-L1 interaction functions to negatively regulate the immune system, attenuating antitumor immunity by maintaining an immunosuppressive tumor microenvironment. PD-L1 expression is upregulated in tumor cells through activation of common oncogenic pathways or exposure to inflammatory cytokines. Assessment of PD-L1 offers information on patient prognosis and also represents a target for immune manipulation in treatment of solid tumors. Clinical trials are currently recruiting patients with various tumor types testing immunomodulatory agents.
PGP	P-glycoprotein (MDR1, ABCB1) is an ATP-dependent, transmembrane drug efflux pump with broad substrate specificity, which pumps antitumor drugs out of cells. Its expression is often induced by chemotherapy drugs and is thought to be a major mechanism of chemotherapy resistance. Overexpression of p-gp is associated with resistance to anthracyclines (doxorubicin, epirubicin). P-gp remains the most important and dominant representative of Multi-Drug Resistance phenotype and is correlated with disease state and resistant phenotype.
PIK3CA	The hot spot missense mutations in the gene PIK3CA are present in various malignancies including breast, colon and NSCLC resulting in activation of the PI3 kinase pathway. This pathway is an active target for drug development. PIK3CA exon 20 mutations have been associated with benefit from mTOR inhibitors (everolimus, temsirolimus). Evidence suggests that breast cancer patients with activation of the PI3K pathway due to PTEN loss or PIK3CA mutation/amplification have a significantly shorter survival following trastuzumab treatment. PIK3CA mutation causes reduced response to EGFR targeted therapies in colorectal cancer and NSCLC patients. Various clinical trials (on www.clinicaltrials.gov) investigating agents which target this gene may be available for PIK3CA mutated patients.
PR	The progesterone receptor (PR or PGR) is an intracellular steroid receptor that specifically binds progesterone, an important hormone that fuels breast cancer growth. PR positivity in a tumor indicates that the tumor is more likely to be responsive to hormone therapy by anti-estrogens, aromatase inhibitors and progestogens.
PTEN	PTEN (phosphatase and tensin homolog) is a tumor suppressor gene that prevents cells from proliferating. Loss of PTEN protein is one of the most common occurrences in multiple advanced human cancers. PTEN is an important mediator in signaling downstream of EGFR, and its loss is associated with reduced benefit to trastuzumab in breast cancer and EGFR-targeted therapies in CRC and NSCLC.
RET	RET or rearranged during transfection gene, located on chromosome 10, activates cell signaling pathways involved in proliferation and cell survival. RET mutations are found in 23-65% of sporadic medullary thyroid cancers (MTC), but RET fusions are common in papillary thyroid cancer, and more recently have been found in 1-2% of lung adenocarcinoma. Amassyst RET mutations in sporadic MTC, 85% involve the M918T mutation which is associated with a higher response rate to vandetanib in comparison to M918T negative patients. Further, a 10-year study notes that medullary thyroid cancer patients with somatic RET mutations have a poorer prognosis. Various clinical trials (on www.clinicaltrials.gov) investigating multikinase inhibitors which include RET as one of the targets may be available for RET mutated patients. Germline activating mutations of RET are associated with multiple endocrine neoplasia type 2 (MEN2), which is characterized by the presence of medullary thyroid carcinoma, bilateral pheochromocytoma, and primary hyperparathyroidism. Germline inactivating mutations of RET are associated with Hirschsprung's disease.
RRM1	Ribonucleotide reductase subunit M1 (RRM1) is a component of the ribonucleotide reductase holoenzyme consisting of M1 and M2 subunits. The ribonucleotide reductase is a rate-limiting enzyme involved in the production of nucleotides required for DNA synthesis. Gemcitabine is a deoxycytidine analogue which inhibits ribonucleotide reductase activity. High RRM1 level is associated with resistance to gemcitabine.

SAMPLE REPORT FOR ILLUSTRATIVE PURPOSES ONLY

FIG. 29V

BIOMARKER DESCRIPTION

Target	Biomarker Description
SMO	SMO (smoothened) is a G protein-coupled receptor which plays an important role in the Hedgehog signaling pathway. It is a key regulator of cell growth and differentiation during development, and is important in epithelial and mesenchymal interaction in many tissues during embryogenesis. Dysregulation of the Hedgehog pathway is found in cancers including basal cell carcinomas (12%) and medulloblastoma (1%). A gain-of-function mutation in SMO results in constitutive activation of hedgehog pathway signaling, contributing to the genesis of basal cell carcinoma. SMO mutations have been associated with the resistance to SMO antagonist GDC-0449 in medulloblastoma patients by blocking the binding to SMO. SMO mutation may also contribute partially to resistance to SMO antagonist LDE225 in BCC. Various clinical trials (on www.clinicaltrials.gov) investigating SMO antagonists may be available for SMO mutated patients.
SPARC Monoclonal	SPARC Monoclonal (secreted protein acidic and rich in cysteine) is a calcium-binding matricellular glycoprotein secreted by many types of cells. It has a normal role in wound repair, cell migration, and cell-matrix interactions. Its over-expression is thought to have a role in tumor invasion and angiogenesis. A few studies indicate that SPARC over-expression improves the response to the anti cancer drug, nab-paclitaxel. The improved response is thought to be related to SPARC's role in accumulating albumin and albumin targeted agents within tumor tissue.
SPARC Polyclonal	SPARC Polyclonal (secreted protein acidic and rich in cysteine) is a calcium-binding matricellular glycoprotein secreted by many types of cells. It has a normal role in wound repair, cell migration, and cell-matrix interactions. Its over-expression is thought to have a role in tumor invasion and angiogenesis. A few studies indicate that SPARC over-expression improves the response to the anti cancer drug, nab-paclitaxel. The improved response is thought to be related to SPARC's role in accumulating albumin and albumin targeted agents within tumor tissue.
TLE3	TLE3 is a member of the transducin-like enhancer of split (TLE) family of proteins that have been implicated in tumorigenesis. It acts downstream of APC and beta-catenin to repress transcription of a number of oncogenes, which influence growth and microtubule stability. Studies indicate that TLE3 expression is associated with response to taxane therapy.
TOP2A	TOPOIIA is an enzyme that alters the supercoiling of double-stranded DNA and allows chromosomal segregation into daughter cells. Due to its essential role in DNA synthesis and repair, and frequent overexpression in tumors, TOPOIIA is an ideal target for antineoplastic agents. Amplification of TOPOIIA with or without HER2 co-amplification, as well as high protein expression of TOPOIIA, have been associated with benefit from anthracycline based therapy.
TOPO1	Topoisomerase I is an enzyme that alters the supercoiling of double-stranded DNA. TOPO1 acts by transiently cutting one strand of the DNA to relax the coil and extend the DNA molecule. High expression of TOPO1 has been associated with response to TOPO1 inhibitors including irinotecan and topotecan.
TP53	TP53, or p53, plays a central role in modulating response to cellular stress through transcriptional regulation of genes involved in cell-cycle arrest, DNA repair, apoptosis, and senescence. Inactivation of the p53 pathway is essential for the formation of the majority of human tumors. Mutation in p53 (TP53) remains one of the most commonly described genetic events in human neoplasia, estimated to occur in 30-50% of all cancers. Generally, presence of a disruptive p53 mutation is associated with a poor prognosis in all types of cancers, and diminished sensitivity to radiation and chemotherapy. In addition, various clinical trials (on www.clinicaltrials.gov) investigating agents which target p53's downstream or upstream effectors may have clinical utility depending on the p53 status. Germline p53 mutations are associated with the Li-Fraumeni syndrome (LFS) which may lead to early-onset of several forms of cancer currently known to occur in the syndrome, including sarcomas of the bone and soft tissues, carcinomas of the breast and adrenal cortex (hereditary adrenocortical carcinoma), brain tumors and acute leukemias.
TS	Thymidylate synthase (TS) is an enzyme involved in DNA synthesis that generates thymidine monophosphate (dTMP), which is subsequently phosphorylated to thymidine triphosphate for use in DNA synthesis and repair. Low levels of TS are predictive of response to fluoropyrimidines and other folate analogues.
TUBB3	Class III β-Tubulin (TUBB3) is part of a class of proteins that provide the framework for microtubules, major structural components of the cytoskeleton. Due to their importance in maintaining structural integrity of the cell, microtubules are ideal targets for anti-cancer agents. Low expression of TUBB3 is associated with potential clinical benefit to taxane therapy.
VHL	VHL or von Hippel-Lindau gene encodes for tumor suppressor protein pVHL, which polyubiquitylates hypoxia-inducible factor. Absence of pVHL causes stabilization of HIF and expression of its target genes, many of which are important in regulating angiogenesis, cell growth and cell survival. VHL somatic mutation has been seen in 20-76% of patients with sporadic clear cell renal cell carcinoma (ccRCC) and the mutation may imply a poor prognosis, adverse pathological features, and increased tumor grade or lymph-node involvement. Renal cell cancer patients with a 'loss of function' mutation in VHL show a higher response rate to therapy (bevacizumab or sorafenib) than is seen in patients with wild type VHL, however the mutation is not associated with improvement in progression free survival or overall survival. Various clinical trials (on www.clinicaltrials.gov) investigating angiogenesis inhibitors in various cancer types may be available for VHL mutated patients. Germline mutations in VHL cause von Hippel-Lindau syndrome, associated with clear-cell renal-cell carcinomas, central nervous system hemangioblastomas, pheochromocytomas and pancreatic tumors.

SAMPLE

FIG. 29W

LITERATURE LEVEL OF EVIDENCE ASSESSMENT FRAMEWORK

Study Design	
Hierarchy of Design	Criteria
I	Evidence obtained from at least one properly designed **randomized controlled trial**.
II-1	Evidence obtained from well-designed controlled trials **without randomization**.
II-2	Evidence obtained from well-designed **cohort** or **case-control** analytic studies, preferably from more than one center or research group.
II-3	Evidence obtained from multiple time series with or without the intervention. Dramatic results in uncontrolled trials might also be regarded as this type of evidence.
III	Opinions of respected authorities, based on clinical experience, descriptive studies, or reports of expert committees.

Study Validity	
Grade	Criteria
Good	The study is judged to be valid and relevant as regards results, statistical analysis, and conclusions and shows no significant flaws.
Fair	The study is judged to be valid and relevant as regards results, statistical analysis, and conclusions, but contains at least one significant but not fatal flaw.
Poor	The study is judged to have a fatal flaw such that the conclusions are not valid for the purposes of this test.

[1] Adapted from Harris, T., G. Atkins, et al. (2001). "Current Methods of the U.S. Preventive Services Task Force." Am J Prev Med 2008.[2]

SAMPLE REPORT. ILLUSTRATIVE PURPOSES ONLY. NOT FOR CLINICAL USE.

FIG. 29X

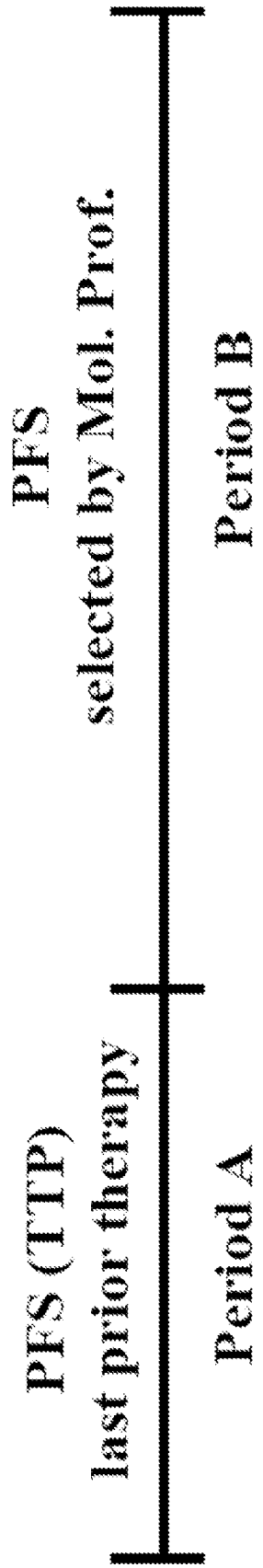

PFS (TTP)
last prior therapy
PFS
selected by Mol. Prof.
Period A
Period B

FIG. 30

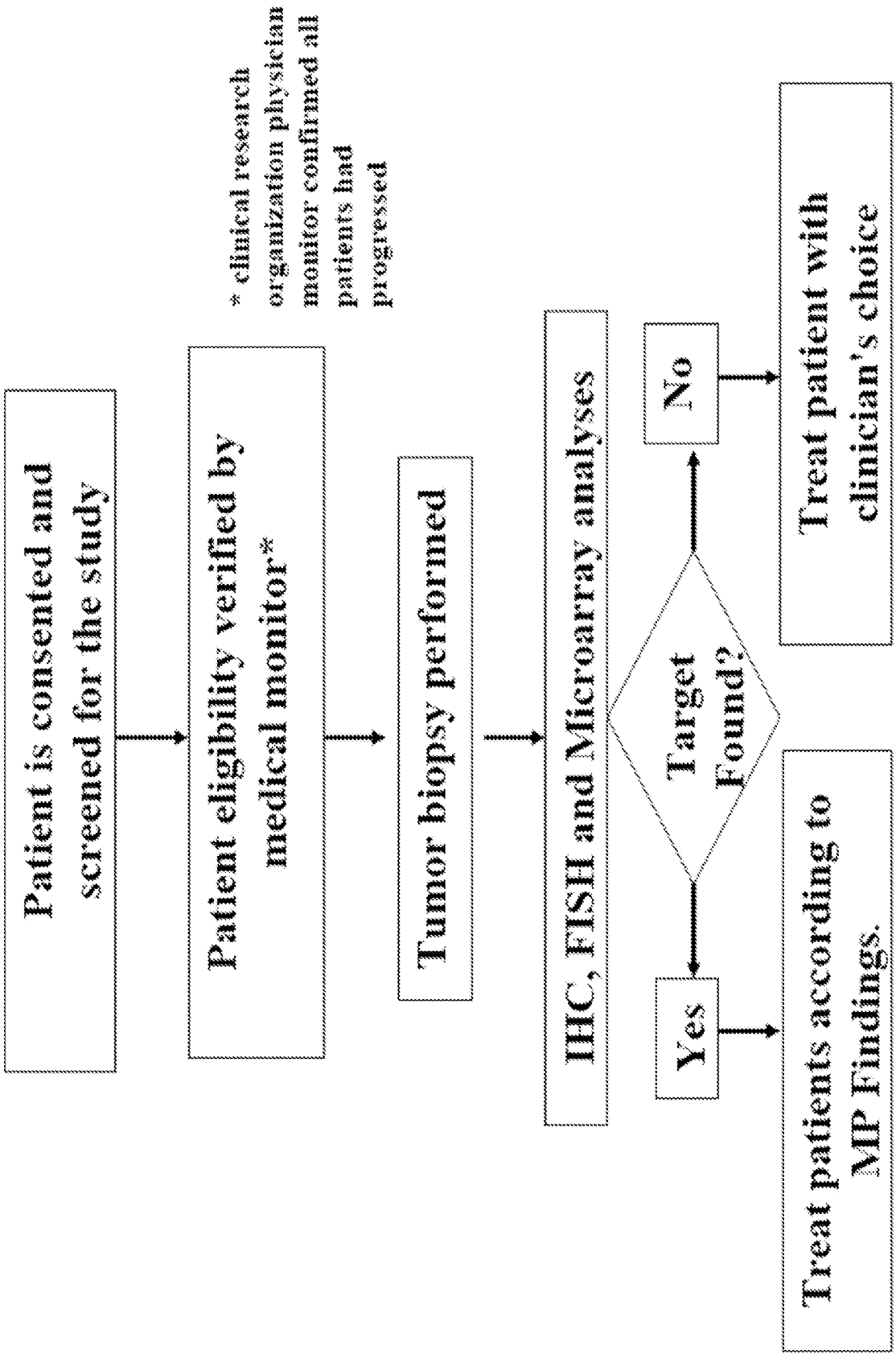
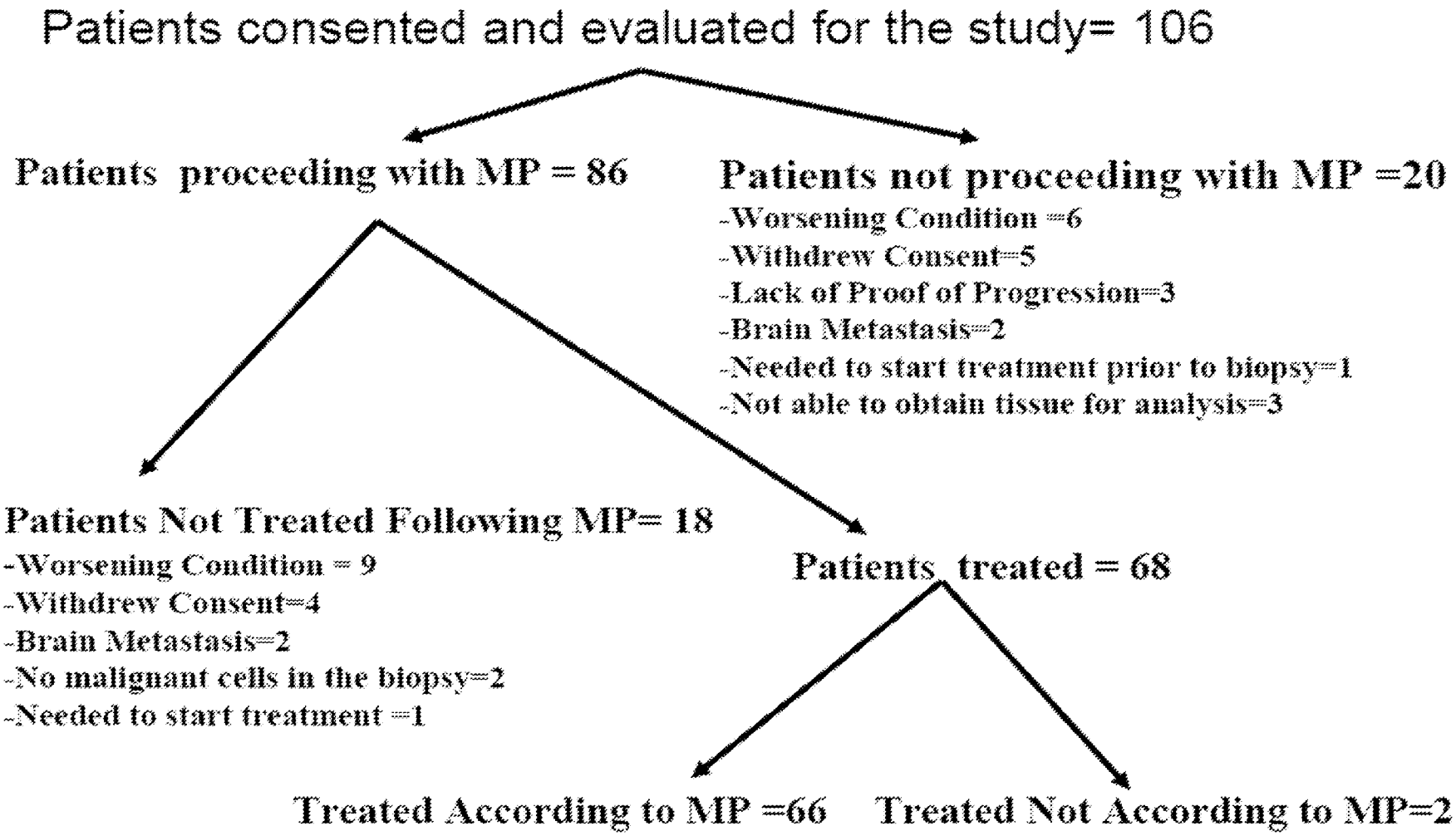

Patient is consented and screened for the study
Patient eligibility verified by medical monitor*
* clinical research organization physician monitor confirmed all patients had progressed
Tumor biopsy performed
IHC, FISH and Microarray analyses
Target Found?
Yes
No
Treat patients according to MP Findings.
Treat patient with clinician's choice

FIG. 31



Note: Median time for MP results available to a clinician = 16 days from biopsy and 8 days from reception of tissue samples for analyses

FIG. 32

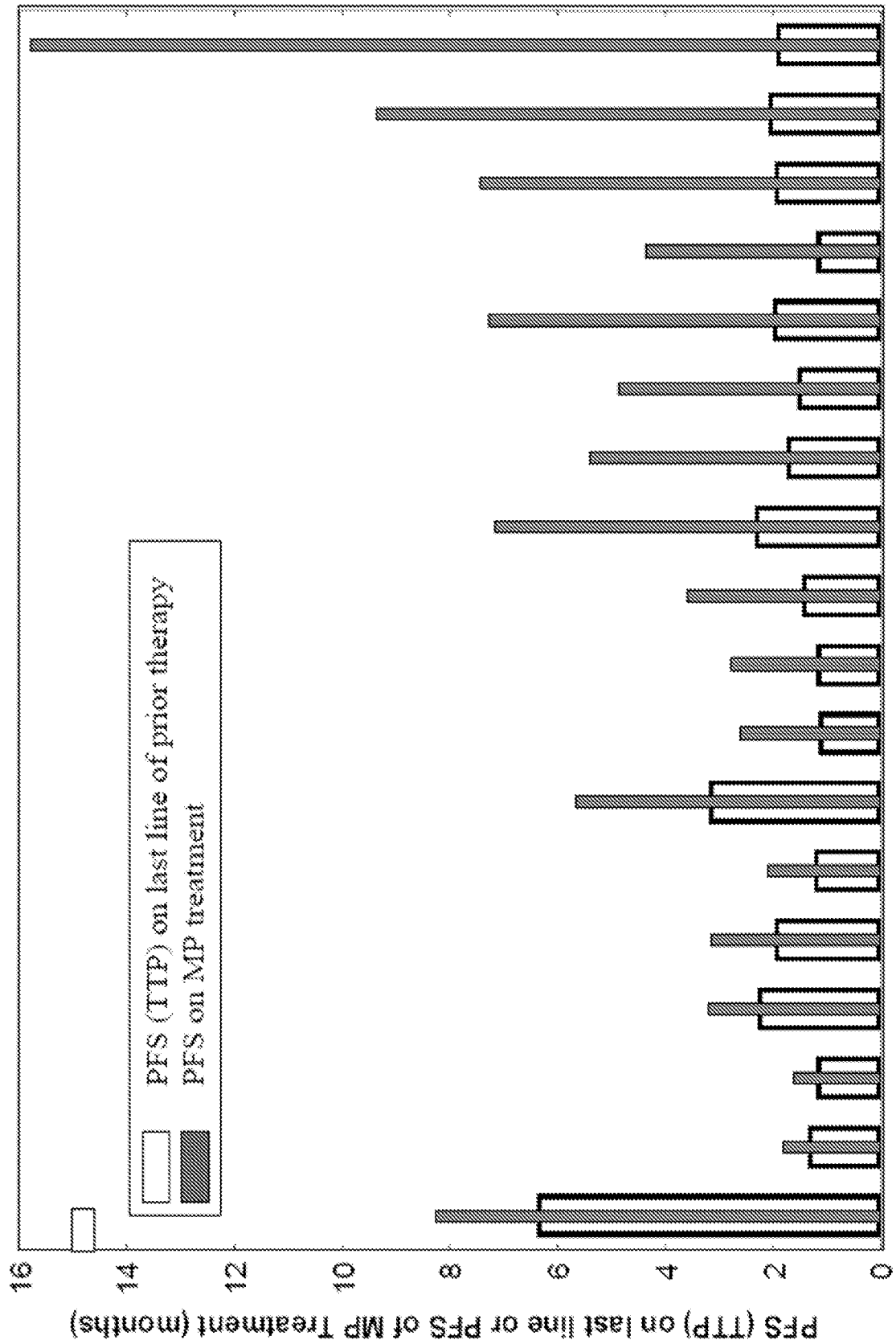

PFS (TTP) on last line or PFS of MP Treatment (months)
0
2
4
6
8
10
12
14
16
PFS (TTP) on last line of prior therapy
PFS on MP treatment
Patients with PFS ratio ≥ 1.3

FIG. 33

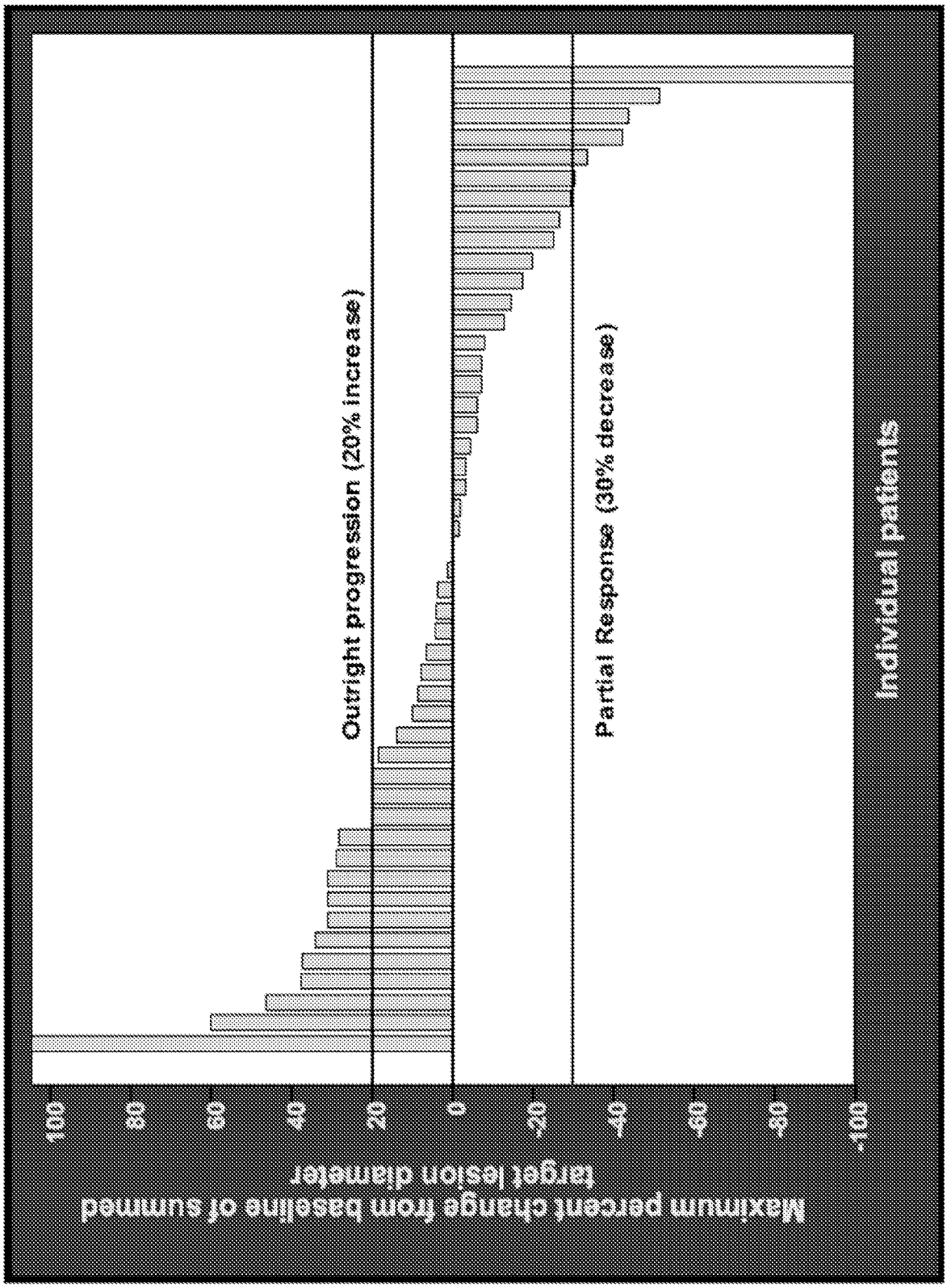

Maximum percent change from baseline of summed target lesion diameter
100
80
60
40
20
0
-20
-40
-60
-80
-100
Outright progression (20% increase)
Partial Response (30% decrease)
Individual patients

FIG. 34

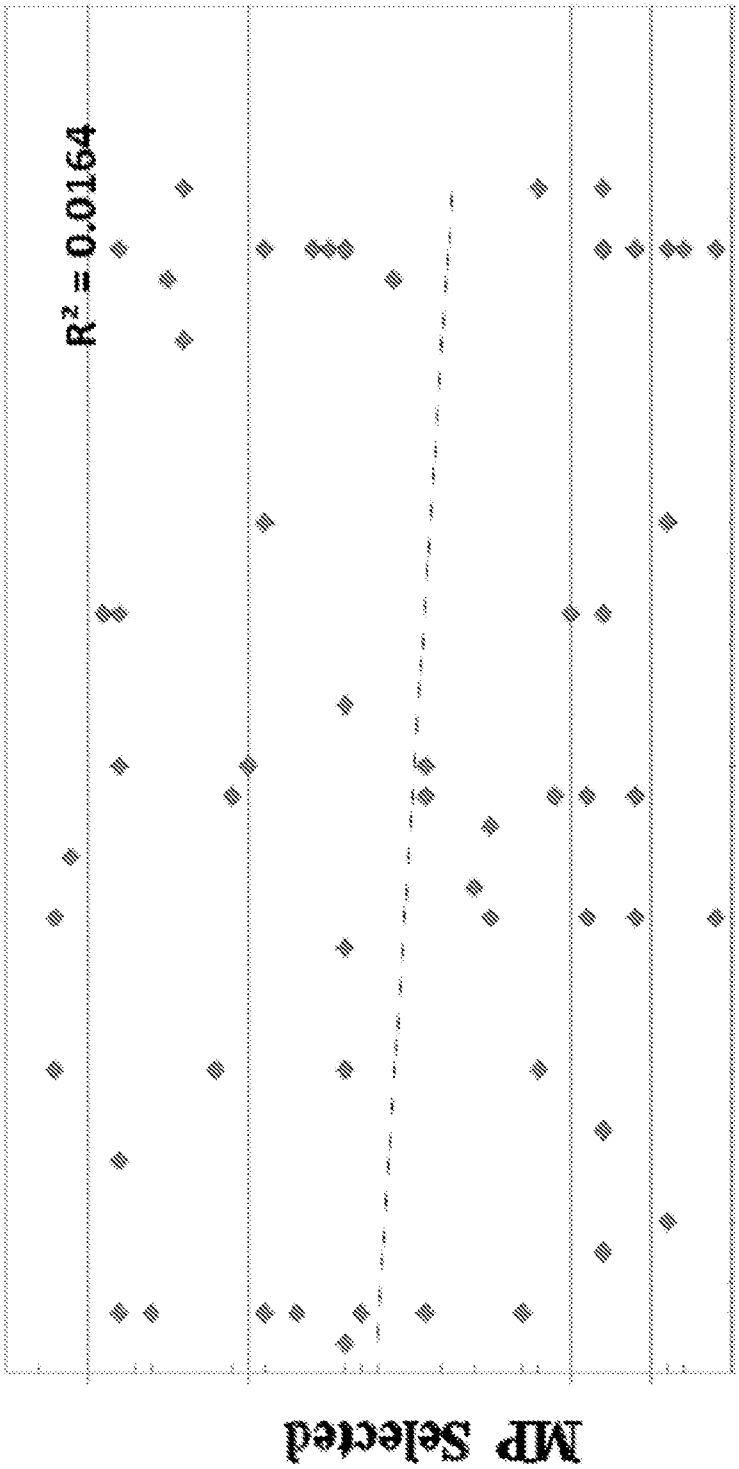


FIG. 35

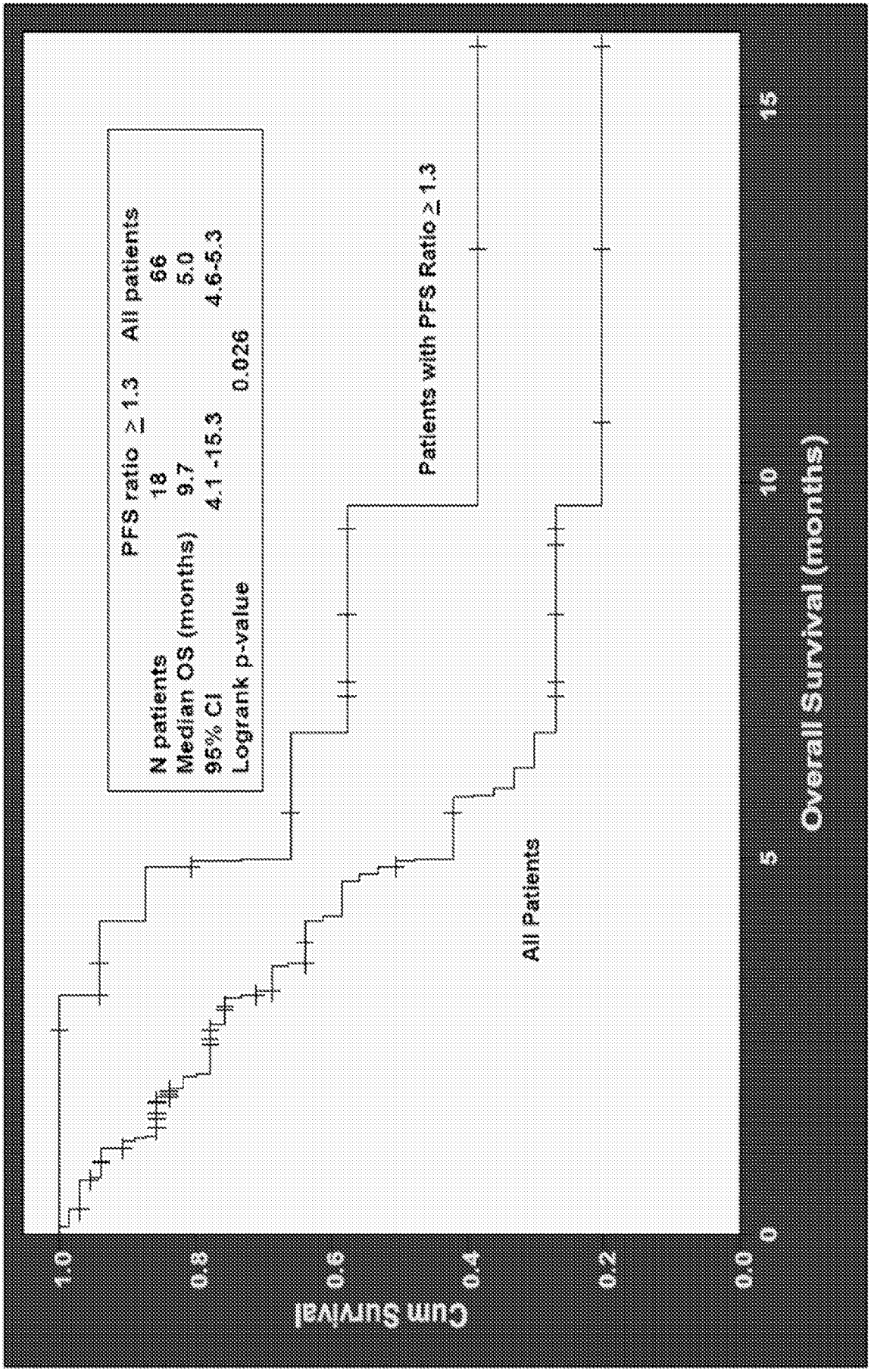

PFS ratio ≥ 1.3
All patients
N patients
18
66
Median OS (months)
9.7
5.0
95% CI
4.1 -15.3
4.6-5.3
Logrank p-value
0.026
Patients with PFS Ratio ≥ 1.3
All Patients
Cum Survival
1.0
0.8
0.6
0.4
0.2
0.0
0
5
10
15
Overall Survival (months)

FIG. 36

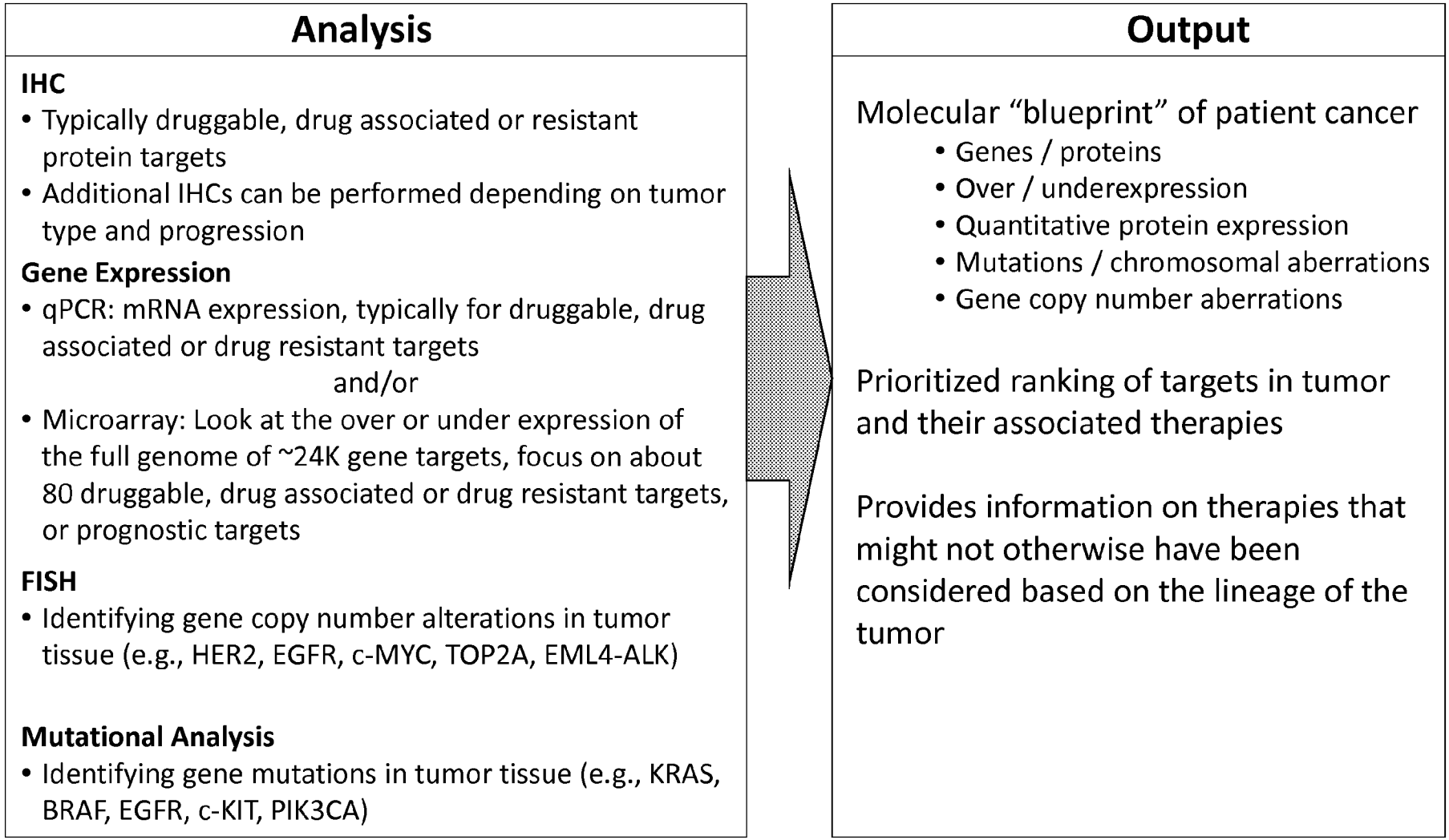
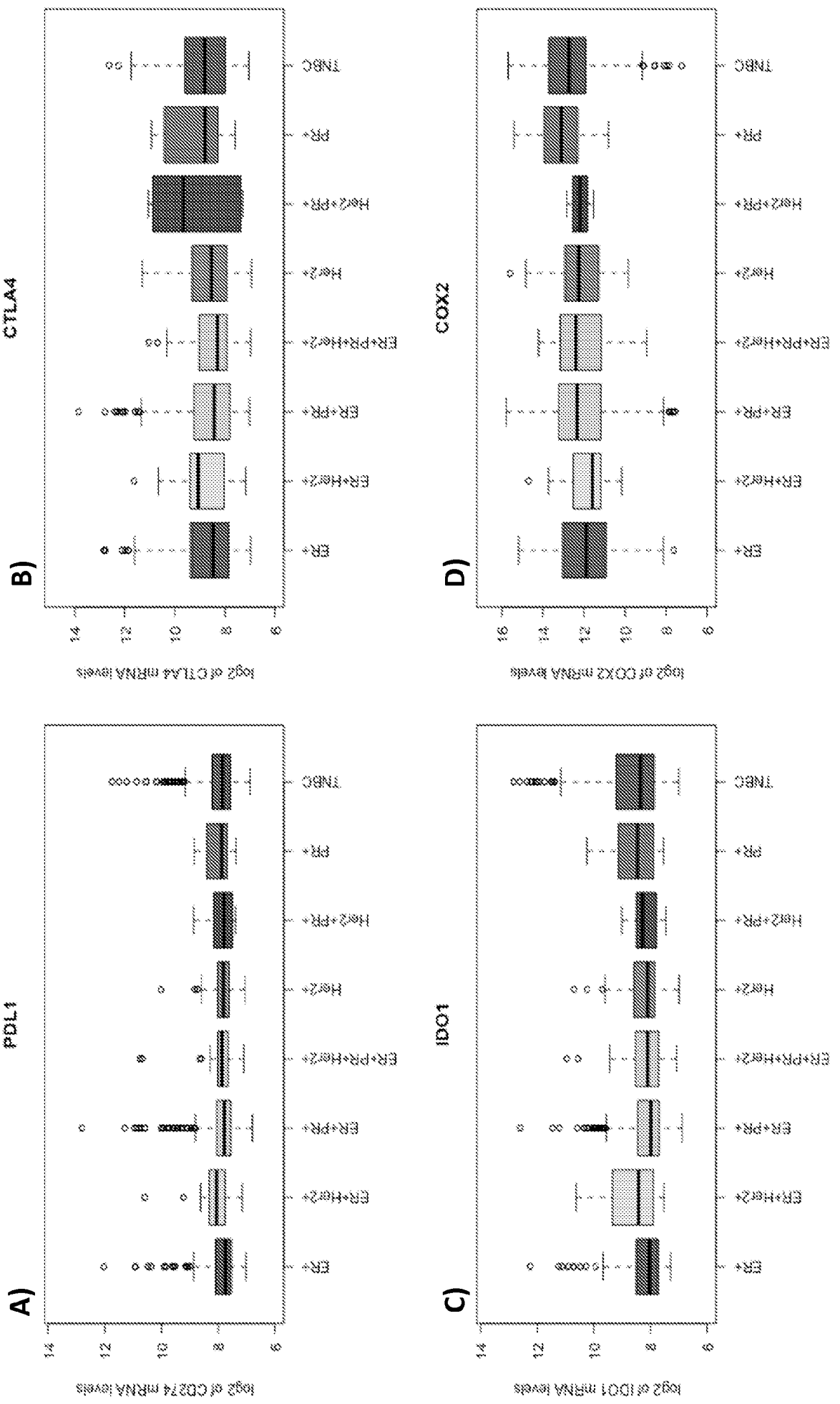

Analysis
IHC
• Typically druggable, drug associated or resistant protein targets
• Additional IHCs can be performed depending on tumor type and progression
Gene Expression
• qPCR: mRNA expression, typically for druggable, drug associated or drug resistant targets
and/or
• Microarray: Look at the over or under expression of the full genome of ~24K gene targets, focus on about 80 druggable, drug associated or drug resistant targets, or prognostic targets
FISH
• Identifying gene copy number alterations in tumor tissue (e.g., HER2, EGFR, c-MYC, TOP2A, EML4-ALK)
Mutational Analysis
• Identifying gene mutations in tumor tissue (e.g., KRAS, BRAF, EGFR, c-KIT, PIK3CA)
Output
Molecular "blueprint" of patient cancer
• Genes / proteins
• Over / underexpression
• Quantitative protein expression
• Mutations / chromosomal aberrations
• Gene copy number aberrations
Prioritized ranking of targets in tumor and their associated therapies
Provides information on therapies that might not otherwise have been considered based on the lineage of the tumor

FIG. 37

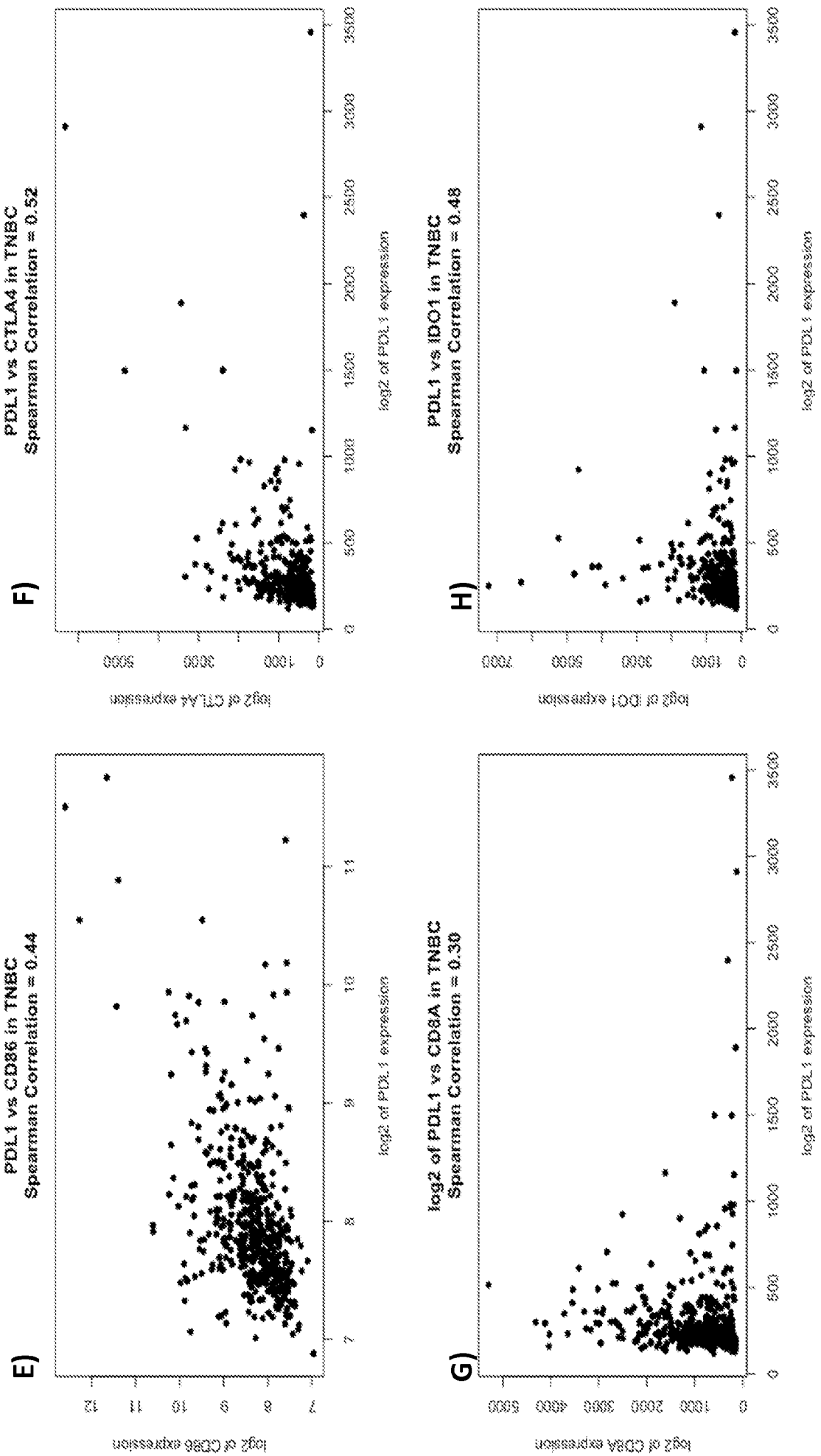
Microarray Analysis on Formalin-Fixed Tissue for RNA Expression

Gene	Ratio	Expression	Result
KIT	0.10	Indeterminate	
TOP2B	0.22	Under Expressed*	
EGFR	0.23	Indeterminate	
MLH1	0.29	Indeterminate	
CD33	0.31	Indeterminate	
SSTR2	0.31	Indeterminate	
AR	0.42	Indeterminate	
ERCC3	0.43	Indeterminate	
PDGFRA	0.44	Under Expressed*	
SPARC	0.45	Under Expressed*	
PGR	0.50	Indeterminate	
RAF1	0.50	Under Expressed*	
GART	0.55	Under Expressed*	
FOLR2	0.57	Under Expressed*	
ADA	0.57	Under Expressed*	
POLA1	0.59	Indeterminate	
ZAP70	0.59	Indeterminate	
ESR1	0.63	Indeterminate	
LCK	0.63	Indeterminate	
TXNRD1	0.64	Indeterminate	
SSTR1	0.65	Indeterminate	
KDR	0.66	Indeterminate	
PDGFC	0.68	No Change	
FYN	0.71	No Change	
IDCK	0.71	No Change	
ASNS	0.75	No Change	
BCL2	0.77	Indeterminate	
RRM1	0.79	No Change	
PDGFRB	0.81	No Change	
NFKB1	0.93	No Change	
LYN	0.94	No Change	
DNMT3A	0.94	Indeterminate	
CD52	0.95	Indeterminate	
TOP2A	0.97	Indeterminate	
RXRG	0.97	Indeterminate	
HCK	0.97	No Change	
PTEN	1.01	No Change	
BRCA1	1.01	Indeterminate	
FLT1	1.04	Indeterminate	
DNMT3B	1.05	Indeterminate	
SSTR3	1.06	Indeterminate	
DNMT1	1.11	No Change	
IL2RA	1.12	Indeterminate	
SSTR4	1.13	Indeterminate	
BRCA2	1.16	Indeterminate	
HDAC1	1.17	No Change	
GNRH1	1.18	No Change	
ERCC1	1.19	Indeterminate	
RRM2B	1.19	No Change	
RXRB	1.20	No Change	
MS4A1	1.20	Indeterminate	
ERBB2	1.23	No Change	
TOP1	1.25	Indeterminate	
YES1	1.25	No Change	
DHFR	1.27	No Change	
ABCG2	1.28	No Change	
TNF	1.31	Indeterminate	
NFKB2	1.32	Indeterminate	
CDA	1.33	No Change	
HSP90AA1	1.34	No Change	
RARA	1.42	Indeterminate	
CES2	1.44	No Change	
TYMS	1.50	Indeterminate	
HIF1A	1.64	Over Expressed	✓
SRC	1.64	Indeterminate	
MGMT	1.73	Over Expressed	✓
VDR	1.83	Indeterminate	
GSTP1	1.84	Over Expressed	
EGFR	1.94	Indeterminate	
TK1	1.94	Over Expressed	
BIRC5	2.14	Indeterminate	
MSH2	2.17	Indeterminate	
VEGFA	2.22	Over Expressed	
EPHA2	2.35	Indeterminate	
VHL	2.75	Over Expressed	
SSTR5	3.43	Indeterminate	
ABCC1	3.59	Indeterminate	
ECGF1	4.08	Over Expressed	
RRM2	9.01	Indeterminate	
PTGS2	9.21	Over Expressed	✓

FIG. 38


A)
PDL1
log2 of CD274 mRNA levels
14
12
10
8
6
ER+
ER+Her2+
ER+PR+
Her2+PR+
Her2+
ER+PR+Her2+
ER+PR+
Her2+
PR+
TNBC
B)
CTLA4
log2 of CTLA4 mRNA levels
14
12
10
8
6
ER+
ER+Her2+
ER+PR+
ER+PR+Her2+
Her2+
Her2+PR+
PR+
TNBC
C)
IDO1
log2 of IDO1 mRNA levels
14
12
10
8
6
ER+
ER+Her2+
ER+PR+
ER+PR+Her2+
Her2+
Her2+PR+
PR+
TNBC
D)
COX2
log2 of COX2 mRNA levels
16
14
12
10
8
6
ER+
ER+Her2+
ER+PR+
ER+PR+Her2+
Her2+
Her2+PR+
PR+
TNBC

FIGs. 39A-D


E)
PDL1 vs CD86 in TNBC
Spearman Correlation = 0.44
log2 of CD86 expression
log2 of PDL1 expression
F)
PDL1 vs CTLA4 in TNBC
Spearman Correlation = 0.52
log2 of CTLA4 expression
log2 of PDL1 expression
G)
log2 of PDL1 vs CD8A in TNBC
Spearman Correlation = 0.30
log2 of CD8A expression
log2 of PDL1 expression
H)
PDL1 vs IDO1 in TNBC
Spearman Correlation = 0.48
log2 of IDO1 expression
log2 of PDL1 expression

FIGs. 39E-H

Spearman correlation among 11 immune markers

	CD274	PTGS2	CTLA4	CD86	GZMA	CD8A	CD19	GZMB	CCR7	CD276	IDO1
CD274	1	-0.06967	0.52867	0.444961	0.299881	0.302173	0.177866	0.237077	0.172093	0.299227	0.48127
PTGS2	-0.06967	1	-0.12848	-0.10428	-0.14545	-0.21119	-0.05478	0.039093	-0.07109	-0.00075	-0.2324
CTLA4	0.52867	-0.12848	1	0.473677	0.408701	0.43154	0.415147	0.354364	0.504019	0.388966	0.523525
CD86	0.444961	-0.10428	0.473677	1	0.232901	0.325973	0.146208	0.063683	0.18083	0.393691	0.38288
GZMA	0.299881	-0.14545	0.408701	0.232901	1	0.726489	0.553377	0.67415	0.651042	-0.13157	0.587415
CD8A	0.302173	-0.21119	0.43154	0.325973	0.726489	1	0.471923	0.445949	0.521245	-0.10644	0.594916
CD19	0.177866	-0.05478	0.415147	0.146208	0.553377	0.471923	1	0.536941	0.749291	-0.07455	0.367209
GZMB	0.237077	0.039093	0.354364	0.063683	0.67415	0.445949	0.536941	1	0.662158	-0.13443	0.493636
CCR7	0.172093	-0.07109	0.504019	0.18083	0.651042	0.521245	0.749291	0.662158	1	-0.03657	0.420981
CD276	0.299227	-0.00075	0.388966	0.393691	-0.13157	-0.10644	-0.07455	-0.13443	-0.03657	1	0.021884
IDO1	0.48127	-0.2324	0.523525	0.38288	0.587415	0.594916	0.367209	0.493636	0.420981	0.021884	1

FIG. 39I

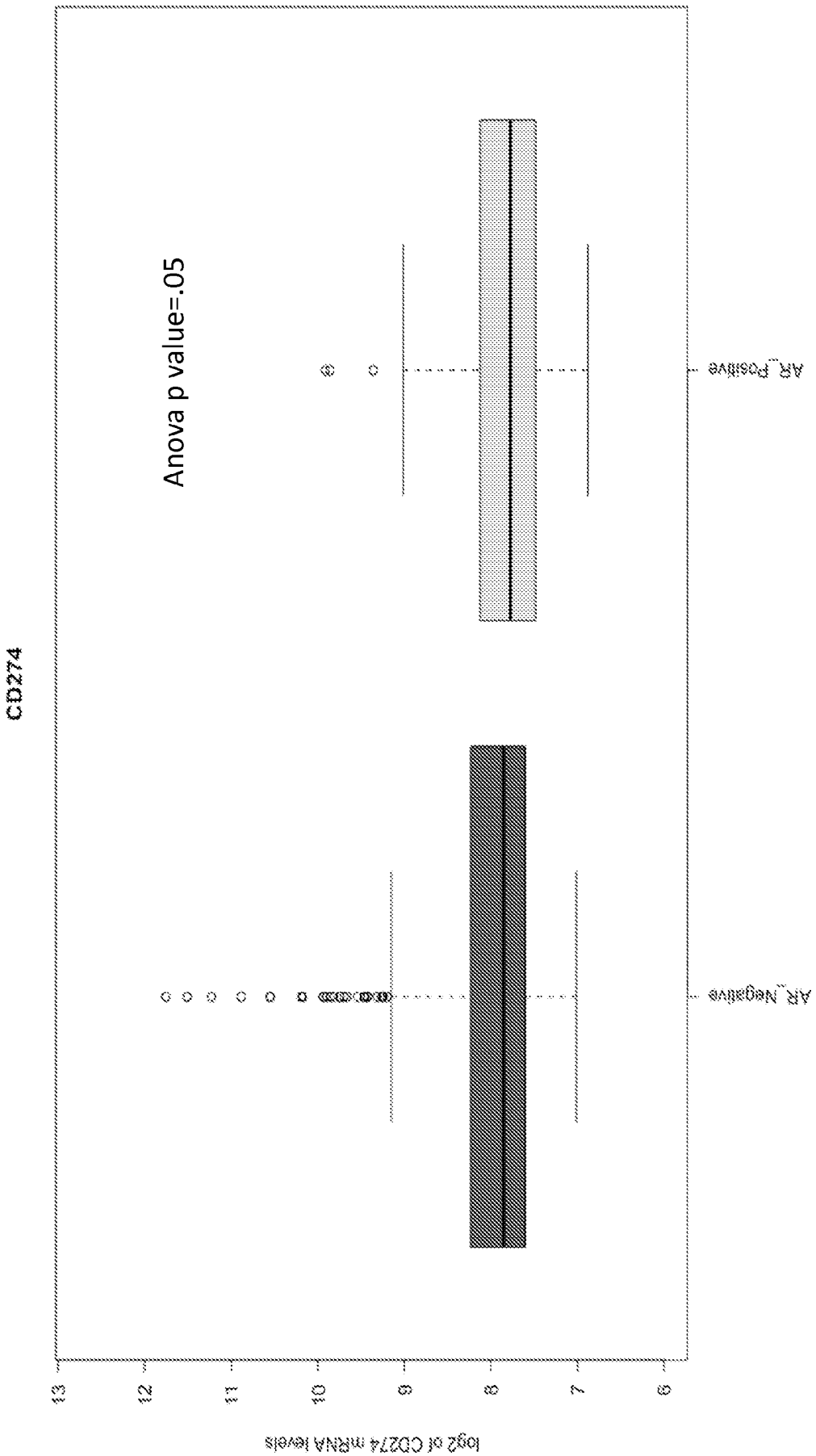

CD274
Anova p value=.05
AR_Positive
AR_Negative
log2 of CD274 mRNA levels
13
12
11
10
9
8
7
6

FIG. 39J

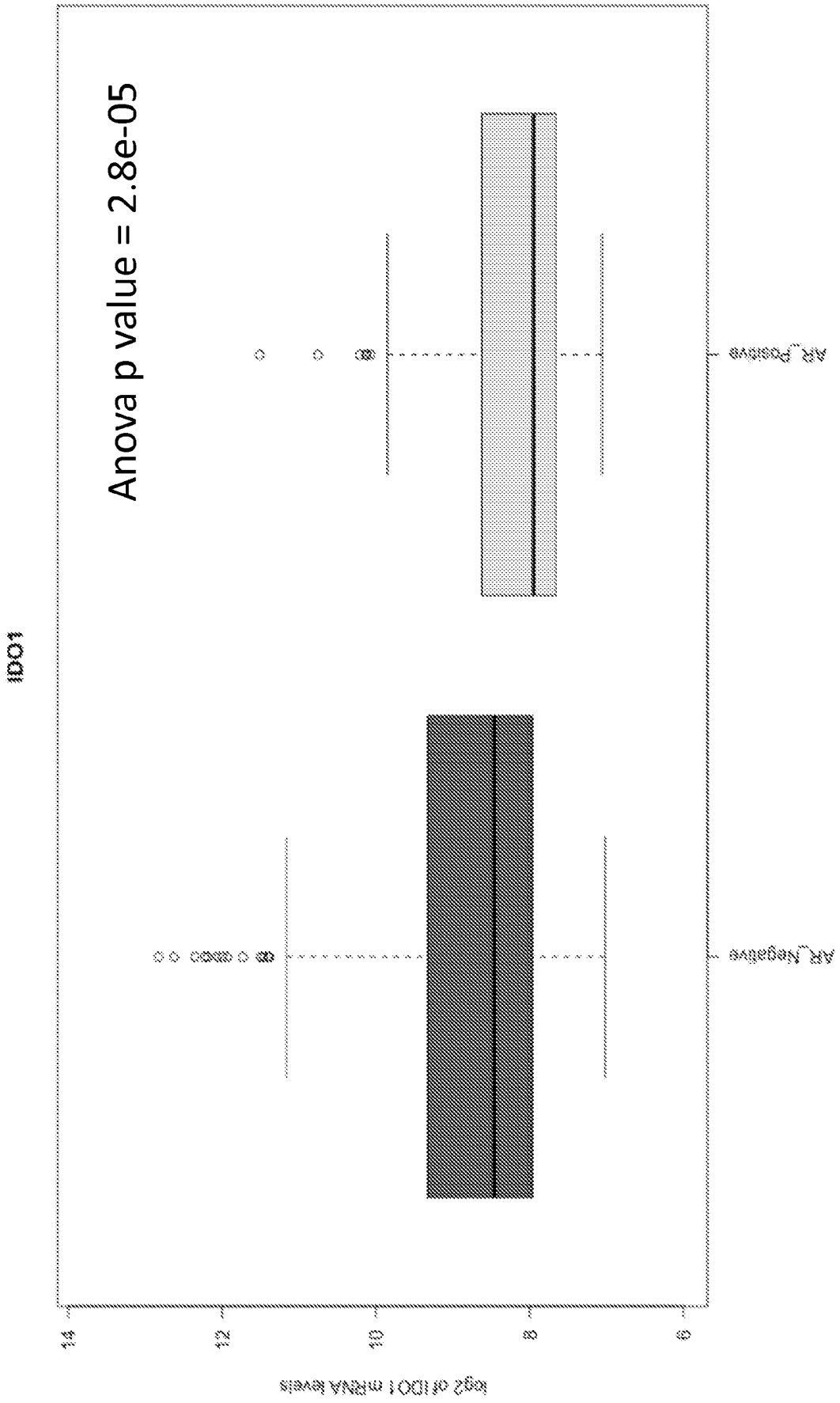

IDO1
Anova p value = 2.8e-05
AR_Positive
AR_Negative
14
12
10
8
6
log2 of IDO1 mRNA levels

FIG. 39K

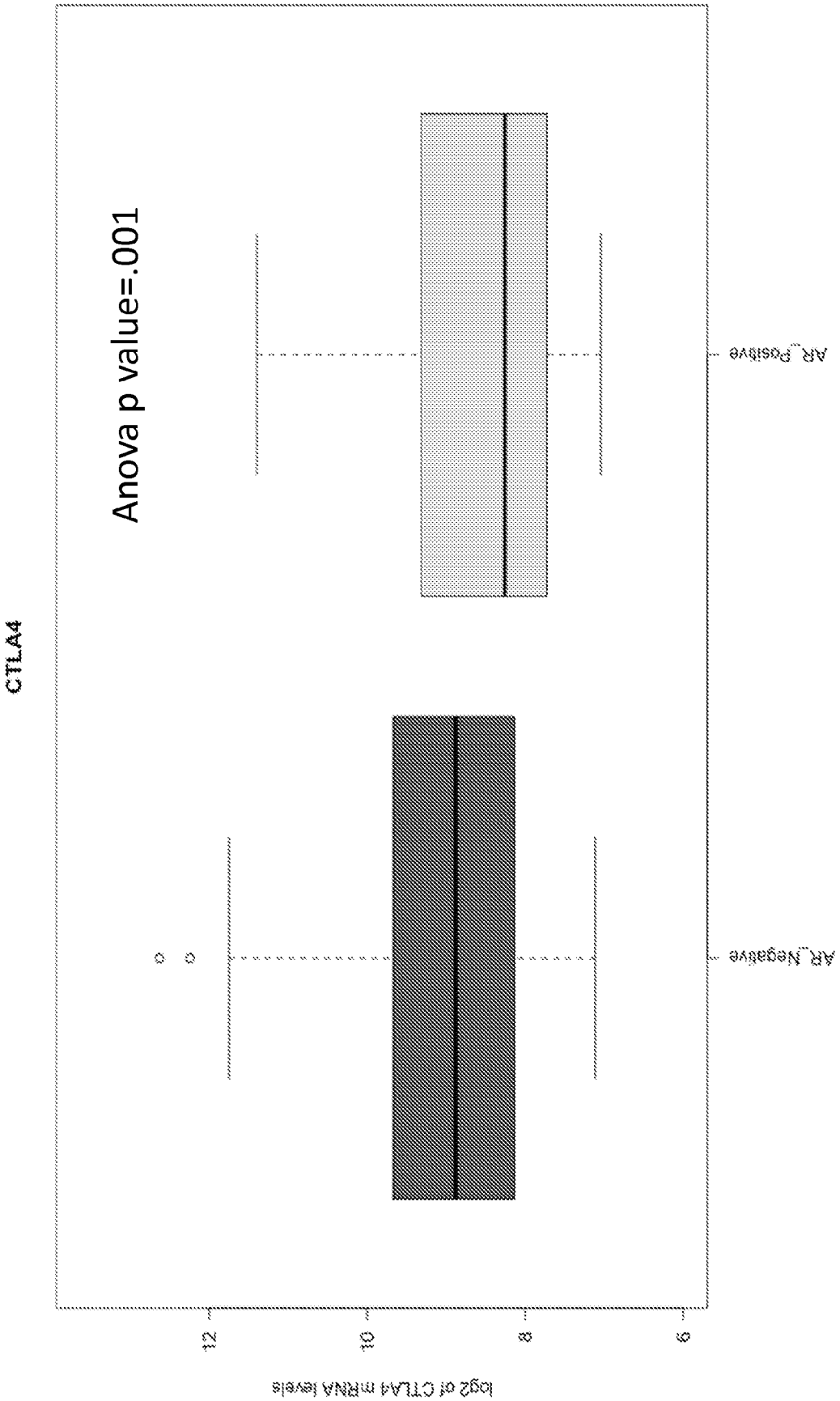

CTLA4
Anova p value=.001
log2 of CTLA4 mRNA levels
12
10
8
6
AR_Negative
AR_Positive

FIG. 39L

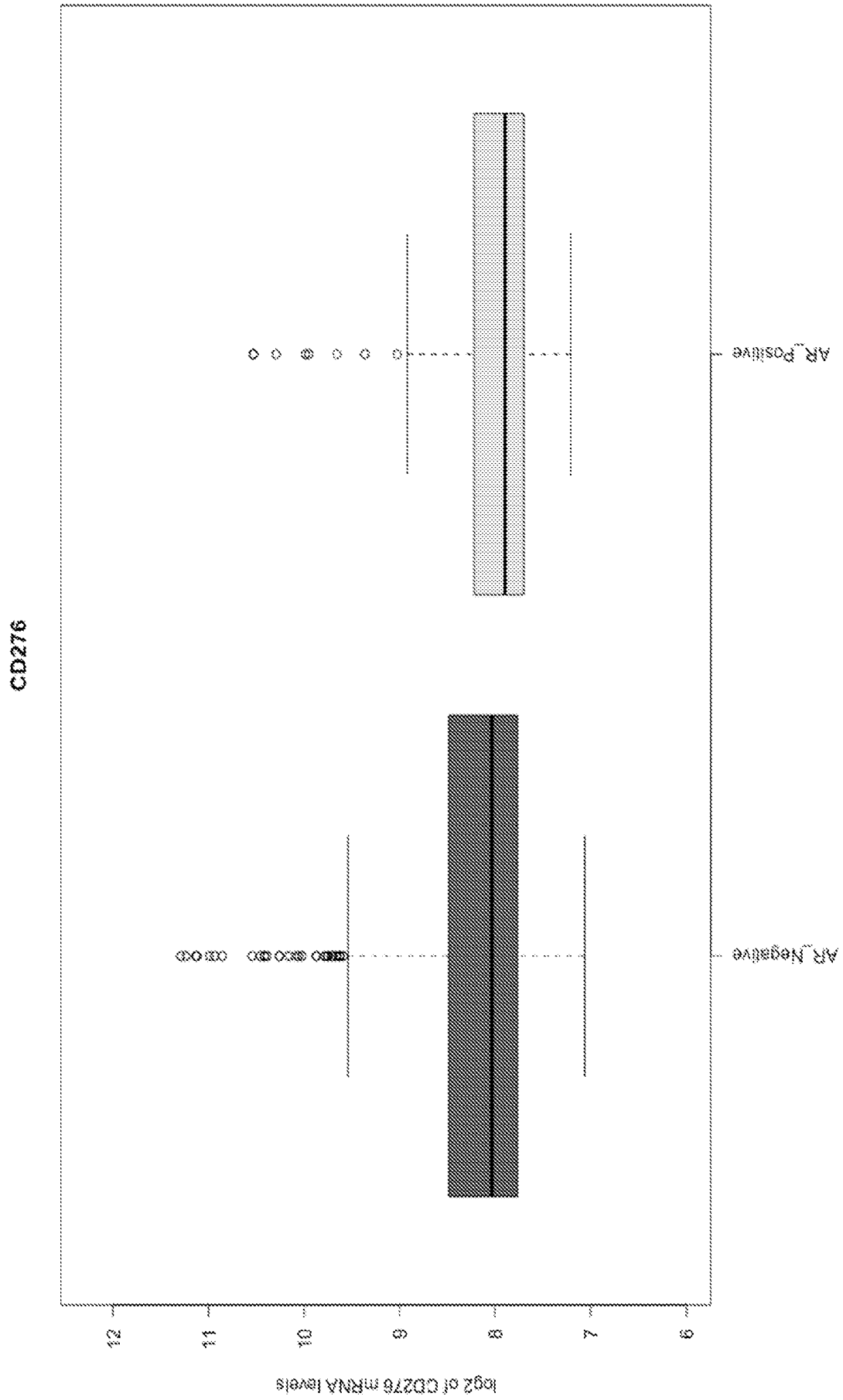

CD276
log2 of CD276 mRNA levels
12
11
10
9
8
7
6
AR_Negative
AR_Positive

FIG. 39M

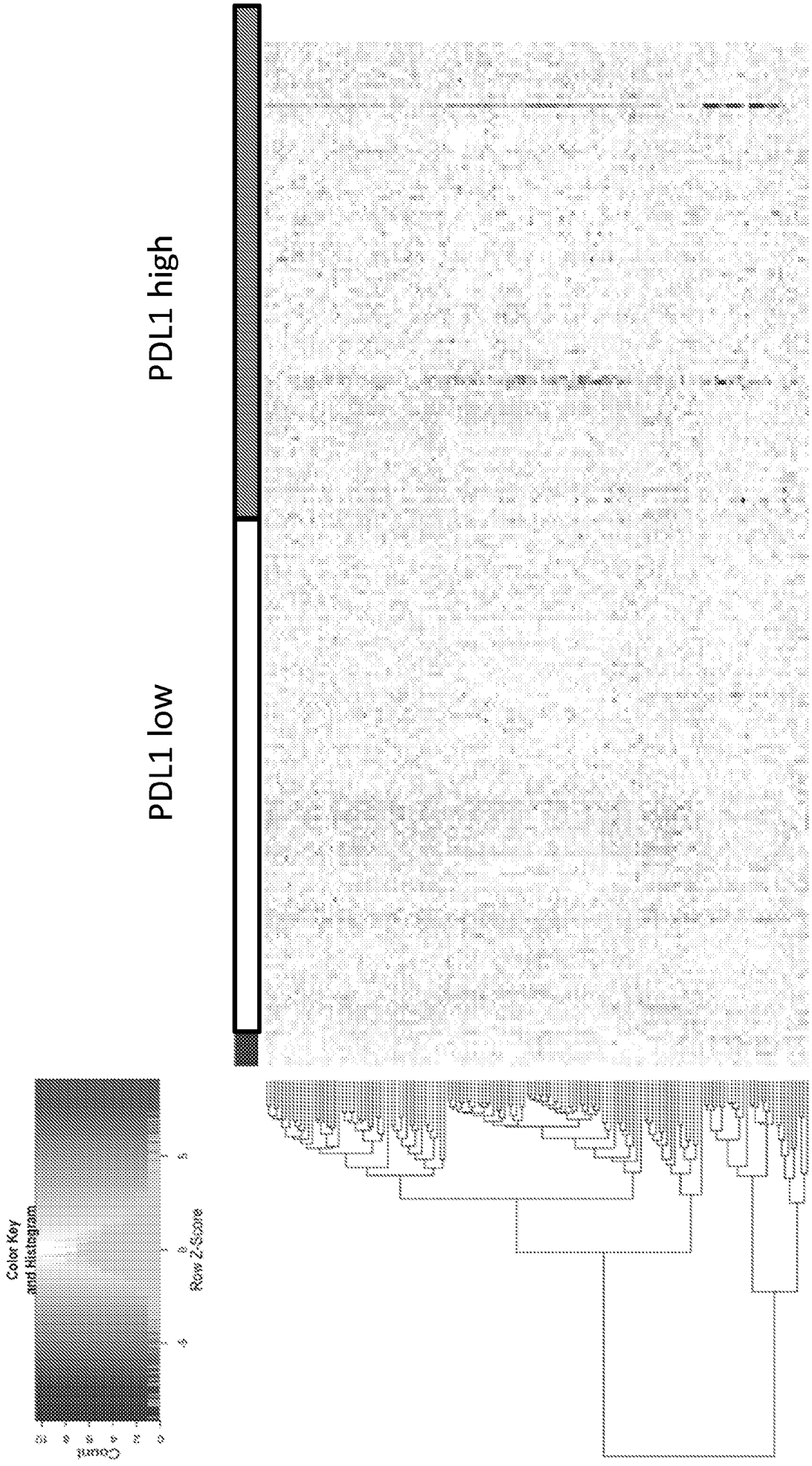


FIG. 39N

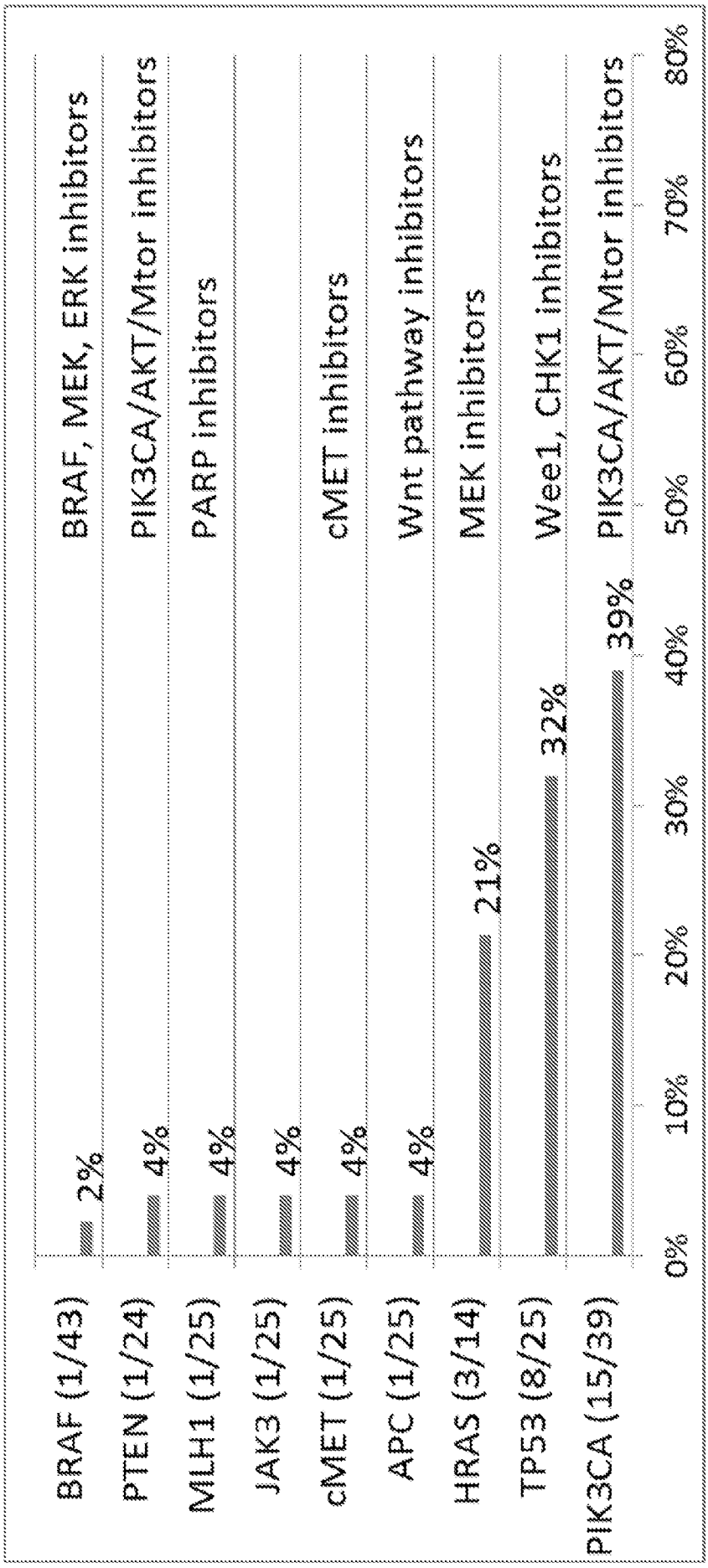

BRAF (1/43)
PTEN (1/24)
MLH1 (1/25)
JAK3 (1/25)
cMET (1/25)
APC (1/25)
HRAS (3/14)
TP53 (8/25)
PIK3CA (15/39)
2%
4%
4%
4%
4%
4%
21%
32%
39%
0%
10%
20%
30%
40%
50%
60%
70%
80%
BRAF, MEK, ERK inhibitors
PIK3CA/AKT/Mtor inhibitors
PARP inhibitors
cMET inhibitors
Wnt pathway inhibitors
MEK inhibitors
Wee1, CHK1 inhibitors
PIK3CA/AKT/Mtor inhibitors

FIG. 40A

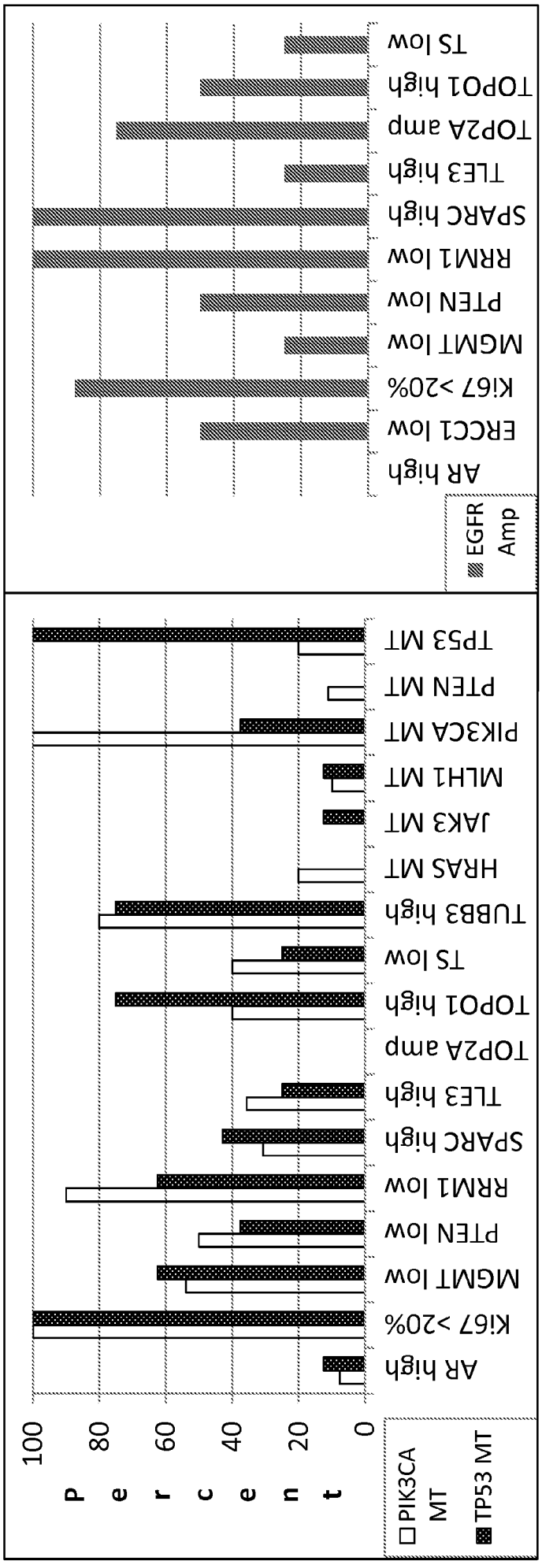

Percent
100
80
60
40
20
0
PIK3CA MT
TP53 MT
AR high
Ki67 >20%
MGMT low
PTEN low
RRM1 low
SPARC high
TLE3 high
TOP2A amp
TOP01 high
TS low
TUBB3 high
HRAS MT
JAK3 MT
MLH1 MT
PIK3CA MT
PTEN MT
TP53 MT
EGFR Amp
AR high
ERCC1 low
Ki67 >20%
MGMT low
PTEN low
RRM1 low
SPARC high
TLE3 high
TOP2A amp
TOP01 high
TS low

FIG. 40B

Demographics					IHC												ISH	DNA Sequencing					
Case #	Histology	AGE	Specimen Site Tested	Met?	AR high	ERCC1 low	Ki67 %	MGMT low	PTEN low	RRM1 low	SPARC high	TLE3 high	TOP2A IHC/ISH	TOPO1 high	TS low	TUBB3 high	EGFR copy #	HRAS	JAK3	MLH1	PIK3CA	PTEN	TP53
1	Metaplastic, NOS	91	Breast	Y		n/t	n/t	n/t	n/t	n/t	✓			n/t	n/t	n/t	n/t	n/t	n/t	n/t	E545K	n/t	n/t
2	Squamous	71	Skin	Y	n/t	n/t	n/t	n/t	n/t	n/t	n/t	✓		n/t	n/t	n/t	n/t	n/t	n/t	n/t	H1047R	n/t	n/t
3	Metaplastic, NOS	68	Breast	Y		n/t	50	✓		n/t		✓		n/t	n/t	n/t	n/t	n/t	n/t	n/t	H1047R	n/t	n/t
4	Squamous	63	Breast	Y		n/t	50		✓	n/t		✓		n/t	n/t	n/t	n/t	n/t	n/t	n/t	H1047R	n/t	n/t
5	Metaplastic, NOS	66	Breast			n/t	n/t	✓		✓							n/t				H1047R	n/t	
6	Metaplastic, NOS	50	Breast	Y		n/t	n/t	✓	✓				n/t	✓		✓	n/t	n/t			H1047R	✓	
7	Sarcomatoid	70	Breast			n/t	n/t			✓					✓		n/t				H1047R		
8	Squamous	57	Breast	Y		n/t	n/t	✓	✓	✓		✓			✓	✓	n/t				H1047L		
9	Cartilage, osseous	78	Pleura	Y		n/t	35			✓	✓						n/t	n/t			H1047L		
10	Squamous	45	Breast	Y		n/t	n/t	✓	✓	✓	✓			✓	✓		n/t	n/t		✓	H1047R		✓
11	Squamous	60	Skin	Y	✓	n/t	n/t	✓		✓							n/t				G106R, H1047R		✓
12	Metaplastic, NOS	65	Breast	Y		n/t	58	✓	✓	✓							n/t	n/t			H1047R		
13	Squamous	50	Breast	Y		n/t	n/t	✓		✓				✓	✓		n/t	✓			H1047R		
14	Sarcomatoid	86	Breast	Y		n/t	20			✓	✓	✓		✓			n/t	n/t			N345K		

FIG. 40C

Demographics					IHC												ISH	DNA Sequencing					
Case #	Histology	AGE	Specimen Site Tested	Met?	AR high	ERCC1 low	Ki67 %	MGMT low	PTEN low	RRM1 low	SPARC high	TLE3 high	TOP2A IHC/ISH	TOPO1 high	TS low	TUBB3 high	EGFR copy #	HRAS	JAK3	MLH1	PIK3CA	PTEN	TP53
TP53 Mutated MpBCs, n=8																							
15	Metaplastic, NOS	44	Breast		n/t	n/t	n/t		✓	✓	✓						n/t						G244S
10	Squamous	45	Breast	Y	n/t	n/t	n/t	✓	✓	✓	✓			✓	✓		n/t	n/t		✓	✓		S106R
11	Squamous	60	Skin	Y	✓	n/t	n/t	✓		✓							n/t				✓		Y236S
16	Metaplastic, NOS	56	Skin	Y		n/t	n/t			✓		✓		✓		✓	n/t	n/t					D281E
17	Metaplastic, NOS	71	Breast	Y		n/t	35	✓	✓		n/t			✓			n/t						R273H
18	Metaplastic, NOS	68	Skin	Y		n/t	81	✓		✓	✓	✓		✓	✓		n/t	n/t					Y163C
19	Metaplastic, NOS	31	Breast	Y		n/t	60	✓	✓					✓		✓	n/t	n/t	✓				R333fs
20	Metaplastic, NOS	33	Lung	Y		n/t	n/t		✓					✓			n/t						R213X
EGFR Amplified MpBCs, n=4																							
21	Squamous	65	Breast				34			✓	✓		✓			n/t	12.7	n/t	n/t	n/t	n/t	n/t	n/t
22	Metaplastic, NOS	60	Breast	Y			54		✓	✓	✓		✓	✓		n/t	4.19	n/t	n/t	n/t	n/t	n/t	n/t
23	Sarcomatoid	47	Breast	Y		✓	45		✓	✓	✓		✓	✓		n/t	3.91	n/t	n/t	n/t	n/t	n/t	n/t
24	Sarcomatoid	76	Breast	Y		✓	15	✓		✓	✓	✓			✓	n/t	3.66	n/t	n/t	n/t	n/t	n/t	n/t

FIG. 40D

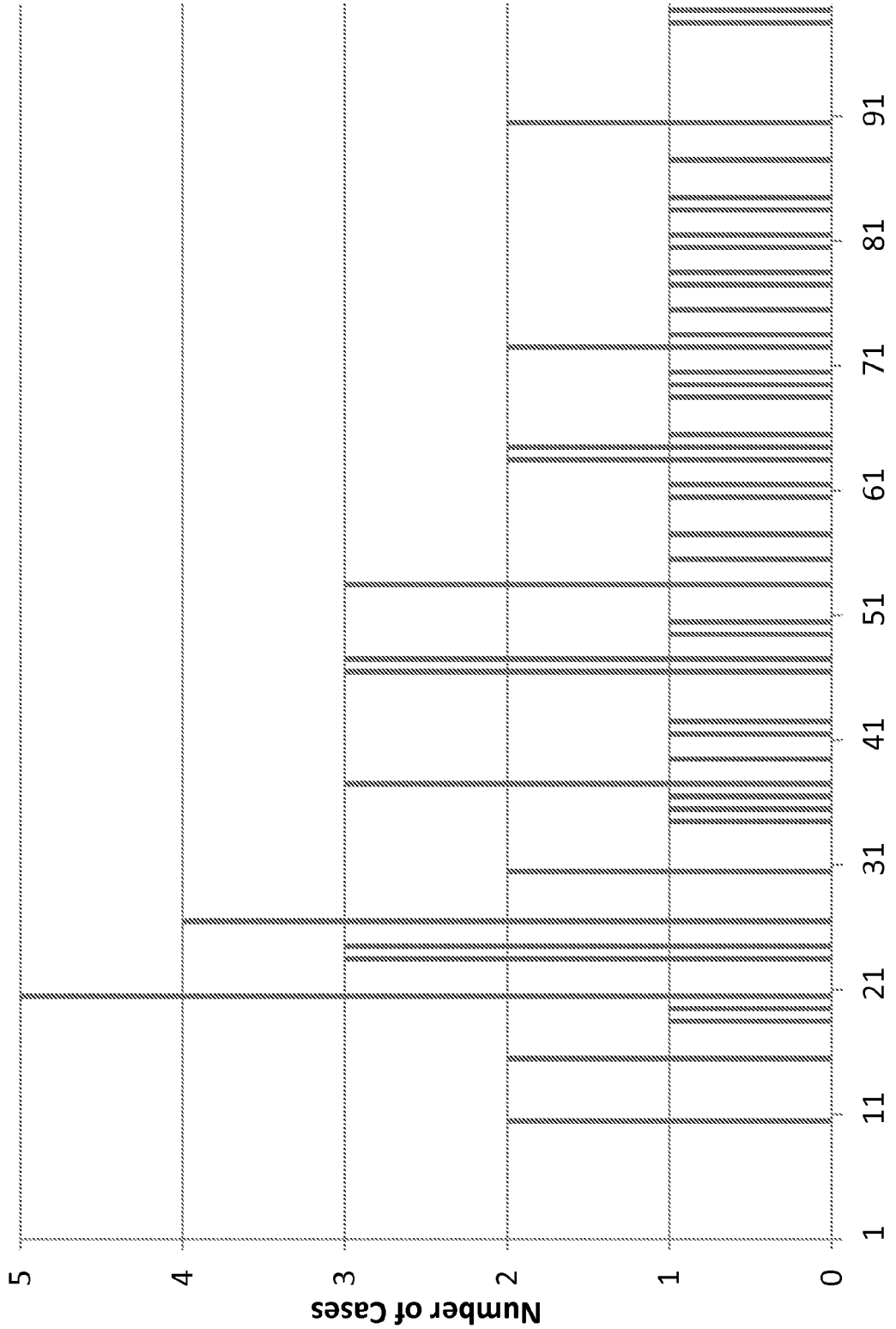

Number of Cases
0
1
2
3
4
5
1
11
21
31
41
51
61
71
81
91
Ki67: Percent Positive Cells

FIG. 40E

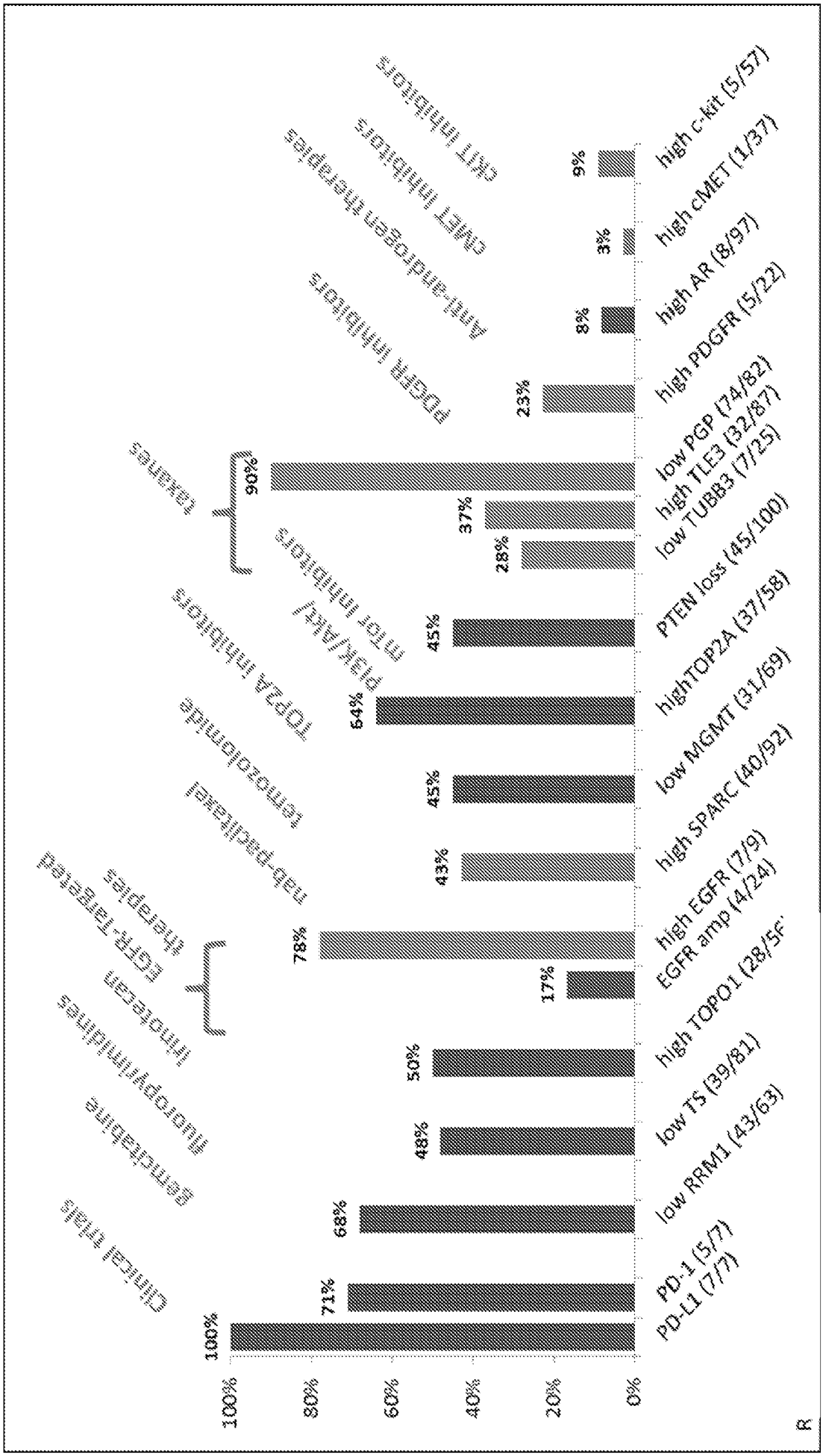

100%
80%
60%
40%
20%
0%
PD-1 (5/7)
PD-L1 (7/7)
low RRM1 (43/63)
low TS (39/81)
high TOPO1 (28/5
EGFR amp (4/24)
high EGFR (7/9)
high SPARC (40/92)
low MGMT (31/69)
highTOP2A (37/58)
PTEN loss (45/100)
low TUBB3 (7/25)
high TLE3 (32/87)
low PGP (74/82)
high PDGFR (5/22)
high AR (8/97)
high cMET (1/37)
high c-Kit (5/57)
71%
68%
48%
50%
17%
78%
43%
45%
45%
64%
28%
37%
23%
8%
3%
9%
Clinical trials
gemcitabine
irinotecan/mitomycin
ixabepilone
EGFR-targeted therapies
nab-paclitaxel
temozolomide
TOP2A inhibitors
PI3K/Akt/ mTOR inhibitors
taxanes
90%
PDGFR inhibitors
Anti-androgen therapies
cMET inhibitors
cKit inhibitors

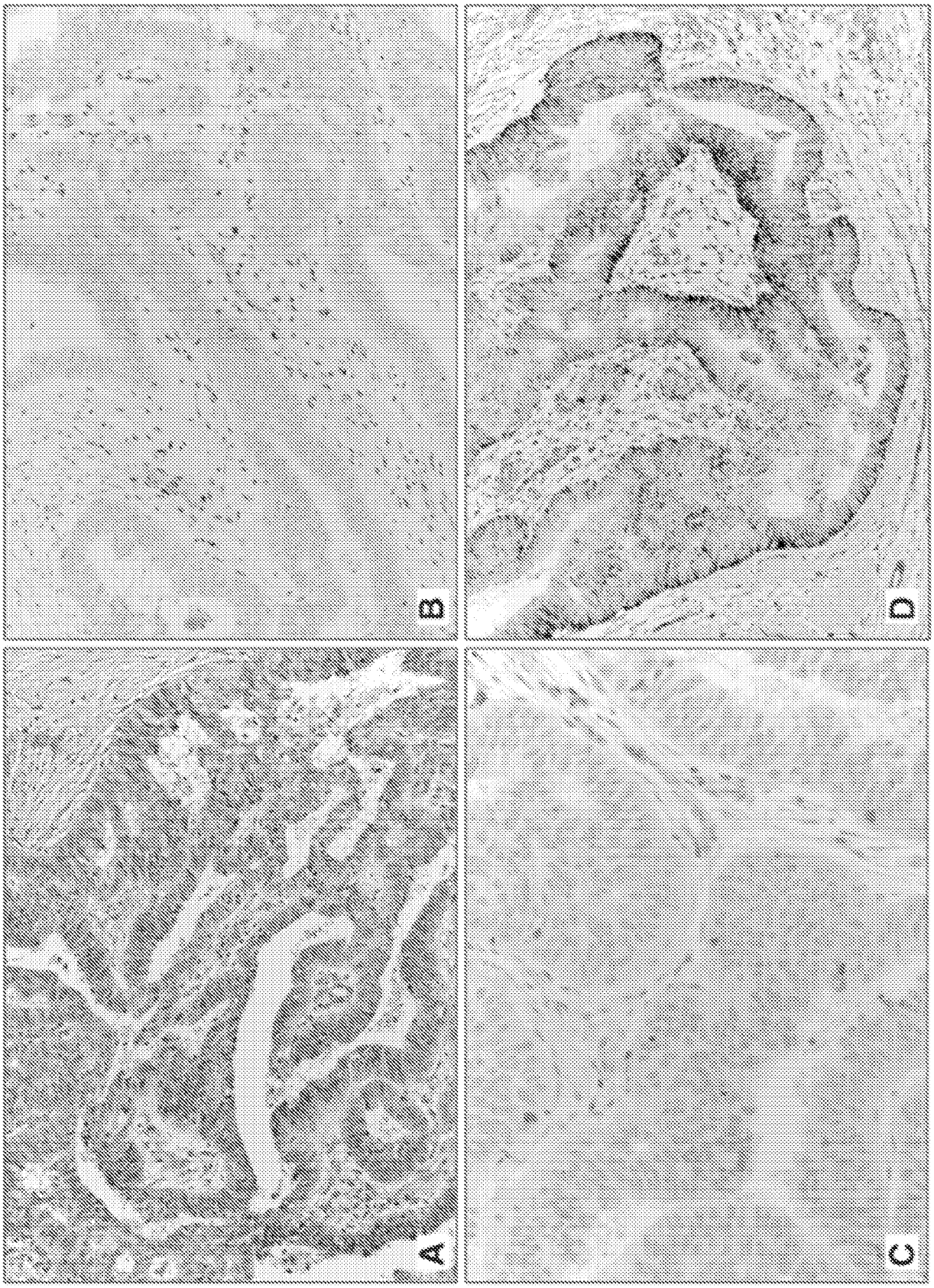
FIG. 40F

immunomodulatory axis	All HNSCC	HPV + HNSCC	HPV - HNSCC	p- value
PD1 + TILs	38%	56%	22%	0.07
PDL1 + tumor cells	24%	13%	33%	0.23
PD1 + TILs / PDL1 + tumor cells	9%	6%	11%	1

HNSCC tumor site	oropharynx (n=18)	non-oropharynx (n=18)	p-value
PD1 + TILs	33%	39%	1
PDL1 + tumor cells	11%	33%	0.22
PD1 + TILs / PDL1 + tumor cells	0%	17%	0.22

metastatic vs. non-metastatic	Metastatic (n=22)	Primary (n=12)	p-value
PD1 + TILs	45%	25%	0.29
PDL1 + tumor cells	27%	17%	0.68
PD1 + TILs / PDL1 + tumor cells	14%	0%	0.5

FIG. 41



FIGs. 42A-D