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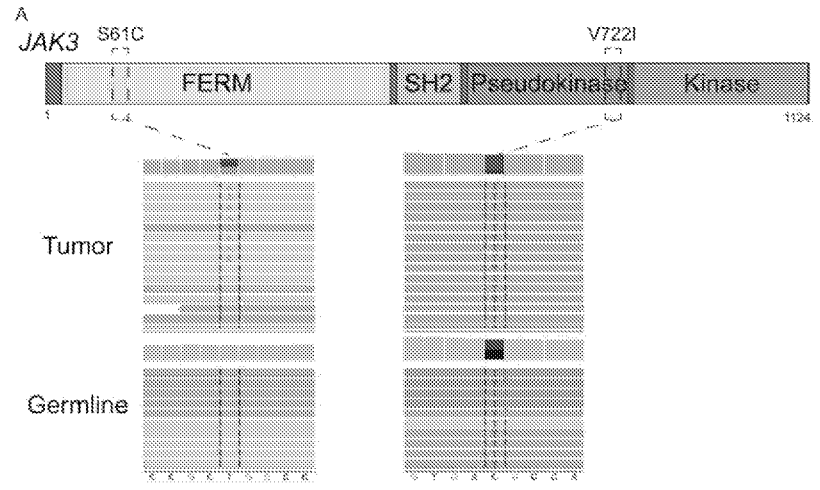
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(54) Title: ACTIVATING JAK KINASE BIOMARKERS PREDICTIVE OF ANTI-IMMUNE CHECKPOINT INHIBITOR RESPONSE

Figure 2



(57) Abstract: The present invention is based on the identification of novel biomarkers predictive of responsiveness to anti-immune checkpoint inhibitor therapies.

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ACTIVATING JAK KINASE BIOMARKERS PREDICTIVE OF ANTI-IMMUNE CHECKPOINT INHIBITOR RESPONSE

Cross-Reference to Related Applications

This application claims the benefit of U.S. Provisional Application No. 62/003,698, filed on 28 May 2014; the entire contents of said application are incorporated herein in their entirety by this reference.

Statement of Rights

This invention was made with government support under Grant Numbers R01 CA122794, R01 CA166480, R01 CA163896, R01 CA140594, U01 CA141576, and K08 CA138918-01A1 awarded by the National Institutes of Health. The U.S. government has certain rights in the invention. This statement is included solely to comply with 37 C.F.R. § 401.14(a)(f)(4) and should not be taken as an assertion or admission that the application discloses and/or claims only one invention.

Background of the Invention

Immune checkpoint blockade targeting the PD-L1/PD-1 receptor interaction has been a major advance in the therapy of melanoma and other solid malignancies, such as non-small cell lung cancer (NSCLC). Although inhibiting such immune checkpoint inhibitors has been demonstrated to generate significant clinical benefit for treating some cancers in some subjects, many subjects do not clinically respond to such inhibition (Wolchok *et al.* (2013) *N. Engl. J. Med.* 369:122-13; Mocellin *et al.* (2013) *Biochim. Biophys. Acta* 1836:187-196; Pardoll *et al.* (2012) *Nat. Rev. Cancer* 12:252-264; Brahmer *et al.* (2012) *N. Engl. J. Med.* 366:2455-2465; and Topalian *et al.* (2012) *N. Engl. J. Med.* 366:2443-2454). For example, only 10-20% of NSCLC patients respond. Accordingly, identifying an accurate biomarker that predicts an effective response has been the subject of intense study. While expression of immune checkpoint inhibitors, such as PD-L1, on tumor cells has been proposed, such expression enriches for response but does not accurately predict sensitivity or responsiveness to anti-immune checkpoint inhibitor therapy. Since therapies that negatively regulate immune checkpoint inhibitors, such as anti-PD-1, anti-PD-L1, and anti-CTLA-4 antibodies, are both significantly toxic in combination and very expensive, there is a great need in the art to identify biomarkers which are predictive of

patient responsiveness to such therapies in order to appropriately determine an efficacious and cost-effective course of therapeutic intervention.

Summary of the Invention

The present invention is based, at least in part, on the discovery that the presence, amount (*e.g.*, copy number or level of expression) and/or activity of activated Jak kinases are predictive of cancer cell responsiveness to anti-immune checkpoint inhibitor therapies.

In one aspect, a method of determining whether a subject afflicted with a cancer or at risk for developing a cancer would benefit from anti-immune checkpoint inhibitor therapy, the method comprising: a) obtaining a biological sample from the subject; b) determining the presence, copy number, amount, and/or activity of at least one biomarker listed in Table 1 in a subject sample; c) determining the presence, copy number, amount, and/or activity of the at least one biomarker in a control; and d) comparing the presence, copy number, amount, and/or activity of said at least one biomarker detected in steps b) and c), wherein the presence or a significant increase in the copy number, amount, and/or activity of the at least one biomarker in the subject sample relative to the control indicates that the subject afflicted with the cancer or at risk for developing the cancer would benefit from anti-immune checkpoint inhibitor therapy, is provided. In one embodiment, the method further comprises recommending, prescribing, or administering anti-immune checkpoint inhibitor therapy if the cancer is determined to benefit from anti-immune checkpoint inhibitor therapy. In another embodiment, the method further comprises recommending, prescribing, or administering anti-cancer therapy other than anti-immune checkpoint inhibitor therapy if the cancer is determined to not benefit from anti-immune checkpoint inhibitor therapy. In still another embodiment, the anti-cancer therapy is selected from the group consisting of targeted therapy, chemotherapy, radiation therapy, and/or hormonal therapy. In yet another embodiment, the control sample is determined from a cancerous or non-cancerous sample from either the patient or a member of the same species to which the patient belongs. In another embodiment, the control sample comprises cells. In still another embodiment, the method further comprises determining responsiveness to anti-immune checkpoint inhibitor therapy measured by at least one criteria selected from the group consisting of clinical benefit rate, survival until mortality, pathological complete response, semi-quantitative measures of pathologic response, clinical complete remission, clinical partial remission, clinical stable disease, recurrence-free

survival, metastasis free survival, disease free survival, circulating tumor cell decrease, circulating marker response, and RECIST criteria.

In another aspect, a method of treating a subject afflicted with a cancer, wherein the cancer comprises at least one activating Janus kinase (JAK) mutation shown in Table 1, comprising administering to the subject anti-immune checkpoint inhibitor therapy, thereby treating the subject afflicted with the cancer, is provided. In one embodiment, the at least one activating JAK mutation comprises an activating JAK3 mutation. In another embodiment, the activating JAK3 mutation is a JH2 domain mutation, optionally a $JAK3^{V722I}$ or $JAK3^{R657Q}$ mutation, and/or a FERM domain mutation, optionally a $JAK3^{S61C}$ mutation. In still another embodiment, the method further comprises administering one or more additional anti-cancer agents. In yet another embodiment, the one or more additional anti-cancer agent is a JAK or activator thereof.

In still another aspect, a method of inhibiting hyperproliferative growth of a cancer cell or cells, wherein the cancer cell or cells comprise at least one activating JAK mutation shown in Table 1, comprising contacting the cancer cell or cells with an anti-immune checkpoint inhibitor agent, thereby inhibiting hyperproliferative growth of the cancer cell or cells, is provided. In one embodiment, the step of contacting occurs *in vivo*, *ex vivo*, or *in vitro*. In another embodiment, the at least one activating JAK mutation comprises an activating JAK3 mutation. In still another embodiment, the activating JAK3 mutation is a JH2 domain mutation, optionally a $JAK3^{V722I}$ or $JAK3^{R657Q}$ mutation, and/or a FERM domain mutation, optionally a $JAK3^{S61C}$ mutation. In yet another embodiment, the method further comprises administering one or more additional anti-cancer agents. In another embodiment, the one or more additional anti-cancer agent is a JAK or activator thereof.

In yet another aspect, a method of assessing the efficacy of an agent for treating a cancer in a subject, wherein the cancer comprises at least one activating JAK mutation, comprising: a) detecting in a first subject sample and maintained in the presence of the agent the presence, copy number, amount and/or activity of at least one biomarker listed in Table 1; b) detecting the presence, copy number, amount and/or activity of the at least one biomarker listed in Table 1 in a second subject sample and maintained in the absence of the test compound; and c) comparing the presence, copy number, amount and/or activity of the at least one biomarker listed in Table 1 from steps a) and b), wherein the presence or a significantly increased copy number, amount, and/or activity of the at least one biomarker

listed in Table 1 in the first subject sample relative to the second subject sample, indicates that the agent treats the cancer in the subject, is provided.

In another aspect, a method of monitoring the progression of a cancer in a subject, wherein the cancer comprises at least one activating JAK mutation, comprising: a) detecting in a subject sample at a first point in time the presence, copy number, amount, and/or activity of at least one biomarker listed in Table 1; b) repeating step a) during at least one subsequent point in time after administration of a therapeutic agent; and c) comparing the presence, copy number, amount, and/or activity detected in steps a) and b), wherein the presence or a significantly increased copy number, amount, and/or activity of the at least one biomarker listed in Table 1 in the first subject sample relative to at least one subsequent subject sample, indicates that the agent treats the cancer in the subject, is provided. In one embodiment, the subject has undergone treatment, completed treatment, and/or is in remission for the cancer in between the first point in time and the subsequent point in time. In another embodiment, the subject has undergone anti-immune checkpoint inhibitor therapy in between the first point in time and the subsequent point in time. In still another embodiment, the first and/or at least one subsequent sample is selected from the group consisting of *ex vivo* and *in vivo* samples. In yet another embodiment, the first and/or at least one subsequent sample is obtained from an animal model of the cancer. In another embodiment, the first and/or at least one subsequent sample is a portion of a single sample or pooled samples obtained from the subject.

In still another aspect, a cell-based method for identifying an agent that inhibits a cancer, the method comprising: a) contacting a cell expressing at least one biomarker listed in Table 1 with a test agent; and b) determining the effect of the test agent on the copy number, level of expression, and/or level of activity of the at least one biomarker in Table 1 to thereby identify an agent that inhibits the cancer, is provided. In one embodiment, the method further comprises determining the effect of the test agent on the copy number, level of expression, and/or level of activity of at least one immune checkpoint inhibitor. In another embodiment, said cells are isolated from a source selected from the group consisting of an animal model of a cancer, a subject afflicted with a cancer, and a cell comprising at least one activating JAK3 mutation. In still another embodiment, said cells are unresponsive to anti-immune checkpoint inhibitor therapy. In yet another embodiment, the step of contacting occurs *in vivo*, *ex vivo*, or *in vitro*. In another embodiment, the method further comprises determining the ability of the test agent to bind to the at least one

biomarker listed in Table 1 before or after determining the effect of the test agent on the copy number, level of expression, or level of activity of the at least one biomarker listed in Table 1.

Numerous embodiments are contemplated for any method, assay, and the like, described herein. For example, in one embodiment, the sample comprises cells, cell lines, histological slides, paraffin embedded tissue, fresh frozen tissue, fresh tissue, biopsies, bronchoalveolar lavage (BAL) fluid, blood, plasma, serum, buccal scrape, saliva, cerebrospinal fluid, urine, stool, mucus, or bone marrow, obtained from the subject. In another embodiment, the presence or copy number is assessed by whole exome sequencing, microarray, quantitative PCR (qPCR), high-throughput sequencing, comparative genomic hybridization (CGH), or fluorescent *in situ* hybridization (FISH). In still another embodiment, the amount of the at least one biomarker listed in Table 1 is assessed by detecting the presence in the samples of a polynucleotide molecule encoding the biomarker or a portion of said polynucleotide molecule. In yet another embodiment, the polynucleotide molecule is a mRNA, cDNA, or functional variants or fragments thereof. In another embodiment, the step of detecting further comprises amplifying the polynucleotide molecule. In still another embodiment, the amount of the at least one biomarker is assessed by annealing a nucleic acid probe with the sample of the polynucleotide encoding the one or more biomarkers or a portion of said polynucleotide molecule under stringent hybridization conditions. In yet another embodiment, the amount of the at least one biomarker is assessed by detecting the presence a polypeptide of the at least one biomarker. In another embodiment, the presence of said polypeptide is detected using a reagent which specifically binds with said polypeptide. In still another embodiment, the reagent is selected from the group consisting of an antibody, an antibody derivative, and an antibody fragment. In yet another embodiment, the activity of the at least one biomarker is assessed by determining the magnitude of cellular proliferation, cell death, or cytokine production.

In some embodiments, the agent or anti-immune checkpoint inhibitor therapy is selected from the group consisting of a blocking antibody, small molecule, antisense nucleic acid, interfering RNA, shRNA, siRNA, aptamer, ribozyme, dominant-negative protein, and combinations thereof. In another embodiment, the agent is selected from the group consisting of a cytokine, an inhibitor of a Jak kinase inhibitor, a Jak kinase harboring an activating mutation, anti-immune checkpoint inhibitor therapy, and combinations thereof. In still another embodiment, the inhibitor of the Jak kinase inhibitor is an inhibitor

of PIAS1, PIAS2, PIAS3, PIAS4, SOCS1, SOCS3, SHP-1, or SHP-2. In yet another embodiment, the agent or anti-immune checkpoint inhibitor therapy is selected from the group consisting of inhibitors of PD-1, PD-L1, PD-L2, CTLA-4, and combinations thereof. In another embodiment, the agent or anti-immune checkpoint inhibitor therapy is a blocking antibody of PD-1, PD-L1, PD-L2, or CTLA-4, and combinations thereof. In still another embodiment, the at least one biomarker is selected from the group consisting of 1, 2, 3, 4, 5, 6, 7, 8, 9, 10, or more biomarkers. In yet another embodiment, the at least one biomarker is an activating JAK3 mutation. In another embodiment, the activating JAK3 mutation is a JH2 domain mutation, optionally a $JAK3^{V722I}$ or $JAK3^{R657Q}$ mutation, and/or a FERM domain mutation, optionally a $JAK3^{S61C}$ mutation. In still another embodiment, the cancer is a solid malignancy. In yet another embodiment, the solid malignancy is selected from the group consisting of lung cancer, non-small cell lung cancer (NSCLC), skin cancer, melanoma, cervical cancer, uterine cancer, ovarian cancer, breast cancer, pancreatic cancer, stomach cancer, esophageal cancer, colorectal cancer, liver cancer, prostate cancer, kidney cancer, bladder cancer, head and neck cancer, sarcoma, lymphoma, and brain cancer. In another embodiment, the subject is a mammal (*e.g.*, an animal model of cancer, or a human).

Brief Description of the Drawings

Figure 1 includes 4 panels, identified as panels A, B, C, and D, which show long-term durable response to PD-L1 blockade in a patient with metastatic lung adenocarcinoma. Panel A shows systemic therapies received by the patient over time. CT = carboplatin/taxol, CPB = carboplatin/pemetrexed/bevacizumab, PB = maintenance pemetrexed/bevacizumab, and PD-L1 inhibitor = MPDL3280A. Figure 1B shows the size of the left paratracheal mass over time, as measured by longest diameter (cm). Panel B shows the change in patient weight (kg) during the same time period. Panel C shows a chest CT scan prior to initiation of MPDL3280A serial chest CT scans demonstrating reduction in size of the paratracheal mass over time (arrows). Panel D shows serial abdominal CT scans demonstrating recurrence and re-treatment response of the right adrenal mass (arrows).

Figure 2 includes 4 panels, identified as panels A, B, C, and D, which show that genomic profiling identified two JAK3 alterations present in the tumor that result in constitutive JAK3 activation. Panel A shows structural organization of JAK3 including the

N-terminal FERM domain, the SH2 domain, and the JH2 or pseudokinase domain, which is adjacent to the kinase domain and contributes to autoinhibition. Sequencing of position 722 of JAK3 in the JH2 domain reveals heterozygosity for alleles in the germline consistent with a single copy of $JAK3^{V722I}$, while the left adrenal metastasis revealed loss of heterozygosity (LOH) and complete acquisition of the $JAK3^{V722I}$ allele (predominant band over coverage band). The somatic $JAK3^{S61C}$ mutation was also observed using the Integrated Genomics Viewer (IGV). Panel B shows the results of whole exome sequencing which data revealed apparent copy number neutrality of the *JAK3* locus on chromosome 19. Panel C shows the results of that the $JAK3^{V722I}$ allele was detected when analyzed at the allelic level clonality, consistent with the focused sequencing results. Panel D shows an immunoblot of total JAK3, and tyrosine phosphorylated (Y980/981) pJAK3, in 293T cells transfected with EGFP control vector, $JAK3^{WT}$, $JAK3^{S61C}$, $JAK3^{V722I}$, $JAK3^{S61C/V722I}$ or $JAK3^{R657Q}$.

Figure 3 includes 2 panels, identified as panels A and B, which show the results of orthogonal sequencing of JAK3 mutations. Polymerase chain reaction (PCR) tracings for V722I (Panel A) and S61C (Panel B) alterations observed in the tumor and germline DNA from the patient are shown.

Figure 4 shows the copy number profile of the patient's tumor across the exome. The profile is organized by chromosome. CR stands for the copy ratio.

Figure 5 shows absolute copy number analyses. After correction for tumor purity, ploidy, and allele specific copy number, the absolute copy number derived from ABSOLUTE (Herbst *et al.* (2014) *Nature* 515:563-567) is shown by chromosome.

Figure 6 shows PHIAL results of the patient's somatic exome. Heuristic analysis of the somatic mutations, short insertion/deletions, and copy number alterations across the exome identified 18 mutations for additional evaluation.

Figure 7 includes 3 panels, identified as panels A, B, and C, which show that deregulated *JAK3* signaling induces PD-L1 expression in lung cells. Panel A shows an immunoblot of total JAK3 levels following stable transduction of $JAK3^{WT}$ or the patient derived $JAK3^{S61C/V722I}$ alleles in BEAS-2B or Calu-1 cells. Panel B shows the levels of cell surface PD-L1 expression on these same BEAS-2B or Calu-1 cells as measured by flow cytometry using a PD-L1 specific monoclonal antibody compared to isotype. The percent change in isotype-normalized mean fluorescence intensity (MFI) relative to control is

highlighted. Panel C shows cell surface PD-L1 expression on Calu-1 cells expressing control vector or the patient derived *JAK3*$^{S861C/V722I}$ allele, stimulated with or without EGF.

Figure 8 includes 4 panels, identified as panels A, B, C, and D, which show the results of germline contribution of *JAK3*^{V722I} to immune cell PD-L1 expression and T cell suppression. Panel A shows the results of PD-L1 and pSTAT3 immunohistochemistry of the patient's adrenal metastasis (arrows denote example tumor cells). Panel B shows levels of tumor cell or immune cell PD-L1 positivity by immunohistochemistry (IHC) across a panel of thoracic malignancies including *JAK3*^{V722I} and *JAK3*^{P132T} positive cases or *JAK3*^{WT} controls (% positive cells and staining intensity, from 0 to 3+, is listed for each tumor and immune cell population from each sample). The case report patient (#4) is marked in bold. Panel C shows PD-L1 MFI on CD14+ myeloid cells from two patients (corresponding to patients 2, 3 and 4 in 3C, denoted with asterisk) or donor PBMCs (n = 14) stimulated with IFN-gamma for 48 hours ($p = 0.02$; t-test). Panel D shows the results of blood samples drawn from the index patient immediately pre- and 1 h post- MPDL3280A infusion, and monocytes -/+ IFNγ stimulation incubated with T cells from the patient (autologous, pre-MPDL3280A) or a donor (allogeneic). T cell proliferation (frequency of positive cells in gate 4) is shown for autologous or allogeneic CD4+ or CD8+ T cells under each condition.

Figure 9 includes 2 panels, identified as panels A and B, which show modified H-scores for tumor and immune cells. A comparison of modified H-scores (% positive cells x staining intensity) between V722I-mutant cases and controls for tumor cells (Panel A) and immune cells (Panel B) is shown. P-values were calculated using the Mann-Whitney test.

Figure 10 shows the results of T cell re-activation following co-culture with JAK3-V722I expressing monocytes in the presence of MPDL3280A. Representative FACS plots of activated autologous CD4 and CD8 T cells (upper panels) or allogeneic CD4 and CD8 T cells (lower panels) following incubation with monocytes primed +/- IFNγ in the absence or presence of MPDL3820A are shown. Highlighted is Gate 4, which was used to quantify the percentage of active T cells.

Figure 11 shows information on all somatic point mutations and short insertion/deletions observed in the tumor sample from this patient. Additional annotations about protein change, allelic fraction, copy ratio (as segment mean), and other information are provided.

Detailed Description of the Invention

The present invention is based, at least in part, on the discovery that the presence, amount (*e.g.*, copy number or level of expression) and/or activity of activated Jak kinases are predictive of cancer cell responsiveness to anti-immune checkpoint inhibitor therapies. In a retrospective analysis of an exceptional responder to the PD-L1 targeted antibody, MPDL3280a (Genentech), it was determined that the responder had an activating JAK3 V722I mutation. It was further determined that activated Jak kinases (*e.g.*, activating mutations in a Jak kinase itself or biological perturbations resulting in Jak kinase hyperactivity) represent a new mechanism that directly contributes to the induction of the PD-L1 immune checkpoint inhibitor expression in tumors and sensitivity to immune checkpoint blockade. Since activating Jak mutations are only present in 5-10% of cancers and are generally restricted to liquid malignancies, it was surprising to identify Jak mutations as being generally predictive of anti-immune checkpoint inhibitor therapy response and also having such an effect in solid cancers.

Accordingly, the present invention relates, in part, to methods for predicting response of a cancer in a subject to anti-immune checkpoint inhibitor therapy based upon a determination and analysis of specific biomarkers described herein. In addition, such analyses can be used in order to provide useful anti-immune checkpoint inhibitor treatment regimens (*e.g.*, based on predictions of subject survival or relapse, timing of adjuvant or neoadjuvant treatment, etc.).

I. Definitions

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

The term "altered amount" or "altered level" refers to increased or decreased copy number (*e.g.*, germline and/or somatic) of a biomarker nucleic acid, *e.g.*, increased or decreased expression level in a cancer sample, as compared to the expression level or copy number of the biomarker nucleic acid in a control sample. The term "altered amount" of a biomarker also includes an increased or decreased protein level of a biomarker protein in a sample, *e.g.*, a cancer sample, as compared to the corresponding protein level in a normal, control sample. Furthermore, an altered amount of a biomarker protein may be determined

by detecting posttranslational modification such as methylation status of the marker, which may affect the expression or activity of the biomarker protein.

The amount of a biomarker in a subject is "significantly" higher or lower than the normal amount of the biomarker, if the amount of the biomarker is greater or less, respectively, than the normal level by an amount greater than the standard error of the assay employed to assess amount, and preferably at least 20%, 30%, 40%, 50%, 60%, 70%, 80%, 90%, 100%, 150%, 200%, 300%, 350%, 400%, 500%, 600%, 700%, 800%, 900%, 1000% or than that amount. Alternately, the amount of the biomarker in the subject can be considered "significantly" higher or lower than the normal amount if the amount is at least about two, and preferably at least about three, four, or five times, higher or lower, respectively, than the normal amount of the biomarker. Such "significance" can also be applied to any other measured parameter described herein, such as for expression, inhibition, cytotoxicity, cell growth, and the like.

The term "altered level of expression" of a biomarker refers to an expression level or copy number of the biomarker in a test sample, *e.g.*, a sample derived from a patient suffering from cancer, that is greater or less than the standard error of the assay employed to assess expression or copy number, and is preferably at least twice, and more preferably three, four, five or ten or more times the expression level or copy number of the biomarker in a control sample (*e.g.*, sample from a healthy subjects not having the associated disease) and preferably, the average expression level or copy number of the biomarker in several control samples. The altered level of expression is greater or less than the standard error of the assay employed to assess expression or copy number, and is preferably at least twice, and more preferably three, four, five or ten or more times the expression level or copy number of the biomarker in a control sample (*e.g.*, sample from a healthy subjects not having the associated disease) and preferably, the average expression level or copy number of the biomarker in several control samples.

The term "altered activity" of a biomarker refers to an activity of the biomarker which is increased or decreased in a disease state, *e.g.*, in a cancer sample, as compared to the activity of the biomarker in a normal, control sample. Altered activity of the biomarker may be the result of, for example, altered expression of the biomarker, altered protein level of the biomarker, altered structure of the biomarker, or, *e.g.*, an altered interaction with other proteins involved in the same or different pathway as the biomarker or altered interaction with transcriptional activators or inhibitors.

The term "altered structure" of a biomarker refers to the presence of mutations or allelic variants within a biomarker nucleic acid or protein, *e.g.*, mutations which affect expression or activity of the biomarker nucleic acid or protein, as compared to the normal or wild-type gene or protein. For example, mutations include, but are not limited to substitutions, deletions, or addition mutations. Mutations may be present in the coding or non-coding region of the biomarker nucleic acid.

Unless otherwise specified here within, the terms "antibody" and "antibodies" broadly encompass naturally-occurring forms of antibodies (*e.g.* IgG, IgA, IgM, IgE) and recombinant antibodies such as single-chain antibodies, chimeric and humanized antibodies and multi-specific antibodies, as well as fragments and derivatives of all of the foregoing, which fragments and derivatives have at least an antigenic binding site. Antibody derivatives may comprise a protein or chemical moiety conjugated to an antibody.

The term "antibody" as used herein also includes an "antigen-binding portion" of an antibody (or simply "antibody portion"). The term "antigen-binding portion", as used herein, refers to one or more fragments of an antibody that retain the ability to specifically bind to an antigen (*e.g.*, a biomarker polypeptide, fragment thereof, or biomarker metabolite). It has been shown that the antigen-binding function of an antibody can be performed by fragments of a full-length antibody. Examples of binding fragments encompassed within the term "antigen-binding portion" of an antibody include (i) a Fab fragment, a monovalent fragment consisting of the VL, VH, CL and CH1 domains; (ii) a $F(ab')_2$ fragment, a bivalent fragment comprising two Fab fragments linked by a disulfide bridge at the hinge region; (iii) a Fd fragment consisting of the VH and CH1 domains; (iv) a Fv fragment consisting of the VL and VH domains of a single arm of an antibody, (v) a dAb fragment (Ward *et al.*, (1989) *Nature* 341:544-546), which consists of a VH domain; and (vi) an isolated complementarity determining region (CDR). Furthermore, although the two domains of the Fv fragment, VL and VH, are coded for by separate genes, they can be joined, using recombinant methods, by a synthetic linker that enables them to be made as a single protein chain in which the VL and VH regions pair to form monovalent polypeptides (known as single chain Fv (scFv); see *e.g.*, Bird *et al.* (1988) *Science* 242:423-426; and Huston *et al.* (1988) *Proc. Natl. Acad. Sci. USA* 85:5879-5883; and Osbourn *et al.* 1998, Nature Biotechnology 16: 778). Such single chain antibodies are also intended to be encompassed within the term "antigen-binding portion" of an antibody. Any VH and VL sequences of specific scFv can be linked to human immunoglobulin constant region cDNA

or genomic sequences, in order to generate expression vectors encoding complete IgG polypeptides or other isotypes. VH and VL can also be used in the generation of Fab, Fv or other fragments of immunoglobulins using either protein chemistry or recombinant DNA technology. Other forms of single chain antibodies, such as diabodies are also encompassed. Diabodies are bivalent, bispecific antibodies in which VH and VL domains are expressed on a single polypeptide chain, but using a linker that is too short to allow for pairing between the two domains on the same chain, thereby forcing the domains to pair with complementary domains of another chain and creating two antigen binding sites (see *e.g.*, Holliger *et al.* (1993) *Proc. Natl. Acad. Sci. U.S.A.* 90:6444-6448; Poljak *et al.* (1994) *Structure* 2:1121-1123).

Still further, an antibody or antigen-binding portion thereof may be part of larger immunoadhesion polypeptides, formed by covalent or noncovalent association of the antibody or antibody portion with one or more other proteins or peptides. Examples of such immunoadhesion polypeptides include use of the streptavidin core region to make a tetrameric scFv polypeptide (Kipriyanov, S.M., *et al.* (1995) *Human Antibodies and Hybridomas* 6:93-101) and use of a cysteine residue, biomarker peptide and a C-terminal polyhistidine tag to make bivalent and biotinylated scFv polypeptides (Kipriyanov, S.M., *et al.* (1994) *Mol. Immunol.* 31:1047-1058). Antibody portions, such as Fab and $F(ab')_2$ fragments, can be prepared from whole antibodies using conventional techniques, such as papain or pepsin digestion, respectively, of whole antibodies. Moreover, antibodies, antibody portions and immunoadhesion polypeptides can be obtained using standard recombinant DNA techniques, as described herein.

Antibodies may be polyclonal or monoclonal; xenogeneic, allogeneic, or syngeneic; or modified forms thereof (*e.g.* humanized, chimeric, etc.). Antibodies may also be fully human. Preferably, antibodies of the invention bind specifically or substantially specifically to a biomarker polypeptide or fragment thereof. The terms "monoclonal antibodies" and "monoclonal antibody composition," as used herein, refer to a population of antibody polypeptides that contain only one species of an antigen binding site capable of immunoreacting with a particular epitope of an antigen, whereas the term "polyclonal antibodies" and "polyclonal antibody composition" refer to a population of antibody polypeptides that contain multiple species of antigen binding sites capable of interacting with a particular antigen. A monoclonal antibody composition typically displays a single binding affinity for a particular antigen with which it immunoreacts.

Antibodies may also be "humanized", which is intended to include antibodies made by a non-human cell having variable and constant regions which have been altered to more closely resemble antibodies that would be made by a human cell. For example, by altering the non-human antibody amino acid sequence to incorporate amino acids found in human germline immunoglobulin sequences. The humanized antibodies of the invention may include amino acid residues not encoded by human germline immunoglobulin sequences (*e.g.*, mutations introduced by random or site-specific mutagenesis *in vitro* or by somatic mutation *in vivo*), for example in the CDRs. The term "humanized antibody", as used herein, also includes antibodies in which CDR sequences derived from the germline of another mammalian species, such as a mouse, have been grafted onto human framework sequences.

The term "assigned score" refers to the numerical value designated for each of the biomarkers after being measured in a patient sample. The assigned score correlates to the absence, presence or inferred amount of the biomarker in the sample. The assigned score can be generated manually (*e.g.*, by visual inspection) or with the aid of instrumentation for image acquisition and analysis. In certain embodiments, the assigned score is determined by a qualitative assessment, for example, detection of a fluorescent readout on a graded scale, or quantitative assessment. In one embodiment, an "aggregate score," which refers to the combination of assigned scores from a plurality of measured biomarkers, is determined. In one embodiment the aggregate score is a summation of assigned scores. In another embodiment, combination of assigned scores involves performing mathematical operations on the assigned scores before combining them into an aggregate score. In certain, embodiments, the aggregate score is also referred to herein as the predictive score."

The term "biomarker" refers to a measurable entity of the present invention that has been determined to be predictive of anti-immune checkpoint inhibitor therapy effects on a cancer. Biomarkers can include, without limitation, nucleic acids, proteins, and metabolites, particularly those shown in Table 1.

For example, "JAKs" are biomarkers of the present invention and refer to a family of non-receptor protein tyrosine kinases known as Janus kinases involved in cytokine receptor signaling. The mammalian JAK protein family consists of four members: JAK1 (Janus kinase-1), JAK2 (Janus kinase-2), JAK3 (also known as Janus kinase leukocyte or JAKL), and TYK2 (protein-tyrosine kinase 2). In some embodiments, JAK1, JAK2, JAK3, TYK2, either alone or in any combination thereof, for use in any aspect of the present

invention is contemplated. The JAK kinases mediate the signaling of all receptors belonging to the hematopoietic cytokine receptor type I and type II superfamily and they are required for the biological responses of interferons, most interleukins and colony stimulating factors, and hormones, such as erythropoietin, thrombopoietin, growth hormone, prolactin, and leptin (see, for example, WO 2011/098673; WO 2013/086196; Rawlings *et al.* (2004) *J. Cell Sci.* 117:1281-1283). JAK3 in particular selectively binds to receptors and is part of the cytokine signaling pathway for IL-2, IL-4, IL-7, IL-9, IL-15, and IL-21, and modulates IL-10 expression (Yamaoka *et al.* (2005) 106:3227-3233). JAK1 interacts with, among others, the receptors for cytokines IL-2, IL-4, IL-7, IL-9, and IL-21, while JAK2 interacts with, among others, the receptors for IL-9 and TNFR1 (Pincheira *et al.* (2008) *J. Immunol.* 181:1288-1298). Upon binding of certain cytokines to their receptors (for example, IL-2, IL-4, IL-7, IL-9, IL-15, and IL-21), receptor oligomerization occurs, resulting in the cytoplasmic tails of associated JAK kinases being brought into proximity and facilitating the trans-phosphorylation of tyrosine residues on the JAK kinase. This trans-phosphorylation results in the activation of the JAK kinase. Phosphorylated JAK kinases bind various STAT (Signal Transducer and Activator of Transcription) proteins. STAT proteins, which are DNA binding proteins activated by phosphorylation of tyrosine residues, function both as signaling molecules and transcription factors and ultimately bind to specific DNA sequences present in the promoters of cytokine-responsive genes (Darnell (1997) *Science* 277:1630-1635; Leonard *et al.* (1998) *Ann. Rev. Immunol.* 16:293-322; Darnell *et al.* (1994) *Science* 264:1415-1421). While JAK1, JAK2, and TYK2 are ubiquitously expressed, JAK3 is preferentially expressed in natural killer (NK) cells and not resting T cells, suggesting a role in lymphoid activation (Kawamura *et al.* (1994) *Proc. Natl. Acad. Sci. U.S.A.* 91:6374-6378). The results described herein are unexpected given the restricted JAK3 expression pattern. However, JAK3 may also be ectopically expressed in cancer (Verbsky *et al.* (1996) *J. Biol. Chem.* 271:13976-13980) and its activity in lung cancer cells is regulated by certain growth factors, such as neuregulin (Liu and Kern (2002) *Am. J. Respir. Cell Mol. Biol.* 27:306-313). Furthermore, both IL-4 and IL-9 have been shown to signal in lung cancer cells in a JAK3 dependent manner to upregulate the expression of certain cell surface glycoproteins (Damera (2006) *Respir Res* 7:39; Damera (2006) *Biosci. Rep.* 1:55-67), indicating that lung cancer cells can aberrantly engage JAK3-mediated signal transduction, which could influence their behavior.

JAK proteins comprise seven different conserved domains (JAK homology domains, JH1-7) and the structure-function relationships of these domains are well known in the art (see, for example, Rane *et al.* (2000) *Oncogene* 19:5662-5679; Scott *et al.* (2002) *Clin. Diagn. Lab. Immunol.* 9:1153-1159). The carboxyl terminus contains two nearly identical domains, an active kinase do- main (JH1) and a catalytically inactive pseudokinase domain (JH2) also termed as a kinase-like domain (KLD). It has been generally acknowledged that JH2 lacks enzymatic activity yet it is involved in regulating the activity of JH1. Both biochemical and cell biological data as well as genetic evidence from human diseases and animal models indicate that JH2 has a dual function in regulation of cytokine signaling. JH2 is required to maintain JAK kinases inactive in the absence of cytokine stimulation, but they are also required for cytokine induced signaling. The region immediately N-terminal to the JH2 is an SH2-like domain consisting of the whole JH3 and a part of JH4. The region immediately N- terminal to the SH2-like domain is a FERM-like domain consisting of a part of JH4 and the whole JH5-JH7. The JAK proteins bind to cytokine receptors through their amino-terminal FERM (Band-4.1, ezrin, radixin, moesin) domains. After the binding of cytokines to their receptors, as stated above, JAKs are activated and phosphorylate the receptors, thereby creating docking sites for signaling molecules, especially for STAT family members (Yamaoka *et al.* (2004) *Genome Biol.* 5:253). Like most kinases, JAKs require autophosphorylation for their full activity. In the case of JAK2, the phosphorylation of the activation loop tyrosines 1007 and 1008 are critical for the activity.

Activation of JAK/STAT in cancers may occur by multiple mechanisms including cytokine stimulation (*e.g.*, IL-6 or GM-CSF) or by a reduction in the endogenous suppressors of JAK signaling, such as SOCS (suppressor or cytokine signaling) or PIAS (protein inhibitor of activated STAT) (Boudny and Kovarik (2002) *J. Neoplasm.* 49:349-355). Traditionally, JAK inhibition has been desired and it is known, for example, that catalytic inactivation of JH2 domain, such as by an inactivating mutation K581A, K581R or N678A in JH2 of JAK2, abolishes aberrant activation of JAK signaling caused by activating point mutations, such as V617F. In contact, however, it has been determined herein that JAK activation is associated with the upregulation of immune checkpoint inhibitors that render cancer cells more susceptible to anti-immune checkpoint inhibitor therapy.

Mutations in a gene such as a JAK kinase that cause increased activity of the Jak kinase gene or encoded product (*e.g.*, polypeptide, RNA, and the like) are known as "activating mutations." Such mutations can be constitutive (*i.e.*, always causing increased activity) or transient (*e.g.*, pulsed for a limited duration or inducible). Such mutations can also cause variable increases in JAK activity. Activating mutations are well known in the art for JAKs. For example, point mutations causing constitutively active (*i.e.*, hyper-activating JAK signaling) include, but are not limited to, JAK1-T478S, JAK1-V623A, JAK1-A634D, JAK1-V658F, JAK1-R724H, JAK1-L683, JAK2-V617F, JAK2-M531 I, JAK2-F537I, JAK2-K539L, JAK2-F537-K539delinsL, JAK2-H538QK539L, JAK2-H538D+K539L+I546S, JAK2-H538-K539del, JAK2-D620E, JAK2-V617FD629E, JAK2-V67FC618R, JAK2-V617FC616Y; JAK2-L611S, JAK2-K607N, JAK2-T875N, JAK3-S61C, JAK3-A572V, JAK3-A573V, JAK3-A593T+A573V, JAK3-V722I, JAK3-P132T or F, TYK2-V678F, and TYK2-P1104A. Other activating JAK mutations are known to a person skilled in the art including, but not limited to, allelic variants, splice variants, derivative variants, substitution variants, deletion variants, and/or insertion variants, fusion polypeptides, orthologs, and interspecies homologs. Any combination of activating JAK mutations is contemplated.

In some embodiments, the term "activating JAK mutations" also encompass biological alterations that result in increased JAK activity. Such biological alterations include, but are not limited to, downregulating or otherwise decreasing or suppressing inhibitors of JAKs, upregulating or otherwise increasing or promoting cytokine signaling through JAKs, and upregulating or otherwise increasing or promoting JAK activity directly or through a direct binding partner in a complex with the JAK. For example, increasing cytokine stimulation (*e.g.*, IL-6 or GM-CSF) or reducing suppressors of JAK signaling, such as SOCS or PIAS.

JAK activity modulators are well known in the art. PIAS proteins, which bind and inhibit at the level of the STAT proteins (Chung *et al.* (1997) *Science* 278:1803-1805), are members of an SH2 domain-containing family of proteins able to bind to JAKs and/or receptors and block signaling (see, for example, Aman and Leonard (1997) *Curr. Biol.* 7:R784-R788; Nicholson and Hilton (1998) *J. Leukocyte Biol.* 63:665-668). Four members of the PIAS family have been identified, PIAS1, PIAS2 (also known as PIASx), PIAS3, and PIAS4 (also known as PIAS4). PIAS1 was found to bind only to activated Statl, and PIAS3 to only activated Stat3 (WO 2001/079555; Chung *et al.* (1997) *Science* 278:1803-1805; Liu

et al. (1998) *Proc. Natl. Acad. Sci. U.S.A.* 95:10626-10631). PIAS-mediated inhibition of the Jak/Stat signaling pathway, unlike SOCS-mediated inhibition of the Jak/Stat signaling pathway, is very specific.

The SOCS family of proteins have been shown to inhibit the Jak/Stat pathway by inhibiting the activity of the Jaks (Hilton *et al.* (1998) *Proc. Natl. Acad. Sci. U.S.A.* 95:114-119; Hilton (1999) *Cell, Mol. Life Sci.* 55:1658-1677; Trengove and Ward (2013) *Am. J. Clin. Exp. Immunol.* 2:1-29). The suppressor of cytokine signaling (SOCS) proteins are a family of eight SH2 domain containing proteins which includes the cytokine-inducible SH2 (CIS) domain-containing protein and SOCS-1 to 7. SOCS1 and SOCS3 directly interact with the Jaks and Tyk2 via their kinase inhibitory region (KIR) and SH2 domains, inhibiting the ability of Jak family members to phosphorylate target substrates (Kershaw *et al.* (2013) *Nat. Struct. Mol. Biol.* 20:469-476; Babon *et al.* (2012) *Immunity* 36:239-250). Once produced, SOCS proteins bind to key components of the signaling apparatus to deactivate and possibly target them for degradation via a conserved C-terminal motif, called the "SOCS Box", that recruits ubiquitin ligases (see Krebs and Hilton (2000) *J. Cell Sci.* 113:2813-2819; Yasukawa *et al.* (2000) *Annu. Rev. Immunol.* 18:143-164; Greenhalgh and Hilton (2001) *J. Leukoc. Biol.* 70:348-356). Cytokine-inducible Src homology 2-containing (CIS) protein, an inhibitor of STAT signaling (Yoshimura *et al.* (1995) *EMBO J.* 14:2816-2826) and CIS-related proteins, which can inhibit STAT signaling and/or directly bind to JAKs, are also SOCS family members (Yoshimura *et al.* (1995) *EMBO J.* 14:2816-2826; Matsumoto *et al.* (1997) *Blood* 89:3148-3154; Starr *et al.* (1997) *Nature* 387:917-921; Endo *et al.* (1997) *Nature* 387:921-924; Naka *et al.* (1997) *Nature* 387:924-929) are contemplated. Suppressor of cytokine signaling-1 protein (SOCS-1, also referred to as JAB or SSI-1) associates with all JAKs to block the downstream activation of STAT3 (Ohya *et al.* (1997) *J. Biol. Chem.* 272:27178-27182). SOCS1 expression inhibits IL-6, LIF, oncostatin M, IFN-γ, IFN-β, T-FN-α, thrombopoeitin, and growth hormone (GH) induced Jak/Stat signaling. SOCS3 expression inhibits IFN-γ, IFN-β, J-FN-α, GH and leptin. SOCS nucleic acid and polypeptide sequences, such as for SOCS1 and SOCS3, are well known in the art (see, for example, Starr *et al.* (1997) *Nature*, 387:917-921; Minamoto *et al.* (1997) *Biochem. Biophys. Res. Commun.* 237:79-83; Masuhara *et al.* (1997) *Biochem. Biophys. Res. Commun.* 239:439-446; Naka *et al.* (1997) *Nature* 387:924-929; Endo *et al.* (1997) *Nature* 387:921-924; WO 1999/028465). Similarly, modulators of SOCS activity are well known in the art (see, for example, U.S. Pat. 6,534,277; WO 2004/108955).

SHP-1 and SHP-2 bind to phosphorylated tyrosine residues on receptors or Jaks, and inactivate signaling by dephosphorylating them (Haque *et al.* (1998) *J. Biol. Chem.* 273:33898-33896; You *et al.* (1999) *Mol. Cell. Biol.* 19:2416-2424). SHP-1, also known as PTPN6, and SHP-2, also known as Syp, SHPTP2, PTP2C, PTPN11, PTP1D, and BPTP3, are members of the family of non-membrane tyrosine phosphatases (U.S. Patent No. 5,589,375, and U.S. Patent No. 5,831,009). The SHP proteins contain two src homology 2 (SH2) domains, conserved regions of approximately 100 amino acids originally identified in Src protein tyrosine kinases, that promote protein-protein interactions through phosphotyrosyl residue binding (Neel (1993) *Semin. Cell. Biol.* 4: 419-432). These two domains have been shown to display differential functions in the regulation of the phosphatase activity and consequently affect different signaling pathways. The N-terminal SH2 domain serves as a regulatory and recruiting domain, producing an autoinhibitory effect through intramolecular interactions with the internal catalytic phosphatase domain. While the C-terminal SH2 domain acts merely to recruit other proteins for intermolecular interactions necessary for signal transduction (Pei *et al.* (1996) *Proc. Natl. Acad. Sci. USA.* 93:1141-1145). The phosphorylation state of the SHP molecule regulates its phosphatase activity. Protein-tyrosine phosphatases, including SH2-containing phosphatases, are highly conserved among eukaryotes from such diverse species as mammals, including humans, to yeast and *Xenopus*. SHP-2 has been shown to play a critical role in aberrant immunological responses (*e.g.*, in the allergic response. (Pazdrak *et al.* (1997) *J. Exp. Med.* 186:561-568). SHP phosphorylation is easily detectable by methods known in the art, including, without limitation, the detection of altered mobility of the SHP molecule on a PAGE gel, phosphorylation assays, and assays which measure the activity of the SHP molecule. Detection of SHP phosphorylation may be direct, or alternatively may be indirect, *e.g.*,. detection of a downstream activity or event.

Other direct JAK inhibitors, whose elimination promotes JAK activity include tyrophostins, which are derivatives of benzylidene malononitrile, resembling tyrosine and erbstatin moieties (Gazit *et al.* (1989) *J. Med. Chem.* 32:2344-2352); AG-490, a member of the tyrophostin family of tyrosine kinase inhibitors (Wang *et al.* (1999) *J. Immunol.* 162:3897-3904; Kirken *et al.* (1999) *J. Leukoc. Biol.* 65:891-899); 4,5-dimethoxy-2-nitrobenzoic acid and 4,5-dimethoxy-2-nitrobenzamide, which specifically inhibit JAK3 (Goodman *et al.* (1998) *J. Biol. Chem.* 273:17742-17748); 4-(phenyl)-amino-6,7-dimethoxyquinazoline (parent compound WHI-258) and derivatives of this compound

which are structurally-derived from dimethoxyquinazoline compounds (Sudbeck *et al.* (1999)); compounds containing a 4′-OH group, including 4-(4′-hydroxyphenyl)-amino-6,7-dimethoxyquinazoline (WH1-P131), 4-(3′-bromo-4′-hydroxylphenyl)-amino-6,7-dimethoxyquinazoline (WH1-P154), and 4-(3′,5′-dibromo-4′-hydroxylphenyl)-amino-6,7-dimethoxyquinazoline (WH1-P97); WH1-P180, another dimethoxyquinazoline compound (Chen *et al.* (1999) *Pharm. Res.* 16:117-122); and cAMP elevating agents, such as forskolin, a direct activator of adenylate cyclase and dibutyryl cAMP, and 3-isobutyl-1-methylxanthine (IBMX), an inhibitor of cAMP phosphodiesterase (Kolenko *et al.* (1999) *Blood* 93:2308-2318).

The increases in JAK activity can be measured in any number of ways (*e.g.*, according to measures described herein, including using controls, ratios, comparisons to baselines, and the like). For example, a JAK activating mutation or an activator of JAK activity can enhance the catalytic activity of the JH2 domain or overall JAK activity as compared to the level of such JAK activity in the absence of a stimulator such as a cytokine.

Representative human Jak1 cDNA and protein sequences are well-known in the art and are publicly available from the National Center for Biotechnology Information (NCBI). For example, Jak1 sequences are available under accession numbers NM_002227.2 and NP_002218.2. Nucleic acid and polypeptide sequences of Jak1 orthologs in organisms other than humans are well known and include, for example, chimpanzee Jak1 (XM_001161205.3, XP_001161205.1, XM_001161242.3, and XP_001161242.1), monkey Jak1 (NM_001257909.1 and NP_001244838.1), dog Jak1 (NM_001287126.1 and NP_001274055.1), cow Jak1 (NM_001206534.1 and NP_001193463.1), mouse Jak1 (NM_146145.2 and NP_666257.2), and chicken Jak1 (NM_204870.1 and NP_990201.1). Representative Jak1 sequences are presented below in Table 1.

Representative human Jak2 cDNA and protein sequences are well-known in the art and are publicly available from the National Center for Biotechnology Information (NCBI). For example, Jak2 sequences are available under accession numbers NM_004972.3 and NP_004963.1. Nucleic acid and polypeptide sequences of Jak2 orthologs in organisms other than humans are well known and include, for example, chimpanzee Jak2 (XM_003311984.2, XP_003312032.1, XM_001139368.2, and XP_001139368.1), monkey Jak2 (NM_001265901.1 and NP_001252830.1), dog Jak2 (XM_541301.4 and XP_541301.2), mouse Jak2 (NM_008413.3, NP_032439.2, NM_001048177.2, and

NP_001041642.1), rat Jak2 (NM_031514.1 and NP_113702.1), and chicken Jak2 (NM_001030538.1 and NP_001025709.1). Representative Jak2 sequences are presented below in Table 1.

Representative human Jak3 cDNA and protein sequences are well-known in the art and are publicly available from the National Center for Biotechnology Information (NCBI). For example, Jak3 sequences are available under accession numbers NM_000215.3 and NP_000206.2. Nucleic acid and polypeptide sequences of Jak3 orthologs in organisms other than humans are well known and include, for example, chimpanzee Jak3 (XM_512502.4 and XP_512502.3), dog Jak3 (XM_005643717.1 and XP_005632774.1), cow Jak3 (XM_002688539.3 and XP_002688585.2), mouse Jak3 (NM_010589.6, NP_034719.2, NM_001190830.1, and NP_001177759.1), rat Jak3 (NM_012855.2 and NP_036987.2), and chicken Jak3 (NM_204996.1 and NP_990327.1). Representative Jak3 sequences are presented below in Table 1.

Representative human Tyk2 cDNA and protein sequences are well-known in the art and are publicly available from the National Center for Biotechnology Information (NCBI). For example, Tyk2 sequences are available under accession numbers NM_000215.3 and NP_000206.2. Nucleic acid and polypeptide sequences of Tyk2 orthologs in organisms other than humans are well known and include, for example, chimpanzee Tyk2 (XM_001165313.2, XP_001165313.2, XM_003316108.1, and XP_003316156.1), monkey Tyk2 (XM_001101130.2 and XP_001101130.2), dog Tyk2 (XM_005633212.1 and XP_005633269.1), cow Tyk2 (NM_001113764.1 and NP_001107236.1), mouse Tyk2 (NM_018793.2, NP_061263.2, NM_001205312.1, and NP_001192241.1), and rat Tyk2 (NM_001257347.1 and NP_001244276.1). Representative Tyk2 sequences are presented below in Table 1.

Representative human PIAS1 cDNA and protein sequences are well-known in the art and are publicly available from the National Center for Biotechnology Information (NCBI). For example, PIAS1 sequences are available under accession numbers NM_016166.1 and NP_057250.1. Nucleic acid and polypeptide sequences of PIAS1 orthologs in organisms other than humans are well known and include, for example, monkey PIAS1 (NM_001266301.2 and NP_001253230.1), cow PIAS1 (NM_001075396.2 and NP_001068864.1), mouse PIAS1 (NM_019663.3 and NP_062637.2), rat PIAS1 (NM_001106829.2 and NP_001100299.2), and chicken PIAS1 (NM_001031456.1 and NP_001026627.1). Representative PIAS1 sequences are presented below in Table 1.

Representative human PIAS2 cDNA and protein sequences are well-known in the art and are publicly available from the National Center for Biotechnology Information (NCBI). For example, human PIAS2 isoform 1 is available under accession numbers NM_173206.3 and NP_775298.1. The transcript variant uses an alternate 3' coding exon compared to variant 2 resulting in a shorter isoform that has a unique C-terminus relative to isoform 2.

Human PIAS2 isoform 2, available under accession numbers NM_004671.3 and NP_004662.2 represents the longer transcript and encodes the longer isoform. Nucleic acid and polypeptide sequences of PIAS2 orthologs in organisms other than humans are well known and include, for example, chimpanzee PIAS2 (XM_001147441.3, XP_001147441.2, XM_003953281.1, and XP_00395330.1), monkey PIAS2 (XM_001085456.2 and XP_001085456.2), mouse PIAS2 (NM_008602.4, NP_032628.3, NM_001164170.1, NP_001157642.1, NM_001164169.1, NP_001157641.1, NM_001164168.1, NP_001157640.1, NM_001164167.1, and NP_001157639.1), rat PIAS2 (NM_053337.1 and NP_445789.1), and chicken PIAS2 (NM_001030626.1 and NP_001025797.1). Representative PIAS2 sequences are presented below in Table 1.

Representative human PIAS3 cDNA and protein sequences are well-known in the art and are publicly available from the National Center for Biotechnology Information (NCBI). For example, PIAS3 sequences are available under accession numbers NM_006099.3 and NP_006090.2. Nucleic acid and polypeptide sequences of PIAS3 orthologs in organisms other than humans are well known and include, for example, chimpanzee PIAS3 (XM_003949491.1 and XP_003949540.1), monkey PIAS3 (XM_001095153.2 and XP_001095153.2), cow PIAS3 (NM_001102185.1 and NP_001095655.1), mouse PIAS3 (NM_146135.2, NP_666247.1, NM_018812.2, NP_061282.2, NM_001165949.1, and NP_001159421.1), and rat PIAS3 (NM_031784.2 and NP_113972.2). Representative PIAS3 sequences are presented below in Table 1.

Representative human PIAS4 cDNA and protein sequences are well-known in the art and are publicly available from the National Center for Biotechnology Information (NCBI). For example, PIAS4 sequences are available under accession numbers NM_015897.2 and NP_056981.2. Nucleic acid and polypeptide sequences of PIAS4 orthologs in organisms other than humans are well known and include, for example, dog PIAS4 (XM_542167.5 and XP_542167.4), cow PIAS4 (NM_001083482.2 and NP_001076951.1), mouse PIAS4 (NM_021501.4 and NP_067476.2), and rat PIAS4

(NM_001100757.1 and NP_001094227.1). Representative PIAS4 sequences are presented below in Table 1.

Representative human SOCS1 cDNA and protein sequences are well-known in the art and are publicly available from the National Center for Biotechnology Information (NCBI). For example, SOCS1 sequences are available under accession numbers NM_003745.1 and NP_003736.1. Nucleic acid and polypeptide sequences of SOCS1 orthologs in organisms other than humans are well known and include, for example, chimpanzee SOCS1 (XM_001141793.3 and XP_001141793.1), monkey SOCS1 (XM_001104595.2 and XP_001104595.1), dog SOCS1 (XM_005622079.1 and XP_005622136.1), cow SOCS1 (XM_002697964.2 and XP_002698010.1), mouse SOCS1 (NM_009896.2, NP_034026.1, NM_001271603.1, and NP_001258532.1), rat SOCS1 (NM_145879.2 and NP_665886.2), and chicken SOCS1 (NM_001137648.1 and NP_001131120.1). Representative SOCS1 sequences are presented below in Table 1.

Representative human SOCS3 cDNA and protein sequences are well-known in the art and are publicly available from the National Center for Biotechnology Information (NCBI). For example, SOCS1 sequences are available under accession numbers NM_003955.4 and NP_003946.3. Nucleic acid and polypeptide sequences of SOCS3 orthologs in organisms other than humans are well known and include, for example, chimpanzee SOCS3 (XM_001157032.3 and XP_001157032.1), monkey SOCS3 (NM_001194326.1 and NP_001181255.1), dog SOCS3 (NM_001031631.1 and NP_001026801.1), cow SOCS3 (NM_174466.2 and NP_776891.1), mouse SOCS3 (NM_007707.3 and NP_031733.1), rat SOCS3 (NM_053565.1 and NP_446017.1), and chicken SOCS3 (NM_204600.1 and NP_989931.1). Representative SOCS3 sequences are presented below in Table 1.

Nucleic acid and polypeptide sequences of other SOCS orthologs in organisms, including humans, are also well known. For example, nucleic acid and polypeptide sequences of cytokine-inducible SH2 (CIS) are well known and include, for example, human CIS (NM_145071.2, NP_659508.1, NM_013324.5, and NP_037456.5), chimpanzee CIS (XM_526202.3, XP_526202.3, XM_003309810.1, and XP_003309858.1), monkey CIS (NM_001258075.1 and NP_001245004.1), dog CIS (XM_541873.4 and XP_541873.3), cow CIS (NM_001046586.1 and NP_001040051.1), mouse CIS (NM_009895.3 and NP_034025.1), rat CIS (NM_031804.1 and NP_113992.1), and chicken CIS (NM_204626.1 and NP_989957.1). Nucleic acid and polypeptide sequences

of SOCS2 are well known and include, for example, human SOCS2 (NM_003877.4, NP_003868.1, NM_001270471.1, NM_001257400.1, NM_001270470.1, NM_001257399.1, NM_001270469.1, NM_001257398.1, NM_001270468.1, NM_001257397.1, NM_001270467.1, and NM_001257396.1), chimpanzee SOCS2 (XM_001139989.3 and XP_001139989.1), monkey SOCS2 (NM_001194762.1 and NP_001181691.1), cow SOCS2 (NM_177523.2 and NP_803489.1), mouse SOCS2 (NM_007706.4, NP_031732.1, NM_001168657.1, NP_001162128.1, NM_001168656.1, NP_001162127.1, NM_001168655.1, and NP_001162126.1), rat SOCS2 (NM_058208.1 and NP_478115.1), and chicken SOCS2 (NM_204540.1 and NP_989871.1). Nucleic acid and polypeptide sequences of SOCS4 are well known and include for example, human SOCS4 (NM_199421.1, NP_955453.1, NM_080867.2, and NP_543143.1), monkey SOCS4 (NM_001193820.1 and NP_001180749.1), dog SOCS4 (XM_003435136.3 and XP_003435184.1), cow SOCS4 (NM_001076218.2 and NP_001069686.1), mouse SOCS4 (NM_080843.2 and NP_543119.2), rat SOCS4 (NM_001107256.2 and NP_001100726.1), and chicken SOCS4 (NM_001199108.1 and NP_001186037.1). Nucleic acid and polypeptide sequences of SOCS5 are well known and include for example, human SOCS5 (NM_144949.2, NP_659198.1, NM_014011.4, and NP_054730.1), chimpanzee SOCS5 (XM_515453.3 and XP_515453.2), monkey SOCS5 (NM_001266928.1 and NP_001253857.1), cow SOCS5 (XM_005626083.1 and XP_005626140.1), cow SOCS5 (NM_001046182.1 and NP_001039647.1), mouse SOCS5 (XM_006524675.1, XP_006524738.1, XM_006524671.1, XP_006524734.1, XM_006524672.1, XP_006524735.1, XM_006524673.1, XP_006524736.1, XM_006524674.1, and XP_006524737.1), rat SOCS5 (NM_001109274.1 and NP_001102744.1), and chicken SOCS5 (NM_001127314.1 and NP_001120786.1). Nucleic acid and polypeptide sequences of SOCS6 are well known and include for example, human SOCS6 (NM_004232.3 and NP_004223.2), mouse SOCS6 (NM_018821.4 and NP_061291.2), rat SOCS6 (NM_001271149.1 and NP_001258078.1), and chicken SOCS6 (NM_001127312.1 and NP_001120784.1). Finally, nucleic acid and polypeptide sequences of SOCS7 are well known and include for example, human SOCS7 (NM_014598.3 and NP_055413.1), chimpanzee SOCS7 (XM_003954433.1 and XP_003954482.1), monkey SOCS7 (XM_001082440.2 and XP_001082440.2), dog SOCS7 (XM_005624981.1 and XP_005625038.1), mouse SOCS7 (NM_138657.3 and NP_619598.1), and rat SOCS7 (XM_006247484.1 and XP_006247546.1).

Representative human SHP-1 cDNA and protein sequences are well-known in the art and are publicly available from the National Center for Biotechnology Information (NCBI). For example, SHP-1 isoform 1 is available under accession numbers NM_002831.5 and NP_002822.2. Transcript variant 1 encoding isoform 1 represents the predominant transcript and encodes the shortest isoform. Transcript variant 2 (NM_080548.4) uses an alternate 5' terminal exon compared to transcript variant 1 resulting in a SHP-1 isoform 2 (NP_536858.1) with a distinct and 2 amino acid longer N-terminus as compared to isoform 1. Finally, transcript variant 3 (NM_0800549.3) uses an alternate 5' terminal exon and an alternate acceptor splice site at the penultimate exon as compared to transcript variant 1 resulting in a longer isoform (SHP-1 isoform 3; NP_536859.1; also known as SHP-1L) with a distinct N- and C-terminus as compared to isoform 1. Nucleic acid and polypeptide sequences of SHP-1 orthologs in organisms other than humans are well known and include, for example, monkey SHP-1 (XM_001110915.2 and XP_001110915.1), dog SHP-1 (XM_005637211.1 and XP_005637268.1), cow SHP-1 (NM_001098017.1 and NP_001091486.1), mouse SHP-1 (NM_013545.3, NP_038573.2, NM_001077705.2, and NP_001071173.1), rat SHP-1 (NM_053908.1 and NP_446360.1), and chicken SHP-1 (NM_001031484.1 and NP_001026655.1). Representative SHP-1 sequences are presented below in Table 1.

Representative human SHP-2 cDNA and protein sequences are well-known in the art and are publicly available from the National Center for Biotechnology Information (NCBI). For example, SHP-2 isoform 1 is available under accession numbers NM_002834.3 and NP_002825.3. Transcript variant 1 encoding isoform 1 represents the longer transcript and encodes the longer isoform. Transcript variant 2 (NM_080601.1) differs in the 3' untranslated region (UTR) and coding sequence as compared to transcript variant 1 resulting in a SHP-2 isoform 2 (NP_542168.1) with a shorter and distinct N-terminus as compared to isoform 1. Nucleic acid and polypeptide sequences of SHP-2 orthologs in organisms other than humans are well known and include, for example, chimpanzee SHP-2 (XM_522535.4 and XP_522535.3), monkey SHP-2 (NM_001261109.1 and NP_001248038.1), dog SHP-2 (XM_005636251.1, XP_005636308.1, XM_005636250.1, and XP_005636307.1), cow SHP-2 (XM_002694590.3 and XP_002694636.2), mouse SHP-2 (NM_011202.3, NP_035332.1, NM_001109992.1, and NP_001103462.1), rat SHP-2 (NM_013088.2, NP_037220.2, NM_001177593.1, and

NP_001171064.1), and chicken SHP-2 (NM_204968.1 and NP_990299.1). Representative SHP-2 sequences are presented below in Table 1.

It is to be noted that the biomarkers described herein can be used to refer to any combination of features described herein regarding any individual or combination of such biomarkers. For example, any combination of sequence composition, percentage identity, sequence length, domain structure, functional activity, mutation status, *etc.* can be used to describe a biomarker molecule of the present invention.

A "blocking" antibody or an antibody "antagonist" is one which inhibits or reduces at least one biological activity of the antigen(s) it binds. In certain embodiments, the blocking antibodies or antagonist antibodies or fragments thereof described herein substantially or completely inhibit a given biological activity of the antigen(s).

The term "body fluid" refers to fluids that are excreted or secreted from the body as well as fluid that are normally not (*e.g.*, bronchoalveolar lavage fluid, amniotic fluid, aqueous humor, bile, blood and blood plasma, cerebrospinal fluid, cerumen and earwax, cowper's fluid or pre-ejaculatory fluid, chyle, chyme, stool, female ejaculate, interstitial fluid, intracellular fluid, lymph, menses, breast milk, mucus, pleural fluid, pus, saliva, sebum, semen, serum, sweat, synovial fluid, tears, urine, vaginal lubrication, vitreous humor, vomit).

The terms "cancer" or "tumor" or "hyperproliferative" refer to the presence of cells possessing characteristics typical of cancer-causing cells, such as uncontrolled proliferation, immortality, metastatic potential, rapid growth and proliferation rate, and certain characteristic morphological features. In some embodiments, such cells exhibit such characteristics in part or in full due to the expression and activity of immune checkpoint inhibitors, such as PD-1, PD-L1, PD-L2, and/or CTLA-4. Cancer cells are often in the form of a tumor, but such cells may exist alone within an animal, or may be a non-tumorigenic cancer cell, such as a leukemia cell. As used herein, the term "cancer" includes premalignant as well as malignant cancers. Cancers include, but are not limited to, B cell cancer, *e.g.*, multiple myeloma, Waldenström's macroglobulinemia, the heavy chain diseases, such as, for example, alpha chain disease, gamma chain disease, and mu chain disease, benign monoclonal gammopathy, and immunocytic amyloidosis, melanomas, breast cancer, lung cancer, bronchus cancer, colorectal cancer, prostate cancer, pancreatic cancer, stomach cancer, ovarian cancer, urinary bladder cancer, brain or central nervous system cancer, peripheral nervous system cancer, esophageal cancer, cervical cancer,

uterine or endometrial cancer, cancer of the oral cavity or pharynx, liver cancer, kidney cancer, testicular cancer, biliary tract cancer, small bowel or appendix cancer, salivary gland cancer, thyroid gland cancer, adrenal gland cancer, osteosarcoma, chondrosarcoma, cancer of hematologic tissues, and the like. Other non-limiting examples of types of cancers applicable to the methods encompassed by the present invention include human sarcomas and carcinomas, *e.g.*, fibrosarcoma, myxosarcoma, liposarcoma, chondrosarcoma, osteogenic sarcoma, chordoma, angiosarcoma, endotheliosarcoma, lymphangiosarcoma, lymphangioendotheliosarcoma, synovioma, mesothelioma, Ewing's tumor, leiomyosarcoma, rhabdomyosarcoma, colon carcinoma, colorectal cancer, pancreatic cancer, breast cancer, ovarian cancer, prostate cancer, squamous cell carcinoma, basal cell carcinoma, adenocarcinoma, sweat gland carcinoma, sebaceous gland carcinoma, papillary carcinoma, papillary adenocarcinomas, cystadenocarcinoma, medullary carcinoma, bronchogenic carcinoma, renal cell carcinoma, hepatoma, bile duct carcinoma, liver cancer, choriocarcinoma, seminoma, embryonal carcinoma, Wilms' tumor, cervical cancer, bone cancer, brain tumor, testicular cancer, lung carcinoma, small cell lung carcinoma, bladder carcinoma, epithelial carcinoma, glioma, astrocytoma, medulloblastoma, craniopharyngioma, ependymoma, pinealoma, hemangioblastoma, acoustic neuroma, oligodendroglioma, meningioma, melanoma, neuroblastoma, retinoblastoma; leukemias, *e.g.*, acute lymphocytic leukemia and acute myelocytic leukemia (myeloblastic, promyelocytic, myelomonocytic, monocytic and erythroleukemia); chronic leukemia (chronic myelocytic (granulocytic) leukemia and chronic lymphocytic leukemia); and polycythemia vera, lymphoma (Hodgkin's disease and non-Hodgkin's disease), multiple myeloma, Waldenstrom's macroglobulinemia, and heavy chain disease. In some embodiments, cancers are epithlelial in nature and include but are not limited to, bladder cancer, breast cancer, cervical cancer, colon cancer, gynecologic cancers, renal cancer, laryngeal cancer, lung cancer, oral cancer, head and neck cancer, ovarian cancer, pancreatic cancer, prostate cancer, or skin cancer. In other embodiments, the cancer is breast cancer, prostate cancer, lung cancer, or colon cancer. In still other embodiments, the epithelial cancer is non-small-cell lung cancer, nonpapillary renal cell carcinoma, cervical carcinoma, ovarian carcinoma (*e.g.*, serous ovarian carcinoma), or breast carcinoma. The epithelial cancers may be characterized in various other ways including, but not limited to, serous, endometrioid, mucinous, clear cell, Brenner, or undifferentiated.

In some embodiments, lung cancer subtypes are included. For example, according to the American Cancer Society, there are two major types of lung cancer: small cell lung cancer (SCLC) and non-small cell lung cancer (NSCLC). SCLC comprises about 15% of all cancers. NSCLC, however, comprises about 85% of all lung cancers and is divided into three distinct sub-types: squamous cell carcinoma (about 25-30% of the cases), large cell carcinomas (about 10-15%), and adenocarcinomas (about 40%). The cells in these sub-types differ in size, shape, and chemical make-up. These lung cancers are inclusive of bronchogenic carcinoma, bronchial carcinoids, chondromatous hamartoma, solitary pulmonary nodules, pulmonary sarcomas, undifferentiated small cell carcinoma, undifferentiated large cell carcinoma, and broncholoalveolar carcinomas. Each such lung cancer subtype is contemplated for use according to the present invention, either alone or in any combination.

The term "coding region" refers to regions of a nucleotide sequence comprising codons which are translated into amino acid residues, whereas the term "noncoding region" refers to regions of a nucleotide sequence that are not translated into amino acids (*e.g.*, 5' and 3' untranslated regions).

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

The term "control" refers to any reference standard suitable to provide a comparison to the expression products in the test sample. In one embodiment, the control comprises obtaining a "control sample" from which expression product levels are detected and compared to the expression product levels from the test sample. Such a control sample may comprise any suitable sample, including but not limited to a sample from a control cancer patient (can be stored sample or previous sample measurement) with a known outcome; normal tissue or cells isolated from a subject, such as a normal patient or the cancer patient, cultured primary cells/tissues isolated from a subject such as a normal subject or the cancer patient, adjacent normal cells/tissues obtained from the same organ or body location of the cancer patient, a tissue or cell sample isolated from a normal subject, or a primary cells/tissues obtained from a depository. In another preferred embodiment, the control may comprise a reference standard expression product level from any suitable source, including but not limited to housekeeping genes, an expression product level range from normal tissue (or other previously analyzed control sample), a previously determined expression product level range within a test sample from a group of patients, or a set of patients with a certain outcome (for example, survival for one, two, three, four years, etc.) or receiving a certain treatment (for example, standard of care cancer therapy). It will be understood by those of skill in the art that such control samples and reference standard expression product levels can be used in combination as controls in the methods of the present invention. In one embodiment, the control may comprise normal or non-cancerous cell/tissue sample. In another preferred embodiment, the control may comprise an expression level for a set of patients, such as a set of cancer patients, or for a set of cancer patients receiving a certain treatment, or for a set of patients with one outcome versus another outcome. In the former case, the specific expression product level of each patient can be assigned to a percentile level of expression, or expressed as either higher or lower than the mean or average of the reference standard expression level. In another preferred embodiment, the control may comprise normal cells, cells from patients treated with combination chemotherapy, and cells from patients having benign cancer. In another embodiment, the control may also comprise a measured value for example, average level of expression of a particular gene in a population compared to the level of expression of a housekeeping gene in the same population. Such a population may comprise normal subjects, cancer patients who have not undergone any treatment (*i.e.*, treatment naive), cancer patients undergoing standard of care therapy, or patients having benign cancer. In another preferred embodiment, the control

comprises a ratio transformation of expression product levels, including but not limited to determining a ratio of expression product levels of two genes in the test sample and comparing it to any suitable ratio of the same two genes in a reference standard; determining expression product levels of the two or more genes in the test sample and determining a difference in expression product levels in any suitable control; and determining expression product levels of the two or more genes in the test sample, normalizing their expression to expression of housekeeping genes in the test sample, and comparing to any suitable control. In particularly preferred embodiments, the control comprises a control sample which is of the same lineage and/or type as the test sample. In another embodiment, the control may comprise expression product levels grouped as percentiles within or based on a set of patient samples, such as all patients with cancer. In one embodiment a control expression product level is established wherein higher or lower levels of expression product relative to, for instance, a particular percentile, are used as the basis for predicting outcome. In another preferred embodiment, a control expression product level is established using expression product levels from cancer control patients with a known outcome, and the expression product levels from the test sample are compared to the control expression product level as the basis for predicting outcome. As demonstrated by the data below, the methods of the invention are not limited to use of a specific cut-point in comparing the level of expression product in the test sample to the control.

The "copy number" of a biomarker nucleic acid refers to the number of DNA sequences in a cell (*e.g.*, germline and/or somatic) encoding a particular gene product. Generally, for a given gene, a mammal has two copies of each gene. The copy number can be increased, however, by gene amplification or duplication, or reduced by deletion. For example, germline copy number changes include changes at one or more genomic loci, wherein said one or more genomic loci are not accounted for by the number of copies in the normal complement of germline copies in a control (*e.g.*, the normal copy number in germline DNA for the same species as that from which the specific germline DNA and corresponding copy number were determined). Somatic copy number changes include changes at one or more genomic loci, wherein said one or more genomic loci are not accounted for by the number of copies in germline DNA of a control (*e.g.*, copy number in germline DNA for the same subject as that from which the somatic DNA and corresponding copy number were determined).

The "normal" copy number (*e.g.*, germline and/or somatic) of a biomarker nucleic acid or "normal" level of expression of a biomarker nucleic acid, protein, or metabolite is the activity/level of expression or copy number in a biological sample, *e.g.*, a sample containing tissue, whole blood, serum, plasma, buccal scrape, saliva, cerebrospinal fluid, urine, stool, and bone marrow, from a subject, *e.g.*, a human, not afflicted with cancer, or from a corresponding non-cancerous tissue in the same subject who has cancer.

The term "determining a suitable treatment regimen for the subject" is taken to mean the determination of a treatment regimen (*i.e.*, a single therapy or a combination of different therapies that are used for the prevention and/or treatment of the cancer in the subject) for a subject that is started, modified and/or ended based or essentially based or at least partially based on the results of the analysis according to the present invention. One example is determining whether to provide targeted therapy against a cancer to provide immunotherapy that generally increases immune responses against the cancer (*e.g.*, anti-immune checkpoint inhibitor therapy). Another example is starting an adjuvant therapy after surgery whose purpose is to decrease the risk of recurrence, another would be to modify the dosage of a particular chemotherapy. The determination can, in addition to the results of the analysis according to the present invention, be based on personal characteristics of the subject to be treated. In most cases, the actual determination of the suitable treatment regimen for the subject will be performed by the attending physician or doctor.

A molecule is "fixed" or "affixed" to a substrate if it is covalently or non-covalently associated with the substrate such that the substrate can be rinsed with a fluid (*e.g.* standard saline citrate, pH 7.4) without a substantial fraction of the molecule dissociating from the substrate.

The term "expression signature" or "signature" refers to a group of two or more coordinately expressed biomarkers. For example, the genes, proteins, metabolites, and the like making up this signature may be expressed in a specific cell lineage, stage of differentiation, or during a particular biological response. The biomarkers can reflect biological aspects of the tumors in which they are expressed, such as the cell of origin of the cancer, the nature of the non-malignant cells in the biopsy, and the oncogenic mechanisms responsible for the cancer. Expression data and gene expression levels can be stored on computer readable media, *e.g.*, the computer readable medium used in

conjunction with a microarray or chip reading device. Such expression data can be manipulated to generate expression signatures.

"Homologous" as used herein, refers to nucleotide sequence similarity between two regions of the same nucleic acid strand or between regions of two different nucleic acid strands. When a nucleotide residue position in both regions is occupied by the same nucleotide residue, then the regions are homologous at that position. A first region is homologous to a second region if at least one nucleotide residue position of each region is occupied by the same residue. Homology between two regions is expressed in terms of the proportion of nucleotide residue positions of the two regions that are occupied by the same nucleotide residue. By way of example, a region having the nucleotide sequence 5'-ATTGCC-3' and a region having the nucleotide sequence 5'-TATGGC-3' share 50% homology. Preferably, the first region comprises a first portion and the second region comprises a second portion, whereby, at least about 50%, and preferably at least about 75%, at least about 90%, or at least about 95% of the nucleotide residue positions of each of the portions are occupied by the same nucleotide residue. More preferably, all nucleotide residue positions of each of the portions are occupied by the same nucleotide residue.

The term "immune cell" refers to cells that play a role in the immune response. Immune cells are of hematopoietic origin, and include lymphocytes, such as B cells and T cells; natural killer cells; myeloid cells, such as monocytes, macrophages, eosinophils, mast cells, basophils, and granulocytes.

The term "immune checkpoint inhibitor" means a group of molecules on the cell surface of CD4+ and/or CD8+ T cells that fine-tune immune responses by down-modulating or inhibiting an anti-tumor immune response. Immune checkpoint proteins are well known in the art and include, without limitation, CTLA-4, PD-1, VISTA, B7-H2, B7-H3, PD-L1, B7-H4, B7-H6, 2B4, ICOS, HVEM, PD-L2, CD160, gp49B, PIR-B, KIR family receptors, TIM-1, TIM-3, TIM-4, LAG-3, BTLA, SIRPalpha (CD47), CD48, 2B4 (CD244), B7.1, B7.2, ILT-2, ILT-4, TIGIT, and A2aR (see, for example, WO 2012/177624). "Anti-immune checkpoint inhibitor therapy" refers to the use of agents that inhibit immune checkpoint inhibitors. Inhibition of one or more immune checkpoint inhibitors can block or otherwise neutralize inhibitory signaling to thereby upregulate an immune response in order to more efficaciously treat cancer. Exemplary agents useful for inhibiting immune checkpoint inhibitors include antibodies, small molecules, peptides, peptidomimetics, natural ligands, and derivatives of natural ligands, that can either bind

and/or inactivate or inhibit immune checkpoint proteins, or fragments thereof; as well as RNA interference, antisense, nucleic acid aptamers, etc. that can downregulate the expression and/or activity of immune checkpoint inhibitor nucleic acids, or fragments thereof. Exemplary agents for upregulating an immune response include antibodies against one or more immune checkpoint inhibitor proteins block the interaction between the proteins and its natural receptor(s); a non-activating form of one or more immune checkpoint inhibitor proteins (*e.g.*, a dominant negative polypeptide); small molecules or peptides that block the interaction between one or more immune checkpoint inhibitor proteins and its natural receptor(s); fusion proteins (*e.g.* the extracellular portion of an immune checkpoint inhibition protein fused to the Fc portion of an antibody or immunoglobulin) that bind to its natural receptor(s); nucleic acid molecules that block immune checkpoint inhibitor nucleic acid transcription or translation; and the like. Such agents can directly block the interaction between the one or more immune checkpoint inhibitors and its natural receptor(s) (*e.g.*, antibodies) to prevent inhibitory signaling and upregulate an immune response. Alternatively, agents can indirectly block the interaction between one or more immune checkpoint proteins and its natural receptor(s) to prevent inhibitory signaling and upregulate an immune response. For example, a soluble version of an immune checkpoint protein ligand such as a stabilized extracellular domain can binding to its receptor to indirectly reduce the effective concentration of the receptor to bind to an appropriate ligand. In one embodiment, anti-PD-1 antibodies, anti-PD-L1 antibodies, and anti-CTLA-4 antibodies, either alone or in combination, are used to inhibit immune checkpoint inhibitors.

"PD-1" is an immune checkpoint inhibitor that refers to a member of the immunoglobulin gene superfamily that functions as a coinhibitory receptor having PD-L1 and PD-L2 as known ligands. PD-1 was previously identified using a subtraction cloning based approach to select for proteins involved in apoptotic cell death. PD-1 is a member of the CD28/CTLA-4 family of molecules based on its ability to bind to PD-L1. Like CTLA-4, PD-1 is rapidly induced on the surface of T-cells in response to anti-CD3 (Agata *et al.* 25 (1996) *Int. Immunol.* 8:765). In contrast to CTLA-4, however, PD-1 is also induced on the surface of B-cells (in response to anti-IgM). PD-1 is also expressed on a subset of thymocytes and myeloid cells (Agata *et al.* (1996) *supra;* Nishimura *et al.* (1996) *Int. Immunol.* 8:773).

The nucleic acid and amino acid sequences of a representative human PD-1 biomarker is available to the public at the GenBank database under NM_005018.2 and NP_005009.2 (see also Ishida *et al.* (1992) 20 *EMBO J* 11:3887; Shinohara *et al.* (1994) *Genomics* 23:704; U.S. Patent 5,698,520). PD-1 has an extracellular region containing immunoglobulin superfamily domain, a transmembrane domain, and an intracellular region including an immunoreceptor tyrosine-based inhibitory motif (ITIM) (Ishida *et al.* (1992) *EMBO J.* 11:3887; Shinohara *et al.* (1994) *Genomics* 23:704; and U.S. Patent 5,698,520). These features also define a larger family of polypeptides, called the immunoinhibitory receptors, which also includes gp49B, PIR-B, and the killer inhibitory receptors (KIRs) (Vivier and Daeron (1997) *Immunol. Today* 18:286). It is often assumed that the tyrosyl phosphorylated ITIM motif of these receptors interacts with SH2-domain containing phosphatases, which leads to inhibitory signals. A subset of these immunoinhibitory receptors bind to MHC polypeptides, for example the KIRs, and CTLA4 binds to B7-1 and B7-2. It has been proposed that there is a phylogenetic relationship between the MHC and B7 genes (Henry *et al.* (1999) *Immunol. Today* 20(6):285-8). Nucleic acid and polypeptide sequences of PD-1 orthologs in organisms other than humans are well known and include, for example, mouse PD-1 (NM_008798.2 and NP_032824.1), rat PD-1 (NM_001106927.1 and NP_001100397.1), dog PD-1 (XM_543338.3 and XP_543338.3), cow PD-1 (NM_001083506.1 and NP_001076975.1), and chicken PD-1 (XM_422723.3 and XP_422723.2).

PD-1 polypeptides are inhibitory receptors capable of transmitting an inhibitory signal to an immune cell to thereby inhibit immune cell effector function, or are capable of promoting costimulation (*e.g.*, by competitive inhibition) of immune cells, *e.g.*, when present in soluble, monomeric form. Preferred PD-1 family members share sequence identity with PD-1 and bind to one or more B7 family members, *e.g.*, B7-1, B7-2, PD-1 ligand, and/or other polypeptides on antigen presenting cells.

The term "PD-1 activity" includes the ability of a PD-1 polypeptide to modulate an inhibitory signal in an activated immune cell, *e.g.*, by engaging a natural PD-1 ligand on an antigen presenting cell. PD-1 transmits an inhibitory signal to an immune cell in a manner similar to CTLA4. Modulation of an inhibitory signal in an immune cell results in modulation of proliferation of, and/or cytokine secretion by, an immune cell. Thus, the term "PD-1 activity" includes the ability of a PD-1 polypeptide to bind its natural ligand(s),

the ability to modulate immune cell costimulatory or inhibitory signals, and the ability to modulate the immune response.

The term "PD-1 ligand" refers to binding partners of the PD-1 receptor and includes both PD-L1 (Freeman *et al.* (2000) *J. Exp. Med.* 192:1027) and PD-L2 (Latchman *et al.* (2001) *Nat. Immunol.* 2:261). At least two types of human PD-1 ligand polypeptides exist. PD-1 ligand proteins comprise a signal sequence, and an IgV domain, an IgC domain, a transmembrane domain, and a short cytoplasmic tail. Both PD-L1 (See Freeman *et al.* (2000) J. Exp. Med. 192:1027 for sequence data) and PD-L2 (See Latchman *et al.* (2001) Nat. Immunol. 2:261 for sequence data) are members of the B7 family of polypeptides. Both PD-L1 and PD-L2 are expressed in placenta, spleen, lymph nodes, thymus, and heart. Only PD-L2 is expressed in pancreas, lung and liver, while only PD-L1 is expressed in fetal liver. Both PD-1 ligands are upregulated on activated monocytes and dendritic cells, although PD-L1 expression is broader. For example, PD-L1 is known to be constitutively expressed and upregulated to higher levels on murine hematopoietic cells (*e.g.*, T cells, B cells, macrophages, dendritic cells (DCs), and bone marrow-derived mast cells) and non-hematopoietic cells (*e.g.*, endothelial, epithelial, and muscle cells), whereas PD-L2 is inducibly expressed on DCs, macrophages, and bone marrow-derived mast cells (see, Butte *et al.* (2007) *Immunity* 27:111).

PD-1 ligands comprise a family of polypeptides having certain conserved structural and functional features. The term "family" when used to refer to proteins or nucleic acid molecules, is intended to mean two or more proteins or nucleic acid molecules having a common structural domain or motif and having sufficient amino acid or nucleotide sequence homology, as defined herein. Such family members can be naturally or non-naturally occurring and can be from either the same or different species. For example, a family can contain a first protein of human origin, as well as other, distinct proteins of human origin or alternatively, can contain homologues of non-human origin. Members of a family may also have common functional characteristics. PD-1 ligands are members of the B7 family of polypeptides. The term "B7 family" or "B7 polypeptides" as used herein includes costimulatory polypeptides that share sequence homology with B7 polypeptides, *e.g.*, with B7-1 (CD80), B7-2 (CD86), inducible costimulatory ligand (ICOS-L), B7-H3, B7-H4, VISTA, B7-H6, B7h (Swallow *et al.* (1999) *Immunity* 11:423), and/or PD-1 ligands (*e.g.*, PD-L1 or PD-L2). For example, human B7-1 and B7-2 share approximately 26% amino acid sequence identity when compared using the BLAST program at NCBI with the

default parameters (Blosum62 matrix with gap penalties set at existence 11 and extension 1 (see the NCBI website). The term B7 family also includes variants of these polypeptides which are capable of modulating immune cell function. The B7 family of molecules share a number of conserved regions, including signal domains, IgV domains and the IgC domains. IgV domains and the IgC domains are art-recognized Ig superfamily member domains. These domains correspond to structural units that have distinct folding patterns called Ig folds. Ig folds are comprised of a sandwich of two β sheets, each consisting of anti-parallel β strands of 5-10 amino acids with a conserved disulfide bond between the two sheets in most, but not all, IgC domains of Ig, TCR, and MHC molecules share the same types of sequence patterns and are called the C1-set within the Ig superfamily. Other IgC domains fall within other sets. IgV domains also share sequence patterns and are called V set domains. IgV domains are longer than IgC domains and contain an additional pair of β strands.

The term "PD-L1" refers to a specific PD-1 ligand. Two forms of human PD-L1 molecules have been identified. One form is a naturally occurring PD-L1 soluble polypeptide, *i.e.*, having a short hydrophilic domain at the COOH-terminal end and no transmembrane domain, and is referred to herein as PD-L1S. The second form is a cell-associated polypeptide, *i.e.*, having a transmembrane and cytoplasmic domain, referred to herein as PD-L1M. The nucleic acid and amino acid sequences of representative human PD-L1 biomarkers regarding PD-L1M are also available to the public at the GenBank database under NM_014143.3 and NP_054862.1. PD-L1 proteins comprise a signal sequence, and an IgV domain and an IgC domain. The signal sequence is from about amino acid 1 to about amino acid 18. The signal sequence is from about amino acid 1 to about amino acid 18. The IgV domain is from about amino acid 19 to about amino acid 134 and the IgV domain is from about amino acid 19 to about amino acid 134. The IgC domain is from about amino acid 135 to about amino acid 227 and the IgC domain of SEQ ID NO: 6 is shown from about amino acid 135 to about amino acid 227. The hydrophilic tail of PD-L1 comprises a hydrophilic tail shown from about amino acid 228 to about amino acid 245. The PD-L1 polypeptide comprises a transmembrane domain shown from about amino acids 239 to about amino acid 259 and a cytoplasmic domain shown of about 30 amino acids from 260 to about amino acid 290. In addition, nucleic acid and polypeptide sequences of PD-L1 orthologs in organisms other than humans are well known and include, for example, mouse PD-L1 (NM_021893.3 and NP_068693.1), rat PD-L1 (NM_001191954.1 and

NP_001178883.1), dog PD-L1 (XM_541302.3 and XP_541302.3), cow PD-L1 (NM_001163412.1 and NP_001156884.1), and chicken PD-L1 (XM_424811.3 and XP_424811.3).

The term "PD-L2" refers to another specific PD-1 ligand. PD-L2 is a B7 family member expressed on various APCs, including dendritic cells, macrophages and bone-marrow derived mast cells (Zhong *et al.* (2007) *Eur. J. Immunol.* 37:2405). APC-expressed PD-L2 is able to both inhibit T cell activation through ligation of PD-1 and costimulate T cell activation, through a PD-1 independent mechanism (Shin *et al.* (2005) *J. Exp. Med.* 201:1531). In addition, ligation of dendritic cell-expressed PD-L2 results in enhanced dendritic cell cytokine expression and survival (Radhakrishnan *et al.* (2003) *J. Immunol.* 37:1827; Nguyen *et al.* (2002) *J. Exp. Med.* 196:1393). The nucleic acid and amino acid sequences of representative human PD-L2 biomarkers are well known in the art and are also available to the public at the GenBank database under NM_025239.3 and NP_079515.2. PD-L2 proteins are characterized by common structural elements. In some embodiments, PD-L2 proteins include at least one or more of the following domains: a signal peptide domain, a transmembrane domain, an IgV domain, an IgC domain, an extracellular domain, a transmembrane domain, and a cytoplasmic domain. For example, amino acids 1-19 comprise a signal sequence. As used herein, a "signal sequence" or "signal peptide" serves to direct a polypeptide containing such a sequence to a lipid bilayer, and is cleaved in secreted and membrane bound polypeptides and includes a peptide containing about 15 or more amino acids which occurs at the N-terminus of secretory and membrane bound polypeptides and which contains a large number of hydrophobic amino acid residues. For example, a signal sequence contains at least about 10-30 amino acid residues, preferably about 15- 25 amino acid residues, more preferably about 18-20 amino acid residues, and even more preferably about 19 amino acid residues, and has at least about 35-65%, preferably about 38-50%, and more preferably about 40-45% hydrophobic amino acid residues (*e.g.*, valine, leucine, isoleucine or phenylalanine). In another embodiment, amino acid residues 220-243 of the native human PD-L2 polypeptide and amino acid residues 201-243 of the mature polypeptide comprise a transmembrane domain. As used herein, the term "transmembrane domain" includes an amino acid sequence of about 15 amino acid residues in length which spans the plasma membrane. More preferably, a transmembrane domain includes about at least 20, 25, 30, 35, 40, or 45 amino acid residues and spans the plasma membrane. Transmembrane domains are rich in

hydrophobic residues, and typically have an alpha-helical structure. In a preferred embodiment, at least 50%, 60%, 70%, 80%, 90%, 95% or more of the amino acids of a transmembrane domain are hydrophobic, *e.g.*, leucines, isoleucines, tyrosines, or tryptophans. Transmembrane domains are described in, for example, Zagotta *et al.* (1996) *Annu. Rev. Neurosci.* 19: 235-263. In still another embodiment, amino acid residues 20-120 of the native human PD-L2 polypeptide and amino acid residues 1-101 of the mature polypeptide comprise an IgV domain. Amino acid residues 121- 219 of the native human PD-L2 polypeptide and amino acid residues 102-200 of the mature polypeptide comprise an IgC domain. As used herein, IgV and IgC domains are recognized in the art as Ig superfamily member domains. These domains correspond to structural units that have distinct folding patterns called Ig folds. Ig folds are comprised of a sandwich of two ß sheets, each consisting of antiparallel (3 strands of 5-10 amino acids with a conserved disulfide bond between the two sheets in most, but not all, domains. IgC domains of Ig, TCR, and MHC molecules share the same types of sequence patterns and are called the C1 set within the Ig superfamily. Other IgC domains fall within other sets. IgV domains also share sequence patterns and are called V set domains. IgV domains are longer than C-domains and form an additional pair of strands. In yet another embodiment, amino acid residues 1-219 of the native human PD-L2 polypeptide and amino acid residues 1-200 of the mature polypeptide comprise an extracellular domain. As used herein, the term "extracellular domain" represents the N-terminal amino acids which extend as a tail from the surface of a cell. An extracellular domain of the present invention includes an IgV domain and an IgC domain, and may include a signal peptide domain. In still another embodiment, amino acid residues 244-273 of the native human PD-L2 polypeptide and amino acid residues 225-273 of the mature polypeptide comprise a cytoplasmic domain. As used herein, the term "cytoplasmic domain" represents the C-terminal amino acids which extend as a tail into the cytoplasm of a cell. In addition, nucleic acid and polypeptide sequences of PD-L2 orthologs in organisms other than humans are well known and include, for example, mouse PD-L2 (NM_021396.2 and NP_067371.1), rat PD-L2 (NM_001107582.2 and NP_001101052.2), dog PD-L2 (XM_847012.2 and XP_852105.2), cow PD-L2 (XM_586846.5 and XP_586846.3), and chimpanzee PD-L2 (XM_001140776.2 and XP_001140776.1).

The term "PD-L2 activity," "biological activity of PD-L2," or "functional activity of PD-L2," refers to an activity exerted by a PD-L2 protein, polypeptide or nucleic acid

molecule on a PD-L2-responsive cell or tissue, or on a PD- L2 polypeptide binding partner, as determined *in vivo*, or *in vitro*, according to standard techniques. In one embodiment, a PD-L2 activity is a direct activity, such as an association with a PD-L2 binding partner. As used herein, a "target molecule" or "binding partner" is a molecule with which a PD-L2 polypeptide binds or interacts in nature, such that PD-L2-mediated function is achieved. In an exemplary embodiment, a PD-L2 target molecule is the receptor RGMb. Alternatively, a PD-L2 activity is an indirect activity, such as a cellular signaling activity mediated by interaction of the PD- L2 polypeptide with its natural binding partner, *e.g.*, RGMb. The biological activities of PD-L2 are described herein. For example, the PD-L2 polypeptides of the present invention can have one or more of the following activities: 1) bind to and/or modulate the activity of the receptor RGMb, PD-1, or other PD-L2 natural binding partners, 2) modulate intra-or intercellular signaling, 3) modulate activation of immune cells, *e.g.* , T lymphocytes, and 4) modulate the immune response of an organism, *e.g.*, a mouse or human organism.

The term "immune response" includes T cell-mediated and/or B cell-mediated immune responses. Exemplary immune responses include T cell responses, *e.g.*, cytokine production and cellular cytotoxicity. In addition, the term immune response includes immune responses that are indirectly effected by T cell activation, *e.g.*, antibody production (humoral responses) and activation of cytokine responsive cells, *e.g.*, macrophages.

The term "immunotherapeutic agent" can include any molecule, peptide, antibody or other agent which can stimulate a host immune system to generate an immune response to a tumor or cancer in the subject. Various immunotherapeutic agents are useful in the compositions and methods described herein.

The term "inhibit" includes the decrease, limitation, or blockage, of, for example a particular action, function, or interaction. In some embodiments, cancer is "inhibited" if at least one symptom of the cancer is alleviated, terminated, slowed, or prevented. As used herein, cancer is also "inhibited" if recurrence or metastasis of the cancer is reduced, slowed, delayed, or prevented.

The term "interaction", when referring to an interaction between two molecules, refers to the physical contact (*e.g.*, binding) of the molecules with one another. Generally, such an interaction results in an activity (which produces a biological effect) of one or both of said molecules.

An "isolated protein" refers to a protein that is substantially free of other proteins, cellular material, separation medium, and culture medium when isolated from cells or produced by recombinant DNA techniques, or chemical precursors or other chemicals when chemically synthesized. An "isolated" or "purified" protein or biologically active portion thereof is substantially free of cellular material or other contaminating proteins from the cell or tissue source from which the antibody, polypeptide, peptide or fusion protein is derived, or substantially free from chemical precursors or other chemicals when chemically synthesized. The language "substantially free of cellular material" includes preparations of a biomarker polypeptide or fragment thereof, in which the protein is separated from cellular components of the cells from which it is isolated or recombinantly produced. In one embodiment, the language "substantially free of cellular material" includes preparations of a biomarker protein or fragment thereof, having less than about 30% (by dry weight) of non-biomarker protein (also referred to herein as a "contaminating protein"), more preferably less than about 20% of non-biomarker protein, still more preferably less than about 10% of non-biomarker protein, and most preferably less than about 5% non-biomarker protein. When antibody, polypeptide, peptide or fusion protein or fragment thereof, *e.g.*, a biologically active fragment thereof, is recombinantly produced, it is also preferably substantially free of culture medium, *i.e.*, culture medium represents less than about 20%, more preferably less than about 10%, and most preferably less than about 5% of the volume of the protein preparation.

A "kit" is any manufacture (*e.g.* a package or container) comprising at least one reagent, *e.g.* a probe or small molecule, for specifically detecting and/or affecting the expression of a marker of the invention. The kit may be promoted, distributed, or sold as a unit for performing the methods of the present invention. The kit may comprise one or more reagents necessary to express a composition useful in the methods of the present invention. In certain embodiments, the kit may further comprise a reference standard, *e.g.*, a nucleic acid encoding a protein that does not affect or regulate signaling pathways controlling cell growth, division, migration, survival or apoptosis. One skilled in the art can envision many such control proteins, including, but not limited to, common molecular tags (*e.g.*, green fluorescent protein and beta-galactosidase), proteins not classified in any of pathway encompassing cell growth, division, migration, survival or apoptosis by GeneOntology reference, or ubiquitous housekeeping proteins. Reagents in the kit may be provided in individual containers or as mixtures of two or more reagents in a single

container. In addition, instructional materials which describe the use of the compositions within the kit can be included.

The term "neoadjuvant therapy" refers to a treatment given before the primary treatment. Examples of neoadjuvant therapy can include chemotherapy, radiation therapy, and hormone therapy. For example, in treating breast cancer, neoadjuvant therapy can allow patients with large breast cancer to undergo breast-conserving surgery.

The "normal" level of expression of a biomarker is the level of expression of the biomarker in cells of a subject, *e.g.*, a human patient, not afflicted with a cancer. An "over-expression" or "significantly higher level of expression" of a biomarker refers to an expression level in a test sample that is greater than the standard error of the assay employed to assess expression, and is preferably at least twice, and more preferably 2.1, 2.2, 2.3, 2.4, 2.5, 2.6, 2.7, 2.8, 2.9, 3, 3.5, 4, 4.5, 5, 5.5, 6, 6.5, 7, 7.5, 8, 8.5, 9, 9.5, 10, 10.5, 11, 12, 13, 14, 15, 16, 17, 18, 19, 20 times or more higher than the expression activity or level of the biomarker in a control sample (*e.g.*, sample from a healthy subject not having the biomarker associated disease) and preferably, the average expression level of the biomarker in several control samples. A "significantly lower level of expression" of a biomarker refers to an expression level in a test sample that is at least twice, and more preferably 2.1, 2.2, 2.3, 2.4, 2.5, 2.6, 2.7, 2.8, 2.9, 3, 3.5, 4, 4.5, 5, 5.5, 6, 6.5, 7, 7.5, 8, 8.5, 9, 9.5, 10, 10.5, 11, 12, 13, 14, 15, 16, 17, 18, 19, 20 times or more lower than the expression level of the biomarker in a control sample (*e.g.*, sample from a healthy subject not having the biomarker associated disease) and preferably, the average expression level of the biomarker in several control samples.

The term "at least one mutation" in a polypeptide or a gene encoding a polypeptide and grammatical variations thereof means a polypeptide or gene encoding a polypeptide having one or more allelic variants, splice variants, derivative variants, substitution variants, deletion variants, truncation variants, and/or insertion variants, fusion polypeptides, orthologs, and/or interspecies homologs. By way of example, at least one mutation of a Jak protein would include a Jak protein in which part of all of the sequence of a polypeptide or gene encoding the Jak protein is absent or not expressed in the cell for at least one Jak protein produced in the cell. For example, a Jak protein may be produced by a cell in a truncated form and the sequence of the truncated form may be wild type over the sequence of the truncate. A deletion may mean the absence of all or part of a gene or protein encoded by a gene. Additionally, some of a protein expressed in or encoded by a

cell may be mutated while other copies of the same protein produced in the same cell may be wild type. By way of another example a mutation in a Jak protein would include a Jak protein having one or more amino acid differences in its amino acid sequence compared with wild type of the same Jak protein. By way of another example, a mutated Jak3 polypeptide is a Jak3 polypeptide having at least one amino acid difference compared to wild type Jak3 polkypeptide. Mutations may be somatic and/or germline.

An "over-expression" or "significantly higher level of expression" of a biomarker refers to an expression level in a test sample that is greater than the standard error of the assay employed to assess expression, and is preferably at least twice, and more preferably 2.1, 2.2, 2.3, 2.4, 2.5, 2.6, 2.7, 2.8, 2.9, 3, 3.5, 4, 4.5, 5, 5.5, 6, 6.5, 7, 7.5, 8, 8.5, 9, 9.5, 10, 10.5, 11, 12, 13, 14, 15, 16, 17, 18, 19, 20 times or more higher than the expression activity or level of the biomarker in a control sample (*e.g.*, sample from a healthy subject not having the biomarker associated disease) and preferably, the average expression level of the biomarker in several control samples. A "significantly lower level of expression" of a biomarker refers to an expression level in a test sample that is at least twice, and more preferably 2.1, 2.2, 2.3, 2.4, 2.5, 2.6, 2.7, 2.8, 2.9, 3, 3.5, 4, 4.5, 5, 5.5, 6, 6.5, 7, 7.5, 8, 8.5, 9, 9.5, 10, 10.5, 11, 12, 13, 14, 15, 16, 17, 18, 19, 20 times or more lower than the expression level of the biomarker in a control sample (*e.g.*, sample from a healthy subject not having the biomarker associated disease) and preferably, the average expression level of the biomarker in several control samples.

The term "predictive" includes the use of a biomarker nucleic acid, protein, and/or metabolite status, *e.g.*, over- or under- activity, emergence, expression, growth, remission, recurrence or resistance of tumors before, during or after therapy, for determining the likelihood of response of a cancer to anti-immune checkpoint inhibitor treatment (*e.g.*, therapeutic antibodies against PD-1, PD-L1, PD-L2, and/or CTLA-4). Such predictive use of the biomarker may be confirmed by, *e.g.*, (1) increased or decreased copy number (*e.g.*, by FISH, FISH plus SKY, single-molecule sequencing, *e.g.*, as described in the art at least at J. Biotechnol., 86:289-301, or qPCR), overexpression or underexpression of a biomarker nucleic acid (*e.g.*, by ISH, Northern Blot, or qPCR), increased or decreased biomarker protein (*e.g.*, by IHC) and/or biomarker metabolite, or increased or decreased activity (determined by, for example, modulation of biomarkers, *e.g.*, in more than about 5%, 6%, 7%, 8%, 9%, 10%, 11%, 12%, 13%, 14%, 15%, 20%, 25%, 30%, 40%, 50%, 60%, 70%, 80%, 90%, 95%, 100%, or more of assayed human cancers types or cancer samples; (2) its

absolute or relatively modulated presence or absence in a biological sample, *e.g.*, a sample containing tissue, whole blood, serum, plasma, buccal scrape, saliva, cerebrospinal fluid, urine, stool, or bone marrow, from a subject, *e.g.* a human, afflicted with cancer; (3) its absolute or relatively modulated presence or absence in clinical subset of patients with cancer (*e.g.*, those responding to a particular anti-immune checkpoint inhibitor therapy or those developing resistance thereto).

The terms "prevent," "preventing," "prevention," "prophylactic treatment," and the like refer to reducing the probability of developing a disease, disorder, or condition in a subject, who does not have, but is at risk of or susceptible to developing a disease, disorder, or condition.

The term "probe" refers to any molecule which is capable of selectively binding to a specifically intended target molecule, for example, a nucleotide transcript or protein encoded by or corresponding to a biomarker nucleic acid. Probes can be either synthesized by one skilled in the art, or derived from appropriate biological preparations. For purposes of detection of the target molecule, probes may be specifically designed to be labeled, as described herein. Examples of molecules that can be utilized as probes include, but are not limited to, RNA, DNA, proteins, antibodies, and organic molecules.

The term "prognosis" includes a prediction of the probable course and outcome of cancer or the likelihood of recovery from the disease. In some embodiments, the use of statistical algorithms provides a prognosis of cancer in an individual. For example, the prognosis can be surgery, development of a clinical subtype of cancer (*e.g.*, solid tumors, such as lung cancer, melanoma, and renal cell carcinoma), development of one or more clinical factors, development of intestinal cancer, or recovery from the disease.

The term "response to anti-immune checkpoint inhibitor therapy" relates to any response of the hyperproliferative disorder (*e.g.*, cancer) to an anti-immune checkpoint inhibitor therapy, such as anti-immune checkpoint inhibitor therapy, preferably to a change in tumor mass and/or volume after initiation of neoadjuvant or adjuvant chemotherapy. Hyperproliferative disorder response may be assessed , for example for efficacy or in a neoadjuvant or adjuvant situation, where the size of a tumor after systemic intervention can be compared to the initial size and dimensions as measured by CT, PET, mammogram, ultrasound or palpation. Responses may also be assessed by caliper measurement or pathological examination of the tumor after biopsy or surgical resection. Response may be recorded in a quantitative fashion like percentage change in tumor volume or in a

qualitative fashion like "pathological complete response" (pCR), "clinical complete remission" (cCR), "clinical partial remission" (cPR), "clinical stable disease" (cSD), "clinical progressive disease" (cPD) or other qualitative criteria. Assessment of hyperproliferative disorder response may be done early after the onset of neoadjuvant or adjuvant therapy, *e.g.*, after a few hours, days, weeks or preferably after a few months. A typical endpoint for response assessment is upon termination of neoadjuvant chemotherapy or upon surgical removal of residual tumor cells and/or the tumor bed. This is typically three months after initiation of neoadjuvant therapy. In some embodiments, clinical efficacy of the therapeutic treatments described herein may be determined by measuring the clinical benefit rate (CBR). The clinical benefit rate is measured by determining the sum of the percentage of patients who are in complete remission (CR), the number of patients who are in partial remission (PR) and the number of patients having stable disease (SD) at a time point at least 6 months out from the end of therapy. The shorthand for this formula is CBR=CR+PR+SD over 6 months. In some embodiments, the CBR for a particular cancer therapeutic regimen is at least 25%, 30%, 35%, 40%, 45%, 50%, 55%, 60%, 65%, 70%, 75%, 80%, 85%, or more. Additional criteria for evaluating the response to cancer therapies are related to "survival," which includes all of the following: survival until mortality, also known as overall survival (wherein said mortality may be either irrespective of cause or tumor related); "recurrence-free survival" (wherein the term recurrence shall include both localized and distant recurrence); metastasis free survival; disease free survival (wherein the term disease shall include cancer and diseases associated therewith). The length of said survival may be calculated by reference to a defined start point (*e.g.*, time of diagnosis or start of treatment) and end point (*e.g.*, death, recurrence or metastasis). In addition, criteria for efficacy of treatment can be expanded to include response to chemotherapy, probability of survival, probability of metastasis within a given time period, and probability of tumor recurrence. For example, in order to determine appropriate threshold values, a particular cancer therapeutic regimen can be administered to a population of subjects and the outcome can be correlated to biomarker measurements that were determined prior to administration of any cancer therapy. The outcome measurement may be pathologic response to therapy given in the neoadjuvant setting. Alternatively, outcome measures, such as overall survival and disease-free survival can be monitored over a period of time for subjects following cancer therapy for whom biomarker measurement values are known. In certain embodiments, the doses administered are standard doses

known in the art for cancer therapeutic agents. The period of time for which subjects are monitored can vary. For example, subjects may be monitored for at least 2, 4, 6, 8, 10, 12, 14, 16, 18, 20, 25, 30, 35, 40, 45, 50, 55, or 60 months. Biomarker measurement threshold values that correlate to outcome of a cancer therapy can be determined using well-known methods in the art, such as those described in the Examples section.

The term "resistance" refers to an acquired or natural resistance of a cancer sample or a mammal to a cancer therapy (*i.e.*, being nonresponsive to or having reduced or limited response to the therapeutic treatment), such as having a reduced response to a therapeutic treatment by 25% or more, for example, 30%, 40%, 50%, 60%, 70%, 80%, or more, to 2-fold, 3-fold, 4-fold, 5-fold, 10-fold, 15-fold, 20-fold or more. The reduction in response can be measured by comparing with the same cancer sample or mammal before the resistance is acquired, or by comparing with a different cancer sample or a mammal who is known to have no resistance to the therapeutic treatment. A typical acquired resistance to chemotherapy is called "multidrug resistance." The multidrug resistance can be mediated by P-glycoprotein or can be mediated by other mechanisms, or it can occur when a mammal is infected with a multi-drug-resistant microorganism or a combination of microorganisms. The determination of resistance to a therapeutic treatment is routine in the art and within the skill of an ordinarily skilled clinician, for example, can be measured by cell proliferative assays and cell death assays as described herein as "sensitizing." In some embodiments, the term "reverses resistance" means that the use of a second agent in combination with a primary cancer therapy (*e.g.*, chemotherapeutic or radiation therapy) is able to produce a significant decrease in tumor volume at a level of statistical significance (*e.g.*, $p<0.05$) when compared to tumor volume of untreated tumor in the circumstance where the primary cancer therapy (*e.g.*, chemotherapeutic or radiation therapy) alone is unable to produce a statistically significant decrease in tumor volume compared to tumor volume of untreated tumor. This generally applies to tumor volume measurements made at a time when the untreated tumor is growing log rhythmically.

The terms "response" or "responsiveness" refers to an anti-cancer response, *e.g.* in the sense of reduction of tumor size or inhibiting tumor growth. The terms can also refer to an improved prognosis, for example, as reflected by an increased time to recurrence, which is the period to first recurrence censoring for second primary cancer as a first event or death without evidence of recurrence, or an increased overall survival, which is the period from treatment to death from any cause. To respond or to have a response means there is a

beneficial endpoint attained when exposed to a stimulus. Alternatively, a negative or detrimental symptom is minimized, mitigated or attenuated on exposure to a stimulus. It will be appreciated that evaluating the likelihood that a tumor or subject will exhibit a favorable response is equivalent to evaluating the likelihood that the tumor or subject will not exhibit favorable response (*i.e.*, will exhibit a lack of response or be non-responsive).

An "RNA interfering agent" as used herein, is defined as any agent which interferes with or inhibits expression of a target biomarker gene by RNA interference (RNAi). Such RNA interfering agents include, but are not limited to, nucleic acid molecules including RNA molecules which are homologous to the target biomarker gene of the invention, or a fragment thereof, short interfering RNA (siRNA), and small molecules which interfere with or inhibit expression of a target biomarker nucleic acid by RNA interference (RNAi).

"RNA interference (RNAi)" is an evolutionally conserved process whereby the expression or introduction of RNA of a sequence that is identical or highly similar to a target biomarker nucleic acid results in the sequence specific degradation or specific post-transcriptional gene silencing (PTGS) of messenger RNA (mRNA) transcribed from that targeted gene (*see* Coburn, G. and Cullen, B. (2002) *J. of Virology* 76(18):9225), thereby inhibiting expression of the target biomarker nucleic acid. In one embodiment, the RNA is double stranded RNA (dsRNA). This process has been described in plants, invertebrates, and mammalian cells. In nature, RNAi is initiated by the dsRNA-specific endonuclease Dicer, which promotes processive cleavage of long dsRNA into double-stranded fragments termed siRNAs. siRNAs are incorporated into a protein complex that recognizes and cleaves target mRNAs. RNAi can also be initiated by introducing nucleic acid molecules, *e.g.*, synthetic siRNAs or RNA interfering agents, to inhibit or silence the expression of target biomarker nucleic acids. As used herein, "inhibition of target biomarker nucleic acid expression" or "inhibition of marker gene expression" includes any decrease in expression or protein activity or level of the target biomarker nucleic acid or protein encoded by the target biomarker nucleic acid. The decrease may be of at least 30%, 40%, 50%, 60%, 70%, 80%, 90%, 95% or 99% or more as compared to the expression of a target biomarker nucleic acid or the activity or level of the protein encoded by a target biomarker nucleic acid which has not been targeted by an RNA interfering agent.

The term "sample" used for detecting or determining the presence or level of at least one biomarker is typically whole blood, plasma, serum, saliva, urine, stool (*e.g.*, feces), tears, and any other bodily fluid (*e.g.*, as described above under the definition of "body

fluids"), or a tissue sample (*e.g.*, biopsy) such as a small intestine, colon sample, or surgical resection tissue. In certain instances, the method of the present invention further comprises obtaining the sample from the individual prior to detecting or determining the presence or level of at least one marker in the sample.

The term "sensitize" means to alter cancer cells or tumor cells in a way that allows for more effective treatment of the associated cancer with a cancer therapy (*e.g.*, anti-immune checkpoint inhibitor, chemotherapeutic, and/or radiation therapy). In some embodiments, normal cells are not affected to an extent that causes the normal cells to be unduly injured by the anti-immune checkpoint inhibitor therapy. An increased sensitivity or a reduced sensitivity to a therapeutic treatment is measured according to a known method in the art for the particular treatment and methods described herein below, including, but not limited to, cell proliferative assays (Tanigawa N, Kern D H, Kikasa Y, Morton D L, Cancer Res 1982; 42: 2159-2164), cell death assays (Weisenthal L M, Shoemaker R H, Marsden J A, Dill P L, Baker J A, Moran E M, Cancer Res 1984; 94: 161-173; Weisenthal L M, Lippman M E, Cancer Treat Rep 1985; 69: 615-632; Weisenthal L M, In: Kaspers G J L, Pieters R, Twentyman P R, Weisenthal L M, Veerman A J P, eds. Drug Resistance in Leukemia and Lymphoma. Langhorne, P A: Harwood Academic Publishers, 1993: 415-432; Weisenthal L M, Contrib Gynecol Obstet 1994; 19: 82-90). The sensitivity or resistance may also be measured in animal by measuring the tumor size reduction over a period of time, for example, 6 month for human and 4-6 weeks for mouse. A composition or a method sensitizes response to a therapeutic treatment if the increase in treatment sensitivity or the reduction in resistance is 25% or more, for example, 30%, 40%, 50%, 60%, 70%, 80%, or more, to 2-fold, 3-fold, 4-fold, 5-fold, 10-fold, 15-fold, 20-fold or more, compared to treatment sensitivity or resistance in the absence of such composition or method. The determination of sensitivity or resistance to a therapeutic treatment is routine in the art and within the skill of an ordinarily skilled clinician. It is to be understood that any method described herein for enhancing the efficacy of a cancer therapy can be equally applied to methods for sensitizing hyperproliferative or otherwise cancerous cells (*e.g.*, resistant cells) to the cancer therapy.

The term "synergistic effect" refers to the combined effect of two or more anti-immune checkpoint inhibitor agents can be greater than the sum of the separate effects of the anticancer agents alone.

"Short interfering RNA" (siRNA), also referred to herein as "small interfering RNA" is defined as an agent which functions to inhibit expression of a target biomarker nucleic acid, *e.g.*, by RNAi. An siRNA may be chemically synthesized, may be produced by *in vitro* transcription, or may be produced within a host cell. In one embodiment, siRNA is a double stranded RNA (dsRNA) molecule of about 15 to about 40 nucleotides in length, preferably about 15 to about 28 nucleotides, more preferably about 19 to about 25 nucleotides in length, and more preferably about 19, 20, 21, or 22 nucleotides in length, and may contain a 3' and/or 5' overhang on each strand having a length of about 0, 1, 2, 3, 4, or 5 nucleotides. The length of the overhang is independent between the two strands, *i.e.*, the length of the overhang on one strand is not dependent on the length of the overhang on the second strand. Preferably the siRNA is capable of promoting RNA interference through degradation or specific post-transcriptional gene silencing (PTGS) of the target messenger RNA (mRNA).

In another embodiment, an siRNA is a small hairpin (also called stem loop) RNA (shRNA). In one embodiment, these shRNAs are composed of a short (*e.g.*, 19-25 nucleotide) antisense strand, followed by a 5-9 nucleotide loop, and the analogous sense strand. Alternatively, the sense strand may precede the nucleotide loop structure and the antisense strand may follow. These shRNAs may be contained in plasmids, retroviruses, and lentiviruses and expressed from, for example, the pol III U6 promoter, or another promoter (*see, e.g.*, Stewart, *et al.* (2003) *RNA* Apr;9(4):493-501 incorporated by reference herein).

RNA interfering agents, *e.g.*, siRNA molecules, may be administered to a patient having or at risk for having cancer, to inhibit expression of a biomarker gene which is overexpressed in cancer and thereby treat, prevent, or inhibit cancer in the subject.

The term "subject" refers to any healthy animal, mammal or human, or any animal, mammal or human afflicted with a cancer, *e.g.*, lung, ovarian, pancreatic, liver, breast, prostate, and colon carcinomas, as well as melanoma and multiple myeloma. The term "subject" is interchangeable with "patient."

The term "survival" includes all of the following: survival until mortality, also known as overall survival (wherein said mortality may be either irrespective of cause or tumor related); "recurrence-free survival" (wherein the term recurrence shall include both localized and distant recurrence); metastasis free survival; disease free survival (wherein the term disease shall include cancer and diseases associated therewith). The length of said

survival may be calculated by reference to a defined start point (*e.g.* time of diagnosis or start of treatment) and end point (*e.g.* death, recurrence or metastasis). In addition, criteria for efficacy of treatment can be expanded to include response to chemotherapy, probability of survival, probability of metastasis within a given time period, and probability of tumor recurrence.

The term "therapeutic effect" refers to a local or systemic effect in animals, particularly mammals, and more particularly humans, caused by a pharmacologically active substance. The term thus means any substance intended for use in the diagnosis, cure, mitigation, treatment or prevention of disease or in the enhancement of desirable physical or mental development and conditions in an animal or human. The phrase "therapeutically-effective amount" means that amount of such a substance that produces some desired local or systemic effect at a reasonable benefit/risk ratio applicable to any treatment. In certain embodiments, a therapeutically effective amount of a compound will depend on its therapeutic index, solubility, and the like. For example, certain compounds discovered by the methods of the present invention may be administered in a sufficient amount to produce a reasonable benefit/risk ratio applicable to such treatment.

The terms "therapeutically-effective amount" and "effective amount" as used herein means that amount of a compound, material, or composition comprising a compound of the present invention which is effective for producing some desired therapeutic effect in at least a sub-population of cells in an animal at a reasonable benefit/risk ratio applicable to any medical treatment. Toxicity and therapeutic efficacy of subject compounds may be determined by standard pharmaceutical procedures in cell cultures or experimental animals, *e.g.*, for determining the LD_{50} and the ED_{50}. Compositions that exhibit large therapeutic indices are preferred. In some embodiments, the LD_{50} (lethal dosage) can be measured and can be, for example, at least 10%, 20%, 30%, 40%, 50%, 60%, 70%, 80%, 90%, 100%, 200%, 300%, 400%, 500%, 600%, 700%, 800%, 900%, 1000% or more reduced for the agent relative to no administration of the agent. Similarly, the ED_{50} (*i.e.*, the concentration which achieves a half-maximal inhibition of symptoms) can be measured and can be, for example, at least 10%, 20%, 30%, 40%, 50%, 60%, 70%, 80%, 90%, 100%, 200%, 300%, 400%, 500%, 600%, 700%, 800%, 900%, 1000% or more increased for the agent relative to no administration of the agent. Also, Similarly, the IC_{50} (*i.e.*, the concentration which achieves half-maximal cytotoxic or cytostatic effect on cancer cells) can be measured and can be, for example, at least 10%, 20%, 30%, 40%, 50%, 60%, 70%, 80%, 90%, 100%,

200%, 300%, 400%, 500%, 600%, 700%, 800%, 900%, 1000% or more increased for the agent relative to no administration of the agent. In some embodiments, cancer cell growth in an assay can be inhibited by at least about 10%, 15%, 20%, 25%, 30%, 35%, 40%, 45%, 50%, 55%, 60%, 65%, 70%, 75%, 80%, 85%, 90%, 95%, or even 100%. In another embodiment, at least about a 10% , 15%, 20%, 25%, 30%, 35%, 40%, 45%, 50%, 55%, 60%, 65%, 70%, 75%, 80%, 85%, 90%, 95%, or even 100% decrease in a solid malignancy can be achieved.

A "transcribed polynucleotide" or "nucleotide transcript" is a polynucleotide (*e.g.* an mRNA, hnRNA, a cDNA, or an analog of such RNA or cDNA) which is complementary to or homologous with all or a portion of a mature mRNA made by transcription of a biomarker nucleic acid and normal post-transcriptional processing (*e.g.* splicing), if any, of the RNA transcript, and reverse transcription of the RNA transcript.

There is a known and definite correspondence between the amino acid sequence of a particular protein and the nucleotide sequences that can code for the protein, as defined by the genetic code (shown below). Likewise, there is a known and definite correspondence between the nucleotide sequence of a particular nucleic acid and the amino acid sequence encoded by that nucleic acid, as defined by the genetic code.

GENETIC CODE

Alanine (Ala, A)	GCA, GCC, GCG, GCT
Arginine (Arg, R)	AGA, ACG, CGA, CGC, CGG, CGT
Asparagine (Asn, N)	AAC, AAT
Aspartic acid (Asp, D)	GAC, GAT
Cysteine (Cys, C)	TGC, TGT
Glutamic acid (Glu, E)	GAA, GAG
Glutamine (Gln, Q)	CAA, CAG
Glycine (Gly, G)	GGA, GGC, GGG, GGT
Histidine (His, H)	CAC, CAT
Isoleucine (Ile, I)	ATA, ATC, ATT
Leucine (Leu, L)	CTA, CTC, CTG, CTT, TTA, TTG
Lysine (Lys, K)	AAA, AAG
Methionine (Met, M)	ATG
Phenylalanine (Phe, F)	TTC, TTT
Proline (Pro, P)	CCA, CCC, CCG, CCT

Serine (Ser, S)	AGC, AGT, TCA, TCC, TCG, TCT
Threonine (Thr, T)	ACA, ACC, ACG, ACT
Tryptophan (Trp, W)	TGG
Tyrosine (Tyr, Y)	TAC, TAT
Valine (Val, V)	GTA, GTC, GTG, GTT
Termination signal (end)	TAA, TAG, TGA

An important and well known feature of the genetic code is its redundancy, whereby, for most of the amino acids used to make proteins, more than one coding nucleotide triplet may be employed (illustrated above). Therefore, a number of different nucleotide sequences may code for a given amino acid sequence. Such nucleotide sequences are considered functionally equivalent since they result in the production of the same amino acid sequence in all organisms (although certain organisms may translate some sequences more efficiently than they do others). Moreover, occasionally, a methylated variant of a purine or pyrimidine may be found in a given nucleotide sequence. Such methylations do not affect the coding relationship between the trinucleotide codon and the corresponding amino acid.

In view of the foregoing, the nucleotide sequence of a DNA or RNA encoding a biomarker nucleic acid (or any portion thereof) can be used to derive the polypeptide amino acid sequence, using the genetic code to translate the DNA or RNA into an amino acid sequence. Likewise, for polypeptide amino acid sequence, corresponding nucleotide sequences that can encode the polypeptide can be deduced from the genetic code (which, because of its redundancy, will produce multiple nucleic acid sequences for any given amino acid sequence). Thus, description and/or disclosure herein of a nucleotide sequence which encodes a polypeptide should be considered to also include description and/or disclosure of the amino acid sequence encoded by the nucleotide sequence. Similarly, description and/or disclosure of a polypeptide amino acid sequence herein should be considered to also include description and/or disclosure of all possible nucleotide sequences that can encode the amino acid sequence.

Finally, nucleic acid and amino acid sequence information for the loci and biomarkers of the present invention (*e.g.*, biomarkers listed in Table 1) are well known in the art and readily available on publicly available databases, such as the National Center for Biotechnology Information (NCBI). For example, exemplary nucleic acid and amino acid sequences derived from publicly available sequence databases are provided below.

Table 1

SEQ ID NO: 1 Human Jak1 cDNA sequence

```
   1 atgcagtatc taaatataaa agaggactgc aatgccatgg ctttctgtgc taaaatgagg
  61 agctccaaga agactgaggt gaacctggag gcccctgagc caggggtgga agtgatcttc
 121 tatctgtcgg acagggagcc cctccggctg ggcagtggag agtacacagc agaggaactg
 181 tgcatcaggg ctgcacaggc atgccgtatc tctcctcttt gtcacaacct ctttgccctg
 241 tatgacgaga acaccaagct ctggtatgct ccaaatcgca ccatcaccgt tgatgacaag
 301 atgtccctcc ggctccacta ccggatgagg ttctatttca ccaattggca tggaaccaac
 361 gacaatgagc agtcagtgtg gcgtcattct ccaaagaagc agaaaaatgg ctacgagaaa
 421 aaaaagattc cagatgcaac ccctctcctt gatgccagct cactggagta tctgtttgct
 481 cagggacagt atgatttggt gaaatgcctg gctcctattc gagaccccaa gaccgagcag
 541 gatggacatg atattgagaa cgagtgtcta gggatggctg tcctggccat ctcacactat
 601 gccatgatga agaagatgca gttgccagaa ctgcccaagg acatcagcta caagcgatat
 661 attccagaaa cattgaataa gtccatcaga cagaggaacc ttctcaccag gatgcggata
 721 aataatgttt tcaaggattt cctaaaggaa tttaacaaca agaccatttg tgacagcagc
 781 gtgtccacgc atgacctgaa ggtgaaatac ttggctacct tggaaacttt gacaaaacat
 841 tacggtgctg aaatatttga gacttccatg ttactgattt catcagaaaa tgagatgaat
 901 tggtttcatt cgaatgacgg tggaaacgtt ctctactacg aagtgatggt gactgggaat
 961 cttggaatcc agtggaggca taaaccaaat gttgtttctg ttgaaaagga aaaaaataaa
1021 ctgaagcgga aaaaactgga aaataaacac aagaaggatg aggagaaaaa caagatccgg
1081 gaagagtgga acaatttttc ttacttccct gaaatcactc acattgtaat aaaggagtct
1141 gtggtcagca ttaacaagca ggacaacaag aaaatggaac tgaagctctc ttcccacgag
1201 gaggccttgt cctttgtgtc cctggtagat ggctacttcc ggctcacagc agatgcccat
1261 cattacctct gcaccgacgt ggccccccgg ttgatcgtcc acaacataca gaatggctgt
1321 catggtccaa tctgtacaga atacgccatc aataaattgc ggcaagaagg aagcgaggag
1381 gggatgtacg tgctgaggtg gagctgcacc gactttgaca acatcctcat gaccgtcacc
1441 tgctttgaga agtctgagca ggtgcagggt gcccagaagc agttcaagaa ctttcagatc
1501 gaggtgcaga agggccgcta cagtctgcac ggttcggacc gcagcttccc cagcttggga
1561 gacctcatga gccacctcaa gaagcagatc ctgcgcacgg ataacatcag cttcatgcta
1621 aaacgctgct gccagcccaa gccccgagaa atctccaacc tgctggtggc tactaagaaa
1681 gcccaggagt ggcagcccgt ctaccccatg agccagctga gtttcgatcg gatcctcaag
1741 aaggatctgg tgcagggcga gcaccttggg agaggcacga gaacacacat ctatttctgg
1801 accctgatgg attacaagga tgacgaagga actttctgaag agaagaagat aaaagtgatc
1861 ctcaaagtct tagaccccag ccacagggat atttccctgg ccttcttcga ggcagccagc
1921 atgatgagac aggtctccca caaacacatc gtgtacctct atggcgtctg tgtccgcgac
1981 gtggagaata tcatggtgga agagtttgtg gaaggggtc ctctggatct cttcatgcac
2041 cggaaaagcg atgtccttac cacaccatgg aaattcaaag ttgccaaaca gctggccagt
2101 gccctgagct acttggagga taaagacctg gtccatggaa atgtgtgtac taaaaacctc
2161 ctcctggccc gtgagggcat cgacagtgag tgtggcccat tcatcaagct cagtgacccc
2221 ggcatcccca ttacggtgct gtctaggcaa gaatgcattg aacgaatccc atggattgct
2281 cctgagtgtg ttgaggactc caagaacctg agtgtggctg ctgacaagtg gagctttgga
2341 accacgctct gggaaatctg ctacaatggc gagatcccct tgaaagacaa gacgctgatt
2401 gagaaagaga gattctatga aagccggtgc aggccagtga caccatcatg taaggagctg
2461 gctgacctca tgacccgctg catgaactat gaccccaatc agaggccttt cttccgagcc
2521 atcatgagag acattaataa gcttgaagag cagaatccag atattgtttc agaaaaaaaa
2581 ccagcaactg aagtggaccc cacacatttt gaaaagcgct tcctaaagag gatccgtgac
2641 ttgggagagg gccactttgg gaaggttgag ctctgcaggt atgaccccga aggggacaat
2701 acaggggagc aggtggctgt taaatctctg aagcctgaga gtggaggtaa ccacatagct
2761 gatctgaaaa aggaaatcga gatcttaagg aacctctatc atgagaacat tgtgaagtac
2821 aaaggaatct gcacagaaga cggaggaaat ggtattaagc tcatcatgga atttctgcct
2881 tcgggaagcc ttaaggaata tcttccaaag aataagaaca aaataaacct caaacagcag
2941 ctaaaatatg ccgttcagat ttgtaagggg atggactatt tgggttctcg gcaatacgtt
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3001 caccgggact tggcagcaag aaatgtcctt gttgagagtg aacaccaagt gaaaattgga
3061 gacttcggtt taaccaaagc aattgaaacc gataaggagt attacaccgt caaggatgac
3121 cgggacagcc ctgtgttttg gtatgctcca gaatgtttaa tgcaatctaa attttatatt
3181 gcctctgacg tctggtcttt tggagtcact ctgcatgagc tgctgactta ctgtgattca
3241 gattctagtc ccatggcttt gttcctgaaa atgataggcc caacccatgg ccagatgaca
3301 gtcacaagac ttgtgaatac gttaaaagaa ggaaaacgcc tgccgtgccc acctaactgt
3361 ccagatgagg tttatcaact tatgaggaaa tgctgggaat tccaaccatc caatcggaca
3421 agctttcaga accttattga aggatttgaa gcacttttaa aataa
```

SEQ ID NO: 2 Human Jak1 amino acid sequence

```
   1 mqylnikedc namafcakmr sskktevnle apepgvevif ylsdreplrl gsgeytaeel
  61 ciraaqacri splchnlfal ydentklwya pnrtitvddk mslrlhyrmr fyftnwhgtn
 121 dneqsvwrhs pkkqkngyek kkipdatpll dassleylfa qgqydlvkcl apirdpkteq
 181 dghdienecl gmavlaishy ammkkmqlpe lpkdisykry ipetlnksir qrnlltrmri
 241 nnvfkdflke fnnktiedss vsthdlkvky latletltkh ygaeifetsm llissenemn
 301 wfhsndggnv lyyevmvtgn lgiqwrhkpn vvsvekeknk lkrkklenkh kkdeeknkir
 361 eewnnfsyfp eithivikes vvsinkqdnk kmelklsshe ealsfvslvd gyfrltadah
 421 hylctdvapp livhniqngc hgpicteyai nklrqegsee gmyvlrwsct dfdnilmtvt
 481 cfekseqvqg aqkqfknfqi evqkgryslh gsdrsfpslg dlmshlkkqi lrtdnisfml
 541 krccqpkpre isnllvatkk aqewqpvypm sqlsfdrilk kdlvqgehlg rgtrthiysg
 601 tlmdykddeg tseekkikvi lkvldpshrd islaffeaas mmrqvshkhi vylygvcvrd
 661 venimveefv eggpldlfmh rksdvlttpw kfkvakqlas alsyledkdl vhgnvctknl
 721 llaregidse cgpfiklsdp gipitvlsrq ecieripwia pecvedsknl svaadkwsfg
 781 ttlweicyng eiplkdktli ekerfyesrc rpvtpsckel adlmtrcmny dpnqrpffra
 841 imrdinklee qnpdivsekk patevdpthf ekrflkrird lgeghfgkve lcrydpegdn
 901 tgeqvavksl kpesggnhia dlkkeieilr nlyhenivky kgictedggn giklimeflp
 961 sgslkeylpk nknkinlkqq lkyavqickg mdylgsrqyv hrdlaarnvl vesehqvkig
1021 dfgltkaiet dkeyytvkdd rdspvfwyap eclmqskfyi asdvwsfgvt lhelltycds
1081 dsspmalflk migpthgqmt vtrlvntlke gkrlpcppnc pdevyqlmrk cwefqpsnrt
1141 sfqnliegfe allk
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SEQ ID NO: 3 Human Jak2 cDNA sequence

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   1 atgggaatgg cctgccttac gatgacagaa atggagggaa catccacctc ttctatatat
  61 cagaatggtg atatttctgg aaatgccaat tctatgaagc aaatagatcc agttcttcag
 121 gtgtatcttt accattccct tgggaaatct gaggcagatt atctgacctt tccatctggg
 181 gagtatgttg cagaagaaat ctgtattgct gcttctaaag cttgtggtat cacacctgtg
 241 tatcataata tgtttgcttt aatgagtgaa acagaaagga tctggtatcc acccaaccat
 301 gtcttccata tagatgagtc aaccaggcat aatgtactct acagaataag attttacttt
 361 cctcgttggt attgcagtgg cagcaacaga gcctatcggc atggaatatc tcgaggtgct
 421 gaagctccta ttcttgatga ctttgtcatg tcttacctct ttgctcagtg gcggcatgat
 481 tttgtgcacg gatggataaa agtacctgtg actcatgaaa cacaggaaga atgtcttggg
 541 atggcagtgt tagatatgat gagaatagcc aaagaaaacg atcaaacccc actggccatc
 601 tataactcta tcagctacaa gacattctta ccaaaatgta ttcgagcaaa gatccaagac
 661 tatcatattt tgacaaggaa gcgaataagg tacagatttc gcagatttat tcagcaattc
 721 agccaatgca aagccactgc cagaaacttg aaacttaagt atcttataaa tctggaaact
 781 ctgcagtctg ccttctacac agagaaattt gaagtaaaag aacctggaag tggtccttca
 841 ggtgaggaga tttttgcaac cattataata actggaaacg gtggaattca gtggtcaaga
 901 gggaaacata aagaaagtga gacactgaca gaacaggatt tacagttata ttgcgatttt
 961 cctaatatta ttgatgtcag tattaagcaa gcaaaccaag agggttcaaa tgaaagccga
1021 gttgtaacta tccataagca agatggtaaa aatctggaaa ttgaacttag ctcattaagg
1081 gaagctttgt ctttcgtgtc attaattgat ggatattata gattaactgc agatgcacat
1141 cattacctct gtaaagaagt agcacctcca gccgtgcttg aaaatataca aagcaactgt
1201 catggcccaa tttcgatgga ttttgccatt agtaaactga agaaagcagg taatcagact
1261 ggactgtatg tacttcgatg cagtcctaag gactttaata aatatttttt gacttttgct
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1321 gtcgagcgag aaaatgtcat tgaatataaa cactgtttga ttacaaaaaa tgagaatgaa
1381 gagtacaacc tcagtgggac aaagaagaac ttcagcagtc ttaaagatct tttgaattgt
1441 taccagatgg aaactgttcg ctcagacaat ataatttttcc agtttactaa atgctgtccc
1501 ccaaagccaa aagataaatc aaaccttcta gtcttcagaa cgaatggtgt ttctgatgta
1561 ccaacctcac caacattaca gaggcctact catatgaacc aaatggtgtt tcacaaaatc
1621 agaaatgaag atttgatatt taatgaaagc cttggccaag gcacttttac aaagatttttt
1681 aaaggcgtac gaagagaagt aggagactac ggtcaactgc atgaaacaga agttctttta
1741 aaagttctgg ataaagcaca cagaaactat tcagagtctt tctttgaagc agcaagtatg
1801 atgagcaagc tttctcacaa gcatttggtt ttaaatttatg gagtatgtgt ctgtggagac
1861 gagaatattc tggttcagga gtttgtaaaa tttggatcac tagatacata tctgaaaaag
1921 aataaaaatt gtataaatat attatggaaa cttgaagttg ctaaacagtt ggcatgggcc
1981 atgcattttc tagaagaaaa caccccttatt catgggaatg tatgtgccaa aaatattctg
2041 cttatcagag aagaagacag gaagacagga aatcctcctt tcatcaaact tagtgatcct
2101 ggcattagta ttacagtttt gccaaaggac attcttcagg agagaatacc atgggtacca
2161 cctgaatgca ttgaaaatcc taaaaattta aatttggcaa cagacaaatg gagttttggt
2221 accactttgt gggaaatctg cagtggagga gataaacctc taagtgctct ggattctcaa
2281 agaaagctac aattttatga agataggcat cagcttcctg caccaaagtg ggcagaatta
2341 gcaaacctta taaataattg tatggattat gaaccagatt tcaggccttc tttcagagcc
2401 atcatacgag atcttaacag tttgtttact ccagattatg aactattaac agaaaatgac
2461 atgttaccaa atatgaggat aggtgccctg gggttttctg gtgcctttga agaccgggat
2521 cctacacagt ttgaagagag acatttgaaa tttctacagc aacttggcaa gggtaatttt
2581 gggagtgtgg agatgtgccg gtatgaccct ctacaggaca acactgggga ggtggtcgct
2641 gtaaaaaagc ttcagcatag tactgaagag cacctaagag actttgaaag ggaaattgaa
2701 atcctgaaat ccctacagca tgacaacatt gtaaagtaca agggagtgtg ctacagtgct
2761 ggtcggcgta atctaaaatt aattatggaa tatttaccat atggaagttt acgagactat
2821 cttcaaaaac ataaagaacg gatagatcac ataaaacttc tgcagtacac atctcagata
2881 tgcaagggta tggagtatct tggtacaaaa aggtatatcc acagggatct ggcaacgaga
2941 aatatattgg tggagaacga gaacagagtt aaaattggag attttgggtt aaccaaagtc
3001 ttgccacaag acaaagaata ctataaagta aaagaacctg gtgaaagtcc catattctgg
3061 tatgctccag aatcactgac agagagcaag ttttctgtgg cctcagatgt ttggagcttt
3121 ggagtggttc tgtatgaact tttcacatac attgagaaga gtaaaagtcc accagcggaa
3181 tttatgcgta tgattggcaa tgacaaacaa ggacagatga tcgtgttcca tttgatagaa
3241 cttttgaaga ataatggaag attaccaaga ccagatggat gcccagatga gatctatatg
3301 atcatgacag aatgctggaa caataatgta aatcaacgcc cctcctttag ggatctagct
3361 cttcgagtgg atcaaataag ggataacatg gctggatga
```

SEQ ID NO: 4 Human Jak2 amino acid sequence

```
   1 mgmacltmte megtstssiy qngdisgnan smkqidpvlq vylyhslgks eadyltfpsq
  61 eyvaeeicia askacgitpv yhnmfalmse teriwyppnh vfhidestrh nvlyrirfyf
 121 prwycsgsnr ayrhgisrga eaplldfvm sylfaqwrhd fvhgwikvpv thetqeeclg
 181 mavldmmria kendqtplai ynsisyktfl pkcirakiqd yhiltnkrir yrfrrfiqqf
 241 sqckatarnl klkylinlet lqsafytekf evkepgsgps geeifatiii tgnggiqwsr
 301 gkhkesetlt eqdlqlycdf pniidvsikq anqegsnesr vvtihkqdgk nleielsslr
 361 ealsfvslid gyyrltadah hylckevapp avleniqsnc hgpismdfai sklkkagnqt
 421 glyvlrcspk dfnkyfltfa verenvieyk hclitknene eynlsgtkkn fsslkdllnc
 481 yqmetvrsdn iifqftkccp pkpkdksnll vfrtngvsdv ptsptlqrpt hmnqmvfhki
 541 rnedlifnes lgqgtftkif kgvrrevgdy gqlhetevll kvldkahrny sesffeaasm
 601 msklshkhlv lnygvcvcgd enilvqefvk fgsldtylkk nkncinilwk levakqlawa
 661 mhfleentli hgnvcaknil lireedrktg nppfiklsdp gisitvlpkd ilqeripwvp
 721 pecienpknl nlatdkwsfg ttlweicsgg dkplsaldsq rklqfyedrh qlpapkwael
 781 anlinncmdy epdfrpsfra iirdlnslft pdyelltend mlpnmrigal gfsgafedrd
 841 ptqfeerhlk flqqlgkgnf gsvemcrydp lqdntgevva vkklqhstee hlrdfereie
 901 ilkslqhdni vkykgvcysa grrnlklime ylpygslrdy lqkhkeridh ikllqytsqi
 961 ckgmeylgtk ryihrdlatr nilvenenrv kigdfgltkv lpqdkeyykv kepgespifw
```

```
1021 yapesltesk fsvasdvwsf gvvlyelfty iekskspppae fmrmigndkq gqmivfhlie
1081 llknngrlpr pdgcpdeiym imtecwnnnv nqrpsfrdla lrvdqirdnm ag
```

SEQ ID NO: 5 Human Jak3 cDNA sequence

```
   1 atggcacctc caagtgaaga gacgcccctg atccctcagc gttcatgcag cctcttgtcc
  61 acggaggctg gtgccctgca tgtgctgctg cccgctcggg gccccgggcc cccccagcgc
 121 ctatctttct cctttggggа ccacttggct gaggacctgt gcgtgcaggc tgccaaggcc
 181 agcggcatcc tgcctgtgta ccactcccтc tttgctctgg ccacggagga cctgtcctgc
 241 tggttccccc cgagccacat cttctccgtg gaggatgcca gcacccaagt cctgctgtac
 301 aggattcgct tttacttccc caattggttt gggctggaga agtgccaccg ctttcgggcta
 361 cgcaaggatt tggccagtgc tatccttgac ctgccagtcc tggagcacct ctttgcccag
 421 caccgcagtg acctggtgag tgggcgcctc cccgtgggcc tcagtctcaa ggagcagggt
 481 gagtgtctca gcctggccgt gttggacctg gccccggatgg cgcgagagca ggcccagcgg
 541 ccgggagagc tgctgaagac tgtcagctac aaggcctgcc tacccccaag cctgcgcgac
 601 ctgatccagg gcctgagctt cgtgacgcgg aggcgtattc ggaggacggt gcgcagagcc
 661 ctgcgccgcg tggccgcctg ccaggcagac cggcactcgc tcatggccaa gtacatcatg
 721 gacctggagc ggctggatcc agccggggcc gccgagacct tccacgtggg cctccctggg
 781 gccccttggtg gccacgacgg gctggggctg ctccgcgtgg ctggtgacgg cggcatcgcc
 841 tggacccagg gagaacagga ggtcctccag ccctttctgcg actttccaga aatcgtagac
 901 attagcatca agcaggcccc gcgcgttggc ccggccggag agcaccgcct ggtcactgtt
 961 accaggacag acaaccagat tttagaggcc gagttcccag ggctgcccga ggctctgtcg
1021 ttcgtggcgc tcgtggacgg ctacttccgg ctgaccacgg actcccagca cttcttctgc
1081 aaggaggtgg caccgccgag gctgctggag gaagtggccg agcagtgcca cggccccatc
1141 actctggact ttgccatcaa caagctcaag actgggggct cacgtcctgg ctcctatgtt
1201 ctccgccgca gccccccagga ctttgacagc ttcctcctca ctgtctgtgt ccagaacccc
1261 cttggtcctg attataaggg ctgcctcatc cggcgcagcc ccacaggaac cttccttctg
1321 gttggcctca gccgacccca cagcagtctt cgagagctcc tggcaacctg ctgggatggg
1381 gggctgcacg tagatggggt ggcagtgacc ctcacttcct gctgtatccc cagacccaaa
1441 gaaaagtcca acctgatcgt ggtccagaga ggtcacagcc cacccacatc atccttggtt
1501 cagccccaat cccaatacca gctgagtcag atgacatttc acaagatccc tgctgacagc
1561 ctggagtggc atgagaacct gggccatggg tccttcacca agatttaccg gggctgtcgc
1621 catgaggtgg tggatggggа ggcccgaaag acagaggtgc tgctgaaggt catggatgcc
1681 aagcacaaga actgcatgga gtcattcctg gaagcagcga gcttgatgag ccaagtgtcg
1741 taccggcatc tcgtgctgct ccacggcgtg tgcatggctg gagacagcac catggtgcag
1801 gaattttgtac acctgggggc catagacatg tatctgcgaa aacgtggcca cctggtgcca
1861 gccagctgga agctgcaggt ggtcaaacag ctggcctacg ccctcaacta tctggaggac
1921 aaaggcctgc cccatggcaa tgtctctgcc cggaaggtgc tcctggctcg ggaggggggct
1981 gatgggagcc cgcccttcat caagctgagt gaccctgggg tcagccccgc tgtgttaagc
2041 ctggagatgc tcaccgacag gatcccctgg gtggcccccg agtgtctccg ggaggcgcag
2101 acacttagct tggaagctga caagtggggc ttcggcgcca cggtctggga agtgtttagt
2161 ggcgtcacca tgcccatcag tgccctggat cctgctaaga aactccaatt ttatgaggac
2221 cggcagcagc tgccggcccc caagtggaca gagctggccc tgctgattca acagtgcatg
2281 gcctatgagc cggtccagag gccctccttc cgagccgtca ttcgtgacct caatagcctc
2341 atctcttcag actatgagct cctctcagac cccacacctg gtgccctggc acctcgtgat
2401 gggctgtgga atggtgccca gctctatgcc tgccaagacc ccacgatctt cgaggagaga
2461 cacctcaagt acatctcaca gctgggcaag ggcaactttg gcagcgtgga gctgtgccgc
2521 tatgacccgc taggcgacaa tacaggtgcc ctggtggccg tgaaacagct gcagcacagc
2581 gggccagacc agcagaggga ctttcagcgg gagattcaga tcctcaaagc actgcacagt
2641 gatttcattg tcaagtatcg tggtgtcagc tatggcccgg gccgccagag cctgcggctg
2701 gtcatggagt acctgcccag cggctgcttg cgcgacttcc tgcagcggca ccgcgcgcgc
2761 ctcgatgcca gccgcctcct tctctattcc tcgcagatct gcaagggcat ggagtacctg
2821 ggctcccgcc gctgcgtgca ccgcgacctg gccgccccgaa acatcctcgt ggagagcgag
2881 gcacacgtca agatcgctga cttcggccta gctaagctgc tgccgcttga caaagactac
2941 tacgtggtcc gcgagccagg ccagagcccc attttctggt atgcccccga atccctctcg
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3001 gacaacatct tctctcgcca gtcagacgtc tggagcttcg gggtcgtcct gtacgagctc
3061 ttcacctact gcgacaaaag ctgcagcccc tcggccgagt tcctgcggat gatgggatgt
3121 gagcgggatg tcccccgccct ctgccgcctc ttggaactgc tggaggaggg ccagaggctg
3181 ccggcgcctc ctgcctgccc tgctgaggtt cacgagctca tgaagctgtg ctgggccccct
3241 agcccacagg accggccatc attcagcgcc ctgggccccc agctggacat gctgtggagc
3301 ggaagccggg ggtgtgagac tcatgccttc actgctcacc cagagggcaa acaccactcc
3361 ctgtcctttt catag
```

SEQ ID NO: 6 Human Jak3 amino acid sequence

```
   1 mappseetpl ipqrscslls teagalhvll pargpgpppqr lsfsfgdhla edlcvqaaka
  61 sgilpvyhsl falatedlsc wfppshifsv edastqvlly rirfyfpnwf glekchrfgl
 121 rkdlasaild lpvlehlfaq hrsdlvsgrl pvglslkeqg eclslavldl armareqaqr
 181 pgellktvsy kaclppslrd liqglsfvtr rrirrtvrra lrrvaacqad rhslmakyim
 241 dlerldpaga aetfhvglpg alggphdglgl lrvagdggia wtqgeqevlq pfcdfpeivd
 301 isikqaprvg pagehrlvtv trtdnqilea efpglpeals fvalvdgyfr lttdsqhffc
 361 kevapprlle evaeqchgpi tldfainklk tggsrpgsyv lrrspqdfds flltvcvqnp
 421 lgpdykgcli rrsptgtfll vglsrphssl rellatcwdg glhvdgvavt ltscciprpk
 481 eksnlivvqr ghspptsslv qpqsqyqlsq mtfhkipads lewhenlghg sftkiyrgcr
 541 hevvdgeark tevllkvmda khknemesfl eaaslmsqvs yrhlvllhgv cmagdstmvq
 601 efvhlgaidm ylrkrghlvp aswklqvvkq layalnyled kglphgnvsa rkvllarega
 661 dgsppfikls dpgvspavls lemltdripw vapeclreaq tlsleadkwg fgatvwevfs
 721 gvtmpisald pakklqfyed rqqlpapkwt elalliqqcm ayepvqrpsf ravirdlnsl
 781 issdyellsd ptpgalaprd glwngaqlya cqdptifeer hlkyisqlgk gnfgsvelcr
 841 ydplgdntga lvavkqlqhs gpdqqrdfqr eiqilkalhs dfivkyrgvs ygpgrqslrl
 901 vmeylpsgcl rdflqrhrar ldasrlllys sqickgmeyl gsrrcvhrdl aarnilvese
 961 ahvkiadfgl akllpldkdy yvvrepgqsp ifwyapesls dnifsrqsdv wsfgvvlyel
1021 ftycdkscsp saeflrmmgc erdvpalcrl lelleegqrl pappacpaev helmklcwap
1081 spqdrpsfsa lgpqldmlws gsrgcethaf tahpegkhhs lsfs
```

SEQ ID NO: 7 Human Tyk2 cDNA sequence

```
   1 atgcctctgc gccactgggg gatggccagg ggcagtaagc ccgttgggga tggagcccag
  61 cccatggctg ccatgggagg cctgaaggtg cttctgcact gggctggtcc aggcggcggg
 121 gagccctggg tcactttcag tgagtcatcg ctgacagctg aggaagtctg catccacatt
 181 gcacataaag ttggtatcac tcctccttgc ttcaatctct ttgccctctt cgatgctcag
 241 gcccaagtct ggttgccccc aaaccacatc ctagagatcc ccagagatgc aagcctgatg
 301 ctatatttcc gcataaggtt ttatttcccgg aactggcatg gcatgaatcc tcgggaaccg
 361 gctgtgtacc gttgtgggcc cccaggaacc gaggcatcct cagatcagac agcacagggg
 421 atgcaactcc tggacccagc ctcatttgag tacctctttg agcagggcaa gcatgagttt
 481 gtgaatgacg tggcatcact gtgggagctg tcgaccgagg aggagatcca ccactttaag
 541 aatgagagcc tgggcatggc ctttctgcac ctctgtcacc tcgctctccg ccatggcatc
 601 ccctggagg aggtggccaa gaagaccagc ttcaaggact gcatccccgcg ctccttccgc
 661 cggcatatcc ggcagcacag cgccctgacc cggctgcgcc ttcggaacgt cttccgcagg
 721 ttcctgcggg acttccagcc gggccgactc tcccagcaga tggtcatggt caaatacctа
 781 gccacactcg agcggctggc accccgcttc ggcacagagc gtgtgcccgt gtgccacctg
 841 aggctgctgg cccaggccga gggggagccc tgctacatcc gggacagtgg ggtggccct
 901 acagaccctg gccctgagtc tgctgctggg ccccccaacc acgaggtgct ggtgacaggc
 961 actggtggca tccagtggtg gccagtagag gaggaggtga acaaggagga gggttctagt
1021 ggcagcagtg gcaggaaccc ccaagccagc ctgtttggga agaaggccaa ggctcacaag
1081 gcagtcggcc agccggcaga caggccgcgg gagccactgt gggcctactt ctgtgacttc
1141 cgggacatca cccacgtggt gctgaaagag cactgtgtca gcatccaccg gcaggacaac
1201 aagtgcctgg agctgagctt gccttcccgg gctgcggcgc tgtccttcgt gtcgctggtg
1261 gacggctatt tccgcctgac ggccgactcc agccactacc tgtgccacga ggtggctccc
1321 ccacggctgg tgatgagcat ccgggatggg atccacggac ccctgctgga gccatttgtg
1381 caggccaagc tgcggcccga ggacggcctg tacctcattc actggagcac cagccacccc
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1441 taccgcctga tcctcacagt ggcccagcgt agccaggcac cagacggcat gcagagcttg
1501 cggctccgaa agttccccat tgagcagcag gacggggcct tcgtgctgga gggctggggc
1561 cggtccttcc ccagcgttcg ggaacttggg gctgccttgc agggctgctt gctgagggcc
1621 ggggatgact gcttctctct gcgtcgctgt tgcctgcccc aaccaggaga aacctccaat
1681 ctcatcatca tgcgggggcc tcgggccagc cccaggacac tcaacctcag ccagctcagc
1741 ttccaccggg ttgaccagaa ggagatcacc cagctgtccc acttgggcca gggcacaagg
1801 accaacgtgt atgagggccg cctgcgagtg gagggcagcg gggaccctga ggagggcaag
1861 atggatgacg aggacccccc cgtgcctggc agggaccgtg ggcaggagct acgagtggtg
1921 ctcaaagtgc tggaccctag tcaccatgac atcgccctgg cctttctacga gacagccagc
1981 ctcatgagcc aggtctccca cacgcacctg gccttcgtgc atggcgtctg tgtgcggcgc
2041 cctgaaaata tcatggtgac agagtacgtg gagcacggac ccctggatgt gtggctgcgg
2101 agggagcggg gccatgtgcc catggcttgg aagatggtgg tggcccagca gctggccagc
2161 gccctcagct acctggagaa caagaacctg gttcatggta atgtgtgtgg ccggaacatc
2221 ctgctggccc ggctggggtt ggcagagggc accagccccc tcatcaagct gagtgatcct
2281 ggcgtgggcc tgggcgccct ctccagggag gagcgggtgg agaggatccc ctggctggcc
2341 cccgaatgcc taccaggtgg ggccaacagc ctaagcaccg ccatggacaa gtgggggttt
2401 ggcgccaccc tcctggagat ctgctttgac ggagaggccc ctctgcagag ccgcagtccc
2461 tccgagaagg agcatttcta ccagaggcag caccggctgc ccgagccctc ctgcccacag
2521 ctggccacac tcaccagcca gtgtctgacc tatgagccaa cccagaggcc atcattccgc
2581 accatcctgc gtgacctcac ccggctgcag ccccacaatc ttgctgacgt ctttgactgtg
2641 aaccccggact caccggcgtc ggaccctacg gttttccaca agcgctattt gaaaaagatc
2701 cgagatctgg gcgagggtca cttcggcaag gtcagcttgt actgctacga tccgaccaac
2761 gacggcactg gcgagatggt ggcggtgaaa gccctcaagg cagactgcgg ccccccagcac
2821 cgctcgggct ggaagcagga gattgacatt ctgcgcacgc tctaccacga gcacatcatc
2881 aagtacaagg gctgctgcga ggaccaaggc gagaagtcgc tgcagctggt catggagtac
2941 gtgccccctgg gcagcctccg agactacctg ccccggcaca gcatcgggct ggccccagctg
3001 ctgctcttcg cccagcagat ctgcgagggc atggcctatc tgcacgcgca gcactacatc
3061 caccgagacc tagccgcgcg caacgtgctg ctggacaacg acaggctggt caagatcggg
3121 gactttggcc tagccaaggc cgtgccccgaa ggccacgagt actaccgcgt gcgcgaggat
3181 ggggacagcc ccgtgttctg gtatgccccca gagtgcctga aggagtataa gttctactat
3241 gcgtcagatg tctggtcctt cggggtgacc ctgtatgagc tgctgacgca ctgtgactcc
3301 agccagagcc ccccccacgaa attccttgag ctcataggca ttgctcaggg tcagatgaca
3361 gttctgagac tcactgagtt gctggaacga ggggagaggc tgccacggcc cgacaaatgt
3421 ccctgtgagg tctatcatct catgaagaac tgctgggaga cagaggcgtc ctttcgccca
3481 accttcgaga acctcatacc cattctgaag acagtccatg agaagtacca aggccaggcc
3541 ccttcagtgt tcagcgtgtg ctga
```

SEQ ID NO: 8 Human Tyk2 amino acid sequence

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   1 mplrhwgmar gskpvgdgaq pmaamgglkv llhwagpggg epwvtfsess ltaeevcihi
  61 ahkvgitppc fnlfalfdaq aqvwlppnhi leiprdaslm lyfrirfyfr nwhgmnprep
 121 avyrcgppgt eassdqtaqg mqlldpasfe ylfeqgkhef vndvaslwel steeeihhfk
 181 neslgmaflh lchlalrhgi pleevakkts fkdciprsfr rhirqhsalt rlrlrnvfrr
 241 flrdfqpgrl sqqmvmvkyl atlerlaprf gtervpvchl rllaqaegep cyirdsgvap
 301 tdpgpesaag ppthevlvtg tggiqwwpve eevnkeegss gssgrnpqas lfgkkakahk
 361 avgqpadrpr eplwayfcdf rdithvvlke hcvsihrqdn kclelslpsr aaalsfvslv
 421 dgyfrltads shylchevap prlvmsirdg ihgpllepfv qaklrpedgl ylihwstshp
 481 yrliltvaqr sqapdgmqsl rlrkfpieqq dgafvlegwg rsfpsvrelg aalqgcllra
 541 gddcfslrrc clpqpgetsn liimrgaras prtlnlsqls fhrvdqkeit qlshlgqgtr
 601 tnvyegrlrv egsgdpeegk mddedplvpg rdrggelrvv lkvldpshhd ialafyetas
 661 lmsqvshthl afvhgvcvrg penimvteyv ehgpldvwlr rerghvpmaw kmvvaqqlas
 721 alsylenknl vhgnvcgrni llarlglaeg tspfiklsdp gvglgalsre erveripwla
 781 peclpggans lstamdkwgf gatlleicfd geaplqsrsp sekehfyqrq hrlpepspcpq
 841 latltsqclt yeptqrpsfr tilrdltrlq phnladvltv npdspasdpt vfhkrylkki
 901 rdlgeghfgk vslycydptn dgtgemvavk alkadcgpqh rsgwkqeidi lrtlyhehii
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```
 961 kykgccedqg ekslqlvmey vplgslrdyl prhsiglaql llfaqqiceg maylhaqhyi
1021 hrdlaarnvl ldndrlvkig dfglakavpe gheyyrvred gdspvfwyap eclkeykfyy
1081 asdvwsfgvt lyellthcds sqspptkfle ligiaqgqmt vlrlteller gerlprpdkc
1141 pcevyhlmkn cweteasfrp tfenlipilk tvhekyqgqa psvfsvc
```

SEQ ID NO: 9 Human PIAS1 cDNA sequence

```
   1 atggcggaca gtgcggaact aaagcaaatg gttatgagcc ttagagtttc tgaactccaa
  61 gtactgttgg gctacgccgg gagaaacaag cacggacgca aacacgaact tctcacaaaa
 121 gccctgcatt tgctaaaggc tggctgtagt cctgctgtgc aaatgaaaat taaggaactc
 181 tataggcggc ggttcccaca gaaaatcatg acgcctgcag acttgtccat ccccaacgta
 241 cattcaagtc ctatgccagc aactttgtct ccatctacca ttccacaact cacttacgat
 301 ggtcaccctg catcatcgcc attactccct gtttctcttc tgggacctaa acatgaactg
 361 gaactcccac atcttacatc agctcttcac ccagtccatc cggatataaa acttcaaaaa
 421 ttaccatttt atgatttact ggatgaactg ataaaaccca ccagtctagc atcagacaac
 481 agtcagcgct ttcgagaaac ctgttttgca tttgccttga caccacaaca agtgcagcaa
 541 atcagtagtt ccatggatat ttctgggacc aaatgtgact tcacagtaca ggtccagtta
 601 aggttttgtt tatcagaaac cagttgtcca caagaagatc acttcccacc caatctttgt
 661 gtgaaagtga atacaaaacc ttgcagcctt ccaggttacc ttccacctac aaaaaatggc
 721 gtggaaccaa agcgacccag ccgaccaatt aatatcacct cacttgtccg actgtccaca
 781 acagtaccaa acacgattgt tgtttcttgg actgcagaaa ttggaagaaa ctattccatg
 841 gcagtatatc ttgtaaaaca gttgtcctca acagttcttc ttcagaggtt acgagcaaag
 901 ggaataagga atccggatca ttctagagct ttaattaaag agaagttgac tgcggatcca
 961 gacagtgaaa tagctacaac cagcctaagg gtttctctac tatgtccact tggtaaaatg
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1081 ctttacattc agatgaatga gaaaaaacca acctgggttt gtcctgtctg tgataagaag
1141 gctccatatg aacaccttat tattgatggc ttgtttatgg aaatcctaaa gtactgtaca
1201 gactgtgatg aaatacaatt taaggaggat ggcacttggg caccgatgag atcaaaaaag
1261 gaagtacagg aagtttctgc ctcttacaat ggagtcgatg gatgcttgag ctccacattg
1321 gagcatcagg tagcgtctca ccaccagtcc tcaaataaaa acaagaaagt agaagtgatt
1381 gacctaacca tagacagttc atctgatgaa gaggaagaag agccatctgc caagaggacc
1441 tgtccttccc tatctcccac atcaccacta aataataaag gcattttaag tcttccacat
1501 caagcatctc cagtatcccg caccccaagc cttcctgctg tagacacaag ctacattaat
1561 acctccctca tccaagacta taggcatcct ttccacatga cacccatgcc ttacgactta
1621 caaggattag atttctttcc tttcttatca ggagacaatc agcattacaa cacctccttg
1681 cttgccgctg cagcagcagc agtttcagat gatcaagacc tcctacactc gtctcggttt
1741 ttcccgtata cctcctcaca gatgtttctt gatcagttaa gtgcaggagg cagtacttct
1801 ctgccaacca ccaatggaag cagtagtggc agtaacagca gcctggtttc ttccaacagc
1861 ctaagggaaa gccatagcca cacccgtcaca aacaggagca gcacggacac ggcatccatc
1921 tttggcatca taccagacat tatttcattg gactga
```

SEQ ID NO: 10 Human PIAS1 amino acid sequence

```
   1 madsaelkqm vmslrvselq vllgyagrnk hgrkhelltk alhllkagcs pavqmkikel
  61 yrrrfpqkim tpadlsipnv hsspmpatls pstipqltyd ghpasspllp vsllgpkhel
 121 elphltsalh pvhpdiklqk lpfydlldel ikptslasdn sqrfretcfa faltpqqvqq
 181 isssmdisgt kcdftvqvql rfclsetscp qedhfppnlc vkvntkpcsl pgylpptkng
 241 vepkrpsrpi nitslvrlst tvpntivvsw taeigrnysm avylvkqlss tvllqrlrak
 301 girnpdhsra likekltadp dseiattslr vsllcplgkm rltipcralt cshlqcfdat
 361 lyiqmnekkp twvcpvcdkk apyehliidg lfmeilkyct dcdeiqfked gtwapmrskk
 421 evqevsasyn gvdgclsstl ehqvashhqs snknkkvevi dltidsssde eeeepsakrt
 481 cpslsptspl nnkgilslph qaspvsrtps lpavdtsyin tsliqdyrhp fhmtpmpydl
 541 qgldffpfls gdnqhyntsl laaaaaavsd dqdllhssrf fpytssqmfl dqlsaggsts
 601 lpttngsssg snsslvssns lreshshtvt nrsstdtasi fgiipdiisl d
```

SEQ ID NO: 11 Human PIAS2 (transcript variant 1) cDNA sequence

```
   1 atggcggatt tcgaagagtt gaggaatatg gtttctagtt ttagggtttc tgaactacaa
  61 gtattactag gctttgctgg acggaataaa agtggacgca agcatgacct cctgatgagg
 121 gcgctgcatt tattgaagag cggctgcagc cctgcggttc agattaaaat ccgagaattg
 181 tatagacgcc gatatccacg aactcttgaa ggactttctg atttatccac aatcaaatca
 241 tcggttttca gtttggatgg tggctcatca cctgtagaac ctgacttggc cgtggctgga
 301 atccactcgt tgccttccac ttcagttaca cctcactcac catcctctcc tgttggttct
 361 gtgctgcttc aagatactaa gcccacattt gagatgcagc agccatctcc cccaattcct
 421 cctgtccatc ctgatgtgca gttaaaaaat ctgccctttt atgatgtcct tgatgttctc
 481 atcaagccca cgagtttagt tcaaagcagt attcagcgat ttcaagagaa gtttttttatt
 541 tttgctttga cacctcaaca agttagagag atatgcatat ccagggattt tttgccaggt
 601 ggtaggagag attatacagt ccaagttcag ttgagacttt gcctggcaga gacaagttgc
 661 cctcaagaag ataactatcc aaatagtcta tgtataaaag taaatgggaa gctatttcct
 721 ttgcctggct atgcaccacc gcctaaaaat gggattgaac agaagcgccc tggacgcccc
 781 ttgaatatta catctttagt taggttatct tcagctgtgc caaaccaaat ttccatttct
 841 tgggcatcag aaattgggaa gaattactct atgtctgtat atcttgtacg gcagcttaca
 901 tcagccatgt tattacagag attaaaaatg aaaggtatta gaaaccctga tcattccaga
 961 gcactaatta aagaaaaact tactgcagat cctgatagtg aaattgctac aactagcctt
1021 cgggtatcct tgatgtgccc tttaggaaaa atgaggctga caatcccatg ccgtgcagtg
1081 acttgtacac atctgcagtg ttttgatgct gccctctatc tacaaatgaa tgagaaaaag
1141 cccacctgga tttgtcctgt gtgtgacaaa aaagctgcct atgaaagtct aatattagat
1201 gggctttttta tggaaatttct caatgactgt tctgatgtag atgagatcaa attccaagaa
1261 gatggttctt ggtgtccaat gagaccgaag aaagaagcta tgaaagtatc cagccaaccg
1321 tgtacaaaaa tagaaagttc aagcgtcctc agtaagcctt gttcagtgac tgtagccagt
1381 gaggcaagca agaagaaagt agatgttatt gatcttacaa tagaaagctc ttctgacgaa
1441 gaggaagacc ctcctgccaa aaggaaatgc atctttatgt cagaaacaca aagcagccca
1501 accaaagggg ttctcatgta tcagccatct tctgtaaggg tgcccagtgt gacttcggtt
1561 gatcctgctg ctattccgcc ttcattaaca gactactcag taccattcca ccatacgcca
1621 atatcaagca tgtcatcaga tttgccagga gaacaaagaa gaaatgatat taataatgaa
1681 ctgaagcttg gaacatcttc tgatactgtg caacagtga
```

SEQ ID NO: 12 Human PIAS2 (isoform 1) amino acid sequence

```
   1 madfeelrnm vssfrvselq vllgfagrnk sgrkhdllmr alhllksgcs pavqikirel
  61 yrrryprtle glsdlstiks svfsldggss pvepdlavag ihslpstsvt phspsspvgs
 121 vllqdtkptf emqqpsppip pvhpdvqlkn lpfydvldvl ikptslvqss iqrfqekffi
 181 faltpqqvre icisrdflpg grrdytvqvq lrlclaetsc pqednypnsl cikvngklfp
 241 lpgyapppkn gieqkrpgrp lnitslvrls savpnqisis waseigknys msvylvrqlt
 301 samllqrlkm kgirnpdhsr alikekltad pdseiattsl rvslmcplgk mrltipcrav
 361 tcthlqcfda alylqmnekk ptwicpvcdk kaayeslild glfmeilndc sdvdeikfqe
 421 dgswcpmrpk keamkvssqp ctkiesssvl skpcsvtvas easkkkvdvi dltiesssde
 481 eedppakrkc ifmsetqssp tkgvlmyqps svrvpsvtsv dpsaippslt dysvpfhhtp
 541 issmssdlpg eqrrrndinne lklgtssdtv qq
```

SEQ ID NO: 13 Human PIAS2 (transcript variant 2) cDNA sequence

```
   1 atggcggatt tcgaagagtt gaggaatatg gtttctagtt ttagggtttc tgaactacaa
  61 gtattactag gctttgctgg acggaataaa agtggacgca agcatgacct cctgatgagg
 121 gcgctgcatt tattgaagag cggctgcagc cctgcggttc agattaaaat ccgagaattg
 181 tatagacgcc gatatccacg aactcttgaa ggactttctg atttatccac aatcaaatca
 241 tcggttttca gtttggatgg tggctcatca cctgtagaac ctgacttggc cgtggctgga
 301 atccactcgt tgccttccac ttcagttaca cctcactcac catcctctcc tgttggttct
 361 gtgctgcttc aagatactaa gcccacattt gagatgcagc agccatctcc cccaattcct
 421 cctgtccatc ctgatgtgca gttaaaaaat ctgccctttt atgatgtcct tgatgttctc
 481 atcaagccca cgagtttagt tcaaagcagt attcagcgat ttcaagagaa gtttttttatt
```

```
 541 tttgctttga cacctcaaca agttagagag atatgcatat ccagggattt tttgccaggt
 601 ggtaggagag attatacagt ccaagttcag ttgagacttt gcctggcaga gacaagttgc
 661 cctcaagaag ataactatcc aaatagtcta tgtataaaag taaatgggaa gctatttcct
 721 ttgcctggct atgcaccacc gcctaaaaat gggattgaac agaagcgccc tggacgcccc
 781 ttgaatatta catctttagt taggttatct tcagctgtgc caaaccaaat ttccatttct
 841 tgggcatcag aaattgggaa gaattactct atgtctgtat atcttgtacg gcagcttaca
 901 tcagccatgt tattacagag attaaaaatg aaaggtatta gaaaccctga tcattccaga
 961 gcactaatta aagaaaaact tactgcagat cctgatagtg aaattgctac aactagcctt
1021 cgggtatcct tgatgtgccc tttaggaaaa atgaggctga caatcccatg ccgtgcagtg
1081 acttgtacac atctgcagtg ttttgatgct gccctctatc tacaaatgaa tgagaaaaag
1141 cccacctgga tttgtcctgt gtgtgacaaa aaagctgcct atgaaagtct aatattagat
1201 gggctttta tggaaattct caatgactgt tctgatgtag atgagatcaa attccaagaa
1261 gatggttctt ggtgtccaat gagaccgaag aaagaagcta tgaaagtatc cagccaaccg
1321 tgtacaaaaa tagaaagttc aagcgtcctc agtaagcctt gttcagtgac tgtagccagt
1381 gaggcaagca agaagaaagt agatgttatt gatcttacaa tagaaagctc ttctgacgaa
1441 gaggaagacc ctcctgccaa aaggaaatgc atctttatgt cagaaacaca aagcagccca
1501 accaaagggg ttctcatgta tcagccatct tctgtaaggg tgcccagtgt gacttcggtt
1561 gatcctgctg ctattccgcc ttcattaaca gactactcag taccattcca ccatacgcca
1621 atatcaagca tgtcatcaga tttgccaggt ttggattttc tttcccttat tccagttgat
1681 ccccagtact gtcctcctat gtttttggat agtctcacct cacccttaac agcaagcagt
1741 acgtctgtca ccaccaccag ctcccatgaa agcagtactc atgttagttc atccagcagc
1801 aggagtgaga caggggtcat aaccagcagt ggaagtaaca ttcctgacat catctcattg
1861 gactaa
```

SEQ ID NO: 14 Human PIAS2 (isoform 2) amino acid sequence

```
   1 madfeelrnm vssfrvselq vllgfagrnk sgrkhdllmr alhllksgcs pavqikirel
  61 yrrrypртle glsdlstiks svfsldggss pvepdlavag ihslpstsvt phspsspvgs
 121 vllqdtkptf emqqpsppip pvhpdvqlkn lpfydvldvl ikptslvqss iqrfqekffi
 181 faltpqqvre icisrdflpg grrdytvqvq lrlclaetsc pqednypnsl cikvngklfp
 241 lpgyapppkn gieqkrpgrp lnitslvrls savpnqisis waseigknys msvylvrqlt
 301 samllqrlkm kgirnpdhsr alikekltad pdseiattsl rvslmcplgk mrltipcrav
 361 tcthlqcfda alylqmnekk ptwicpvcdk kaayeslild glfmeilndc sdvdeikfqe
 421 dgswcpmrpk keamkvssqp ctkiesssvl skpcsvtvas easkkkvdvi dltiesssde
 481 eedppakrkc ifmsetqssp tkgvlmyqps svrvpsvtsv dpaaippslt dysvpfhhtp
 541 issmssdlpg ldflslipvd pqycppmfld sltspltass tsvtttsshe ssthvsssss
 601 rsetqvitss gsnipdiisl d
```

SEQ ID NO: 15 Human PIAS3 cDNA sequence

```
   1 atggcggagc tgggcgaatt aaagcacatg gtgatgagtt tccgggtgtc tgagctccag
  61 gtgcttcttg gctttgctgg ccggaacaag agtggacgga agcacgagct cctggccaag
 121 gctctgcacc tcctgaagtc cagctgtgcc cctagtgtcc agatgaagat caaagagctt
 181 taccgacgac gttttccccg gaagaccctg gggccctctg atctctccct tctctctttg
 241 ccccctggca cctctcctgt aggctccccт ggtcctctag ctcccattcc cccaacgctg
 301 ttggcccctg gcaccctgct gggccccaag cgtgaggtgg acatgcaccc ccctctgccc
 361 cagcctgtgc accctgatgt caccatgaaa ccattgccct tctatgaagt ctatggggag
 421 ctcatccggc ccaccaccct tgcatccact tctagccagc ggtttgagga agcgcacttt
 481 acctttgccc tcacacccca gcaagtgcag cagattctta catccagaga ggttctgcca
 541 ggagccaaat gtgattatac catacaggtg cagctaaggt tctgtctctg tgagaccagc
 601 tgccccccagg aagattattt tccccccaac ctctttgtca aggtcaatgg gaaactgtgc
 661 ccctgccgg gttaccttcc cccaaccaag aatgggccg agcccaagag gcccagccgc
 721 cccatcaaca tcacaccccт ggctcgactc tcagccactg ttcccaacac cattgtggtc
 781 aattggtcat ctgagttcgg acggaattac tccttgtctg tgtacctggt gaggcagttg
 841 actgcaggaa cccttctaca aaaactcaga gcaaagggta tccggaaccc agaccactcg
 901 cgggcactga tcaaggagaa attgactgct gaccctgaca gtgaggtggc cactacaagt
```

```
 961 ctccgggtgt cactcatgtg cccgctaggg aagatgcgcc tgactgtccc ttgtcgtgcc
1021 ctcacctgcg cccacctgca gagcttcgat gctgcccttt atctacagat gaatgagaag
1081 aagcctacat ggacatgtcc tgtgtgtgac aagaaggctc cctatgaatc tcttatcatt
1141 gatggtttat ttatggagat tcttagttcc tgttcagatt gtgatgagat ccaattcatg
1201 gaagatggat cctggtgccc aatgaaaccc aagaaggagg catctgaggt ttgccccccg
1261 ccagggtatg ggctggatgg cctccagtac agcccagtcc agggggggaga tccatcagag
1321 aataagaaga aggtcgaagt tattgacttg acaatagaaa gctcatcaga tgaggaggat
1381 ctgccccccta ccaagaagca ctgttctgtc acctcagctg ccatcccggc cctacctgga
1441 agcaaaggag tcctgacatc tggccaccag ccatcctcgg tgctaaggag ccctgctatg
1501 ggcacgtttgg gtggggattt cctgtccagt ctcccactac atgagtaccc acctgccttc
1561 ccactgggag ccgacatcca aggtttagat ttatttttcat ttcttcagac agagagtcag
1621 cactatggcc cctctgtcat cacctcacta gatgaacagg atgcccttgg ccacttcttc
1681 cagtaccgag ggacccccttc tcactttctg ggcccactgg ccccacgct ggggagctcc
1741 cactgcagcg ccactccggc gccccctcct ggccgtgtca gcagcattgt ggccccctggg
1801 ggggccttga gggaggggca tggaggaccc ctgccctcag gtccctcttt gactggctgt
1861 cggtcagaca tcatttccct ggactga
```

SEQ ID NO: 16 Human PIAS3 amino acid sequence

```
   1 maelgelkhm vmsfrvselq vllgfagrnk sgrkhellak alhllkssca psvqmkikel
  61 yrrrfprktl gpsdlsllsl ppgtspvgsp gplapipptl lapgtllgpk revdmhpplp
 121 qpvhpdvtmk plpfyevyge lirpttlast ssqrfeeahf tfaltpqqvq qiltsrevlp
 181 gakcdytiqv qlrfclcets cpqedyfppn lfvkvngklc plpgylpptk ngaepkrpsr
 241 pinitplarl satvpntivv nwssefgrny slsvylvrql tagtllqklr akgirnpdhs
 301 ralikeklta dpdsevatts lrvslmcplg kmrltvpcra ltcahlqsfd aalylqmnek
 361 kptwtcpvcd kkapyeslii dglfmeilss csdcdeiqfm edgswcpmkp kheasevcpp
 421 pgyyldglqy spvqqydpse nkkkvevidl tiesssdeed lpptkkhcsv tsaaipalpg
 481 skgvltsghq pssvlrspam gtlggdflss lplheyppaf plgadiqgld lfsflqtesq
 541 hygpsvitsl deqdalghff qyrgtpshfl gplaptlgss hcsatpappp grvssivapg
 601 galreghgyp lpsgpsltgc rsdiisld
```

SEQ ID NO: 17 Human PIAS4 cDNA sequence

```
   1 atggcggcgg agctggtgga ggccaaaaac atggtgatga gttttcgagt ctccgacctt
  61 cagatgctcc tgggtttcgt gggccggagt aagagtggac tgaagcacga gctcgtcacc
 121 agggccctcc agctggtgca gtttgactgt agccctgagc tgttcaagaa gatcaaggag
 181 ctgtacgaga cccgctacgc caagaagaac tcggagcctg ccccacagcc gcaccggccc
 241 ctggaccccc tgaccatgca ctccacctac gaccgggccg gcgctgtgcc caggactccg
 301 ctggcaggcc ccaatattga ctaccccgtg ctctacggaa agtacttaaa cggactggga
 361 cggttgcccg ccaagaccct caagccagaa gtccgcctgg tgaagctgcc gttctttaat
 421 atgctggatg agctgctgaa gcccaccgaa ttagtcccac agaacaacga gaagcttcag
 481 gagagcccgt gcatcttcgc attgacgcca agacaggtgg agttgatccg gaactccagg
 541 gaactgcagc ccggagttaa agccgtgcag gtcgtcctga gaatctgtta ctcagacacc
 601 agctgccctc aggaggacca gtacccgccc aacatcgctg tgaaggtcaa ccacagctac
 661 tgctccgtcc cgggctacta ccctctccaat aagccccgggg tggagcccaa gaggccgtgc
 721 cgccccatca acctcactca cctcatgtac ctgtcctcgg ccaccaaccg catcactgtc
 781 acctgggggga actacggcaa gagctactcg gtggccctgt acctggtgcg gcagctgacc
 841 tcatcggagc tgctgcagag gctgaagacc attggggtaa agcacccgga gctgtgcaag
 901 gcactggtca aggagaagct gcgccttgat cctgacagcg agatcgccac caccggtgtg
 961 cgggtgtccc tcatctgtcc gctggtgaag atgcggctct ccgtgccctg ccgggcagag
1021 acctgcgccc acctgcagtg cttcgacgcc gtcttctacc tgcagatgaa cgagaagaag
1081 cccacctgga tgtgccccgt gtgcgacaag ccagcccct acgaccagct catcatcgac
1141 gggctcctct cgaagatcct gagcgagtgt gaggacgccg acgagatcga gtacctggtg
1201 gacggctcgt ggtgcccgat ccgcgccgaa aaggagcgca gctgcagccc gcagggcgcc
1261 atcctcgtgc tgggccccctc ggacgccaat gggctcctgc ccgccccccag cgtcaacggg
1321 agcggtgccc tgggcagcac gggtggcggc ggccccggtgg gcagcatgga gaatgggaag
```

```
1381 ccgggcgccg atgtggtgga cctcacgctg gacagctcat cgtcctcgga ggatgaggag
1441 gaggaggaag aggaggagga agacgaggac gaagaggggc cccggcccaa gcgccgctgc
1501 cccttccaga agggcctggt gccggcctgc tga
```

SEQ ID NO: 18 Human PIAS4 amino acid sequence

```
   1 maaelveakn mvmsfrvsdl qmllgfvgrs ksglkhelvt ralqlvqfdc spelfkkike
  61 lyetryakkn sepapqphrp ldpltmhsty dragavprtp lagpnidypv lygkylnglg
 121 rlpaktlkpe vrlvklpffn mldellkpte lvpqnneklq espcifaltp rqvelirnsr
 181 elqpgvkavq vvlricysdt scpqedqypp niavkvnhsy csvpgyypsn kpgvepkrpc
 241 rpinlthlmy lssatnritv twgnygksys valylvrqlt ssellqrlkt igvkhpelck
 301 alvkeklrld pdseiattgv rvslicplvk mrlsvpcrae tcahlqcfda vfylqmnekk
 361 ptwmcpvcdk papydqliid gllskilsec edadeieylv dgswcpirae kerscspqga
 421 ilvlgpsdan gllpapsvng sgalgstggg gpvgsmengk pgadvvdltl dsssssedee
 481 eeeeeeeded eegprpkrrc pfqkglvpac
```

SEQ ID NO: 19 Human SOCS1 cDNA sequence

```
   1 atggtagcac acaaccaggt ggcagccgac aatgcagtct ccacagcagc agagccccga
  61 cggcggccag aaccttcctc ctcttcctcc tcctcgcccg cggccccccc gcgccccgcgg
 121 ccgtgccccg cggtccccgc cccggccccc ggcgacacgc acttccgcac attccgttcg
 181 cacgccgatt accggcgcat cacgcgcgcc agcgcgctcc tggacgcctg cggattctac
 241 tgggggcccc tgagcgtgca cgggggcgcac gagcggctgc gcgccgagcc cgtgggcacc
 301 ttcctggtgc gcgacagccg ccagcggaac tgcttttttcg cccttagcgt gaagatggcc
 361 tcgggaccca cgagcatccg cgtgcacttt caggccggcc gctttcacct ggatggcagc
 421 cgcgagagct tcgactgcct cttcgagctg ctggagcact acgtggcggc gccgcgccgc
 481 atgctggggg ccccgctgcg ccagcgccgc gtgcggccgc tgcaggagct gtgccgccag
 541 cgcatcgtgg ccaccgtggg ccgcgagaac ctggctcgca tccccctcaa ccccgtcctc
 601 cgcgactacc tgagctcctt ccccttccag atttga
```

SEQ ID NO: 20 Human SOCS1 amino acid sequence

```
   1 mvahnqvaad navstaaepr rrpepsssss sspaaparpr pcpavpapap gdthfrtfrs
  61 hadyrritra salldacgfy wgplsvhgah erlraepvgt flvrdsrqrn cffalsvkma
 121 sgptsirvhf qagrfhldgs resfdclfel lehyvaaprr mlgaplrqrr vrplqelcrq
 181 rivatvgren lariplnpvl rdylssfpfq i
```

SEQ ID NO: 21 Human SOCS3 cDNA sequence

```
   1 atggtcaccc acagcaagtt tcccgccgcc gggatgagcc gccccctgga caccagcctg
  61 cgcctcaaga ccttcagctc caagagcgag taccagctgg tggtgaacgc agtgcgcaag
 121 ctgcaggaga gcggcttcta ctggagcgca gtgaccggcg gcgaggcgaa cctgctgctc
 181 agtgccgagc ccgccggcac ctttctgatc cgcgacagct cggaccagcg ccacttcttc
 241 acgctcagcg tcaagaccca gtctgggacc aagaacctgc gcatccagtg tgaggggggc
 301 agcttctctc tgcagagcga tccccggagc acgcagcccg tgccccgctt cgactgcgtg
 361 ctcaagctgg tgcaccacta catgccgccc cctggagccc cctccttccc ctcgccacct
 421 actgaaccct cctccgaggt gcccgagcag ccgtctgccc agccactccc tgggagtccc
 481 cccagaagag cctattacat ctactccggg ggcgagaaga tccccctggt gttgagccgg
 541 ccctctcct ccaacgtggc cactcttcag catctctgtc ggaagaccgt caacggccac
 601 ctggactcct atgagaaagt cacccagctg ccggggcccca ttcgggagtt cctggaccag
 661 tacgatgccc cgctttaa
```

SEQ ID NO: 22 Human SOCS3 amino acid sequence

```
   1 mvthskfpaa gmsrpldtsl rlktfsskse yqlvvnavrk lqesgfywsa vtggeanlll
  61 saepagtfli rdssdqrhff tlsvktqsgt knlriqcegg sfslqsdprs tqpvprfdcv
```

```
 121 lklvhhympp pgapsfpspp tepssevpeq psaqplpgsp prrayyiysg gekiplvlsr
 181 plssnvatlq hlcrktvngh ldsyekvtql pgpirefldq ydapl
```

SEQ ID NO: 23 Human SHP-1 (transcript variant 1) cDNA sequence

```
   1 atggtgaggt ggtttcaccg agacctcagt gggctggatg cagagaccct gctcaagggc
  61 cgaggtgtcc acggtagctt cctggctcgg cccagtcgca agaaccaggg tgacttctcg
 121 ctctccgtca gggtgggggg tcaggtgacc catattcgga tccagaactc aggggatttc
 181 tatgacctgt atggaggggg gaagtttgcg actctgacag agctggtgga gtactacact
 241 cagcagcagg gtgtcctgca ggaccgcgac ggcaccatca tccacctcaa gtacccgctg
 301 aactgctccg atcccactag tgagaggtgg taccatggcc acatgtctgg cgggcaggca
 361 gagacgctgc tgcaggccaa gggcgagccc tggacgtttc ttgtgcgtga gagcctcagc
 421 cagcctggag acttcgtgct ttctgtgctc agtgaccagc ccaaggctgg cccaggctcc
 481 ccgctcaggg tcacccacat caaggtcatg tgcgagggtg gacgctacac agtgggtggt
 541 ttggagacct tcgacagcct cacggacctg gtggagcatt tcaagaagac ggggattgag
 601 gaggcctcag gcgcctttgt ctacctgcgg cagccgtact atgccacgag ggtgaatgcg
 661 gctgacattg agaaccgagt gttggaactg aacaagaagc aggagtccga ggatacagcc
 721 aaggctggct tctgggagga gtttgagagt ttgcagaagc aggaggtgaa gaacttgcac
 781 cagcgtctgg aagggcagcg gccagagaac aagggcaaga accgctacaa gaacattctc
 841 cctttgacc acagccgagt gatcctgcag ggacgggaca gtaacatccc cgggtccgac
 901 tacatcaatg ccaactacat caagaaccag ctgctaggcc ctgatgagaa cgctaagacc
 961 tacatcgcca gccagggctg tctggaggcc acggtcaatg acttctggca gatggcgtgg
1021 caggagaaca gccgtgtcat cgtcatgacc acccgagagg tggagaaagg ccggaacaaa
1081 tgcgtcccat actggcccga ggtgggcatg cagcgtgctt atgggcccta ctctgtgacc
1141 aactgcgggg agcatgacac aaccgaatac aaactccgta ccttacaggt ctccccgctg
1201 gacaatggag acctgattcg ggagatctgg cattaccagt acctgagctg gccccgaccat
1261 ggggtccccc gtgagcctgg gggtgtcctc agcttcctgg accagatcaa ccagcggcag
1321 gaaagtctgc ctcacgcagg gcccatcatc gtgcactgca gcgccggcat cggccgcaca
1381 ggcaccatca ttgtcatcga catgctcatg gagaacatct ccaccaaggg cctggactgt
1441 gacattgaca tccagaagac catccagatg gtgcgggcgc agcgctcggg catggtgcag
1501 acggaggcgc agtacaagtt catctacgtg gccatcgccc agttcattga aaccactaag
1561 aagaagctgg aggtcctgca gtcgcagaag ggccaggagt cggagtacgg gaacatcacc
1621 tatcccccag ccatgaagaa tgcccatgcc aaggcctccc gcaccctcgtc caaaacacaag
1681 gaggatgtgt atgagaacct gcacactaag aacaagaggg aggagaaagt gaagaagcag
1741 cggtcagcag acaaggagaa gagcaagggt tccctcaaga ggaagtga
```

SEQ ID NO: 24 Human SHP-1 (isoform 1) amino acid sequence

```
   1 mvrwfhrdls gldaetllkg rgvhgsflar psrknqgdfs lsvrvgdqvt hiriqnsgdf
  61 ydlyggekfa tltelveyyt qqqgvlqdrd gtiihlkypl ncsdptserw yhghmsgggq
 121 etllqakgep wtflvresls qpgdfvlsvl sdqpkagpgs plrvthikvm ceggrytvgg
 181 letfdsltdl vehfkktgie easgafvylr qpyyatrvna adienrvlel nkkqesedta
 241 kagfweefes lqkqevknlh qrlegqrpen kgknryknil pfdhsrvilq grdsnipgsd
 301 yinanyikng llgpdenakt yiasqgclea tvndfwqmaw qensrvivmt trevekgrnk
 361 cvpywpevgm qraygpysvt ncgehdttey klrtlqvspl dngdlireiw hyqylswpdh
 421 gvpsepggvl sfldqinqrq eslphagpii vhcsagigrt gtiividmlm enistkgldc
 481 didiqktiqm vraqrsgmvq teaqykfiyv aiaqfiettk kklevlqsqk ggeseygnit
 541 yppamknaha kasrtsskhk edvyenlhtk nkreekvkkq rsadkekskg slkrk
```

SEQ ID NO: 25 Human SHP-1 (transcript variant 2) cDNA sequence

```
   1 atgctgtccc gtgggtggtt tcaccgagac ctcagtgggc tggatgcaga gaccctgctc
  61 aagggccgag gtgtccacgg tagcttcctg gctcggccca gtcgcaagaa ccagggtgac
 121 ttctcgctct ccgtcagggt gggggatcag gtgacccata ttcggatcca gaactcaggg
 181 gatttctatg acctgtatgg aggggagaag tttgcgactc tgacagagct ggtggagtac
 241 tacactcagc agcagggtgt cctgcaggac cgcgacggca ccatcatcca cctcaagtac
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 301 ccgctgaact gctccgatcc cactagtgag aggtggtacc atggccacat gtctggcggg
 361 caggcagaga cgctgctgca ggccaagggc gagccctgga cgtttcttgt gcgtgagagc
 421 ctcagccagc ctggagactt cgtgctttct gtgctcagtg accagcccaa ggctggccca
 481 ggctcccccgc tcagggtcac ccacatcaag gtcatgtgcg agggtggacg ctacacagtg
 541 ggtggtttgg agaccttcga cagcctcacg gacctggtgg agcatttcaa gaagacgggg
 601 attgaggagg cctcaggcgc ctttgtctac ctgcggcagc cgtactatgc cacgagggtg
 661 aatgcggctg acattgagaa ccgagtgttg gaactgaaca agaagcagga gtccgaggat
 721 acagccaagg ctggcttctg ggaggagttt gagagtttgc agaagcagga ggtgaagaac
 781 ttgcaccagc gtctggaagg gcagcggcca gagaacaagg gcaagaaccg ctacaagaac
 841 attctccccct ttgaccacag ccgagtgatc ctgcagggac gggacagtaa catccccggg
 901 tccgactaca tcaatgccaa ctacatcaag aaccagctgc taggccctga tgagaacgct
 961 aagacctaca tcgccagcca gggctgtctg gaggccacgg tcaatgactt ctggcagatg
1021 gcgtggcagg agaacagccg tgtcatcgtc atgaccaccc gagaggtgga gaaaggccgg
1081 aacaaatgcg tcccatactg gccccgaggtg ggcatgcagc gtgcttatgg gccctactct
1141 gtgaccaact gcggggagca tgacacaacc gaatacaaac tccgtacctt acaggtctcc
1201 ccgctggaca atggagacct gattcgggag atctggcatt accagtacct gagctggccc
1261 gaccatgggg tccccagtga gcctgggggt gtcctcagct tcctggacca gatcaaccag
1321 cggcaggaaa gtctgcctca cgcagggccc atcatcgtgc actgcagcgc cggcatcggc
1381 cgcacaggca ccatcattgt catcgacatg ctcatggaga acatctccac caagggcctg
1441 gactgtgaca ttgacatcca gaagaccatc cagatggtgc gggcgcagcg ctcgggcatg
1501 gtgcagacgg aggcgcagta caagttcatc tacgtggcca tcgcccagtt cattgaaacc
1561 actaagaaga agctggaggt cctgcagtcg cagaagggcc aggagtcgga gtacgggaac
1621 atcacctatc ccccagccat gaagaatgcc catgccaagg cctcccgcac ctcgtccaaa
1681 cacaaggagg atgtgtatga gaacctgcac actaagaaca agagggagga gaaagtgaag
1741 aagcagcggt cagcagacaa ggagaagagc aagggttccc tcaagaggaa gtga
```

SEQ ID NO: 26 Human SHP-1 (isoform 2) amino acid sequence

```
   1 mlsrgwfhrd lsgldaetll kgrgvhgsfl arpsrknqgd fslsvrvgdq vthiriqnsg
  61 dfydlyggek fatltelvey ytqqqgvlqd rdgtiihlky plncsdptse rwyhghmsgg
 121 qaetllqakg epwtflvres lsqpgdfvls vlsdqpkagp gsplrvthik vmceggrytv
 181 ggletfdslt dlvehfkktg ieeasgafvy lrqpyyatrv naadienrvl elnkkqesed
 241 takagfweef eslqkqevkn lhqrlegqrp enkgknrykn ilpfdhsrvi lqgrdsnipg
 301 sdyinanyik nqllgpdena ktyiasqgcl eatvndfwqm awqensrviv mttrevekgr
 361 nkcvpywpev gmqraygpys vtncgehdtt eyklrtlqvs pldngdlire iwhyqylswp
 421 dhgvpsepgg vlsfldqinq rqeslphagp iivhcsagig rtgtiividm lmenistkgl
 481 dcdidiqkti qmvraqrsgm vqteaqykfi yvaiaqfiet tkkklevlqs qkgqeseygn
 541 ityppamkna hakasrtssk hkedvyenlh tknkreekvk kqrsadkeks kqsikrk
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SEQ ID NO: 27 Human SHP-1 (transcript variant 3) cDNA sequence

```
   1 atggtgaggt ggtttcaccg agacctcagt gggctggatg cagagaccct gctcaagggc
  61 cgaggtgtcc acggtagctt cctggctcgg cccagtcgca agaaccaggg tgacttctcg
 121 ctctccgtca gggtgggggga tcaggtgacc catattcgga tccagaactc aggggatttc
 181 tatgacctgt atggaggggga gaagtttgcg actctgacag agctggtgga gtactacact
 241 cagcagcagg gtgtcctgca ggaccgcgac ggcaccatca tccacctcaa gtacccgctg
 301 aactgctccg atcccactag tgagaggtgg taccatggcc acatgtctgg cgggcaggca
 361 gagacgctgc tgcaggccaa gggcgagccc tggacgtttc ttgtgcgtga gagcctcagc
 421 cagcctggag acttcgtgct ttctgtgctc agtgaccagc ccaaggctgg cccaggctcc
 481 ccgctcaggg tcacccacat caaggtcatg tgcgagggtg gacgctacac agtgggtggt
 541 ttggagacct tcgacagcct cacggacctg gtggagcatt tcaagaagac ggggattgag
 601 gaggcctcag gcgcctttgt ctacctgcgg cagccgtact atgccacgag ggtgaatgcg
 661 gctgacattg agaaccgagt gttggaactg aacaagaagc aggagtccga ggatacagcc
 721 aaggctggct tctgggagga gtttgagagt ttgcagaagc aggaggtgaa gaacttgcac
 781 cagcgtctgg aagggcagcg gccagagaac aagggcaaga accgctacaa gaacattctc
 841 cctttgacc acagccgagt gatcctgcag ggacgggaca gtaacatccc cgggtccgac
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 901 tacatcaatg ccaactacat caagaaccag ctgctaggcc ctgatgagaa cgctaagacc
 961 tacatcgcca gccagggctg tctggaggcc acggtcaatg acttctggca gatggcgtgg
1021 caggagaaca gccgtgtcat cgtcatgacc acccgagagg tggagaaagg ccggaacaaa
1081 tgcgtcccat actggcccga ggtgggcatg cagcgtgctt atgggcccta ctctgtgacc
1141 aactgcgggg agcatgacac aaccgaatac aaactccgta ccttacaggt ctccccgctg
1201 gacaatggag acctgattcg ggagatctgg cattaccagt acctgagctg gcccgaccat
1261 ggggtcccca gtgagcctgg gggtgtcctc agcttcctgg accagatcaa ccagcggcag
1321 gaaagtctgc ctcacgcagg gcccatcatc gtgcactgca gcgccggcat cggccgcaca
1381 ggcaccatca ttgtcatcga catgctcatg gagaacatct ccaccaaggg cctggactgt
1441 gacattgaca tccagaagac catccagatg gtgcgggcgc agcgctcggg catggtgcag
1501 acggaggcgc agtacaagtt catctacgtg gccatcgccc agttcattga aaccactaag
1561 aagaagctgg aggtcctgca gtcgcagaag ggccaggagt cggagtacgg gaacatcacc
1621 tatcccccag ccatgaagaa tgcccatgcc aaggcctccc gcacctcgtc caagagcttg
1681 gagtctagtg cagggaccgt ggctgcgtca cctgtgagac ggggtggcca gaggggactg
1741 ccagtgcccgg gtccccctgt gctgtctcct gacctgcacc aactgcctgt acttgccccc
1801 ctgcacccgg ctgcagacac aaggaggatg tgtatgagaa cctgcacact aagaacaaga
1861 gggaggagaa agtga
```

SEQ ID NO: 28 Human SHP-1 (isoform 3) amino acid sequence

```
   1 mvrwfhrdls gldaetllkg rgvhgsflar psrknqgdfs lsvrvgdqvt hiriqnsgdf
  61 ydlyggekfa tltelveyyt qqqgvlqdrd gtiihlkypl ncsdptserw yhghmsgggq
 121 etllqakgep wtflvresls qpgdfvlsvl sdqpkagpgs plrvthikvm ceggrytvgg
 181 letfdsltdl vehfkktgie easgafvylr qpyyatrvna adienrvlel nkkqesedta
 241 kagfweefes lqkqevknlh qrleggqrpen kgknryknil pfdhsrvilq grdsnipgsd
 301 yinanyikng llgpdenakt yiasqgclea tvndfwqmaw qensrvivmt trevekgrnk
 361 cvpywpevgm qraygpysvt ncgehdttey klrtlqvspl dngdlireiw hyqylswpdh
 421 gvpsepggvl sfldqinqrq eslphagpii vhcsagigrt gtiividmlm enistkgldc
 481 didiqktiqm vraqrsgmvq teaqykfiyv aiaqfiettk kklevlqsqk gqeseygnit
 541 yppamknaha kasrtssksl essagtvaas pvrrgyqryl pvpgppvlsp dlhqlpvlap
 601 lhpaadtrrm cmrtctlrtr grrk
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SEQ ID NO: 29 Human SHP-2 (transcript variant 1) cDNA sequence

```
   1 atgacatcgc ggagatggtt tcacccaaat atcactggtg tggaggcaga aaacctactg
  61 ttgacaagag gagttgatgg cagtttttg gcaaggccta gtaaaagtaa ccctggagac
 121 ttcacacttt ccgttagaag aaatggagct gtcacccaca tcaagattca gaacactggt
 181 gattactatg acctgtatgg aggggagaaa tttgccactt tggctgagtt ggtccagtat
 241 tacatggaac atcacgggca attaaaagag aagaatggag atgtcattga gcttaaatat
 301 cctctgaact gtgcagatcc tacctctgaa aggtggtttc atggacatct ctctgggaaa
 361 gaagcagaga aattattaac tgaaaaagga aaacatggta gttttcttgt acgagagagc
 421 cagagccacc ctggagattt tgttctttct gtgcgcactg gtgatgacaa aggggagagc
 481 aatgacggca agtctaaagt gacccatgtt atgattcgct gtcaggaact gaaatacgac
 541 gttggtggag gagaacggtt tgattctttg acagatcttg tggaacatta taagaagaat
 601 cctatggtgg aaacattggg tacagtacta caactcaagc agccccttaa cacgactcgt
 661 ataaatgctg ctgaaataga aagcagagtt cgagaactaa gcaaattagc tgagaccaca
 721 gataaagtca aacaaggctt ttgggaagaa tttgagacac tacaacaaca ggagtgcaaa
 781 cttctctaca gccgaaaaga gggtcaaagg caagaaaaca aaaacaaaaa tagatataaa
 841 aacatcctgc cctttgatca taccagggtt gtcctacacg atggtgatcc caatgagcct
 901 gtttcagatt acatcaatgc aaatatcatc atgcctgaat ttgaaaccaa gtgcaacaat
 961 tcaaagccca aaaagagtta cattgccaca caaggctgcc tgcaaaacac ggtgaatgac
1021 ttttggcgga tggtgttcca agaaaactcc cgagtgattg tcatgacaac gaaagaagtg
1081 gagagaggaa agagtaaatg tgtcaaatac tggcctgatg agtatgctct aaaagaatat
1141 ggcgtcatgc gtgttaggaa cgtcaaagaa agcgccgctc atgactatac gctaagagaa
1201 cttaaacttt caaaggttgg acaagggaat acggagagaa cggtctggca ataccacttt
1261 cggacctggc cggaccacgg cgtgcccagc gaccctgggg gcgtgctgga cttcctggag
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1321 gaggtgcacc ataagcagga gagcatcatg gatgcagggc cggtcgtggt gcactgcagt
1381 gctggaattg gccggacagg gacgttcatt gtgattgata ttcttattga catcatcaga
1441 gagaaaggtg ttgactgcga tattgacgtt cccaaaacca tccagatggt gcggtctcag
1501 aggtcaggga tggtccagac agaagcacag taccgattta tctatatggc ggtccagcat
1561 tatattgaaa cactacagcg caggattgaa gaagagcaga aaagcaagag gaaagggcac
1621 gaatatacaa atattaagta ttctctagcg gaccagacga gtggagatca gagccctctc
1681 ccgccttgta ctccaacgcc accctgtgca gaaatgagag aagacagtgc tagagtctat
1741 gaaaacgtgg gcctgatgca acagcagaaa agtttcagat ga
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SEQ ID NO: 30 Human SHP-2 (isoform 1) amino acid sequence

```
   1 mtsrrwfhpn itgveaenll ltrgvdgsfl arpsksnpgd ftlsvrrnga vthikiqntg
  61 dyydlyggek fatlaelvqy ymehhgqlke kngdvielky plncadptse rwfhghlsgk
 121 eaeklltekg khgsflvres qshpgdfvls vrtgddkges ndgkskvthv mircqelkyd
 181 vgggerfdsl tdlvehykkn pmvetlgtvl qlkqplnttr inaaeiesrv relsklaett
 241 dkvkqgfwee fetlqqqeck llysrkegqr qenknknryk nilpfdhtrv vlhdgdpnep
 301 vsdyinanii mpefetkcnn skpkksyiat qgclqntvnd fwrmvfqens rvivmttkev
 361 ergkskcvky wpdeyalkey gvmrvrnvke saahdytlre lklskvgqgn tertvwqyhf
 421 rtwpdhgvps dpggvldfle evhhkqesim dagpvvvhcs agigrtgtfi vidilidiir
 481 ekgvdcdidv pktiqmvrsq rsgmvqteaq yrfiymavqh yietlqrrie eeqkskrkgh
 541 eytnikysla dqtsgdqspl ppctptppca emredsarvy envglmqqqk sfr
```

SEQ ID NO: 31 Human SHP-2 (transcript variant 1) cDNA sequence

```
   1 atgacatcgc ggagatggtt tcacccaaat atcactggtg tggaggcaga aaacctactg
  61 ttgacaagag gagttgatgg cagtttttg gcaaggccta gtaaaagtaa ccctggagac
 121 ttcacacttt ccgttagaag aaatggagct gtcacccaca tcaagattca gaacactggt
 181 gattactatg acctgtatgg aggggagaaa tttgccactt tggctgagtt ggtccagtat
 241 tacatggaac atcacgggca attaaaagag aagaatggag atgtcattga gcttaaatat
 301 cctctgaact gtgcagatcc tacctctgaa aggtggtttc atggacatct ctctgggaaa
 361 gaagcagaga aattattaac tgaaaaagga aaacatggta gttttcttgt acgagagagc
 421 cagagccacc ctggagattt tgttctttct gtgcgcactg gtgatgacaa aggggagagc
 481 aatgacggca agtctaaagt gacccatgtt atgattcgct gtcaggaact gaaatacgac
 541 gttggtggag gagaacggtt tgattctttg acagatcttg tggaacatta taagaagaat
 601 cctatggtgg aaacattggg tacagtacta caactcaagc agccccttaa cacgactcgt
 661 ataaatgctg ctgaaataga aagcagagtt cgagaactaa gcaaattagc tgagaccaca
 721 gataaagtca aacaaggctt ttgggaagaa tttgagacac tacaacaaca ggagtgcaaa
 781 cttctctaca gccgaaaaga gggtcaaagg caagaaaaca aaaacaaaaa tagatataaa
 841 aacatcctgc cctttgatca taccagggtt gtcctacacg atggtgatcc caatgagcct
 901 gtttcagatt acatcaatgc aaatatcatc atgcctgaat ttgaaaccaa gtgcaacaat
 961 tcaaagccca aaaagagtta cattgccaca caaggctgcc tgcaaaacac ggtgaatgac
1021 ttttggcgga tggtgttcca agaaaactcc cgagtgattg tcatgacaac gaaagaagtg
1081 gagagaggaa agagtaaatg tgtcaaatac tggcctgatg agtatgctct aaaagaatat
1141 ggcgtcatgc gtgttaggaa cgtcaaagaa agcgccgctc atgactatac gctaagagaa
1201 cttaaacttt caaaggttgg acaagggaat acggagagaa cggtctggca ataccacttt
1261 cggacctggc cggaccacgg cgtgcccagc gaccctgggg gcgtgctgga cttcctggag
1321 gaggtgcacc ataagcagga gagcatcatg gatgcagggc cggtcgtggt gcactgcagg
1381 tga
```

SEQ ID NO: 32 Human SHP-2 (isoform 2) amino acid sequence

```
   1 mtsrrwfhpn itgveaenll ltrgvdgsfl arpsksnpgd ftlsvrrnga vthikiqntg
  61 dyydlyggek fatlaelvqy ymehhgqlke kngdvielky plncadptse rwfhghlsgk
 121 eaeklltekg khgsflvres qshpgdfvls vrtgddkges ndgkskvthv mircqelkyd
 181 vgggerfdsl tdlvehykkn pmvetlgtvl qlkqplnttr inaaeiesrv relsklaett
 241 dkvkqgfwee fetlqqqeck llysrkegqr qenknknryk nilpfdhtrv vlhdgdpnep
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301 vsdyinanii mpefetkcnn skpkksyiat qgclqntvnd fwrmvfqens rvivmttkev
361 ergkskcvky wpdeyalkey gvmrvrnvke saahdytlre lklskvgqgn tertvwqyhf
421 rtwpdhgvps dpggvldfle evhhkqesim dagpvvvhcr
```

* Included in Table 1 are RNA nucleic acid molecules (*e.g.*, thymines replaced with uredines), nucleic acid molecules encoding orthologs of the encoded proteins, as well as DNA or RNA nucleic acid sequences comprising a nucleic acid sequence having at least 80%, 81%, 82%, 83%, 84%, 85%, 86%, 87%, 88%, 89%, 90%, 91%, 92%, 93%, 94%, 95%, 96%, 97%, 98%, 99%, 99.5%, or more identity across their full length with the nucleic acid sequence of any SEQ ID NO listed in Table 1, or a portion thereof. Such nucleic acid molecules can have a function of the full-length nucleic acid as described further herein, but harbor one or more activating mutations or one or more inhibiting mutations to thereby, for example, activate a Jak kinase or inhibit a Jak kinase inhibitor.

* Included in Table 1 are orthologs of the proteins, as well as polypeptide molecules comprising an amino acid sequence having at least 80%, 81%, 82%, 83%, 84%, 85%, 86%, 87%, 88%, 89%, 90%, 91%, 92%, 93%, 94%, 95%, 96%, 97%, 98%, 99%, 99.5%, or more identity across their full length with an amino acid sequence of any SEQ ID NO listed in Table 1, or a portion thereof. Such polypeptides can have a function of the full-length polypeptide as described further herein, but harbor one or more activating mutations or one or more inhibiting mutations to thereby, for example, activate a Jak kinase or inhibit a Jak kinase inhibitor.

* Included in Table 1 are the well known SOCS family members other than SOCS1 and SOCS3, such as CIS and SOCS2 and SOCS4-7. In addition, any Jak kinase modulator, direct Jak kinase binding protein, cytokine, and cytokine receptor described herein is also included in Table 1. The nucleic acid and polypeptide descriptions provided above in the asterisked sections of Table 1 also apply.

II. Subjects

In one embodiment, the subject for whom predicted likelihood of efficacy of an anti-immune checkpoint inhibitor therapy is determined, is a mammal (*e.g.*, mouse, rat, primate, non-human mammal, domestic animal such as dog, cat, cow, horse), and is preferably a human.

In another embodiment of the methods of the invention, the subject has not undergone treatment, such as chemotherapy, radiation therapy, targeted therapy, and/or anti-immune checkpoint inhibitor therapy. In still another embodiment, the subject has undergone treatment, such as chemotherapy, radiation therapy, targeted therapy, and/or anti-immune checkpoint inhibitor therapy.

In certain embodiments, the subject has had surgery to remove cancerous or precancerous tissue. In other embodiments, the cancerous tissue has not been removed, *e.g.*, the cancerous tissue may be located in an inoperable region of the body, such as in a tissue that is essential for life, or in a region where a surgical procedure would cause considerable risk of harm to the patient.

The methods of the invention can be used to determine the responsiveness to anti-immune checkpoint inhibitor therapies of many different cancers in subjects such as those described above. In one embodiment, the cancers are solid tumors, such as lung cancer or lung cancer subtypes (*e.g.*, squamous cell carcinoma), melanoma, and/or renal cell carcinoma. In another embodiment, the cancer is an epithelial cancer such as, but not limited to, brain cancer (*e.g.*, glioblastomas) bladder cancer, breast cancer, cervical cancer, colon cancer, gynecologic cancers, renal cancer, laryngeal cancer, lung cancer, oral cancer, head and neck cancer, ovarian cancer, pancreatic cancer, prostate cancer, or skin cancer. In still other embodiments, the cancer is breast cancer, prostate cancer, lung cancer, or colon cancer. In still other embodiments, the epithelial cancer is non-small-cell lung cancer, nonpapillary renal cell carcinoma, cervical carcinoma, ovarian carcinoma (*e.g.*, serous ovarian carcinoma), or breast carcinoma. The epithelial cancers may be characterized in various other ways including, but not limited to, serous, endometrioid, mucinous, clear cell, brenner, or undifferentiated.

III. Sample Collection, Preparation and Separation

In some embodiments, biomarker amount and/or activity measurement(s) in a sample from a subject is compared to a predetermined control (standard) sample. The sample from the subject is typically from a diseased tissue, such as cancer cells or tissues. The control sample can be from the same subject or from a different subject. The control sample is typically a normal, non-diseased sample. However, in some embodiments, such as for staging of disease or for evaluating the efficacy of treatment, the control sample can be from a diseased tissue. The control sample can be a combination of samples from

several different subjects. In some embodiments, the biomarker amount and/or activity measurement(s) from a subject is compared to a pre-determined level. This pre-determined level is typically obtained from normal samples. As described herein, a "pre-determined" biomarker amount and/or activity measurement(s) may be a biomarker amount and/or activity measurement(s) used to, by way of example only, evaluate a subject that may be selected for treatment, evaluate a response to an anti-immune checkpoint inhibitor therapy, and/or evaluate a response to a combination anti-immune checkpoint inhibitor therapy. A pre-determined biomarker amount and/or activity measurement(s) may be determined in populations of patients with or without cancer. The pre-determined biomarker amount and/or activity measurement(s) can be a single number, equally applicable to every patient, or the pre-determined biomarker amount and/or activity measurement(s) can vary according to specific subpopulations of patients. Age, weight, height, and other factors of a subject may affect the pre-determined biomarker amount and/or activity measurement(s) of the individual. Furthermore, the pre-determined biomarker amount and/or activity can be determined for each subject individually. In one embodiment, the amounts determined and/or compared in a method described herein are based on absolute measurements. In another embodiment, the amounts determined and/or compared in a method described herein are based on relative measurements, such as ratios (*e.g.*, expression and/or activity of biomarkers to that of wild type biomarkers and expression and/or activity of a biomarker of interest normalized to that of a housekeeping gene).

The pre-determined biomarker amount and/or activity measurement(s) can be any suitable standard. For example, the pre-determined biomarker amount and/or activity measurement(s) can be obtained from the same or a different human for whom a patient selection is being assessed. In one embodiment, the pre-determined biomarker amount and/or activity measurement(s) can be obtained from a previous assessment of the same patient. In such a manner, the progress of the selection of the patient can be monitored over time. In addition, the control can be obtained from an assessment of another human or multiple humans, *e.g.*, selected groups of humans, if the subject is a human. In such a manner, the extent of the selection of the human for whom selection is being assessed can be compared to suitable other humans, *e.g.*, other humans who are in a similar situation to the human of interest, such as those suffering from similar or the same condition(s) and/or of the same ethnic group.

In some embodiments of the present invention the change of biomarker amount and/or activity measurement(s) from the pre-determined level is about 0.5 fold, about 1.0 fold, about 1.5 fold, about 2.0 fold, about 2.5 fold, about 3.0 fold, about 3.5 fold, about 4.0 fold, about 4.5 fold, or about 5.0 fold or greater. In some embodiments, the fold change is less than about 1, less than about 5, less than about 10, less than about 20, less than about 30, less than about 40, or less than about 50. In other embodiments, the fold change in biomarker amount and/or activity measurement(s) compared to a predetermined level is more than about 1, more than about 5, more than about 10, more than about 20, more than about 30, more than about 40, or more than about 50.

Biological samples can be collected from a variety of sources from a patient including a body fluid sample, cell sample, or a tissue sample comprising nucleic acids and/or proteins. "Body fluids" refer to fluids that are excreted or secreted from the body as well as fluids that are normally not (*e.g.*, bronchoalevolar lavage fluid, amniotic fluid, aqueous humor, bile, blood and blood plasma, cerebrospinal fluid, cerumen and earwax, cowper's fluid or pre-ejaculatory fluid, chyle, chyme, stool, female ejaculate, interstitial fluid, intracellular fluid, lymph, menses, breast milk, mucus, pleural fluid, pus, saliva, sebum, semen, serum, sweat, synovial fluid, tears, urine, vaginal lubrication, vitreous humor, vomit). In a preferred embodiment, the subject and/or control sample is selected from the group consisting of cells, cell lines, histological slides, paraffin embedded tissues, biopsies, whole blood, nipple aspirate, serum, plasma, buccal scrape, saliva, cerebrospinal fluid, urine, stool, and bone marrow. In one embodiment, the sample is serum, plasma, or urine. IN another embodiment, the sample is serum.

The samples can be collected from individuals repeatedly over a longitudinal period of time (*e.g.*, once or more on the order of days, weeks, months, annually, biannually, etc.). Obtaining numerous samples from an individual over a period of time can be used to verify results from earlier detections and/or to identify an alteration in biological pattern as a result of, for example, disease progression, drug treatment, *etc.* For example, subject samples can be taken and monitored every month, every two months, or combinations of one, two, or three month intervals according to the invention. In addition, the biomarker amount and/or activity measurements of the subject obtained over time can be conveniently compared with each other, as well as with those of normal controls during the monitoring period, thereby providing the subject's own values, as an internal, or personal, control for long-term monitoring.

Sample preparation and separation can involve any of the procedures, depending on the type of sample collected and/or analysis of biomarker measurement(s). Such procedures include, by way of example only, concentration, dilution, adjustment of pH, removal of high abundance polypeptides (*e.g.*, albumin, gamma globulin, and transferrin, etc.), addition of preservatives and calibrants, addition of protease inhibitors, addition of denaturants, desalting of samples, concentration of sample proteins, extraction and purification of lipids.

The sample preparation can also isolate molecules that are bound in non-covalent complexes to other protein (*e.g.*, carrier proteins). This process may isolate those molecules bound to a specific carrier protein (*e.g.*, albumin), or use a more general process, such as the release of bound molecules from all carrier proteins via protein denaturation, for example using an acid, followed by removal of the carrier proteins.

Removal of undesired proteins (*e.g.*, high abundance, uninformative, or undetectable proteins) from a sample can be achieved using high affinity reagents, high molecular weight filters, ultracentrifugation and/or electrodialysis. High affinity reagents include antibodies or other reagents (*e.g.*, aptamers) that selectively bind to high abundance proteins. Sample preparation could also include ion exchange chromatography, metal ion affinity chromatography, gel filtration, hydrophobic chromatography, chromatofocusing, adsorption chromatography, isoelectric focusing and related techniques. Molecular weight filters include membranes that separate molecules on the basis of size and molecular weight. Such filters may further employ reverse osmosis, nanofiltration, ultrafiltration and microfiltration.

Ultracentrifugation is a method for removing undesired polypeptides from a sample. Ultracentrifugation is the centrifugation of a sample at about 15,000-60,000 rpm while monitoring with an optical system the sedimentation (or lack thereof) of particles. Electrodialysis is a procedure which uses an electromembrane or semipermable membrane in a process in which ions are transported through semi-permeable membranes from one solution to another under the influence of a potential gradient. Since the membranes used in electrodialysis may have the ability to selectively transport ions having positive or negative charge, reject ions of the opposite charge, or to allow species to migrate through a semipermable membrane based on size and charge, it renders electrodialysis useful for concentration, removal, or separation of electrolytes.

Separation and purification in the present invention may include any procedure known in the art, such as capillary electrophoresis (*e.g.*, in capillary or on-chip) or chromatography (*e.g.*, in capillary, column or on a chip). Electrophoresis is a method which can be used to separate ionic molecules under the influence of an electric field. Electrophoresis can be conducted in a gel, capillary, or in a microchannel on a chip. Examples of gels used for electrophoresis include starch, acrylamide, polyethylene oxides, agarose, or combinations thereof. A gel can be modified by its cross-linking, addition of detergents, or denaturants, immobilization of enzymes or antibodies (affinity electrophoresis) or substrates (zymography) and incorporation of a pH gradient. Examples of capillaries used for electrophoresis include capillaries that interface with an electrospray.

Capillary electrophoresis (CE) is preferred for separating complex hydrophilic molecules and highly charged solutes. CE technology can also be implemented on microfluidic chips. Depending on the types of capillary and buffers used, CE can be further segmented into separation techniques such as capillary zone electrophoresis (CZE), capillary isoelectric focusing (CIEF), capillary isotachophoresis (cITP) and capillary electrochromatography (CEC). An embodiment to couple CE techniques to electrospray ionization involves the use of volatile solutions, for example, aqueous mixtures containing a volatile acid and/or base and an organic such as an alcohol or acetonitrile.

Capillary isotachophoresis (cITP) is a technique in which the analytes move through the capillary at a constant speed but are nevertheless separated by their respective mobilities. Capillary zone electrophoresis (CZE), also known as free-solution CE (FSCE), is based on differences in the electrophoretic mobility of the species, determined by the charge on the molecule, and the frictional resistance the molecule encounters during migration which is often directly proportional to the size of the molecule. Capillary isoelectric focusing (CIEF) allows weakly-ionizable amphoteric molecules, to be separated by electrophoresis in a pH gradient. CEC is a hybrid technique between traditional high performance liquid chromatography (HPLC) and CE.

Separation and purification techniques used in the present invention include any chromatography procedures known in the art. Chromatography can be based on the differential adsorption and elution of certain analytes or partitioning of analytes between mobile and stationary phases. Different examples of chromatography include, but not limited to, liquid chromatography (LC), gas chromatography (GC), high performance liquid chromatography (HPLC), etc.

IV. Biomarker Nucleic Acids and Polypeptides

One aspect of the invention pertains to the use of isolated nucleic acid molecules that correspond to biomarker nucleic acids that encode a biomarker polypeptide or a portion of such a polypeptide. As used herein, the term "nucleic acid molecule" is intended to include DNA molecules (*e.g.*, cDNA or genomic DNA) and RNA molecules (*e.g.*, mRNA) and analogs of the DNA or RNA generated using nucleotide analogs. The nucleic acid molecule can be single-stranded or double-stranded, but preferably is double-stranded DNA.

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

A biomarker nucleic acid molecule of the present invention can be isolated using standard molecular biology techniques and the sequence information in the database records described herein. Using all or a portion of such nucleic acid sequences, nucleic acid molecules of the invention can be isolated using standard hybridization and cloning techniques (*e.g.*, as described in Sambrook *et al.*, ed., *Molecular Cloning: A Laboratory Manual, 2nd ed.*, Cold Spring Harbor Laboratory Press, Cold Spring Harbor, NY, 1989).

A nucleic acid molecule of the invention can be amplified using cDNA, mRNA, or genomic DNA as a template and appropriate oligonucleotide primers according to standard PCR amplification techniques. The nucleic acid molecules so amplified can be cloned into an appropriate vector and characterized by DNA sequence analysis. Furthermore, oligonucleotides corresponding to all or a portion of a nucleic acid molecule of the

invention can be prepared by standard synthetic techniques, *e.g.*, using an automated DNA synthesizer.

Moreover, a nucleic acid molecule of the invention can comprise only a portion of a nucleic acid sequence, wherein the full length nucleic acid sequence comprises a marker of the invention or which encodes a polypeptide corresponding to a marker of the invention. Such nucleic acid molecules can be used, for example, as a probe or primer. The probe/primer typically is used as one or more substantially purified oligonucleotides. The oligonucleotide typically comprises a region of nucleotide sequence that hybridizes under stringent conditions to at least about 7, preferably about 15, more preferably about 25, 50, 75, 100, 125, 150, 175, 200, 250, 300, 350, or 400 or more consecutive nucleotides of a biomarker nucleic acid sequence. Probes based on the sequence of a biomarker nucleic acid molecule can be used to detect transcripts or genomic sequences corresponding to one or more markers of the invention. The probe comprises a label group attached thereto, *e.g.*, a radioisotope, a fluorescent compound, an enzyme, or an enzyme co-factor.

A biomarker nucleic acid molecules that differ, due to degeneracy of the genetic code, from the nucleotide sequence of nucleic acid molecules encoding a protein which corresponds to the biomarker, and thus encode the same protein, are also contemplated.

In addition, it will be appreciated by those skilled in the art that DNA sequence polymorphisms that lead to changes in the amino acid sequence can exist within a population (*e.g.*, the human population). Such genetic polymorphisms can exist among individuals within a population due to natural allelic variation. An allele is one of a group of genes which occur alternatively at a given genetic locus. In addition, it will be appreciated that DNA polymorphisms that affect RNA expression levels can also exist that may affect the overall expression level of that gene (*e.g.*, by affecting regulation or degradation).

The term "allele," which is used interchangeably herein with "allelic variant," refers to alternative forms of a gene or portions thereof. Alleles occupy the same locus or position on homologous chromosomes. When a subject has two identical alleles of a gene, the subject is said to be homozygous for the gene or allele. When a subject has two different alleles of a gene, the subject is said to be heterozygous for the gene or allele. For example, biomarker alleles can differ from each other in a single nucleotide, or several nucleotides, and can include substitutions, deletions, and insertions of nucleotides. An allele of a gene can also be a form of a gene containing one or more mutations.

The term "allelic variant of a polymorphic region of gene" or "allelic variant", used interchangeably herein, refers to an alternative form of a gene having one of several possible nucleotide sequences found in that region of the gene in the population. As used herein, allelic variant is meant to encompass functional allelic variants, non-functional allelic variants, SNPs, mutations and polymorphisms.

The term "single nucleotide polymorphism" (SNP) refers to a polymorphic site occupied by a single nucleotide, which is the site of variation between allelic sequences. The site is usually preceded by and followed by highly conserved sequences of the allele (*e.g.*, sequences that vary in less than 1/100 or 1/1000 members of a population). A SNP usually arises due to substitution of one nucleotide for another at the polymorphic site. SNPs can also arise from a deletion of a nucleotide or an insertion of a nucleotide relative to a reference allele. Typically the polymorphic site is occupied by a base other than the reference base. For example, where the reference allele contains the base "T" (thymidine) at the polymorphic site, the altered allele can contain a "C" (cytidine), "G" (guanine), or "A" (adenine) at the polymorphic site. SNP's may occur in protein-coding nucleic acid sequences, in which case they may give rise to a defective or otherwise variant protein, or genetic disease. Such a SNP may alter the coding sequence of the gene and therefore specify another amino acid (a "missense" SNP) or a SNP may introduce a stop codon (a "nonsense" SNP). When a SNP does not alter the amino acid sequence of a protein, the SNP is called "silent." SNP's may also occur in noncoding regions of the nucleotide sequence. This may result in defective protein expression, *e.g.*, as a result of alternative spicing, or it may have no effect on the function of the protein.

As used herein, the terms "gene" and "recombinant gene" refer to nucleic acid molecules comprising an open reading frame encoding a polypeptide corresponding to a marker of the invention. Such natural allelic variations can typically result in 1-5% variance in the nucleotide sequence of a given gene. Alternative alleles can be identified by sequencing the gene of interest in a number of different individuals. This can be readily carried out by using hybridization probes to identify the same genetic locus in a variety of individuals. Any and all such nucleotide variations and resulting amino acid polymorphisms or variations that are the result of natural allelic variation and that do not alter the functional activity are intended to be within the scope of the invention.

In another embodiment, a biomarker nucleic acid molecule is at least 7, 15, 20, 25, 30, 40, 60, 80, 100, 150, 200, 250, 300, 350, 400, 450, 550, 650, 700, 800, 900, 1000, 1100,

1200, 1300, 1400, 1500, 1600, 1700, 1800, 1900, 2000, 2200, 2400, 2600, 2800, 3000, 3500, 4000, 4500, or more nucleotides in length and hybridizes under stringent conditions to a nucleic acid molecule corresponding to a marker of the invention or to a nucleic acid molecule encoding a protein corresponding to a marker of the invention. As used herein, the term "hybridizes under stringent conditions" is intended to describe conditions for hybridization and washing under which nucleotide sequences at least 60% (65%, 70%, 75%, 80%, preferably 85%) identical to each other typically remain hybridized to each other. Such stringent conditions are known to those skilled in the art and can be found in sections 6.3.1-6.3.6 of *Current Protocols in Molecular Biology*, John Wiley & Sons, N.Y. (1989). A preferred, non-limiting example of stringent hybridization conditions are hybridization in 6X sodium chloride/sodium citrate (SSC) at about 45°C, followed by one or more washes in 0.2X SSC, 0.1% SDS at 50-65°C.

In addition to naturally-occurring allelic variants of a nucleic acid molecule of the invention that can exist in the population, the skilled artisan will further appreciate that sequence changes can be introduced by mutation thereby leading to changes in the amino acid sequence of the encoded protein, without altering the biological activity of the protein encoded thereby. For example, one can make nucleotide substitutions leading to amino acid substitutions at "non-essential" amino acid residues. A "non-essential" amino acid residue is a residue that can be altered from the wild-type sequence without altering the biological activity, whereas an "essential" amino acid residue is required for biological activity. For example, amino acid residues that are not conserved or only semi-conserved among homologs of various species may be non-essential for activity and thus would be likely targets for alteration. Alternatively, amino acid residues that are conserved among the homologs of various species (*e.g.*, murine and human) may be essential for activity and thus would not be likely targets for alteration.

Accordingly, another aspect of the invention pertains to nucleic acid molecules encoding a polypeptide of the invention that contain changes in amino acid residues that are not essential for activity. Such polypeptides differ in amino acid sequence from the naturally-occurring proteins which correspond to the markers of the invention, yet retain biological activity. In one embodiment, a biomarker protein has an amino acid sequence that is at least about 40% identical, 50%, 60%, 70%, 75%, 80%, 83%, 85%, 87.5%, 90%, 91%, 92%, 93%, 94%, 95%, 96%, 97%, 98%, 99% or identical to the amino acid sequence of a biomarker protein described herein.

An isolated nucleic acid molecule encoding a variant protein can be created by introducing one or more nucleotide substitutions, additions or deletions into the nucleotide sequence of nucleic acids of the invention, such that one or more amino acid residue substitutions, additions, or deletions are introduced into the encoded protein. Mutations can be introduced by standard techniques, such as site-directed mutagenesis and PCR-mediated mutagenesis. Preferably, conservative amino acid substitutions are made at one or more predicted non-essential amino acid residues. A "conservative amino acid substitution" is one in which the amino acid residue is replaced with an amino acid residue having a similar side chain. Families of amino acid residues having similar side chains have been defined in the art. These families include amino acids with basic side chains (*e.g.*, lysine, arginine, histidine), acidic side chains (*e.g.*, aspartic acid, glutamic acid), uncharged polar side chains (*e.g.*, glycine, asparagine, glutamine, serine, threonine, tyrosine, cysteine), non-polar side chains (*e.g.*, alanine, valine, leucine, isoleucine, proline, phenylalanine, methionine, tryptophan), beta-branched side chains (*e.g.*, threonine, valine, isoleucine) and aromatic side chains (*e.g.*, tyrosine, phenylalanine, tryptophan, histidine). Alternatively, mutations can be introduced randomly along all or part of the coding sequence, such as by saturation mutagenesis, and the resultant mutants can be screened for biological activity to identify mutants that retain activity. Following mutagenesis, the encoded protein can be expressed recombinantly and the activity of the protein can be determined.

In some embodiments, the present invention further contemplates the use of anti-biomarker antisense nucleic acid molecules, *i.e.*, molecules which are complementary to a sense nucleic acid of the invention, *e.g.*, complementary to the coding strand of a double-stranded cDNA molecule corresponding to a marker of the invention or complementary to an mRNA sequence corresponding to a marker of the invention. Accordingly, an antisense nucleic acid molecule of the invention can hydrogen bond to (*i.e.* anneal with) a sense nucleic acid of the invention. The antisense nucleic acid can be complementary to an entire coding strand, or to only a portion thereof, *e.g.*, all or part of the protein coding region (or open reading frame). An antisense nucleic acid molecule can also be antisense to all or part of a non-coding region of the coding strand of a nucleotide sequence encoding a polypeptide of the invention. The non-coding regions ("5' and 3' untranslated regions") are the 5' and 3' sequences which flank the coding region and are not translated into amino acids.

An antisense oligonucleotide can be, for example, about 5, 10, 15, 20, 25, 30, 35, 40, 45, or 50 or more nucleotides in length. An antisense nucleic acid can be constructed using chemical synthesis and enzymatic ligation reactions using procedures known in the art. For example, an antisense nucleic acid (*e.g.*, an antisense oligonucleotide) can be chemically synthesized using naturally occurring nucleotides or variously modified nucleotides designed to increase the biological stability of the molecules or to increase the physical stability of the duplex formed between the antisense and sense nucleic acids, *e.g.*, phosphorothioate derivatives and acridine substituted nucleotides can be used. Examples of modified nucleotides which can be used to generate the antisense nucleic acid include 5-fluorouracil, 5-bromouracil, 5-chlorouracil, 5-iodouracil, hypoxanthine, xanthine, 4-acetylcytosine, 5-(carboxyhydroxylmethyl) uracil, 5-carboxymethylaminomethyl-2-thiouridine, 5-carboxymethylaminomethyluracil, dihydrouracil, beta-D-galactosylqueosine, inosine, N6-isopentenyladenine, 1-methylguanine, 1-methylinosine, 2,2-dimethylguanine, 2- methyladenine, 2-methylguanine, 3-methylcytosine, 5-methylcytosine, N6-adenine, 7-methylguanine, 5-methylaminomethyluracil, 5-methoxyaminomethyl-2-thiouracil, beta-D-mannosylqueosine, 5'-methoxycarboxymethyluracil, 5-methoxyuracil, 2-methylthio-N6-isopentenyladenine, uracil-5-oxyacetic acid (v), wybutoxosine, pseudouracil, queosine, 2-thiocytosine, 5-methyl-2-thiouracil, 2-thiouracil, 4-thiouracil, 5-methyluracil, uracil-5-oxyacetic acid methylester, uracil-5-oxyacetic acid (v), 5-methyl-2-thiouracil, 3-(3-amino-3-N-2-carboxypropyl) uracil, (acp3)w, and 2,6-diaminopurine. Alternatively, the antisense nucleic acid can be produced biologically using an expression vector into which a nucleic acid has been sub-cloned in an antisense orientation (*i.e.*, RNA transcribed from the inserted nucleic acid will be of an antisense orientation to a target nucleic acid of interest, described further in the following subsection).

The antisense nucleic acid molecules of the invention are typically administered to a subject or generated *in situ* such that they hybridize with or bind to cellular mRNA and/or genomic DNA encoding a polypeptide corresponding to a selected marker of the invention to thereby inhibit expression of the marker, *e.g.*, by inhibiting transcription and/or translation. The hybridization can be by conventional nucleotide complementarity to form a stable duplex, or, for example, in the case of an antisense nucleic acid molecule which binds to DNA duplexes, through specific interactions in the major groove of the double helix. Examples of a route of administration of antisense nucleic acid molecules of the invention includes direct injection at a tissue site or infusion of the antisense nucleic acid

into a blood- or bone marrow-associated body fluid. Alternatively, antisense nucleic acid molecules can be modified to target selected cells and then administered systemically. For example, for systemic administration, antisense molecules can be modified such that they specifically bind to receptors or antigens expressed on a selected cell surface, *e.g.*, by linking the antisense nucleic acid molecules to peptides or antibodies which bind to cell surface receptors or antigens. The antisense nucleic acid molecules can also be delivered to cells using the vectors described herein. To achieve sufficient intracellular concentrations of the antisense molecules, vector constructs in which the antisense nucleic acid molecule is placed under the control of a strong pol II or pol III promoter are preferred.

An antisense nucleic acid molecule of the invention can be an α-anomeric nucleic acid molecule. An α-anomeric nucleic acid molecule forms specific double-stranded hybrids with complementary RNA in which, contrary to the usual α-units, the strands run parallel to each other (Gaultier *et al.*, 1987, *Nucleic Acids Res.* 15:6625-6641). The antisense nucleic acid molecule can also comprise a 2'-o-methylribonucleotide (Inoue *et al.*, 1987, *Nucleic Acids Res.* 15:6131-6148) or a chimeric RNA-DNA analogue (Inoue *et al.*, 1987, *FEBS Lett.* 215:327-330).

The present invention also encompasses ribozymes. Ribozymes are catalytic RNA molecules with ribonuclease activity which are capable of cleaving a single-stranded nucleic acid, such as an mRNA, to which they have a complementary region. Thus, ribozymes (*e.g.*, hammerhead ribozymes as described in Haselhoff and Gerlach, 1988, *Nature* 334:585-591) can be used to catalytically cleave mRNA transcripts to thereby inhibit translation of the protein encoded by the mRNA. A ribozyme having specificity for a nucleic acid molecule encoding a polypeptide corresponding to a marker of the invention can be designed based upon the nucleotide sequence of a cDNA corresponding to the marker. For example, a derivative of a *Tetrahymena* L-19 IVS RNA can be constructed in which the nucleotide sequence of the active site is complementary to the nucleotide sequence to be cleaved (see Cech *et al.* U.S. Patent No. 4,987,071; and Cech *et al.* U.S. Patent No. 5,116,742). Alternatively, an mRNA encoding a polypeptide of the invention can be used to select a catalytic RNA having a specific ribonuclease activity from a pool of RNA molecules (see, *e.g.*, Bartel and Szostak, 1993, *Science* 261:1411-1418).

The present invention also encompasses nucleic acid molecules which form triple helical structures. For example, expression of a biomarker protein can be inhibited by targeting nucleotide sequences complementary to the regulatory region of the gene

encoding the polypeptide (*e.g.*, the promoter and/or enhancer) to form triple helical structures that prevent transcription of the gene in target cells. See generally Helene (1991) *Anticancer Drug Des.* 6(6):569-84; Helene (1992) *Ann. N.Y. Acad. Sci.* 660:27-36; and Maher (1992) *Bioassays* 14(12):807-15.

In various embodiments, the nucleic acid molecules of the present invention can be modified at the base moiety, sugar moiety or phosphate backbone to improve, *e.g.*, the stability, hybridization, or solubility of the molecule. For example, the deoxyribose phosphate backbone of the nucleic acid molecules can be modified to generate peptide nucleic acid molecules (see Hyrup *et al.*, 1996, *Bioorganic & Medicinal Chemistry* 4(1): 5-23). As used herein, the terms "peptide nucleic acids" or "PNAs" refer to nucleic acid mimics, *e.g.*, DNA mimics, in which the deoxyribose phosphate backbone is replaced by a pseudopeptide backbone and only the four natural nucleobases are retained. The neutral backbone of PNAs has been shown to allow for specific hybridization to DNA and RNA under conditions of low ionic strength. The synthesis of PNA oligomers can be performed using standard solid phase peptide synthesis protocols as described in Hyrup *et al.* (1996), *supra*; Perry-O'Keefe *et al.* (1996) *Proc. Natl. Acad. Sci. USA* 93:14670-675.

PNAs can be used in therapeutic and diagnostic applications. For example, PNAs can be used as antisense or antigene agents for sequence-specific modulation of gene expression by, *e.g.*, inducing transcription or translation arrest or inhibiting replication. PNAs can also be used, *e.g.*, in the analysis of single base pair mutations in a gene by, *e.g.*, PNA directed PCR clamping; as artificial restriction enzymes when used in combination with other enzymes, *e.g.*, S1 nucleases (Hyrup (1996), *supra*; or as probes or primers for DNA sequence and hybridization (Hyrup, 1996, *supra*; Perry-O'Keefe *et al.*, 1996, *Proc. Natl. Acad. Sci. USA* 93:14670-675).

In another embodiment, PNAs can be modified, *e.g.*, to enhance their stability or cellular uptake, by attaching lipophilic or other helper groups to PNA, by the formation of PNA-DNA chimeras, or by the use of liposomes or other techniques of drug delivery known in the art. For example, PNA-DNA chimeras can be generated which can combine the advantageous properties of PNA and DNA. Such chimeras allow DNA recognition enzymes, *e.g.*, RNASE H and DNA polymerases, to interact with the DNA portion while the PNA portion would provide high binding affinity and specificity. PNA-DNA chimeras can be linked using linkers of appropriate lengths selected in terms of base stacking, number of bonds between the nucleobases, and orientation (Hyrup, 1996, *supra*). The

synthesis of PNA-DNA chimeras can be performed as described in Hyrup (1996), *supra*, and Finn *et al.* (1996) *Nucleic Acids Res.* 24(17):3357-63. For example, a DNA chain can be synthesized on a solid support using standard phosphoramidite coupling chemistry and modified nucleoside analogs. Compounds such as 5'-(4-methoxytrityl)amino-5'-deoxy-thymidine phosphoramidite can be used as a link between the PNA and the 5' end of DNA (Mag *et al.*, 1989, *Nucleic Acids Res.* 17:5973-88). PNA monomers are then coupled in a step-wise manner to produce a chimeric molecule with a 5' PNA segment and a 3' DNA segment (Finn *et al.*, 1996, *Nucleic Acids Res.* 24(17):3357-63). Alternatively, chimeric molecules can be synthesized with a 5' DNA segment and a 3' PNA segment (Peterser *et al.*, 1975, *Bioorganic Med. Chem. Lett.* 5:1119-11124).

In other embodiments, the oligonucleotide can include other appended groups such as peptides (*e.g.*, for targeting host cell receptors *in vivo*), or agents facilitating transport across the cell membrane (see, *e.g.*, Letsinger *et al.*, 1989, *Proc. Natl. Acad. Sci. USA* 86:6553-6556; Lemaitre *et al.*, 1987, *Proc. Natl. Acad. Sci. USA* 84:648-652; PCT Publication No. WO 88/09810) or the blood-brain barrier (see, *e.g.*, PCT Publication No. WO 89/10134). In addition, oligonucleotides can be modified with hybridization-triggered cleavage agents (see, *e.g.*, Krol *et al.*, 1988, *Bio/Techniques* 6:958-976) or intercalating agents (see, *e.g.*, Zon, 1988, *Pharm. Res.* 5:539-549). To this end, the oligonucleotide can be conjugated to another molecule, *e.g.*, a peptide, hybridization triggered cross-linking agent, transport agent, hybridization-triggered cleavage agent, *etc.*

Another aspect of the invention pertains to the use of biomarker proteins and biologically active portions thereof. In one embodiment, the native polypeptide corresponding to a marker can be isolated from cells or tissue sources by an appropriate purification scheme using standard protein purification techniques. In another embodiment, polypeptides corresponding to a marker of the invention are produced by recombinant DNA techniques. Alternative to recombinant expression, a polypeptide corresponding to a marker of the invention can be synthesized chemically using standard peptide synthesis techniques.

An "isolated" or "purified" protein or biologically active portion thereof is substantially free of cellular material or other contaminating proteins from the cell or tissue source from which the protein is derived, or substantially free of chemical precursors or other chemicals when chemically synthesized. The language "substantially free of cellular material" includes preparations of protein in which the protein is separated from cellular

components of the cells from which it is isolated or recombinantly produced. Thus, protein that is substantially free of cellular material includes preparations of protein having less than about 30%, 20%, 10%, or 5% (by dry weight) of heterologous protein (also referred to herein as a "contaminating protein"). When the protein or biologically active portion thereof is recombinantly produced, it is also preferably substantially free of culture medium, *i.e.*, culture medium represents less than about 20%, 10%, or 5% of the volume of the protein preparation. When the protein is produced by chemical synthesis, it is preferably substantially free of chemical precursors or other chemicals, *i.e.*, it is separated from chemical precursors or other chemicals which are involved in the synthesis of the protein. Accordingly such preparations of the protein have less than about 30%, 20%, 10%, 5% (by dry weight) of chemical precursors or compounds other than the polypeptide of interest.

Biologically active portions of a biomarker polypeptide include polypeptides comprising amino acid sequences sufficiently identical to or derived from a biomarker protein amino acid sequence described herein, but which includes fewer amino acids than the full length protein, and exhibit at least one activity of the corresponding full-length protein. Typically, biologically active portions comprise a domain or motif with at least one activity of the corresponding protein. A biologically active portion of a protein of the invention can be a polypeptide which is, for example, 10, 25, 50, 100 or more amino acids in length. Moreover, other biologically active portions, in which other regions of the protein are deleted, can be prepared by recombinant techniques and evaluated for one or more of the functional activities of the native form of a polypeptide of the invention.

Preferred polypeptides have an amino acid sequence of a biomarker protein encoded by a nucleic acid molecule described herein. Other useful proteins are substantially identical (*e.g.*, at least about 40%, preferably 50%, 60%, 70%, 75%, 80%, 83%, 85%, 88%, 90%, 91%, 92%, 93%, 94%, 95%, 96%, 97%, 98%, or 99%) to one of these sequences and retain the functional activity of the protein of the corresponding naturally-occurring protein yet differ in amino acid sequence due to natural allelic variation or mutagenesis.

To determine the percent identity of two amino acid sequences or of two nucleic acids, the sequences are aligned for optimal comparison purposes (*e.g.*, gaps can be introduced in the sequence of a first amino acid or nucleic acid sequence for optimal alignment with a second amino or nucleic acid sequence). The amino acid residues or nucleotides at corresponding amino acid positions or nucleotide positions are then

compared. When a position in the first sequence is occupied by the same amino acid residue or nucleotide as the corresponding position in the second sequence, then the molecules are identical at that position. The percent identity between the two sequences is a function of the number of identical positions shared by the sequences (*i.e.*, % identity = # of identical positions/total # of positions (*e.g.*, overlapping positions) x100). In one embodiment the two sequences are the same length.

The determination of percent identity between two sequences can be accomplished using a mathematical algorithm. A preferred, non-limiting example of a mathematical algorithm utilized for the comparison of two sequences is the algorithm of Karlin and Altschul (1990) *Proc. Natl. Acad. Sci. USA* 87:2264-2268, modified as in Karlin and Altschul (1993) *Proc. Natl. Acad. Sci. USA* 90:5873-5877. Such an algorithm is incorporated into the NBLAST and XBLAST programs of Altschul, *et al.* (1990) *J. Mol. Biol.* 215:403-410. BLAST nucleotide searches can be performed with the NBLAST program, score = 100, wordlength = 12 to obtain nucleotide sequences homologous to a nucleic acid molecules of the invention. BLAST protein searches can be performed with the XBLAST program, score = 50, wordlength = 3 to obtain amino acid sequences homologous to a protein molecules of the invention. To obtain gapped alignments for comparison purposes, Gapped BLAST can be utilized as described in Altschul *et al.* (1997) *Nucleic Acids Res.* 25:3389-3402. Alternatively, PSI-Blast can be used to perform an iterated search which detects distant relationships between molecules. When utilizing BLAST, Gapped BLAST, and PSI-Blast programs, the default parameters of the respective programs (*e.g.*, XBLAST and NBLAST) can be used. See http://www.ncbi.nlm.nih.gov. Another preferred, non-limiting example of a mathematical algorithm utilized for the comparison of sequences is the algorithm of Myers and Miller, (1988) *Comput Appl Biosci,* 4:11-7. Such an algorithm is incorporated into the ALIGN program (version 2.0) which is part of the GCG sequence alignment software package. When utilizing the ALIGN program for comparing amino acid sequences, a PAM120 weight residue table, a gap length penalty of 12, and a gap penalty of 4 can be used. Yet another useful algorithm for identifying regions of local sequence similarity and alignment is the FASTA algorithm as described in Pearson and Lipman (1988) *Proc. Natl. Acad. Sci. USA* 85:2444-2448. When using the FASTA algorithm for comparing nucleotide or amino acid sequences, a PAM120 weight residue table can, for example, be used with a *k*-tuple value of 2.

The percent identity between two sequences can be determined using techniques similar to those described above, with or without allowing gaps. In calculating percent identity, only exact matches are counted.

The invention also provides chimeric or fusion proteins corresponding to a biomarker protein. As used herein, a "chimeric protein" or "fusion protein" comprises all or part (preferably a biologically active part) of a polypeptide corresponding to a marker of the invention operably linked to a heterologous polypeptide (*i.e.*, a polypeptide other than the polypeptide corresponding to the marker). Within the fusion protein, the term "operably linked" is intended to indicate that the polypeptide of the invention and the heterologous polypeptide are fused in-frame to each other. The heterologous polypeptide can be fused to the amino-terminus or the carboxyl-terminus of the polypeptide of the invention.

One useful fusion protein is a GST fusion protein in which a polypeptide corresponding to a marker of the invention is fused to the carboxyl terminus of GST sequences. Such fusion proteins can facilitate the purification of a recombinant polypeptide of the invention.

In another embodiment, the fusion protein contains a heterologous signal sequence, immunoglobulin fusion protein, toxin, or other useful protein sequence. Chimeric and fusion proteins of the invention can be produced by standard recombinant DNA techniques. In another embodiment, the fusion gene can be synthesized by conventional techniques including automated DNA synthesizers. Alternatively, PCR amplification of gene fragments can be carried out using anchor primers which give rise to complementary overhangs between two consecutive gene fragments which can subsequently be annealed and re-amplified to generate a chimeric gene sequence (see, *e.g.*, Ausubel *et al., supra*). Moreover, many expression vectors are commercially available that already encode a fusion moiety (*e.g.*, a GST polypeptide). A nucleic acid encoding a polypeptide of the invention can be cloned into such an expression vector such that the fusion moiety is linked in-frame to the polypeptide of the invention.

A signal sequence can be used to facilitate secretion and isolation of the secreted protein or other proteins of interest. Signal sequences are typically characterized by a core of hydrophobic amino acids which are generally cleaved from the mature protein during secretion in one or more cleavage events. Such signal peptides contain processing sites that allow cleavage of the signal sequence from the mature proteins as they pass through the

secretory pathway. Thus, the invention pertains to the described polypeptides having a signal sequence, as well as to polypeptides from which the signal sequence has been proteolytically cleaved (*i.e.*, the cleavage products). In one embodiment, a nucleic acid sequence encoding a signal sequence can be operably linked in an expression vector to a protein of interest, such as a protein which is ordinarily not secreted or is otherwise difficult to isolate. The signal sequence directs secretion of the protein, such as from a eukaryotic host into which the expression vector is transformed, and the signal sequence is subsequently or concurrently cleaved. The protein can then be readily purified from the extracellular medium by art recognized methods. Alternatively, the signal sequence can be linked to the protein of interest using a sequence which facilitates purification, such as with a GST domain.

The present invention also pertains to variants of the biomarker polypeptides described herein. Such variants have an altered amino acid sequence which can function as either agonists (mimetics) or as antagonists. Variants can be generated by mutagenesis, *e.g.*, discrete point mutation or truncation. An agonist can retain substantially the same, or a subset, of the biological activities of the naturally occurring form of the protein. An antagonist of a protein can inhibit one or more of the activities of the naturally occurring form of the protein by, for example, competitively binding to a downstream or upstream member of a cellular signaling cascade which includes the protein of interest. Thus, specific biological effects can be elicited by treatment with a variant of limited function. Treatment of a subject with a variant having a subset of the biological activities of the naturally occurring form of the protein can have fewer side effects in a subject relative to treatment with the naturally occurring form of the protein.

Variants of a biomarker protein which function as either agonists (mimetics) or as antagonists can be identified by screening combinatorial libraries of mutants, *e.g.*, truncation mutants, of the protein of the invention for agonist or antagonist activity. In one embodiment, a variegated library of variants is generated by combinatorial mutagenesis at the nucleic acid level and is encoded by a variegated gene library. A variegated library of variants can be produced by, for example, enzymatically ligating a mixture of synthetic oligonucleotides into gene sequences such that a degenerate set of potential protein sequences is expressible as individual polypeptides, or alternatively, as a set of larger fusion proteins (*e.g.*, for phage display). There are a variety of methods which can be used to produce libraries of potential variants of the polypeptides of the invention from a

degenerate oligonucleotide sequence. Methods for synthesizing degenerate oligonucleotides are known in the art (see, *e.g.*, Narang, 1983, *Tetrahedron* 39:3; Itakura *et al.*, 1984, *Annu. Rev. Biochem.* 53:323; Itakura *et al.*, 1984, *Science* 198:1056; Ike *et al.*, 1983 *Nucleic Acid Res.* 11:477).

In addition, libraries of fragments of the coding sequence of a polypeptide corresponding to a marker of the invention can be used to generate a variegated population of polypeptides for screening and subsequent selection of variants. For example, a library of coding sequence fragments can be generated by treating a double stranded PCR fragment of the coding sequence of interest with a nuclease under conditions wherein nicking occurs only about once per molecule, denaturing the double stranded DNA, renaturing the DNA to form double stranded DNA which can include sense/antisense pairs from different nicked products, removing single stranded portions from reformed duplexes by treatment with S1 nuclease, and ligating the resulting fragment library into an expression vector. By this method, an expression library can be derived which encodes amino terminal and internal fragments of various sizes of the protein of interest.

Several techniques are known in the art for screening gene products of combinatorial libraries made by point mutations or truncation, and for screening cDNA libraries for gene products having a selected property. The most widely used techniques, which are amenable to high throughput analysis, for screening large gene libraries typically include cloning the gene library into replicable expression vectors, transforming appropriate cells with the resulting library of vectors, and expressing the combinatorial genes under conditions in which detection of a desired activity facilitates isolation of the vector encoding the gene whose product was detected. Recursive ensemble mutagenesis (REM), a technique which enhances the frequency of functional mutants in the libraries, can be used in combination with the screening assays to identify variants of a protein of the invention (Arkin and Yourvan, 1992, *Proc. Natl. Acad. Sci. USA* 89:7811-7815; Delgrave *et al.*, 1993, *Protein Engineering* 6(3):327- 331).

The production and use of biomarker nucleic acid and/or biomarker polypeptide molecules described herein can be facilitated by using standard recombinant techniques. In some embodiments, such techniques use vectors, preferably expression vectors, containing a nucleic acid encoding a biomarker polypeptide or a portion of such a polypeptide. As used herein, the term "vector" refers to a nucleic acid molecule capable of transporting another nucleic acid to which it has been linked. One type of vector is a "plasmid", which

refers to a circular double stranded DNA loop into which additional DNA segments can be ligated. Another type of vector is a viral vector, wherein additional DNA segments can be ligated into the viral genome. Certain vectors are capable of autonomous replication in a host cell into which they are introduced (*e.g.*, bacterial vectors having a bacterial origin of replication and episomal mammalian vectors). Other vectors (*e.g.*, non-episomal mammalian vectors) are integrated into the genome of a host cell upon introduction into the host cell, and thereby are replicated along with the host genome. Moreover, certain vectors, namely expression vectors, are capable of directing the expression of genes to which they are operably linked. In general, expression vectors of utility in recombinant DNA techniques are often in the form of plasmids (vectors). However, the present invention is intended to include such other forms of expression vectors, such as viral vectors (*e.g.*, replication defective retroviruses, adenoviruses and adeno-associated viruses), which serve equivalent functions.

The recombinant expression vectors of the invention comprise a nucleic acid of the invention in a form suitable for expression of the nucleic acid in a host cell. This means that the recombinant expression vectors include one or more regulatory sequences, selected on the basis of the host cells to be used for expression, which is operably linked to the nucleic acid sequence to be expressed. Within a recombinant expression vector, "operably linked" is intended to mean that the nucleotide sequence of interest is linked to the regulatory sequence(s) in a manner which allows for expression of the nucleotide sequence (*e.g.*, in an *in vitro* transcription/translation system or in a host cell when the vector is introduced into the host cell). The term "regulatory sequence" is intended to include promoters, enhancers and other expression control elements (*e.g.*, polyadenylation signals). Such regulatory sequences are described, for example, in Goeddel, *Methods in Enzymology: Gene Expression Technology* vol.185, Academic Press, San Diego, CA (1991). Regulatory sequences include those which direct constitutive expression of a nucleotide sequence in many types of host cell and those which direct expression of the nucleotide sequence only in certain host cells (*e.g.*, tissue-specific regulatory sequences). It will be appreciated by those skilled in the art that the design of the expression vector can depend on such factors as the choice of the host cell to be transformed, the level of expression of protein desired, and the like. The expression vectors of the invention can be introduced into host cells to thereby produce proteins or peptides, including fusion proteins or peptides, encoded by nucleic acids as described herein.

The recombinant expression vectors for use in the invention can be designed for expression of a polypeptide corresponding to a marker of the invention in prokaryotic (*e.g.*, *E. coli*) or eukaryotic cells (*e.g.*, insect cells {using baculovirus expression vectors}, yeast cells or mammalian cells). Suitable host cells are discussed further in Goeddel, *supra*. Alternatively, the recombinant expression vector can be transcribed and translated *in vitro*, for example using T7 promoter regulatory sequences and T7 polymerase.

Expression of proteins in prokaryotes is most often carried out in *E. coli* with vectors containing constitutive or inducible promoters directing the expression of either fusion or non-fusion proteins. Fusion vectors add a number of amino acids to a protein encoded therein, usually to the amino terminus of the recombinant protein. Such fusion vectors typically serve three purposes: 1) to increase expression of recombinant protein; 2) to increase the solubility of the recombinant protein; and 3) to aid in the purification of the recombinant protein by acting as a ligand in affinity purification. Often, in fusion expression vectors, a proteolytic cleavage site is introduced at the junction of the fusion moiety and the recombinant protein to enable separation of the recombinant protein from the fusion moiety subsequent to purification of the fusion protein. Such enzymes, and their cognate recognition sequences, include Factor Xa, thrombin and enterokinase. Typical fusion expression vectors include pGEX (Pharmacia Biotech Inc; Smith and Johnson, 1988, *Gene* 67:31-40), pMAL (New England Biolabs, Beverly, MA) and pRIT5 (Pharmacia, Piscataway, NJ) which fuse glutathione S-transferase (GST), maltose E binding protein, or protein A, respectively, to the target recombinant protein.

Examples of suitable inducible non-fusion *E. coli* expression vectors include pTrc (Amann *et al.*, 1988, *Gene* 69:301-315) and pET 11d (Studier *et al.*, p. 60-89, In *Gene Expression Technology: Methods in Enzymology* vol.185, Academic Press, San Diego, CA, 1991). Target biomarker nucleic acid expression from the pTrc vector relies on host RNA polymerase transcription from a hybrid trp-lac fusion promoter. Target biomarker nucleic acid expression from the pET 11d vector relies on transcription from a T7 gn10-lac fusion promoter mediated by a co-expressed viral RNA polymerase (T7 gn1). This viral polymerase is supplied by host strains BL21 (DE3) or HMS174(DE3) from a resident prophage harboring a T7 gn1 gene under the transcriptional control of the lacUV 5 promoter.

One strategy to maximize recombinant protein expression in *E. coli* is to express the protein in a host bacterium with an impaired capacity to proteolytically cleave the

recombinant protein (Gottesman, p. 119-128, In *Gene Expression Technology: Methods in Enzymology* vol. 185, Academic Press, San Diego, CA, 1990. Another strategy is to alter the nucleic acid sequence of the nucleic acid to be inserted into an expression vector so that the individual codons for each amino acid are those preferentially utilized in *E. coli* (Wada *et al.*, 1992, *Nucleic Acids Res.* 20:2111-2118). Such alteration of nucleic acid sequences of the invention can be carried out by standard DNA synthesis techniques.

In another embodiment, the expression vector is a yeast expression vector. Examples of vectors for expression in yeast *S. cerevisiae* include pYepSec1 (Baldari *et al.*, 1987, *EMBO J.* 6:229-234), pMFa (Kurjan and Herskowitz, 1982, *Cell* 30:933-943), pJRY88 (Schultz *et al.*, 1987, *Gene* 54:113-123), pYES2 (Invitrogen Corporation, San Diego, CA), and pPicZ (Invitrogen Corp, San Diego, CA).

Alternatively, the expression vector is a baculovirus expression vector. Baculovirus vectors available for expression of proteins in cultured insect cells (*e.g.*, Sf 9 cells) include the pAc series (Smith *et al.*, 1983, *Mol. Cell Biol.* 3:2156-2165) and the pVL series (Lucklow and Summers, 1989, *Virology* 170:31-39).

In yet another embodiment, a nucleic acid of the invention is expressed in mammalian cells using a mammalian expression vector. Examples of mammalian expression vectors include pCDM8 (Seed, 1987, *Nature* 329:840) and pMT2PC (Kaufman *et al.*, 1987, *EMBO J.* 6:187-195). When used in mammalian cells, the expression vector's control functions are often provided by viral regulatory elements. For example, commonly used promoters are derived from polyoma, Adenovirus 2, cytomegalovirus and Simian Virus 40. For other suitable expression systems for both prokaryotic and eukaryotic cells see chapters 16 and 17 of Sambrook *et al.*, *supra.*

In another embodiment, the recombinant mammalian expression vector is capable of directing expression of the nucleic acid preferentially in a particular cell type (*e.g.*, tissue-specific regulatory elements are used to express the nucleic acid). Tissue-specific regulatory elements are known in the art. Non-limiting examples of suitable tissue-specific promoters include the albumin promoter (liver-specific; Pinkert *et al.*, 1987, *Genes Dev.* 1:268-277), lymphoid-specific promoters (Calame and Eaton, 1988, *Adv. Immunol.* 43:235-275), in particular promoters of T cell receptors (Winoto and Baltimore, 1989, *EMBO J.* 8:729-733) and immunoglobulins (Banerji *et al.*, 1983, *Cell* 33:729-740; Queen and Baltimore, 1983, *Cell* 33:741-748), neuron-specific promoters (*e.g.*, the neurofilament promoter; Byrne and Ruddle, 1989, *Proc. Natl. Acad. Sci. USA* 86:5473-5477), pancreas-

specific promoters (Edlund *et al.*, 1985, *Science* 230:912-916), and mammary gland-specific promoters (*e.g.*, milk whey promoter; U.S. Patent No. 4,873,316 and European Application Publication No. 264,166). Developmentally-regulated promoters are also encompassed, for example the murine hox promoters (Kessel and Gruss, 1990, *Science* 249:374-379) and the α-fetoprotein promoter (Camper and Tilghman, 1989, *Genes Dev.* 3:537-546).

The invention further provides a recombinant expression vector comprising a DNA molecule cloned into the expression vector in an antisense orientation. That is, the DNA molecule is operably linked to a regulatory sequence in a manner which allows for expression (by transcription of the DNA molecule) of an RNA molecule which is antisense to the mRNA encoding a polypeptide of the invention. Regulatory sequences operably linked to a nucleic acid cloned in the antisense orientation can be chosen which direct the continuous expression of the antisense RNA molecule in a variety of cell types, for instance viral promoters and/or enhancers, or regulatory sequences can be chosen which direct constitutive, tissue-specific or cell type specific expression of antisense RNA. The antisense expression vector can be in the form of a recombinant plasmid, phagemid, or attenuated virus in which antisense nucleic acids are produced under the control of a high efficiency regulatory region, the activity of which can be determined by the cell type into which the vector is introduced. For a discussion of the regulation of gene expression using antisense genes (see Weintraub *et al.*, 1986, *Trends in Genetics*, Vol. 1(1)).

Another aspect of the invention pertains to host cells into which a recombinant expression vector of the invention has been introduced. The terms "host cell" and "recombinant host cell" are used interchangeably herein. It is understood that such terms refer not only to the particular subject cell but to the progeny or potential progeny of such a cell. Because certain modifications may occur in succeeding generations due to either mutation or environmental influences, such progeny may not, in fact, be identical to the parent cell, but are still included within the scope of the term as used herein.

A host cell can be any prokaryotic (*e.g.*, *E. coli*) or eukaryotic cell (*e.g.*, insect cells, yeast or mammalian cells).

Vector DNA can be introduced into prokaryotic or eukaryotic cells via conventional transformation or transfection techniques. As used herein, the terms "transformation" and "transfection" are intended to refer to a variety of art-recognized techniques for introducing foreign nucleic acid into a host cell, including calcium phosphate or calcium chloride co-

precipitation, DEAE-dextran-mediated transfection, lipofection, or electroporation. Suitable methods for transforming or transfecting host cells can be found in Sambrook, *et al.* (*supra*), and other laboratory manuals.

For stable transfection of mammalian cells, it is known that, depending upon the expression vector and transfection technique used, only a small fraction of cells may integrate the foreign DNA into their genome. In order to identify and select these integrants, a gene that encodes a selectable marker (*e.g.*, for resistance to antibiotics) is generally introduced into the host cells along with the gene of interest. Preferred selectable markers include those which confer resistance to drugs, such as G418, hygromycin and methotrexate. Cells stably transfected with the introduced nucleic acid can be identified by drug selection (*e.g.*, cells that have incorporated the selectable marker gene will survive, while the other cells die).

V. Analyzing Biomarker Nucleic Acids and Polypeptides

Biomarker nucleic acids and/or biomarker polypeptides can be analyzed according to the methods described herein and techniques known to the skilled artisan to identify such genetic or expression alterations useful for the present invention including, but not limited to, 1) an alteration in the level of a biomarker transcript or polypeptide, 2) a deletion or addition of one or more nucleotides from a biomarker gene, 4) a substitution of one or more nucleotides of a biomarker gene, 5) aberrant modification of a biomarker gene, such as an expression regulatory region, and the like.

a. Methods for Detection of Copy Number

Methods of evaluating the copy number of a biomarker nucleic acid are well known to those of skill in the art. The presence or absence of chromosomal gain or loss can be evaluated simply by a determination of copy number of the regions or markers identified herein.

In one embodiment, a biological sample is tested for the presence of copy number changes in genomic loci containing the genomic marker. A copy number of at least 3, 4, 5, 6, 7, 8, 9, or 10 is predictive of poorer outcome of anti-immune checkpoint inhibitor treatment.

Methods of evaluating the copy number of a biomarker locus include, but are not limited to, hybridization-based assays. Hybridization-based assays include, but are not limited to, traditional "direct probe" methods, such as Southern blots, *in situ* hybridization

(*e.g.*, FISH and FISH plus SKY) methods, and "comparative probe" methods, such as comparative genomic hybridization (CGH), *e.g.*, cDNA-based or oligonucleotide-based CGH. The methods can be used in a wide variety of formats including, but not limited to, substrate (*e.g.* membrane or glass) bound methods or array-based approaches.

In one embodiment, evaluating the biomarker gene copy number in a sample involves a Southern Blot. In a Southern Blot, the genomic DNA (typically fragmented and separated on an electrophoretic gel) is hybridized to a probe specific for the target region. Comparison of the intensity of the hybridization signal from the probe for the target region with control probe signal from analysis of normal genomic DNA (*e.g.*, a non-amplified portion of the same or related cell, tissue, organ, *etc.*) provides an estimate of the relative copy number of the target nucleic acid. Alternatively, a Northern blot may be utilized for evaluating the copy number of encoding nucleic acid in a sample. In a Northern blot, mRNA is hybridized to a probe specific for the target region. Comparison of the intensity of the hybridization signal from the probe for the target region with control probe signal from analysis of normal RNA (*e.g.*, a non-amplified portion of the same or related cell, tissue, organ, *etc.*) provides an estimate of the relative copy number of the target nucleic acid. Alternatively, other methods well known in the art to detect RNA can be used, such that higher or lower expression relative to an appropriate control (*e.g.*, a non-amplified portion of the same or related cell tissue, organ, etc.) provides an estimate of the relative copy number of the target nucleic acid.

An alternative means for determining genomic copy number is *in situ* hybridization (*e.g.*, Angerer (1987) *Meth. Enzymol* 152: 649). Generally, *in situ* hybridization comprises the following steps: (1) fixation of tissue or biological structure to be analyzed; (2) prehybridization treatment of the biological structure to increase accessibility of target DNA, and to reduce nonspecific binding; (3) hybridization of the mixture of nucleic acids to the nucleic acid in the biological structure or tissue; (4) post-hybridization washes to remove nucleic acid fragments not bound in the hybridization and (5) detection of the hybridized nucleic acid fragments. The reagent used in each of these steps and the conditions for use vary depending on the particular application. In a typical *in situ* hybridization assay, cells are fixed to a solid support, typically a glass slide. If a nucleic acid is to be probed, the cells are typically denatured with heat or alkali. The cells are then contacted with a hybridization solution at a moderate temperature to permit annealing of labeled probes specific to the nucleic acid sequence encoding the protein. The targets (*e.g.*,

cells) are then typically washed at a predetermined stringency or at an increasing stringency until an appropriate signal to noise ratio is obtained. The probes are typically labeled, *e.g.*, with radioisotopes or fluorescent reporters. In one embodiment, probes are sufficiently long so as to specifically hybridize with the target nucleic acid(s) under stringent conditions. Probes generally range in length from about 200 bases to about 1000 bases. In some applications it is necessary to block the hybridization capacity of repetitive sequences. Thus, in some embodiments, tRNA, human genomic DNA, or Cot-I DNA is used to block non-specific hybridization.

An alternative means for determining genomic copy number is comparative genomic hybridization. In general, genomic DNA is isolated from normal reference cells, as well as from test cells (*e.g.*, tumor cells) and amplified, if necessary. The two nucleic acids are differentially labeled and then hybridized *in situ* to metaphase chromosomes of a reference cell. The repetitive sequences in both the reference and test DNAs are either removed or their hybridization capacity is reduced by some means, for example by prehybridization with appropriate blocking nucleic acids and/or including such blocking nucleic acid sequences for said repetitive sequences during said hybridization. The bound, labeled DNA sequences are then rendered in a visualizable form, if necessary. Chromosomal regions in the test cells which are at increased or decreased copy number can be identified by detecting regions where the ratio of signal from the two DNAs is altered. For example, those regions that have decreased in copy number in the test cells will show relatively lower signal from the test DNA than the reference compared to other regions of the genome. Regions that have been increased in copy number in the test cells will show relatively higher signal from the test DNA. Where there are chromosomal deletions or multiplications, differences in the ratio of the signals from the two labels will be detected and the ratio will provide a measure of the copy number. In another embodiment of CGH, array CGH (aCGH), the immobilized chromosome element is replaced with a collection of solid support bound target nucleic acids on an array, allowing for a large or complete percentage of the genome to be represented in the collection of solid support bound targets. Target nucleic acids may comprise cDNAs, genomic DNAs, oligonucleotides (*e.g.*, to detect single nucleotide polymorphisms) and the like. Array-based CGH may also be performed with single-color labeling (as opposed to labeling the control and the possible tumor sample with two different dyes and mixing them prior to hybridization, which will yield a ratio due to competitive hybridization of probes on the arrays). In single color

CGH, the control is labeled and hybridized to one array and absolute signals are read, and the possible tumor sample is labeled and hybridized to a second array (with identical content) and absolute signals are read. Copy number difference is calculated based on absolute signals from the two arrays. Methods of preparing immobilized chromosomes or arrays and performing comparative genomic hybridization are well known in the art (see, *e.g.*, U.S. Pat. Nos: 6,335,167; 6,197,501; 5,830,645; and 5,665,549 and Albertson (1984) *EMBO J.* 3: 1227-1234; Pinkel (1988) *Proc. Natl. Acad. Sci. USA* 85: 9138-9142; EPO Pub. No. 430,402; *Methods in Molecular Biology*, Vol. 33: *In situ* Hybridization Protocols, Choo, ed., Humana Press, Totowa, N.J. (1994), *etc.*) In another embodiment, the hybridization protocol of Pinkel, *et al.* (1998) *Nature Genetics* 20: 207-211, or of Kallioniemi (1992) *Proc. Natl Acad Sci USA* 89:5321-5325 (1992) is used.

In still another embodiment, amplification-based assays can be used to measure copy number. In such amplification-based assays, the nucleic acid sequences act as a template in an amplification reaction (*e.g.*, Polymerase Chain Reaction (PCR). In a quantitative amplification, the amount of amplification product will be proportional to the amount of template in the original sample. Comparison to appropriate controls, *e.g.* healthy tissue, provides a measure of the copy number.

Methods of "quantitative" amplification are well known to those of skill in the art. For example, quantitative PCR involves simultaneously co-amplifying a known quantity of a control sequence using the same primers. This provides an internal standard that may be used to calibrate the PCR reaction. Detailed protocols for quantitative PCR are provided in Innis, *et al.* (1990) PCR Protocols, *A Guide to Methods and Applications*, Academic Press, Inc. N.Y.). Measurement of DNA copy number at microsatellite loci using quantitative PCR analysis is described in Ginzonger, *et al.* (2000) *Cancer Research* 60:5405-5409. The known nucleic acid sequence for the genes is sufficient to enable one of skill in the art to routinely select primers to amplify any portion of the gene. Fluorogenic quantitative PCR may also be used in the methods of the invention. In fluorogenic quantitative PCR, quantitation is based on amount of fluorescence signals, *e.g.*, TaqMan and SYBR green.

Other suitable amplification methods include, but are not limited to, ligase chain reaction (LCR) (see Wu and Wallace (1989) *Genomics* 4: 560, Landegren, *et al.* (1988) *Science* 241:1077, and Barringer *et al.* (1990) *Gene* 89: 117), transcription amplification (Kwoh, *et al.* (1989) *Proc. Natl. Acad. Sci. USA* 86: 1173), self-sustained sequence

replication (Guatelli, *et al.* (1990) *Proc. Nat. Acad. Sci. USA* 87: 1874), dot PCR, and linker adapter PCR, *etc.*

Loss of heterozygosity (LOH) and major copy proportion (MCP) mapping (Wang, Z.C., *et al.* (2004) *Cancer Res* 64(1):64-71; Seymour, A. B., *et al.* (1994) *Cancer Res* 54, 2761-4; Hahn, S. A., *et al.* (1995) *Cancer Res* 55, 4670-5; Kimura, M., *et al.* (1996) *Genes Chromosomes Cancer* 17, 88-93; Li *et al.*, (2008) *MBC Bioinform.* 9, 204-219) may also be used to identify regions of amplification or deletion.

b. Methods for Detection of Biomarker Nucleic Acid Expression

Biomarker expression may be assessed by any of a wide variety of well known methods for detecting expression of a transcribed molecule or protein. Non-limiting examples of such methods include immunological methods for detection of secreted, cell-surface, cytoplasmic, or nuclear proteins, protein purification methods, protein function or activity assays, nucleic acid hybridization methods, nucleic acid reverse transcription methods, and nucleic acid amplification methods.

In preferred embodiments, activity of a particular gene is characterized by a measure of gene transcript (*e.g.* mRNA), by a measure of the quantity of translated protein, or by a measure of gene product activity. Marker expression can be monitored in a variety of ways, including by detecting mRNA levels, protein levels, or protein activity, any of which can be measured using standard techniques. Detection can involve quantification of the level of gene expression (*e.g.*, genomic DNA, cDNA, mRNA, protein, or enzyme activity), or, alternatively, can be a qualitative assessment of the level of gene expression, in particular in comparison with a control level. The type of level being detected will be clear from the context.

In another embodiment, detecting or determining expression levels of a biomarker and functionally similar homologs thereof, including a fragment or genetic alteration thereof (*e.g.*, in regulatory or promoter regions thereof) comprises detecting or determining RNA levels for the marker of interest. In one embodiment, one or more cells from the subject to be tested are obtained and RNA is isolated from the cells. In a preferred embodiment, a sample of breast tissue cells is obtained from the subject.

In one embodiment, RNA is obtained from a single cell. For example, a cell can be isolated from a tissue sample by laser capture microdissection (LCM). Using this technique, a cell can be isolated from a tissue section, including a stained tissue section, thereby assuring that the desired cell is isolated (*see, e.g.*, Bonner *et al.* (1997) Science 278:

1481; Emmert-Buck *et al.* (1996) Science 274:998; Fend *et al.* (1999) Am. J. Path. 154: 61 and Murakami *et al.* (2000) Kidney Int. 58:1346). For example, Murakami *et al.*, *supra*, describe isolation of a cell from a previously immunostained tissue section.

It is also be possible to obtain cells from a subject and culture the cells *in vitro*, such as to obtain a larger population of cells from which RNA can be extracted. Methods for establishing cultures of non-transformed cells, *i.e.*, primary cell cultures, are known in the art.

When isolating RNA from tissue samples or cells from individuals, it may be important to prevent any further changes in gene expression after the tissue or cells has been removed from the subject. Changes in expression levels are known to change rapidly following perturbations, *e.g.*, heat shock or activation with lipopolysaccharide (LPS) or other reagents. In addition, the RNA in the tissue and cells may quickly become degraded. Accordingly, in a preferred embodiment, the tissue or cells obtained from a subject is snap frozen as soon as possible.

RNA can be extracted from the tissue sample by a variety of methods, *e.g.*, the guanidium thiocyanate lysis followed by CsCl centrifugation (Chirgwin *et al.*, 1979, Biochemistry 18:5294-5299). RNA from single cells can be obtained as described in methods for preparing cDNA libraries from single cells, such as those described in Dulac, C. (1998) Curr. Top. Dev. Biol. 36, 245 and Jena *et al.* (1996) J. Immunol. Methods 190:199. Care to avoid RNA degradation must be taken, *e.g.*, by inclusion of RNAsin.

The RNA sample can then be enriched in particular species. In one embodiment, poly(A)+ RNA is isolated from the RNA sample. In general, such purification takes advantage of the poly-A tails on mRNA. In particular and as noted above, poly-T oligonucleotides may be immobilized within on a solid support to serve as affinity ligands for mRNA. Kits for this purpose are commercially available, *e.g.*, the MessageMaker kit (Life Technologies, Grand Island, NY).

In a preferred embodiment, the RNA population is enriched in marker sequences. Enrichment can be undertaken, *e.g.*, by primer-specific cDNA synthesis, or multiple rounds of linear amplification based on cDNA synthesis and template-directed *in vitro* transcription (*see, e.g.*, Wang *et al.* (1989) PNAS 86, 9717; Dulac *et al.*, *supra*, and Jena *et al.*, *supra*).

The population of RNA, enriched or not in particular species or sequences, can further be amplified. As defined herein, an "amplification process" is designed to

strengthen, increase, or augment a molecule within the RNA. For example, where RNA is mRNA, an amplification process such as RT-PCR can be utilized to amplify the mRNA, such that a signal is detectable or detection is enhanced. Such an amplification process is beneficial particularly when the biological, tissue, or tumor sample is of a small size or volume.

Various amplification and detection methods can be used. For example, it is within the scope of the present invention to reverse transcribe mRNA into cDNA followed by polymerase chain reaction (RT-PCR); or, to use a single enzyme for both steps as described in U.S. Pat. No. 5,322,770, or reverse transcribe mRNA into cDNA followed by symmetric gap ligase chain reaction (RT-AGLCR) as described by R. L. Marshall, *et al.*, PCR Methods and Applications 4: 80-84 (1994). Real time PCR may also be used.

Other known amplification methods which can be utilized herein include but are not limited to the so-called "NASBA" or "3SR" technique described in PNAS USA 87: 1874-1878 (1990) and also described in Nature 350 (No. 6313): 91-92 (1991); Q-beta amplification as described in published European Patent Application (EPA) No. 4544610; strand displacement amplification (as described in G. T. Walker *et al.*, Clin. Chem. 42: 9-13 (1996) and European Patent Application No. 684315; target mediated amplification, as described by PCT Publication WO9322461; PCR; ligase chain reaction (LCR) (*see, e.g.*, Wu and Wallace, Genomics 4, 560 (1989), Landegren *et al.*, Science 241, 1077 (1988)); self-sustained sequence replication (SSR) (*see, e.g.*, Guatelli *et al.*, Proc. Nat. Acad. Sci. USA, 87, 1874 (1990)); and transcription amplification (*see, e.g.*, Kwoh *et al.*, Proc. Natl. Acad. Sci. USA 86, 1173 (1989)).

Many techniques are known in the state of the art for determining absolute and relative levels of gene expression, commonly used techniques suitable for use in the present invention include Northern analysis, RNase protection assays (RPA), microarrays and PCR-based techniques, such as quantitative PCR and differential display PCR. For example, Northern blotting involves running a preparation of RNA on a denaturing agarose gel, and transferring it to a suitable support, such as activated cellulose, nitrocellulose or glass or nylon membranes. Radiolabeled cDNA or RNA is then hybridized to the preparation, washed and analyzed by autoradiography.

In situ hybridization visualization may also be employed, wherein a radioactively labeled antisense RNA probe is hybridized with a thin section of a biopsy sample, washed, cleaved with RNase and exposed to a sensitive emulsion for autoradiography. The samples

may be stained with hematoxylin to demonstrate the histological composition of the sample, and dark field imaging with a suitable light filter shows the developed emulsion. Non-radioactive labels such as digoxigenin may also be used.

Alternatively, mRNA expression can be detected on a DNA array, chip or a microarray. Labeled nucleic acids of a test sample obtained from a subject may be hybridized to a solid surface comprising biomarker DNA. Positive hybridization signal is obtained with the sample containing biomarker transcripts. Methods of preparing DNA arrays and their use are well known in the art (see, *e.g.*, U.S. Pat. Nos: 6,618,6796; 6,379,897; 6,664,377; 6,451,536; 548,257; U.S. 20030157485 and Schena *et al.* (1995) *Science* 20, 467-470; Gerhold *et al.* (1999) *Trends In Biochem. Sci.* 24, 168-173; and Lennon *et al.* (2000) *Drug Discovery Today* 5, 59-65, which are herein incorporated by reference in their entirety). Serial Analysis of Gene Expression (SAGE) can also be performed (See for example U.S. Patent Application 20030215858).

To monitor mRNA levels, for example, mRNA is extracted from the biological sample to be tested, reverse transcribed, and fluorescently-labeled cDNA probes are generated. The microarrays capable of hybridizing to marker cDNA are then probed with the labeled cDNA probes, the slides scanned and fluorescence intensity measured. This intensity correlates with the hybridization intensity and expression levels.

Types of probes that can be used in the methods described herein include cDNA, riboprobes, synthetic oligonucleotides and genomic probes. The type of probe used will generally be dictated by the particular situation, such as riboprobes for *in situ* hybridization, and cDNA for Northern blotting, for example. In one embodiment, the probe is directed to nucleotide regions unique to the RNA. The probes may be as short as is required to differentially recognize marker mRNA transcripts, and may be as short as, for example, 15 bases; however, probes of at least 17, 18, 19 or 20 or more bases can be used. In one embodiment, the primers and probes hybridize specifically under stringent conditions to a DNA fragment having the nucleotide sequence corresponding to the marker. As herein used, the term "stringent conditions" means hybridization will occur only if there is at least 95% identity in nucleotide sequences. In another embodiment, hybridization under "stringent conditions" occurs when there is at least 97% identity between the sequences.

The form of labeling of the probes may be any that is appropriate, such as the use of radioisotopes, for example, ^{32}P and ^{35}S. Labeling with radioisotopes may be achieved,

whether the probe is synthesized chemically or biologically, by the use of suitably labeled bases.

In one embodiment, the biological sample contains polypeptide molecules from the test subject. Alternatively, the biological sample can contain mRNA molecules from the test subject or genomic DNA molecules from the test subject.

In another embodiment, the methods further involve obtaining a control biological sample from a control subject, contacting the control sample with a compound or agent capable of detecting marker polypeptide, mRNA, genomic DNA, or fragments thereof, such that the presence of the marker polypeptide, mRNA, genomic DNA, or fragments thereof, is detected in the biological sample, and comparing the presence of the marker polypeptide, mRNA, genomic DNA, or fragments thereof, in the control sample with the presence of the marker polypeptide, mRNA, genomic DNA, or fragments thereof in the test sample.

c. Methods for Detection of Biomarker Protein Expression

The activity or level of a biomarker protein can be detected and/or quantified by detecting or quantifying the expressed polypeptide. The polypeptide can be detected and quantified by any of a number of means well known to those of skill in the art. Aberrant levels of polypeptide expression of the polypeptides encoded by a biomarker nucleic acid and functionally similar homologs thereof, including a fragment or genetic alteration thereof (*e.g.*, in regulatory or promoter regions thereof) are associated with the likelihood of response of a cancer to an anti-immune checkpoint inhibitor therapy. Any method known in the art for detecting polypeptides can be used. Such methods include, but are not limited to, immunodiffusion, immunoelectrophoresis, radioimmunoassay (RIA), enzyme-linked immunosorbent assays (ELISAs), immunofluorescent assays, Western blotting, binder-ligand assays, immunohistochemical techniques, agglutination, complement assays, high performance liquid chromatography (HPLC), thin layer chromatography (TLC), hyperdiffusion chromatography, and the like (*e.g.*, Basic and Clinical Immunology, Sites and Terr, eds., Appleton and Lange, Norwalk, Conn. pp 217-262, 1991 which is incorporated by reference). Preferred are binder-ligand immunoassay methods including reacting antibodies with an epitope or epitopes and competitively displacing a labeled polypeptide or derivative thereof.

For example, ELISA and RIA procedures may be conducted such that a desired biomarker protein standard is labeled (with a radioisotope such as ^{125}I or ^{35}S, or an assayable enzyme, such as horseradish peroxidase or alkaline phosphatase), and, together

with the unlabelled sample, brought into contact with the corresponding antibody, whereon a second antibody is used to bind the first, and radioactivity or the immobilized enzyme assayed (competitive assay). Alternatively, the biomarker protein in the sample is allowed to react with the corresponding immobilized antibody, radioisotope- or enzyme-labeled anti-biomarker proteinantibody is allowed to react with the system, and radioactivity or the enzyme assayed (ELISA-sandwich assay). Other conventional methods may also be employed as suitable.

The above techniques may be conducted essentially as a "one-step" or "two-step" assay. A "one-step" assay involves contacting antigen with immobilized antibody and, without washing, contacting the mixture with labeled antibody. A "two-step" assay involves washing before contacting, the mixture with labeled antibody. Other conventional methods may also be employed as suitable.

In one embodiment, a method for measuring biomarker protein levels comprises the steps of: contacting a biological specimen with an antibody or variant (*e.g.*, fragment) thereof which selectively binds the biomarker protein, and detecting whether said antibody or variant thereof is bound to said sample and thereby measuring the levels of the biomarker protein.

Enzymatic and radiolabeling of biomarker protein and/or the antibodies may be effected by conventional means. Such means will generally include covalent linking of the enzyme to the antigen or the antibody in question, such as by glutaraldehyde, specifically so as not to adversely affect the activity of the enzyme, by which is meant that the enzyme must still be capable of interacting with its substrate, although it is not necessary for all of the enzyme to be active, provided that enough remains active to permit the assay to be effected. Indeed, some techniques for binding enzyme are non-specific (such as using formaldehyde), and will only yield a proportion of active enzyme.

It is usually desirable to immobilize one component of the assay system on a support, thereby allowing other components of the system to be brought into contact with the component and readily removed without laborious and time-consuming labor. It is possible for a second phase to be immobilized away from the first, but one phase is usually sufficient.

It is possible to immobilize the enzyme itself on a support, but if solid-phase enzyme is required, then this is generally best achieved by binding to antibody and affixing

the antibody to a support, models and systems for which are well-known in the art. Simple polyethylene may provide a suitable support.

Enzymes employable for labeling are not particularly limited, but may be selected from the members of the oxidase group, for example. These catalyze production of hydrogen peroxide by reaction with their substrates, and glucose oxidase is often used for its good stability, ease of availability and cheapness, as well as the ready availability of its substrate (glucose). Activity of the oxidase may be assayed by measuring the concentration of hydrogen peroxide formed after reaction of the enzyme-labeled antibody with the substrate under controlled conditions well-known in the art.

Other techniques may be used to detect biomarker protein according to a practitioner's preference based upon the present disclosure. One such technique is Western blotting (Towbin et at., Proc. Nat. Acad. Sci. 76:4350 (1979)), wherein a suitably treated sample is run on an SDS-PAGE gel before being transferred to a solid support, such as a nitrocellulose filter. Anti-biomarker protein antibodies (unlabeled) are then brought into contact with the support and assayed by a secondary immunological reagent, such as labeled protein A or anti-immunoglobulin (suitable labels including ^{125}I, horseradish peroxidase and alkaline phosphatase). Chromatographic detection may also be used.

Immunohistochemistry may be used to detect expression of biomarker protein, *e.g.*, in a biopsy sample. A suitable antibody is brought into contact with, for example, a thin layer of cells, washed, and then contacted with a second, labeled antibody. Labeling may be by fluorescent markers, enzymes, such as peroxidase, avidin, or radiolabelling. The assay is scored visually, using microscopy.

Anti- biomarker protein antibodies, such as intrabodies, may also be used for imaging purposes, for example, to detect the presence of biomarker protein in cells and tissues of a subject. Suitable labels include radioisotopes, iodine (^{125}I, ^{121}I), carbon (^{14}C), sulphur (^{35}S), tritium (^{3}H), indium (^{112}In), and technetium (^{99}mTc), fluorescent labels, such as fluorescein and rhodamine, and biotin.

For *in vivo* imaging purposes, antibodies are not detectable, as such, from outside the body, and so must be labeled, or otherwise modified, to permit detection. Markers for this purpose may be any that do not substantially interfere with the antibody binding, but which allow external detection. Suitable markers may include those that may be detected by X-radiography, NMR or MRI. For X-radiographic techniques, suitable markers include any radioisotope that emits detectable radiation but that is not overtly harmful to the

subject, such as barium or cesium, for example. Suitable markers for NMR and MRI generally include those with a detectable characteristic spin, such as deuterium, which may be incorporated into the antibody by suitable labeling of nutrients for the relevant hybridoma, for example.

The size of the subject, and the imaging system used, will determine the quantity of imaging moiety needed to produce diagnostic images. In the case of a radioisotope moiety, for a human subject, the quantity of radioactivity injected will normally range from about 5 to 20 millicuries of technetium-99. The labeled antibody or antibody fragment will then preferentially accumulate at the location of cells which contain biomarker protein. The labeled antibody or antibody fragment can then be detected using known techniques.

Antibodies that may be used to detect biomarker protein include any antibody, whether natural or synthetic, full length or a fragment thereof, monoclonal or polyclonal, that binds sufficiently strongly and specifically to the biomarker protein to be detected. An antibody may have a K_d of at most about 10^{-6}M, 10^{-7}M, 10^{-8}M, 10^{-9}M, 10^{-10}M, 10^{-11}M, 10^{-12}M. The phrase "specifically binds" refers to binding of, for example, an antibody to an epitope or antigen or antigenic determinant in such a manner that binding can be displaced or competed with a second preparation of identical or similar epitope, antigen or antigenic determinant. An antibody may bind preferentially to the biomarker protein relative to other proteins, such as related proteins.

Antibodies are commercially available or may be prepared according to methods known in the art.

Antibodies and derivatives thereof that may be used encompass polyclonal or monoclonal antibodies, chimeric, human, humanized, primatized (CDR-grafted), veneered or single-chain antibodies as well as functional fragments, *i.e.*, biomarker protein binding fragments, of antibodies. For example, antibody fragments capable of binding to a biomarker protein or portions thereof, including, but not limited to, Fv, Fab, Fab' and F(ab') 2 fragments can be used. Such fragments can be produced by enzymatic cleavage or by recombinant techniques. For example, papain or pepsin cleavage can generate Fab or F(ab') 2 fragments, respectively. Other proteases with the requisite substrate specificity can also be used to generate Fab or F(ab') 2 fragments. Antibodies can also be produced in a variety of truncated forms using antibody genes in which one or more stop codons have been introduced upstream of the natural stop site. For example, a chimeric gene encoding a F(ab') 2 heavy chain portion can be designed to include DNA sequences encoding the CH, domain

and hinge region of the heavy chain.

Synthetic and engineered antibodies are described in, *e.g.*, Cabilly *et al.*, U.S. Pat. No. 4,816,567 Cabilly *et al.*, European Patent No. 0,125,023 B1; Boss *et al.*, U.S. Pat. No. 4,816,397; Boss *et al.*, European Patent No. 0,120,694 B1; Neuberger, M. S. *et al.*, WO 86/01533; Neuberger, M. S. *et al.*, European Patent No. 0,194,276 B1; Winter, U.S. Pat. No. 5,225,539; Winter, European Patent No. 0,239,400 B1; Queen *et al.*, European Patent No. 0451216 B1; and Padlan, E. A. *et al.*, EP 0519596 A1. See also, Newman, R. *et al.*, BioTechnology, 10: 1455-1460 (1992), regarding primatized antibody, and Ladner *et al.*, U.S. Pat. No. 4,946,778 and Bird, R. E. *et al.*, Science, 242: 423-426 (1988)) regarding single-chain antibodies. Antibodies produced from a library, *e.g.*, phage display library, may also be used.

In some embodiments, agents that specifically bind to a biomarker protein other than antibodies are used, such as peptides. Peptides that specifically bind to a biomarker protein can be identified by any means known in the art. For example, specific peptide binders of a biomarker protein can be screened for using peptide phage display libraries.

d. Methods for Detection of Biomarker Structural Alterations

The following illustrative methods can be used to identify the presence of a structural alteration in a biomarker nucleic acid and/or biomarker polypeptide molecule in order to, for example, identify biomarkers.

In certain embodiments, detection of the alteration involves the use of a probe/primer in a polymerase chain reaction (PCR) (see, *e.g.*, U.S. Pat. Nos. 4,683,195 and 4,683,202), such as anchor PCR or RACE PCR, or, alternatively, in a ligation chain reaction (LCR) (see, *e.g.*, Landegran *et al.* (1988) Science 241:1077-1080; and Nakazawa *et al.* (1994) Proc. Natl. Acad. Sci. USA 91:360-364), the latter of which can be particularly useful for detecting point mutations in a biomarker nucleic acid such as a biomarker gene (see Abravaya *et al.* (1995) Nucleic Acids Res. 23:675-682). This method can include the steps of collecting a sample of cells from a subject, isolating nucleic acid (*e.g.*, genomic, mRNA or both) from the cells of the sample, contacting the nucleic acid sample with one or more primers which specifically hybridize to a biomarker gene under conditions such that hybridization and amplification of the biomarker gene (if present) occurs, and detecting the presence or absence of an amplification product, or detecting the size of the amplification product and comparing the length to a control sample. It is anticipated that PCR and/or

LCR may be desirable to use as a preliminary amplification step in conjunction with any of the techniques used for detecting mutations described herein.

Alternative amplification methods include: self sustained sequence replication (Guatelli, J. C. *et al.* (1990) Proc. Natl. Acad. Sci. USA 87:1874-1878), transcriptional amplification system (Kwoh, D. Y. *et al.* (1989) Proc. Natl. Acad. Sci. USA 86:1173-1177), Q-Beta Replicase (Lizardi, P. M. *et al.* (1988) Bio-Technology 6:1197), or any other nucleic acid amplification method, followed by the detection of the amplified molecules using techniques well known to those of skill in the art. These detection schemes are especially useful for the detection of nucleic acid molecules if such molecules are present in very low numbers.

In an alternative embodiment, mutations in a biomarker nucleic acid from a sample cell can be identified by alterations in restriction enzyme cleavage patterns. For example, sample and control DNA is isolated, amplified (optionally), digested with one or more restriction endonucleases, and fragment length sizes are determined by gel electrophoresis and compared. Differences in fragment length sizes between sample and control DNA indicates mutations in the sample DNA. Moreover, the use of sequence specific ribozymes (see, for example, U.S. Pat. No. 5,498,531) can be used to score for the presence of specific mutations by development or loss of a ribozyme cleavage site.

In other embodiments, genetic mutations in biomarker nucleic acid can be identified by hybridizing a sample and control nucleic acids, *e.g.*, DNA or RNA, to high density arrays containing hundreds or thousands of oligonucleotide probes (Cronin, M. T. *et al.* (1996) Hum. Mutat. 7:244-255; Kozal, M. J. *et al.* (1996) Nat. Med. 2:753-759). For example, biomarker genetic mutations can be identified in two dimensional arrays containing light-generated DNA probes as described in Cronin *et al.* (1996) supra. Briefly, a first hybridization array of probes can be used to scan through long stretches of DNA in a sample and control to identify base changes between the sequences by making linear arrays of sequential, overlapping probes. This step allows the identification of point mutations. This step is followed by a second hybridization array that allows the characterization of specific mutations by using smaller, specialized probe arrays complementary to all variants or mutations detected. Each mutation array is composed of parallel probe sets, one complementary to the wild-type gene and the other complementary to the mutant gene. Such biomarker genetic mutations can be identified in a variety of contexts, including, for example, germline and somatic mutations.

In yet another embodiment, any of a variety of sequencing reactions known in the art can be used to directly sequence a biomarker gene and detect mutations by comparing the sequence of the sample biomarker with the corresponding wild-type (control) sequence. Examples of sequencing reactions include those based on techniques developed by Maxam and Gilbert (1977) *Proc. Natl. Acad. Sci. USA* 74:560 or Sanger (1977) *Proc. Natl. Acad Sci. USA* 74:5463. It is also contemplated that any of a variety of automated sequencing procedures can be utilized when performing the diagnostic assays (Naeve (1995) *Biotechniques* 19:448-53), including sequencing by mass spectrometry (see, *e.g.*, PCT International Publication No. WO 94/16101; Cohen *et al.* (1996) *Adv. Chromatogr.* 36:127-162; and Griffin *et al.* (1993) *Appl. Biochem. Biotechnol.* 38:147-159).

Other methods for detecting mutations in a biomarker gene include methods in which protection from cleavage agents is used to detect mismatched bases in RNA/RNA or RNA/DNA heteroduplexes (Myers *et al.* (1985) Science 230:1242). In general, the art technique of "mismatch cleavage" starts by providing heteroduplexes formed by hybridizing (labeled) RNA or DNA containing the wild-type biomarker sequence with potentially mutant RNA or DNA obtained from a tissue sample. The double-stranded duplexes are treated with an agent which cleaves single-stranded regions of the duplex such as which will exist due to base pair mismatches between the control and sample strands. For instance, RNA/DNA duplexes can be treated with RNase and DNA/DNA hybrids treated with SI nuclease to enzymatically digest the mismatched regions. In other embodiments, either DNA/DNA or RNA/DNA duplexes can be treated with hydroxylamine or osmium tetroxide and with piperidine in order to digest mismatched regions. After digestion of the mismatched regions, the resulting material is then separated by size on denaturing polyacrylamide gels to determine the site of mutation. See, for example, Cotton *et al.* (1988) Proc. Natl. Acad. Sci. USA 85:4397 and Saleeba *et al.* (1992) Methods Enzymol. 217:286-295. In a preferred embodiment, the control DNA or RNA can be labeled for detection.

In still another embodiment, the mismatch cleavage reaction employs one or more proteins that recognize mismatched base pairs in double-stranded DNA (so called "DNA mismatch repair" enzymes) in defined systems for detecting and mapping point mutations in biomarker cDNAs obtained from samples of cells. For example, the mutY enzyme of *E. coli* cleaves A at G/A mismatches and the thymidine DNA glycosylase from HeLa cells cleaves T at G/T mismatches (Hsu *et al.* (1994) *Carcinogenesis* 15:1657-1662). According

to an exemplary embodiment, a probe based on a biomarker sequence, *e.g.*, a wild-type biomarker treated with a DNA mismatch repair enzyme, and the cleavage products, if any, can be detected from electrophoresis protocols or the like (*e.g.*, U.S. Pat. No. 5,459,039.)

In other embodiments, alterations in electrophoretic mobility can be used to identify mutations in biomarker genes. For example, single strand conformation polymorphism (SSCP) may be used to detect differences in electrophoretic mobility between mutant and wild type nucleic acids (Orita *et al.* (1989) *Proc Natl. Acad. Sci USA* 86:2766; see also Cotton (1993) *Mutat. Res.* 285:125-144 and Hayashi (1992) *Genet. Anal. Tech. Appl.* 9:73-79). Single-stranded DNA fragments of sample and control biomarker nucleic acids will be denatured and allowed to renature. The secondary structure of single-stranded nucleic acids varies according to sequence, the resulting alteration in electrophoretic mobility enables the detection of even a single base change. The DNA fragments may be labeled or detected with labeled probes. The sensitivity of the assay may be enhanced by using RNA (rather than DNA), in which the secondary structure is more sensitive to a change in sequence. In a preferred embodiment, the subject method utilizes heteroduplex analysis to separate double stranded heteroduplex molecules on the basis of changes in electrophoretic mobility (Keen *et al.* (1991) *Trends Genet.* 7:5).

In yet another embodiment the movement of mutant or wild-type fragments in polyacrylamide gels containing a gradient of denaturant is assayed using denaturing gradient gel electrophoresis (DGGE) (Myers *et al.* (1985) *Nature* 313:495). When DGGE is used as the method of analysis, DNA will be modified to ensure that it does not completely denature, for example by adding a GC clamp of approximately 40 bp of high-melting GC-rich DNA by PCR. In a further embodiment, a temperature gradient is used in place of a denaturing gradient to identify differences in the mobility of control and sample DNA (Rosenbaum and Reissner (1987) *Biophys. Chem.* 265:12753).

Examples of other techniques for detecting point mutations include, but are not limited to, selective oligonucleotide hybridization, selective amplification, or selective primer extension. For example, oligonucleotide primers may be prepared in which the known mutation is placed centrally and then hybridized to target DNA under conditions which permit hybridization only if a perfect match is found (Saiki *et al.* (1986) *Nature* 324:163; Saiki *et al.* (1989) *Proc. Natl. Acad. Sci. USA* 86:6230). Such allele specific oligonucleotides are hybridized to PCR amplified target DNA or a number of different

mutations when the oligonucleotides are attached to the hybridizing membrane and hybridized with labeled target DNA.

Alternatively, allele specific amplification technology which depends on selective PCR amplification may be used in conjunction with the instant invention. Oligonucleotides used as primers for specific amplification may carry the mutation of interest in the center of the molecule (so that amplification depends on differential hybridization) (Gibbs *et al.* (1989) *Nucleic Acids Res.* 17:2437-2448) or at the extreme 3' end of one primer where, under appropriate conditions, mismatch can prevent, or reduce polymerase extension (Prossner (1993) *Tibtech* 11:238). In addition it may be desirable to introduce a novel restriction site in the region of the mutation to create cleavage-based detection (Gasparini *et al.* (1992) *Mol. Cell Probes* 6:1). It is anticipated that in certain embodiments amplification may also be performed using Taq ligase for amplification (Barany (1991) *Proc. Natl. Acad. Sci USA* 88:189). In such cases, ligation will occur only if there is a perfect match at the 3' end of the 5' sequence making it possible to detect the presence of a known mutation at a specific site by looking for the presence or absence of amplification.

Protein expression and activity can also be assessed according to functional assays described further below.

3. Anti-Cancer Therapies and Combination Therapies

The efficacy of anti-immune checkpoint inhibitor therapy is predicted according to biomarker amount and/or activity associated with a cancer in a subject according to the methods described herein. In one embodiment, such anti-immune checkpoint inhibitor therapy or combinations of therapies (*e.g.*, anti-PD-1, anti-PD-L1, anti-PD-L2, and anti-CTLA4 therapies) can be administered once a subject is indicated as being a likely responder to anti-immune checkpoint inhibitor therapy. In another embodiment, such anti-immune checkpoint inhibitor therapy can be avoided once a subject is indicated as not being a likely responder to anti-immune checkpoint inhibitor therapy and an alternative treatment regimen, such as targeted and/or untargeted anti-cancer therapies can be administered. Combination therapies are also contemplated and can comprise, for example, one or more chemotherapeutic agents and radiation, one or more chemotherapeutic agents and immunotherapy, or one or more chemotherapeutic agents, radiation and chemotherapy, each combination of which can be with or without anti-immune checkpoint inhibitor therapy.

The term "targeted therapy" refers to administration of agents that selectively interact with a chosen biomolecule to thereby treat cancer.

Immunotherapy is one form of targeted therapy that may comprise, for example, the use of cancer vaccines and/or sensitized antigen presenting cells. For example, an oncolytic virus is a virus that is able to infect and lyse cancer cells, while leaving normal cells unharmed, making them potentially useful in cancer therapy. Replication of oncolytic viruses both facilitates tumor cell destruction and also produces dose amplification at the tumor site. They may also act as vectors for anticancer genes, allowing them to be specifically delivered to the tumor site. The immunotherapy can involve passive immunity for short-term protection of a host, achieved by the administration of pre-formed antibody directed against a cancer antigen or disease antigen (*e.g.*, administration of a monoclonal antibody, optionally linked to a chemotherapeutic agent or toxin, to a tumor antigen). Immunotherapy can also focus on using the cytotoxic lymphocyte-recognized epitopes of cancer cell lines. Alternatively, antisense polynucleotides, ribozymes, RNA interference molecules, triple helix polynucleotides and the like, can be used to selectively modulate biomolecules that are linked to the initiation, progression, and/or pathology of a tumor or cancer.

In one embodiment, the immunotherapy can comprise the use of a Jak kinase nucleic acid or polypeptide or other Jak kinase stimulator (*e.g.*, a small molecule, an inhibitor of a Jak kinase inhibitor, and the like) in order to increase or overexpress Jak kinase activity. Without being bound by theory, it is believed that promoting Jak kinase activity, as opposed to the standard method in the art of inhibiting Jak kinase activity, increases expression of immune checkpoint inhibitory molecules thereby rendering cancer cells more susceptible to anti-immune checkpoint inhibitor therapy. Such Jak kinase stimulation can be transient (*e.g.*, inducible at will for repeated exposure) or constitutive. Such Jak kinase stimulation can also be systemic (*e.g.*, by generally administering Jak kinase-activating cytokine(s) or expressing a Jak kinase nucleic acid with a general promoter) or targeted (*e.g.*, locally administering a Jak kinase activating cytokine(s) or expressing a Jak kinase nucleic acid using a tissue-specific promoter).

The term "untargeted therapy" referes to administration of agents that do not selectively interact with a chosen biomolecule yet treat cancer. Representative examples of untargeted therapies include, without limitation, chemotherapy, gene therapy, and radiation therapy.

In one embodiment, chemotherapy is used. Chemotherapy includes the administration of a chemotherapeutic agent. Such a chemotherapeutic agent may be, but is not limited to, those selected from among the following groups of compounds: platinum compounds, cytotoxic antibiotics, antimetabolities, anti-mitotic agents, alkylating agents, arsenic compounds, DNA topoisomerase inhibitors, taxanes, nucleoside analogues, plant alkaloids, and toxins; and synthetic derivatives thereof. Exemplary compounds include, but are not limited to, alkylating agents: cisplatin, carboplatin, treosulfan, and trofosfamide; plant alkaloids: vinblastine, paclitaxel, docetaxol; DNA topoisomerase inhibitors: teniposide, crisnatol, and mitomycin; anti-folates: methotrexate, mycophenolic acid, and hydroxyurea; pyrimidine analogs: 5-fluorouracil, doxifluridine, and cytosine arabinoside; purine analogs: mercaptopurine and thioguanine; DNA antimetabolites: 2'-deoxy-5-fluorouridine, aphidicolin glycinate, pemetrexed, and pyrazoloimidazole; and antimitotic agents: halichondrin, colchicine, and rhizoxin. Compositions comprising one or more chemotherapeutic agents (*e.g.*, FLAG, CHOP) may also be used. FLAG comprises fludarabine, cytosine arabinoside (Ara-C) and G-CSF. CHOP comprises cyclophosphamide, vincristine, doxorubicin, and prednisone. In another embodiments, PARP (*e.g.*, PARP-1 and/or PARP-2) inhibitors are used and such inhibitors are well known in the art (*e.g.*, Olaparib, ABT-888, BSI-201, BGP-15 (N-Gene Research Laboratories, Inc.); INO-1001 (Inotek Pharmaceuticals Inc.); PJ34 (Soriano *et al.*, 2001; Pacher *et al.*, 2002b); 3-aminobenzamide (Trevigen); 4-amino-1,8-naphthalimide; (Trevigen); 6(5H)-phenanthridinone (Trevigen); benzamide (U.S. Pat. Re. 36,397); and NU1025 (Bowman *et al.*). The mechanism of action is generally related to the ability of PARP inhibitors to bind PARP and decrease its activity. PARP catalyzes the conversion of .beta.-nicotinamide adenine dinucleotide (NAD+) into nicotinamide and poly-ADP-ribose (PAR). Both poly (ADP-ribose) and PARP have been linked to regulation of transcription, cell proliferation, genomic stability, and carcinogenesis (Bouchard V. J. et.al. Experimental Hematology, Volume 31, Number 6, June 2003, pp. 446-454(9); Herceg Z.; Wang Z.-Q. Mutation Research/Fundamental and Molecular Mechanisms of Mutagenesis, Volume 477, Number 1, 2 Jun. 2001, pp. 97-110(14)). Poly(ADP-ribose) polymerase 1 (PARP1) is a key molecule in the repair of DNA single-strand breaks (SSBs) (de Murcia J. *et al.* 1997. Proc Natl Acad Sci USA 94:7303-7307; Schreiber V, Dantzer F, Ame J C, de Murcia G (2006) Nat Rev Mol Cell Biol 7:517-528; Wang Z Q, *et al.* (1997) Genes Dev 11:2347-2358). Knockout of SSB repair by inhibition of PARP1 function induces DNA double-strand

breaks (DSBs) that can trigger synthetic lethality in cancer cells with defective homology-directed DSB repair (Bryant H E, *et al.* (2005) Nature 434:913-917; Farmer H, *et al.* (2005) Nature 434:917-921). The foregoing examples of chemotherapeutic agents are illustrative, and are not intended to be limiting.

In another embodiment, radiation therapy is used. The radiation used in radiation therapy can be ionizing radiation. Radiation therapy can also be gamma rays, X-rays, or proton beams. Examples of radiation therapy include, but are not limited to, external-beam radiation therapy, interstitial implantation of radioisotopes (I-125, palladium, iridium), radioisotopes such as strontium-89, thoracic radiation therapy, intraperitoneal P-32 radiation therapy, and/or total abdominal and pelvic radiation therapy. For a general overview of radiation therapy, see Hellman, Chapter 16: Principles of Cancer Management: Radiation Therapy, 6th edition, 2001, DeVita *et al.*, eds., J. B. Lippencott Company, Philadelphia. The radiation therapy can be administered as external beam radiation or teletherapy wherein the radiation is directed from a remote source. The radiation treatment can also be administered as internal therapy or brachytherapy wherein a radioactive source is placed inside the body close to cancer cells or a tumor mass. Also encompassed is the use of photodynamic therapy comprising the administration of photosensitizers, such as hematoporphyrin and its derivatives, Vertoporfin (BPD-MA), phthalocyanine, photosensitizer Pc4, demethoxy-hypocrellin A; and 2BA-2-DMHA.

In another embodiment, hormone therapy is used. Hormonal therapeutic treatments can comprise, for example, hormonal agonists, hormonal antagonists (*e.g.*, flutamide, bicalutamide, tamoxifen, raloxifene, leuprolide acetate (LUPRON), LH-RH antagonists), inhibitors of hormone biosynthesis and processing, and steroids (*e.g.*, dexamethasone, retinoids, deltoids, betamethasone, cortisol, cortisone, prednisone, dehydrotestosterone, glucocorticoids, mineralocorticoids, estrogen, testosterone, progestins), vitamin A derivatives (*e.g.*, all-trans retinoic acid (ATRA)); vitamin D3 analogs; antigestagens (*e.g.*, mifepristone, onapristone), or antiandrogens (*e.g.*, cyproterone acetate).

In another embodiment, hyperthermia, a procedure in which body tissue is exposed to high temperatures (up to 106°F.) is used. Heat may help shrink tumors by damaging cells or depriving them of substances they need to live. Hyperthermia therapy can be local, regional, and whole-body hyperthermia, using external and internal heating devices. Hyperthermia is almost always used with other forms of therapy (*e.g.*, radiation therapy, chemotherapy, and biological therapy) to try to increase their effectiveness. Local

hyperthermia refers to heat that is applied to a very small area, such as a tumor. The area may be heated externally with high-frequency waves aimed at a tumor from a device outside the body. To achieve internal heating, one of several types of sterile probes may be used, including thin, heated wires or hollow tubes filled with warm water; implanted microwave antennae; and radiofrequency electrodes. In regional hyperthermia, an organ or a limb is heated. Magnets and devices that produce high energy are placed over the region to be heated. In another approach, called perfusion, some of the patient's blood is removed, heated, and then pumped (perfused) into the region that is to be heated internally. Whole-body heating is used to treat metastatic cancer that has spread throughout the body. It can be accomplished using warm-water blankets, hot wax, inductive coils (like those in electric blankets), or thermal chambers (similar to large incubators). Hyperthermia does not cause any marked increase in radiation side effects or complications. Heat applied directly to the skin, however, can cause discomfort or even significant local pain in about half the patients treated. It can also cause blisters, which generally heal rapidly.

In still another embodiment, photodynamic therapy (also called PDT, photoradiation therapy, phototherapy, or photochemotherapy) is used for the treatment of some types of cancer. It is based on the discovery that certain chemicals known as photosensitizing agents can kill one-celled organisms when the organisms are exposed to a particular type of light. PDT destroys cancer cells through the use of a fixed-frequency laser light in combination with a photosensitizing agent. In PDT, the photosensitizing agent is injected into the bloodstream and absorbed by cells all over the body. The agent remains in cancer cells for a longer time than it does in normal cells. When the treated cancer cells are exposed to laser light, the photosensitizing agent absorbs the light and produces an active form of oxygen that destroys the treated cancer cells. Light exposure must be timed carefully so that it occurs when most of the photosensitizing agent has left healthy cells but is still present in the cancer cells. The laser light used in PDT can be directed through a fiber-optic (a very thin glass strand). The fiber-optic is placed close to the cancer to deliver the proper amount of light. The fiber-optic can be directed through a bronchoscope into the lungs for the treatment of lung cancer or through an endoscope into the esophagus for the treatment of esophageal cancer. An advantage of PDT is that it causes minimal damage to healthy tissue. However, because the laser light currently in use cannot pass through more than about 3 centimeters of tissue (a little more than one and an eighth inch), PDT is mainly used to treat tumors on or just under the skin or on the lining of internal organs.

Photodynamic therapy makes the skin and eyes sensitive to light for 6 weeks or more after treatment. Patients are advised to avoid direct sunlight and bright indoor light for at least 6 weeks. If patients must go outdoors, they need to wear protective clothing, including sunglasses. Other temporary side effects of PDT are related to the treatment of specific areas and can include coughing, trouble swallowing, abdominal pain, and painful breathing or shortness of breath. In December 1995, the U.S. Food and Drug Administration (FDA) approved a photosensitizing agent called porfimer sodium, or Photofrin®, to relieve symptoms of esophageal cancer that is causing an obstruction and for esophageal cancer that cannot be satisfactorily treated with lasers alone. In January 1998, the FDA approved porfimer sodium for the treatment of early nonsmall cell lung cancer in patients for whom the usual treatments for lung cancer are not appropriate. The National Cancer Institute and other institutions are supporting clinical trials (research studies) to evaluate the use of photodynamic therapy for several types of cancer, including cancers of the bladder, brain, larynx, and oral cavity.

In yet another embodiment, laser therapy is used to harness high-intensity light to destroy cancer cells. This technique is often used to relieve symptoms of cancer such as bleeding or obstruction, especially when the cancer cannot be cured by other treatments. It may also be used to treat cancer by shrinking or destroying tumors. The term "laser" stands for light amplification by stimulated emission of radiation. Ordinary light, such as that from a light bulb, has many wavelengths and spreads in all directions. Laser light, on the other hand, has a specific wavelength and is focused in a narrow beam. This type of high-intensity light contains a lot of energy. Lasers are very powerful and may be used to cut through steel or to shape diamonds. Lasers also can be used for very precise surgical work, such as repairing a damaged retina in the eye or cutting through tissue (in place of a scalpel). Although there are several different kinds of lasers, only three kinds have gained wide use in medicine: Carbon dioxide (CO_2) laser--This type of laser can remove thin layers from the skin's surface without penetrating the deeper layers. This technique is particularly useful in treating tumors that have not spread deep into the skin and certain precancerous conditions. As an alternative to traditional scalpel surgery, the CO_2 laser is also able to cut the skin. The laser is used in this way to remove skin cancers. Neodymium:yttrium-aluminum-garnet (Nd:YAG) laser-- Light from this laser can penetrate deeper into tissue than light from the other types of lasers, and it can cause blood to clot quickly. It can be carried through optical fibers to less accessible parts of the body. This

type of laser is sometimes used to treat throat cancers. Argon laser--This laser can pass through only superficial layers of tissue and is therefore useful in dermatology and in eye surgery. It also is used with light-sensitive dyes to treat tumors in a procedure known as photodynamic therapy (PDT). Lasers have several advantages over standard surgical tools, including: Lasers are more precise than scalpels. Tissue near an incision is protected, since there is little contact with surrounding skin or other tissue. The heat produced by lasers sterilizes the surgery site, thus reducing the risk of infection. Less operating time may be needed because the precision of the laser allows for a smaller incision. Healing time is often shortened; since laser heat seals blood vessels, there is less bleeding, swelling, or scarring. Laser surgery may be less complicated. For example, with fiber optics, laser light can be directed to parts of the body without making a large incision. More procedures may be done on an outpatient basis. Lasers can be used in two ways to treat cancer: by shrinking or destroying a tumor with heat, or by activating a chemical--known as a photosensitizing agent--that destroys cancer cells. In PDT, a photosensitizing agent is retained in cancer cells and can be stimulated by light to cause a reaction that kills cancer cells. CO_2 and Nd:YAG lasers are used to shrink or destroy tumors. They may be used with endoscopes, tubes that allow physicians to see into certain areas of the body, such as the bladder. The light from some lasers can be transmitted through a flexible endoscope fitted with fiber optics. This allows physicians to see and work in parts of the body that could not otherwise be reached except by surgery and therefore allows very precise aiming of the laser beam. Lasers also may be used with low-power microscopes, giving the doctor a clear view of the site being treated. Used with other instruments, laser systems can produce a cutting area as small as 200 microns in diameter--less than the width of a very fine thread. Lasers are used to treat many types of cancer. Laser surgery is a standard treatment for certain stages of glottis (vocal cord), cervical, skin, lung, vaginal, vulvar, and penile cancers. In addition to its use to destroy the cancer, laser surgery is also used to help relieve symptoms caused by cancer (palliative care). For example, lasers may be used to shrink or destroy a tumor that is blocking a patient's trachea (windpipe), making it easier to breathe. It is also sometimes used for palliation in colorectal and anal cancer. Laser-induced interstitial thermotherapy (LITT) is one of the most recent developments in laser therapy. LITT uses the same idea as a cancer treatment called hyperthermia; that heat may help shrink tumors by damaging cells or depriving them of substances they need to live. In this treatment, lasers are directed to interstitial areas (areas between organs) in the body.

The laser light then raises the temperature of the tumor, which damages or destroys cancer cells.

The duration and/or dose of treatment with anti-immune checkpoint inhibitor therapies may vary according to the particular anti-immune checkpoint inhibitor agent or combination thereof (*e.g.*, Jak kinase stimulating agents in combination with inhibitors of PD-1, PD-L1, PD-L2, CTLA-4, and the like). An appropriate treatment time for a particular cancer therapeutic agent will be appreciated by the skilled artisan. The invention contemplates the continued assessment of optimal treatment schedules for each cancer therapeutic agent, where the phenotype of the cancer of the subject as determined by the methods of the invention is a factor in determining optimal treatment doses and schedules.

Any means for the introduction of a polynucleotide into mammals, human or non-human, or cells thereof may be adapted to the practice of this invention for the delivery of the various constructs of the invention into the intended recipient. In one embodiment of the invention, the DNA constructs are delivered to cells by transfection, *i.e.*, by delivery of "naked" DNA or in a complex with a colloidal dispersion system. A colloidal system includes macromolecule complexes, nanocapsules, microspheres, beads, and lipid-based systems including oil-in-water emulsions, micelles, mixed micelles, and liposomes. The preferred colloidal system of this invention is a lipid-complexed or liposome-formulated DNA. In the former approach, prior to formulation of DNA, *e.g.*, with lipid, a plasmid containing a transgene bearing the desired DNA constructs may first be experimentally optimized for expression (*e.g.*, inclusion of an intron in the 5' untranslated region and elimination of unnecessary sequences (Felgner, *et al.*, Ann NY Acad Sci 126-139, 1995). Formulation of DNA, *e.g.* with various lipid or liposome materials, may then be effected using known methods and materials and delivered to the recipient mammal. See, *e.g.*, Canonico et al, Am J Respir Cell Mol Biol 10:24-29, 1994; Tsan et al, Am J Physiol 268; Alton *et al.*, Nat Genet. 5:135-142, 1993 and U.S. patent No. 5,679,647 by Carson *et al.*

The targeting of liposomes can be classified based on anatomical and mechanistic factors. Anatomical classification is based on the level of selectivity, for example, organ-specific, cell-specific, and organelle-specific. Mechanistic targeting can be distinguished based upon whether it is passive or active. Passive targeting utilizes the natural tendency of liposomes to distribute to cells of the reticulo-endothelial system (RES) in organs, which contain sinusoidal capillaries. Active targeting, on the other hand, involves alteration of the liposome by coupling the liposome to a specific ligand such as a monoclonal antibody,

sugar, glycolipid, or protein, or by changing the composition or size of the liposome in order to achieve targeting to organs and cell types other than the naturally occurring sites of localization.

The surface of the targeted delivery system may be modified in a variety of ways. In the case of a liposomal targeted delivery system, lipid groups can be incorporated into the lipid bilayer of the liposome in order to maintain the targeting ligand in stable association with the liposomal bilayer. Various linking groups can be used for joining the lipid chains to the targeting ligand. Naked DNA or DNA associated with a delivery vehicle, *e.g.*, liposomes, can be administered to several sites in a subject (see below).

Nucleic acids can be delivered in any desired vector. These include viral or non-viral vectors, including adenovirus vectors, adeno-associated virus vectors, retrovirus vectors, lentivirus vectors, and plasmid vectors. Exemplary types of viruses include HSV (herpes simplex virus), AAV (adeno associated virus), HIV (human immunodeficiency virus), BIV (bovine immunodeficiency virus), and MLV (murine leukemia virus). Nucleic acids can be administered in any desired format that provides sufficiently efficient delivery levels, including in virus particles, in liposomes, in nanoparticles, and complexed to polymers.

The nucleic acids encoding a protein or nucleic acid of interest may be in a plasmid or viral vector, or other vector as is known in the art. Such vectors are well known and any can be selected for a particular application. In one embodiment of the invention, the gene delivery vehicle comprises a promoter and a demethylase coding sequence. Preferred promoters are tissue-specific promoters and promoters which are activated by cellular proliferation, such as the thymidine kinase and thymidylate synthase promoters. Other preferred promoters include promoters which are activatable by infection with a virus, such as the α- and β-interferon promoters, and promoters which are activatable by a hormone, such as estrogen. Other promoters which can be used include the Moloney virus LTR, the CMV promoter, and the mouse albumin promoter. A promoter may be constitutive or inducible.

In another embodiment, naked polynucleotide molecules are used as gene delivery vehicles, as described in WO 90/11092 and U.S. Patent 5,580,859. Such gene delivery vehicles can be either growth factor DNA or RNA and, in certain embodiments, are linked to killed adenovirus. Curiel *et al.*, Hum. Gene. Ther. 3:147-154, 1992. Other vehicles which can optionally be used include DNA-ligand (Wu *et al.*, J. Biol. Chem.

264:16985-16987, 1989), lipid-DNA combinations (Felgner *et al.*, Proc. Natl. Acad. Sci. USA 84:7413 7417, 1989), liposomes (Wang *et al.*, Proc. Natl. Acad. Sci. 84:7851-7855, 1987) and microprojectiles (Williams *et al.*, Proc. Natl. Acad. Sci. 88:2726-2730, 1991).

A gene delivery vehicle can optionally comprise viral sequences such as a viral origin of replication or packaging signal. These viral sequences can be selected from viruses such as astrovirus, coronavirus, orthomyxovirus, papovavirus, paramyxovirus, parvovirus, picornavirus, poxvirus, retrovirus, togavirus or adenovirus. In a preferred embodiment, the growth factor gene delivery vehicle is a recombinant retroviral vector. Recombinant retroviruses and various uses thereof have been described in numerous references including, for example, Mann *et al.*, Cell 33:153, 1983, Cane and Mulligan, Proc. Nat'l. Acad. Sci. USA 81:6349, 1984, Miller *et al.*, Human Gene Therapy 1:5-14, 1990, U.S. Patent Nos. 4,405,712, 4,861,719, and 4,980,289, and PCT Application Nos. WO 89/02,468, WO 89/05,349, and WO 90/02,806. Numerous retroviral gene delivery vehicles can be utilized in the present invention, including for example those described in EP 0,415,731; WO 90/07936; WO 94/03622; WO 93/25698; WO 93/25234; U.S. Patent No. 5,219,740; WO 9311230; WO 9310218; Vile and Hart, Cancer Res. 53:3860-3864, 1993; Vile and Hart, Cancer Res. 53:962-967, 1993; Ram *et al.*, Cancer Res. 53:83-88, 1993; Takamiya *et al.*, J. Neurosci. Res. 33:493-503, 1992; Baba *et al.*, J. Neurosurg. 79:729-735, 1993 (U.S. Patent No. 4,777,127, GB 2,200,651, EP 0,345,242 and WO91/02805).

Other viral vector systems that can be used to deliver a polynucleotide of the invention have been derived from herpes virus, *e.g.*, Herpes Simplex Virus (U.S. Patent No. 5,631,236 by Woo *et al.*, issued May 20, 1997 and WO 00/08191 by Neurovex), vaccinia virus (Ridgeway (1988) Ridgeway, "Mammalian expression vectors," In: Rodriguez R L, Denhardt D T, ed. Vectors: A survey of molecular cloning vectors and their uses. Stoneham: Butterworth,; Baichwal and Sugden (1986) "Vectors for gene transfer derived from animal DNA viruses: Transient and stable expression of transferred genes," In: Kucherlapati R, ed. Gene transfer. New York: Plenum Press; Coupar *et al.* (1988) Gene, 68:1-10), and several RNA viruses. Preferred viruses include an alphavirus, a poxivirus, an arena virus, a vaccinia virus, a polio virus, and the like. They offer several attractive features for various mammalian cells (Friedmann (1989) Science, 244:1275-1281; Ridgeway, 1988, supra; Baichwal and Sugden, 1986, supra; Coupar *et al.*, 1988; Horwich *et al.*(1990) J.Virol., 64:642-650).

In other embodiments, target DNA in the genome can be manipulated using well-known methods in the art. For example, the target DNA in the genome can be manipulated by deletion, insertion, and/or mutation are retroviral insertion, artificial chromosome techniques, gene insertion, random insertion with tissue specific promoters, gene targeting, transposable elements and/or any other method for introducing foreign DNA or producing modified DNA/modified nuclear DNA. Other modification techniques include deleting DNA sequences from a genome and/or altering nuclear DNA sequences. Nuclear DNA sequences, for example, may be altered by site-directed mutagenesis.

In other embodiments, recombinant biomarker polypeptides, and fragments thereof, can be administered to subjects. In some embodiments, fusion proteins can be constructed and administered which have enhanced biological properties. In addition, the biomarker polypeptides, and fragment thereof, can be modified according to well-known pharmacological methods in the art (*e.g.*, pegylation, glycosylation, oligomerization, *etc.*) in order to further enhance desirable biological activities, such as increased bioavailability and decreased proteolytic degradation.

4. Clincal Efficacy

Clinical efficacy can be measured by any method known in the art. For example, the response to a therapy, such as anti-immune checkpoint inhibitor therapies, relates to any response of the cancer, *e.g.*, a tumor, to the therapy, preferably to a change in tumor mass and/or volume after initiation of neoadjuvant or adjuvant chemotherapy. Tumor response may be assessed in a neoadjuvant or adjuvant situation where the size of a tumor after systemic intervention can be compared to the initial size and dimensions as measured by CT, PET, mammogram, ultrasound or palpation and the cellularity of a tumor can be estimated histologically and compared to the cellularity of a tumor biopsy taken before initiation of treatment. Response may also be assessed by caliper measurement or pathological examination of the tumor after biopsy or surgical resection. Response may be recorded in a quantitative fashion like percentage change in tumor volume or cellularity or using a semi-quantitative scoring system such as residual cancer burden (Symmans *et al.*, *J. Clin. Oncol.* (2007) 25:4414-4422) or Miller-Payne score (Ogston *et al.*, (2003) *Breast* (Edinburgh, Scotland) 12:320-327) in a qualitative fashion like "pathological complete response" (pCR), "clinical complete remission" (cCR), "clinical partial remission" (cPR), "clinical stable disease" (cSD), "clinical progressive disease" (cPD) or other qualitative

criteria. Assessment of tumor response may be performed early after the onset of neoadjuvant or adjuvant therapy, *e.g.*, after a few hours, days, weeks or preferably after a few months. A typical endpoint for response assessment is upon termination of neoadjuvant chemotherapy or upon surgical removal of residual tumor cells and/or the tumor bed.

In some embodiments, clinical efficacy of the therapeutic treatments described herein may be determined by measuring the clinical benefit rate (CBR). The clinical benefit rate is measured by determining the sum of the percentage of patients who are in complete remission (CR), the number of patients who are in partial remission (PR) and the number of patients having stable disease (SD) at a time point at least 6 months out from the end of therapy. The shorthand for this formula is CBR=CR+PR+SD over 6 months. In some embodiments, the CBR for a particular anti-immune checkpoint inhibitor therapeutic regimen is at least 25%, 30%, 35%, 40%, 45%, 50%, 55%, 60%, 65%, 70%, 75%, 80%, 85%, or more.

Additional criteria for evaluating the response to anti-immune checkpoint inhibitor therapies are related to "survival," which includes all of the following: survival until mortality, also known as overall survival (wherein said mortality may be either irrespective of cause or tumor related); "recurrence-free survival" (wherein the term recurrence shall include both localized and distant recurrence); metastasis free survival; disease free survival (wherein the term disease shall include cancer and diseases associated therewith). The length of said survival may be calculated by reference to a defined start point (*e.g.*, time of diagnosis or start of treatment) and end point (*e.g.*, death, recurrence or metastasis). In addition, criteria for efficacy of treatment can be expanded to include response to chemotherapy, probability of survival, probability of metastasis within a given time period, and probability of tumor recurrence.

For example, in order to determine appropriate threshold values, a particular anti-immune checkpoint inhibitor therapeutic regimen can be administered to a population of subjects and the outcome can be correlated to biomarker measurements that were determined prior to administration of any anti-immune checkpoint inhibitor therapy. The outcome measurement may be pathologic response to therapy given in the neoadjuvant setting. Alternatively, outcome measures, such as overall survival and disease-free survival can be monitored over a period of time for subjects following anti-immune checkpoint inhibitor therapy for whom biomarker measurement values are known. In certain

embodiments, the same doses of anti-immune checkpoint inhibitor agents are administered to each subject. In related embodiments, the doses administered are standard doses known in the art for anti-immune checkpoint inhibitor agents. The period of time for which subjects are monitored can vary. For example, subjects may be monitored for at least 2, 4, 6, 8, 10, 12, 14, 16, 18, 20, 25, 30, 35, 40, 45, 50, 55, or 60 months. Biomarker measurement threshold values that correlate to outcome of an anti-immune checkpoint inhibitor therapy can be determined using methods such as those described in the Examples section.

5. Further Uses and Methods of the Present Invention

The compositions described herein can be used in a variety of diagnostic, prognostic, and therapeutic applications.

a. Screening Methods

One aspect of the present invention relates to screening assays, including non-cell based assays. In one embodiment, the assays provide a method for identifying whether a cancer is likely to respond to anti-immune checkpoint inhibitor therapy and/or whether an agent can inhibit the growth of or kill a cancer cell that is unlikely to respond to anti-immune checkpoint inhibitor therapy.

In one embodiment, the invention relates to assays for screening test agents which bind to, or modulate the biological activity of, at least one biomarker listed in Table 1. In one embodiment, a method for identifying such an agent entails determining the ability of the agent to modulate, *e.g.* inhibit, the at least one biomarker listed in Table 1.

In one embodiment, an assay is a cell-free or cell-based assay, comprising contacting at least one biomarker listed in Table 1, with a test agent, and determining the ability of the test agent to modulate (*e.g.* inhibit) the enzymatic activity of the biomarker, such as by measuring direct binding of substrates or by measuring indirect parameters as described below.

For example, in a direct binding assay, biomarker protein (or their respective target polypeptides or molecules) can be coupled with a radioisotope or enzymatic label such that binding can be determined by detecting the labeled protein or molecule in a complex. For example, the targets can be labeled with ^{125}I, ^{35}S, ^{14}C, or ^{3}H, either directly or indirectly, and the radioisotope detected by direct counting of radioemmission or by scintillation counting. Alternatively, the targets can be enzymatically labeled with, for example,

horseradish peroxidase, alkaline phosphatase, or luciferase, and the enzymatic label detected by determination of conversion of an appropriate substrate to product. Determining the interaction between biomarker and substrate can also be accomplished using standard binding or enzymatic analysis assays. In one or more embodiments of the above described assay methods, it may be desirable to immobilize polypeptides or molecules to facilitate separation of complexed from uncomplexed forms of one or both of the proteins or molecules, as well as to accommodate automation of the assay.

Binding of a test agent to a target can be accomplished in any vessel suitable for containing the reactants. Non-limiting examples of such vessels include microtiter plates, test tubes, and micro-centrifuge tubes. Immobilized forms of the antibodies of the present invention can also include antibodies bound to a solid phase like a porous, microporous (with an average pore diameter less than about one micron) or macroporous (with an average pore diameter of more than about 10 microns) material, such as a membrane, cellulose, nitrocellulose, or glass fibers; a bead, such as that made of agarose or polyacrylamide or latex; or a surface of a dish, plate, or well, such as one made of polystyrene.

In an alternative embodiment, determining the ability of the agent to modulate the interaction between the biomarker and a substrate or a biomarker metabolite and its natural binding partner can be accomplished by determining the ability of the test agent to modulate the activity of a polypeptide or other product that functions downstream or upstream of its position within the pathway (*e.g.*, feedback loops).

The present invention further pertains to novel agents identified by the above-described screening assays. Accordingly, it is within the scope of this invention to further use an agent identified as described herein in an appropriate animal model. For example, an agent identified as described herein can be used in an animal model to determine the efficacy, toxicity, or side effects of treatment with such an agent. Alternatively, an antibody identified as described herein can be used in an animal model to determine the mechanism of action of such an agent.

In some embodiments, detecting Jak kinase autophosphorylation is useful and methods for such detection are well known in the art. In an autophosphorylation assay, a test compound suspected of being a Jak kinase modultator is contacted or reacted with a suitable reaction mixture comprising JAK polypeptide as a source of tyrosine and/or serine kinase activity under conditions and for a time sufficient to allow phosphorylation of a

tyrosine and/or serine residue. The tyrosine kinase reaction may be initiated in the presence of ATP or an analog thereof and Mn^{2+} or Mg^{2+} (*e.g.*, as $MnCl_2$ or a mixture of divalent cations comprising Mn^{2+}or Mg^{2+}), whereas the serine kinase reaction may be initiated in the presence of ATP and divalent cations, such as Mn^{2+} (*e.g.*, as $MnCl_2$ or a mixture of divalent cations comprising Mn^{2+}) or Mg^{2+} (*e.g.*, as $MgCl_2$ or a mixture of divalent cations comprising Mg^{2+}), or mixtures thereof. Subsequently, the presence or absence of autophosphorylated tyrosine and/or serine residues may be determined by standard methods known in the art. Such methods include, but are not limited to mass spectrometry, microscopy, spectroscopy, Western blotting, and immunoassays such as SPR, RIA, EIA, and ELISA, wherein phosphotyrosine or phosphoserine specific antibodies (including polyclonal, monoclonal, chimeric, and single chain antibodies, as well as FAb fragments) available in the art may be used. The antibody may be directly or indirectly labelled, for example, with a radiolabel, fluorescent label, luminescent label, or enzymatic label capable of producing a detectable signal.

The assay may comprise a step, wherein the level of serine and/or tyrosine phosphorylation in the presence of a test substance is compared to that in the absence of said test substance. In some embodiments, if the level of serine and/or tyrosine phosporylation is increased as compared to the control (no test substance present), the test substance is a Jak kinase activator. In other embodiments, if the level of serine and/or tyrosine phosphorylation is decreased as compared to the control, the test substance is a Jak kinase inhibitor. IN still other embodiments, an inhibitor of autophosphorylation of the JH2 domain may act as an activator for JH1 domain catalytic activity and signaling, and in some specific embodiments the inhibitor may inhibit JH1 activity and signaling.

In other embodiments, the assay is based on the capability of a test compound to modulate the ability of a Jak kinase to bind a substrate or transphosphorylate tyrosine and/or serine residues of a substrate. The term "substrate" refers to a protein or a peptide which is acted on by the tyrosine and/or serine kinase activity of the Jak kinase such that it is phosphoryiated on tyrosine and/or serine residues, respectively.

In a transphosphorylation assay, a test compound is contacted or reacted with a suitable reaction mixture comprising Jak polypeptide comprising a catalytically active JH2 domain as a source of tyrosine and/or serine kinase activity and a substrate. Suitable tyrosine and serine substrates are available in the art and include, but are not limited to, Poly-Gly-Tyr peptide. The kinase reaction is initiated in the presence of ATP and divalent

cations such as Mn^{2+} or Mg^{2+} as described above. The reaction is carried out under conditions and for a time sufficient to allow phosphorylation of a tyrosine and/or serine residue. Subsequently, the presence or absence of phosphoryiated tyrosine and/or serine residues in the substrate may be determined by standard methods known in the art as described above for autophophorylation assays. Further, the assay may comprise a step, wherein the level of transphosphorylation in the presence of a test substance is compared to that in the absence of said test substance. If the level of serine and/or tyrosine transphosporylation is increased as compared to the control (no test substance present), the test substance is an activator of Jak kinase activity. On the other hand, if the level of serine and/or tyrosine transphosphorylation is decreased as compared to the control, the test substance is an inhibitor of Jak kinase activity.

Jak kinase modulators can also be screened, identified, and characterized by employing calorimetric methods such as differential scanning calorimetry or fluorimetry, or isothermal titration calorimetry or fluorimetry, where the binding of the modulator is analysed with respect to a change in the melting temperature of the Jak kinase. Such methods are known to a person skilled in the art and include measurement of surface plasmon resonance or spectrocopical methods including fluorescence, UV/visible light, CD, NMR based methods and microscopy methods including atom force microscopy, as well as crystallography.

In cell-based assays, cells can be used that lackthe specified biomarker of interest, such as a Jak kinase having an activating mutation. Receptor activation may be employed and the readout may be based on detection of tyrosine or serine phosphorylation in the context of Jak kinase autophosphorylation activation or Jak kinase catalysis of transphosphorylation or as activation of downstream signalling cascades/proteins, such as STAT transcription factors, PI-3K/Akt cascade, MAP kinase pathway, and the like. Furthermore, colony formation, cellular mobility, proliferation, other cellular functions can be used as a readout for the assays. In one embodiment, the expression of at least one immune checkpoint inhibitor is analyzed (*e.g.*, PD-L1 expression).

b. Predictive Medicine

The present invention also pertains to the field of predictive medicine in which diagnostic assays, prognostic assays, and monitoring clinical trials are used for prognostic (predictive) purposes to thereby treat an individual prophylactically. Accordingly, one aspect of the present invention relates to diagnostic assays for determining the amount

and/or activity level of a biomarker listed in Table 1 in the context of a biological sample (*e.g.*, blood, serum, cells, or tissue) to thereby determine whether an individual afflicted with a cancer is likely to respond to anti-immune checkpoint inhibitor therapy, whether in an original or recurrent cancer. Such assays can be used for prognostic or predictive purpose to thereby prophylactically treat an individual prior to the onset or after recurrence of a disorder characterized by or associated with biomarker polypeptide, nucleic acid expression or activity. The skilled artisan will appreciate that any method can use one or more (*e.g.*, combinations) of biomarkers listed in Table 1.

Another aspect of the present invention pertains to monitoring the influence of agents (*e.g.*, drugs, compounds, and small nucleic acid-based molecules) on the expression or activity of a biomarker listed in Table 1. These and other agents are described in further detail in the following sections.

The skilled artisan will also appreciated that, in certain embodiments, the methods of the present invention implement a computer program and computer system. For example, a computer program can be used to perform the algorithms described herein. A computer system can also store and manipulate data generated by the methods of the present invention which comprises a plurality of biomarker signal changes/profiles which can be used by a computer system in implementing the methods of this invention. In certain embodiments, a computer system receives biomarker expression data; (ii) stores the data; and (iii) compares the data in any number of ways described herein (*e.g.*, analysis relative to appropriate controls) to determine the state of informative biomarkers from cancerous or pre-cancerous tissue. In other embodiments, a computer system (i) compares the determined expression biomarker level to a threshold value; and (ii) outputs an indication of whether said biomarker level is significantly modulated (*e.g.*, above or below) the threshold value, or a phenotype based on said indication.

In certain embodiments, such computer systems are also considered part of the present invention. Numerous types of computer systems can be used to implement the analytic methods of this invention according to knowledge possessed by a skilled artisan in the bioinformatics and/or computer arts. Several software components can be loaded into memory during operation of such a computer system. The software components can comprise both software components that are standard in the art and components that are special to the present invention (*e.g.*, dCHIP software described in Lin *et al.* (2004)

Bioinformatics 20, 1233-1240; radial basis machine learning algorithms (RBM) known in the art).

The methods of the invention can also be programmed or modeled in mathematical software packages that allow symbolic entry of equations and high-level specification of processing, including specific algorithms to be used, thereby freeing a user of the need to procedurally program individual equations and algorithms. Such packages include, *e.g.*, Matlab from Mathworks (Natick, Mass.), Mathematica from Wolfram Research (Champaign, Ill.) or S-Plus from MathSoft (Seattle, Wash.).

In certain embodiments, the computer comprises a database for storage of biomarker data. Such stored profiles can be accessed and used to perform comparisons of interest at a later point in time. For example, biomarker expression profiles of a sample derived from the non-cancerous tissue of a subject and/or profiles generated from population-based distributions of informative loci of interest in relevant populations of the same species can be stored and later compared to that of a sample derived from the cancerous tissue of the subject or tissue suspected of being cancerous of the subject.

In addition to the exemplary program structures and computer systems described herein, other, alternative program structures and computer systems will be readily apparent to the skilled artisan. Such alternative systems, which do not depart from the above described computer system and programs structures either in spirit or in scope, are therefore intended to be comprehended within the accompanying claims.

c. Diagnostic Assays

The present invention provides, in part, methods, systems, and code for accurately classifying whether a biological sample is associated with a cancer that is likely to respond to anti-immune checkpoint inhibitor therapy. In some embodiments, the present invention is useful for classifying a sample (*e.g.*, from a subject) as associated with or at risk for responding to or not responding to anti-immune checkpoint inhibitor therapy using a statistical algorithm and/or empirical data (*e.g.*, the amount or activity of a biomarker listed in Table 1).

An exemplary method for detecting the amount or activity of a biomarker listed in Table 1, and thus useful for classifying whether a sample is likely or unlikely to respond to anti-immune checkpoint inhibitor therapy involves obtaining a biological sample from a test subject and contacting the biological sample with an agent, such as a protein-binding agent like an antibody or antigen-binding fragment thereof, or a nucleic acid-binding agent

like an oligonucleotide, capable of detecting the amount or activity of the biomarker in the biological sample. In some embodiments, at least one antibody or antigen-binding fragment thereof is used, wherein two, three, four, five, six, seven, eight, nine, ten, or more such antibodies or antibody fragments can be used in combination (*e.g.*, in sandwich ELISAs) or in serial. In certain instances, the statistical algorithm is a single learning statistical classifier system. For example, a single learning statistical classifier system can be used to classify a sample as a based upon a prediction or probability value and the presence or level of the biomarker. The use of a single learning statistical classifier system typically classifies the sample as, for example, a likely anti-immune checkpoint inhibitor therapy responder or progressor sample with a sensitivity, specificity, positive predictive value, negative predictive value, and/or overall accuracy of at least about 75%, 76%, 77%, 78%, 79%, 80%, 81%, 82%, 83%, 84%, 85%, 86%, 87%, 88%, 89%, 90%, 91%, 92%, 93%, 94%, 95%, 96%, 97%, 98%, or 99%.

Other suitable statistical algorithms are well known to those of skill in the art. For example, learning statistical classifier systems include a machine learning algorithmic technique capable of adapting to complex data sets (*e.g.*, panel of markers of interest) and making decisions based upon such data sets. In some embodiments, a single learning statistical classifier system such as a classification tree (*e.g.*, random forest) is used. In other embodiments, a combination of 2, 3, 4, 5, 6, 7, 8, 9, 10, or more learning statistical classifier systems are used, preferably in tandem. Examples of learning statistical classifier systems include, but are not limited to, those using inductive learning (*e.g.*, decision/classification trees such as random forests, classification and regression trees (C&RT), boosted trees, etc.), Probably Approximately Correct (PAC) learning, connectionist learning (*e.g.*, neural networks (NN), artificial neural networks (ANN), neuro fuzzy networks (NFN), network structures, perceptrons such as multi-layer perceptrons, multi-layer feed-forward networks, applications of neural networks, Bayesian learning in belief networks, etc.), reinforcement learning (*e.g.*, passive learning in a known environment such as naive learning, adaptive dynamic learning, and temporal difference learning, passive learning in an unknown environment, active learning in an unknown environment, learning action-value functions, applications of reinforcement learning, etc.), and genetic algorithms and evolutionary programming. Other learning statistical classifier systems include support vector machines (*e.g.*, Kernel methods), multivariate adaptive regression splines (MARS), Levenberg-Marquardt algorithms, Gauss-Newton algorithms,

mixtures of Gaussians, gradient descent algorithms, and learning vector quantization (LVQ). In certain embodiments, the method of the present invention further comprises sending the sample classification results to a clinician, *e.g.*, an oncologist.

In another embodiment, the diagnosis of a subject is followed by administering to the individual a therapeutically effective amount of a defined treatment based upon the diagnosis.

In one embodiment, the methods further involve obtaining a control biological sample (*e.g.*, biological sample from a subject who does not have a cancer or whose cancer is susceptible to anti-immune checkpoint inhibitor therapy), a biological sample from the subject during remission, or a biological sample from the subject during treatment for developing a cancer progressing despite anti-immune checkpoint inhibitor therapy.

d. Prognostic Assays

The diagnostic methods described herein can furthermore be utilized to identify subjects having or at risk of developing a cancer that is likely or unlikely to be responsive to anti-immune checkpoint inhibitor therapy. The assays described herein, such as the preceding diagnostic assays or the following assays, can be utilized to identify a subject having or at risk of developing a disorder associated with a misregulation of the amount or activity of at least one biomarker described in Table 1, such as in cancer. Alternatively, the prognostic assays can be utilized to identify a subject having or at risk for developing a disorder associated with a misregulation of the at least one biomarker described in Table 1, such as in cancer. Furthermore, the prognostic assays described herein can be used to determine whether a subject can be administered an agent (*e.g.*, an agonist, antagonist, peptidomimetic, polypeptide, peptide, nucleic acid, small molecule, or other drug candidate) to treat a disease or disorder associated with the aberrant biomarker expression or activity.

e. Treatment Methods

The compositions described herein (including dual binding antibodies and derivatives and conjugates thereof) can be used in a variety of *in vitro* and *in vivo* therapeutic applications using the formulations and/or combinations described herein. In one embodiment, anti-immune checkpoint inhibitor agents can be used to treat cancers determined to be responsive thereto. For example, antibodies that block the interaction between PD-L1, PD-L2, and/or CTLA-4 and their receptors (*e.g.*, PD-L1 binding to PD-1,

PD-L2 binding to PD-1, and the like) can be used to treat cancer in subjects identified as likely responding thereto.

6. Pharmaceutical Compositions

In another aspect, the present invention provides pharmaceutically acceptable compositions which comprise a therapeutically-effective amount of an agent that modulates biomarker expression and/or activity (*e.g.*, increases Jak kinase activity and/or decreases the activity of Jak kinase inhibitors), one or more anti-immune checkpoint inhibitors, or a combination thereof, formulated together with one or more pharmaceutically acceptable carriers (additives) and/or diluents. As described in detail below, the pharmaceutical compositions of the present invention may be specially formulated for administration in solid or liquid form, including those adapted for the following: (1) oral administration, for example, drenches (aqueous or non-aqueous solutions or suspensions), tablets, boluses, powders, granules, pastes; (2) parenteral administration, for example, by subcutaneous, intramuscular or intravenous injection as, for example, a sterile solution or suspension; (3) topical application, for example, as a cream, ointment or spray applied to the skin; (4) intravaginally or intrarectally, for example, as a pessary, cream or foam; or (5) aerosol, for example, as an aqueous aerosol, liposomal preparation or solid particles containing the compound.

The phrase "therapeutically-effective amount" as used herein means that amount of an agent that modulates biomarker expression and/or activity, or expression and/or activity of the complex, or composition comprising an agent that modulates biomarker expression and/or activity, or expression and/or activity of the complex, which is effective for producing some desired therapeutic effect, *e.g.*, cancer treatment, at a reasonable benefit/risk ratio.

The phrase "pharmaceutically acceptable" is employed herein to refer to those agents, materials, compositions, and/or dosage forms which are, within the scope of sound medical judgment, suitable for use in contact with the tissues of human beings and animals without excessive toxicity, irritation, allergic response, or other problem or complication, commensurate with a reasonable benefit/risk ratio.

The phrase "pharmaceutically-acceptable carrier" as used herein means a pharmaceutically-acceptable material, composition or vehicle, such as a liquid or solid filler, diluent, excipient, solvent or encapsulating material, involved in carrying or

transporting the subject chemical from one organ, or portion of the body, to another organ, or portion of the body. Each carrier must be "acceptable" in the sense of being compatible with the other ingredients of the formulation and not injurious to the subject. Some examples of materials which can serve as pharmaceutically-acceptable carriers include: (1) sugars, such as lactose, glucose and sucrose; (2) starches, such as corn starch and potato starch; (3) cellulose, and its derivatives, such as sodium carboxymethyl cellulose, ethyl cellulose and cellulose acetate; (4) powdered tragacanth; (5) malt; (6) gelatin; (7) talc; (8) excipients, such as cocoa butter and suppository waxes; (9) oils, such as peanut oil, cottonseed oil, safflower oil, sesame oil, olive oil, corn oil and soybean oil; (10) glycols, such as propylene glycol; (11) polyols, such as glycerin, sorbitol, mannitol and polyethylene glycol; (12) esters, such as ethyl oleate and ethyl laurate; (13) agar; (14) buffering agents, such as magnesium hydroxide and aluminum hydroxide; (15) alginic acid; (16) pyrogen-free water; (17) isotonic saline; (18) Ringer's solution; (19) ethyl alcohol; (20) phosphate buffer solutions; and (21) other non-toxic compatible substances employed in pharmaceutical formulations.

The term "pharmaceutically-acceptable salts" refers to the relatively non-toxic, inorganic and organic acid addition salts of the agents that modulates biomarker expression and/or activity, or expression and/or activity of the complex encompassed by the invention. These salts can be prepared *in situ* during the final isolation and purification of the agents, or by separately reacting a purified agent in its free base form with a suitable organic or inorganic acid, and isolating the salt thus formed. Representative salts include the hydrobromide, hydrochloride, sulfate, bisulfate, phosphate, nitrate, acetate, valerate, oleate, palmitate, stearate, laurate, benzoate, lactate, phosphate, tosylate, citrate, maleate, fumarate, succinate, tartrate, napthylate, mesylate, glucoheptonate, lactobionate, and laurylsulphonate salts and the like (See, for example, Berge *et al.* (1977) "Pharmaceutical Salts", *J. Pharm. Sci.* 66:1-19).

In other cases, the agents useful in the methods of the present invention may contain one or more acidic functional groups and, thus, are capable of forming pharmaceutically-acceptable salts with pharmaceutically-acceptable bases. The term "pharmaceutically-acceptable salts" in these instances refers to the relatively non-toxic, inorganic and organic base addition salts of agents that modulates biomarker expression and/or activity, or expression and/or activity of the complex. These salts can likewise be prepared in situ during the final isolation and purification of the agents, or by separately reacting the

purified agent in its free acid form with a suitable base, such as the hydroxide, carbonate or bicarbonate of a pharmaceutically-acceptable metal cation, with ammonia, or with a pharmaceutically-acceptable organic primary, secondary or tertiary amine. Representative alkali or alkaline earth salts include the lithium, sodium, potassium, calcium, magnesium, and aluminum salts and the like. Representative organic amines useful for the formation of base addition salts include ethylamine, diethylamine, ethylenediamine, ethanolamine, diethanolamine, piperazine and the like (see, for example, Berge *et al.*, *supra*).

Wetting agents, emulsifiers and lubricants, such as sodium lauryl sulfate and magnesium stearate, as well as coloring agents, release agents, coating agents, sweetening, flavoring and perfuming agents, preservatives and antioxidants can also be present in the compositions.

Examples of pharmaceutically-acceptable antioxidants include: (1) water soluble antioxidants, such as ascorbic acid, cysteine hydrochloride, sodium bisulfate, sodium metabisulfite, sodium sulfite and the like; (2) oil-soluble antioxidants, such as ascorbyl palmitate, butylated hydroxyanisole (BHA), butylated hydroxytoluene (BHT), lecithin, propyl gallate, alpha-tocopherol, and the like; and (3) metal chelating agents, such as citric acid, ethylenediamine tetraacetic acid (EDTA), sorbitol, tartaric acid, phosphoric acid, and the like.

Formulations useful in the methods of the present invention include those suitable for oral, nasal, topical (including buccal and sublingual), rectal, vaginal, aerosol and/or parenteral administration. The formulations may conveniently be presented in unit dosage form and may be prepared by any methods well known in the art of pharmacy. The amount of active ingredient which can be combined with a carrier material to produce a single dosage form will vary depending upon the host being treated, the particular mode of administration. The amount of active ingredient, which can be combined with a carrier material to produce a single dosage form will generally be that amount of the compound which produces a therapeutic effect. Generally, out of one hundred per cent, this amount will range from about 1 per cent to about ninety-nine percent of active ingredient, preferably from about 5 per cent to about 70 per cent, most preferably from about 10 per cent to about 30 per cent.

Methods of preparing these formulations or compositions include the step of bringing into association an agent that modulates biomarker expression and/or activity, with the carrier and, optionally, one or more accessory ingredients. In general, the formulations

are prepared by uniformly and intimately bringing into association a agent with liquid carriers, or finely divided solid carriers, or both, and then, if necessary, shaping the product.

Formulations suitable for oral administration may be in the form of capsules, cachets, pills, tablets, lozenges (using a flavored basis, usually sucrose and acacia or tragacanth), powders, granules, or as a solution or a suspension in an aqueous or non-aqueous liquid, or as an oil-in-water or water-in-oil liquid emulsion, or as an elixir or syrup, or as pastilles (using an inert base, such as gelatin and glycerin, or sucrose and acacia) and/or as mouth washes and the like, each containing a predetermined amount of a agent as an active ingredient. A compound may also be administered as a bolus, electuary or paste.

In solid dosage forms for oral administration (capsules, tablets, pills, dragees, powders, granules and the like), the active ingredient is mixed with one or more pharmaceutically-acceptable carriers, such as sodium citrate or dicalcium phosphate, and/or any of the following: (1) fillers or extenders, such as starches, lactose, sucrose, glucose, mannitol, and/or silicic acid; (2) binders, such as, for example, carboxymethylcellulose, alginates, gelatin, polyvinyl pyrrolidone, sucrose and/or acacia; (3) humectants, such as glycerol; (4) disintegrating agents, such as agar-agar, calcium carbonate, potato or tapioca starch, alginic acid, certain silicates, and sodium carbonate; (5) solution retarding agents, such as paraffin; (6) absorption accelerators, such as quaternary ammonium compounds; (7) wetting agents, such as, for example, acetyl alcohol and glycerol monostearate; (8) absorbents, such as kaolin and bentonite clay; (9) lubricants, such a talc, calcium stearate, magnesium stearate, solid polyethylene glycols, sodium lauryl sulfate, and mixtures thereof; and (10) coloring agents. In the case of capsules, tablets and pills, the pharmaceutical compositions may also comprise buffering agents. Solid compositions of a similar type may also be employed as fillers in soft and hard-filled gelatin capsules using such excipients as lactose or milk sugars, as well as high molecular weight polyethylene glycols and the like.

A tablet may be made by compression or molding, optionally with one or more accessory ingredients. Compressed tablets may be prepared using binder (for example, gelatin or hydroxypropylmethyl cellulose), lubricant, inert diluent, preservative, disintegrant (for example, sodium starch glycolate or cross-linked sodium carboxymethyl cellulose), surface-active or dispersing agent. Molded tablets may be made by molding in a suitable machine a mixture of the powdered peptide or peptidomimetic moistened with an inert liquid diluent.

Tablets, and other solid dosage forms, such as dragees, capsules, pills and granules, may optionally be scored or prepared with coatings and shells, such as enteric coatings and other coatings well known in the pharmaceutical-formulating art. They may also be formulated so as to provide slow or controlled release of the active ingredient therein using, for example, hydroxypropylmethyl cellulose in varying proportions to provide the desired release profile, other polymer matrices, liposomes and/or microspheres. They may be sterilized by, for example, filtration through a bacteria-retaining filter, or by incorporating sterilizing agents in the form of sterile solid compositions, which can be dissolved in sterile water, or some other sterile injectable medium immediately before use. These compositions may also optionally contain opacifying agents and may be of a composition that they release the active ingredient(s) only, or preferentially, in a certain portion of the gastrointestinal tract, optionally, in a delayed manner. Examples of embedding compositions, which can be used include polymeric substances and waxes. The active ingredient can also be in micro-encapsulated form, if appropriate, with one or more of the above-described excipients.

Liquid dosage forms for oral administration include pharmaceutically acceptable emulsions, microemulsions, solutions, suspensions, syrups and elixirs. In addition to the active ingredient, the liquid dosage forms may contain inert diluents commonly used in the art, such as, for example, water or other solvents, solubilizing agents and emulsifiers, such as ethyl alcohol, isopropyl alcohol, ethyl carbonate, ethyl acetate, benzyl alcohol, benzyl benzoate, propylene glycol, 1,3-butylene glycol, oils (in particular, cottonseed, groundnut, corn, germ, olive, castor and sesame oils), glycerol, tetrahydrofuryl alcohol, polyethylene glycols and fatty acid esters of sorbitan, and mixtures thereof.

Besides inert diluents, the oral compositions can also include adjuvants such as wetting agents, emulsifying and suspending agents, sweetening, flavoring, coloring, perfuming and preservative agents.

Suspensions, in addition to the active agent may contain suspending agents as, for example, ethoxylated isostearyl alcohols, polyoxyethylene sorbitol and sorbitan esters, microcrystalline cellulose, aluminum metahydroxide, bentonite, agar-agar and tragacanth, and mixtures thereof.

Formulations for rectal or vaginal administration may be presented as a suppository, which may be prepared by mixing one or more agents with one or more suitable nonirritating excipients or carriers comprising, for example, cocoa butter, polyethylene

glycol, a suppository wax or a salicylate, and which is solid at room temperature, but liquid at body temperature and, therefore, will melt in the rectum or vaginal cavity and release the active agent.

Formulations which are suitable for vaginal administration also include pessaries, tampons, creams, gels, pastes, foams or spray formulations containing such carriers as are known in the art to be appropriate.

Dosage forms for the topical or transdermal administration of an agent that modulates (*e.g.*, inhibits) biomarker expression and/or activity include powders, sprays, ointments, pastes, creams, lotions, gels, solutions, patches and inhalants. The active component may be mixed under sterile conditions with a pharmaceutically-acceptable carrier, and with any preservatives, buffers, or propellants which may be required.

The ointments, pastes, creams and gels may contain, in addition to a agent, excipients, such as animal and vegetable fats, oils, waxes, paraffins, starch, tragacanth, cellulose derivatives, polyethylene glycols, silicones, bentonites, silicic acid, talc and zinc oxide, or mixtures thereof.

Powders and sprays can contain, in addition to an agent that modulates (*e.g.*, inhibits) biomarker expression and/or activity, excipients such as lactose, talc, silicic acid, aluminum hydroxide, calcium silicates and polyamide powder, or mixtures of these substances. Sprays can additionally contain customary propellants, such as chlorofluorohydrocarbons and volatile unsubstituted hydrocarbons, such as butane and propane.

The agent that modulates (*e.g.*, inhibits) biomarker expression and/or activity, can be alternatively administered by aerosol. This is accomplished by preparing an aqueous aerosol, liposomal preparation or solid particles containing the compound. A nonaqueous (*e.g.*, fluorocarbon propellant) suspension could be used. Sonic nebulizers are preferred because they minimize exposing the agent to shear, which can result in degradation of the compound.

Ordinarily, an aqueous aerosol is made by formulating an aqueous solution or suspension of the agent together with conventional pharmaceutically acceptable carriers and stabilizers. The carriers and stabilizers vary with the requirements of the particular compound, but typically include nonionic surfactants (Tweens, Pluronics, or polyethylene glycol), innocuous proteins like serum albumin, sorbitan esters, oleic acid, lecithin, amino

acids such as glycine, buffers, salts, sugars or sugar alcohols. Aerosols generally are prepared from isotonic solutions.

Transdermal patches have the added advantage of providing controlled delivery of a agent to the body. Such dosage forms can be made by dissolving or dispersing the agent in the proper medium. Absorption enhancers can also be used to increase the flux of the peptidomimetic across the skin. The rate of such flux can be controlled by either providing a rate controlling membrane or dispersing the peptidomimetic in a polymer matrix or gel.

Ophthalmic formulations, eye ointments, powders, solutions and the like, are also contemplated as being within the scope of this invention.

Pharmaceutical compositions of this invention suitable for parenteral administration comprise one or more agents in combination with one or more pharmaceutically-acceptable sterile isotonic aqueous or nonaqueous solutions, dispersions, suspensions or emulsions, or sterile powders which may be reconstituted into sterile injectable solutions or dispersions just prior to use, which may contain antioxidants, buffers, bacteriostats, solutes which render the formulation isotonic with the blood of the intended recipient or suspending or thickening agents.

Examples of suitable aqueous and nonaqueous carriers which may be employed in the pharmaceutical compositions of the invention include water, ethanol, polyols (such as glycerol, propylene glycol, polyethylene glycol, and the like), and suitable mixtures thereof, vegetable oils, such as olive oil, and injectable organic esters, such as ethyl oleate. Proper fluidity can be maintained, for example, by the use of coating materials, such as lecithin, by the maintenance of the required particle size in the case of dispersions, and by the use of surfactants.

These compositions may also contain adjuvants such as preservatives, wetting agents, emulsifying agents and dispersing agents. Prevention of the action of microorganisms may be ensured by the inclusion of various antibacterial and antifungal agents, for example, paraben, chlorobutanol, phenol sorbic acid, and the like. It may also be desirable to include isotonic agents, such as sugars, sodium chloride, and the like into the compositions. In addition, prolonged absorption of the injectable pharmaceutical form may be brought about by the inclusion of agents which delay absorption such as aluminum monostearate and gelatin.

In some cases, in order to prolong the effect of a drug, it is desirable to slow the absorption of the drug from subcutaneous or intramuscular injection. This may be

accomplished by the use of a liquid suspension of crystalline or amorphous material having poor water solubility. The rate of absorption of the drug then depends upon its rate of dissolution, which, in turn, may depend upon crystal size and crystalline form. Alternatively, delayed absorption of a parenterally-administered drug form is accomplished by dissolving or suspending the drug in an oil vehicle.

Injectable depot forms are made by forming microencapsule matrices of an agent that modulates biomarker expression and/or activity, in biodegradable polymers such as polylactide-polyglycolide. Depending on the ratio of drug to polymer, and the nature of the particular polymer employed, the rate of drug release can be controlled. Examples of other biodegradable polymers include poly(orthoesters) and poly(anhydrides). Depot injectable formulations are also prepared by entrapping the drug in liposomes or microemulsions, which are compatible with body tissue.

When the agents of the present invention are administered as pharmaceuticals, to humans and animals, they can be given per se or as a pharmaceutical composition containing, for example, 0.1 to 99.5% (more preferably, 0.5 to 90%) of active ingredient in combination with a pharmaceutically acceptable carrier.

Actual dosage levels of the active ingredients in the pharmaceutical compositions of this invention may be determined by the methods of the present invention so as to obtain an amount of the active ingredient, which is effective to achieve the desired therapeutic response for a particular subject, composition, and mode of administration, without being toxic to the subject.

The nucleic acid molecules of the invention can be inserted into vectors and used as gene therapy vectors. Gene therapy vectors can be delivered to a subject by, for example, intravenous injection, local administration (see U.S. Pat. No. 5,328,470) or by stereotactic injection (see *e.g.*, Chen *et al.* (1994) *Proc. Natl. Acad. Sci. USA* 91:3054 3057). The pharmaceutical preparation of the gene therapy vector can include the gene therapy vector in an acceptable diluent, or can comprise a slow release matrix in which the gene delivery vehicle is imbedded. Alternatively, where the complete gene delivery vector can be produced intact from recombinant cells, *e.g.*, retroviral vectors, the pharmaceutical preparation can include one or more cells which produce the gene delivery system.

The present invention also encompasses kits for detecting and/or modulating biomarkers described herein. A kit of the present invention may also include instructional materials disclosing or describing the use of the kit or an antibody of the disclosed

invention in a method of the disclosed invention as provided herein. A kit may also include additional components to facilitate the particular application for which the kit is designed. For example, a kit may additionally contain means of detecting the label (*e.g.*, enzyme substrates for enzymatic labels, filter sets to detect fluorescent labels, appropriate secondary labels such as a sheep anti-mouse-HRP, *etc.*) and reagents necessary for controls (*e.g.*, control biological samples or metabolite standards). A kit may additionally include buffers and other reagents recognized for use in a method of the disclosed invention. Non-limiting examples include agents to reduce non-specific binding, such as a carrier protein or a detergent.

Other embodiments of the present invention are described in the following Examples. The present invention is further illustrated by the following examples which should not be construed as further limiting.

EXAMPLES

Example 1: Materials and Methods for Examples 2-4

a. Subject

The programmed cell death-1 (PD-1) protein is a co-inhibitory receptor that restrains immune signaling by inhibiting T cell function. Tumors that express its major inducible ligand, PD-L1, evade immunosurveillance by engaging the PD-1 immune checkpoint (Dong *et al.* (2002) *Nat. Med.* 8:793-800; Freeman *et al.* (2000) *J. Exp. Med.* 192:1027-1034). In preclinical models, blockade of PD-L1 interaction with PD-1 promotes immune-mediated antitumor activity (Iwai *et al.* (2002) *Proc. Natl. Acad. Sci. U.S.A.* 99:12293-12297). Clinical trials of PD-1 and PD-L1 inhibitors have uncovered durable tumor regression in a subset of patients with a variety of aggressive cancers (Brahmer *et al.* (2012) *New Engl. J. Med.* 366:2455-2465; Topalian *et al.* (2012) *New Engl. J. Med.* 366:2443-2454; Ansell *et al.* (2015) *New Engl. J. Med.* 372:311-319; Powles *et al.* (2014) *Nature* 515:558-562). Although studies have suggested that tumors PD-L1 expression in tumors or tumor infiltrating immune cells (Herbst *et al.* (2014) *Nature* 515:563-567) appear more likely to respond to immune checkpoint inhibition, the specific determinants of this enhanced responsiveness remain incompletely characterized.

Identifying genomic mechanisms of inhibitor sensitivity may inform patient selection for agents targeting immune checkpoints and suggest approaches to enhance their efficacy in otherwise resistant patients. Comprehensive genomic profiling of exceptional

responders has revealed the genomic mechanism of extraordinary response to targeted therapies (Iyer *et al.* (2012) *Science* 338:221; Al-Ahmadie *et al.* (2014) *Cancer Disc.* 4:1014-1021; Imielinski *et al.* (2014) *J. Clin. Invest.* 124:1582-1586; Wagle *et al.* (2014) *New Engl. J. Med.* 371:1426-1433; Wagle *et al.* (2014) *Cancer Disc.* 4:546-553), but has not yet been applied to immunotherapies.

A 57 year old male with an 40 pack-year smoking history presented with left shoulder discomfort. Magnetic resonance imaging (MRI) revealed a 1 x 1.4 x 2 cm lytic lesion in the left humeral head. A computed tomography (CT)-guided biopsy of this lesion was obtained, which demonstrated CK7 and TTF-1 positive adenocarcinoma suggestive of primary lung origin and lung cancer. Subsequent CT of the chest demonstrated a 4 x 3.3 x 2 cm mass in the left apex of the lung. PET confirmed that this mass was FDG-avid, and there was left paratracheal lymphadenopathy and lytic metastasis in the proximal left humerus. Brain MRI revealed four small solid enhancing lesions consistent with additional metastatic disease. Thus, the patient was diagnosed with Stage IV metastatic lung adenocarcinoma.

The patient received palliative radiation therapy to the left shoulder and whole brain radiation therapy, followed by a single cycle of carboplatin and paclitaxel, which he tolerated poorly (Figure 1A). He then developed a perirectal abscess and was switched to dose-reduced carboplatin and pemetrexed, together with bevacizumab for three additional cycles and was transitioned to maintenance pemetrexed and bevacizumab.

After 8 months of maintenance therapy, the restaging CT scans demonstrated growth of a left adrenal mass. Laparoscopic left adrenalectomy was performed for palliation of severe flank pain and to obtain tissue for further genetic and immunohistochemical (IHC) testing. Initial clinical testing for oncogenic alterations revealed non-mutated wild-type *EGFR*, *KRAS*, and *ALK*. Three months later the patient developed a new right adrenal mass and worsening mediastinal lymphadenopathy (recurrence of the left paratracheal lymphadenopathy). Hospice was considered in the setting of worsening pain and weight loss (Figure 1B). Immunohistochemistry (IHC) performed on the excised left adrenal tumor demonstrated PD-L1 reactivity, prompting enrollment on Dana-Farber/Harvard Cancer Center (DF/HCC) clinical trial 11-314, a phase I study of MPDL3280A, an engineered anti-PDL1 antibody.

The patient provided written informed consent for research biopsies, genomic profiling, and sequencing of tumor and normal DNA, as approved by the Dana-Farber/Harvard Cancer Center Institutional Review Board (DF/HCC Protocol 11-104).

The patient also consented to enroll on a phase 1 multicenter open-label dose-escalation study to evaluate the safety, tolerability, and pharmacokinetics of MPDL3280A (DF/HCC Protocol 11-314; NCT# 01375842). He met all eligibility criteria, which included histologically or cytologically documented incurable or metastatic solid malignancy that failed to respond to available standard therapy; there was measurable disease by RECIST; ECOG performance status was 1; brain metastasis had been treated and was stable; laboratory testing was within protocol parameters; there was no history of autoimmune disease or evidence of active pneumonitis; no prior anti-CTLA4, anti-PD1, or anti-PD-L1 therapy; no intercurrent infection or illness; no history of hypersensitivity to chimeric or humanized antibodies; and steroids were held for 4 weeks prior to starting. As part of the inclusion criteria, his tumor was also tested for PD-L1 expression and found to be positive. The drug was administered as a single agent at a dose of 20 mg/kg every 3 weeks. After cycle 2 was delayed for 3 weeks due to a series of falls and hospital admissions, he gradually improved and received 16 cycles total. Therapy was tolerated well other than a mild flare of his underlying chronic obstructive pulmonary disease that responded to an albuterol inhaler daily. Tumor response and evaluation was performed by physical examination and serial chest/abdomen/pelvic CT scans, and target lesions were evaluated using RECIST criteria. Following treatment discontinuation at 1 year, the patient has continued to be monitored every 3 months by examination and CT scans.

Thus, after a temporary decline, the patient subsequently received sixteen cycles of MPDL3280A over a one-year period. He achieved a partial response by RECIST criteria (Figure 1C), and, significantly, he experienced complete resolutions of his symptoms, discontinuation of all narcotic pain medication, and return to pre-diagnosis body weight. He completed one year of therapy per protocol and remained without evidence of disease progression for an additional 12 months. At this point, he began to lose weight again and developed regrowth of the right adrenal mass (Figure 1D), leading to re-initiation of MPDL3280A therapy. Restaging scans after another 3 months of MPDL3280A showed rapid improvement of the right adrenal lesion (Figure 1D).

Given his extraordinary and repeated response to PD-L1 immune checkpoint blockade, comprehensive genomic profiling of the patient's tumor and germline sample was

performed. A blood sample was obtained from the patient for germline testing after the complete response was identified, and whole blood was stored at -80°C until DNA extraction was performed. Tumor specimens for PD-L1 immunohistochemistry and blood samples were obtained from additional patients consented through DF/HCC Protocol 11-104. Normal blood donor samples were accessed through DF/HCC Protocol 10-145.

b. Histology and staining

Histopathologic analysis of the surgically resected left adrenal gland revealed metastatic adenocarcinoma, consistent with metastasis from the patient's lung primary. Surgical resection margins were negative for tumor and the tumor was confined by the adrenal capsule. The tumor showed approximately 10% necrosis and fibrosis consistent with partial treatment effect. Immunohistochemistry (IHC) for tumor PD-L1 expression was performed as described in Chen *et al.* (2013) *Clin. Cancer Res.* 19:3462-3473, Choueiri *et al.* (2014) *Annal. Oncol.* 25:2178-2184, and Shi *et al.* (2014) *Amer. J. Surg. Pathol.* 38:1715-1723. In brief, PD-L1 (9A11, 1:125) was performed on the Leica BOND III platform using Bond's Polymer Refine Detection kit. Heat-induced antigen retrieval was performed in Bond ER2 solution for 30 minutes online. Sections were incubated at room temperature in primary antibody for 120 minutes at room temperature. Upon staining completion, slides were dehydrated and coverslipped offline. IHC interpretation was blinded to JAK3 V722I or P132T status when assessing PD-L1 stain. A randomly control set of 9 lung cancers identified by an independent pathologist was stained in parallel to determine relative enrichment. Scoring was performed by measuring the average number of positive cells in a given sample and the average intensity of staining (0 = no staining, 1+ = weak, 2+ = moderate, 3+ = intense positive staining, with all positive staining considered over background). Determination of statistically significant differences between the groups was performed by calculating an adjusted expression (H) score (% positive cells x staining intensity) (Choueiri *et al.* (2014) *Annal. Oncol.* 25:2178-2184; Azuma *et al.* (2014) *Annal. Oncol.* 25:1935-1940). Macrophage cells were identified through morphologic determination in intratumoral or alveolar spaces.

c. Site-directed sequencing

Standard techniques were utilized to extract genomic DNA from tumor within the left adrenalectomy specimen and from blood. Initial sequencing of tumor DNA was performed using the OncoMap assay, which detects mutations in 41 cancer genes at 471

different loci using multiplex PCR to amplify the region containing the variant of interest (MacConail *et al.* (2009) *PLoS One* 4: e7887). Following primer extension of the allele-specific DNA products, DNA analyses were measured using chip-based mass spectrometry (Sequenom MassARRAY 4). Additional tumor samples that harbored variants of interest in JAK3 were identified through the Dana-Farber Cancer Institute (DFCI) PROFILE project using the OncoMap assay, which included V722I and P132T variants. All lung cancer tumor samples from patients who provided written informed consent (DF/HCC Protocol 11-104) with adequate tumor tissue who underwent OncoMap assay testing done between 2010 and 2013 were included in the query. The OncDRS clinical-genomics database system that links genomic data from PROFILE with salient clinical annotations was utilized to provide a listing of patients with lung cancers who harbored *JAK3* variants of interest, and all such patients with available tumor samples were studied with immunohistochemistry for PD-L1, as described above. These samples were then obtained for additional analyses, including histology and staining for PD-L1 described above.

d. Whole exome sequencing

In summary genomic DNA was sheared, end repaired, ligated with barcoded Illumina sequencing adapters, amplified, size selected and subjected to in solution hybrid capture using the Agilent SureSelect® Human All Exon v2.0 bait set (19, 20). Resulting exome Illumina sequencing libraries were then qPCR quantified, pooled, and sequenced with 76 base paired-end reads using HiSeq2500 sequencers (Illumina, USA). Raw BAM files are deposited in phs000694.v1.p1.

Sequence data processing and quality control: Exome sequence data processing was performed using established analytical pipelines at the Broad Institute. Tumor and normal sequences were aligned to the hg19 human genome build from Illumina sequencing reads using the Picard pipeline (available on the World Wide Web at picard.sourceforge.net/). The BAM was uploaded into Firehose (available on the World Wide Web at www.broadinstitute.org/cancer/cga/Firehose), which manages input and output files. Comparison of the origin for tumor and normal genotypes was performed to assess fingerprinting concordance, and cross-contamination of samples was estimated using ContEst (Cibulskis *et al.* (2011) *Bioinform.* 27:2601-2602).

Alteration identification: MuTect (Cibulskis *et al.* (2013) *Nat. Biotech.* 31:213-219) was applied to identify somatic single-nucleotide variants. DNA oxidation artifacts induced during sequencing were computationally removed using a filter-based method (Costello *et*

al. (2013) *Nucl. Acids Res.* 41:e67). Indelocator (available on the World Wide Web at broadinstitute.org/cancer/cga/indelocator) was applied to identify small insertions or deletions. Annotation of identified variants was done using Oncotator (available on the World Wide Web at broadinstitute.org/cancer/cga/oncotator). Copy ratios were calculated for each captured target by dividing the tumor coverage by the median coverage obtained in a set of reference normal samples. The resulting copy ratios were segmented using the circular binary segmentation algorithm. Genes in copy ratio regions with segment means of greater than $\log_2(4)$ were evaluated for focal amplifications, and genes in regions with segment means of less than $\log_2(0.5)$ were evaluated for deletions. Genome wide copy-ratios were estimated from whole-exome sequencing (WES) data by comparison of the observed depth of coverage at each exon to that achieved in normal samples. Allelic copy-ratios were then estimated by analysis of allelic fractions for all heterozygous SNPs identified in the paired normal sample. Purity and ploidy evaluations to derive absolute copy number were made using ABSOLUTE (Carter *et al.* (2012) *Nat. Biotech.* 30:413-421). Heuristic analysis of all somatic alterations was performed using PHIAL (Van Allen *et al.* (2014) *Nat. Med.* 20:682-688). Somatic alterations were manually reviewed using Integrated Genomics Viewer (Robinson *et al.* (2011) *Nat. Biotech.* 29:24-26; Thorvaldsdottir *et al.* (2013) *Brief. Bioinform.* 14:178-92).

e. Experimental analysis

Cell culture: 293T and Calu-1 cells were maintained in DMEM and RPMI 1640 respectively, with 10% FBS. Beas-2B was maintained in keratinocyte SFM, supplemented with human recombinant EGF and BPE (Gibco). All media were supplemented with 1% penicillin/streptomycin.

Plasmids, immunoblotting, flow cytometry: JAK3 mutant alleles were generated from pLX304-JAK3-WT (Broad Institute, TRC) using the Quikchange® Lightning Site-Directed Mutagenesis Kit (Agilent Technologies), and transferred into the pLX304 vector using the Gateway LR Clonase II enzyme mix from Life Technologies. 293T cells were transfected using X-tremeGENE® HP DNA Transfection Reagent (Roche) with pLX304 EGFP, JAK3-WT or JAK3 constructs as described in Zhu *et al.* (2014) *Cancer Discov.* 4:452-465. Lysates were harvested after 48 hours and immunoblotting was also performed according to a standard protocol (Zhu *et al.* (2014) *Cancer Discov.* 4:452-465). JAK3 (#8827) and Y980/981 pJAK3 (#5031) antibodies were from Cell Signaling Technologies. Beas-2B and Calu-1 cells were infected with lentivirus generated from pLX304 empty

control or the same JAK3 constructs and selected in blasticidin to derive stably infected cell lines as described in Zhu *et al.* (2014) *Cancer Discov.* 4:452-465. Flow cytometry for PD-L1 expression was performed 72 hours after plating as described in Akbay *et al.* (2013) *Cancer Discov.* 3:1355-1363. In brief, cells were stained with an anti-PD-L1 antibody (29E.2A3) or isotype control antibody, and levels of PD-L1 expression were quantified using a BD FACSCanto II® flow cytometer equipped with Diva software (BD Biosciences). Levels were compared with isotype control antibodies. PD-L1 mean fluorescence intensity (MFI) was normalized to isotype control. For EGF stimulation cells were incubated with EGF (50 ng/ml) for 72 h prior to FACS analysis.

PBMC isolation and stimulation: Peripheral blood mononuclear cells (PBMCs) from patients identified as having JAK3 V722I or P132T variants (see sequencing section above) and healthy donors were isolated from fresh blood and platelet-depleted blood collars, respectively, by Ficoll method. PBMCs were plated at a density of 7.5×10^5 cells/ml in a 48-well plate and stimulated with 250 ng/ml IFNγ (PBL Interferon Source). No stimulation controls were set-up for each donor. At 48 h post-stimulation, cells were treated with 2mM EDTA and collected for flow cytometry to assess for PD-L1 expression on CD14+ myeloid cells. Cells were stained with Live/Dead yellow viability dye (Invitrogen), as well as antibodies (BD Biosciences) against CD14 (M5E2), CD11b (ICRF44), and PD-L1 (MIH1) for 30 min at 4C, and then fixed in BD Cytofix® buffer prior to analyses on BD LSR Fortessa SORT® HTS flow cytometer. Given the need to compare the difference between two means in relation to the variation in the data, a t-test was used to compare PD-L1 inducibility between V722I and non-V722I monocytes.

T cell proliferation assay: PBMCs were isolated from patient blood samples immediately pre- and 1 h post-treatment with MPDL3280A, as well as from a normal donor by Ficoll and plated in a density of 4×10^6 cells/ml to a 96-well plate. After 2 h at 37°C non-adherent cells (lymphocyte portion) were removed by pipetting and remaining adherent cells (monocyte portion) were cultured with or without 250 ng/ml IFNγ. At 24 h, non-adherent lymphocytes were labeled with CFSE. Monocytes (IFNγ treated or untreated) were harvested by 5 mM EDTA treatment and resuspended in fresh medium. Both lymphocytes and monocytes were then plated to a 96-well plate pre-coated with 10 μg/ml OKT3 antibody. Proliferation of CD4+T cells and CD8+T cells were monitored at 72 h post-stimulation by CSFE dilution.

Example 2: Genomic profiling identified multiple mutations in JAK3

The only variant observed in the mass spectrometric genotyping panel was *JAK3*^{V722I}. This variant, which is located in the pseudokinase or JH2 domain of JAK3 (Figures 2A and 3A), has been described and functionally characterized as an activating allele in patients with acute megakaryocytic leukemia (Walters *et al.* (2006) *Cancer Cell* 10:65-75), acute lymphoblastic leukemia (Yin *et al.* (2015) *Leuk. Lymph.* epub 01/21/15 doi:10.3109/10428194.2014.957204), and extranodal nasal-type natural killer cell lymphoma (Bouchekioua *et al.* (2014) *Leukem.* 28:338-348). *JAK3*^{V722I} has also been identified in peripheral blood from normal subjects (Riera *et al.* (2011) *Leukem. Lymph.* 52:1742-1750), and the population frequency of this hyperactive germline variant is approximately 1% (Exome Variant Server [cited 2014 June] available on the World Wide Web at evs.gs.washington.edu/EVS/).

Whereas tumor WES revealed neutral copy of the JAK3 locus on chromosome 19 in aggregate (Figures 2B and 4), allele-specific copy number segmentation demonstrated near complete conversion to the mutant allele in this region (Figure 2C and 5). The allelic fraction of the *JAK3*^{V722I} locus was 0.88 (131/149 reads) in the tumor and 0.47 (90/190 reads) in the germline sample, consistent with homozygosity of the *JAK3*^{V722I} allele in the tumor sample (which was 76% pure) and similar to the selection that occurs for activating *JAK2*^{V617F} alleles in myeloproliferative neoplasms (MPNs) (Gonzalez *et al.* (2014) *PLoS One* 9:e86401).

Next, the 1,767 non-synonymous somatic alterations observed in the WES data were ranked for clinical and biological relevance (Figures 6 and 11) (Van Allen *et al.* (2014) *Nat. Med.* 20:682-688). Among the clinically relevant events, a second somatic *JAK3* missense mutation at codon 61 (S->C) was observed and orthogonally validated in tumor DNA with PCR (Figures 2A and 3B). This mutation occurred in the FERM domain and has not been described previously. Since two distinct genomic events were identified in *JAK3* undergoing tumor somatic selection in *cis*, a scenario described in hematologic malignancies (Bergmann *et al.* (2014) *Genes Chrom. Cancer* 53:309-316), and Epstein-Barr Virus (EBV) induces PD-L1 expression via JAK3 (Green *et al.* (2012) *Clin. Cancer Res.* 18:1611-1618), whether these *JAK3* mutations were activating and might contribute to PD-L1 mediated immune checkpoint evasion in lung cancer was determined.

Example 3: Molecular basis of JAK3 auto-activation and PD-L1 overexpression

Constructs that express *JAK3*^{WT}, *JAK3*^{S61C}, *JAK3*^{V722I}, and *JAK3*$^{S61C/V722I}$ were generated, and their activity was compared with an additional known activating JH2-domain mutation (*JAK3*^{R657Q}) (Zhu *et al.* (2014) *Cancer Disc.* 4:452-465), identified as a somatic mutation in squamous cell lung cancer (Cancer Genome Atlas Res. Network (2012) *Nature* 489:519-525). Consistent with the known impact of JH2 domain mutation on relieving JAK3 autoinhibition (Walters *et al.* (2006) *Cancer Cell* 10:65-75), transfection of 293T cells revealed that *JAK3*^{V722I}, *JAK3*$^{S61C/V722I}$ or *JAK3*^{R657Q} overexpression resulted in increased JAK3 autophosphorylation and autoactivation compared with the wild-type control as measured by immunoblotting (Figure 2D). Although levels of *JAK3*^{S61C} phosphorylation did not differ significantly from *JAK3*^{WT}, expression of *JAK3*$^{S61C/V722I}$ caused the highest levels of JAK3 phosphorylation among all mutants, consistent with the positive selection observed in the tumor and cooperative gain of function.

The consequences of stable JAK3 transduction on PD-L1 cell surface expression in immortalized lung epithelial cells (BEAS-2B) and lung cancer cells (Calu-1) was also determined (Figure 7A). Low-level *JAK3*$^{S61C/V722I}$ expression in BEAS-2B cells modestly induced surface PD-L1 by flow cytometry relative to control, as compared to no induction at all in *JAK3*^{WT} expressing cells (Figure 7B). In contrast, 5-fold greater expression of *JAK3* in Calu-1 increased PD-L1 levels more demonstrably, irrespective of the allele (Figure 7).

The consequence of exposure to factors in the lung tumor microenvironment, such as EGF, was also determined since activation of EGFR signaling known to enhance PD-L1 expression in lung cancer (Azuma *et al.* (2014) *Annal. Oncol.* 25:1935-1940; Akbay *et al.* (2013) *Cancer Disc.* 3:1355-1363). Levels of PD-L1 in Calu-1 cells expressing *JAK3*$^{S61C/V722I}$ were as high as control cells stimulated with EGF, and an additive further increase of PD-L1 expression in mutant cells upon EGF exposure was observed (Figure 7C). These findings reveal that JAK3 activation in lung airway and cancer cells induces PD-L1, including wild type kinase, when overexpressed at high levels. Furthermore, activated JAK3 cooperates with factors such as EGF to boost PD-L1 expression even further.

Example 4: Dual impact on the tumor and immune microenvironment

Consistent with these results, IHC of the patient's tumor using a validated antibody (Chen *et al.* (2013) *Clin. Cancer Res.* 19:3462-3473) revealed strong positive membrane PD-L1 expression on both tumor and immune cells (macrophages), coupled with increased nuclear pSTAT3 staining, a marker of JAK pathway activation (Figure 8A). To assess the generalizability of this relationship, 10 out of 500 lung adenocarcinoma patients (2%) previously genotyped for the *JAK3*^{V722I} mutation (MacConaill *et al.* (2014) *J. Mol. Diagn.* 16:660-672) at the institution were identified, including this index case. PD-L1 positivity was observed in tumor cells and more strikingly in macrophages in 9/10 *JAK3*^{V722I} mutated cases (Figure 8B). PD-L1 positivity was substantially enriched as compared to a random control set of lung cancers (tumor cells: $p = 0.02$; immune cells: $p < 0.01$; Mann-Whitney) (Figure 9), including 4 patients carrying the *JAK3*^{P132T} variant, with the exception of high level PD-L1 tumor expression associated with an ALK rearrangement and another tumor with high level PD-L1 expression in macrophages (Figure 8B).

Because of the strong activation in the immune compartment, and the presence of *JAK3*^{V722I} in the germline, the inducibility of PD-L1 expression in available matched patient PBMCs was determined. Stimulation with IFN-γ, another cytokine known to trigger PD-L1 in the tumor immune microenvironment, resulted in modest but significantly increased expression of PD-L1 on CD14+ myeloid cells from *JAK3*^{V722I} positive patients compared to a *JAK3*^{P132T} positive patient or negative blood donor controls (Figure 8C). Next, to determine if this increased PD-L1 expression directly inhibits T cells, blood from the index patient immediately pre- and 1 h post- MPDL3280A infusion was collected and monocytes from these samples were exposed to the patient's own activated T cells, or from allogeneic T cells from a different donor. In both instances, it was found that T cell activation was significantly greater in the presence of circulating MPDL3280A, especially when monocytes were primed with IFNγ (Figures 8D and 10). This enhanced T cell activity correlated with the clinical response that was observed upon MPDL3280A rechallenge (Figure 1D). Thus, monocytes/macrophages that carry the *JAK3*^{V722I} allele also express increased levels of PD-L1, which can contribute directly to T-cell suppression.

Taken together, these findings indicate that, in addition to somatic alteration in lung cancer cells, germline expression of the *JAK3*^{V722I} allele in infiltrating immune cells represents a key contributor of PD-L1 tumor immune checkpoint engagement.

Immune targeting of the PD-L1/PD1 interaction is emerging as an effective therapy for multiple aggressive tumor types, including non-small cell lung cancer (Topalian *et al.* (2012) *New Engl. J. Med.* 366:2443-2454), and results in occasional long-term responses. While tumor or immune cell PD-L1 expression may indicate a suppressed immune microenvironment and enrich for clinical activity (Taube *et al.* (2014) *Clin. Cancer Res.* 20:5064-5074), the molecular basis and markers of response remain unclear.

A patient with metastatic lung adenocarcinoma who experienced an exceptional and durable response to PD-L1 inhibition was genomically characterized. One germline *JAK3* variant and one somatic *JAK3* mutation was determined in the patient's tumor in *cis*, and it was demonstrated that these genetic alterations act in concert to activate JAK3. Stable transduction of this double-mutant increased PD-L1 expression in lung cells. Furthermore, the presence of $JAK3^{V722I}$ in the germline, the strong tumor immune cell PD-L1 positivity, and the enhanced PD-L1 induction by IFN-γ in monocytes, which inhibits T cell activation in an MPDL3280A sensitive manner, also indicate a more complex interaction with the tumor microenvironment. It is believed that this is the first report that illustrates how a genomic mechanism that impacts both tumor cells and the host response enhances PD-L1 expression and immune evasion by engaging the PD1 immune checkpoint.

Multiple reports have identified candidate mechanisms that may predict response to immune checkpoint blockade. These may include high levels of tumor-specific neoantigens (Brown *et al.* (2014) *Genome Res.* 24:743-750; Snyder *et al.* (2014) *New Engl. J. Med.* 371:2189-2199) or inherited immune-related characteristics (Breunis *et al.* (2008) *J. Immunother.* 31:586-590). Notably, JAK3 signaling regulates EBV mediated PD-L1 expression in lymphomas (Green *et al.* (2012) *Clin. Cancer Res.* 18:1611-1618) and has been implicated in response to PD-1 blockade in Hodgkin's lymphoma (Ansell *et al.* (2015) *New Engl. J. Med.* 372:311-319), STAT3 binds directly to the PD-L1 promoter (Wolfle *et al.* (2011) *Eur. J. Immunol.* 41:413-424), and other activating mutations, such as $JAK3^{R657Q}$, are found in lung cancer (Cancer Genome Atlas Res. Network (2012) *Nature* 489:519-525), supporting the potential generalizability of the findings described herein.

In light of the determinations described herein that $JAK3^{WT}$ overexpression also induced PD-L1, it is notable that the *JAK2* amplicon, which also contains the PD-L1 locus, is recurrently amplified in lymphoma and EBV-positive gastric cancer (Green *et al.* (2010) *Blood* 116:3268-3277; Cancer Genome Atlas Res. Network (2014) *Nature* 513:202-209). Thus, functional SNP variants, somatic alterations resulting in activation of other JAK

family members (*e.g.*, *JAK1*, *JAK2*, or *TYK2*), or inactivation of negative regulators, such as suppressor of cytokine signaling (*SOCS*) family members may serve as a common pathway for upregulating PD-L1 expression and predicting responsiveness to this immune therapy. Indeed, since this particular $JAK3^{V722I}$ variant is present in the germline at a significantly lower frequency (1-2%) compared with the frequency of PD-L1 positivity in lung cancer overall (at least 20%), it may only explain a small subset of tumors that engage this pathway. But since long-term durable remissions are much rarer, it is possible that studies in patients enriched for this genotype may show similarly impressive responsiveness as seen in this case.

The results described herein expand the concept of studying extraordinary responses to cancer therapeutics beyond classical targeted therapies to include approved or investigational immunotherapies. The identification of these and other genomic mechanisms of sensitivity to immune checkpoint inhibitor blockade will not only help tailor this therapy in a personalized fashion, but may also suggest pharmacologic approaches to induce sensitivity in otherwise resistant patients. Finally, profiling patients who demonstrate initial responses but develop acquired resistance may further illuminate the spectrum of pathways that restrict tumor immunity and implicate additional rational modalities for therapeutic development.

Incorporation by Reference

All publications, patents, and patent applications mentioned herein are hereby incorporated by reference in their entirety as if each individual publication, patent or patent application was specifically and individually indicated to be incorporated by reference. In case of conflict, the present application, including any definitions herein, will control.

Also incorporated by reference in their entirety are any polynucleotide and polypeptide sequences which reference an accession number correlating to an entry in a public database, such as those maintained by The Institute for Genomic Research (TIGR) on the world wide web and/or the National Center for Biotechnology Information (NCBI) on the world wide web.

Equivalents

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

What is claimed is:

1. A method of determining whether a subject afflicted with a cancer or at risk for developing a cancer would benefit from anti-immune checkpoint inhibitor therapy, the method comprising:

a) obtaining a biological sample from the subject;

b) determining the presence, copy number, amount, and/or activity of at least one biomarker listed in Table 1 in a subject sample;

c) determining the presence, copy number, amount, and/or activity of the at least one biomarker in a control; and

d) comparing the presence, copy number, amount, and/or activity of said at least one biomarker detected in steps b) and c);

wherein the presence or a significant increase in the copy number, amount, and/or activity of the at least one biomarker in the subject sample relative to the control indicates that the subject afflicted with the cancer or at risk for developing the cancer would benefit from anti-immune checkpoint inhibitor therapy.

2. The method of claim 1, further comprising recommending, prescribing, or administering anti-immune checkpoint inhibitor therapy if the cancer is determined to benefit from anti-immune checkpoint inhibitor therapy.

3. The method of claim 1, further comprising recommending, prescribing, or administering anti-cancer therapy other than anti-immune checkpoint inhibitor therapy if the cancer is determined to not benefit from anti-immune checkpoint inhibitor therapy.

4. The method of claim 3, wherein the anti-cancer therapy is selected from the group consisting of targeted therapy, chemotherapy, radiation therapy, and/or hormonal therapy.

5. The method of any one of claims 1-4, wherein the control sample is determined from a cancerous or non-cancerous sample from either the patient or a member of the same species to which the patient belongs.

6. The method of any one of claims 1-5, wherein the control sample comprises cells.

7. The method of any one of claims 1-6, further comprising determining responsiveness to anti-immune checkpoint inhibitor therapy measured by at least one criteria selected from the group consisting of clinical benefit rate, survival until mortality, pathological complete response, semi-quantitative measures of pathologic response, clinical complete remission, clinical partial remission, clinical stable disease, recurrence-free survival, metastasis free survival, disease free survival, circulating tumor cell decrease, circulating marker response, and RECIST criteria.

8. A method of treating a subject afflicted with a cancer, wherein the cancer comprises at least one activating Janus kinase (JAK) mutation shown in Table 1, comprising administering to the subject anti-immune checkpoint inhibitor therapy, thereby treating the subject afflicted with the cancer.

9. The method of claim 8, wherein the at least one activating JAK mutation comprises an activating JAK3 mutation.

10. The method of claim 9, wherein the activating JAK3 mutation is a JH2 domain mutation, optionally a $JAK3^{V722I}$ or $JAK3^{R657Q}$ mutation, and/or a FERM domain mutation, optionally a $JAK3^{S61C}$ mutation.

11. The method of claim 8, further comprising administering one or more additional anti-cancer agents.

12. The method of claim 11, wherein the one or more additional anti-cancer agent is a JAK or activator thereof.

13. A method of inhibiting hyperproliferative growth of a cancer cell or cells, wherein the cancer cell or cells comprise at least one activating JAK mutation shown in Table 1, comprising contacting the cancer cell or cells with an anti-immune checkpoint inhibitor agent, thereby inhibiting hyperproliferative growth of the cancer cell or cells.

14. The method of claim 13, wherein the step of contacting occurs *in vivo*, *ex vivo*, or *in vitro*.

15. The method of claim 13, wherein the at least one activating JAK mutation comprises an activating JAK3 mutation.

16. The method of claim 15, wherein the activating JAK3 mutation is a JH2 domain mutation, optionally a $JAK3^{V722I}$ or $JAK3^{R657Q}$ mutation, and/or a FERM domain mutation, optionally a $JAK3^{S61C}$ mutation.

17. The method of claim 13, further comprising administering one or more additional anti-cancer agents.

18. The method of claim 17, wherein the one or more additional anti-cancer agent is a JAK or activator thereof.

19. A method of assessing the efficacy of an agent for treating a cancer in a subject, wherein the cancer comprises at least one activating JAK mutation, comprising:

a) detecting in a first subject sample and maintained in the presence of the agent the presence, copy number, amount and/or activity of at least one biomarker listed in Table 1;

b) detecting the presence, copy number, amount and/or activity of the at least one biomarker listed in Table 1 in a second subject sample and maintained in the absence of the test compound; and

c) comparing the presence, copy number, amount and/or activity of the at least one biomarker listed in Table 1 from steps a) and b), wherein the presence or a significantly increased copy number, amount, and/or activity of the at least one biomarker listed in Table 1 in the first subject sample relative to the second subject sample, indicates that the agent treats the cancer in the subject.

20. A method of monitoring the progression of a cancer in a subject, wherein the cancer comprises at least one activating JAK mutation, comprising:

a) detecting in a subject sample at a first point in time the presence, copy number, amount, and/or activity of at least one biomarker listed in Table 1;

b) repeating step a) during at least one subsequent point in time after administration of a therapeutic agent; and

c) comparing the presence, copy number, amount, and/or activity detected in steps a) and b), wherein the presence or a significantly increased copy number, amount, and/or activity of the at least one biomarker listed in Table 1 in the first subject sample relative to at least one subsequent subject sample, indicates that the agent treats the cancer in the subject.

21. The method of claim 20, wherein the subject has undergone treatment, completed treatment, and/or is in remission for the cancer in between the first point in time and the subsequent point in time.

22. The method of claim 20 or 21, wherein the subject has undergone anti-immune checkpoint inhibitor therapy in between the first point in time and the subsequent point in time.

23. The method of any one of claims 20-22, wherein the first and/or at least one subsequent sample is selected from the group consisting of *ex vivo* and *in vivo* samples.

24. The method of any one of claims 20-23, wherein the first and/or at least one subsequent sample is obtained from an animal model of the cancer.

25. The method of any one of claims 20-24, wherein the first and/or at least one subsequent sample is a portion of a single sample or pooled samples obtained from the subject.

26. A cell-based method for identifying an agent that inhibits a cancer, the method comprising:

a) contacting a cell expressing at least one biomarker listed in Table 1 with a test agent; and

b) determining the effect of the test agent on the copy number, level of expression, and/or level of activity of the at least one biomarker in Table 1 to thereby identify an agent that inhibits the cancer.

27. The method of claim 26, further comprising determining the effect of the test agent on the copy number, level of expression, and/or level of activity of at least one immune checkpoint inhibitor.

28. The method of claim 26 or 27, wherein said cells are isolated from a source selected from the group consisting of an animal model of a cancer, a subject afflicted with a cancer, and a cell comprising at least one activating JAK3 mutation.

29. The method of any one of claims 26-28, wherein said cells are unresponsive to anti-immune checkpoint inhibitor therapy.

30. The method of any one of claims 26-29, wherein the step of contacting occurs *in vivo*, *ex vivo*, or *in vitro*.

31. The method of any one of claims 26-30, further comprising determining the ability of the test agent to bind to the at least one biomarker listed in Table 1 before or after determining the effect of the test agent on the copy number, level of expression, or level of activity of the at least one biomarker listed in Table 1.

32. The method of any one of claims 1-7 and 19-31, wherein the sample comprises cells, cell lines, histological slides, paraffin embedded tissue, fresh frozen tissue, fresh tissue, biopsies, bronchoalveolar lavage (BAL) fluid, blood, plasma, serum, buccal scrape, saliva, cerebrospinal fluid, urine, stool, mucus, or bone marrow, obtained from the subject.

33. The method of any one of claims 1-7 and 19-32, wherein the presence or copy number is assessed by whole exome sequencing, microarray, quantitative PCR (qPCR), high-throughput sequencing, comparative genomic hybridization (CGH), or fluorescent *in situ* hybridization (FISH).

34. The method of any one of claims 1-7 and 19-32, wherein the amount of the at least one biomarker listed in Table 1 is assessed by detecting the presence in the samples of a polynucleotide molecule encoding the biomarker or a portion of said polynucleotide molecule.

35. The method of claim 34, wherein the polynucleotide molecule is a mRNA, cDNA, or functional variants or fragments thereof.

36. The method of claim 34, wherein the step of detecting further comprises amplifying the polynucleotide molecule.

37. The method of any one of claims 1-7 and 19-32, wherein the amount of the at least one biomarker is assessed by annealing a nucleic acid probe with the sample of the polynucleotide encoding the one or more biomarkers or a portion of said polynucleotide molecule under stringent hybridization conditions.

38. The method of any one of claims 1-7 and 19-32, wherein the amount of the at least one biomarker is assessed by detecting the presence a polypeptide of the at least one biomarker.

39. The method of claim 38, wherein the presence of said polypeptide is detected using a reagent which specifically binds with said polypeptide.

40. The method of claim 39, wherein the reagent is selected from the group consisting of an antibody, an antibody derivative, and an antibody fragment.

41. The method of any one of claims 1-7 and 19-32, wherein the activity of the at least one biomarker is assessed by determining the magnitude of cellular proliferation, cell death, or cytokine production.

42. The method of any one of claims 1-41, wherein the agent or anti-immune checkpoint inhibitor therapy is selected from the group consisting of a blocking antibody, small molecule, antisense nucleic acid, interfering RNA, shRNA, siRNA, aptamer, ribozyme, dominant-negative protein, and combinations thereof.

43. The method of claim 42, wherein the agent is selected from the group consisting of a cytokine, an inhibitor of a Jak kinase inhibitor, a Jak kinase harboring an activating mutation, anti-immune checkpoint inhibitor therapy, and combinations thereof.

44. The method of claim 43, wherein the inhibitor of the Jak kinase inhibitor is an inhibitor of PIAS1, PIAS2, PIAS3, PIAS4, SOCS1, SOCS3, SHP-1, or SHP-2.

45. The method of claim 42, wherein the agent or anti-immune checkpoint inhibitor therapy is selected from the group consisting of inhibitors of PD-1, PD-L1, PD-L2, CTLA-4, and combinations thereof.

46. The method of claim 45, wherein the agent or anti-immune checkpoint inhibitor therapy is a blocking antibody of PD-1, PD-L1, PD-L2, or CTLA-4, and combinations thereof.

47. The method of any one of claims 1-46, wherein the at least one biomarker is selected from the group consisting of 1, 2, 3, 4, 5, 6, 7, 8, 9, 10, or more biomarkers.

48. The method of any one of claims 1-47, wherein the at least one biomarker is an activating JAK3 mutation.

49. The method of claim 48, wherein the activating JAK3 mutation is a JH2 domain mutation, optionally a $JAK3^{V722I}$ or $JAK3^{R657Q}$ mutation, and/or a FERM domain mutation, optionally a $JAK3^{S61C}$ mutation.

50. The method of any one of claims 1-49, wherein the cancer is a solid malignancy.

51. The method of claim 50, wherein the solid malignancy is selected from the group consisting of lung cancer, non-small cell lung cancer (NSCLC), skin cancer, melanoma, cervical cancer, uterine cancer, ovarian cancer, breast cancer, pancreatic cancer, stomach cancer, esophageal cancer, colorectal cancer, liver cancer, prostate cancer, kidney cancer, bladder cancer, head and neck cancer, sarcoma, lymphoma, and brain cancer.

52. The method of any one of claims 1-51, wherein the subject is a mammal.

53. The method of claim 52, wherein the mammal is an animal model of cancer.

54. The method of claim 52, wherein the mammal is a human.

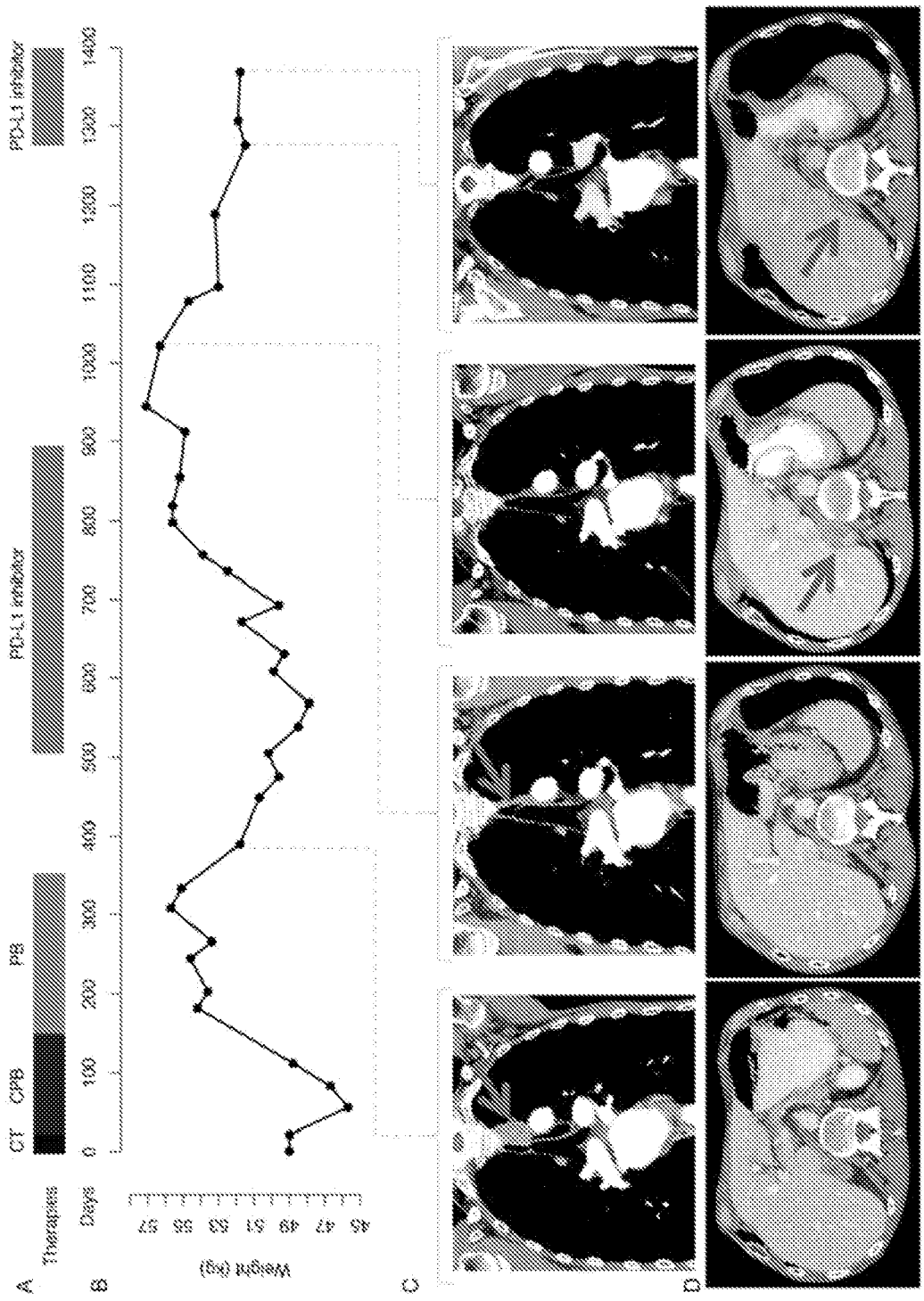

A
Therapies
CT
CPB
PB
PD-L1 inhibitor
B
Days
0
100
200
300
400
500
600
700
800
900
1000
1100
1200
1300
1400
Weight (kg)
45
47
49
51
53
55
57
C
D

Figure 1

Figure 2

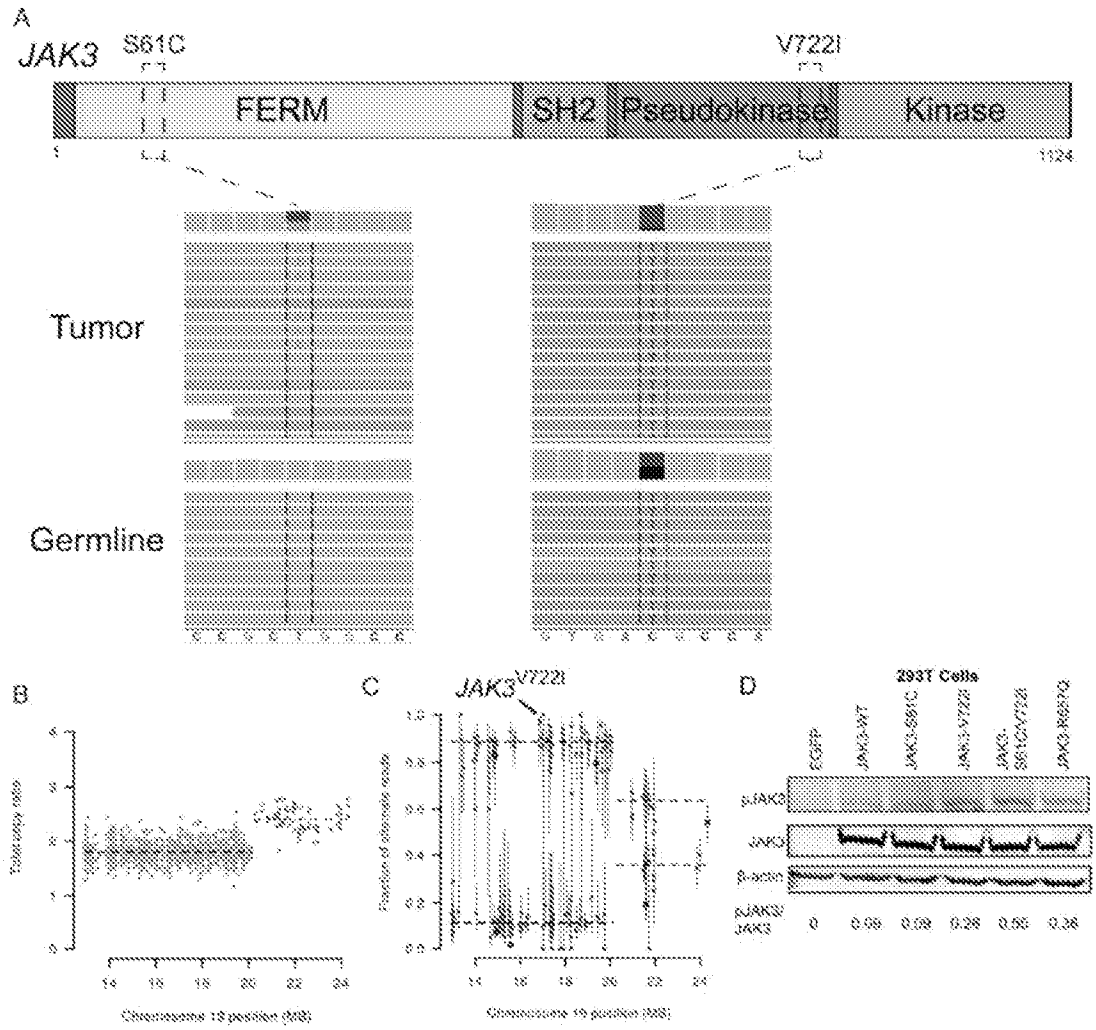

A
JAK3
S61C
V722I
FERM
SH2
Pseudokinase
Kinase
1
1124
Tumor
Germline
B
C
JAK3V722I
D
293T Cells
EGFP
JAK3-WT
JAK3-S61C
JAK3-V722I
JAK3-S61C/V722I
JAK3-R657Q
pJAK3
JAK3
β-actin
pJAK3/JAK3
0
0.09
0.09
0.28
0.56
0.28

Figure 3

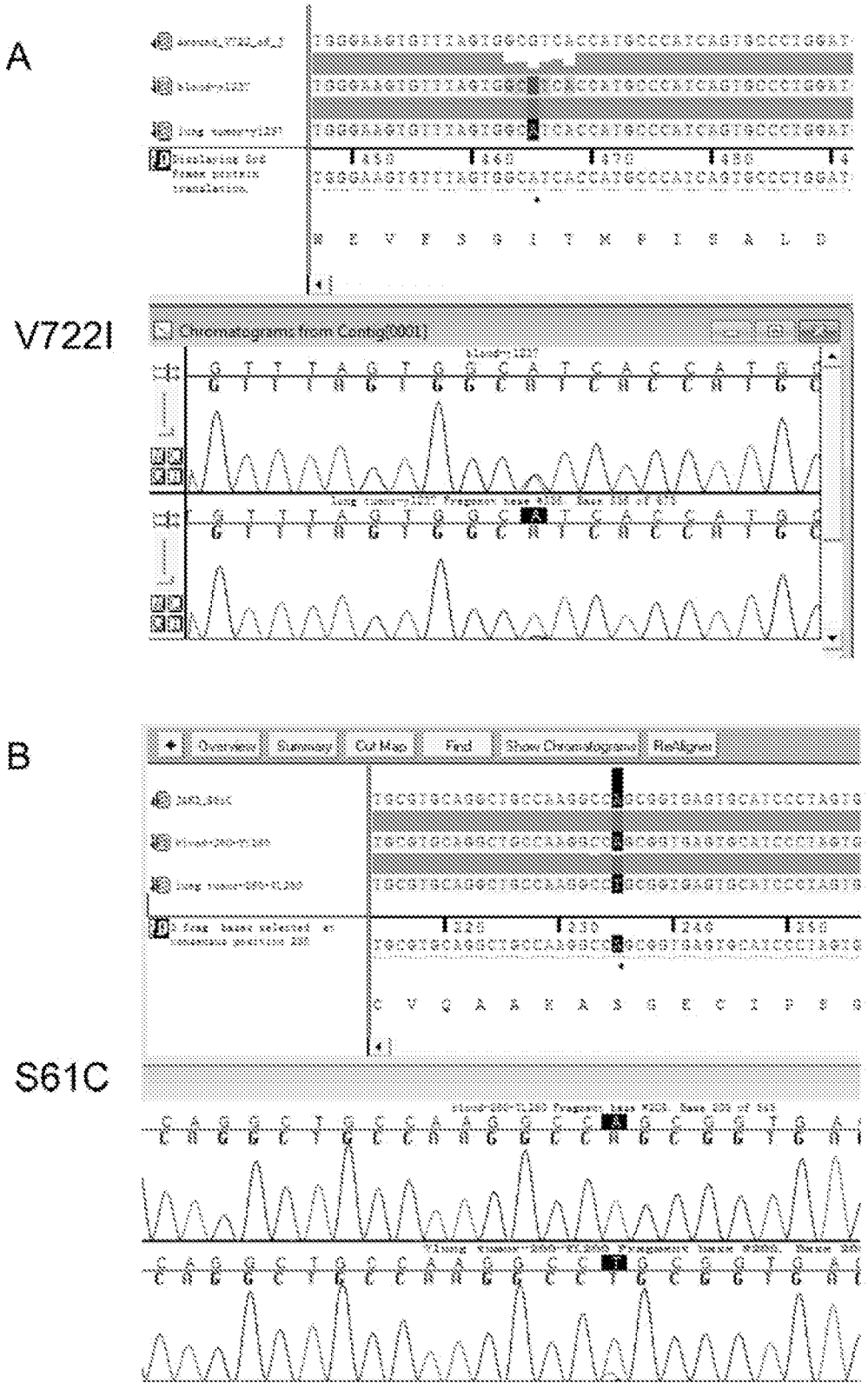


Figure 4

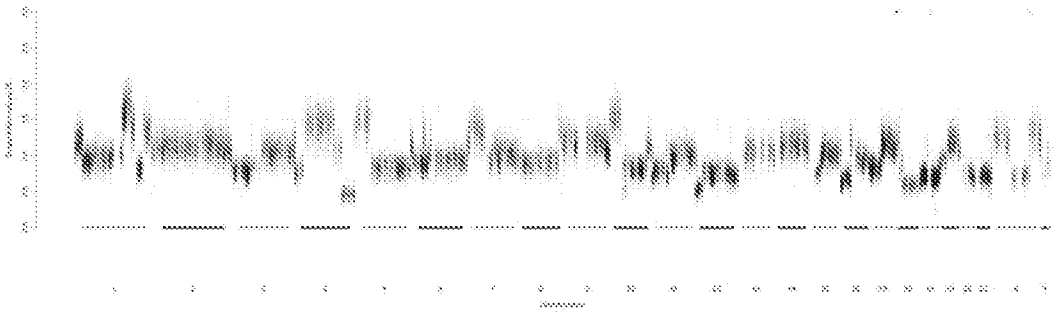


Figure 5

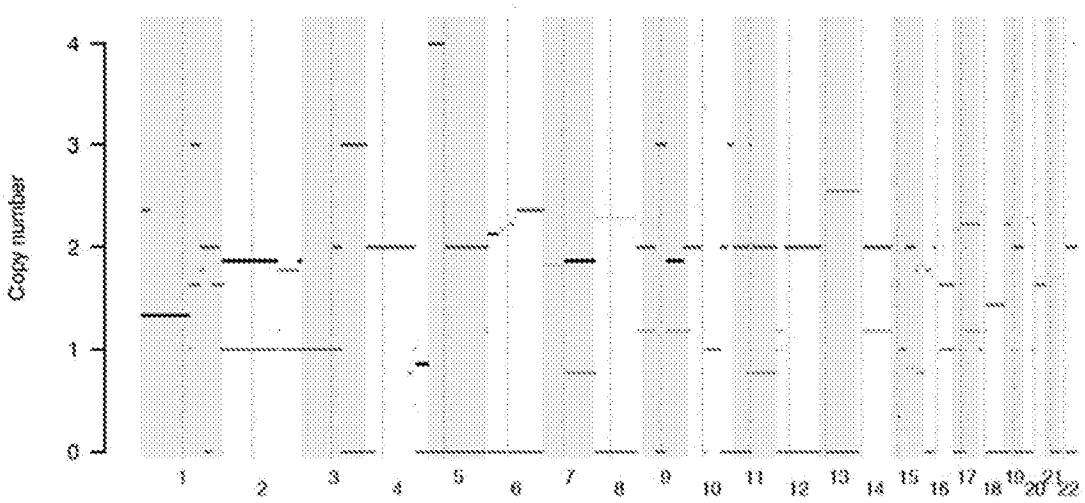


Figure 6

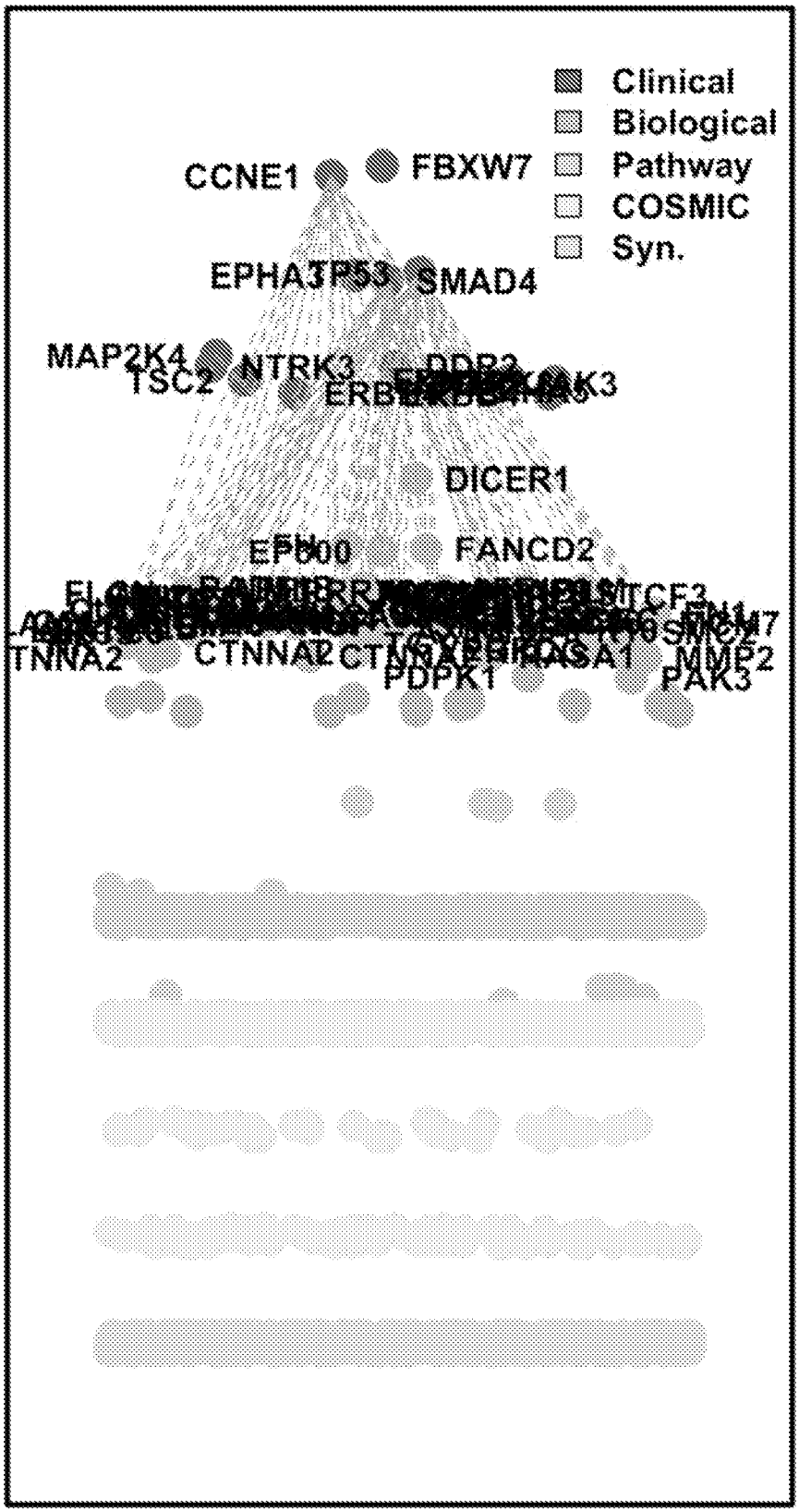
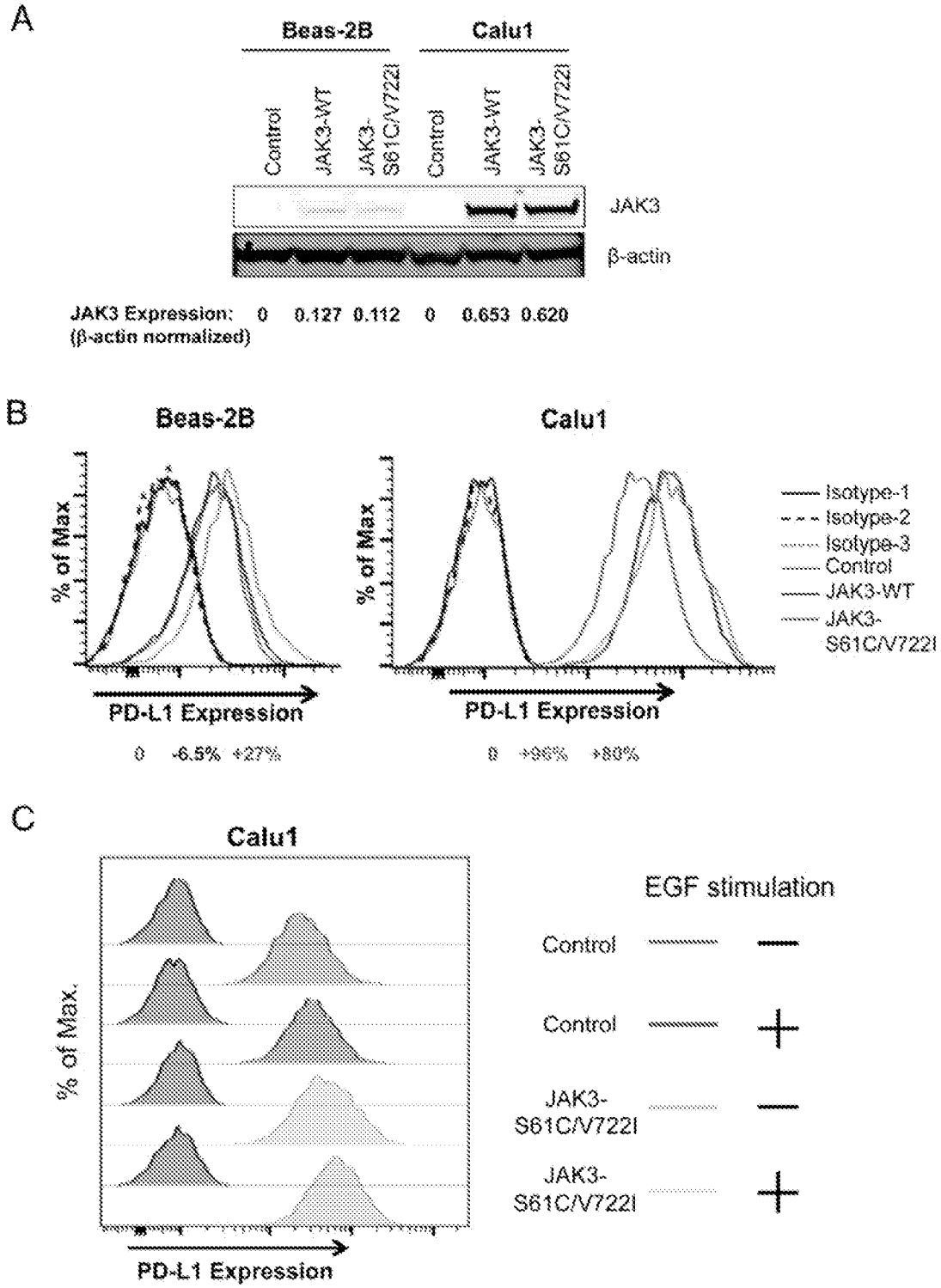

Clinical
Biological
Pathway
COSMIC
Syn.
CCNE1
FBXW7
EPHA3
TP53
SMAD4
MAP2K4
NTRK3
TSC2
DICER1
FANCD2
CTNNA2
PDPK1
PAK3

Figure 7


A
Beas-2B
Calu1
Control
JAK3-WT
JAK3-S61C/V722I
Control
JAK3-WT
JAK3-S61C/V722I
JAK3
β-actin
JAK3 Expression: 0 0.127 0.112 0 0.653 0.620
(β-actin normalized)
B
Beas-2B
Calu1
% of Max
PD-L1 Expression
0 -6.5% +27%
0 +96% +80%
Isotype-1
Isotype-2
Isotype-3
Control
JAK3-WT
JAK3-S61C/V722I
C
Calu1
% of Max.
PD-L1 Expression
EGF stimulation
Control —
Control +
JAK3-S61C/V722I —
JAK3-S61C/V722I +

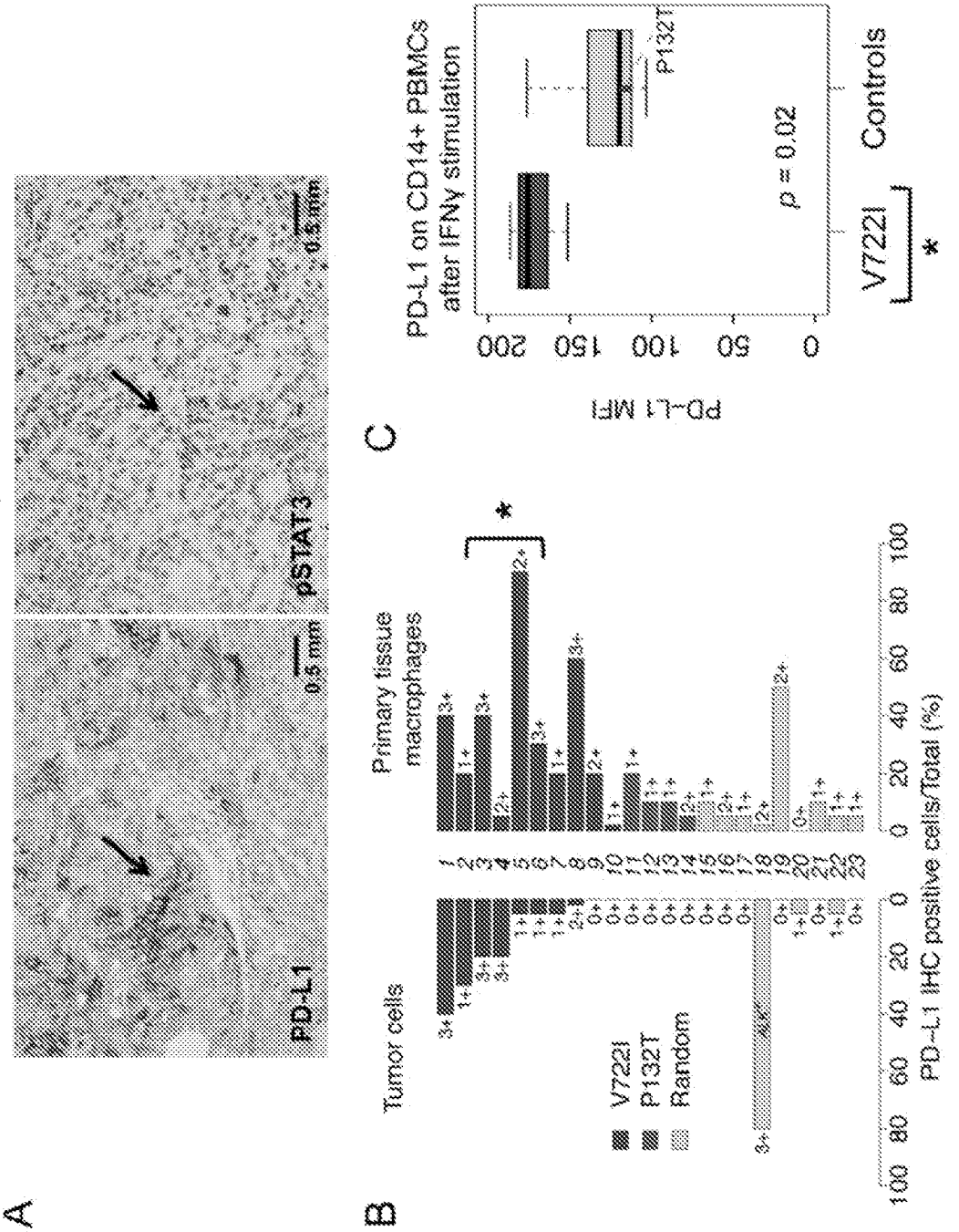

A
Adrenal Tumor PD-L1 Expression
PD-L1
0.5 mm
pSTAT3
0.5 mm
B
Tumor cells
Primary tissue macrophages
V722I
P132T
Random
PD-L1 IHC positive cells/Total (%)
C
PD-L1 on CD14+ PBMCs after IFNγ stimulation
PD-L1 MFI
p = 0.02
V722I
P132T
Controls

Figure 8

Figure 8 (cont.)

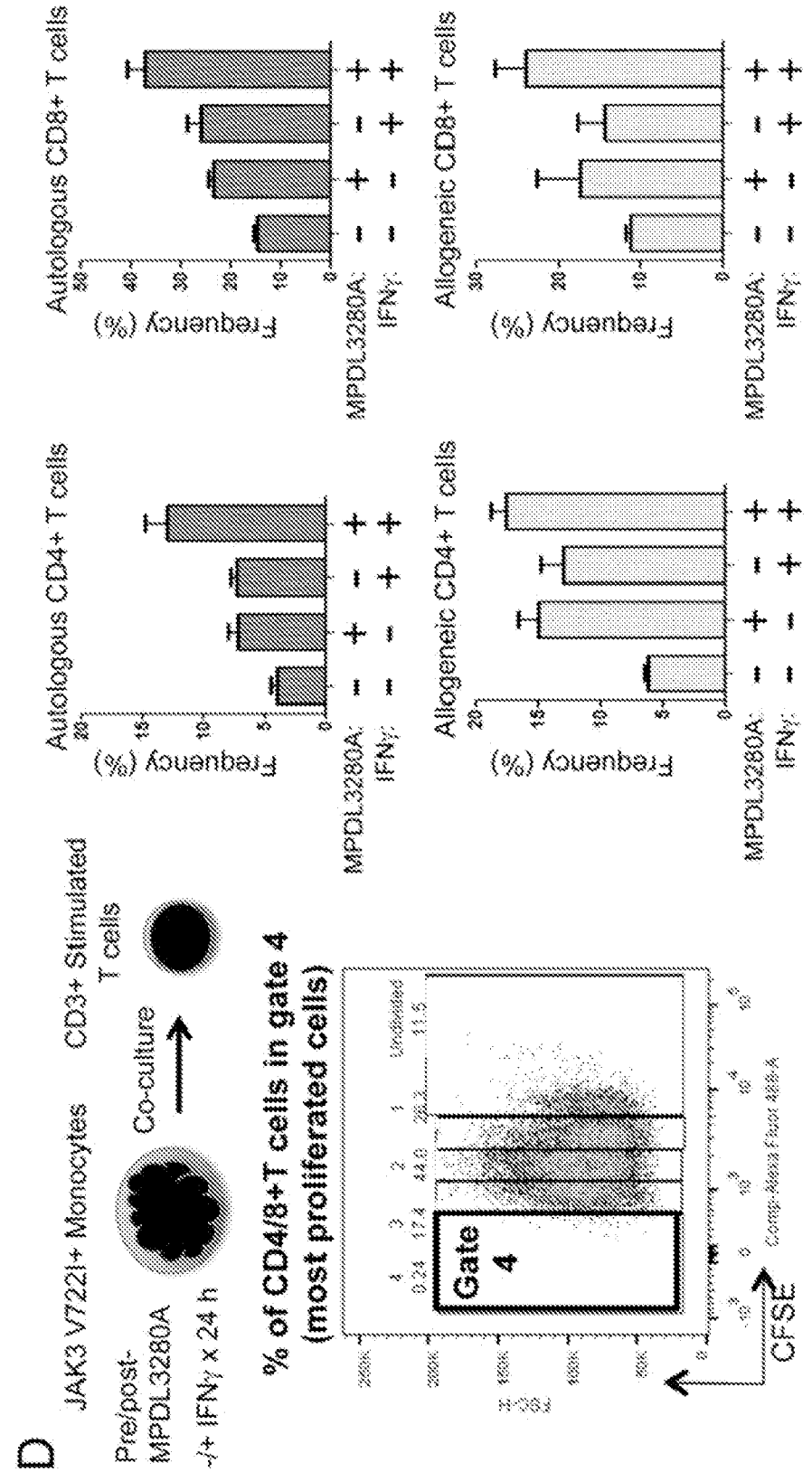


Figure 9

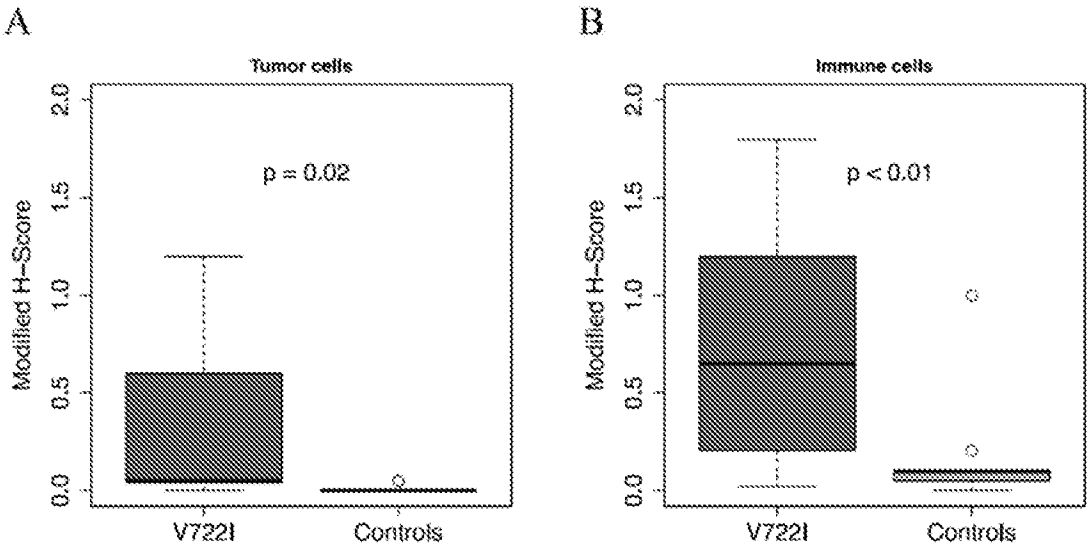

A
Tumor cells
p = 0.02
Modified H-Score
0.0
0.5
1.0
1.5
2.0
V722I
Controls
B
Immune cells
p < 0.01
Modified H-Score
0.0
0.5
1.0
1.5
2.0
V722I
Controls

Figure 10

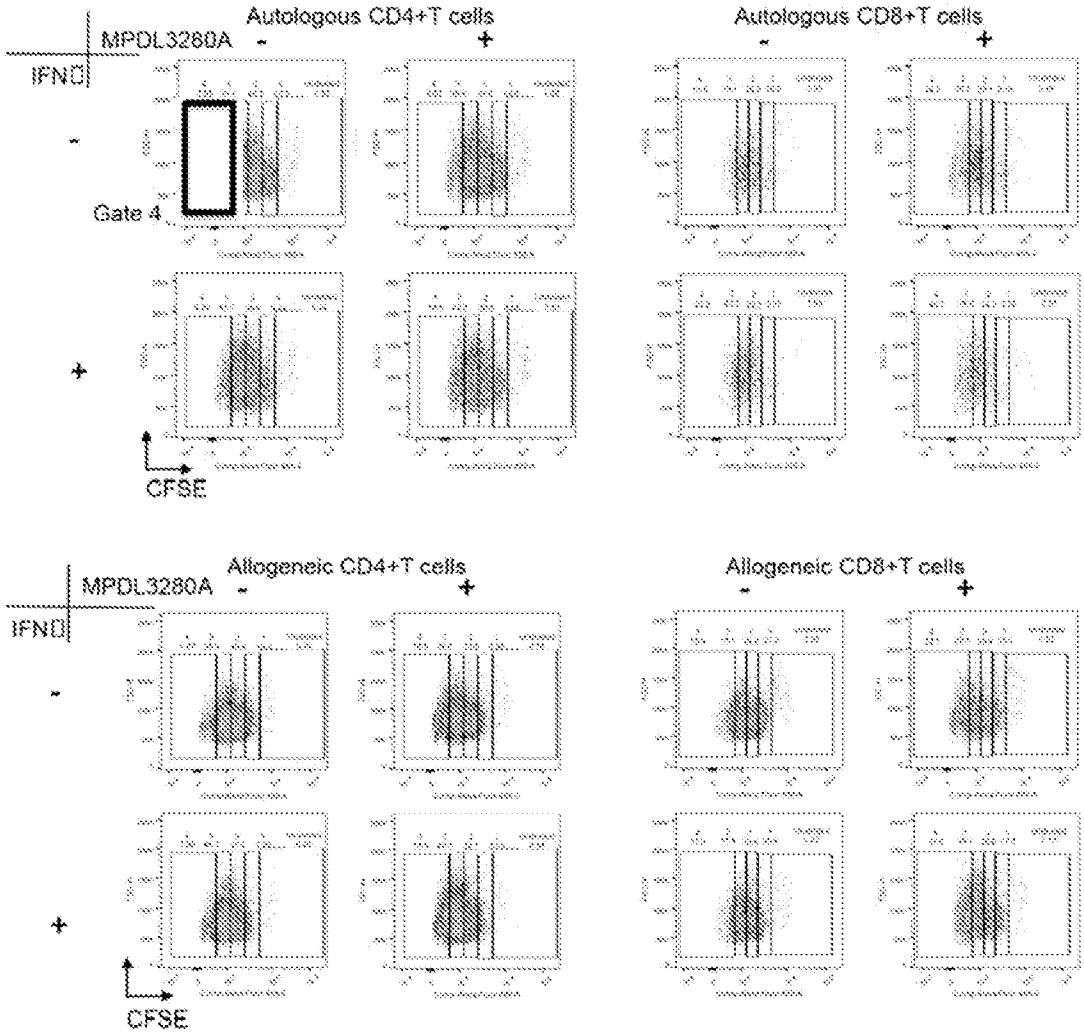

Autologous CD4+T cells
Autologous CD8+T cells
MPDL3280A
IFN
Gate 4
CFSE
Allogeneic CD4+T cells
Allogeneic CD8+T cells
MPDL3280A
IFN
CFSE

Figure 11

Gene	Variant_Classification	Alteration	Tumor_allele	Ref_allele	dbSNP_RS	Chr. #	Start_pos.	End_pos.	Coverage	Allelic_fraction	#_of_Probes	Segment_Mean (Log2(Copy
CCNE1	Copy Number	Amplified	NA	NA	NA	19	30503936	30496617	NA	NA	12	2.9606
FBXW7	Copy Number	Deleted	NA	NA	NA	4	153336744	190873444	NA	NA	1045	-1.1013
EPHA3	Missense_Mutation	p.D604Y	T	G		3	89462338	89462338	340	0.332352941	NA	NA
SMAD4	Missense_Mutation	p.E330K	A	G		18	48591825	48591825	199	0.658291457	NA	NA
TP53	Missense_Mutation	p.V157F	A	C	rs121912654	17	7578461	7578461	186	0.669117647	NA	NA
DDR2	Nonsense_Mutation	p.E670*	T	G		1	162745593	162745593	524	0.507633588	NA	NA
MAP2K4	Missense_Mutation	p.R145Q	A	G		17	11998899	11998899	146	0.301369863	NA	NA
NTRK3	Missense_Mutation	p.E767Q	A	C		15	88420343	88420343	93	0.225806452	NA	NA
DDR2	Missense_Mutation	p.A383S	T	G		1	162735838	162735838	243	0.534979424	NA	NA
EPHA3	Missense_Mutation	p.S419R	T	G		3	89356240	89356240	281	0.495516014	NA	NA
ERBB4	Missense_Mutation	p.A235S	A	C		2	212583839	212583839	236	0.440677966	NA	NA
ERBB4	Missense_Mutation	p.P1052L	A	G		2	212252698	212252698	241	0.265560166	NA	NA
EZH2	Splice_Site	p.H618_splice	A	T		7	148508811	148508811	153	0.274509804	NA	NA
JAK3	Missense_Mutation	p.S63C	A	T		19	17955046	17955046	14	0.5	NA	NA
NF1	Nonsense_Mutation	p.E1238*	T	G		17	29562632	29562632	412	0.735436893	NA	NA
NTRK3	Missense_Mutation	p.P210S	A	G		15	88679835	88679835	54	0.185185185	NA	NA
TSC2	Missense_Mutation	p.E408K	A	G	rs137853988|rs137854127	16	2111877	2111977	64	0.71875	NA	NA
DICER1	Splice_Site		T	C		14	95563051	95563051	52	0.346153846	NA	NA
EP300	Nonsense_Mutation	p.Q476*	T	C		22	41527535	41527535	128	0.40625	NA	NA
FANCD2	Missense_Mutation	p.S882C	G	C		3	10114976	10114976	260	0.357692308	NA	NA
FH	Missense_Mutation	p.H462Y	A	G		1	241663743	241663743	395	0.313924051	NA	NA
AFF1	Missense_Mutation	p.V1190L	T	G		4	88056788	88056788	101	0.405940594	NA	NA
AFF1	Missense_Mutation	p.S415C	G	C		4	88016103	88016103	377	0.183023873	NA	NA
AKAP9	Missense_Mutation	p.P2968S	T	C		7	91714866	91714866	232	0.159482759	NA	NA
ARID1A	Nonsense_Mutation	p.E2078*	T	G		1	27106621	27106621	193	0.398963731	NA	NA
BLM	Missense_Mutation	p.E1314D	T	G		15	91354502	91354502	183	0.234972678	NA	NA
CDK12	Frame_Shift_Del	p.S478fs	-	C		17	37627509	37627509	299	0.267558528	NA	NA
FIP1L1	Frame_Shift_Del	p.L39fs	-	G		4	54245271	54245271	300	0.366666667	NA	NA
FLCN	Missense_Mutation	p.S108C	A	T		17	17129564	17129564	157	0.757961783	NA	NA
FUS	Missense_Mutation	p.G489R	A	G		16	31202355	31202355	257	0.245136187	NA	NA
ITK	Missense_Mutation	p.R193L	T	G	rs17054374	5	156649955	156649955	259	0.760617761	NA	NA
LCP1	Missense_Mutation	p.L390I	T	G		13	46721049	46721049	324	0.802469136	NA	NA
MLL	Copy Number	Deleted	NA	NA	NA	11	118390509	118392889	NA	NA	4	-1.2525
MYCN	Missense_Mutation	p.H46QN	A	C		2	16086202	16086202	338	0.292899408	NA	NA
PMS2	Frame_Shift_Del	p.PVPRP148fs	-	GGGGCGGGGTAG	rs35629870	7	6042165	6042177	485	0.195876289	NA	NA
PRRX1	Nonsense_Mutation	p.Q105*	T	C		1	170688938	170688938	236	0.495762712	NA	NA
RAD51B	Missense_Mutation	p.G288A	C	G		14	68878150	68878150	207	0.270531401	NA	NA
TCF3	Nonsense_Mutation	p.S295*	T	G		19	1621908	1621908	15	0.466666667	NA	NA
TET1	Missense_Mutation	p.A1893T	A	G		10	70450837	70450837	198	0.345360825	NA	NA
TSHR	Nonsense_Mutation	p.E339*	T	G	rs150821978	14	81609417	81609417	272	0.496323529	NA	NA
XPC	Missense_Mutation	p.G388A	G	C		3	14200220	14200220	213	0.126760563	NA	NA
ZNF331	Missense_Mutation	p.A21S	T	G		19	54074909	54074909	484	0.770661157	NA	NA
C6	Nonsense_Mutation	p.C146*	T	A		5	41199877	41199877	437	0.368421053	NA	NA
C6	Splice_Site		-	C		5	41158775	41158775	203	0.408866995	NA	NA
COL4A2	Splice_Site		C	G		13	111088715	111088715	124	0.830645161	NA	NA
COL4A6	Missense_Mutation	p.G1499R	T	C		X	107403723	107403723	140	0.728571429	NA	NA
CUL2	Missense_Mutation	p.D248N	T	C		10	35327992	35327992	438	0.173515982	NA	NA
ETS1	Missense_Mutation	p.A128G	C	G		11	128359337	128359337	306	0.055555556	NA	NA
FI1	Copy Number	Deleted	NA	NA	NA	4	153336744	190873444	NA	NA	1045	-1.1013
FASLG	Missense_Mutation	p.L54I	A	C		1	172628501	172628501	112	0.607142857	NA	NA
FGF14	Missense_Mutation	p.P2Q	T	G		13	102568925	102568925	44	0.840909091	NA	NA
FN1	Nonsense_Mutation	p.G2088*	A	C		2	216237054	216237054	267	0.269662921	NA	NA
FN1	Frame_Shift_Ins	p.R2372fs	C	-		2	216230258	216230259	680	0.276470588	NA	NA
FZD1	Missense_Mutation	p.T337S	T	A		7	90895204	90895204	407	0.535626536	NA	NA
FZD10	Nonsense_Mutation	p.C267*	A	C		12	130648108	130648108	61	0.819672131	NA	NA
GLI3	Missense_Mutation	p.G809E	T	C		7	42007199	42007199	809	0.201483313	NA	NA

Figure 11 (cont.)

Gene	Variant_Classification	Alteration	Tumor_allele	Ref_allele	dbSNP_RS	Chr. #	Start_pos.	End_pos.	Coverage	Allelic_fraction	# of Probes	Segment_Mean (Log2[Copy
HGF	Missense_Mutation	p.E248K	T	C		7	81374320	81374320	159	0.345911135	NA	NA
HGF	Missense_Mutation	p.E682K	T	C		7	81334732	81334732	242	0.128099174	NA	NA
LAMA1	Missense_Mutation	p.R1546M	A	C		18	6999473	6999473	120	0.275	NA	NA
LAMA2	Missense_Mutation	p.S2147I	T	C		6	129774143	129774143	99	0.303030303	NA	NA
LAMA4	Missense_Mutation	p.G1357S	T	C		6	112451142	112451142	100	0.73	NA	NA
LAMA4	Missense_Mutation	p.E845V	A	T		6	112463454	112463454	286	0.762237762	NA	NA
LAMA5	Nonsense_Mutation	p.E1719*	A	C		20	60901980	60901980	226	0.424778761	NA	NA
PTCH2	Missense_Mutation	p.E854D	A	C		1	45282707	45282707	355	0.357746479	NA	NA
WNT5A	Missense_Mutation	p.R202L	A	C		3	55508444	55508444	57	0.350877193	NA	NA
AKIN1	Nonsense_Mutation	p.K807*	A	T		16	347192	347192	222	0.743243243	NA	NA
C3	Missense_Mutation	p.G262W	A	C		19	6713510	6713510	86	0.744186047	NA	NA
C3	Missense_Mutation	p.G262V	A	C		19	6713509	6713509	88	0.75	NA	NA
CASP3	Copy Number	Deleted	NA	NA	NA	4	151336744	190873444	NA	NA	1045	-1.1013
COG6	Missense_Mutation	p.L611F	T	A		13	115037888	115037888	90	0.755555556	NA	NA
EEF1	Missense_Mutation	p.L312I	T	G		4	108991917	108991917	210	0.380952381	NA	NA
MAPK10	Missense_Mutation	p.D17G	C	T		4	87080523	87080523	397	0.453400504	NA	NA
MCM10	Missense_Mutation	p.R318I	T	A		10	13224955	13224955	263	0.435865513	NA	NA
MCM7	Missense_Mutation	p.G275V	A	C		7	99695810	99695810	103	0.524271845	NA	NA
ORC3	Missense_Mutation	p.R367L	T	G		6	88331184	88331184	62	0.758064516	NA	NA
PDGFC	Copy Number	Deleted	NA	NA	NA	4	151336744	190873444	NA	NA	1045	-1.1013
RALB	Missense_Mutation	p.D68Y	T	G		2	121043537	121043537	302	0.539735099	NA	NA
SMC2	Missense_Mutation	p.E880Q	C	G		9	106889611	106889611	246	0.5	NA	NA
SRR	Missense_Mutation	p.H160	G	C		17	2218900	2218900	312	0.378205128	NA	NA
CTNNA2	Missense_Mutation	p.G847V	T	G		2	80874819	80874819	556	0.29676259	NA	NA
CTNNA2	Missense_Mutation	p.G708R	C	G		2	80816543	80816543	217	0.271889401	NA	NA
CTNNA2	Nonsense_Mutation	p.E305*	T	G		2	80136780	80136780	287	0.271777003	NA	NA
HR	Missense_Mutation	p.P66T	T	G		8	21986488	21986488	92	0.695652174	NA	NA
MMP2	Frame_Shift_Ins	p.KE573fs	T	-		16	55532310	55532311	237	0.291139241	NA	NA
PRKCG	Missense_Mutation	p.V884A	C	T		19	54410136	54410136	96	0.71875	NA	NA
RASA1	Copy Number	Deleted	NA	NA	NA	5	86564809	86564872	NA	NA	2	-2.3605
T	Splice_Site		T	C		6	166575934	166575934	99	0.757575758	NA	NA
TG	Missense_Mutation	p.G962E	A	G	rs148894907	8	133906058	133906058	243	0.312757202	NA	NA
VEGFC	Copy Number	Deleted	NA	NA	NA	4	151336744	190873444	NA	NA	1045	-1.1013
PAK3	Missense_Mutation	p.V404M	A	G		X	110439106	110439106	158	0.702531646	NA	NA
PDPK1	Frame_Shift_Del	p.DD151fs	-	CGAC		16	2611896	2611899	6	0.333333333	NA	NA
ACTA1	Splice_Site	p.644_splice	T	C		1	229568627	229568627	189	0.142857143	NA	NA
ADCY1	Missense_Mutation	p.D358N	A	G		7	45632490	45632490	236	0.161016949	NA	NA
AK7	Missense_Mutation	p.S134F	T	C		14	96871200	96871200	152	0.276315789	NA	NA
AK7	Splice_Site		T	G		14	96937814	96937814	154	0.396103896	NA	NA
CD22	Missense_Mutation	p.S395C	G	C		19	35829269	35829269	126	0.746031746	NA	NA
CD4	Copy Number	Deleted	NA	NA	NA	12	6135214	7076942	NA	NA	308	-1.0173
CD9	Copy Number	Deleted	NA	NA	NA	12	6135214	7076942	NA	NA	308	-1.0173
HMGB2	Copy Number	Deleted	NA	NA	NA	4	151336744	190873444	NA	NA	1045	-1.1013
ITGA4	Frame_Shift_Ins	p.R216fs	A	-		2	182344886	182344887	148	0.371621622	NA	NA
KIR2DL4	Copy Number	Deleted	NA	NA	NA	19	55324685	55327991	NA	NA	4	-1.4768
KIR3DL1	Missense_Mutation	p.G97R	C	G		19	55329988	55329988	285	0.789473684	NA	NA
KIR3DL1	Copy Number	Deleted	NA	NA	NA	19	55324685	55327991	NA	NA	4	-1.4768
LILRB2	Splice_Site	p.G12_splice	G	C		19	54784318	54784318	174	0.729885057	NA	NA
LRG1	Missense_Mutation	p.G228E	T	C		19	4538313	4538313	627	0.393939394	NA	NA
GAPDH	Copy Number	Deleted	NA	NA	NA	12	6135214	7076942	NA	NA	308	-1.0173
L1CAM	Splice_Site	p.VA22_splice	T	G		X	153134976	153134976	7	0.857142857	NA	NA
SI	Missense_Mutation	p.L1391F	A	C		3	164727073	164727073	135	0.214814815	NA	NA
TPI1	Copy Number	Deleted	NA	NA	NA	12	6135214	7076942	NA	NA	308	-1.0173
FGFR4	Missense_Mutation	p.D623H	C	G		5	176523103	176523103	101	0.227722772	NA	NA
GRK5	Missense_Mutation	p.R345L	T	G		10	121201577	121201577	150	0.233333333	NA	NA
TRIB2	Missense_Mutation	p.P216Q	A	C		2	12880535	12880535	216	0.240740741	NA	NA
A2M	Missense_Mutation	p.M673I	A	C		12	9247675	9247675	19	0.473684211	NA	NA
A2M	Missense_Mutation	p.R1081K	T	C		12	9230331	9230331	101	0.574257426	NA	NA
AADAC	Missense_Mutation	p.W115C	C	G		3	151535360	151535360	193	0.056994819	NA	NA
AASS	Missense_Mutation	p.L271F	A	G		7	121756770	121756770	161	0.260869565	NA	NA
ABCA6	Missense_Mutation	p.E1253K	T	C		17	67083556	67083556	482	0.304979253	NA	NA

Figure 11 (cont.)

Gene	Variant_Classification	Alteration	Tumor_allele	Ref_allele	dbSNP_RS	Chr. #	Start_pos.	End_pos.	Coverage	Allelic_fraction	#_of_Probes	Segment_Mean (Log2[Copy
ABCC4	Missense_Mutation	p.G652E	C	A		13	95830032	95830032	142	0.028169014	NA	NA
ACACA	Nonsense_Mutation	p.Q976*	A	G		17	35600292	35600292	360	0.261111111	NA	NA
ACSL1	Copy Number	Deleted	NA	NA	NA	4	151336744	190873444	NA	NA	1045	-1.1013
ACTL6B	Missense_Mutation	p.L314M	T	G		7	100244451	100244451	63	0.539682254	NA	NA
ADAM15	Frame_Shift_Ins	p.PP816fs	C	-		1	155034744	155034745	16	0.5	NA	NA
ADAM23	Splice_Site		T	A		2	207436449	207436449	122	0.516393443	NA	NA
ADAMTS1	Missense_Mutation	p.R835Q	G	T		21	28210299	28210299	106	0.773584906	NA	NA
ADAMTS1	Missense_Mutation	p.K504T	G	T		5	33643544	33643544	336	0.833333333	NA	NA
ADAMTS2	Missense_Mutation	p.R968W	A	G		5	178552030	178552030	325	0.516923077	NA	NA
ADAMTS3	Missense_Mutation	p.D674H	G	C		4	73176800	73176800	375	0.445333333	NA	NA
ADAMTS5	Missense_Mutation	p.E689K	T	C		21	28302365	28302365	274	0.332116788	NA	NA
ADAMTS6	Missense_Mutation	p.A277E	T	G		21	28337881	28337881	58	0.655172414	NA	NA
ADCY8	Missense_Mutation	p.N940K	T	G		8	131826381	131826381	368	0.239130435	NA	NA
ADCYAP1	Copy Number	Amplified	NA	NA	NA	18	163455	909638	NA	NA	64	2.2565
ADK	Missense_Mutation	p.C89F	T	G		10	76153942	76153942	256	0.328125	NA	NA
AFAP1	Nonsense_Mutation	p.Q199*	A	G		4	7840382	7840382	303	0.425742574	NA	NA
AFM	Missense_Mutation	p.F139L	A	C		4	74351725	74351725	387	0.42118863	NA	NA
AGA	Copy Number	Deleted	NA	NA	NA	4	151336744	190873444	NA	NA	1045	-1.1013
AHCTF1	Splice_Site	p.S860_splice	A	C		1	247048981	247048981	88	0.284090909	NA	NA
AIM2	Missense_Mutation	p.E275K	T	C		1	159033458	159033458	431	0.169373551	NA	NA
AKAP6	Missense_Mutation	p.R78Q	A	G		14	32902932	32902932	200	0.055	NA	NA
ALDH1L1	Missense_Mutation	p.G265A	G	C		3	125872351	125872351	194	0.278350515	NA	NA
ALOX5	Missense_Mutation	p.R472L	T	G		10	45938628	45938628	438	0.742009132	NA	NA
ALPI	Missense_Mutation	p.P22T	A	C		2	233320973	233320973	213	0.297674419	NA	NA
ALPPL2	Missense_Mutation	p.T116K	A	C		2	233272350	233272350	90	0.211111111	NA	NA
ANGEL1	Missense_Mutation	p.W220R	G	A		14	77274483	77274483	50	0.52	NA	NA
ANGPTL1	Missense_Mutation	p.D17N	T	C		1	178834863	178834863	373	0.142091153	NA	NA
ANK2	Missense_Mutation	p.S2133F	T	C		4	114276172	114276172	144	0.472222222	NA	NA
ANK3	Missense_Mutation	p.V3748L	A	C		10	61829397	61829397	517	0.311411992	NA	NA
ANKRD11	Missense_Mutation	p.R187P	G	C		16	89357134	89357134	145	0.255172414	NA	NA
ANKRD50	Missense_Mutation	p.G83AC	A	C		4	125592532	125592532	351	0.535612536	NA	NA
ANXA10	Copy Number	Deleted	NA	NA	NA	4	151336744	190873444	NA	NA	1045	-1.1013
ANXA2	Splice_Site	p.G120_splice	A	C		15	60649435	60649435	183	0.284153005	NA	NA
ANXA5	Missense_Mutation	p.V240F	A	C		4	122592705	122592705	163	0.380368098	NA	NA
AOX1	Missense_Mutation	p.P29H	A	C		2	201457909	201457909	156	0.25	NA	NA
AP2M1	Missense_Mutation	p.S238L	T	C		3	183899495	183899495	159	0.283018868	NA	NA
APBA2	Missense_Mutation	p.E82E	A	G		15	29346331	29346331	252	0.313492063	NA	NA
APEX2	Nonsense_Mutation	p.Q291*	T	C		X	55033182	55033182	78	0.5	NA	NA
APLN	Splice_Site	p.G43_splice	A	C		X	128782598	128782598	46	0.239130435	NA	NA
APOB	Missense_Mutation	p.S3471T	T	A		2	21229329	21229329	306	0.303663399	NA	NA
APOB	Missense_Mutation	p.T1574N	T	G		2	21235019	21235019	354	0.234463277	NA	NA
APOB	Missense_Mutation	p.R45Q	T	C		2	21265336	21265336	206	0.27184466	NA	NA
APOB	Missense_Mutation	p.S3471C	C	G		2	21229328	21229328	307	0.293159609	NA	NA
APOB	Missense_Mutation	p.P554T	T	G		2	21251368	21251368	281	0.501779359	NA	NA
AQP7	Missense_Mutation	p.M8R	A	C		9	33386995	33386995	399	0.300250627	NA	NA
ARCN1	Missense_Mutation	p.K183N	T	G		11	118454052	118454052	101	0.207920792	NA	NA
ARHGAP2	Missense_Mutation	p.R719M	A	C		11	110451514	110451514	343	0.513119534	NA	NA
ARHGAP28	De_novo_Start_OutOfFrame		A	G		18	6837225	6837225	27	0.444444444	NA	NA
ARHGEF1	Frame_Shift_Del	p.P815fs	-	C		8	1871745	1871745	241	0.746887967	NA	NA
ARID4A	Nonsense_Mutation	p.E714*	T	G		14	58830947	58830947	203	0.305418719	NA	NA
ASB5	Copy Number	Deleted	NA	NA	NA	4	151336744	190873444	NA	NA	1045	-1.1013
ASPA	Missense_Mutation	p.T62S	G	C		17	3379638	3379638	254	0.767716535	NA	NA
ATF6	Missense_Mutation	p.M13I	A	G		1	161736183	161736183	252	0.305555556	NA	NA
ATN1	Copy Number	Deleted	NA	NA	NA	12	6135214	7076942	NA	NA	308	-1.0173
AUTS2	Splice_Site		A	G		7	69583117	69583117	115	0.504347826	NA	NA
AUTS2	Missense_Mutation	p.E127G	G	A		7	69364342	69364342	265	0.230188679	NA	NA
BAI3	Missense_Mutation	p.W834L	T	G		6	69943202	69943202	434	0.790322581	NA	NA
BAI3	Missense_Mutation	p.G462V	T	G		6	69666561	69666561	230	0.739130435	NA	NA
BBOX1	Missense_Mutation	p.N236H	C	A		11	27141262	27141262	116	0.775862069	NA	NA
BBS2	Missense_Mutation	p.D25N	T	C		16	56553702	56553702	58	0.275862069	NA	NA
BBS2	Missense_Mutation	p.Q560N	T	C		16	56581774	56581774	184	0.119565217	NA	NA

Figure 11 (cont.)

Gene	Variant_Classification	Alteration	Tumor_allele	Ref_allele	dbSNP_RS	Chr. #	Start_pos.	End_pos.	Coverage	Allelic_fraction	# of Probes	Segment_Mean (Log2[Copy
BBS7	Missense_Mutation	p.W230L	A	C		4	122775888	122775888	289	0.352941176	NA	NA
BCAN	Missense_Mutation	p.N101K	A	C		1	156616804	156616804	112	0.258928571	NA	NA
BUKRB1	Missense_Mutation	p.P91H	A	C		14	96730291	96730291	315	0.507936508	NA	NA
BMI1	Missense_Mutation	p.R226I	T	G		10	22618167	22618167	252	0.376984127	NA	NA
BMI1	Missense_Mutation	p.R228L	T	G		10	22618173	22618173	238	0.382352941	NA	NA
BMP3	Missense_Mutation	p.A103P	C	G		4	81952745	81952745	33	0.333333333	NA	NA
CA5A	Missense_Mutation	p.R29K	A	C		16	87921784	87921784	111	0.261261261	NA	NA
CABIN1	Missense_Mutation	p.G1873C	T	G		22	24563216	24563216	24	0.625	NA	NA
CACHD1	Missense_Mutation	p.D262N	A	G		1	65098421	65098421	139	0.345323741	NA	NA
CACNA1C	Nonsense_Mutation	p.Y1627*	A	C		12	2786312	2786312	35	0.6	NA	NA
CADPS	Missense_Mutation	p.S1133L	A	G		3	62459927	62459927	451	0.592017738	NA	NA
CALCR	Missense_Mutation	p.V383L	A	C		7	93065368	93065368	147	0.19047619	NA	NA
CALCRL	Missense_Mutation	p.L144Q	T	A		2	188243736	188243736	276	0.518115942	NA	NA
CAND1	Missense_Mutation	p.S744L	T	C	rs144983893	12	67899679	67899679	314	0.414012739	NA	NA
CANX	Frame_Shift_Del	p.M33fs	-	T		5	179149920	179149920	358	0.019774011	NA	NA
CAPNS2	Missense_Mutation	p.M204T	C	T		16	55601279	55601279	648	0.296753643	NA	NA
CASR	Missense_Mutation	p.S106R	T	G		3	122003974	122003974	288	0.548611111	NA	NA
CCKBR	Missense_Mutation	p.R276S	A	C		11	6292048	6292048	77	0.753246753	NA	NA
CCNG2	Nonsense_Mutation	p.Q188*	T	C		4	78082667	78082667	311	0.22829582	NA	NA
CCNO	Nonsense_Mutation	p.E310*	A	C		5	54527328	54527328	116	0.75	NA	NA
CCR8	Missense_Mutation	p.R238K	A	G		3	39374535	39374535	127	0.425196885	NA	NA
CD14	Missense_Mutation	p.L357V	C	G		5	140011500	140011500	157	0.751592357	NA	NA
CD27	Copy Number	Deleted	NA	NA	NA	12	6135214	7076842	NA	NA	308	-1.0173
CDSL	Missense_Mutation	p.V134L	G	C		1	157804515	157804515	235	0.527659574	NA	NA
CDS	Missense_Mutation	p.G563V	T	G		11	60785354	60785354	256	0.39140625	NA	NA
CDS3	Missense_Mutation	p.P437L	A	G		20	23065520	23065520	231	0.562770563	NA	NA
CDC42EP	Missense_Mutation	p.S147C	G	C		22	37962796	37962796	135	0.325925926	NA	NA
CDH2	Missense_Mutation	p.F452L	T	A		18	25570303	25570303	103	0.601941748	NA	NA
CDH2	Missense_Mutation	p.S26L	A	G		18	25616436	25616436	166	0.638554217	NA	NA
CDH23	Missense_Mutation	p.P1982R	G	C		10	73550051	73550051	59	0.372881356	NA	NA
CDON	Missense_Mutation	p.S548L	A	G		11	125875868	125875868	248	0.266129032	NA	NA
CELSR1	Copy Number	Deleted	NA	NA	NA	22	46733843	46860244	NA	NA	30	-1.0829
CES1	Missense_Mutation	p.T297M	A	G		16	55853460	55853460	497	0.018108652	NA	NA
CES1	Missense_Mutation	p.W189R	T	A		16	55855405	55855405	145	0.151724138	NA	NA
CGB	Copy Number	Deleted	NA	NA	NA	19	49526459	49558634	NA	NA	18	-1.9589
CGB1	Copy Number	Deleted	NA	NA	NA	19	49526459	49558634	NA	NA	18	-1.9589
CGB2	Copy Number	Deleted	NA	NA	NA	19	49526459	49558634	NA	NA	18	-1.9589
CHD5B	Frame_Shift_Del	p.T191fs	-	GAGG		10	90966774	90966777	61	0.295081967	NA	NA
CHD4	Copy Number	Deleted	NA	NA	NA	12	6135214	7076842	NA	NA	308	-1.0173
CHL1	Missense_Mutation	p.G821V	T	G		3	431149	431149	113	0.407079646	NA	NA
CHM	Missense_Mutation	p.T141M	A	G	rs149749273	X	85218950	85218950	114	0.640350877	NA	NA
CHML	Nonsense_Mutation	p.Q427*	A	G		1	241797790	241797790	298	0.093645485	NA	NA
CHRND	Missense_Mutation	p.A486D	A	C		2	233399925	233399925	235	0.293617021	NA	NA
CLCN3	Copy Number	Deleted	NA	NA	NA	4	151336744	190873444	NA	NA	1045	-1.1013
CLCN5	Missense_Mutation	p.P744L	T	C		X	49855414	49855414	83	0.78313253	NA	NA
CLCNKB	Nonsense_Mutation	p.E2*	T	G		1	16370991	16370991	14	0.5	NA	NA
CLDN1	Missense_Mutation	p.L131V	C	G		3	190028040	190028040	156	0.262820513	NA	NA
CLUL1	Copy Number	Amplified	NA	NA	NA	18	163455	900638	NA	NA	64	2.2565
CNDP1	Missense_Mutation	p.L237H	A	T		18	72238503	72238503	89	0.640449438	NA	NA
CNGA1	Missense_Mutation	p.R547S	T	G		4	47939079	47939079	653	0.388973966	NA	NA
CNGB1	Frame_Shift_Del	p.G14fs	-	C		16	58001149	58001149	125	0.312	NA	NA
CNKSR1	Missense_Mutation	p.G115C	T	G		1	26511085	26511085	29	0.24137931	NA	NA
CNKSR2	Missense_Mutation	p.L282R	G	T		X	21534637	21534637	282	0.858156028	NA	NA
CNTN5	Missense_Mutation	p.N277Y	T	A		11	99827693	99827693	114	0.456140351	NA	NA
CNTN6	Missense_Mutation	p.G731A	C	G		3	1424651	1424651	144	0.229166667	NA	NA
CNTN6	Missense_Mutation	p.R25L	T	G		3	1262389	1262389	252	0.313492063	NA	NA
CNTN6	Missense_Mutation	p.R135L	T	G		3	1320142	1320142	210	0.323809524	NA	NA
CNTN6	Missense_Mutation	p.G151V	T	G		3	1320190	1320190	142	0.394366197	NA	NA
CNTNAP5	Missense_Mutation	p.A130T	A	G		2	125175026	125175026	109	0.302752294	NA	NA
CNTNAP5	Nonsense_Mutation	p.Y963*	A	C		2	125547618	125547618	144	0.5625	NA	NA
COL11A1	Missense_Mutation	p.P687T	T	G		1	103468323	103468323	232	0.340517241	NA	NA
COL11A1	Nonsense_Mutation	p.Q1032*	A	G		1	103412443	103412443	135	0.296296296	NA	NA
COL11A1	Missense_Mutation	p.G973C	A	C		1	103431078	103431078	258	0.310077519	NA	NA

Figure 11 (cont.)

Gene	Variant_Classification	Alteration	Tumor_allele	Ref_allele	dbSNP_RS	Chr. #	Start_pos.	End_pos.	Coverage	Allelic_fraction	# of Probes	Segment_Mean (Log2[Copy
COL11A1	Missense_Mutation	p.G925D	T	C		1	103444280	103444280	370	0.297297297	NA	NA
COL11A1	Splice_Site		-	C		1	103461419	103461419	144	0.381944444	NA	NA
COL17A1	Missense_Mutation	p.G618C	A	C		10	105812876	105812876	140	0.378571429	NA	NA
COL20A1	Missense_Mutation	p.E97Q	C	G		20	61936864	61936864	50	0.12	NA	NA
COL3A1	Missense_Mutation	p.Q617K	A	C		2	189862095	189862095	223	0.457399103	NA	NA
COL5A1	Frame_Shift_Del	p.G931fs	-	G		9	137687153	137687153	211	0.298388863	NA	NA
COL5A2	Missense_Mutation	p.P701L	A	G		2	189923603	189923603	167	0.179640719	NA	NA
COL5A2	Nonsense_Mutation	p.G885*	A	C		2	189917735	189917735	221	0.285067873	NA	NA
COL5A2	Splice_Site	p.G834_splice	A	C		2	189918203	189918203	171	0.239766082	NA	NA
COL6A2	Missense_Mutation	p.M893I	C	G		21	47549327	47549327	60	0.633333333	NA	NA
COLEC12	Missense_Mutation	p.G557W	A	C		18	334889	334889	90	0.088888889	NA	NA
COLEC12	Copy Number	Amplified	NA	NA	NA	18	163455	903638	NA	NA	64	2.2565
CORS7B	Missense_Mutation	p.H202R	G	A		2	232663643	232663643	188	0.240740741	NA	NA
CORO2B	Missense_Mutation	p.D357H	C	G		15	69011486	69011486	265	0.237735849	NA	NA
CP	Missense_Mutation	p.V543L	A	C		3	148916240	148916240	156	0.801282051	NA	NA
CPE	Copy Number	Deleted	NA	NA	NA	4	151336744	190873444	NA	NA	1045	-1.1013
CPS1	Missense_Mutation	p.H698L	T	A		2	211471542	211471542	351	0.438746439	NA	NA
CPS1	Missense_Mutation	p.R1001L	T	G		2	211507250	211507250	226	0.522123894	NA	NA
CRB1	Missense_Mutation	p.R152K	A	G		1	197403905	197403905	173	0.728323699	NA	NA
CREBZF	Missense_Mutation	p.S79F	A	G		11	85375684	85375684	251	0.243027888	NA	NA
CSMD2	Missense_Mutation	p.A2259D	T	G		1	34066557	34066557	369	0.363143631	NA	NA
CSMD2	Missense_Mutation	p.F3230L	T	G		1	34003151	34003151	102	0.303921569	NA	NA
CSMD2	Missense_Mutation	p.E2410Q	G	C		1	34049254	34049254	514	0.379377432	NA	NA
CSMD3	Missense_Mutation	p.L1663F	A	T		8	113516113	113516113	155	0.75483871	NA	NA
CSMD3	Missense_Mutation	p.S681Y	T	G		8	113702210	113702210	348	0.727011494	NA	NA
CSMD3	Missense_Mutation	p.V2840L	A	C		8	113308158	113308158	175	0.754285714	NA	NA
CTSO	Copy Number	Deleted	NA	NA	NA	4	151336744	190873444	NA	NA	1045	-1.1013
CUX1	Splice_Site		G	A		7	101882598	101882598	95	0.221052632	NA	NA
CYLC2	Splice_Site		T	G		9	105765423	105765423	214	0.252336449	NA	NA
CYP286	Missense_Mutation	p.N287K	A	C	rs141345415	19	41515937	41515937	109	0.706422018	NA	NA
CYYR1	Missense_Mutation	p.S109F	A	G		21	27852599	27852599	297	0.333333333	NA	NA
DACT1	Missense_Mutation	p.V701L	T	G		14	59113553	59113553	301	0.275747508	NA	NA
DARS2	Missense_Mutation	p.D267E	A	T		1	173807358	173807358	317	0.283911672	NA	NA
DCLK1	Missense_Mutation	p.I9SV	C	G		13	36699992	36699992	202	0.297029703	NA	NA
DCX	Missense_Mutation	p.T264S	C	G		X	110644375	110644375	91	0.78021978	NA	NA
DDIT4	Missense_Mutation	p.A94V	T	C		10	74034528	74034528	363	0.308539945	NA	NA
DDO	Missense_Mutation	p.I91S	A	T		6	110729631	110729631	168	0.785714286	NA	NA
DGKI	Splice_Site		T	G		7	15737596	15737596	137	0.525547445	NA	NA
DGKI	Missense_Mutation	p.H548Y	T	C		7	15767219	15767219	177	0.38079096	NA	NA
DGKI	Missense_Mutation	p.R62P	G	C		7	137293794	137293794	142	0.514084507	NA	NA
DHCR7	Missense_Mutation	p.G132V	A	C		11	71153326	71153326	25	0.24	NA	NA
DIDO1	Missense_Mutation	p.Q1970L	A	T		20	61511399	61511399	698	0.446991404	NA	NA
DISC1	Missense_Mutation	p.E597L	T	C		1	231935954	231935954	130	0.346153846	NA	NA
DKFZP434I0714	Copy Number	Deleted	NA	NA	NA	4	151336744	190873444	NA	NA	1045	-1.1013
DKK2	Missense_Mutation	p.W208L	A	C		4	107845292	107845292	419	0.415274463	NA	NA
DLC1	Missense_Mutation	p.K125Q	G	T		8	13357208	13357208	600	0.008333333	NA	NA
DMD	Missense_Mutation	p.N122K	T	A		X	32834749	32834749	180	0.783333333	NA	NA
DMD	Missense_Mutation	p.H2006Y	A	G		X	32328300	32328300	114	0.403508772	NA	NA
DMD	Missense_Mutation	p.R70I	A	C		X	32862955	32862955	133	0.842105263	NA	NA
DMXL2	Nonsense_Mutation	p.Q539*	A	G		15	51829687	51829687	121	0.099173554	NA	NA
DNAH11	Missense_Mutation	p.Y1864N	A	T		7	21723510	21723510	463	0.416846652	NA	NA
DNAH5	Missense_Mutation	p.G2218V	A	C		5	13823406	13823406	492	0.851626016	NA	NA
DNAH7	Frame_Shift_Del	p.D1809fs	-	C		2	196750978	196750978	262	0.320610687	NA	NA
DNAJB12	Missense_Mutation	p.M8M	C	G		10	74114614	74114614	91	0.285714286	NA	NA
DOCK2	Missense_Mutation	p.L1001V	G	C		5	169423097	169423097	140	0.721428571	NA	NA
DOCK2	Missense_Mutation	p.T1243M	T	C		5	169468121	169468121	119	0.756302521	NA	NA
DPP6	Missense_Mutation	p.E799V	T	A		7	154684180	154684180	190	0.452631579	NA	NA
DPP6	Nonsense_Mutation	p.E800*	T	G		7	154684182	154684182	191	0.45026178	NA	NA
DSC3	Splice_Site		T	C		18	28598223	28598223	76	0.736842105	NA	NA
DSCAM	Missense_Mutation	p.G381C	A	C		21	41719666	41719666	688	0.713662791	NA	NA
DSCAM	Missense_Mutation	p.D132Y	A	C		21	42064850	42064850	146	0.712328767	NA	NA
DSP	Nonsense_Mutation	p.E1833*	T	G		6	7582992	7582992	246	0.756097561	NA	NA

Figure 11 (cont.)

Gene	Variant_Classification	Alteration	Tumor_allele	Ref_allele	dbSNP_RS	Chr. #	Start_pos.	End_pos.	Coverage	Allelic_fraction	#_of_Probes	Segment_Mean (Log2[Copy
DST	Missense_Mutation	p.S2287I	A	C		6	56481405	56481405	320	0.6375	NA	NA
DST	Missense_Mutation	p.D3902N	T	C		6	56463405	56463405	154	0.194805195	NA	NA
DZIP3	Missense_Mutation	p.V567L	C	G		3	108363568	108363568	246	0.325203252	NA	NA
ECHS1	Missense_Mutation	p.S198L	A	G		10	135180416	135180416	144	0.375	NA	NA
EDNRA	Copy Number	Deleted	NA	NA	NA	4	148407255	151000602	NA	NA	56	-1.1334
EFTUD2	Missense_Mutation	p.K355Q	G	T		17	42945261	42945261	45	0.555555556	NA	NA
EIF2S3	Missense_Mutation	p.G4V	T	G		X	24073096	24073096	85	0.847058824	NA	NA
EIF3M	Missense_Mutation	p.R166T	C	G		11	32611151	32611151	155	0.75483871	NA	NA
EIF4G2	Missense_Mutation	p.M342V	C	T		11	10823955	10823955	378	0.73015873	NA	NA
ELAVL4	Splice_Site	p.G170_splice	T	A		1	50661234	50661234	235	0.391489362	NA	NA
ELAVL4	Splice_Site	p.G170_splice	T	G		1	50661233	50661233	233	0.386266094	NA	NA
EMG1	Copy Number	Deleted	NA	NA	NA	12	6135214	7076942	NA	NA	308	-1.0173
EMILIN1	Missense_Mutation	p.E526K	A	G		2	27306015	27306015	65	0.323076923	NA	NA
ENAH	Missense_Mutation	p.E194K	T	C		1	225707122	225707122	129	0.19379845	NA	NA
ENO2	Copy Number	Deleted	NA	NA	NA	12	6135214	7076942	NA	NA	308	-1.0173
ENPEP	Missense_Mutation	p.S68L	T	C		4	111397773	111397773	261	0.380076628	NA	NA
ENPEP	Missense_Mutation	p.R628L	T	G	rs147351196	4	111463982	111463982	321	0.442367601	NA	NA
ENTPD1	Missense_Mutation	p.D6E	A	C		10	97471731	97471731	195	0.379487179	NA	NA
EPB41	Frame_Shift_Del	p.R538fs	-	G		1	29365915	29365915	181	0.325966851	NA	NA
EPHB6	Frame_Shift_Del	p.Y571fs	-	C		7	142564789	142564789	179	0.553072626	NA	NA
EPN2	Missense_Mutation	p.Y75F	T	A		17	19186656	19186656	186	0.741935484	NA	NA
EPRS	Missense_Mutation	p.D255N	T	C		1	220197786	220197786	233	0.231759657	NA	NA
ERO1L	Missense_Mutation	p.V441E	T	A		14	53112896	53112896	241	0.32780083	NA	NA
ERO1LB	Nonsense_Mutation	p.Q405*	A	G		1	236384262	236384262	111	0.198198198	NA	NA
ESRRG	Missense_Mutation	p.G70V	A	C		1	216850612	216850612	399	0.483709273	NA	NA
ETFDH	Copy Number	Deleted	NA	NA	NA	4	151336744	190873444	NA	NA	1045	-1.1013
EXPH5	Missense_Mutation	p.E1551A	G	T		11	108381582	108381582	230	0.191304348	NA	NA
FAM134B	Missense_Mutation	p.D6N	T	C		5	16508706	16508706	182	0.164835165	NA	NA
FAM135B	Missense_Mutation	p.T488I	A	G		8	139165255	139165255	138	0.297101449	NA	NA
FAM5B	Missense_Mutation	p.D204H	C	G		1	177226461	177226461	125	0.48	NA	NA
FASN:NARF	Rearrangement	Exon 43 of FASN(-) : IGR: 3Kb before NARF(+)	NA	NA	NA	17:17	80037104	80412637	NA	NA	NA	NA
FBF1	Missense_Mutation	p.E888K	T	C		17	73910944	73910944	98	0.255102041	NA	NA
FBN2	Missense_Mutation	p.G2470V	A	C		5	127613634	127613634	163	0.828220859	NA	NA
FBN2	Missense_Mutation	p.T271N	T	G		5	127800431	127800431	203	0.743842365	NA	NA
FBN2	Missense_Mutation	p.K1612N	G	C		5	127648369	127648369	812	0.774630542	NA	NA
FBXL4	Missense_Mutation	p.G227V	A	C		6	99365428	99365428	201	0.825870647	NA	NA
FCGR3B	Missense_Mutation	p.Y35C	C	T		1	161599783	161599783	317	0.362776025	NA	NA
FCGRT	Missense_Mutation	p.A259S	T	G		19	50027937	50027937	76	0.631578947	NA	NA
FEZ1	Missense_Mutation	p.G229E	T	C		11	125325984	125325984	79	0.544303797	NA	NA
FEZ1	Missense_Mutation	p.R16L	A	C	rs138856048	11	125359627	125359627	193	0.569948187	NA	NA
FGA	Copy Number	Deleted	NA	NA	NA	4	151336744	190873444	NA	NA	1045	-1.1013
FGB	Copy Number	Deleted	NA	NA	NA	4	151336744	190873444	NA	NA	1045	-1.1013
FGG	Copy Number	Deleted	NA	NA	NA	4	151336744	190873444	NA	NA	1045	-1.1013
FLNC	Missense_Mutation	p.G1617A	C	G		7	128488959	128488959	169	0.680473373	NA	NA
FLNC	Missense_Mutation	p.A2533V	T	C		7	128497208	128497208	454	0.011013216	NA	NA
FUT1	Missense_Mutation	p.T96S	A	T		13	29041142	29041142	376	0.763297872	NA	NA
FRG1	Copy Number	Deleted	NA	NA	NA	4	151336744	190873444	NA	NA	1045	-1.1013
FUNDC2	Splice_Site		T	G		X	154282869	154282869	248	0.866935484	NA	NA
FYN	Missense_Mutation	p.T82K	T	G		6	112041010	112041010	115	0.756521739	NA	NA
GAA	Splice_Site		T	G		17	78091398	78091398	175	0.228571429	NA	NA
GAB2	Missense_Mutation	p.S83I	A	C		11	77991775	77991775	292	0.517123288	NA	NA
GAPVD1	Missense_Mutation	p.S502F	T	C		9	128074794	128074794	149	0.302013423	NA	NA
GBE1	Missense_Mutation	p.M587I	G	C		3	81586104	81586104	150	0.386666667	NA	NA
GC	Missense_Mutation	p.T41A	C	T		4	72635055	72635055	137	0.394160584	NA	NA
GCM2	Missense_Mutation	p.R67S	T	G		6	10877517	10877517	119	0.756302521	NA	NA
GDI2	Missense_Mutation	p.Q86L	A	T		10	5836979	5836979	318	0.462264151	NA	NA
GEMIN4	Missense_Mutation	p.R30C	A	G		17	651162	651162	59	0.271186441	NA	NA

Figure 11 (cont.)

Gene	Variant_Classification	Alteration	Tumor_allele	Ref_allele	dbSNP_RS	Chr. #	Start_pos.	End_pos.	Coverage	Allelic_fraction	# of Probes	Segment_Mean (Log2[Copy
GFPT2	Nonsense_Mutation	p.Y223*	T	G		5	179751823	179751823	396	0.318606061	NA	NA
GGPS1	Missense_Mutation	p.E43Q	C	G		1	235505079	235505079	187	0.229946524	NA	NA
GK2	Missense_Mutation	p.G533V	A	C		4	80327757	80327757	250	0.38	NA	NA
GLRA3	Copy Number	Deleted	NA	NA	NA	4	151336744	190873444	NA	NA	1045	-1.1013
GLRB	Missense_Mutation	p.W289L	T	G		4	158065073	158065073	104	0.490384615	NA	NA
GLRB	Copy Number	Deleted	NA	NA	NA	4	151336744	190873444	NA	NA	1045	-1.1013
GLTSD2	Missense_Mutation	p.V32M	T	C		12	104408809	104408809	85	0.658823529	NA	NA
GLYAT	Missense_Mutation	p.G189C	A	C		11	58477565	58477565	200	0.245	NA	NA
GMPR	Missense_Mutation	p.G47W	T	G		6	16247124	16247124	213	0.765258216	NA	NA
GNGT2	Missense_Mutation	p.Q3E	C	G		17	47284778	47284778	368	0.277173913	NA	NA
GON4L	Missense_Mutation	p.L1012V	C	G		1	155736230	155736230	346	0.202312139	NA	NA
GPCPD1:CHGB	Rearrangement	Exon 20 of GPCPD1(-) : Intron of CHGB(+); 2Kb before exon 4	NA	NA	NA	20:20	5928348	5930975	NA	NA	NA	NA
GPM6A	Missense_Mutation	p.W141L	A	C		4	176573104	176573104	53	0.528301887	NA	NA
GPM6A	Copy Number	Deleted	NA	NA	NA	4	151336744	190873444	NA	NA	1045	-1.1013
GPR137	Missense_Mutation	p.G430C	T	G		11	64056697	64056697	178	0.185955172	NA	NA
GPR4	Missense_Mutation	p.M57I	A	C		19	46094954	46094954	117	0.752136752	NA	NA
GRIA2	Copy Number	Deleted	NA	NA	NA	4	151336744	190873444	NA	NA	1045	-1.1013
GRIA4	Missense_Mutation	p.G793E	A	G		11	105836757	105836757	130	0.192307692	NA	NA
GRIK1	Missense_Mutation	p.F824L	T	G		21	30927463	30927463	183	0.710382514	NA	NA
GRIK1	Missense_Mutation	p.V826F	A	C		21	30927459	30927459	182	0.714285714	NA	NA
GRM1	Missense_Mutation	p.P598L	T	C		6	146720268	146720268	275	0.810909091	NA	NA
GRM2	Missense_Mutation	p.R445C	T	C		3	51749122	51749122	118	0.36440678	NA	NA
GRM3	Missense_Mutation	p.M918I	A	G		7	86468384	86468384	301	0.235880399	NA	NA
GRM4	Missense_Mutation	p.R437C	A	G		6	34008385	34008385	98	0.173469388	NA	NA
GRM8	Missense_Mutation	p.H506N	T	G		7	126173920	126173920	186	0.569892473	NA	NA
GRM8	Missense_Mutation	p.A455D	T	G		7	126249546	126249546	125	0.56	NA	NA
GTF3A1	Missense_Mutation	p.G196E	T	C		14	81662477	81662477	306	0.098039216	NA	NA
GTF2E1	Missense_Mutation	p.C74F	T	G		3	120469620	120469620	204	0.475490196	NA	NA
GTF3C2	Missense_Mutation	p.D35Y	A	C		2	27566319	27566319	478	0.487447699	NA	NA
GTSE1	Copy Number	Deleted	NA	NA	NA	22	46733843	46860244	NA	NA	30	-1.0829
GUCY1A2	Missense_Mutation	p.L19M	T	G		11	106888727	106888727	51	0.235294118	NA	NA
GUCY1A2	Missense_Mutation	p.D526E	T	G		11	106680833	106680833	230	0.160869565	NA	NA
GUCY1A3	Copy Number	Deleted	NA	NA	NA	4	151336744	190873444	NA	NA	1045	-1.1013
GUCY1B3	Copy Number	Deleted	NA	NA	NA	4	151336744	190873444	NA	NA	1045	-1.1013
GUCY2F	Missense_Mutation	p.S9F	A	G		X	108719140	108719140	67	0.71641791	NA	NA
GYS2	Nonsense_Mutation	p.S683*	T	G		12	21689952	21689952	217	0.599078341	NA	NA
HAL	Missense_Mutation	p.H358Y	A	G		12	96379918	96379918	113	0.265486726	NA	NA
HAND2	Missense_Mutation	p.H9Q	T	G		4	174450414	174450414	5	1	NA	NA
HAND2	Copy Number	Deleted	NA	NA	NA	4	151336744	190873444	NA	NA	1045	-1.1013
HAO1	Splice_Site	p.G325_splice	A	C		20	7866237	7866237	142	0.718309859	NA	NA
HELZ	Splice_Site	p.Q1306_splice	G	T		17	65110441	65110441	168	0.273809524	NA	NA
HEPH	Missense_Mutation	p.P391H	A	C		X	65409727	65409727	12	0.75	NA	NA
HEPH	Missense_Mutation	p.M730I	A	G		X	65420545	65420545	62	0.870967742	NA	NA
HERC2	Missense_Mutation	p.A4349P	G	C		15	28377371	28377371	59	0.271186441	NA	NA
HERC3	Missense_Mutation	p.R44T	G	C		10	69832735	69832735	269	0.342007435	NA	NA
HERC3	Missense_Mutation	p.T451I	A	G		10	69750752	69750752	126	0.373015873	NA	NA
HIST1H2B	Missense_Mutation	p.S38F	A	G		6	27100414	27100414	572	0.335664336	NA	NA
HIST1H4B	Missense_Mutation	p.R46P	G	C		6	26027344	26027344	236	0.741525424	NA	NA
HOXA4	Missense_Mutation	p.P40H	T	G		7	27170234	27170234	45	0.466666667	NA	NA
HOXB8	Missense_Mutation	p.F13S	T	G		17	46691662	46691662	35	0.228571429	NA	NA
HPGD	Copy Number	Deleted	NA	NA	NA	4	151336744	190873444	NA	NA	1045	-1.1013
HPS1	Missense_Mutation	p.E450K	T	C		10	100184119	100184119	70	0.4	NA	NA
HPS3	Missense_Mutation	p.R30L	T	G		3	148847599	148847599	77	0.246753247	NA	NA
HSBP1	Missense_Mutation	p.R44L	T	G		16	83842928	83842928	27	0.185185185	NA	NA
HSD17B3	Missense_Mutation	p.R45L	A	C		9	99064253	99064253	223	0.461883408	NA	NA
HSD17B7	Missense_Mutation	p.S173N	A	G		1	162769603	162769603	320	0.015625	NA	NA
HSPA5	Missense_Mutation	p.E51Q	G	C		9	128003156	128003156	162	0.277777778	NA	NA
HSPA8	Missense_Mutation	p.G348N	T	C		9	128031061	128031061	292	0.270547945	NA	NA

Figure 11 (cont.)

Gene	Variant_Classification	Alteration	Tumor_allele	Ref_allele	dbSNP_RS	Chr. #	Start_pos.	End_pos.	Coverage	Allelic_fraction	# of Probes	Segment_Mean (Log2[Copy
HSPG2	Missense_Mutation	p.G3799C	A	C		1	22158013	22158013	166	0.277108434	NA	NA
HSPH1	Missense_Mutation	p.E659K	T	C		13	31714326	31714326	194	0.221649485	NA	NA
HYDIN	Missense_Mutation	p.K87E	C	T		16	71218770	71218770	407	0.530712531	NA	NA
IBSP	Missense_Mutation	p.A186E	A	C		4	88732665	88732665	125	0.368	NA	NA
IFI16	Missense_Mutation	p.E80K	A	G		1	158984708	158984708	391	0.163682864	NA	NA
IL12RB1	Missense_Mutation	p.F243L	T	G		19	18184381	18184381	53	0.698113208	NA	NA
IL15	Copy Number	Deleted	NA	NA	NA	4	142643163	142654303	NA	NA	3	-1.1402
IL3	Missense_Mutation	p.S37I	T	A		5	131396509	131396509	143	0.741258741	NA	NA
ING2	Copy Number	Deleted	NA	NA	NA	4	151336744	190873444	NA	NA	1045	-1.1013
INMT	Missense_Mutation	p.R200L	T	G		7	30795274	30795274	217	0.414746544	NA	NA
INPPL1	Missense_Mutation	p.G232W	A	C		1	38397423	38397423	45	0.355555556	NA	NA
INTS1	Missense_Mutation	p.V332M	T	C		7	1539594	1539594	126	0.412698413	NA	NA
IQGAP1	Missense_Mutation	p.E1115K	A	G		15	91025217	91025217	198	0.292929293	NA	NA
IQSEC1	Nonsense_Mutation	p.Y300*	T	G		3	12977658	12977658	216	0.486111111	NA	NA
IRF2	Copy Number	Deleted	NA	NA	NA	4	151336744	190873444	NA	NA	1045	-1.1013
ITGA5	Missense_Mutation	p.E658Q	G	C		12	54796777	54796777	195	0.712820513	NA	NA
ITGA8	Missense_Mutation	p.A138V	A	G		10	15729968	15729968	570	0.431578947	NA	NA
ITGA8	Missense_Mutation	p.P194T	T	G		10	15720771	15720771	258	0.403100775	NA	NA
ITGAX	Missense_Mutation	p.G740V	T	G		16	31383757	31383757	209	0.375358852	NA	NA
ITGBL1	Missense_Mutation	p.G837R	C	G		13	102250643	102250643	66	0.878787879	NA	NA
ITIH4	Missense_Mutation	p.T350I	A	G		3	52858328	52858328	284	0.330985915	NA	NA
ITM2A	Missense_Mutation	p.F181L	T	G		X	78616854	78616854	92	0.663043478	NA	NA
KCNA4	Missense_Mutation	p.S581F	A	G		11	30032454	30032454	102	0.352941176	NA	NA
KCNB1	Missense_Mutation	p.G401E	T	C		20	47990895	47990895	251	0.111553785	NA	NA
KCNB2	Missense_Mutation	p.T59K	A	C		8	73480145	73480145	190	0.752631579	NA	NA
KCNH2	Missense_Mutation	p.A608D	T	G		7	150656665	150656665	160	0.23125	NA	NA
KCNK3	Missense_Mutation	p.R142C	T	C		2	26950675	26950675	192	0.364583333	NA	NA
KCNK5	Missense_Mutation	p.A211P	G	C		6	39161948	39161948	97	0.742268041	NA	NA
KCNV1	Missense_Mutation	p.G38V	A	C		8	110986505	110986505	11	0.727272727	NA	NA
KEL	Missense_Mutation	p.L196I	T	G		7	142655000	142655000	124	0.161290323	NA	NA
KIAA0922	Copy Number	Deleted	NA	NA	NA	4	151336744	190873444	NA	NA	1045	-1.1013
KIAA1324	Splice_Site		T	G		1	109715191	109715191	65	0.446153846	NA	NA
KIAA1462	Nonsense_Mutation	p.E877*	A	C		10	30316448	30316448	258	0.379844961	NA	NA
KIAA1524	Missense_Mutation	p.I180L	A	T		3	108300273	108300273	158	0.246835443	NA	NA
KIF5A	Missense_Mutation	p.G86E	A	G		12	57957442	57957442	140	0.457142857	NA	NA
KLF7	Missense_Mutation	p.R227L	A	C		2	207988551	207988551	259	0.28957529	NA	NA
KLHL2	Copy Number	Deleted	NA	NA	NA	4	151336744	190873444	NA	NA	1045	-1.1013
KLHL25	Missense_Mutation	p.D195N	T	C		15	86312459	86312459	165	0.187878788	NA	NA
KLK6	Missense_Mutation	p.D178K	T	G		19	51465050	51465050	120	0.733333333	NA	NA
KLK81	Copy Number	Deleted	NA	NA	NA	4	151336744	190873444	NA	NA	1045	-1.1013
KMO	Missense_Mutation	p.Q283K	A	C		1	241731837	241731837	270	0.392592593	NA	NA
KRT14	Missense_Mutation	p.G26C	A	C		17	39743011	39743011	24	0.625	NA	NA
KRT9	Missense_Mutation	p.D400E	T	G		17	39724608	39724608	308	0.558441558	NA	NA
LACTB	Missense_Mutation	p.M241V	G	A		15	63419657	63419657	264	0.556818182	NA	NA
LAG3	Copy Number	Deleted	NA	NA	NA	12	6135214	7076942	NA	NA	308	-1.0173
LCE2B	Missense_Mutation	p.P36T	A	C		1	152659407	152659407	634	0.299684543	NA	NA
LEPR	Missense_Mutation	p.G345V	T	G		1	66067114	66067114	195	0.328205128	NA	NA
LEPR	Splice_Site		T	G		1	66088665	66088665	111	0.369369369	NA	NA
LEPREL2	Copy Number	Deleted	NA	NA	NA	12	6135214	7076942	NA	NA	308	-1.0173
LGR5	Missense_Mutation	p.G232W	T	G		12	71950448	71950448	223	0.771300448	NA	NA
LHB	Copy Number	Deleted	NA	NA	NA	19	49526459	49558634	NA	NA	18	-1.9589
LILRA4	Missense_Mutation	p.S398L	A	G		19	54848174	54848174	207	0.31884058	NA	NA
LIPC	Missense_Mutation	p.Q63H	T	G		15	58830632	58830632	553	0.262206148	NA	NA
LMOD1	Missense_Mutation	p.K153E	C	T		1	201869684	201869684	211	0.023696682	NA	NA
LMTK3	Missense_Mutation	p.S335Y	T	G		19	49004400	49004400	31	0.774193548	NA	NA
LPA	Missense_Mutation	p.P1567R	C	G		6	160978535	160978535	257	0.785992218	NA	NA
LPHN1	Splice_Site		A	T		19	14268866	14268866	117	0.760683761	NA	NA
LPHN3	Missense_Mutation	p.D693H	C	G		4	62453076	62453076	137	0.445255474	NA	NA
LRBA	Copy Number	Deleted	NA	NA	NA	4	151336744	190873444	NA	NA	1045	-1.1013
LRCH4	Missense_Mutation	p.T407S	A	T		7	100174972	100174972	45	0.577777778	NA	NA
LRP1B	Missense_Mutation	p.E71K	T	C	rs145250710	2	142238097	142238097	294	0.238095238	NA	NA
LRP1B	Nonsense_Mutation	p.Q4087*	A	G		2	141092046	141092046	285	0.326315789	NA	NA
LRP1B	Missense_Mutation	p.V3971E	T	A		2	141819666	141819666	385	0.275324675	NA	NA
LRP1B	Missense_Mutation	p.P7886A	C	G		2	141771140	141771140	189	0.412698413	NA	NA

Figure 11 (cont.)

Gene	Variant_Classification	Alteration	Tumor_allele	Ref_allele	dbSNP_RS	Chr. #	Start_pos.	End_pos.	Coverage	Allelic_fraction	# of Probes	Segment_Mean (Log2[Copy
LRP1B	Missense_Mutation	p.R2605L	A	C	rs147420499	2	141283856	141283856	190	0.273684211	NA	NA
LRPPRC	Missense_Mutation	p.K941N	A	T		2	44152279	44152279	473	0.283298097	NA	NA
LRPPRC	Nonsense_Mutation	p.E1168*	A	C		2	44128523	44128523	351	0.249014245	NA	NA
LRPPRC	Missense_Mutation	p.V441H	G	A		2	44200794	44200794	390	0.284615385	NA	NA
LRRC23	Copy Number	Deleted	NA	NA	NA	12	6135214	7076942	NA	NA	308	-1.0173
LTBR	Copy Number	Deleted	NA	NA	NA	12	6135214	7076942	NA	NA	308	-1.0173
LZTR1	Frame_Shift_Del	p.A504fs	-	C		22	21348454	21348454	17	0.764705882	NA	NA
MAGEC1	Nonsense_Mutation	p.W1020*	A	G		X	140996249	140996249	251	0.035856574	NA	NA
MAGEC1	Missense_Mutation	p.P26H	A	C		X	140993267	140993267	246	0.841463415	NA	NA
MAGI1	Missense_Mutation	p.R1179C	A	G	rs141617754	3	65349184	65349184	430	0.372093023	NA	NA
MAGI1	Missense_Mutation	p.R1057L	A	C		3	65361530	65361530	248	0.362903226	NA	NA
MAP3K11	Missense_Mutation	p.G632C	A	C		11	65367177	65367177	23	0.652173913	NA	NA
MAP9	Copy Number	Deleted	NA	NA	NA	4	151336744	190873444	NA	NA	1045	-1.1013
MAPT	Missense_Mutation	p.S171L	T	C		17	44060682	44060682	51	0.333333333	NA	NA
MCPH1	Missense_Mutation	p.P313S	T	C		8	6302180	6302180	109	0.688073394	NA	NA
MED29	Missense_Mutation	p.L95F	C	G		19	39883109	39883109	127	0.307086614	NA	NA
MEF2A	Missense_Mutation	p.R3W	T	C		15	100173331	100173331	42	0.095238095	NA	NA
MEOX1	Missense_Mutation	p.H192Y	A	G		17	41720924	41720924	37	0.324324324	NA	NA
MERTK	Missense_Mutation	p.G507C	T	G		2	112754968	112754968	561	0.249554367	NA	NA
MFHAS1	Missense_Mutation	p.D477N	T	C		8	8749140	8749140	127	0.307086614	NA	NA
MGA	Missense_Mutation	p.E2886K	A	G		15	42058936	42058936	263	0.330798479	NA	NA
MGAM	Frame_Shift_Ins	p.R2608fs	T	-		7	141799486	141799487	151	0.503311258	NA	NA
MLF1IP	Missense_Mutation	p.Q160H	A	T		4	185637689	185637689	107	0.579439252	NA	NA
MLF1IP	Copy Number	Deleted	NA	NA	NA	4	151336744	190873444	NA	NA	1045	-1.1013
MMP10	Missense_Mutation	p.R315G	C	G	rs150825082	11	102646042	102646042	142	0.183098592	NA	NA
MMP16	Splice_Site		A	C		8	89198704	89198704	478	0.759414226	NA	NA
MMP27	Missense_Mutation	p.F235L	T	G		11	102567481	102567481	180	0.372222222	NA	NA
MMP27	Missense_Mutation	p.P236T	T	G		11	102567480	102567480	181	0.171270718	NA	NA
MMP3	Missense_Mutation	p.D131N	T	C		11	102713270	102713270	341	0.170087977	NA	NA
MMRN1	Missense_Mutation	p.T423S	G	C		4	90856099	90856099	699	0.363376252	NA	NA
MORF4	Copy Number	Deleted	NA	NA	NA	4	151336744	190873444	NA	NA	1045	-1.1013
MPP2	Missense_Mutation	p.S43F	A	G		17	41981836	41981836	168	0.238095238	NA	NA
MST1R	Missense_Mutation	p.L704I	T	G		3	49934786	49934786	90	0.377777778	NA	NA
MTMR1	Missense_Mutation	p.A528E	A	C		X	149919212	149919212	119	0.798319328	NA	NA
MUC16	Missense_Mutation	p.K13539Q	G	T	rs77501519	19	8999560	8999560	157	0.738853503	NA	NA
MYBPC1	Missense_Mutation	p.G894N	A	G		12	102067253	102067253	332	0.298192771	NA	NA
MYH1	Missense_Mutation	p.E1166D	G	C		17	10404667	10404667	388	0.75257732	NA	NA
MYH2	Splice_Site		A	C		17	10428767	10428767	208	0.807692308	NA	NA
MYH2	Nonsense_Mutation	p.Q28*	A	G		17	10451156	10451156	236	0.745762712	NA	NA
MYH2	Missense_Mutation	p.F122L	T	G		17	10448802	10448802	216	0.759259259	NA	NA
MYH8	Missense_Mutation	p.F41I	T	A		17	10323424	10323424	680	0.752941176	NA	NA
MYOM1	Missense_Mutation	p.A1381G	C	G		18	3086145	3086145	74	0.148648649	NA	NA
MYT1	Missense_Mutation	p.T422I	T	C		20	62839814	62839814	53	0.358490566	NA	NA
N8EA	Missense_Mutation	p.E903K	A	G		13	35731288	35731288	300	0.166666667	NA	NA
NBEA	Missense_Mutation	p.A1518V	T	C		13	35747724	35747724	124	0.790322581	NA	NA
NCAPD2	Copy Number	Deleted	NA	NA	NA	12	6135214	7076942	NA	NA	308	-1.0173
NCAPG2	Missense_Mutation	p.K806N	A	C		7	158468277	158468277	184	0.195652174	NA	NA
NDST1	Splice_Site	p.M479_splice	T	G		5	149915447	149915447	39	0.717948718	NA	NA
NEB	Missense_Mutation	p.P1872L	A	G		2	152520210	152520210	216	0.537037037	NA	NA
NEB	Missense_Mutation	p.V196I	T	C		2	152580800	152580800	61	0.262295082	NA	NA
NEB	Nonsense_Mutation	p.Q1888*	A	G		2	152520172	152520172	213	0.305164319	NA	NA
NEK3	Copy Number	Deleted	NA	NA	NA	4	151336744	190873444	NA	NA	1045	-1.1013
NEK3	Copy Number	Deleted	NA	NA	NA	4	151336744	190873444	NA	NA	1045	-1.1013
NEK4	Missense_Mutation	p.D744H	G	C		3	52773514	52773514	356	0.345505618	NA	NA
NEUROD	Missense_Mutation	p.D311N	T	C		7	31377952	31377952	348	0.146551724	NA	NA
NID2	Missense_Mutation	p.P1188T	T	G		14	52477754	52477754	263	0.501901141	NA	NA
NIPSNAP	Missense_Mutation	p.R206L	T	G		9	107521402	107521402	76	0.289473684	NA	NA
NKTR	Missense_Mutation	p.E985K	A	G		3	42680179	42680179	147	0.340136054	NA	NA
NLRC5	Missense_Mutation	p.E247Q	C	G		16	57059594	57059594	272	0.290441176	NA	NA
NOV	Missense_Mutation	p.C140F	T	G		8	120430406	120430406	172	0.76744186	NA	NA
NOX4	Missense_Mutation	p.E18K	T	C		11	89223655	89223655	265	0.233962264	NA	NA

Figure 11 (cont.)

Gene	Variant_Classification	Alteration	Tumor_allele	Ref_allele	dbSNP_RS	Chr. #	Start_pos.	End_pos.	Coverage	Allelic_fraction	# of Probes	Segment_Mean (Log2[Copy
NPAS1	Missense_Mutation	p.G344R	A	G		19	47543769	47543769	16	0.25	NA	NA
NPTX2	Missense_Mutation	p.L174P	C	T		7	98249049	98249049	151	0.251655629	NA	NA
NPY1R	Copy Number	Deleted	NA	NA	NA	4	151336744	190873444	NA	NA	1045	-1.1013
NPY2R	Copy Number	Deleted	NA	NA	NA	4	151336744	190873444	NA	NA	1045	-1.1013
NPY5R	Copy Number	Deleted	NA	NA	NA	4	151336744	190873444	NA	NA	1045	-1.1013
NR3C2	Copy Number	Deleted	NA	NA	NA	4	148407255	151000602	NA	NA	56	-1.1334
NRG1	Missense_Mutation	p.V384L	T	G		8	32617782	32617782	338	0.724852071	NA	NA
NRG1	Missense_Mutation	p.S142F	T	C		8	31497925	31497925	8	0.625	NA	NA
NRP1	Splice_Site		A	C		10	33619635	33619635	133	0.37593985	NA	NA
NRXN1	Missense_Mutation	p.A1380G	C	G		2	50280518	50280518	367	0.261580381	NA	NA
NRXN3	Missense_Mutation	p.S152Y	A	C		14	79175912	79175912	320	0.5125	NA	NA
NRXN3	Missense_Mutation	p.P23H	A	C		14	79117635	79117635	275	0.236363636	NA	NA
NSUN2	Missense_Mutation	p.G255C	A	C	rs140003855	5	6620271	6620271	375	0.408	NA	NA
NTSR1	Missense_Mutation	p.G137S	A	G		20	61340968	61340968	196	0.37244898	NA	NA
NUP12	Missense_Mutation	p.G131V	T	G		7	23226712	23226712	216	0.328703704	NA	NA
OSR1	Missense_Mutation	p.L246F	A	G		2	19552101	19552101	253	0.529644269	NA	NA
OTC	Missense_Mutation	p.D312Y	T	G		X	38271181	38271181	197	0.827411168	NA	NA
PALLD	Copy Number	Deleted	NA	NA	NA	4	151336744	190873444	NA	NA	1045	-1.1013
PARP1	Missense_Mutation	p.R330Q	T	C		1	226573227	226573227	272	0.202205882	NA	NA
PASK	Missense_Mutation	p.R795W	A	T		2	242065947	242065947	130	0.423076923	NA	NA
PCDH9	Missense_Mutation	p.E1127K	T	C		13	66879122	66879122	183	0.224043716	NA	NA
PELO	Missense_Mutation	p.A43355	A	C		7	82544299	82544299	254	0.208661417	NA	NA
PCSK5	Missense_Mutation	p.K1787M	T	A		9	78973615	78973615	319	0.294670846	NA	NA
PCYOX1	Missense_Mutation	p.K205N	T	G		2	70502211	70502211	538	0.293680297	NA	NA
PDE1B	Splice_Site		T	G		12	54967033	54967053	151	0.721854305	NA	NA
PDE6A	Missense_Mutation	p.E748Q	G	C		5	149247317	149247317	47	0.744680851	NA	NA
PDLIM3	Copy Number	Deleted	NA	NA	NA	4	151336744	190873444	NA	NA	1045	-1.1013
PET112L	Copy Number	Deleted	NA	NA	NA	4	151336744	190873444	NA	NA	1045	-1.1013
PHB2	Copy Number	Deleted	NA	NA	NA	12	6135214	7076942	NA	NA	308	-1.0173
PIGG	Frame_Shift_Del	p.Q471fs	-	G		4	515796	515796	83	0.361445783	NA	NA
PIGO	Missense_Mutation	p.G188C	A	C		9	35094306	35094306	175	0.537142857	NA	NA
PKD2	Missense_Mutation	p.D693E	A	C		4	88983115	88983115	364	0.403846154	NA	NA
PLAU	Missense_Mutation	p.S229P	C	T		10	75673793	75673793	99	0.383838384	NA	NA
PLAU	Missense_Mutation	p.S229F	T	C		10	75673794	75673794	99	0.383838384	NA	NA
PLCXD1	Copy Number	Deleted	NA	NA	NA	X	200983	322327	NA	NA	19	-1.1979
PLEK	Missense_Mutation	p.W21C	T	G		2	68607480	68607480	164	0.225609756	NA	NA
PLSCR1	Missense_Mutation	p.H12Y	A	G		3	146251317	146251317	234	0.329059829	NA	NA
PLXNB1	Nonsense_Mutation	p.E1915*	A	C		3	129775190	129775190	268	0.776515152	NA	NA
POLR2D	Missense_Mutation	p.D7V	A	T		2	128615655	128615655	22	0.227272727	NA	NA
PQN3	Missense_Mutation	p.K242N	G	C		7	94992123	94992123	165	0.6	NA	NA
POU2F2	Missense_Mutation	p.P140Q	T	G		19	42803761	42803761	44	0.840909091	NA	NA
POU3F4	Missense_Mutation	p.W113C	T	G		X	82763674	82763674	27	0.592592593	NA	NA
PPAT	Missense_Mutation	p.M428K	T	A		4	57262859	57262859	224	0.40625	NA	NA
PPP1R12	Missense_Mutation	p.R863Q	A	G		1	202531986	202531986	322	0.354037267	NA	NA
PROM1	Missense_Mutation	p.I240F	A	T		4	16024988	16024988	40	0.725	NA	NA
PRTFDC1	Missense_Mutation	p.R13P	G	C	rs138349148	10	25241467	25241467	41	0.195121951	NA	NA
PSD	Missense_Mutation	p.G781R	T	C		10	104165148	104165148	314	0.407643312	NA	NA
PSEN2	Missense_Mutation	p.L265F	T	C		1	227076657	227076657	160	0.1375	NA	NA
PTGER4	Missense_Mutation	p.R220L	T	G		5	40681754	40681754	63	0.396825397	NA	NA
PTMS	Copy Number	Deleted	NA	NA	NA	12	6135214	7076942	NA	NA	308	-1.0173
PTPN13	Missense_Mutation	p.G1831R	A	G		4	87895652	87895652	48	0.4375	NA	NA
PTPN13	Missense_Mutation	p.I2251V	G	A		4	87726513	87726513	70	0.357142857	NA	NA
PTPN3	Missense_Mutation	p.V720M	T	C		9	112144045	112144045	255	0.31372549	NA	NA
PTPN6	Copy Number	Deleted	NA	NA	NA	12	6135214	7076942	NA	NA	308	-1.0173
PTPRD	Missense_Mutation	p.T487K	T	G		9	8517931	8517931	384	0.299479167	NA	NA
PTPRO	Splice_Site		A	T		9	8471087	8471087	278	0.309352518	NA	NA
PTPRS	Missense_Mutation	p.E1442K	A	G		3	62278968	62278968	121	0.330578512	NA	NA
PTPRN2	Frame_Shift_Del	p.E493fs	-	C		7	157903573	157903573	26	0.461538462	NA	NA
PTPRO	Missense_Mutation	p.S109Y	A	C		12	15637158	15637158	82	0.62195122	NA	NA
PTPRT	Missense_Mutation	p.V1211L	A	C		20	40730913	40730913	143	0.398601399	NA	NA
PTPRT	Missense_Mutation	p.R524N	A	C	rs6016756	20	40980914	40980914	186	0.403225806	NA	NA
PTPRZ1	Missense_Mutation	p.P1748T	A	C		7	121674390	121674390	209	0.210526316	NA	NA
PVRL3	Missense_Mutation	p.G144E	A	G		3	110831147	110831147	235	0.234042553	NA	NA

Figure 11 (cont.)

Gene	Variant_Classification	Alteration	Tumor_allele	Ref_allele	dbSNP_RS	Chr. #	Start_pos.	End_pos.	Coverage	Allelic_fraction	# of Probes	Segment_Mean (Log2[Copy
PYGM	Missense_Mutation	p.L393F	G	C		11	64521411	64521411	23	0.739130435	NA	NA
QKI	Missense_Mutation	p.D46E	G	C		6	163836363	163836363	161	0.701863354	NA	NA
RALGPS1	Missense_Mutation	p.K180I	T	A		9	129831564	129831564	366	0.491803279	NA	NA
RAMP1	Missense_Mutation	p.E53G	G	A		2	238785971	238785971	178	0.449438202	NA	NA
RAPGEF6	Missense_Mutation	p.G1363R	T	C		5	130766930	130766930	243	0.732510288	NA	NA
RASL12	Missense_Mutation	p.S102R	C	G		15	65350884	65350884	151	0.549668874	NA	NA
RASSF2	Missense_Mutation	p.R165G	C	T		20	4771141	4771141	120	0.816666667	NA	NA
RAX2	Missense_Mutation	p.Q76K	T	G		19	3770948	3770948	9	0.555555556	NA	NA
RBM12	Missense_Mutation	p.G851A	G	C		20	34240893	34240893	247	0.380566802	NA	NA
RBM6	Missense_Mutation	p.D79Y	T	G		3	50005093	50005093	209	0.349282297	NA	NA
RCVRN	Missense_Mutation	p.P148S	A	G		17	9804357	9804357	228	0.785087719	NA	NA
RECQL5	Missense_Mutation	p.W453R	T	A		17	73627621	73627621	204	0.558921569	NA	NA
RELN	Missense_Mutation	p.R1453M	A	C		7	103214602	103214602	187	0.534759358	NA	NA
REPIN1	Missense_Mutation	p.Q47L	T	A		7	150066940	150066940	183	0.475728155	NA	NA
RFX2	Missense_Mutation	p.G156W	A	C		19	6040047	6040047	27	0.851851852	NA	NA
RFX3	Missense_Mutation	p.A532G	C	G		9	3262945	3262945	485	0.319587629	NA	NA
RFX8	Missense_Mutation	p.G394V	A	C		8	3271024	3271024	256	0.44140625	NA	NA
RGS18	Nonsense_Mutation	p.S86*	A	C		1	192129543	192129543	94	0.734042553	NA	NA
RGS4	Missense_Mutation	p.K267N	T	G		1	163044242	163044242	1022	0.298434442	NA	NA
RGS7	Missense_Mutation	p.A280T	T	C		1	240978023	240978023	221	0.42081448	NA	NA
RGS8	Missense_Mutation	p.G18E	T	C		1	182636082	182636082	362	0.157458564	NA	NA
RNF216	Splice_Site		A	C		7	5792611	5792611	169	0.502958858	NA	NA
RNF40	Missense_Mutation	p.A732S	T	G		16	30780225	30780225	196	0.357142857	NA	NA
RPL28	Missense_Mutation	p.G118W	T	G		19	55899444	55899444	84	0.726190476	NA	NA
RPS3A	Copy Number	Deleted	NA	NA	NA	4	151336744	190873444	NA	NA	1045	-1.1013
RSPH1	Missense_Mutation	p.T223R	T	G		21	43897466	43897466	130	0.738461538	NA	NA
RSRC1	Missense_Mutation	p.R170K	A	G		3	158254811	158254911	123	0.235772358	NA	NA
RTN1	Missense_Mutation	p.E234K	T	C		14	60212741	60212741	573	0.240837696	NA	NA
RYR1	Missense_Mutation	p.E1090Q	C	G		19	38958339	38958339	178	0.314606742	NA	NA
RYR2	Missense_Mutation	p.E2861D	T	G		1	237831251	237831251	26	0.153846154	NA	NA
RYR2	Missense_Mutation	p.E171K	A	G		1	237540670	237540670	106	0.216981132	NA	NA
RYR3	Nonsense_Mutation	p.E1881*	T	G		1	237778099	237778099	155	0.329032258	NA	NA
RYR3	Missense_Mutation	p.V4319L	T	G		15	34131136	34131136	100	0.37	NA	NA
SAP30	Copy Number	Deleted	NA	NA	NA	4	151336744	190873444	NA	NA	1045	-1.1013
SCAMP1	Copy Number	Deleted	NA	NA	NA	4	151336744	190873444	NA	NA	1045	-1.1013
SCN7A	Missense_Mutation	p.V1396L	A	C		2	167262953	167262953	278	0.258992806	NA	NA
SCNN1A	Copy Number	Deleted	NA	NA	NA	12	6135214	7076842	NA	NA	308	-1.0173
SCRG1	Splice_Site		G	C		4	174312323	174312323	178	0.522247191	NA	NA
SCRG1	Copy Number	Deleted	NA	NA	NA	4	151336744	190873444	NA	NA	1045	-1.1013
SEC23A	Missense_Mutation	p.M158I	T	C		14	39543642	39543642	308	0.240259974	NA	NA
SERPINA4	Missense_Mutation	p.G192V	T	G		14	95030394	95030394	407	0.469287469	NA	NA
SERPINB3	Missense_Mutation	p.M275I	T	C		18	61323239	61323239	150	0.36	NA	NA
SERPINE1	Missense_Mutation	p.H186N	A	C		7	100773926	100773926	395	0.212658228	NA	NA
SERPINI2	Missense_Mutation	p.Q191L	A	T		3	167183368	167183368	140	0.771428571	NA	NA
SEZ6L	Missense_Mutation	p.E868D	T	G		22	26761342	26761342	285	0.705263158	NA	NA
SEZ6L2	Nonsense_Mutation	p.Q600*	A	G		16	29888703	29888703	116	0.379310345	NA	NA
SH3RF1	Copy Number	Deleted	NA	NA	NA	4	151336744	190873444	NA	NA	1045	-1.1013
SH3YL1	Splice_Site		A	C		2	219002	219002	143	0.230769231	NA	NA
SH3YL1	Missense_Mutation	p.R13L	A	C		2	234238	234238	234	0.264957265	NA	NA
SIPA1L3	Missense_Mutation	p.V981M	A	G		19	38621210	38621210	144	0.402777778	NA	NA
SLC12A4	Missense_Mutation	p.E660K	T	C		16	67981334	67981334	94	0.202703704	NA	NA
SLC12A8	Missense_Mutation	p.G84W	A	C		3	124909254	124909254	255	0.305882353	NA	NA
SLC18A3	Missense_Mutation	p.R148H	A	G		10	30819229	30819229	401	0.012219959	NA	NA
SLC22A6	Splice_Site	p.0418_splice	A	G		11	62747067	62747067	99	0.232323232	NA	NA
SLC25A13	Missense_Mutation	p.E298K	T	C		7	95818647	95818647	117	0.307692308	NA	NA
SLC25A14	Missense_Mutation	p.W162C	T	G		X	129492706	129492706	137	0.868613139	NA	NA
SLC25A4	Copy Number	Deleted	NA	NA	NA	4	151336744	190873444	NA	NA	1045	-1.1013
SLC25A46	In_Frame_Del	p.L259del	-	CTT		5	110096997	110096999	272	0.080882353	NA	NA
SLC2A9	Splice_Site	p.0444_splice	A	T		4	9836506	9836506	51	0.078431373	NA	NA
SLC35D1	Missense_Mutation	p.S155F	A	G		1	67515522	67515522	49	0.367755102	NA	NA
SLC37A4	Missense_Mutation	p.D72H	G	C		11	118899071	118899071	34	0.235294118	NA	NA
SLC39A10	Missense_Mutation	p.L77F	T	A		2	196544997	196544997	220	0.481818182	NA	NA
SLC4A3	Missense_Mutation	p.A1176S	T	G		2	220505589	220505589	86	0.534883721	NA	NA

Figure 11 (cont.)

Gene	Variant_Classification	Alteration	Tumor_allele	Ref_allele	dbSNP_RS	Chr. #	Start_pos.	End_pos.	Coverage	Allelic_fraction	# of Probes	Segment_Mean (Log2[Copy
SLC4A4	Missense_Mutation	p.E1079K	A	G		4	72433560	72433560	35	0.285714286	NA	NA
SLC7A2	Missense_Mutation	p.V249M	A	G		8	17406267	17406267	347	0.383285303	NA	NA
SLCO1A2	Copy Number	Deleted	NA	NA	NA	12	21036538	21448728	NA	NA	31	-1.0917
SLIT2	Splice_Site		-	A		4	20512116	20512116	296	0.023648649	NA	NA
SLIT2	Frame_Shift_Ins	p.R1431fs	G	-		4	20619217	20619218	55	0.672727273	NA	NA
SLIT3	Missense_Mutation	p.K1198I	A	T		5	168111064	168111064	115	0.747826087	NA	NA
SLIT3	Missense_Mutation	p.R1310G	C	G		5	168098402	168098402	36	0.777777778	NA	NA
SLIT3	Missense_Mutation	p.R137K	T	C		5	168620486	168620486	147	0.326530612	NA	NA
SLIT3	Missense_Mutation	p.Q720R	C	T		5	168175418	168175418	80	0.7	NA	NA
SMARCC1	Missense_Mutation	p.G20W	A	C		3	47823230	47823230	24	0.25	NA	NA
SNCA	Nonsense_Mutation	p.E13*	A	C		4	90756782	90756782	399	0.431077694	NA	NA
SORBS2	Copy Number	Deleted	NA	NA	NA	4	151336744	190873444	NA	NA	1045	-1.1013
SOX9	Missense_Mutation	p.H310N	A	C		17	70110926	70110926	103	0.368932039	NA	NA
SP110	Missense_Mutation	p.Q397E	C	G		2	231050800	231050800	271	0.184501845	NA	NA
SPOCK1	Missense_Mutation	p.A3S	A	C		5	136834241	136834241	14	0.928571429	NA	NA
SPOCK3	Copy Number	Deleted	NA	NA	NA	4	151336744	190873444	NA	NA	1045	-1.1013
SPTA1	Missense_Mutation	p.G1309S	T	C		1	158615356	158615356	370	0.132432432	NA	NA
SPTA1	Missense_Mutation	p.M316I	T	C		1	158647489	158647489	248	0.294354839	NA	NA
SPTBN2	Missense_Mutation	p.D1752H	G	C		11	66459066	66459066	133	0.330827068	NA	NA
SRCAP	Missense_Mutation	p.R3188L	T	G		16	30750924	30750924	23	0.173913043	NA	NA
SRRM2	Missense_Mutation	p.R2689W	T	C		16	2820396	2820396	25	0.4	NA	NA
ST8GAL1	Missense_Mutation	p.A220S	A	C		8	134477046	134477046	239	0.29707113	NA	NA
STOML1	Missense_Mutation	p.W208L	A	C		15	74277826	74277826	8	0.625	NA	NA
STRN	Missense_Mutation	p.V519L	A	C		2	37088389	37088389	175	0.234285714	NA	NA
SYCP1	Missense_Mutation	p.V376M	A	G		1	115428866	115428866	271	0.372693727	NA	NA
SYCP1	Missense_Mutation	p.E342Q	C	G		1	115428764	115428764	162	0.345679012	NA	NA
SYNE1	Missense_Mutation	p.Q6548V	A	T		6	152565721	152565721	192	0.828125	NA	NA
SYNE1	Missense_Mutation	p.H8678Y	A	G		6	152560703	152560703	250	0.38	NA	NA
SYTA	Missense_Mutation	p.L125V	C	G		18	40854021	40854021	179	0.027932961	NA	NA
TACR2	Nonsense_Mutation	p.Q168*	A	G		10	71174792	71174792	105	0.428571429	NA	NA
TACR2	Missense_Mutation	p.A467V	T	C		16	29994963	29994963	946	0.162790698	NA	NA
TBC1D1A	Missense_Mutation	p.D196N	A	G		4	6925702	6925702	55	0.490909091	NA	NA
TBC1D8B	Missense_Mutation	p.D603N	A	G		X	106091509	106091509	106	0.688679245	NA	NA
TDO2	Copy Number	Deleted	NA	NA	NA	4	151336744	190873444	NA	NA	1045	-1.1013
TEK	Missense_Mutation	p.E109G	G	A		9	27158102	27158102	227	0.471365639	NA	NA
TEP1	Missense_Mutation	p.A2153T	T	C		14	20842602	20842602	112	0.303571429	NA	NA
THOC1	Copy Number	Amplified	NA	NA	NA	18	163455	909638	NA	NA	64	2.2565
THRA	Missense_Mutation	p.G303R	C	G		17	38244678	38244678	418	0.526315789	NA	NA
TIAM1	Missense_Mutation	p.A430P	G	C		21	32624181	32624181	152	0.796052632	NA	NA
TJP2	Missense_Mutation	p.G50E	A	G		9	71831289	71831289	209	0.272727273	NA	NA
TKTL2	Copy Number	Deleted	NA	NA	NA	4	151336744	190873444	NA	NA	1045	-1.1013
TLL1	Copy Number	Deleted	NA	NA	NA	4	151336744	190873444	NA	NA	1045	-1.1013
TLR10	Missense_Mutation	p.P347S	A	G		4	38776173	38776173	405	0.449382716	NA	NA
TLR2	Copy Number	Deleted	NA	NA	NA	4	151336744	190873444	NA	NA	1045	-1.1013
TLR3	Missense_Mutation	p.Y383F	T	A		4	187003988	187003988	79	0.670886076	NA	NA
TLR3	Copy Number	Deleted	NA	NA	NA	4	151336744	190873444	NA	NA	1045	-1.1013
TLR9	Missense_Mutation	p.T566K	T	G		3	52256707	52256707	75	0.28	NA	NA
TMED5	Missense_Mutation	p.E98K	T	C		1	93622036	93622036	106	0.443396226	NA	NA
TMEM14A	Copy Number	Deleted	NA	NA	NA	4	151336744	190873444	NA	NA	1045	-1.1013
TMEM2	Missense_Mutation	p.K733N	A	T		9	74337411	74337411	222	0.306306306	NA	NA
TMEM40	Missense_Mutation	p.R208S	T	G	rs142014087	3	12777111	12777111	120	0.266666667	NA	NA
TMEM63	Missense_Mutation	p.N308K	A	C		6	44115168	44115168	110	0.781818182	NA	NA
TNFRSF1	Copy Number	Deleted	NA	NA	NA	12	6135214	7076942	NA	NA	308	-1.0173
TNFSF4	Missense_Mutation	p.G99W	A	C		1	173155912	173155912	394	0.489847716	NA	NA
TNR	Splice_Site		A	C		1	175360575	175360575	152	0.052631579	NA	NA
TNS1	Missense_Mutation	p.M1215Y	A	G		2	218683100	218683100	118	0.347457627	NA	NA
TOMM13	Nonsense_Mutation	p.E333*	T	G		17	53014551	53014551	397	0.251889169	NA	NA
TOP3A	Missense_Mutation	p.R815Q	T	C		17	38557322	38557322	136	0.257352941	NA	NA
TP63	Missense_Mutation	p.Q182L	T	A		3	189526281	189526281	208	0.259615385	NA	NA
TPTE	Missense_Mutation	p.Y404F	A	T		21	10916381	10916381	299	0.073578595	NA	NA
TRIM3	Copy Number	Deleted	NA	NA	NA	4	151336744	190873444	NA	NA	1045	-1.1013
TRIM48	Missense_Mutation	p.D219G	G	A		11	55036795	55036795	203	0.517241379	NA	NA
TRMU	Copy Number	Deleted	NA	NA	NA	22	46733843	46860244	NA	NA	30	-1.0829
TRPC6	Missense_Mutation	p.S503Y	T	G		11	101353691	101353691	285	0.49122807	NA	NA

Figure 11 (cont.)

Gene	Variant_Classification	Alteration	Tumor_allele	Ref_allele	dbSNP_RS	Chr. #	Start_pos.	End_pos.	Coverage	Allelic_fraction	# of Probes	Segment_Mean (Log2[Copy
TRPM1	Missense_Mutation	p.K517N	A	C		15	31341650	31341650	197	0.390862944	NA	NA
TRPM1	Missense_Mutation	p.P568T	T	G		15	31339432	31339442	116	0.336206897	NA	NA
TRPS1	Missense_Mutation	p.T1059N	T	G		8	116426960	116426960	273	0.736263736	NA	NA
TSPAN3	Missense_Mutation	p.G71R	G	C		15	77348450	77348450	367	0.267029973	NA	NA
TTN	Missense_Mutation	p.D9400Y	A	C		2	179575626	179575626	283	0.275618375	NA	NA
TTN	Missense_Mutation	p.P232475	A	G		2	179441120	179441120	96	0.40625	NA	NA
TTN	Missense_Mutation	p.E20478	T	C		2	179640452	179640452	552	0.293478261	NA	NA
TTN	Missense_Mutation	p.I5527T	G	A		2	179610547	179610547	365	0.531506849	NA	NA
TTN	Missense_Mutation	p.E3764K	T	C		2	179617872	179617872	166	0.253012048	NA	NA
TTN	Missense_Mutation	p.P21318T	T	G		2	178451986	178451986	167	0.562874251	NA	NA
TTN	Missense_Mutation	p.Y17629N	T	A		2	179472629	179472629	384	0.5	NA	NA
TTN	Missense_Mutation	p.P223788I	T	G		2	179444881	179444881	332	0.265060241	NA	NA
TTN	Missense_Mutation	p.S35077A	C	A		2	179396113	179396113	459	0.026143791	NA	NA
TTN	Missense_Mutation	p.V8739L	A	C		2	179579286	179579286	181	0.435643564	NA	NA
TTN	Frame_Shift_Del	p.G14726f	-	C		2	179495072	179495072	144	0.4375	NA	NA
TXNDC15	Missense_Mutation	p.V376L	A	C		14	52949666	52949666	98	0.5	NA	NA
TYMS	Copy Number	Amplified	NA	NA	NA	18	163455	909638	NA	NA	64	2.2565
TYW1	Missense_Mutation	p.G301V	T	G		7	66489927	66489927	142	0.549295775	NA	NA
UBE2Y	Frame_Shift_Del	p.G170fs	-	C		1	202301048	202301048	208	0.711538462	NA	NA
UGT2A1	Frame_Shift_Ins	p.L509fs	T	-	rs4148310	4	70455149	70455150	462	0.404761905	NA	NA
UGT2B7	Missense_Mutation	p.S157T	C	G		4	69962708	69962708	510	0.031372549	NA	NA
UNC119B	Nonsense_Mutation	p.Q143*	T	C		12	121154499	121154499	325	0.316923077	NA	NA
UQCRFS1	Missense_Mutation	p.E187K	T	C		19	29698721	29698721	1535	0.112052117	NA	NA
USH2A	Missense_Mutation	p.H2160Q	C	G		1	216173750	216173750	241	0.348547718	NA	NA
USH2A	Missense_Mutation	p.I2975N	T	A		1	216019297	216019297	231	0.376623377	NA	NA
USH2A	Missense_Mutation	p.P2245R	C	G		1	216166433	216166433	707	0.414427157	NA	NA
USH2A	Missense_Mutation	p.L1769M	T	G		1	216251698	216251698	1006	0.331013917	NA	NA
USH2A	Splice_Site	p.Y1105_splice	T	G		1	216380616	216380616	313	0.357827476	NA	NA
USP14	Copy Number	Amplified	NA	NA	NA	18	163455	909638	NA	NA	64	2.2565
USP5	Copy Number	Deleted	NA	NA	NA	12	6135214	7076942	NA	NA	308	-1.0173
UTRN	Missense_Mutation	p.G1617C	T	G		6	144832164	144832164	356	0.769662921	NA	NA
VAMP1	Copy Number	Deleted	NA	NA	NA	12	6135214	7076942	NA	NA	308	-1.0173
VCAN	Missense_Mutation	p.Q961K	A	C		5	82817006	82817006	218	0.683486239	NA	NA
VPS13D	Missense_Mutation	p.S4218N	A	G		1	12520442	12520442	144	0.236111111	NA	NA
VRK1	Missense_Mutation	p.K359N	C	G		14	97342375	97342375	398	0.547738693	NA	NA
VWF	Copy Number	Deleted	NA	NA	NA	12	6135214	7076942	NA	NA	308	-1.0173
WIZ	Missense_Mutation	p.D1632Y	A	C		19	15533949	15533949	101	0.811881188	NA	NA
WWC3	Missense_Mutation	p.R315L	T	G		X	10078030	10078030	44	0.772727273	NA	NA
WWTR1	Nonsense_Mutation	p.Q109*	A	G		3	149374769	149374769	52	0.288461538	NA	NA
YES1	Copy Number	Amplified	NA	NA	NA	18	163455	909638	NA	NA	64	2.2565
YTHDF2	Missense_Mutation	p.L194F	T	G		1	29069364	29069364	166	0.36746988	NA	NA
ZBTB48	Missense_Mutation	p.S228N	A	G		1	6641352	6641352	111	0.342342342	NA	NA
ZC3H14	Missense_Mutation	p.D575N	A	G		14	89069380	89069380	127	0.220472441	NA	NA
ZD8F2	Missense_Mutation	p.D93Y	T	G		2	207169547	207169547	128	0.515625	NA	NA
ZFHX3	Missense_Mutation	p.M1243V	C	T		16	72845613	72845613	212	0.29245283	NA	NA
ZFP36L2	Missense_Mutation	p.R286L	A	C		2	43453086	43453086	71	0.577464789	NA	NA
ZFR	Missense_Mutation	p.R718G	C	G		5	32388771	32388771	225	0.373333333	NA	NA
ZKSCAN3	Missense_Mutation	p.S158L	T	C		6	28329171	28329171	221	0.180995475	NA	NA
ZMYM4	Missense_Mutation	p.M502K	A	T		1	35847295	35847295	281	0.387900356	NA	NA
ZNF18	Missense_Mutation	p.E142K	T	C		17	11894444	11894444	495	0.37979798	NA	NA
ZNF189	Missense_Mutation	p.D250N	A	G		9	104170840	104170840	789	0.276299113	NA	NA
ZNF202	Nonstop_Mutation	p.*849L	A	T		11	123396706	123396706	131	0.549618321	NA	NA
ZNF367	Missense_Mutation	p.C341F	T	G		16	31926892	31926892	259	0.388795367	NA	NA
ZNF267	Missense_Mutation	p.E539K	A	G		16	31927185	31927185	150	0.233333333	NA	NA
ZNF267	Missense_Mutation	p.D301N	A	G		16	31926471	31926471	181	0.248618785	NA	NA
ZNF384	Copy Number	Deleted	NA	NA	NA	12	6135214	7076942	NA	NA	308	-1.0173
ZNF423	Missense_Mutation	p.P551S	A	G		16	49671412	49671412	148	0.236486486	NA	NA
ZNF516	Missense_Mutation	p.S622C	C	G		18	74092205	74092205	115	0.304347826	NA	NA
ZNF559	Missense_Mutation	p.S13L	T	C		19	9449873	9449873	335	0.304477612	NA	NA
ZNF638	Missense_Mutation	p.Q72L	T	A		2	71576299	71576299	462	0.480519481	NA	NA
ZP4	Missense_Mutation	p.V283F	A	C		1	238049179	238049179	174	0.379310345	NA	NA
ZXDC	Missense_Mutation	p.P616A	C	G		3	126180659	126180659	292	0.839041096	NA	NA
CAMKK2	Missense_Mutation	p.K364N	A	C		12	121691091	121691091	84	0.738095238	NA	NA
CDX17	Missense_Mutation	p.P408E	A	G		12	96678921	96678921	190	0.342105263	NA	NA

Figure 11 (cont.)

Gene	Variant_Classification	Alteration	Tumor_allele	Ref_allele	dbSNP_RS	Chr. #	Start_pos.	End_pos.	Coverage	Allelic_fraction	# of Probes	Segment_Mean (Log2[Copy
DYRK4	Missense_Mutation	p.E192K	A	G		12	4708207	4708207	321	0.348909657	NA	NA
OBSCN	Missense_Mutation	p.W7704C	T	G		1	228558454	228558454	266	0.353383459	NA	NA
TBK1	Missense_Mutation	p.R80I	T	G		12	64858124	64858124	149	0.697986577	NA	NA
YLK2	Missense_Mutation	p.D557G	G	A		17	60689790	60689790	266	0.022556391	NA	NA
AADAT	Copy Number	Deleted	NA	NA	NA	4	151336744	190873444	NA	NA	1045	-1.1013
ABCA9	Missense_Mutation	p.L406M	T	A		17	67029927	67029927	346	0.274566474	NA	NA
ABCA9	Missense_Mutation	p.G1329R	T	C		17	66985230	66985230	257	0.038910506	NA	NA
ABCB5	Missense_Mutation	p.Q150H	T	G		7	20682942	20682942	150	0.38	NA	NA
ABHD12	Missense_Mutation	p.L92Q	T	A		20	25319904	25319904	160	0.18125	NA	NA
ACAN	Missense_Mutation	p.G82W	T	G		15	89382097	89382097	256	0.54296875	NA	NA
ACCN5	Copy Number	Deleted	NA	NA	NA	4	151336744	190873444	NA	NA	1045	-1.1013
ACER1	Nonsense_Mutation	p.Y60*	T	G		19	6312424	6312424	178	0.769662921	NA	NA
ACOT11	Nonsense_Mutation	p.K422*	T	A		1	55070776	55070776	158	0.398585859	NA	NA
ACR8P	Copy Number	Deleted	NA	NA	NA	12	6135214	7076942	NA	NA	308	-1.0173
ACTL9	Missense_Mutation	p.D43N	T	C		19	8808925	8808925	170	0.370588235	NA	NA
ACTRT1	Missense_Mutation	p.V156E	T	A		X	127185719	127185719	210	0.823809524	NA	NA
ACVR1C	Missense_Mutation	p.P104T	T	G		2	158412839	158412839	193	0.481865285	NA	NA
ADAM21	Missense_Mutation	p.P474L	T	C		14	70925637	70925637	201	0.303482587	NA	NA
ADAM22	Missense_Mutation	p.D443Y	T	G		7	87774446	87774446	162	0.487654321	NA	NA
ADAM28	Copy Number	Deleted	NA	NA	NA	4	151336744	190873444	NA	NA	1045	-1.1013
ADAMTS1	Missense_Mutation	p.G609C	T	G		11	130340919	130340919	265	0.022641509	NA	NA
ADAMTS17	Missense_Mutation	p.R268P	G	C	rs143880636	15	100802567	100802567	426	0.495305164	NA	NA
ADAMTS1	Missense_Mutation	p.Q1144L	A	T		16	77323280	77323280	244	0.319672131	NA	NA
ADAMTS1	Frame_Shift_Del	p.Q822fs	-	T		16	77359930	77359930	138	0.5	NA	NA
ADAMTS2	Missense_Mutation	p.W1211G	C	A		12	43822461	43822461	82	0.707317073	NA	NA
ADAMTS7	Missense_Mutation	p.L357M	T	G		15	79082140	79082140	25	0.48	NA	NA
ADAMTS7	Missense_Mutation	p.H93AN	T	G		15	79083040	79083040	149	0.483221477	NA	NA
ADAMTSL	Missense_Mutation	p.A1283E	A	C		15	84659841	84659841	559	0.211091234	NA	NA
AGAP3	Missense_Mutation	p.C8R	G	A		10	75457492	75457492	169	0.029585799	NA	NA
AGBL1	Missense_Mutation	p.H492Q	A	C		15	86808016	86808016	269	0.215613383	NA	NA
AGXT2	Missense_Mutation	p.P21SH	T	G		5	35033587	35033587	270	0.377777778	NA	NA
AKAP17A	Missense_Mutation	p.R416P	C	G		X	1719646	1719646	17	0.411764706	NA	NA
AMOTL1	Missense_Mutation	p.P426L	T	C		11	94554851	94554851	131	0.175572519	NA	NA
AMPD2	Frame_Shift_Del	p.L95fs	-	G		1	110167956	110167956	120	0.308333333	NA	NA
AMPD3	Missense_Mutation	p.R3W	T	C		11	10503667	10503667	325	0.32	NA	NA
ANKRD30	Missense_Mutation	p.C181F	T	G		10	37422936	37422936	686	0.380813953	NA	NA
ANKRD30	Missense_Mutation	p.M340T	C	T		10	37431012	37431012	591	0.475465313	NA	NA
ANKRD30	Missense_Mutation	p.G1120R	A	C		18	14851658	14851658	108	0.453703704	NA	NA
ANKRD36	Missense_Mutation	p.I668T	C	T		2	97851209	97851209	77	0.272727273	NA	NA
ANKRD37	Copy Number	Deleted	NA	NA	NA	4	151336744	190873444	NA	NA	1045	-1.1013
ANKRD42	Missense_Mutation	p.R293H	A	G		11	82938882	82938882	233	0.244635193	NA	NA
ANP32C	Copy Number	Deleted	NA	NA	NA	4	151336744	190873444	NA	NA	1045	-1.1013
AP1G1	Splice_Site		-	A		16	71803440	71803440	109	0.064220183	NA	NA
APIS	Missense_Mutation	p.I97L	T	G		11	43342429	43342429	306	0.561688312	NA	NA
ARFGEF1	Splice_Site		G	C		8	68214670	68214670	100	0.6	NA	NA
ARFIP1	Copy Number	Deleted	NA	NA	NA	4	151336744	190873444	NA	NA	1045	-1.1013
ARHGAP1	Copy Number	Deleted	NA	NA	NA	4	148407255	191000602	NA	NA	56	-1.1334
ARHGAP3	Missense_Mutation	p.D1671Y	A	C		11	128840055	128840055	190	0.563157895	NA	NA
ARHGEF3	Nonsense_Mutation	p.E32*	A	C		3	56835733	56835733	13	0.307692308	NA	NA
ARL10	Missense_Mutation	p.V19L	C	G		5	175792621	175792621	11	0.454545455	NA	NA
ASAP1	Missense_Mutation	p.V776F	A	C		8	131124415	131124415	250	0.496	NA	NA
ASB10	Missense_Mutation	p.L398Q	T	A		7	150878072	150878072	56	0.196428571	NA	NA
ASB17	Missense_Mutation	p.S4F	A	G		1	76397966	76397966	117	0.341880342	NA	NA
ASB2	Missense_Mutation	p.D303Y	A	C		14	94413840	94413840	154	0.292207792	NA	NA
ASH1L	Missense_Mutation	p.G415C	A	C		1	155451418	155451418	665	0.607518797	NA	NA
ASH1L	Missense_Mutation	p.LA08V	C	G		1	155451439	155451439	719	0.598052851	NA	NA
ASPRV1	Missense_Mutation	p.V59M	T	C		2	70188646	70188646	101	0.316831683	NA	NA
ASTL	Missense_Mutation	p.R287M	A	C		2	96795577	96795577	163	0.245398773	NA	NA
ASTN1	Missense_Mutation	p.W518R	G	A		1	176934369	176934369	376	0.295212766	NA	NA
ASTN2	Missense_Mutation	p.C1158Y	T	C		9	119249662	119249662	291	0.491408935	NA	NA
ASTN2	Missense_Mutation	p.S476R	T	G		9	119770534	119770534	141	0.546099291	NA	NA
ASTN2	Missense_Mutation	p.C1158S	T	A		9	119249663	119249663	289	0.494809689	NA	NA
ASTN2	In_Frame_Del	p.L221del	-	CAG		9	119976989	119976991	158	0.056962025	NA	NA
ATP13A2	Nonsense_Mutation	p.Q480*	A	G		1	17322560	17322560	286	0.479020979	NA	NA

Figure 11 (cont.)

Gene	Variant_Classification	Alteration	Tumor_allele	Ref_allele	dbSNP_RS	Chr. #	Start_pos.	End_pos.	Coverage	Allelic_fraction	#_of_Probes	Segment_Mean (Log2[Copy
ATP6V0C	Missense_Mutation	p.V13L	C	G		8	87111238	87111238	146	0.7191780882	NA	NA
ATP6V1E	Missense_Mutation	p.E37Q	G	C		2	46739742	46739742	309	0.2815533398	NA	NA
ATP8B3	Missense_Mutation	p.A723E	T	G		19	1792016	1792016	6	0.6666666667	NA	NA
AXDND1	Splice_Site	p.W905_splice	T	G		1	179497565	179497565	138	0.318840058	NA	NA
BCL9L	Missense_Mutation	p.G1442V	A	C		11	118769299	118769299	78	0.6025641103	NA	NA
BCO2	Missense_Mutation	p.R410M	T	G		11	112084481	112084481	468	0.2393162239	NA	NA
BHLHE23	Missense_Mutation	p.E160K	T	C		20	61637601	61637601	57	0.3333333333	NA	NA
BIRC6	Splice_Site	p.Q317_splice	T	G		2	32617218	32617218	27	0.4444444444	NA	NA
BIRC6	Splice_Site		T	G		2	32617219	32617219	27	0.4444444444	NA	NA
BRD7	Missense_Mutation	p.R145K	T	C		16	50388348	50388348	190	0.4631578895	NA	NA
BTBD17	Missense_Mutation	p.A28S	A	C		17	72357877	72357877	73	0.5890410966	NA	NA
C10orf76	Missense_Mutation	p.D539Y	A	C		10	103716444	103716444	128	0.3125	NA	NA
C10orf90	Missense_Mutation	p.Q333K	T	G		10	128192772	128192772	232	0.224137931	NA	NA
C11orf40	Missense_Mutation	p.A155D	T	G		11	4592843	4592843	112	0.75	NA	NA
C11orf92	Missense_Mutation	p.S66I	A	C	rs149987959	11	111167007	111167007	181	0.5496183271	NA	NA
C11orf92	Missense_Mutation	p.S66C	A	T		11	111167008	111167008	181	0.5496183271	NA	NA
C12orf57	Copy Number	Deleted	NA	NA	NA	12	6135214	7076942	NA	NA	308	-1.0173
C13orf35	Missense_Mutation	p.G19A	C	G		13	113333749	113333749	76	0.8289473684	NA	NA
C14orf15	Missense_Mutation	p.W94C	T	G		14	91633837	91633837	44	0.25	NA	NA
C14orf39	Missense_Mutation	p.R136S	T	G	rs340882126	14	60938375	60938375	154	0.2207792221	NA	NA
C17orf67	Missense_Mutation	p.E12K	T	C		17	54893210	54893210	196	0.2857142866	NA	NA
C18orf56	Copy Number	Amplified	NA	NA	NA	18	168455	909638	NA	NA	64	2.2565
C19orf2	Copy Number	Amplified	NA	NA	NA	19	30303946	30496617	NA	NA	12	2.9606
C1orf173	Missense_Mutation	p.G328C	A	C		1	75086436	75086436	201	0.4029850754	NA	NA
C20orf19	Missense_Mutation	p.D139N	T	C		20	3355767	3355767	364	0.2719780223	NA	NA
C2orf44	Missense_Mutation	p.L652F	A	G		2	24254015	24254015	124	0.2338709684	NA	NA
C2orf44	Missense_Mutation	p.G255C	A	C		2	24261602	24261602	206	0.5631067964	NA	NA
C2orf66	Missense_Mutation	p.P95	A	G		2	197674087	197674087	314	0.5159235674	NA	NA
C4orf22	Missense_Mutation	p.D11Y	T	G		4	81256953	81256953	224	0.4017857143	NA	NA
C4orf27	Copy Number	Deleted	NA	NA	NA	4	151336744	190873444	NA	NA	1045	-1.1013
C4orf39	Copy Number	Deleted	NA	NA	NA	4	151336744	190873444	NA	NA	1045	-1.1013
C4orf41	Copy Number	Deleted	NA	NA	NA	4	151336744	190873444	NA	NA	1045	-1.1013
C4orf43	Copy Number	Deleted	NA	NA	NA	4	151336744	190873444	NA	NA	1045	-1.1013
C4orf46	Copy Number	Deleted	NA	NA	NA	4	151336744	190873444	NA	NA	1045	-1.1013
C5orf38	Missense_Mutation	p.A127T	A	G	rs150419625	5	2753444	2753444	243	0.427983539	NA	NA
C6orf120	Missense_Mutation	p.E37K	A	G		6	170102664	170102664	83	0.4457831133	NA	NA
C7orf60	Missense_Mutation	p.E91K	T	C		7	112555392	112555392	269	0.1858736059	NA	NA
C7orf60	Missense_Mutation	p.Y352F	A	T		7	112461962	112461962	126	0.5476190476	NA	NA
C7orf63	Nonsense_Mutation	p.Y186*	A	C		7	89900865	89900865	243	0.4855967078	NA	NA
C7orf63	Missense_Mutation	p.D410N	A	G		7	89909063	89909063	283	0.5689045936	NA	NA
C7orf66	Missense_Mutation	p.H72Q	T	G		7	108524196	108524196	394	0.5329949239	NA	NA
CA851	Missense_Mutation	p.E350K	A	G		4	71201804	71201804	240	0.220833333	NA	NA
CACNA1B	Nonsense_Mutation	p.R963*	T	C		9	140997245	140997245	178	0.2471910112	NA	NA
CACNA1D	Missense_Mutation	p.R1392S	T	G	rs138000029	3	53804531	53804531	160	0.4	NA	NA
CACNA1E	Missense_Mutation	p.G834W	T	G		1	181701779	181701779	22	0.3636363364	NA	NA
CACNA1S	Splice_Site	p.R1447_splice	A	C		1	201017811	201017811	161	0.7453416149	NA	NA
CAL83	Missense_Mutation	p.R80L	A	C		19	16801336	16801336	193	0.7202072539	NA	NA
CAMK1D	Missense_Mutation	p.S343C	T	A		10	12867677	12867677	568	0.0176056338	NA	NA
CAMTA1	Missense_Mutation	p.L470I	A	C		1	7724015	7724015	209	0.2918660287	NA	NA
CAMTA1	Missense_Mutation	p.M1397I	A	G		1	7804903	7804903	204	0.2549019608	NA	NA
CAMTA2	Missense_Mutation	p.A530V	A	G		17	4883025	4883025	201	0.3681592039	NA	NA
CAPN13	Missense_Mutation	p.P41T	T	G		2	31010071	31010071	38	0.5526315789	NA	NA
CAPSL	Missense_Mutation	p.H144Q	T	G		5	35910061	35910061	387	0.3953488372	NA	NA
CARD16	Splice_Site		A	T		11	104915387	104915387	650	0.5492307692	NA	NA
CATSPER	Missense_Mutation	p.P341H	T	G		11	65792829	65792829	94	0.5425531915	NA	NA
CBR4	Copy Number	Deleted	NA	NA	NA	4	151336744	190873444	NA	NA	1045	-1.1013
CCDC110	Copy Number	Deleted	NA	NA	NA	4	151336744	190873444	NA	NA	1045	-1.1013
CCDC111	Copy Number	Deleted	NA	NA	NA	4	151336744	190873444	NA	NA	1045	-1.1013
CCDC146	Missense_Mutation	p.V137M	A	G		7	76871177	76871177	158	0.5253164026	NA	NA

Figure 11 (cont.)

Gene	Variant_Classification	Alteration	Tumor_allele	Ref_allele	dbSNP_RS	Chr. #	Start_pos.	End_pos.	Coverage	Allelic_fraction	# of Probes	Segment_Mean (Log2[Copy
CCDC159	Missense_Mutation	p.E75K	A	G		19	11460872	11460872	54	0.314814815	NA	NA
CCDC18	Missense_Mutation	p.V68F	T	G		1	93649602	93649602	160	0.3375	NA	NA
CCDC22	Splice_Site		T	A		X	49099350	49099350	13	0.692307692	NA	NA
CCDC57	Missense_Mutation	p.D350H	G	C		17	80146099	80146099	19	0.263157895	NA	NA
CCDC74A	Missense_Mutation	p.G231V	T	G		2	132288242	132288242	115	0.095652174	NA	NA
CCDC74B	Missense_Mutation	p.G231V	A	C		2	130899864	130899864	70	0.114285714	NA	NA
CCDC84	Splice_Site	p.S95_splic	G	C		11	118869721	118869721	85	0.317647059	NA	NA
CD1E	Missense_Mutation	p.P245H	A	C		1	158326550	158326550	348	0.316091954	NA	NA
CD300LD	Missense_Mutation	p.V37M	T	C		17	72584920	72584920	393	0.246819338	NA	NA
CDCA3	Copy Number	Deleted	NA	NA	NA	12	6135214	7076942	NA	NA	308	-1.0173
CDH24	Splice_Site	p.E64_spli ce	-	GCTG CGCG		14	23517688	23517698	213	0.38028169	NA	NA
CDH7	Missense_Mutation	p.G658V	T	G		18	63547745	63547745	137	0.605839416	NA	NA
CDK5RAP	Missense_Mutation	p.D209N	T	C		9	123298687	123298687	184	0.494565217	NA	NA
CDK5RAP	Frame_Shift_Ins	p.F167Ifs	T	-		9	123166344	123166345	204	0.191176471	NA	NA
CDKN2A	Copy Number	Deleted	NA	NA	NA	9	19133674A	190873444	NA	NA	1045	-1.1013
CEP350	Missense_Mutation	p.E425C	G	C		1	179981091	179981091	41	0.243902439	NA	NA
CEP85	Frame_Shift_Del	p.G371fs	-	C		1	26584709	26584709	246	0.369918699	NA	NA
CETN1	Copy Number	Amplified	NA	NA	NA	18	163455	909638	NA	NA	64	2.2565
CFTR	Missense_Mutation	p.G27E	A	G		7	117144333	117144333	130	0.230769231	NA	NA
CGB5	Copy Number	Deleted	NA	NA	NA	19	49526459	49558634	NA	NA	18	-1.9589
CGB7	Copy Number	Deleted	NA	NA	NA	19	49526459	49558634	NA	NA	18	-1.9589
CGB8	Copy Number	Deleted	NA	NA	NA	19	49526459	49558634	NA	NA	18	-1.9589
CGNL1	Missense_Mutation	p.R284Q	A	G		15	57731078	57731078	72	0.236111111	NA	NA
CHMP1A	Nonsense_Mutation	p.R51*	A	G		16	89715840	89715840	176	0.545454545	NA	NA
CHRM5	Missense_Mutation	p.L449M	A	C		15	34356263	34356263	143	0.377622378	NA	NA
CLDN2	Missense_Mutation	p.I4F	T	C		X	106171468	106171468	63	0.73015873	NA	NA
CLDN22	Copy Number	Deleted	NA	NA	NA	4	19133674A	190873444	NA	NA	1045	-1.1013
CLNK	Missense_Mutation	p.Q272E	T	G		4	10515180	10515180	25	0.6	NA	NA
CLNK	Missense_Mutation	p.Q272R	C	T		4	10515179	10515179	24	0.583333333	NA	NA
CNOT1	Missense_Mutation	p.Q1230K	T	G		16	58581152	58581152	583	0.288164666	NA	NA
CNST	Missense_Mutation	p.G319S	A	G		1	246810458	246810458	196	0.25	NA	NA
CNTLN	Missense_Mutation	p.S8L	T	C		9	17135086	17135086	20	0.3	NA	NA
CNTN4	Missense_Mutation	p.R76L	T	G		3	2787250	2787250	268	0.361940299	NA	NA
CNTNAP4	Missense_Mutation	p.D872E	A	C		16	76556018	76556018	363	0.272727273	NA	NA
COG8	Missense_Mutation	p.I61Q	T	A		16	69373274	69373274	30	0.666666667	NA	NA
COL22A1	Missense_Mutation	p.G1188V	A	C		8	139648992	139648992	702	0.00997151	NA	NA
COL22A1	Missense_Mutation	p.P1202A	C	G		8	139647258	139647258	130	0.569230769	NA	NA
COL2A1	Missense_Mutation	p.I755K	T	G		1	86482880	86482880	48	0.0625	NA	NA
COL24A1	Missense_Mutation	p.P1478T	T	G		1	86227084	86227084	104	0.403846154	NA	NA
COL25A1	Missense_Mutation	p.A310S	A	C		4	109810868	109810868	243	0.308641975	NA	NA
COL27A1	Missense_Mutation	p.G1003R	C	G		9	117012922	117012922	734	0.490463215	NA	NA
COL27A1	Missense_Mutation	p.L131F	T	C	rs111569026	9	116930226	116930226	258	0.282945736	NA	NA
COL4A3	Splice_Site	p.Q182_sp lice	G	A		2	228113235	228113235	163	0.435582822	NA	NA
COL4A3	Nonsense_Mutation	p.G242*	T	G		2	228118813	228118813	412	0.279126214	NA	NA
COL4A3	Missense_Mutation	p.E1374K	A	G		2	228168827	228168827	99	0.272727273	NA	NA
COPS7A	Copy Number	Deleted	NA	NA	NA	12	6135214	7076942	NA	NA	308	-1.0173
CORO2A	Missense_Mutation	p.V332L	A	C		9	100890975	100890975	120	0.241666667	NA	NA
CPSF2	Missense_Mutation	p.E38D	T	G		14	92597442	92597442	209	0.421052632	NA	NA
CSGALNACT2	Copy Number	Deleted	NA	NA	NA	10	43693645	43871519	NA	NA	10	-1.2948
CSMD1	Missense_Mutation	p.Q3038K	T	G		8	3611461	3611461	86	0.686046512	NA	NA
CSNK1G1	Missense_Mutation	p.T37I	A	G		15	64481669	64481669	11	0.272727273	NA	NA
CTNND2	Missense_Mutation	p.M733I	T	C		5	11117640	11117640	519	0.202312139	NA	NA
CTNND2	Missense_Mutation	p.S1193F	A	G		5	10973665	10973665	282	0.212765957	NA	NA
CTNND2	Missense_Mutation	p.W809C	A	C		5	11111006	11111006	1360	0.380882353	NA	NA
CUBN	Missense_Mutation	p.E2446K	T	C		10	16957046	16957046	187	0.28342246	NA	NA
CWC25	Missense_Mutation	p.R389L	A	C		17	36958457	36958457	101	0.237623762	NA	NA
CXXC4	Missense_Mutation	p.E355K	T	C		4	105411903	105411903	545	0.214678899	NA	NA
CYLC1	Missense_Mutation	p.D383Y	T	G	rs143448675	X	83128863	83128863	23	0.826086957	NA	NA
CYP2W1	Missense_Mutation	p.V358L	T	G		7	1027114	1027114	17	0.470588235	NA	NA
CYP4V2	Copy Number	Deleted	NA	NA	NA	4	19133674A	190873444	NA	NA	1045	-1.1013

Figure 11 (cont.)

Gene	Variant_Classification	Alteration	Tumor_allele	Ref_allele	dbSNP_RS	Chr. #	Start_pos.	End_pos.	Coverage	Allelic_fraction	#_of_Probes	Segment_Mean (Log2[Copy
DAPP1	Missense_Mutation	p.G176C	T	G		4	100782771	100782771	43	0.511627907	NA	NA
DCAF12L	Missense_Mutation	p.R342L	A	C		X	125685567	125685567	113	0.82300885	NA	NA
DCAF12L	Missense_Mutation	p.D189Y	A	C		X	125686027	125686027	85	0.870588235	NA	NA
DCAF4L2	Missense_Mutation	p.G261V	A	C		8	88885418	88885418	199	0.798994975	NA	NA
DCHS2	Copy Number	Deleted	NA	NA	NA	4	151336744	190873444	NA	NA	1045	-1.1013
DCLK2	Missense_Mutation	p.W165C	T	G		4	151023703	151023703	219	0.168949772	NA	NA
DCLK2	Copy Number	Deleted	NA	NA	NA	4	148407255	151000602	NA	NA	56	-1.1334
DCP1B	Missense_Mutation	p.G487V	A	C		12	2061646	2061646	284	0.345070423	NA	NA
DCST2	Missense_Mutation	p.G58C	A	C		1	155006006	155006006	291	0.127147766	NA	NA
DCTD	Copy Number	Deleted	NA	NA	NA	4	151336744	190873444	NA	NA	1045	-1.1013
DDX19B	Splice_Site		G	A		16	70349870	70349870	412	0.45631068	NA	NA
DDX60	Missense_Mutation	p.G89V	A	C		4	169229245	169229245	113	0.451327434	NA	NA
DDX60	Copy Number	Deleted	NA	NA	NA	4	151336744	190873444	NA	NA	1045	-1.1013
DDX60L	Copy Number	Deleted	NA	NA	NA	4	151336744	190873444	NA	NA	1045	-1.1013
DEFB121	Missense_Mutation	p.P54T	T	G		20	29992787	29992787	346	0.384393064	NA	NA
DENND2A	Missense_Mutation	p.P227R	C	G		7	140301518	140301518	391	0.227621483	NA	NA
DFNB59	Missense_Mutation	p.P305T	A	C		2	179325855	179325855	411	0.513381995	NA	NA
DGKQ	Missense_Mutation	p.A543V	A	G		4	959076	959076	103	0.485436893	NA	NA
DNAH10	Missense_Mutation	p.E392D	C	G		12	124270421	124270421	120	0.05	NA	NA
DNAH8	Missense_Mutation	p.W2708L	T	G		6	38863835	38863835	209	0.736842105	NA	NA
DNAH8	Missense_Mutation	p.M2647K	A	T		6	38862484	38862484	160	0.71875	NA	NA
DNHD1	Frame_Shift_Del	p.R4411fs	-	DG		11	6591973	6591974	25	0.72	NA	NA
DOCK5	Splice_Site	p.Y1425_splice	T	C		8	25247723	25247723	20	0.85	NA	NA
DOPEY1	Nonsense_Mutation	p.Q2219*	T	C		6	83866951	83866951	230	0.313043478	NA	NA
DOT1L	Nonsense_Mutation	p.S1121*	A	C		19	2222530	2222530	63	0.746031746	NA	NA
DPRX	Missense_Mutation	p.C162S	A	T		19	54140150	54140150	202	0.737623762	NA	NA
DQX1	Missense_Mutation	p.A397S	A	C		2	74750097	74750097	243	0.259259259	NA	NA
DYNLRB2	Missense_Mutation	p.T20I	T	C		16	80577228	80577228	195	0.020512821	NA	NA
DZIP1L	Missense_Mutation	p.D276Y	A	C		3	137811269	137811269	378	0.788359788	NA	NA
EBF3	Missense_Mutation	p.L130I	T	G		10	131760458	131760458	204	0.480392157	NA	NA
ECI1	Missense_Mutation	p.R259Q	T	C		16	2290113	2290113	93	0.41509434	NA	NA
EDA2R	Missense_Mutation	p.V204L	A	C		X	65819610	65819610	21	0.476190476	NA	NA
EIF3A	Missense_Mutation	p.R939L	A	C		10	120802216	120802216	564	0.507092199	NA	NA
EIF4G3	Missense_Mutation	p.S347R	C	A		1	21268456	21268456	563	0.214920071	NA	NA
ELF2	Missense_Mutation	p.D57E	T	A		4	140046385	140046385	294	0.629251701	NA	NA
ELFN2	Missense_Mutation	p.G659C	A	C		22	37769600	37769600	18	0.888888889	NA	NA
ELFN2	Missense_Mutation	p.A263T	T	C	rs140787341	22	37770782	37770782	98	0.775510204	NA	NA
ELFN2	Missense_Mutation	p.S234I	A	C		22	37770874	37770874	68	0.855072464	NA	NA
EMILIN2	Missense_Mutation	p.A578T	A	G	rs144174650	18	2891848	2891848	137	0.218978102	NA	NA
ENAM	Missense_Mutation	p.Q813E	G	C		4	71508080	71508080	278	0.424460432	NA	NA
ENGASE	Missense_Mutation	p.V245M	A	G		17	77077016	77077016	162	0.339506173	NA	NA
ENOSF1	Copy Number	Amplified	NA	NA	NA	18	163455	9096638	NA	NA	64	2.2565
ENPP3	Missense_Mutation	p.S674F	T	C		6	132054795	132054795	323	0.433436533	NA	NA
ENPP6	Copy Number	Deleted	NA	NA	NA	4	151336744	190873444	NA	NA	1045	-1.1013
EPHA6	Missense_Mutation	p.E367K	A	G		3	96706822	96706822	109	0.211009174	NA	NA
FAM107B	Missense_Mutation	p.E246K	T	C		10	14563936	14563936	363	0.206219178	NA	NA
FAM110B	Missense_Mutation	p.S318L	T	C		8	59059736	59059736	98	0.459183673	NA	NA
FAM110C	Missense_Mutation	p.G266C	A	C		2	45590	45590	95	0.431578947	NA	NA
FAM111A	Missense_Mutation	p.G372E	A	G		11	58920271	58920271	438	0.289954338	NA	NA
FAM111A	Missense_Mutation	p.Q451L	T	A		11	58920493	58920493	219	0.557077626	NA	NA
FAM115C	Missense_Mutation	p.H326N	A	C		7	143417128	143417128	183	0.245901639	NA	NA
FAM129A	Missense_Mutation	p.G67SV	A	C		1	184764874	184764874	177	0.344632768	NA	NA
FAM149A	Copy Number	Deleted	NA	NA	NA	4	151336744	190873444	NA	NA	1045	-1.1013
FAM154B	Missense_Mutation	p.E287K	A	G	rs71650979	15	82575065	82575065	241	0.323651452	NA	NA
FAM181B	Nonsense_Mutation	p.Q390*	A	G		11	82443604	82443604	53	0.169811321	NA	NA
FAM198B	Copy Number	Deleted	NA	NA	NA	4	151336744	190873444	NA	NA	1045	-1.1013
FAM26D	Missense_Mutation	p.R247C	T	C		6	116879168	116879168	256	0.328125	NA	NA
FAM43A	Missense_Mutation	p.N219Q	A	C		3	194408212	194408212	11	0.909090909	NA	NA
FAM5C	Missense_Mutation	p.F66W	T	A		1	190067444	190067444	473	0.236786469	NA	NA
FAM83H	Frame_Shift_Ins	p.L872fs	G	-		8	144809017	144809018	90	0.477777778	NA	NA
FARSB	Missense_Mutation	p.D137H	G	C	rs138598722	2	223804345	223804345	142	0.5	NA	NA

Figure 11 (cont.)

Gene	Variant_Classification	Alteration	Tumor_allele	Ref_allele	dbSNP_RS	Chr. #	Start_pos.	End_pos.	Coverage	Allelic_fraction	# of Probes	Segment_Mean (Log2[Copy
FAT1	Copy Number	Deleted	NA	NA	NA	4	151336744	190873444	NA	NA	1045	-1.1013
FAT3	Missense_Mutation	p.W77C	T	G		11	92085508	92085508	144	0.465277778	NA	NA
FAT3	Missense_Mutation	p.P2701H	A	C		11	92534281	92534281	104	0.596153846	NA	NA
FAT4	Nonsense_Mutation	p.Y1457*	A	C		4	126241937	126241937	295	0.549152542	NA	NA
FBXL22	Frame_Shift_Del	p.DHSEF1S2fs	-	ACCACTCGGAGTT		15	63893598	63893608	65	0.123076923	NA	NA
FBXO10	Missense_Mutation	p.Q529E	C	G		9	37529251	37529251	40	0.075	NA	NA
FBXO40	Missense_Mutation	p.K397N	T	G	rs149191160	3	121341467	121341467	341	0.228739003	NA	NA
FBXO8	Copy Number	Deleted	NA	NA	NA	4	151336744	190873444	NA	NA	1045	-1.1013
FBXW8	Splice_Site		T	G		12	117462126	117462126	72	0.694444444	NA	NA
FFAR3	Missense_Mutation	p.S60L	T	C		19	35849971	35849971	254	0.200787402	NA	NA
FMOC1	Copy Number	Deleted	NA	NA	NA	4	151336744	190873444	NA	NA	1045	-1.1013
FHOD1	Missense_Mutation	p.E451K	T	C		16	67268344	67268344	36	0.194444444	NA	NA
FIGN	Missense_Mutation	p.F733L	C	G		2	164466143	164466143	293	0.453924915	NA	NA
FIGN	Missense_Mutation	p.A732G	C	G		2	164466147	164466147	290	0.455172414	NA	NA
FIS1	Missense_Mutation	p.Q22E	C	G		7	100887402	100887402	80	0.3	NA	NA
FMO2	Missense_Mutation	p.G191E	A	G		1	171168572	171168572	374	0.109625668	NA	NA
FMO6P	Missense_Mutation	p.L297M	A	C		1	171121110	171121110	9	0.666666667	NA	NA
FNIP1	Nonsense_Mutation	p.Q258*	A	G		5	131042162	131042162	270	0.340740741	NA	NA
FNIP2	Copy Number	Deleted	NA	NA	NA	4	151336744	190873444	NA	NA	1045	-1.1013
FOXD4	Missense_Mutation	p.G83R	G	C	rs146632717	9	117873	117873	435	0.098850575	NA	NA
FOXO4L1	Missense_Mutation	p.G86R	C	G		2	114257089	114257089	230	0.126086957	NA	NA
FPR2	Missense_Mutation	p.E14K	A	G		19	52271951	52271951	155	0.309677419	NA	NA
FRAS1	Missense_Mutation	p.P1218R	G	C		4	79308533	79308533	199	0.316582915	NA	NA
FRAS1	Missense_Mutation	p.G49F	T	G		4	79158701	79158701	190	0.352631579	NA	NA
FRMPD4	Splice_Site	p.6537_splice	T	G		X	12728656	12728656	228	0.846491228	NA	NA
FRYL	Missense_Mutation	p.E2974K	T	C		4	48501561	48501561	366	0.232240437	NA	NA
FSCB	Missense_Mutation	p.E402V	A	T		14	44974986	44974986	325	0.541538462	NA	NA
FSCB	Missense_Mutation	p.A555P	G	C		14	44974528	44974528	193	0.435233161	NA	NA
FSD2	Missense_Mutation	p.E228K	T	C		15	83455313	83455313	60	0.25	NA	NA
FSTL5	Copy Number	Deleted	NA	NA	NA	4	151336744	190873444	NA	NA	1045	-1.1013
FTMT	Frame_Shift_Del	p.R47fs	-	G		5	121187795	121187795	33	0.727272727	NA	NA
FUT8	Missense_Mutation	p.A17S	T	G		14	66028330	66028330	467	0.233404711	NA	NA
FXR1	Missense_Mutation	p.R40H	A	G		3	180652940	180652940	215	0.255813953	NA	NA
FXYD4	Copy Number	Deleted	NA	NA	NA	10	43693645	43871519	NA	NA	10	-1.2948
G2E3	Missense_Mutation	p.G50E	A	G		14	31058602	31058602	267	0.288389513	NA	NA
GAB4	Missense_Mutation	p.R438I	A	C		22	17446134	17446134	131	0.671755725	NA	NA
GABRA4	Missense_Mutation	p.W321C	A	C		4	46967158	46967158	403	0.399503722	NA	NA
GABRG1	Missense_Mutation	p.V21L	A	C		4	46125870	46125870	253	0.42687747	NA	NA
GABRG1	Missense_Mutation	p.M378I	T	C		4	46043259	46043259	192	0.1875	NA	NA
GALNT5	Missense_Mutation	p.R514I	T	G		2	158140880	158140880	194	0.273195876	NA	NA
GALNT7	Copy Number	Deleted	NA	NA	NA	4	151336744	190873444	NA	NA	1045	-1.1013
GALNTL6	Copy Number	Deleted	NA	NA	NA	4	151336744	190873444	NA	NA	1045	-1.1013
GCNT4	Missense_Mutation	p.K415N	A	C		5	74324618	74324618	235	0.727659574	NA	NA
GDF10	Missense_Mutation	p.L50M	T	G		10	48438563	48438563	17	0.764705882	NA	NA
GGCX	Missense_Mutation	p.S741F	A	G		2	85777112	85777112	159	0.308176101	NA	NA
GHSR	Missense_Mutation	p.R150W	A	G		3	172165756	172165756	99	0.181818182	NA	NA
GJA8	Nonsense_Mutation	p.E110*	T	G		1	147380410	147380410	219	0.515981735	NA	NA
GLB1L3	Missense_Mutation	p.G259V	T	G		11	134162072	134162072	60	0.616666667	NA	NA
GLI3	Splice_Site	p.G870_splice	T	C		9	3829492	3829492	97	0.298969072	NA	NA
GLTSD1	Missense_Mutation	p.A117T	T	C		9	138516425	138516425	243	0.374485597	NA	NA
GLUD2	Missense_Mutation	p.A173S	T	G		X	120182055	120182055	276	0.862318841	NA	NA
GNB3	Copy Number	Deleted	NA	NA	NA	12	6135214	7076942	NA	NA	308	-1.0173
GOLGA2	Missense_Mutation	p.A361T	T	C		9	131024009	131024009	179	0.340782123	NA	NA
GOSR2	Nonsense_Mutation	p.Q201*	T	C		17	45017938	45017938	383	0.266318538	NA	NA
GPAA1	Missense_Mutation	p.R134L	T	G		8	145138651	145138651	139	0.496402878	NA	NA
GPBP1L1	Missense_Mutation	p.L346F	G	A		1	46099168	46099168	156	0.320512821	NA	NA

Figure 11 (cont.)

Gene	Variant_Classification	Alteration	Tumor_allele	Ref._allele	dbSNP_RS	Chr. #	Start_pos.	End_pos.	Coverage	Allelic_fraction	#_of_Probes	Segment_Mean (Log2[Copy
GPCPD1:C20orf196	Rearrangement	Intron of GPCPD1(-): 2Kb before exon 20 : Intron of C20orf196(+): 27Kb after exon	NA	NA	NA	20:20	5530117	5780711	NA	NA	NA	NA
GPR112	Missense_Mutation	p.I2475F	T	A		X	135453313	135453313	59	0.86440678	NA	NA
GPR151	Missense_Mutation	p.H275Y	A	G		5	145894854	145894854	174	0.689655172	NA	NA
GPR158	Missense_Mutation	p.R396K	A	G		10	25701254	25701254	675	0.462222222	NA	NA
GPR158	Missense_Mutation	p.F345L	A	C		10	25684866	25684866	150	0.32	NA	NA
GPR158	Missense_Mutation	p.D710Y	T	G		10	25885701	25885701	293	0.400556314	NA	NA
GPR162	Copy Number	Deleted	NA	NA	NA	12	6135214	7076942	NA	NA	308	-1.0173
GPRC6A	Missense_Mutation	p.S133I	A	C		6	117130871	117130871	198	0.772727273	NA	NA
GREM2	Frame_Shift_Del	p.R79fs	-	C		1	240656539	240656539	224	0.40625	NA	NA
GRIA1	Frame_Shift_Del	p.P114fs	-	C		5	153026607	153026607	356	0.735955056	NA	NA
GRID2	Missense_Mutation	p.P678H	A	C		4	94436402	94436402	135	0.414814815	NA	NA
GRID2	Missense_Mutation	p.E201K	A	G		4	94031970	94031970	380	0.197368421	NA	NA
GRM5	Missense_Mutation	p.D97Y	A	C		11	88780752	88780752	101	0.207920792	NA	NA
GRM7	Missense_Mutation	p.T737K	A	C		3	7620803	7620803	208	0.365384615	NA	NA
GRM7	Missense_Mutation	p.Q207H	T	G		3	7188240	7188240	196	0.403061224	NA	NA
GSDMC	Splice_Site		-	C		8	130788347	130788347	159	0.389937107	NA	NA
GTF3C5	Missense_Mutation	p.R239T	C	G		9	135926313	135926313	80	0.3	NA	NA
GUF1	Missense_Mutation	p.L198M	A	C		4	44685258	44685258	186	0.440860215	NA	NA
GZF1	Missense_Mutation	p.K587N	C	G		20	23350354	23350354	203	0.527093596	NA	NA
HACE1	Missense_Mutation	p.Q189N	T	C		6	105259238	105259238	163	0.306748466	NA	NA
HAUS8	Missense_Mutation	p.A131S	A	C		19	17169430	17169430	113	0.778761062	NA	NA
HCAR3	Missense_Mutation	p.W142L	A	C		12	123200860	123200860	140	0.457142857	NA	NA
HCN1	Nonsense_Mutation	p.Q408*	A	G		5	45396602	45396602	96	0.239583333	NA	NA
HECTD1	Missense_Mutation	p.D955E	T	A		14	31611072	31611072	156	0.314102564	NA	NA
HECW1	Missense_Mutation	p.L338R	G	T		7	43482190	43482190	124	0.419354839	NA	NA
HECW2	Missense_Mutation	p.A330T	T	C		2	197184626	197184626	130	0.107692308	NA	NA
HELT	Copy Number	Deleted	NA	NA	NA	4	151336744	190873444	NA	NA	1045	-1.1013
HHIPL2	Missense_Mutation	p.M222I	A	C		1	222717187	222717187	116	0.198275862	NA	NA
HIP1R	Missense_Mutation	p.G706C	T	G		12	123343450	123343450	91	0.703296703	NA	NA
HKR1	Copy Number	Deleted	NA	NA	NA	19	37784568	38190798	NA	NA	29	-1.0976
HMCN1	Missense_Mutation	p.A2271G	G	C		1	186023068	186023068	329	0.386018237	NA	NA
HNRNPF	Copy Number	Deleted	NA	NA	NA	10	43693645	43871519	NA	NA	10	-1.2948
HOXA3	Missense_Mutation	p.A138D	T	G		7	27149847	27149847	258	0.395348837	NA	NA
HS3ST4	Missense_Mutation	p.H220L	T	A		16	25704397	25704397	51	0.647058824	NA	NA
HTR1A	Missense_Mutation	p.R227S	T	G		5	63256868	63256868	254	0.744094488	NA	NA
HTR3E	Missense_Mutation	p.H334L	T	A		3	183823991	183823991	138	0.442028986	NA	NA
HTRSA	Nonsense_Mutation	p.G182*	T	G		7	154863153	154863153	108	0.592592593	NA	NA
ICAM5	Missense_Mutation	p.G75W	T	G		19	10401888	10401888	65	0.650793651	NA	NA
IFFO1	Copy Number	Deleted	NA	NA	NA	12	6135214	7076942	NA	NA	308	-1.0173
IFNA14	Missense_Mutation	p.K157T	G	T		9	21239465	21239465	1355	0.292250923	NA	NA
IFNA14	Missense_Mutation	p.L90P	G	A		9	21239666	21239666	489	0.505112474	NA	NA
IGDCC3	Missense_Mutation	p.S750Y	T	G		15	65621443	65621443	18	0.555555556	NA	NA
IGSF9B	Missense_Mutation	p.P1077T	T	G		11	133790391	133790391	132	0.227272727	NA	NA
IKZF3	Nonsense_Mutation	p.E360*	A	C	rs144257863	17	37922495	37922495	246	0.304878049	NA	NA
INCENP	Missense_Mutation	p.E769V	T	A		11	61916233	61916233	31	0.483870968	NA	NA
ING4	Copy Number	Deleted	NA	NA	NA	12	6135214	7076942	NA	NA	308	-1.0173
INO80D	Missense_Mutation	p.R737H	T	C		2	206869966	206869966	165	0.078787879	NA	NA
INPP4B	Missense_Mutation	p.Q13H	G	C		4	143352374	143352374	165	0.2	NA	NA
INTS4	Missense_Mutation	p.R249L	A	C		11	77669843	77669843	65	0.630769231	NA	NA
INTS4	Missense_Mutation	p.G201V	A	C		11	77672054	77672054	482	0.18879668	NA	NA
IPO9	Missense_Mutation	p.K195N	C	G		1	201821302	201821302	115	0.486956522	NA	NA
IQCE	Nonsense_Mutation	p.E603*	T	G		7	2645573	2645573	121	0.165289256	NA	NA
IRS4	Missense_Mutation	p.M924I	A	C		X	107976803	107976803	318	0.691823899	NA	NA
IRX2	Nonsense_Mutation	p.Y470*	T	G		5	2747684	2747684	159	0.396226415	NA	NA
JPH2	Missense_Mutation	p.H324R	C	T		20	42788456	42788456	130	0.338461538	NA	NA
KANK4	Missense_Mutation	p.K130N	A	C		1	62740386	62740386	150	0.373333333	NA	NA
KCNA10	Missense_Mutation	p.R152W	A	G		1	111060956	111060956	211	0.369668246	NA	NA

Figure 11 (cont.)

Gene	Variant_Classification	Alteration	Tumor_allele	Ref_allele	dbSNP_RS	Chr. #	Start_pos.	End_pos.	Coverage	Allelic_fraction	# of Probes	Segment_Mean (Log2[Copy
KCNA7	Copy Number	Deleted	NA	NA	NA	19	49526459	49558634	NA	NA	18	-1.9589
KCNH1	Missense_Mutation	p.R564W	A	G		1	210970994	210970994	155	0.044444444	NA	NA
KCNH5	Missense_Mutation	p.R662M	A	C		14	63246480	63246480	414	0.326086957	NA	NA
KCNH8	Missense_Mutation	p.I387T	T	G		3	195750778	195750778	189	0.354497354	NA	NA
KCNIP3	Missense_Mutation	p.N143K	G	C		2	96040938	96040938	152	0.407894737	NA	NA
KIAA0947	Missense_Mutation	p.A333T	A	G		5	5457750	5457750	550	0.44	NA	NA
KIAA1143	Missense_Mutation	p.E70K	T	C		3	44795761	44795761	207	0.314009662	NA	NA
KIAA1210	Missense_Mutation	p.L1532F	A	G		X	118220599	118220599	54	0.685185185	NA	NA
KIAA1211	Missense_Mutation	p.Q472H	T	G		4	57181084	57181084	20	0.3	NA	NA
KIAA1407	Missense_Mutation	p.G491D	T	C		3	113723483	113723483	499	0.056112224	NA	NA
KIAA1430	Copy Number	Deleted	NA	NA	NA	4	151336744	190873444	NA	NA	1045	-1.1013
KIAA1755	Missense_Mutation	p.T519K	T	G		20	36868121	36868121	143	0.363636364	NA	NA
KIAA1755	Missense_Mutation	p.E1036K	T	C		20	36841941	36841941	51	0.254901961	NA	NA
KIAA1755	Frame_Shift_Del	p.E345fs	-	C		20	36869503	36869503	916	0.459340659	NA	NA
KIF21A	Splice_Site	p.K1366_splice	A	T		12	39705220	39705220	135	0.681481481	NA	NA
KIF21A	Missense_Mutation	p.D1042Y	A	C		12	39725521	39725521	282	0.762411348	NA	NA
KIF26A	Frame_Shift_Del	p.V114fs	-	G		14	104624115	104624115	77	0.311688312	NA	NA
KIN	Missense_Mutation	p.K158N	G	C		10	7820885	7820885	758	0.216358839	NA	NA
KLC4	Missense_Mutation	p.L473V	G	C		6	43039922	43039922	234	0.290598291	NA	NA
KLF17	Missense_Mutation	p.R18L	T	G		1	44584632	44584632	57	0.368421053	NA	NA
KLHL30	Missense_Mutation	p.A514V	T	C		2	239059510	239059510	39	0.666666667	NA	NA
KLHL30	Missense_Mutation	p.E75K	A	G		2	239049618	239049618	465	0.301075269	NA	NA
KLHL5	Missense_Mutation	p.S561T	C	G		4	39109345	39109345	222	0.396396396	NA	NA
KPRP	Missense_Mutation	p.R492W	T	A		1	152733538	152733538	259	0.212355212	NA	NA
KRTAP17-	Missense_Mutation	p.N17K	C	G		17	39471852	39471852	78	0.551282051	NA	NA
KRTAP21-	Missense_Mutation	p.C10F	A	C		21	32119482	32119492	206	0.655339806	NA	NA
KRTAP21-	Missense_Mutation	p.G22C	A	C		21	32119457	32119457	174	0.620689655	NA	NA
KRTAP23-	Missense_Mutation	p.G17C	A	C		21	31720876	31720876	116	0.689655172	NA	NA
KRTAP5-5	Missense_Mutation	p.G134V	T	G		11	1651471	1651471	82	0.048780488	NA	NA
L3MBTL3	Nonsense_Mutation	p.W408*	A	G		6	130392252	130392252	483	0.763975155	NA	NA
LCE1A	Missense_Mutation	p.C110W	G	C		1	152800278	152800278	96	0.177083333	NA	NA
LCE1B	Missense_Mutation	p.P27T	A	C		1	152785001	152785001	586	0.309701493	NA	NA
LETMD1	Missense_Mutation	p.R200Q	A	G		12	51449704	51449704	237	0.016877637	NA	NA
LMX1A	Missense_Mutation	p.R207M	A	C		1	165182927	165182927	865	0.549132948	NA	NA
LPAR5	Copy Number	Deleted	NA	NA	NA	12	6135214	7076942	NA	NA	308	-1.0173
LPCAT3	Copy Number	Deleted	NA	NA	NA	12	6135214	7076942	NA	NA	308	-1.0173
LPCAT4	Missense_Mutation	p.R188P	G	C		15	34656423	34656423	300	0.286666667	NA	NA
LRAT	Copy Number	Deleted	NA	NA	NA	4	151336744	190873444	NA	NA	1045	-1.1013
LRFN5	Missense_Mutation	p.L158P	C	T		14	42356304	42356304	277	0.299638989	NA	NA
LRP2BP	Copy Number	Deleted	NA	NA	NA	4	151336744	190873444	NA	NA	1045	-1.1013
LRRC14B	Nonsense_Mutation	p.Q48S*	T	C		5	195376	195376	300	0.1	NA	NA
LRRC35	Splice_Site	p.S118_splice	A	G		17	79982639	79982639	102	0.333333333	NA	NA
LRRC52	Missense_Mutation	p.V251I	T	G		1	165532900	165532900	180	0.572222222	NA	NA
LRRC66	Missense_Mutation	p.E707K	T	C		4	52861069	52861069	350	0.168571429	NA	NA
LRRTM4	Missense_Mutation	p.T21R	T	G		2	77746933	77746933	121	0.545454545	NA	NA
LYST	Splice_Site	p.V3816_splice	A	C		1	235892956	235892956	155	0.361290323	NA	NA
MAB21L2	Copy Number	Deleted	NA	NA	NA	4	151336744	190873444	NA	NA	1045	-1.1013
MAGEC2	Missense_Mutation	p.P8S	A	G		X	141291752	141291752	204	0.485294118	NA	NA
MAML3	Splice_Site	p.Q36_splice	T	C		4	140811099	140811099	115	0.043478261	NA	NA
MAP3K4	Missense_Mutation	p.E24D	T	G		6	161413035	161413035	11	0.454545455	NA	NA
MAP7D3	Missense_Mutation	p.E745K	T	C		X	135304606	135304606	225	0.382222222	NA	NA
1-Mar	Copy Number	Deleted	NA	NA	NA	4	151336744	190873444	NA	NA	1045	-1.1013
MASP1	Missense_Mutation	p.Y71H	G	A		3	187003639	187003639	255	0.262745098	NA	NA
MDGA2	Missense_Mutation	p.T210K	T	G		14	47530454	47530454	301	0.26910299	NA	NA
MON1	Missense_Mutation	p.G498E	T	C		6	90368471	90368471	506	0.364356436	NA	NA
MEGF8	Missense_Mutation	p.G512C	T	G		19	42847649	42847649	114	0.745614035	NA	NA
MEIS3	Missense_Mutation	p.G305R	G	C		19	47910718	47910718	94	0.765957447	NA	NA
METAP1D	Missense_Mutation	p.T75M	T	C		2	172928464	172928464	108	0.259259259	NA	NA
METTL2A	Missense_Mutation	p.S43A	G	T		17	60501596	60501596	68	0.264705882	NA	NA
MFAP3L	Copy Number	Deleted	NA	NA	NA	4	151336744	190873444	NA	NA	1045	-1.1013
MFSD6	Missense_Mutation	p.P165L	T	C		2	191301249	191301249	246	0.199186992	NA	NA

Figure 11 (cont.)

Gene	Variant_Classification	Alteration	Tumor_allele	Ref_allele	dbSNP_RS	Chr. #	Start_pos.	End_pos.	Coverage	Allelic_fraction	# of Probes	Segment_Mean (Log2[Copy
MITD1	Missense_Mutation	p.D43N	T	C		2	99797318	99797318	292	0.325342466	NA	NA
MLF2	Copy Number	Deleted	NA	NA	NA	12	6135214	7076942	NA	NA	308	-1.0173
MND1	Copy Number	Deleted	NA	NA	NA	4	151336744	190873444	NA	NA	1045	-1.1013
MOGAT3	Missense_Mutation	p.H217R	G	A		2	223558252	223559252	85	0.470588235	NA	NA
MOCS	Missense_Mutation	p.G135V	A	C		2	74691828	74691828	111	0.468468468	NA	NA
MORC1	Missense_Mutation	p.A366S	A	C		3	108776269	108776269	336	0.476190476	NA	NA
MPV17L	Missense_Mutation	p.T184S	T	A		16	15501928	15501928	49	0.653061224	NA	NA
MRC1L1	Missense_Mutation	p.P359S	T	C	rs146543862	10	17891594	17891594	517	0.03868472	NA	NA
MRGPRF	Missense_Mutation	p.P25Q	T	G		11	68773704	68773704	115	0.52173913	NA	NA
MRPL51	Copy Number	Deleted	NA	NA	NA	12	6135214	7076942	NA	NA	308	-1.0173
MS4A12	Missense_Mutation	p.A10D	A	C	rs12788393	11	60264820	60264820	415	0.053012048	NA	NA
MTL5	Missense_Mutation	p.A340V	A	G		11	68480850	68480850	738	0.588075881	NA	NA
MTNR1A	Copy Number	Deleted	NA	NA	NA	4	151336744	190873444	NA	NA	1045	-1.1013
MTUS2	Missense_Mutation	p.P205T	A	C		13	29599418	29599418	181	0.790055249	NA	NA
MUC17	Missense_Mutation	p.A2941V	T	C		7	100683519	100683519	854	0.007025761	NA	NA
MUC17	Missense_Mutation	p.S264R	G	C		7	100675489	100675489	567	0.035273369	NA	NA
MUC17	Missense_Mutation	p.T3654I	T	C		7	100685658	100685658	391	0.217391304	NA	NA
MYF5	Missense_Mutation	p.Q141H	T	G		12	81111265	81111265	309	0.741100324	NA	NA
MYH13	Missense_Mutation	p.E833K	T	C		17	10227476	10227476	122	0.745901639	NA	NA
MYH13	Missense_Mutation	p.R1849S	T	G		17	10206737	10206737	252	0.742063492	NA	NA
MYH15	Missense_Mutation	p.G729E	A	C		3	108175653	108175653	443	0.270880361	NA	NA
MYLK3	Missense_Mutation	p.T302K	T	G		16	46771719	46771719	195	0.528205128	NA	NA
MYO18B	Missense_Mutation	p.L2228M	A	C		22	26422622	26422622	62	0.709677419	NA	NA
MYO1D	Missense_Mutation	p.R318L	A	C		17	31091978	31091978	232	0.771551724	NA	NA
NAA15	Missense_Mutation	p.W375C	T	G		4	140278577	140278577	176	0.238636364	NA	NA
NACA2	Missense_Mutation	p.A135S	A	C		17	59668139	59668139	652	0.26993865	NA	NA
NAF1	Copy Number	Deleted	NA	NA	NA	4	151336744	190873444	NA	NA	1045	-1.1013
NAV3	Missense_Mutation	p.C652F	T	G		12	78415574	78415574	143	0.741258741	NA	NA
NBPF10	Missense_Mutation	p.E349K	A	G		1	145311796	145311796	123	0.146341463	NA	NA
NDST3	Missense_Mutation	p.R528Q	A	G		4	119145689	119145689	465	0.182795699	NA	NA
NEDD1	Missense_Mutation	p.S408F	T	C		12	97337530	97337530	75	0.426666667	NA	NA
NEK5	Missense_Mutation	p.M481I	T	C		13	52660449	52660449	230	0.252173913	NA	NA
NEK9	Missense_Mutation	p.E448D	G	C	rs142097968	13	52661522	52661522	210	0.685714286	NA	NA
NEXN	Missense_Mutation	p.R348I	T	G		1	78399148	78399148	62	0.35483871	NA	NA
NGEF	Missense_Mutation	p.R804P	G	C		2	233758544	233758544	277	0.537906137	NA	NA
NIT2	Missense_Mutation	p.V197A	C	T		3	100071253	100071253	221	0.271493213	NA	NA
NKIRAS2	Missense_Mutation	p.Q131N	A	G		17	40175726	40175726	185	0.27027027	NA	NA
NLGN3	Missense_Mutation	p.A535D	A	C		X	70387611	70387611	143	0.699300699	NA	NA
NLGN4X	Missense_Mutation	p.R173G	C	G		X	5950747	5950747	31	0.838709677	NA	NA
NLRP9	Missense_Mutation	p.E576Q	G	C		19	56243471	56243471	99	0.828282828	NA	NA
NOD1	Missense_Mutation	p.R657P	G	C		7	30491063	30491063	93	0.333333333	NA	NA
NOP14	Missense_Mutation	p.A717T	T	C		4	2943359	2943359	21	0.285714286	NA	NA
NOP2	Copy Number	Deleted	NA	NA	NA	12	6135214	7076942	NA	NA	308	-1.0173
NOTCH4	Missense_Mutation	p.R1924W	A	G		6	32163456	32163456	153	0.366013072	NA	NA
NOTCH4	Missense_Mutation	p.S458F	A	G		6	32187506	32187506	43	0.837209302	NA	NA
NPAS4	Missense_Mutation	p.R595Q	A	G		11	66192145	66192145	282	0.571817808	NA	NA
NPSR1	Missense_Mutation	p.N192K	A	C		7	34867110	34867110	456	0.418859649	NA	NA
NTF4	Copy Number	Deleted	NA	NA	NA	19	49526459	49558634	NA	NA	18	-1.9589
NUMB	Nonsense_Mutation	p.Q408*	A	G		14	73743588	73743588	92	0.239130435	NA	NA
OBSCN	Missense_Mutation	p.R6452C	T	C		1	228547947	228547947	129	0.325581395	NA	NA
OBSCN	Frame_Shift_Del	p.A708fs	-	C		1	228404140	228404140	6	0.5	NA	NA
OOZ3	Copy Number	Deleted	NA	NA	NA	4	151336744	190873444	NA	NA	1045	-1.1013
OLFM3	Missense_Mutation	p.E408Q	A	C		1	102269947	102269947	424	0.370283019	NA	NA
OR10G4	Frame_Shift_Del	p.P182fs	-	C		11	123886825	123886825	421	0.586698337	NA	NA
OR10G7	Frame_Shift_Del	p.P182fs	-	G		11	123909163	123909163	842	0.079572447	NA	NA
OR10R2	Missense_Mutation	p.L325F	C	G		1	158450642	158450642	281	0.516014235	NA	NA
OR10S1	Missense_Mutation	p.P185L	A	G		11	123847845	123847845	121	0.049586777	NA	NA
OR10W1	Missense_Mutation	p.S208C	C	G		11	58034708	58034708	114	0.561403509	NA	NA
OR11G2	Missense_Mutation	p.P231T	A	C		14	20666185	20666185	413	0.554479419	NA	NA
OR13C5	Missense_Mutation	p.V158L	A	C		9	107361223	107361223	528	0.515151515	NA	NA
OR14A16	Missense_Mutation	p.G168V	A	C		1	247978529	247978529	253	0.320158103	NA	NA
OR14A16	Nonsense_Mutation	p.Q181*	A	G		1	247978491	247978491	215	0.465116279	NA	NA
OR1N2	Nonsense_Mutation	p.Y140*	A	T		9	125315868	125315868	415	0.272289157	NA	NA

Figure 11 (cont.)

Gene	Variant_Classification	Alteration	Tumor_allele	Ref_allele	dbSNP_RS	Chr. #	Start_pos.	End_pos.	Coverage	Allelic_fraction	# of Probes	Segment_Mean (Log2[Copy
OR1S2	Missense_Mutation	p.F153L	G	A		11	57971197	57971197	321	0.470404984	NA	NA
OR2A14	Missense_Mutation	p.T39K	A	C		7	143826809	143826809	243	0.288950617	NA	NA
OR2A25	Missense_Mutation	p.S63L	T	C		7	143771500	143771500	252	0.346825397	NA	NA
OR2A7	Missense_Mutation	p.M257I	T	C		7	143955951	143955951	401	0.351620948	NA	NA
OR2L13	Missense_Mutation	p.A107E	A	C		1	248262997	248262997	527	0.290322581	NA	NA
OR2M2	Frame_Shift_Del	p.A38fs	-	C		1	248343400	248343400	558	0.370967742	NA	NA
OR2M3	Missense_Mutation	p.D155Y	T	G		1	248366832	248366832	561	0.315508021	NA	NA
OR2M5	Missense_Mutation	p.R172G	G	C		1	248308963	248308963	730	0.026027397	NA	NA
OR2M7	Missense_Mutation	p.R172G	C	G		1	248487357	248487357	526	0.5	NA	NA
OR2T10	Missense_Mutation	p.A125V	A	G		1	248756696	248756696	242	0.272727273	NA	NA
OR2T11	Missense_Mutation	p.P284H	T	G		1	248789579	248789579	272	0.433823529	NA	NA
OR2T27	Missense_Mutation	p.S229R	T	G	rs141276385	1	248813499	248813499	75	0.266666667	NA	NA
OR2T33	Missense_Mutation	p.G96S	T	C		1	248436831	248436831	489	0.051124744	NA	NA
OR2T33	Missense_Mutation	p.S265C	C	G		1	248436323	248436323	513	0.249512671	NA	NA
OR2T8	Missense_Mutation	p.G96S	A	G		1	248084605	248084605	19	0.210526316	NA	NA
OR2W3	Missense_Mutation	p.Q100H	T	G		1	248059188	248059188	263	0.479087452	NA	NA
OR3A1	Missense_Mutation	p.T163I	A	G		17	3195389	3195389	245	0.685714286	NA	NA
OR4A47	Start_Codon_SNP	p.M1I	A	G		11	48510347	48510347	129	0.062015504	NA	NA
OR4C13	Missense_Mutation	p.Q291H	T	A		11	49974847	49974847	162	0.666666667	NA	NA
OR4C15	Missense_Mutation	p.L108M	A	C		11	55322104	55322104	460	0.232608696	NA	NA
OR4D11	Missense_Mutation	p.M36I	A	G		11	59271156	59271156	275	0.243636364	NA	NA
OR4P4	Missense_Mutation	p.I180I	A	C		11	55406311	55406311	302	0.552980132	NA	NA
OR5I84	Missense_Mutation	p.A116S	A	C		11	5322831	5322831	192	0.671875	NA	NA
OR5I86	Missense_Mutation	p.S306F	T	C		11	5373654	5373654	176	0.357954545	NA	NA
OR5IQ1	Missense_Mutation	p.L311F	T	C		11	5444361	5444361	72	0.916666667	NA	NA
OR5Z82	Missense_Mutation	p.L148M	T	G		11	6191115	6191115	85	0.611764706	NA	NA
OR5D13	Missense_Mutation	p.T112S	T	A		11	55541247	55541247	482	0.209543568	NA	NA
OR5H15	Nonsense_Mutation	p.C97*	A	C		3	97887834	97887834	517	0.232108317	NA	NA
OR5I1	Missense_Mutation	p.F101L	A	C		11	55579245	55579245	518	0.22972973	NA	NA
OR5R1	Missense_Mutation	p.S267T	T	A		11	56184910	56184910	414	0.239130435	NA	NA
OR6N2	Missense_Mutation	p.S108Y	T	G		1	158747109	158747109	387	0.209741602	NA	NA
OR6R1	Missense_Mutation	p.S92F	A	G		11	56058264	56058264	883	0.285390713	NA	NA
OS9P1A	Frame_Shift_Del	p.N793fs	-	T		18	21750331	21750331	293	0.023890785	NA	NA
OSBPL1A	Missense_Mutation	p.L431V	G	C		2	179236856	179236856	130	0.253846154	NA	NA
OSBPL6	Missense_Mutation	p.S344F	T	C		2	179213994	179213994	593	0.180438449	NA	NA
OSBPL8	Splice_Site	p.S1A_splice	A	C		12	76881290	76881290	104	0.653846154	NA	NA
OSTC	Splice_Site		T	G		4	109578804	109578804	272	0.400735294	NA	NA
PALM3	Missense_Mutation	p.P498S	T	C		1	100155311	100155311	223	0.349775785	NA	NA
PAPPA	Nonsense_Mutation	p.Q1398*	T	C		9	118115917	118115917	306	0.297385621	NA	NA
PAPPA2	Nonsense_Mutation	p.Q835*	T	C		1	176661333	176661333	611	0.134206219	NA	NA
PARD3B	Missense_Mutation	p.E890K	A	G		2	206265774	206265774	27	0.185185185	NA	NA
PARD3B	Missense_Mutation	p.G736V	T	G		2	206165275	206165275	471	0.468598726	NA	NA
PCDH10	Missense_Mutation	p.G723C	T	G		4	134073462	134073462	238	0.281512605	NA	NA
PCDH10	Missense_Mutation	p.L43M	A	C		4	134071422	134071422	337	0.537091988	NA	NA
PCDH11X	Missense_Mutation	p.P687R	G	C		X	91133299	91133299	143	0.79020979	NA	NA
PCDH11Y	Missense_Mutation	p.T603R	G	C		Y	4967478	4967478	114	0.692982456	NA	NA
PCDH15	Missense_Mutation	p.T413I	A	G		10	55855510	55955510	176	0.347088824	NA	NA
PCDH15	Splice_Site		T	C		10	56287638	56287638	150	0.433333333	NA	NA
PCDH18	Missense_Mutation	p.Q385L	A	T		4	138452089	138452089	271	0.254612546	NA	NA
PCDH18	Missense_Mutation	p.V261F	A	C		4	138452462	138452462	337	0.237388724	NA	NA
PCDH19	Missense_Mutation	p.D102LY	A	C		X	99551661	99551661	88	0.693181818	NA	NA
PCDH7	Missense_Mutation	p.E1046K	A	G		4	30726180	30726180	207	0.236714976	NA	NA
PCDH8B	Missense_Mutation	p.R518S	C	G		5	140559169	140559169	984	0.219512195	NA	NA
PCDHGB1	Missense_Mutation	p.R32P	C	G		5	140729922	140729922	44	0.340909091	NA	NA
PCMTD1	Missense_Mutation	p.G267R	T	C		8	52733186	52733186	339	0.064896755	NA	NA
PDE1C	Missense_Mutation	p.N49SI	A	T		7	31862785	31862785	319	0.420062696	NA	NA
PDE3A	Missense_Mutation	p.D282Y	T	G		12	20523062	20523062	90	0.433333333	NA	NA
PDHA2	Missense_Mutation	p.A134S	T	G		4	96761701	96761701	463	0.43412527	NA	NA
PDSS1	Missense_Mutation	p.R163L	T	G		10	27009167	27009167	224	0.40625	NA	NA
PDZRN4	Missense_Mutation	p.K617M	T	A		12	41967211	41967211	232	0.754310345	NA	NA
PFN4	Missense_Mutation	p.G83R	T	C		2	24344101	24344101	951	0.228675136	NA	NA
PGAS	Missense_Mutation	p.I324M	G	C		11	61017441	61017441	63	0.587301587	NA	NA
PGBD3	Missense_Mutation	p.S166R	A	C		1	248212034	248212034	114	0.280701754	NA	NA
PHF20L1	Missense_Mutation	p.Q885Y	T	G		8	133855025	133855025	347	0.33814121	NA	NA

Figure 11 (cont.)

Gene	Variant_Classification	Alteration	Tumor_allele	Ref_allele	dbSNP_RS	Chr. #	Start_pos.	End_pos.	Coverage	Allelic_fraction	# of Probes	Segment_Mean (Log2(Copy
PHF23	De_novo_Start_OutOfFrame		A	C		22	45312331	45312331	78	0.679487179	NA	NA
PHF23	Missense_Mutation	p.N185I	A	T		17	7139692	7139692	144	0.722222222	NA	NA
PIKFYVE	Missense_Mutation	p.H489Y	T	C		16	47674973	47674973	267	0.243445693	NA	NA
PIWIL3	Missense_Mutation	p.R701S	A	C		12	130847595	130847595	206	0.432038835	NA	NA
PKD1L2	Missense_Mutation	p.P102Q	T	G		16	81250008	81250008	126	0.293650794	NA	NA
PKHD1	Missense_Mutation	p.S3916L	A	G		6	51491833	51491833	283	0.201413428	NA	NA
PLA2G4E	Splice_Site	p.E370_splice	A	C		15	42289322	42289322	18	0.333333333	NA	NA
PLEKHG6	Copy Number	Deleted	NA	NA	NA	12	6135214	7076942	NA	NA	308	-1.0173
PLEKHH1	Missense_Mutation	p.R344L	T	G		14	68029379	68029379	46	0.369565217	NA	NA
PLRG1	Copy Number	Deleted	NA	NA	NA	4	151336744	190873444	NA	NA	1045	-1.1013
PNKD	Missense_Mutation	p.D212V	T	A		2	219206721	219206721	107	0.46728972	NA	NA
POM121L	Missense_Mutation	p.P235A	G	C		7	53104067	53104067	159	0.415094434	NA	NA
POP1	Missense_Mutation	p.E784K	A	G		8	99168570	99168570	224	0.375	NA	NA
POP4	Missense_Mutation	p.Q16H	C	G		19	30099602	30099602	818	0.216381418	NA	NA
POU4F3	Missense_Mutation	p.R298C	T	C		5	145719882	145719882	110	0.127272727	NA	NA
PPARGC1	Missense_Mutation	p.L409P	C	T		5	149212862	149212862	132	0.795454545	NA	NA
PPID	Copy Number	Deleted	NA	NA	NA	4	151336744	190873444	NA	NA	1045	-1.1013
PPP1R13L	Missense_Mutation	p.S584F	A	G		19	45895172	45895172	68	0.75	NA	NA
PPP1R13L	Missense_Mutation	p.Q284L	A	T		19	45899478	45899478	31	0.709677419	NA	NA
PPP1R18	Missense_Mutation	p.P53T	A	C		17	37785807	37785807	148	0.296551724	NA	NA
PPP2R3B	Copy Number	Deleted	NA	NA	NA	X	300983	322327	NA	NA	19	-1.1979
PPP4R2	Nonsense_Mutation	p.G157*	T	G		3	73112873	73112873	223	0.358744395	NA	NA
PRDM2	Missense_Mutation	p.G1109C	T	G		1	14107615	14107615	337	0.296735905	NA	NA
PRDM9	Missense_Mutation	p.K21N	T	G		5	23509205	23509205	250	0.348	NA	NA
PRDM9	Missense_Mutation	p.G800V	T	G		5	23527596	23527596	473	0.361522199	NA	NA
PREX2	Missense_Mutation	p.W352C	T	G		8	68965444	68965444	225	0.791111111	NA	NA
PRMT10	Copy Number	Deleted	NA	NA	NA	4	148407255	151000602	NA	NA	56	-1.1334
PROKR2	Missense_Mutation	p.E319K	T	C		20	5282886	5282886	323	0.281733746	NA	NA
PROM2	Missense_Mutation	p.G17V	T	G		2	95940383	95940383	136	0.286764706	NA	NA
PRRG1	Missense_Mutation	p.G70A	C	G		X	37312426	37312426	78	0.871794872	NA	NA
PRSS48	Copy Number	Deleted	NA	NA	NA	4	151336744	190873444	NA	NA	1045	-1.1013
PRUNE2	Missense_Mutation	p.P2010A	C	G		9	79320085	79320085	437	0.244851259	NA	NA
PSPC1	Missense_Mutation	p.G504C	A	C		13	20277377	20277377	183	0.180327869	NA	NA
PSPC1	Missense_Mutation	p.G504V	A	C		13	20277376	20277376	182	0.181318681	NA	NA
PSPC1	Missense_Mutation	p.G512V	A	C		13	20277352	20277352	167	0.143712575	NA	NA
PTPDC1	Missense_Mutation	p.E70K	A	G		9	96847020	96847020	296	0.206081081	NA	NA
PTPN4	Missense_Mutation	p.A21D	A	C		2	120567491	120567491	308	0.256493506	NA	NA
PTPRB	Missense_Mutation	p.T2335	C	G		12	71016180	71016180	205	0.72195122	NA	NA
PTPRB	Missense_Mutation	p.E1769K	T	C		12	70946639	70946639	184	0.293478261	NA	NA
PUS7	Missense_Mutation	p.N317H	G	T		7	105122859	105122859	350	0.585714286	NA	NA
PYROXD2	Missense_Mutation	p.I169F	A	C		10	100157220	100157220	137	0.306569343	NA	NA
QRICH2	Missense_Mutation	p.Q960H	G	C		17	74287430	74287430	141	0.553191489	NA	NA
RAD9B	Missense_Mutation	p.P306Q	T	G		7	4874737	4874737	53	0.339622642	NA	NA
RANBP6; RANBP6	Rearrangement	Exon 1 of RANBP6(-) : IGR: 26Kb before RANBP6(-)	NA	NA	NA	9.09	6014819	6037359	NA	NA	NA	NA
RAPGEF2	Copy Number	Deleted	NA	NA	NA	4	151336744	190873444	NA	NA	1045	-1.1013
RAPH1	Missense_Mutation	p.R46M	A	T		2	204356026	204356026	191	0.251308901	NA	NA
RASGEF1	Copy Number	Deleted	NA	NA	NA	10	43893645	43871519	NA	NA	10	-1.2948
RASSF8	Splice_Site		-	A		12	26220500	26220500	74	0.77027027	NA	NA
RBM44	Nonsense_Mutation	p.R732*	T	A		2	238730456	238730456	60	0.416666667	NA	NA
RBM44	Missense_Mutation	p.R732M	T	A		2	238730457	238730457	57	0.403508772	NA	NA
RBM44	Splice_Site		G	A		2	238722233	238722233	90	0.533333333	NA	NA
RBM46	Copy Number	Deleted	NA	NA	NA	4	151336744	190873444	NA	NA	1045	-1.1013
RDH14	Missense_Mutation	p.E309V	A	T		2	18736542	18736542	388	0.290488432	NA	NA
RGPD4	Missense_Mutation	p.R1699K	A	G		2	108499159	108499159	584	0.030821918	NA	NA
RIC3	Missense_Mutation	p.E52K	T	C		11	8148236	8148236	167	0.341317365	NA	NA
RNF149	Missense_Mutation	p.P398L	A	G		2	101893710	101893710	125	0.272	NA	NA
RNF175	Copy Number	Deleted	NA	NA	NA	4	151336744	190873444	NA	NA	1045	-1.1013
RNF6	Missense_Mutation	p.E380D	A	T		13	26788879	26788879	233	0.042918455	NA	NA
RPN2	Missense_Mutation	p.E202K	A	G		20	35833218	35833218	374	0.136363636	NA	NA

Figure 11 (cont.)

Gene	Variant_Classification	Alteration	Tumor_allele	Ref_allele	dbSNP_RS	Chr. #	Start_pos.	End_pos.	Coverage	Allelic_fraction	# of Probes	Segment_Mean (Log2[Copy
RPTN	Missense_Mutation	p.G504R	G	C		1	152128865	152128865	2804	0.296836234	NA	NA
RSC1A1	Missense_Mutation	p.D288H	C	G		1	15987210	15987210	328	0.262195122	NA	NA
RTNA	Missense_Mutation	p.L590V	C	G		2	55253467	55253467	304	0.447368421	NA	NA
RYR3	Missense_Mutation	p.P177L	T	C		15	46542220	46542220	182	0.346153846	NA	NA
RUNDC1	Missense_Mutation	p.S465L	T	C		17	41143285	41143285	202	0.282178218	NA	NA
RUVBL2	Copy Number	Deleted	NA	NA	NA	19	49526459	49558684	NA	NA	18	-1.9589
RWDD3	Frame_Shift_Ins	p.S2A5fs	A	-		1	95712356	95712357	99	0.353535354	NA	NA
RXFP1	Copy Number	Deleted	NA	NA	NA	4	151336744	190873444	NA	NA	1045	-1.1013
RXFP4	Missense_Mutation	p.L40I	A	C		1	155911618	155911618	434	0.011520737	NA	NA
RXFP4	Nonsense_Mutation	p.L58*	A	T		1	155911673	155911673	338	0.180473373	NA	NA
S100P8P	Missense_Mutation	p.D284G	G	A		1	33293613	33293613	223	0.031390135	NA	NA
SAL11	Nonsense_Mutation	p.S1049*	T	G		16	51172696	51172696	113	0.477876106	NA	NA
SAMD3	Missense_Mutation	p.Q140E	C	G		6	130505728	130505728	104	0.75	NA	NA
SCARF1	Missense_Mutation	p.G778W	A	C		17	1538210	1538210	135	0.777777778	NA	NA
SCD5	Missense_Mutation	p.P33T	T	G		4	83719594	83719594	116	0.439655172	NA	NA
SCMH1	Missense_Mutation	p.S19F	A	G		1	41618291	41618291	110	0.390909091	NA	NA
SDX1	Frame_Shift_Ins	p.MG1558	G	-		7	4198131	4198132	247	0.340080972	NA	NA
SEC16B	Missense_Mutation	p.G988W	A	C		1	177901672	177901672	18	0.333333333	NA	NA
SEC16B	Missense_Mutation	p.E905K	T	C		15	49285034	49285034	475	0.294736842	NA	NA
SEL1L2	Missense_Mutation	p.D443E	C	G		20	13847428	13847428	250	0.02	NA	NA
SEMA3D	Missense_Mutation	p.C128R	G	A		7	84702391	84702391	310	0.251612903	NA	NA
SEMA3D	Frame_Shift_Del	p.A86fs	-	G		7	84727175	84727175	536	0.208178439	NA	NA
SEMA4G	Missense_Mutation	p.S781N	A	G		10	102743728	102743728	109	0.394495413	NA	NA
SEMA6D	Missense_Mutation	p.E767Q	C	G		15	48063059	48063059	219	0.219178082	NA	NA
SENP5	Missense_Mutation	p.Q707H	C	G		3	196656518	196656618	262	0.175572519	NA	NA
SERPINA1	Missense_Mutation	p.D219N	T	C		14	94962960	94962960	213	0.225352113	NA	NA
SERPINH1	Missense_Mutation	p.L83V	G	C		11	75277641	75277641	38	0.342105263	NA	NA
SESTD1	Missense_Mutation	p.G230V	A	C		2	180008479	180008479	347	0.328530259	NA	NA
SETX	Nonsense_Mutation	p.W2282*	T	C		9	135152507	135152507	155	0.264516129	NA	NA
SF3B3	Missense_Mutation	p.E752Q	C	G		16	70595653	70595653	313	0.319488818	NA	NA
SFRP2	Copy Number	Deleted	NA	NA	NA	4	151336744	190873444	NA	NA	1045	-1.1013
SH3BGRL	Missense_Mutation	p.V37L	T	G		6	80383394	80383394	226	0.752212389	NA	NA
SH3D19	Copy Number	Deleted	NA	NA	NA	4	151336744	190873444	NA	NA	1045	-1.1013
SH3TC2	Missense_Mutation	p.S96L	A	G		5	148424194	148424194	108	0.407407407	NA	NA
SHOX	Missense_Mutation	p.E255K	A	G		X	605255	605255	13	0.846153846	NA	NA
SHPRH	Missense_Mutation	p.T629S	A	T		6	146264632	146264632	346	0.774566474	NA	NA
SHROOM	Missense_Mutation	p.C842F	A	C		X	50378048	50378048	75	0.866666667	NA	NA
SIGLEC15	Missense_Mutation	p.R122G	G	C		18	43417729	43417729	8	1	NA	NA
SIGLEC15	Splice_Site		T	G		18	43416993	43416993	94	0.574468085	NA	NA
SIX2	Missense_Mutation	p.G205C	A	C		2	45233572	45233572	481	0.286902287	NA	NA
SLC10A4	Missense_Mutation	p.A167S	T	G		4	48486077	48486077	115	0.556521739	NA	NA
SLC10A4	Frame_Shift_Del	p.G138fs	-	GC		4	48485991	48485992	36	0.333333333	NA	NA
SLC13A4	Missense_Mutation	p.C509Y	T	C		7	135370349	135370349	415	0.21686747	NA	NA
SLC24A2	Missense_Mutation	p.E359K	T	C		9	19619585	19619585	372	0.137096774	NA	NA
SLC25A10	Missense_Mutation	p.R287L	T	G		17	79687076	79687076	213	0.258215962	NA	NA
SLC25A25	Missense_Mutation	p.G85V	T	G		9	130863409	130863409	197	0.294416244	NA	NA
SLC25A44	Missense_Mutation	p.R44L	T	G		1	156169769	156169769	273	0.534798535	NA	NA
SLC26A4	Missense_Mutation	p.S119C	G	C		7	107312634	107312634	323	0.354798762	NA	NA
SLC2A11	Missense_Mutation	p.R260L	T	G		22	24224739	24224739	22	0.727272727	NA	NA
SLC30A2	Missense_Mutation	p.Q282K	T	G		1	26365779	26365779	37	0.216216216	NA	NA
SLC34A2	Missense_Mutation	p.T543M	T	C		4	25677926	25677926	284	0.735915493	NA	NA
SLC39A12	Missense_Mutation	p.L545M	A	C		10	18289628	18289628	567	0.398589065	NA	NA
SLC39A12	Missense_Mutation	p.P13A	G	C		10	18242242	18242242	256	0.37890625	NA	NA
SLC45A1	Missense_Mutation	p.P295Q	A	C		1	8390437	8390437	136	0.272058824	NA	NA
SLC4A7	Nonsense_Mutation	p.E363*	A	C	rs145052230	3	27472825	27472825	286	0.384615385	NA	NA
SLC6A13	Missense_Mutation	p.V442L	A	C		12	332388	332388	26	0.346153846	NA	NA
SLC6A20	Missense_Mutation	p.V336A	G	A		3	45811792	45811792	145	0.358620689	NA	NA
SLCO1B1	Copy Number	Deleted	NA	NA	NA	12	21036538	21448728	NA	NA	31	-1.0917
SLCO1B3	Copy Number	Deleted	NA	NA	NA	12	21036538	21448728	NA	NA	31	-1.0917
SLCO4A1	Missense_Mutation	p.T324N	A	C		20	61291847	61291847	227	0.356828194	NA	NA
SLCO6A1	Missense_Mutation	p.P287T	T	G		5	101811441	101811441	367	0.719346049	NA	NA
SLFN5	Missense_Mutation	p.R357L	T	G		17	33588047	33588047	369	0.807588076	NA	NA
SLMO2	Splice_Site		A	C		20	57613690	57613690	373	0.474530831	NA	NA
SMOC1	Missense_Mutation	p.P198T	A	C		14	70461128	70461128	650	0.275384615	NA	NA

Figure 11 (cont.)

Gene	Variant_Classification	Alteration	Tumor_allele	Ref._allele	dbSNP_RS	Chr. #	Start_pos.	End_pos.	Coverage	Allelic_fraction	#_of_Probes	Segment_Mean (Log2[Copy
SNAPIN	Missense_Mutation	p.A12T	A	G		1	153631253	153631253	71	0.633802817	NA	NA
SNX25	Copy Number	Deleted	NA	NA	NA	4	151336744	190873444	NA	NA	1045	-1.1013
SNX7	Missense_Mutation	p.E329K	A	G		1	99225661	99225661	186	0.306451613	NA	NA
SORCS3	Frame_Shift_Ins	p.E402fs	T	-		10	108466331	108466332	205	0.336585366	NA	NA
SORCS3	Splice_Site	p.Q709_splice	T	G		10	106959874	106959874	170	0.382352941	NA	NA
SPATA4	Copy Number	Deleted	NA	NA	NA	4	151336744	190873444	NA	NA	1045	-1.1013
SPCS3	Copy Number	Deleted	NA	NA	NA	4	151336744	190873444	NA	NA	1045	-1.1013
SPG7	Missense_Mutation	p.R475M	T	G		16	89603272	89603272	350	0.26	NA	NA
SPHKAP	Nonsense_Mutation	p.E55*	A	C		2	228973631	228973631	338	0.474874372	NA	NA
SPOCD1	Missense_Mutation	p.T544R	C	G		1	32265714	32265714	50	0.34	NA	NA
SPRY3	Missense_Mutation	p.R242P	C	G		X	155004258	155004258	683	0.221083455	NA	NA
SPSB2	Copy Number	Deleted	NA	NA	NA	12	6135214	7076842	NA	NA	308	-1.0173
SSTR4	Missense_Mutation	p.G218R	C	G		20	23016775	23016775	373	0.536193029	NA	NA
ST8SIA6	Missense_Mutation	p.N278I	A	T		10	17363247	17363247	561	0.456327986	NA	NA
STCH2	Copy Number	Deleted	NA	NA	NA	4	151336744	190873444	NA	NA	1045	-1.1013
STT3A	Missense_Mutation	p.L468F	T	C		11	125482920	125482920	488	0.291546392	NA	NA
STX18	Missense_Mutation	p.E210K	T	C		4	4436571	4436571	130	0.261538462	NA	NA
SUCLG2	Missense_Mutation	p.Q192H	A	T		3	67568755	67568755	168	0.357142857	NA	NA
SUSP2	Missense_Mutation	p.K958N	A	C		19	19115029	19115029	132	0.060606061	NA	NA
SYT3	Missense_Mutation	p.Y498N	T	A		19	51128732	51128732	33	0.757575758	NA	NA
SYT3	Missense_Mutation	p.E262K	T	C		19	51133319	51133319	132	0.333333333	NA	NA
SZT2	Splice_Site		T	G		1	43897195	43897195	271	0.420664207	NA	NA
TAF1L	Missense_Mutation	p.E1317K	T	C		9	32631629	32631629	634	0.260252366	NA	NA
TAF3	Missense_Mutation	p.Y83F	T	A		10	7866362	7866362	544	0.426470588	NA	NA
TAPBPL	Copy Number	Deleted	NA	NA	NA	12	6135214	7076842	NA	NA	308	-1.0173
TARDBP	Missense_Mutation	p.I257M	G	A		1	11082237	11082237	281	0.523131673	NA	NA
TAS2R14	Missense_Mutation	p.T215I	A	G		12	11091163	11091163	80	0.6625	NA	NA
TBC1D25	Missense_Mutation	p.M334I	A	G		X	48418298	48418298	62	0.064516129	NA	NA
TBCK	Missense_Mutation	p.E419K	T	C		4	107157642	107157642	109	0.229357798	NA	NA
TBX20	Missense_Mutation	p.S19C	C	G		7	35293176	35293176	213	0.474178404	NA	NA
TCHH	Missense_Mutation	p.L1830H	T	A		1	152080204	152080204	371	0.296495957	NA	NA
TDRD3	Missense_Mutation	p.F27I	A	T		13	61084847	61084847	133	0.030075188	NA	NA
TECPR1	Nonsense_Mutation	p.Q827*	A	G		7	97857360	97857360	57	0.49122807	NA	NA
TET3	Missense_Mutation	p.S17L	T	C		2	74273499	74273499	176	0.306818182	NA	NA
THSD7B	Missense_Mutation	p.D1453N	A	G		2	138414712	138414712	97	0.278350515	NA	NA
THUMPD	Missense_Mutation	p.V262M	T	C		3	39993216	39993216	226	0.530973451	NA	NA
TIGD4	Copy Number	Deleted	NA	NA	NA	4	151336744	190873444	NA	NA	1045	-1.1013
TMEM51	Missense_Mutation	p.S218I	A	G		7	219141809	219141809	120	0.133333333	NA	NA
TMC2	Splice_Site		A	G		20	2597717	2597717	110	0.236363636	NA	NA
TMCC1	Missense_Mutation	p.K264M	A	T		3	129389893	129389893	578	0.795847751	NA	NA
TMEM10	Missense_Mutation	p.S208I	T	G		7	12270055	12270055	400	0.4025	NA	NA
TMEM13	Missense_Mutation	p.S88N	A	G		2	98418894	98418894	83	0.084337349	NA	NA
TMEM13	Missense_Mutation	p.R1062G	C	T		2	98412697	98412697	26	0.230769231	NA	NA
TMEM15	Missense_Mutation	p.T104S	A	T		12	129558575	129558575	198	0.035353535	NA	NA
TMEM17	Nonsense_Mutation	p.W127*	T	C		12	130184942	130184942	106	0.320754717	NA	NA
TMEM17	Missense_Mutation	p.G133W	T	G		7	142983668	142983668	232	0.530172414	NA	NA
TMEM15	Copy Number	Deleted	NA	NA	NA	4	151336744	190873444	NA	NA	1045	-1.1013
TMEM16	Missense_Mutation	p.W552C	A	C		7	112407690	112407690	388	0.525773196	NA	NA
TMEM18	Copy Number	Deleted	NA	NA	NA	4	148407255	151000602	NA	NA	56	-1.1334
TMEM19	Copy Number	Deleted	NA	NA	NA	4	151336744	190873444	NA	NA	1045	-1.1013
TMEM20	Missense_Mutation	p.S3L	T	C		16	67262453	67262453	74	0.202702703	NA	NA
TMEM25	Copy Number	Deleted	NA	NA	NA	11	118390509	118392889	NA	NA	4	-1.2525
TMEM63	Missense_Mutation	p.G803V	T	G		14	77723050	77723050	145	0.524137931	NA	NA
TMIGD2	Missense_Mutation	p.P250T	T	G		19	4292697	4292697	118	0.788135966	NA	NA
TMPRSS1	Missense_Mutation	p.A382S	T	G		4	69362394	69362394	1403	0.402410549	NA	NA
TMTC1	Missense_Mutation	p.A60V	A	G		12	29911688	29911688	105	0.723809524	NA	NA
TMTC2	Missense_Mutation	p.H593Y	T	C		12	83359449	83359449	225	0.275555556	NA	NA
TNKS1BP	Missense_Mutation	p.R1201T	G	C		11	57076583	57076583	363	0.305785124	NA	NA
TOR1AIP2	Missense_Mutation	p.T156A	C	T		1	179820067	179820067	378	0.417989418	NA	NA
TP53BP1	Nonsense_Mutation	p.E1551*	A	C		15	43712518	43712518	545	0.256880734	NA	NA
TREX2	Missense_Mutation	p.S38F	A	G		X	152710776	152710776	30	0.4	NA	NA
TRIM54	Splice_Site		C	G		2	27529323	27529323	29	0.206896552	NA	NA
TRIM58	Missense_Mutation	p.S441F	T	C		1	248039652	248039652	552	0.184782609	NA	NA
TRIM58	Missense_Mutation	p.P280H	A	C		1	248031333	248031333	51	0.411764706	NA	NA

Figure 11 (cont.)

Gene	Variant_Classification	Alteration	Tumor_allele	Ref_allele	dbSNP_RS	Chr. #	Start_pos.	End_pos.	Coverage	Allelic_fraction	# of Probes	Segment_Mean (Log2[Copy
TRIM60	Copy Number	Deleted	NA	NA	NA	4	151336744	190873444	NA	NA	1045	-1.1013
TRIM61	Copy Number	Deleted	NA	NA	NA	4	151336744	190873444	NA	NA	1045	-1.1013
TRIM73	Missense_Mutation	p.R745N	C	G		3	32932931	32932931	77	0.363636364	NA	NA
TRIML1	Copy Number	Deleted	NA	NA	NA	4	151336744	190873444	NA	NA	1045	-1.1013
TRIML2	Copy Number	Deleted	NA	NA	NA	4	151336744	190873444	NA	NA	1045	-1.1013
TRPC5	Missense_Mutation	p.H370N	T	G		X	111097127	111097127	103	0.747572816	NA	NA
TRPM5	Missense_Mutation	p.Q283E	C	G		11	2439462	2439462	21	0.857142857	NA	NA
TRPM8	Nonsense_Mutation	p.Q571*	T	C		2	234871983	234871983	225	0.328888889	NA	NA
TRPV5	Missense_Mutation	p.L218Q	T	A		7	142625895	142625895	216	0.518518519	NA	NA
TRRAP	Splice_Site	p.T2607_splice	A	C		7	98573773	98573773	185	0.389189189	NA	NA
TSHZ3	Missense_Mutation	p.K1044Q	G	T		19	31767569	31767569	112	0.741071429	NA	NA
TSHZ3	Missense_Mutation	p.D113Y	A	C		19	31770362	31770362	180	0.033333333	NA	NA
TSPY2	Missense_Mutation	p.G5C	T	G		Y	6114322	6114322	58	0.465517241	NA	NA
TTC23L	Nonsense_Mutation	p.Y307*	A	T		5	34869090	34869090	64	0.890625	NA	NA
TTC24	Missense_Mutation	p.R403S	T	G		1	156553645	156553645	41	0.536585366	NA	NA
TTC33	Missense_Mutation	p.E12K	T	C		8	40747087	40747087	268	0.205223881	NA	NA
TTC36	Copy Number	Deleted	NA	NA	NA	11	118390509	118392889	NA	NA	4	-1.2525
TXNDC2	Missense_Mutation	p.P369T	A	C		18	9887581	9887581	426	0.286384977	NA	NA
UBAP2L	Missense_Mutation	p.A638S	T	G		1	154227630	154227630	315	0.39047619	NA	NA
UBAP2L	Missense_Mutation	p.R134L	T	G		1	154207188	154207188	104	0.605769231	NA	NA
UBE2E2	Missense_Mutation	p.R193K	A	G		3	23631294	23631294	158	0.316455696	NA	NA
UBR4	Missense_Mutation	p.R2272H	T	C		1	19479812	19479812	307	0.016286645	NA	NA
UFSP2	Copy Number	Deleted	NA	NA	NA	4	151336744	190873444	NA	NA	1045	-1.1013
UGT2B11	Missense_Mutation	p.E293D	A	C		4	70074192	70074192	912	0.387061404	NA	NA
UGT2B28	Missense_Mutation	p.R49G	G	A		4	70146363	70146363	877	0.015963512	NA	NA
UGT2B28	Nonsense_Mutation	p.W262*	A	G		4	70148295	70148295	713	0.192145863	NA	NA
UNC13C	Missense_Mutation	p.M1762I	A	G		15	54793161	54793161	374	0.240641711	NA	NA
UNC45B	Missense_Mutation	p.T832K	A	C		17	33510561	33510561	64	0.765625	NA	NA
UNC80	Missense_Mutation	p.R33M	T	G		2	210837829	210837829	165	0.430303303	NA	NA
UNCX	Missense_Mutation	p.T110N	A	C		7	1273210	1273210	133	0.54887218	NA	NA
USP12	Frame_Shift_Ins	p.L7fs	T	-	rs150054920	13	27745758	27745759	89	0.674157303	NA	NA
USP20	Missense_Mutation	p.N49S	T	A		9	132632042	132632042	259	0.49034749	NA	NA
USP28	Missense_Mutation	p.S2F	A	G		11	113700097	113700097	140	0.257142857	NA	NA
USP32	Missense_Mutation	p.W709C	A	C		17	58289437	58289437	231	0.147186147	NA	NA
USP38	Nonsense_Mutation	p.R173*	T	C		4	144107120	144107120	345	0.286956522	NA	NA
UTP20	Missense_Mutation	p.A628S	T	G		12	101699787	101699787	257	0.404669261	NA	NA
VWA3B	Missense_Mutation	p.Y105N	A	T		2	98736000	98736000	361	0.274238227	NA	NA
WDR17	Nonsense_Mutation	p.E1041*	T	G		4	177089908	177089908	74	0.445945946	NA	NA
WDR17	Copy Number	Deleted	NA	NA	NA	4	151336744	190873444	NA	NA	1045	-1.1013
WDR59	Missense_Mutation	p.T518K	T	G	rs142986172	16	74943488	74943488	316	0.234177215	NA	NA
WIPF3	Missense_Mutation	p.R60L	T	G	rs145862657	7	29915534	29915534	184	0.461956522	NA	NA
WLS	Missense_Mutation	p.W106C	G	C		1	68659699	68659699	161	0.385093168	NA	NA
WSCD2	Missense_Mutation	p.R217L	T	G		12	108604050	108604050	14	0.714285714	NA	NA
WWC3	Copy Number	Deleted	NA	NA	NA	4	151336744	190873444	NA	NA	1045	-1.1013
XIRP2	Missense_Mutation	p.E2919Q	C	G		2	168106657	168106657	262	0.103053435	NA	NA
XIRP2	Missense_Mutation	p.A901V	T	C		2	168100604	168100604	195	0.292307692	NA	NA
XIRP2	Nonsense_Mutation	p.W2065*	A	G		2	168104096	168104096	203	0.512315271	NA	NA
XIRP2	Missense_Mutation	p.G1150W	T	G		2	168101380	168101380	272	0.503676471	NA	NA
XKR6	Missense_Mutation	p.E294K	T	C		8	10782225	10782225	189	0.798816568	NA	NA
ZADH2	Missense_Mutation	p.I39R	T	C		18	72914210	72914210	194	0.659793814	NA	NA
ZAP70	Missense_Mutation	p.E136K	A	G		2	98341558	98341558	110	0.381818182	NA	NA
ZBED2	De_novo_Start_OutOfFrame		T	C		3	111313187	111313187	21	0.285714286	NA	NA
ZCCHC11	Nonsense_Mutation	p.Q1468*	A	G		1	52896991	52896991	238	0.331932773	NA	NA
ZCCHC17	Missense_Mutation	p.A148S	T	G		1	31821684	31821684	206	0.359223301	NA	NA
ZDHHC6	Missense_Mutation	p.W131L	A	C		10	114202077	114202077	72	0.236111111	NA	NA
ZEAT	Missense_Mutation	p.E601V	A	T		8	135614124	135614124	289	0.3183391	NA	NA
ZFHX3	Missense_Mutation	p.Q1807K	A	C		8	77764576	77764576	11	0.636363636	NA	NA
ZFHX4	Missense_Mutation	p.H234L	T	A		8	77617024	77617024	124	0.814516129	NA	NA
ZFHX4	Missense_Mutation	p.Q1781K	A	C		8	77764498	77764498	47	0.808510638	NA	NA
ZFP30	Copy Number	Deleted	NA	NA	NA	19	37734568	38180798	NA	NA	29	-1.0976

Figure 11 (cont.)

Gene	Variant_Classification	Alteration	Tumor_allele	Ref_allele	dbSNP_RS	Chr. #	Start_pos.	End_pos.	Coverage	Allelic_fraction	# of Probes	Segment_Mean (Log2[Copy
ZFP42	Copy Number	Deleted	NA	NA	NA	4	151336744	190873444	NA	NA	1045	-1.1013
ZFR2	Missense_Mutation	p.C280F	A	C		19	3831314	3831314	17	0.882352941	NA	NA
ZFYVE20	Missense_Mutation	p.R483H	T	C		3	15116196	15116196	158	0.360759494	NA	NA
ZNF138	Splice_Site		T	G		7	64276032	64276032	193	0.191709845	NA	NA
ZNF138	Splice_Site	p.A13_splice	A	G		7	64276031	64276031	194	0.195876289	NA	NA
ZNF276	Missense_Mutation	p.R200Q	A	G		16	89789935	89789935	68	0.260889565	NA	NA
ZNF280A	Missense_Mutation	p.G332N	T	C		22	22868861	22868961	236	0.677966102	NA	NA
ZNF383	Copy Number	Deleted	NA	NA	NA	19	37734568	38190798	NA	NA	29	-1.0976
ZNF420	Missense_Mutation	p.D55N	A	G		19	37618056	37618056	161	0.074534161	NA	NA
ZNF454	Missense_Mutation	p.P327H	A	C		5	178392385	178392385	196	0.224489796	NA	NA
ZNF479	Missense_Mutation	p.A249G	C	G		7	57188376	57188376	168	0.392857143	NA	NA
ZNF492	Missense_Mutation	p.Y84C	G	A		19	22846722	22846722	68	0.220588235	NA	NA
ZNF496	Missense_Mutation	p.P29L	A	G		1	247492795	247492795	203	0.201970443	NA	NA
ZNF503	Missense_Mutation	p.P556T	T	G		10	77158782	77158782	37	0.405405405	NA	NA
ZNF527	Copy Number	Deleted	NA	NA	NA	19	37734568	38190798	NA	NA	29	-1.0976
ZNF536	Missense_Mutation	p.E95R	A	C		19	30934754	30934754	566	0.09540636	NA	NA
ZNF536	Missense_Mutation	p.Q435E	A	C		19	30935772	30935772	1024	0.107421875	NA	NA
ZNF536	Missense_Mutation	p.R592K	A	G		19	30936244	30936244	2014	0.174627607	NA	NA
ZNF536	Frame_Shift_Del	p.A422fs	-	C		19	30935734	30935734	336	0.029761905	NA	NA
ZNF540	Copy Number	Deleted	NA	NA	NA	19	37734568	38190798	NA	NA	29	-1.0976
ZNF548	Missense_Mutation	p.M259I	A	G		19	57910462	57910462	101	0.405940594	NA	NA
ZNF568	Copy Number	Deleted	NA	NA	NA	19	37734568	38190798	NA	NA	29	-1.0976
ZNF570	Copy Number	Deleted	NA	NA	NA	19	37734568	38190798	NA	NA	29	-1.0976
ZNF571	Copy Number	Deleted	NA	NA	NA	19	37734568	38190798	NA	NA	29	-1.0976
ZNF607	Copy Number	Deleted	NA	NA	NA	19	37734568	38190798	NA	NA	29	-1.0976
ZNF609	Missense_Mutation	p.G1121C	T	G		15	64968414	64968414	65	0.6	NA	NA
ZNF658	Splice_Site		A	C		9	40784604	40784604	165	0.460606061	NA	NA
ZNF665	Nonsense_Mutation	p.W37*	T	C		19	53669437	53669437	388	0.368556701	NA	NA
ZNF676	Nonsense_Mutation	p.R132*	A	T		19	22364125	22364125	311	0.549839228	NA	NA
ZNF716	Missense_Mutation	p.P463S	T	C		7	57529554	57529554	64	0.265625	NA	NA
ZNF716	Frame_Shift_Del	p.S54fs	-	C		7	57522287	57522287	411	0.374695864	NA	NA
ZNF781	Copy Number	Deleted	NA	NA	NA	19	37734568	38190798	NA	NA	29	-1.0976
ZNF793	Copy Number	Deleted	NA	NA	NA	19	37734568	38190798	NA	NA	29	-1.0976
ZNF804A	Missense_Mutation	p.L37M	A	C		2	185463795	185463795	115	0.591304348	NA	NA
ZNF804B	Missense_Mutation	p.G865C	T	G		7	88964289	88964289	279	0.555555556	NA	NA
ZNF804B	Missense_Mutation	p.L1348F	T	G		7	88966340	88966340	168	0.255952381	NA	NA
ZNF835	Missense_Mutation	p.G388S	T	C		19	57175411	57175411	89	0.08988764	NA	NA
ZNF879	Missense_Mutation	p.C346Y	A	G		5	178459986	178459986	22	0.5	NA	NA
ZNF91	Missense_Mutation	p.S1028V	A	C		19	23542698	23542698	308	0.266233766	NA	NA
ZNRF4	Missense_Mutation	p.V35GL	T	G		19	5456550	5456550	159	0.729559748	NA	NA
ZSCAN4	Nonsense_Mutation	p.Q10*	T	C		19	58187541	58187541	162	0.351851852	NA	NA
ZSWIM3	Missense_Mutation	p.F61L	A	C		20	44511414	44511414	191	0.209424084	NA	NA
ANXA2P1	Copy Number	Deleted	NA	NA	NA	4	151336744	190873444	NA	NA	1045	-1.1013
C12orf53	Copy Number	Deleted	NA	NA	NA	12	6135214	7076942	NA	NA	308	-1.0173
C4orf38	Copy Number	Deleted	NA	NA	NA	4	151336744	190873444	NA	NA	1045	-1.1013
C4orf48	Copy Number	Deleted	NA	NA	NA	4	151336744	190873444	NA	NA	1045	-1.1013
C4orf47	Copy Number	Deleted	NA	NA	NA	4	151336744	190873444	NA	NA	1045	-1.1013
CLDN24	Copy Number	Deleted	NA	NA	NA	4	151336744	190873444	NA	NA	1045	-1.1013
FAM160A	Copy Number	Deleted	NA	NA	NA	4	151336744	190873444	NA	NA	1045	-1.1013
FAM91A3	Copy Number	Deleted	NA	NA	NA	4	151336744	190873444	NA	NA	1045	-1.1013
GK3P	Copy Number	Deleted	NA	NA	NA	4	151336744	190873444	NA	NA	1045	-1.1013
KIAA1712	Copy Number	Deleted	NA	NA	NA	4	151336744	190873444	NA	NA	1045	-1.1013
KIR3DP1	Copy Number	Deleted	NA	NA	NA	19	55324685	55327991	NA	NA	4	-1.4768
LOC1712	Copy Number	Deleted	NA	NA	NA	12	6135214	7076942	NA	NA	308	-1.0173
LOC2844	Copy Number	Deleted	NA	NA	NA	19	37734568	38190798	NA	NA	29	-1.0976
LOC2854	Copy Number	Deleted	NA	NA	NA	4	151336744	190873444	NA	NA	1045	-1.1013
LOC2855	Copy Number	Deleted	NA	NA	NA	4	151336744	190873444	NA	NA	1045	-1.1013
LOC3400	Copy Number	Deleted	NA	NA	NA	4	151336744	190873444	NA	NA	1045	-1.1013
LOC3892	Copy Number	Deleted	NA	NA	NA	4	151336744	190873444	NA	NA	1045	-1.1013
LOC4011	Copy Number	Deleted	NA	NA	NA	4	151336744	190873444	NA	NA	1045	-1.1013
LOC6786	Copy Number	Deleted	NA	NA	NA	12	6135214	7076942	NA	NA	308	-1.0173
LOC7280	Copy Number	Deleted	NA	NA	NA	4	151336744	190873444	NA	NA	1045	-1.1013
LST-	Copy Number	Deleted	NA	NA	NA	12	21036538	21448728	NA	NA	31	-1.0917
MGC8580	Copy Number	Deleted	NA	NA	NA	4	151336744	190873444	NA	NA	1045	-1.1013

Figure 11 (cont.)

Gene	Variant_Classification	Alteration	Tumor_allele	Ref_allele	dbSNP_RS	Chr. #	Start_pos.	End_pos.	Coverage	Allelic_fraction	# of Probes	Segment_Mean (Log2)Copy
MIR1305	Copy Number	Deleted	NA	NA	NA	4	151336744	190873444	NA	NA	1045	-1.1013
MIR141	Copy Number	Deleted	NA	NA	NA	12	6135214	7076942	NA	NA	308	-1.0173
MIR1979	Copy Number	Deleted	NA	NA	NA	4	151336744	190873444	NA	NA	1045	-1.1013
MIR200C	Copy Number	Deleted	NA	NA	NA	12	6135214	7076942	NA	NA	308	-1.0173
MIR3140	Copy Number	Deleted	NA	NA	NA	4	151336744	190873444	NA	NA	1045	-1.1013
MIR4276	Copy Number	Deleted	NA	NA	NA	4	151336744	190873444	NA	NA	1045	-1.1013
MIR578	Copy Number	Deleted	NA	NA	NA	4	151336744	190873444	NA	NA	1045	-1.1013
NBLA003	Copy Number	Deleted	NA	NA	NA	4	151336744	190873444	NA	NA	1045	-1.1013
NCRNA00107	Copy Number	Deleted	NA	NA	NA	X	200983	322327	NA	NA	19	-1.1979
RPL13P5	Copy Number	Deleted	NA	NA	NA	12	6135214	7076942	NA	NA	308	-1.0173
RWDD4A	Copy Number	Deleted	NA	NA	NA	4	151336744	190873444	NA	NA	1045	-1.1013
SCARNA1	Copy Number	Deleted	NA	NA	NA	12	6135214	7076942	NA	NA	308	-1.0173
SCARNA1	Copy Number	Deleted	NA	NA	NA	12	6135214	7076942	NA	NA	308	-1.0173
SCARNA1	Copy Number	Deleted	NA	NA	NA	12	6135214	7076942	NA	NA	308	-1.0173
SLED1	Copy Number	Deleted	NA	NA	NA	4	151336744	190873444	NA	NA	1045	-1.1013
SNAR-G1	Copy Number	Deleted	NA	NA	NA	19	49526459	49558634	NA	NA	18	-1.9589
SNAR-G2	Copy Number	Deleted	NA	NA	NA	19	49526459	49558634	NA	NA	18	-1.9589
SNORD73	Copy Number	Deleted	NA	NA	NA	4	151336744	190873444	NA	NA	1045	-1.1013
AAR2	Missense_Mutation	p.P163L	T	C		20	34828278	34828278	308	0.214285714	NA	NA
AC00949 9.1	lincRNA		A	C	rs114804891	2	33952248	33952248	36	0.611111111	NA	NA
AK9	Nonsense_Mutation	p.G195*	A	C		6	109980478	109980478	135	0.814814815	NA	NA
AL445989	Missense_Mutation	p.G71R	C	G		13	64321144	64321144	133	0.804511278	NA	NA
AL928742 .12	lincRNA		A	C		14	106066249	106066249	27	0.444444444	NA	NA
AMN1	Missense_Mutation	p.M44I	A	C		12	31862266	31862266	100	0.66	NA	NA
ASUN	Missense_Mutation	p.C331Y	T	C		12	27070362	27070362	75	0.253333333	NA	NA
ATP6V0E	Missense_Mutation	p.S162N	A	G		7	1495766609	1495766609	43	0.279069767	NA	NA
C4orf51	Splice_Site	p.Q78_spli ce	T	G		4	146617711	146617711	116	0.301724138	NA	NA
CACTIN	Missense_Mutation	p.R456H	T	C		19	3613573	3613573	26	0.730769231	NA	NA
CACUL1	Missense_Mutation	p.E353K	T	C		10	120446122	120446122	146	0.239726027	NA	NA
CAMSAP2	Missense_Mutation	p.A806V	T	C		1	200818314	200818314	168	0.023809524	NA	NA
CCDC180	Missense_Mutation	p.V526L	C	G		9	100087373	100087373	136	0.198529412	NA	NA
CCER1	Missense_Mutation	p.G132C	A	C		12	91348126	91348126	58	0.810344828	NA	NA
CEP1708	Missense_Mutation	p.R1093W	T	C		14	105353850	105353850	56	0.464285714	NA	NA
CHP1	Splice_Site	p.G74_spli ce	C	G		15	41549188	41549188	220	0.404545455	NA	NA
CLPSL1	Missense_Mutation	p.R114E	G	A		6	35755761	35755761	263	0.216730038	NA	NA
CROCCP2	lincRNA		T	C	rs2779441	1	16958063	16958063	33	0.151515152	NA	NA
CTD-2066L21.	lincRNA		A	G		5	33162298	33162298	126	0.261904762	NA	NA
DCDC2B	Missense_Mutation	p.V198M	A	G		1	32678155	32678155	146	0.369863014	NA	NA
DNAAF2	Missense_Mutation	p.V82I	A	C		14	50092313	50092313	147	0.489795918	NA	NA
DNAAF2	Nonsense_Mutation	p.Q829*	A	G		14	50092289	50092289	122	0.204918033	NA	NA
DRAXIN	Nonsense_Mutation	p.C274*	A	C	rs61744690	1	11772461	11772461	182	0.21978022	NA	NA
ELMSAN1	Missense_Mutation	p.R205R	C	T	rs1A32293780	14	74206098	74206098	188	0.35106383	NA	NA
FAM212A	Missense_Mutation	p.R241L	T	G		3	49842278	49842278	215	0.404651163	NA	NA
FAM214A	Missense_Mutation	p.N823K	C	G		15	52901248	52901248	388	0.198453608	NA	NA
GCSAML	Missense_Mutation	p.L75I	A	C		1	247737559	247737559	143	0.27972028	NA	NA
GSE1	Missense_Mutation	p.S100SF	T	C		16	85699837	85699837	134	0.223880597	NA	NA
HCCAT5	lincRNA		T	C		16	73127383	73127383	27	0.148148148	NA	NA
HCCAT5	lincRNA		C	G		16	73126857	73126857	193	0.481865285	NA	NA
HCG17	lincRNA		A	G		6	30231309	30231309	39	0.179487179	NA	NA
HLA-J	Missense_Mutation	p.A152S	A	C	rs35275462	6	32726819	32726819	255	0.325490196	NA	NA
ISY1-RAB43	Splice_Site	p.E27_spli ce	A	C		3	128875763	128875763	120	0.583333333	NA	NA
KIAA1211	Missense_Mutation	p.E359K	T	C		2	99439661	99439661	17	0.647058824	NA	NA
KMT2A	Missense_Mutation	p.Q844E	G	C		11	118344404	118344404	116	0.275862069	NA	NA
KMT2C	Missense_Mutation	p.P837S	A	G		7	151945010	151945010	565	0.217699115	NA	NA
KMT2C	Missense_Mutation	p.Q318H	G	C		7	151970848	151970848	757	0.034346103	NA	NA
KRTAP5-4	Missense_Mutation	p.S73Y	T	G		11	1643106	1643106	288	0.617449664	NA	NA
LINC0088	lincRNA		T	C		3	156527936	156527936	295	0.257627119	NA	NA

Figure 11 (cont.)

Gene	Variant_Classification	Alteration	Tumor_allele	Ref_allele	dbSNP_RS	Chr. #	Start_pos.	End_pos.	Coverage	Allelic_fraction	# of Probes	Segment_Mean (Log2[Copy
LINC00096	lincRNA		G	A	rs121907841	3	195400728	195400728	17	0.235294118	NA	NA
LPPR5	Frame_Shift_Del	p.P65fs	-	G		1	99470033	99470083	111	0.351351351	NA	NA
LRRC73	Missense_Mutation	p.E79Q	G	C		6	43477289	43477289	54	0.425925926	NA	NA
MALAT1	lincRNA		G	T		11	65271320	65271320	58	0.568965517	NA	NA
MALAT1	lincRNA		T	G		11	65268179	65268179	197	0.177664975	NA	NA
MROH2B	De_novo_Start_OutOfFrame		A	C		5	41070990	41070990	45	0.377777778	NA	NA
MROH9	Missense_Mutation	p.S235I	T	G		1	170952662	170952662	120	0.366666667	NA	NA
MUC3A	Missense_Mutation	p.T171I	T	C		7	100551761	100551761	812	0.233990148	NA	NA
MUC5B	Missense_Mutation	p.T4545A	G	A		11	1271734	1271734	240	0.033333333	NA	NA
NME8	Missense_Mutation	p.E341K	A	G		7	37923931	37923931	385	0.228571429	NA	NA
NMRK1	Missense_Mutation	p.M162I	T	C		9	77683834	77683934	178	0.264044944	NA	NA
NPAP1	Missense_Mutation	p.E148K	A	G		15	24921456	24921456	96	0.354166667	NA	NA
NSG1	Missense_Mutation	p.G5E	A	G		4	4389370	4389370	77	0.246753247	NA	NA
NSG2	Splice_Site		G	A		5	173531229	173531229	140	0.521428571	NA	NA
NYAP1	Missense_Mutation	p.E491K	A	G		7	100086815	100086815	42	0.261904762	NA	NA
PCDHGA4	Missense_Mutation	p.A321P	C	G		5	140735728	140735728	64	0.765625	NA	NA
PEAK1	Missense_Mutation	p.S236I	A	C		15	77473562	77473562	400	0.205	NA	NA
PLEKHS1	Missense_Mutation	p.E145Q	C	G		10	115528647	115528647	124	0.395161129	NA	NA
POTEE	Missense_Mutation	p.G897S	A	G		2	132021717	132021717	448	0.261160714	NA	NA
PPM1N	Missense_Mutation	p.A100V	T	C		19	46002263	46002263	26	0.346153846	NA	NA
PPME1	Splice_Site		T	G		11	73950302	73950302	60	0.483333333	NA	NA
PPP1R26	Missense_Mutation	p.E329K	A	G		9	138377341	138377341	234	0.290598291	NA	NA
PRRC2C	Missense_Mutation	p.R1700L	T	G		1	171519351	171519351	63	0.111111111	NA	NA
RP11-204M4.2	lincRNA		A	C		9	41962565	41962565	153	0.130718954	NA	NA
RP11-3P17.4	lincRNA		T	C		3	161146953	161146953	330	0.203030303	NA	NA
RP11-417J8.6	lincRNA		A	T		1	142713133	142713133	359	0.384401114	NA	NA
RP11-702L6.4	lincRNA		A	T		3	109128891	109128891	39	0.58974359	NA	NA
RP11-812E19.9	Missense_Mutation	p.T110K	T	G		16	33647271	33647271	811	0.410604192	NA	NA
SH3D7	Missense_Mutation	p.S427R	A	C		15	78393876	78393876	63	0.571428571	NA	NA
SMCO4	Missense_Mutation	p.Q28H	A	C		11	93212272	93212272	172	0.534883721	NA	NA
SPATA31	Missense_Mutation	p.R1067L	T	G		9	39890213	39890213	342	0.529239766	NA	NA
SPATA31	Missense_Mutation	p.A1785	T	G		9	84805917	84805917	259	0.50965251	NA	NA
SPATA31	Missense_Mutation	p.V45M	A	G		9	84603866	84603866	240	0.429166667	NA	NA
SSUH2	Missense_Mutation	p.R248L	A	C		3	8667298	8667298	153	0.294117647	NA	NA
STRIP2	Missense_Mutation	p.V739A	C	T		7	129122849	129122849	116	0.181034483	NA	NA
SUCO	Missense_Mutation	p.S750C	T	A		1	172558033	172558033	204	0.328431373	NA	NA
TENM1	Missense_Mutation	p.S1398P	G	A		X	123556401	123556401	247	0.020242915	NA	NA
TENM2	Nonsense_Mutation	p.S2224*	A	C		5	167674978	167674978	158	0.805031447	NA	NA
TICRR	Missense_Mutation	p.I1893V	G	A		15	90170261	90170251	382	0.219387755	NA	NA
TMEM23	Missense_Mutation	p.V284L	A	C		16	75575315	75575315	65	0.4	NA	NA
TPGS2	Missense_Mutation	p.E146V	A	T		18	34380220	34380220	289	0.716262976	NA	NA
TRIM51	Missense_Mutation	p.C93R	C	T		11	55653181	55653181	116	0.220338983	NA	NA
TRIM51	Missense_Mutation	p.P37H	A	C		11	55653014	55653014	173	0.520231214	NA	NA
TRIM51	Missense_Mutation	p.I125V	G	A		11	55653277	55653277	292	0.616438356	NA	NA
TSIX	lincRNA		T	A		X	73042944	73042944	39	0.769230769	NA	NA
TSIX	lincRNA		C	A		X	73047379	73047379	14	0.642857143	NA	NA
UBR5	Nonsense_Mutation	p.E393*	T	G		17	21731532	21731532	75	0.76	NA	NA
UNC79	Missense_Mutation	p.P1263S	T	C		14	94071274	94071274	36	0.166666667	NA	NA
UNC79	Missense_Mutation	p.D492Y	T	G		14	94007127	94007127	384	0.494791667	NA	NA
URI1	Missense_Mutation	p.E382K	A	G		19	30503385	30503385	2417	0.035581299	NA	NA
UTS2B	Splice_Site		A	C		3	190994634	190994634	103	0.621359223	NA	NA
XIST	lincRNA		C	G		X	73071958	73071958	10	0.4	NA	NA
XYLT1	Splice_Site		A	C		3	194877177	194877177	147	0.829931973	NA	NA
YBX3	Missense_Mutation	p.Q231K	T	G		12	10862596	10862596	32	0.59375	NA	NA
ZNF286B	Nonsense_Mutation	p.E154*	A	C		17	18566359	18566359	178	0.735955056	NA	NA
ZNF433	Missense_Mutation	p.R460K	T	C		19	12126198	12126198	156	0.429487179	NA	NA
ZNF845	Missense_Mutation	p.C945R	C	T		19	53856761	53856761	96	0.041666667	NA	NA
AAAS	Intron		-	CA		12	53708526	53708527	49	0.714285714	NA	NA
ABCA1	Silent	p.I756T	A	C		9	107589298	107589298	162	0.450617284	NA	NA

Figure 11 (cont.)

Gene	Variant_Classification	Alteration	Tumor_allele	Ref_allele	dbSNP_RS	Chr. #	Start_pos.	End_pos.	Coverage	Allelic_fraction	# of Probes	Segment_Mean (Log2[Copy
ABCA13	Silent	p.R594R	T	G		7	48287958	48287958	436	0.415137615	NA	NA
ABCA13	Silent	p.P4648P	A	C		7	48559783	48559783	201	0.388059701	NA	NA
ABCA2	Intron		T	C		9	139918060	139918060	10	0.4	NA	NA
ABCB6	Silent	p.L23L	T	C		2	220083827	220083827	67	0.238805987	NA	NA
AC008626	RNA		A	C		19	46717099	46717099	11	1	NA	NA
AC09364	RNA		A	G		2	243037145	243037145	310	0.212903226	NA	NA
AC09383	RNA		T	G		2	132273208	132273208	40	0.5	NA	NA
AC09657	RNA		T	G		2	89161464	89161464	188	0.574468085	NA	NA
ACBD3	Silent	p.L171L	C	G		1	226352546	226352546	322	0.177018634	NA	NA
ACBD4	Intron		A	G		17	43213612	43213612	31	0.322580645	NA	NA
ACOT11	3'UTR		T	C		1	55075728	55075728	6	0.666666667	NA	NA
ACOT6	Silent	p.L2L	T	C		14	74083882	74083882	396	0.535353535	NA	NA
ACOT7	Silent	p.V38V	A	G		1	6453346	6453346	33	0.272727273	NA	NA
ACVR1C	Silent	p.P111P	A	G		2	158412816	158412816	196	0.326530612	NA	NA
ADAM10	Intron		A	G		15	58985279	58985279	136	0.397058824	NA	NA
ADAM10	Intron		A	C		15	58983653	58983653	58	0.189655172	NA	NA
ADAM12	Silent	p.V215V	G	A		10	127798377	127798377	361	0.536845789	NA	NA
ADAMTS1	Silent	p.T213T	T	G		21	28216635	28216635	29	0.689655172	NA	NA
ADAMTS1	Silent	p.C1024C	C	T		10	72517843	72517843	44	0.409090909	NA	NA
ADAMTS1	Silent	p.S638S	G	C		11	130340918	130340918	268	0.02238806	NA	NA
ADAT3	Silent	p.V59V	A	G		19	1912223	1912223	16	0.8125	NA	NA
ADCY10	Silent	p.V828V	A	C		1	167815048	167815048	361	0.210526316	NA	NA
ADCY2	Silent	p.T542T	A	C		5	7717273	7717273	262	0.374045802	NA	NA
ADCYAP1	Silent	p.I45I	T	C		7	31146259	31146259	82	0.182926829	NA	NA
ADRM1	Intron		-	TTGT		20	60881460	60881463	75	0.12	NA	NA
AE000661.37	RNA		G	C		14	22935082	22935082	42	0.238095238	NA	NA
AE000661.37	RNA		T	A		14	22938184	22938184	297	0.481481481	NA	NA
AGAP11	RNA		A	G		10	88754649	88754649	82	0.317073171	NA	NA
AGAP5	Silent	p.Q20Q	C	T		10	75457454	75457454	235	0.017021277	NA	NA
AGL	Silent	p.A1128A	T	G	rs148393587	1	100366213	100366213	409	0.364303178	NA	NA
AGPAT5	3'UTR		T	G		8	6614934	6614934	99	0.757575758	NA	NA
AIP	Silent	p.I230I	T	C		11	67257831	67257831	83	0.265060241	NA	NA
AK7	Intron		-	G		14	96913000	96913000	91	0.43956044	NA	NA
AKR1A1	Silent	p.G131G	T	G	rs146246275	1	46033690	46033690	55	0.363636364	NA	NA
AKR1D1	De_novo_Start_InFrame		A	G		7	137761228	137761228	72	0.527777778	NA	NA
ALOX15	Silent	p.S615S	A	C	rs140716949	17	4535039	4535039	250	0.768	NA	NA
AMT	3'UTR		T	C		3	49454662	49454662	33	0.515151515	NA	NA
ANAPC5	Silent	p.A36A	T	C		12	121790036	121790036	131	0.305343511	NA	NA
ANGPT2	3'UTR		A	G		8	6357305	6357305	64	0.375	NA	NA
ANK1	Silent	p.L1878L	T	C		8	41519074	41519074	148	0.344594595	NA	NA
ANKK1	Silent	p.E638E	A	G		11	113270605	113270605	70	0.242857143	NA	NA
ANKMY2	3'UTR		A	C		7	16640233	16640233	14	0.428571429	NA	NA
ANKRD2	3'UTR		T	G		10	99343556	99343556	22	0.5	NA	NA
ANKRD20A8P	RNA		A	C		2	95494796	95494796	364	0.516483516	NA	NA
ANKRD26	5'UTR		T	C		10	27389391	27389391	20	0.5	NA	NA
ANKRD30	3'UTR		G	C		10	37520451	37520451	118	0.457627119	NA	NA
ANKRD30	Intron		A	-		10	37507783	37507784	13	0.384615385	NA	NA
ANKS1B	Silent	p.L294L	A	G		12	100169405	100169405	55	0.327272727	NA	NA
AOC3	3'UTR		T	G		17	41008704	41008704	23	0.47826087	NA	NA
AP8A2	Silent	p.G158G	T	C		15	29346555	29346555	62	0.370967742	NA	NA
APBB1IP	3'UTR		A	G		10	26790189	26790189	25	0.4	NA	NA
APBB1IP	3'UTR		A	C		10	26790190	26790190	23	0.434782609	NA	NA
APCDD1L	Silent	p.V285V	T	G		20	57036497	57036497	21	0.523809524	NA	NA
APOBEC3	Intron		T	G		22	39441707	39441707	17	0.764705882	NA	NA
ARHGEF4	Silent	p.G905G	T	G		2	131688677	131688677	91	0.494505495	NA	NA
ARID3C	Silent	p.A185A	A	G		9	34623881	34623881	56	0.410714286	NA	NA
ARL8	5'UTR		A	C	rs150178049	4	57377452	57377452	141	0.333333333	NA	NA

Figure 11 (cont.)

Gene	Variant_Classification	Alteration	Tumor_allele	Ref_allele	dbSNP_RS	Chr. #	Start_pos.	End_pos.	Coverage	Allelic_fraction	# of Probes	Segment_Mean (Log2[Copy
ARMC5	Silent	p.G418G	T	G		16	31474122	31474122	135	0.488888889	NA	NA
ASB4	5'UTR		A	T		7	95157791	95157791	19	0.578947368	NA	NA
ASNS	Intron		-	AC		7	97488125	97488126	172	0.31627907	NA	NA
ASTN2	Silent	p.T1134T	C	T		9	119249733	119249733	201	0.313432836	NA	NA
ASXL3	Silent	p.R1066R	A	C		18	31323005	31323005	65	0.707692308	NA	NA
ATP4P4	RNA		T	C		11	113661718	113661718	92	0.619565217	NA	NA
ATP10D	Intron		-	A	rs1317713423	4	47537335	47537335	5	0.8	NA	NA
ATP13A4	Intron		TG	-		3	193156676	193156677	92	0.293478261	NA	NA
ATP1A2	Silent	p.V661V	A	G		1	160104953	160104953	135	0.471794872	NA	NA
ATP2C1	Silent	p.L355L	A	G		3	130683832	130683832	140	0.242857143	NA	NA
ATP5G3	5'UTR		T	C		2	176046133	176046133	69	0.333333333	NA	NA
ATP8A2	Silent	p.L1011L	G	C		13	26104218	26104218	145	0.179310345	NA	NA
ATP8B3	5'UTR		T	G		19	1811803	1811803	14	0.428571429	NA	NA
ATRNL1	Intron		T	-		10	117228898	117228899	73	0.232876712	NA	NA
B3GAT2	3'UTR		T	C		6	715700S6	715700S6	103	0.475728155	NA	NA
B3GNT2	Silent	p.A147A	C	G		2	62449796	62449796	368	0.535326087	NA	NA
BACE2	Intron		T	G		21	42551038	42551038	18	0.722222222	NA	NA
BAI3	Silent	p.V626V	A	G		6	69703803	69703803	226	0.384955752	NA	NA
BBC3	Intron		-	AC		19	47733300	47733301	6	0.333333333	NA	NA
BBS2	5'UTR		A	C		16	56553843	56553843	27	0.407407407	NA	NA
BCAT2	Silent	p.G298S	A	G		19	49299883	49299883	150	0.733333333	NA	NA
BCL11B	Silent	p.P311P	A	C		14	99642027	99642027	38	0.342105263	NA	NA
BCLAF1	Silent	p.V415V	C	G		6	136597418	136597418	809	0.187886279	NA	NA
BECN1	Intron		A	C		17	40970978	40970978	67	0.208955224	NA	NA
BICD2	Silent	p.A644A	T	G		9	95480995	95480995	490	0.512244898	NA	NA
BMPER	3'UTR		T	G		7	34192967	34192967	51	0.352941176	NA	NA
BNC2	Intron		A	C		9	16728138	16728138	54	0.296296296	NA	NA
BOLL	Intron		-	T		2	198621388	198621388	8	0.375	NA	NA
BPIFA3	Silent	p.L12L	A	C		20	31805378	31805378	197	0.395939086	NA	NA
BTNL9	Silent	p.I229I	A	C		5	180477320	180477320	248	0.52016129	NA	NA
BUB3	3'UTR		T	C		10	124924679	124924679	91	0.274725275	NA	NA
C10orf32	3'UTR		A	T		10	104622805	104622805	28	0.357142857	NA	NA
C15orf57	3'UTR		T	C		15	40824334	40824334	12	0.416666667	NA	NA
C16orf58	Silent	p.S71S	A	G		16	31519482	31519482	27	0.333333333	NA	NA
C19orf10	Intron		-	C		19	4670125	4670125	26	0.615384615	NA	NA
C1orf87	Intron		-	C		1	60475913	60475813	24	0.458333333	NA	NA
C1QA	Silent	p.P97P	T	G		1	22965453	22965453	27	0.259259259	NA	NA
C2orf70	5'UTR		A	C		2	26802356	26802356	183	0.240437158	NA	NA
C3orf55	Intron		-	T	rs150188021; rs72589381	3	157295938	157295938	9	0.333333333	NA	NA
C5orf3	Silent	p.V584V	T	G		9	97717549	97717549	199	0.477386935	NA	NA
C5orf72	Silent	p.S234S	T	G		9	27560261	27560261	82	0.231707317	NA	NA
C9orf78	Intron		-	C		9	132593092	132593092	65	0.261538462	NA	NA
CACNA1C	Silent	p.P2000P	T	C		12	2797828	2797828	91	0.571428571	NA	NA
CACNA1H	Silent	p.G2211G	A	C		16	1270565	1270565	44	0.795454545	NA	NA
CACNA1S	Silent	p.R1447R	A	C		1	201017810	201017810	163	0.736196319	NA	NA
CACNA2D	Intron		-	G		3	50410950	50410950	10	0.5	NA	NA
CACNG7	Silent	p.V2aV	T	A		19	54416157	54416157	77	0.792207792	NA	NA
CAD	Silent	p.C1529C	T	C		2	27460609	27460609	179	0.569832402	NA	NA
CADM2	Silent	p.V347V	G	A		3	86114825	86114825	118	0.338983051	NA	NA
CADPS	Silent	p.A178A	A	G		3	62751576	62751576	380	0.268421053	NA	NA
CADPS2	Silent	p.L247L	A	C		7	122303336	122303336	115	0.2	NA	NA
CAGE1	Silent	p.L513L	A	G		6	7373513	7373513	59	0.389830508	NA	NA
CAND1	Intron		-	C		12	67886623	67886623	85	0.647058824	NA	NA
CANT1	5'UTR		T	C		17	76993764	76993764	29	0.24137931	NA	NA
CAPN3	Intron		-	A		15	42652566	42652566	6	0.333333333	NA	NA
CAPN9	Intron		-	TC		1	230937295	230937296	328	0.027439024	NA	NA
CARD11	3'UTR		G	C		7	2946110	2946110	19	0.315789474	NA	NA
CCAR1	Intron		C	-		10	70520714	70520715	87	0.103448276	NA	NA
CCDC13	Silent	p.L662L	A	C		3	42750574	42750574	115	0.382608696	NA	NA
CCDC173	5'UTR		G	C		2	170550917	170550917	36	0.583333333	NA	NA
CCDC84	Silent	p.F105F	T	C		11	118869751	118869751	83	0.325842697	NA	NA
CCR2	Silent	p.F201F	T	C		3	46399621	46399621	478	0.336820084	NA	NA

Figure 11 (cont.)

Gene	Variant_Classification	Alteration	Tumor_allele	Ref_allele	dbSNP_RS	Chr. #	Start_pos.	End_pos.	Coverage	Allelic_fraction	# of Probes	Segment_Mean (Log2[Copy
CCR5	3'UTR		T	C		3	46415461	46415461	220	0.336363636	NA	NA
CCT8L2	Silent	p.L34L	T	C		22	17073339	17073339	110	0.654545455	NA	NA
CD177	RNA		A	G		19	43859687	43859687	25	0.28	NA	NA
CD1B	5'UTR		A	C		1	158301228	158301228	132	0.5	NA	NA
CD1E	3'UTR		T	G	rs138530827	1	158326205	158326205	78	0.58974359	NA	NA
CD36	Intron		-	T		7	80300275	80300275	250	0.024137931	NA	NA
CD5L	3'UTR		A	G		1	157801678	157801678	191	0.183246073	NA	NA
CDC42BP	Silent	p.C1413C	G	A		1	227216446	227216446	451	0.403547672	NA	NA
CDCA2	Silent	p.K389K	A	G		8	25341528	25341528	229	0.048034934	NA	NA
CDH26	Silent	p.E679E	G	A		20	58574658	58574658	322	0.338509317	NA	NA
CDH8	Silent	p.R566R	T	G		16	61689582	61689582	163	0.263803681	NA	NA
CDK19	Silent	p.N406N	A	G		6	110942466	110942466	237	0.818565401	NA	NA
CDK5RAP	Silent	p.Q282Q	T	C		9	123291055	123291055	171	0.111111111	NA	NA
CDS2	Intron		-	A		20	5165756	5165756	17	0.352941176	NA	NA
CEACAM3	3'UTR		T	C		19	42092805	42092805	158	0.335443038	NA	NA
CELP	RNA		G	C		8	135962436	135962436	11	0.454545455	NA	NA
CENPF	Intron		G	A		1	214782026	214782026	48	0.125	NA	NA
CER1	Silent	p.T108T	C	G		9	14722344	14722344	171	0.50877193	NA	NA
CFC1	Silent	p.A65A	T	G		2	131356267	131356267	293	0.160409556	NA	NA
CFTR	Silent	p.F1090F	T	C		7	117251765	117251765	230	0.217391304	NA	NA
CGB7	5'UTR		T	A		19	49558776	49558776	16	0.375	NA	NA
CGB7	5'UTR		C	G		19	49558653	49558653	85	0.223529412	NA	NA
CGB7	5'UTR		G	T	rs35000456	19	49558678	49558678	68	0.147058824	NA	NA
CGB7	5'UTR		G	T	rs55995744	19	49558690	49558690	58	0.103448276	NA	NA
CGB7	5'UTR		G	A		19	49558772	49558772	15	0.4	NA	NA
CGN	Silent	p.F22F	T	C		1	151491061	151491061	502	0.135458167	NA	NA
CHRDL1	3'UTR		C	G		1	203148468	203148468	55	0.509090909	NA	NA
CHRNA4	3'UTR		T	G		20	61978081	61978081	54	0.444444444	NA	NA
CHST12	5'UTR		A	G		7	2472248	2472248	74	0.243243243	NA	NA
CHST15	Silent	p.I342I	A	G		10	125801824	125801824	268	0.235074627	NA	NA
CIT	Silent	p.P1911P	G	C		12	120135613	120135613	173	0.74566474	NA	NA
CLCN1	5'UTR		A	C		7	143013265	143013265	63	0.222222222	NA	NA
CLCN6	Silent	p.P607P	T	C		1	11896051	11896051	56	0.589285714	NA	NA
CLCN7	Silent	p.V663V	A	C		16	1498380	1498380	70	0.685714286	NA	NA
CLSTN2	3'UTR		A	C		3	140285107	140285107	11	0.545454545	NA	NA
CNIH2	Silent	p.V158V	T	G		11	66051059	66051059	23	0.217391304	NA	NA
CNNM4	Silent	p.L312L	T	C		2	97427672	97427672	473	0.231078947	NA	NA
CNNM4	Silent	p.F93F	T	C		2	97427015	97427015	95	0.252631579	NA	NA
CNNM4	Silent	p.R140R	A	G		2	97427156	97427156	263	0.231939163	NA	NA
CNTF	3'UTR		T	C		11	58392067	58392067	125	0.208	NA	NA
CNTNAP4	Intron		A	C		16	76528972	76528972	243	0.316872428	NA	NA
CNTNAP5	Silent	p.R683R	C	G		2	125405390	125405390	68	0.308823529	NA	NA
CNTRL	Intron		-	T		9	123924715	123924715	7	0.428571429	NA	NA
COG2	5'UTR		G	C		1	230778279	230778279	12	0.5	NA	NA
COL20A1	Silent	p.C179C	T	C		20	61938861	61938861	95	0.305263158	NA	NA
COL24A1	Intron		-	A		1	86529309	86529309	57	0.438596491	NA	NA
COL27A1	Silent	p.V1602V	T	C		9	117012921	117012921	742	0.489218329	NA	NA
COL4A3	Silent	p.L1129L	T	C		2	228159255	228159255	313	0.158730159	NA	NA
COL6A6	Silent	p.T1882T	A	C		3	130368319	130368319	57	0.192982456	NA	NA
COX11	Intron		-	TTTA ACT	rs3217151|rs72299663	17	53041893	53041899	9	0.333333333	NA	NA
CPSF3L	5'UTR		G	C		1	1280012	1280012	93	0.247311828	NA	NA
CRHR2	Silent	p.R148R	A	C		7	30704785	30704785	90	0.311111111	NA	NA
CSMD1	Silent	p.P173P	T	A		8	3889518	3889518	98	0.785714286	NA	NA
CSMD2	Silent	p.R2603R	T	G		1	34037282	34037282	217	0.387096774	NA	NA
CSMD3	Silent	p.T3232T	T	G		8	113276034	113276034	162	0.728395062	NA	NA
CSPG4	Silent	p.L1344L	A	G		15	75977800	75977800	46	0.217391304	NA	NA
CST6	Intron		A	G		11	65779795	65779795	16	0.375	NA	NA
CTD-2049I23.2	RNA		A	C		3	159819579	159819579	448	0.75	NA	NA
CTNNO1	5'UTR		T	G		5	11904005	11904005	12	0.916666667	NA	NA
CTTNBP2	Silent	p.S1257S	A	G		7	117375072	117375072	143	0.216783217	NA	NA
CUL4B	Silent	p.H80H	A	G		X	119666301	119666301	93	0.419354839	NA	NA
CWC25	Intron		-	C		17	36958585	36958585	7	0.571428571	NA	NA

Figure 11 (cont.)

Gene	Variant_Classification	Alteration	Tumor_allele	Ref_allele	dbSNP_RS	Chr. #	Start_pos.	End_pos.	Coverage	Allelic_fraction	# of Probes	Segment_Mean (Log2[Copy
CWH43	Intron		A	-		4	48063796	48063797	52	0.442307692	NA	NA
CXCL6	Silent	p.L25L	T	G		4	74702542	74702542	86	0.441860465	NA	NA
CYP11A1	Silent	p.L344L	A	G		15	74635402	74635402	327	0.235474006	NA	NA
CYP11A1	Intron		-	C		15	74632207	74632207	16	0.375	NA	NA
CYP11B1	Silent	p.F280F	A	G		8	143957771	143957771	102	0.294117647	NA	NA
CYP3A43	3'UTR		T	C		7	99463644	99463644	49	0.183673469	NA	NA
CYP4F24P	RNA		A	C		19	15882960	15882960	119	0.722689076	NA	NA
CYP4Z1	3'UTR		A	G		1	47583667	47583667	41	0.317073171	NA	NA
DACH1	5'UTR		T	A		13	72441001	72441001	7	0.857142857	NA	NA
DBX2	3'UTR		A	C		12	45409834	45409834	5	0.6	NA	NA
DCAF4L1	5'UTR		T	C		4	41983728	41983728	41	0.43902439	NA	NA
DCAF4L1	5'UTR		A	C		4	41983729	41983729	38	0.447368421	NA	NA
DCAKD	Silent	p.G208G	A	C		17	43101873	43101873	130	0.523076923	NA	NA
DCHS2	Intron		G	-	rs70947159	4	155287703	155287704	6	0.5	NA	NA
DDX1	Intron		-	T		2	15739606	15739606	6	0.333333333	NA	NA
DDX55	Intron		G	C		12	124090424	124090424	137	0.394160584	NA	NA
DDX60	Silent	p.V453V	T	C		4	169206630	169206630	88	0.5	NA	NA
DEF6	Silent	p.T16T	A	C		6	35265681	35265681	28	0.285714286	NA	NA
DEGS2	Intron		-	C	rs67172600|rs66550225|rs10709140	14	100613996	100613996	7	0.428571429	NA	NA
DEPTOR	Silent	p.L113L	T	C		8	120942092	120942092	260	0.453846154	NA	NA
DEPTOR	3'UTR		A	G		8	121061985	121061985	116	0.448275862	NA	NA
DFNB59	Intron		GGTCCCTCCCACC	-	rs143243813|rs113195419|rs75521356	2	179322311	179322312	10	0.5	NA	NA
DGKB	5'UTR		T	C		7	14880955	14880955	41	0.292682927	NA	NA
DHX40	Intron		-	A		17	57665581	57665581	6	0.5	NA	NA
DHX58	Silent	p.I557I	A	G		17	40255709	40255709	180	0.233333333	NA	NA
DHX58	Silent	p.T26T	A	C		17	40263833	40263833	271	0.306273063	NA	NA
DICER1	Silent	p.L708L	A	C		14	95577786	95577786	402	0.480099502	NA	NA
DIDO1	Intron		-	A	rs112847171|rs79926948|rs11088145	20	61526812	61526812	7	0.714285714	NA	NA
DLC1	Intron		-	TT	rs71207122	8	12958414	12958415	6	0.333333333	NA	NA
DLEC1	Silent	p.V827V	A	C		3	38139044	38139044	102	0.431372549	NA	NA
DMRT3	Intron		G	-		9	989918	989919	80	0.325	NA	NA
DNAH3	Silent	p.V2527V	G	C	rs148693918	16	20996483	20996483	123	0.731707317	NA	NA
DNAH9	Silent	p.T317T	A	C		17	11515144	11515144	128	0.7734375	NA	NA
DNAJB12	5'UTR		-	C	rs72808155	10	74114778	74114778	37	0.405405405	NA	NA
DNAJB9	Silent	p.K135K	A	G		7	108213530	108213530	220	0.227272727	NA	NA
DNASE1L	Silent	p.L72L	T	C		16	2287110	2287110	2	1	NA	NA
DNM1P4	RNA		T	G		15	100332276	100332276	163	0.54601227	NA	NA
DNM1P4	RNA		G	A		15	100340104	100340104	88	0.204819277	NA	NA
DNM1P4	RNA		G	C		15	102292829	102292829	50	0.06	NA	NA
DOCK1	Silent	p.L817L	T	C		10	128904563	128904563	52	0.307692308	NA	NA
DOCK8	Silent	p.L460L	T	G		9	340226	340226	343	0.486880466	NA	NA
DOPEY2	Silent	p.T2275T	T	A		21	37665797	37665797	199	0.758793844	NA	NA
DPF3	Silent	p.S318S	A	T		14	73137964	73137964	184	0.489130435	NA	NA
DPP4	Silent	p.A652A	A	G		2	162865103	162865103	244	0.352459016	NA	NA
DPP8	Silent	p.V589V	A	G		19	4688887	4688887	145	0.365517241	NA	NA
DPY5	Silent	p.T235T	T	G		8	105456564	105456564	172	0.784883721	NA	NA
DRD3	Silent	p.R274R	T	G		11	113283596	113283596	68	0.661764706	NA	NA
DRD2	Silent	p.N422N	A	G		11	113281915	113281915	339	0.185840708	NA	NA
DSC3	Silent	p.P884P	T	G		18	28574180	28574180	97	0.577319588	NA	NA
DSN1	Intron		-	A		20	35396636	35396636	8	0.375	NA	NA
DST	Silent	p.L5567L	T	C		6	56400067	56400067	160	0.56875	NA	NA
DUSP13	Silent	p.V263V	A	C		10	76854629	76854629	50	0.26	NA	NA
DUSP22	5'UTR		T	C		6	292471	292471	47	0.255319149	NA	NA
DUSP27	Silent	p.G330G	T	G		1	167095358	167095358	28	0.285714286	NA	NA

Figure 11 (cont.)

Gene	Variant_Classification	Alteration	Tumor_allele	Ref_allele	dbSNP_RS	Chr. #	Start_pos.	End_pos.	Coverage	Allelic_fraction	# of Probes	Segment_Mean (Log2[Copy
ECHS1	3'UTR		A	C		10	135176282	135176282	91	0.274509804	NA	NA
EFCAB13	Intron		T	C		17	45503187	45503187	248	0.258012048	NA	NA
EGR2	Silent	p.L200L	A	G		10	64573800	64573800	188	0.356382979	NA	NA
EHBP1	Intron		-	C		2	63223910	63223910	178	0.415730337	NA	NA
EIF2AK3	Silent	p.L238L	A	G		2	88892845	88892845	391	0.250639386	NA	NA
EIF2AK3	Silent	p.L236L	A	G		2	88892851	88892851	397	0.234256927	NA	NA
EIF3I	3'UTR		G	C		15	44853361	44853361	324	0.338888889	NA	NA
ELMO1	Silent	p.V484V	T	C		7	36934608	36934608	455	0.210989011	NA	NA
ELOVL6	Silent	p.Y254Y	A	G		4	110972530	110972530	327	0.422018349	NA	NA
ELTD1	3'UTR		G	C		1	79356783	79356783	71	0.352112676	NA	NA
EMC3	Silent	p.L61L	A	G		3	10019103	10019103	307	0.319218241	NA	NA
EML4	Silent	p.P122P	T	A		2	42488288	42488288	108	0.296296296	NA	NA
ENPP2	3'UTR		A	C	rs74007148	16	10626664	10626664	19	0.736842105	NA	NA
ENPP7	Silent	p.L135L	A	G		17	77708847	77708847	67	0.253731343	NA	NA
EP400NL	3'UTR		GGCA GA	-	rs141780032	12	132603415	132603416	16	0.375	NA	NA
EPDR1	5'UTR		A	G		7	37960951	37960951	24	0.458333333	NA	NA
EPG5	3'UTR		-	AC		18	43432246	43432247	6	0.333333333	NA	NA
EPHA10	Silent	p.S557S	C	T		1	38192875	38192875	42	0.238095238	NA	NA
EPHB4	Silent	p.L188L	A	G		7	100420139	100420139	49	0.306122449	NA	NA
EPN1	Silent	p.P367P	A	C		19	56203200	56203200	150	0.733333333	NA	NA
EPRS	Intron		A	T		1	220198465	220198465	191	0.214659686	NA	NA
ERBB4	3'UTR		A	G		2	212248336	212248336	150	0.233333333	NA	NA
ERC2	Silent	p.L625L	T	C		3	56044522	56044522	225	0.368888889	NA	NA
ERCC6	Intron		C	T		10	50714167	50714167	30	0.8	NA	NA
F13B	Intron		A	-		1	197009613	197009614	37	0.297297297	NA	NA
F5	5'UTR		T	C		1	169555688	169555688	35	0.257142857	NA	NA
FADD	Silent	p.L137L	A	G		11	70052363	70052363	85	0.152941176	NA	NA
FADD	Silent	p.S167S	T	C		11	70052453	70052453	110	0.354545455	NA	NA
FAM120C	Intron		-	T		X	54099867	54099867	10	0.4	NA	NA
FAM47C	Silent	p.P707P	A	C		X	37028604	37028604	197	0.766497462	NA	NA
FAM48A	Silent	p.L52L	G	C		2	16746950	16746950	556	0.537769784	NA	NA
FAM58	Silent	p.P409P	A	C		1	177247913	177247913	250	0.524	NA	NA
FAM58	Silent	p.P14P	T	G		1	177199054	177199054	191	0.403141361	NA	NA
FAM5C	Silent	p.T398T	A	G		1	190068255	190068255	129	0.310077519	NA	NA
FAM86C1	Intron		T	C		11	71505670	71505670	15	0.333333333	NA	NA
FAT3	Silent	p.I3275I	A	C		11	92565131	92565131	25	0.6	NA	NA
FAT3	Silent	p.C3018C	T	C		11	92600302	92600302	41	0.317073171	NA	NA
FBN1	Silent	p.S2714S	T	C		15	48791207	48791207	419	0.560859189	NA	NA
FBRS	Silent	p.F700F	T	C		16	30679906	30679906	495	0.177777778	NA	NA
FBXO42	Intron		A	G		1	16580012	16580012	27	0.222222222	NA	NA
FGD3	Silent	p.P711P	T	G		9	95797826	95797826	100	0.52	NA	NA
FGF12	Silent	p.N130N	G	A		3	192053174	192053174	278	0.508960573	NA	NA
FGF17	De_novo_Start_InFrame		A	G		8	21900439	21900439	39	0.769230769	NA	NA
FGF19	Silent	p.L141L	T	G		11	69514258	69514258	186	0.591397849	NA	NA
FHL5	Intron		T	-		6	97054120	97054121	8	0.375	NA	NA
FKBP3	5'UTR		A	G		14	45603698	45603698	71	0.464788732	NA	NA
FMN2	5'UTR		T	C		1	240635629	240635629	162	0.080246914	NA	NA
FN1	Silent	p.T748T	T	G		2	216274347	216274347	193	0.424870466	NA	NA
FOCAD	Silent	p.R558R	A	C		9	20820943	20820943	587	0.480787018	NA	NA
FOLH1B	RNA		G	C		11	89395374	89395374	143	0.244755245	NA	NA
FOLH1B	RNA		T	G		11	89403303	89403303	219	0.520547945	NA	NA
FOXL2	3'UTR		T	G		3	138664340	138664340	20	0.5	NA	NA
FPGT-TNNI3K	Silent	p.H453H	T	C		1	74819692	74819692	290	0.348275862	NA	NA
FREM1	Silent	p.R941R	T	C		9	14812883	14812883	633	0.309636651	NA	NA
FRMD3	Silent	p.V458V	A	C		9	85863253	85863253	456	0.50877193	NA	NA
FRMD3	3'UTR		A	T		9	85862667	85862667	110	0.445454545	NA	NA
FRMD4A	Silent	p.T171T	T	G		10	13789771	13789771	225	0.422222222	NA	NA
FRMD4B	Silent	p.P190P	A	C		3	69267503	69267503	35	0.4	NA	NA
FRYL	Intron		G	-		4	48584963	48584963	9	0.333333333	NA	NA
FSCN3	Silent	p.L227L	T	G		7	127235897	127235897	229	0.585152838	NA	NA
FUNDC2P	RNA		A	C		2	84518563	84518563	12	0.416666667	NA	NA

Figure 11 (cont.)

Gene	Variant_Classification	Alteration	Tumor_allele	Ref_allele	dbSNP_RS	Chr. #	Start_pos.	End_pos.	Coverage	Allelic_fraction	# of Probes	Segment_Mean (Log2[Copy
FYCO1	Silent	p.Q1246Q	T	C		3	45999961	45999961	87	0.413793103	NA	NA
GABBR1	Intron		-	AAG	rs68479771[rs67779259	4	47034102	47034104	69	0.111111111	NA	NA
GABRG1	5'UTR		A	C	rs146918422	4	46125931	46125931	186	0.403225806	NA	NA
GABRR1	3'UTR		T	G		6	89888383	89888383	22	0.818181818	NA	NA
GABRR3	RNA		C	T		3	97705574	97705574	35	0.514285714	NA	NA
GAD2	5'UTR		A	C		10	26505655	26505655	37	0.297297297	NA	NA
GAD2	5'UTR		A	C		10	26505659	26505659	45	0.555555556	NA	NA
GALNT11	Silent	p.A233A	T	G		7	151802442	151802442	275	0.283636364	NA	NA
GALNT18	Silent	p.P538P	A	G		11	11314639	11314639	141	0.361702128	NA	NA
GALNT2	Silent	p.L270L	A	G		1	230381889	230381889	278	0.23381295	NA	NA
GAS2L2	Silent	p.L735L	A	G	rs147442768	17	34072253	34072253	266	0.406015038	NA	NA
GAS7	3'UTR		T	G		17	9820463	9820463	42	0.738095238	NA	NA
GBP5	Intron		A	T		1	89735371	89735371	24	0.291666667	NA	NA
GCDH	Silent	p.T193T	T	C		19	13006879	13006879	97	0.701030928	NA	NA
GCK	5'UTR		A	G		7	44198736	44198736	127	0.377952756	NA	NA
GCN1L1	Silent	p.R212R	T	C		12	120597642	120597642	64	0.71875	NA	NA
GDI1	Intron		-	TC		X	153667085	153667086	506	0.015810277	NA	NA
GGT6	5'UTR		A	C		17	4463121	4463121	24	0.666666667	NA	NA
GIMAP1	Silent	p.V208V	A	C		7	150417716	150417716	27	0.481481481	NA	NA
GIMAP5	Intron		-	AC		7	150438187	150438188	6	0.333333333	NA	NA
GIP	Intron		-	GAAA		17	47041501	47041504	6	0.333333333	NA	NA
GLI3	Silent	p.I1421I	A	G		7	42004408	42004408	384	0.200520833	NA	NA
GLIPR1L2	3'UTR		A	G		12	75816929	75816929	24	0.416666667	NA	NA
GLIS3	Silent	p.H406H	A	G		9	4117795	4117795	480	0.31875	NA	NA
GLYCTK	3'UTR		T	C		3	52328086	52328086	6	0.5	NA	NA
GNGT2	Silent	p.A2A	C	G		17	47284779	47284779	362	0.273480663	NA	NA
GOLGA4	Silent	p.R68R	T	G		3	37323490	37323490	247	0.392712551	NA	NA
GOLGA5	Silent	p.T578T	C	T		14	93289481	93289481	353	0.02266289	NA	NA
GOLGA8E	RNA		G	C		15	23443567	23443567	47	0.234042553	NA	NA
GOLGB1	Silent	p.R676S	A	G		3	121417342	121417342	323	0.046439628	NA	NA
GPATCH1	Intron		-	A		2	37315480	37315480	17	0.470588235	NA	NA
GPATCH8	Silent	p.G659G	G	C		17	42477234	42477234	317	0.299684543	NA	NA
GPBP1L1	Silent	p.L346L	A	C		1	46099167	46099167	158	0.316455696	NA	NA
GPN1	Silent	p.L268L	T	C		2	27862954	27862954	662	0.285498489	NA	NA
GPR39	Silent	p.I39I	T	C		2	133174732	133174732	163	0.294478528	NA	NA
GPR4	5'UTR		T	C		19	46095136	46095136	56	0.714285714	NA	NA
GPR85	3'UTR		G	A		7	112723656	112723656	329	0.197568389	NA	NA
GPR88	Silent	p.L294L	T	C		1	101005402	101005402	81	0.333333333	NA	NA
GRB14	Intron		-	T		2	165476397	165476397	74	0.297297297	NA	NA
GREM2	3'UTR		A	C		1	240656219	240656219	34	0.588235294	NA	NA
GREM2	Silent	p.V160V	A	C		1	240656296	240656296	186	0.408602151	NA	NA
GRIK3	3'UTR		C	G		1	37267413	37267413	78	0.410256A1	NA	NA
GRIN3A	Intron		A	C		9	104357296	104357296	72	0.583333333	NA	NA
GRM1	Silent	p.P368P	A	C		6	146625894	146625894	134	0.78358209	NA	NA
GRM4	Intron		T	G		6	34029960	34029960	10	0.6	NA	NA
GRN	Intron		T	G		17	42427579	42427579	181	0.480662983	NA	NA
GRN	Intron		T	G		17	42427580	42427580	182	0.483516484	NA	NA
GSTM5	3'UTR		C	G		1	110258475	110258475	9	0.333333333	NA	NA
GSTM5	5'UTR		C	T		1	110255585	110255585	15	0.466666667	NA	NA
GTF2IRD2	Intron		-	A		7	74239418	74239418	10	0.4	NA	NA
GTF3C5	Silent	p.R239R	A	G		9	135926314	135926314	79	0.278481013	NA	NA
GUCA2A	Silent	p.P101P	A	C		1	42628622	42628622	113	0.389380531	NA	NA
GUCA2B	Silent	p.L62L	A	C		1	42620446	42620446	104	0.394230769	NA	NA
GUCY1A2	Silent	p.A538A	T	G		11	106680806	106680806	205	0.395121951	NA	NA
GYPC	Intron		A	C		2	127413940	127413940	21	0.333333333	NA	NA
GZF1	Silent	p.K314K	A	G		20	23345962	23345962	253	0.158798283	NA	NA
H2AFX	Silent	p.A48A	A	C		11	118965961	118965961	149	0.496644295	NA	NA
H2AFZ	5'UTR		A	C		4	100871406	100871406	93	0.408602151	NA	NA
HBS1L	Intron		A	-		6	135306238	135306239	8	0.75	NA	NA
HCAR3	Silent	p.V83V	G	C		12	123201036	123201036	114	0.368421053	NA	NA
HCFC2	Silent	p.P651P	T	C		12	104495820	104495820	210	0.3	NA	NA

Figure 11 (cont.)

Gene	Variant_Classification	Alteration	Tumor_allele	Ref_allele	dbSNP_RS	Chr. #	Start_pos.	End_pos.	Coverage	Allelic_fraction	# of Probes	Segment_Mean (Log2[Copy
HCN1	Silent	p.L779L	T	C		5	45262359	45262359	263	0.197718631	NA	NA
HDAC5	Silent	p.L809L	A	G		17	42160954	42160954	82	0.073170732	NA	NA
HEATR5A	Silent	p.Q1420Q	C	T		14	31782355	31782355	531	0.540489642	NA	NA
HGSNAT	Intron		AGA	-	rs144111342	8	43050798	43050799	9	0.333333333	NA	NA
HNIP	Silent	p.N614N	T	C	rs141591140	4	145655974	145655974	210	0.028571429	NA	NA
HMCN1	3'UTR		A	T		1	185962539	185962539	84	0.464285714	NA	NA
HMGB1P	RNA		A	G		3	22424078	22424078	45	0.4	NA	NA
HMGB3	Intron		A	C		X	150156222	150156222	157	0.796178344	NA	NA
HMGX84	Silent	p.K391K	A	G		22	35661554	35661554	67	0.059701493	NA	NA
HOXA10	Silent	p.L79L	A	C		7	27213701	27213701	28	0.392857143	NA	NA
HOXA11	5'UTR		A	C		7	27224816	27224816	112	0.455357143	NA	NA
HOXA2	5'UTR		A	G		7	27142120	27142120	207	0.362318841	NA	NA
HOX8-	RNA		G	-		17	46623853	46623854	6	0.333333333	NA	NA
HOXB2	5'UTR		A	C		17	46622354	46622354	28	0.678571429	NA	NA
HOXD12	Silent	p.A105A	T	G		2	176964844	176964844	72	0.291666667	NA	NA
HOXD4	Silent	p.V133V	T	C		2	177016760	177016760	114	0.271929825	NA	NA
HOXD8	Intron		T	-	rs4972804|rs774035049	2	176996022	176996023	224	0.03125	NA	NA
HSPE1	3'UTR		A	G		2	198368050	198368050	132	0.060606061	NA	NA
HSPH1	5'UTR		T	C		13	31735746	31735746	97	0.192982456	NA	NA
HTR2C	Intron		A	C		X	113865287	113865287	56	0.607142857	NA	NA
HTR3A	5'UTR		G	C		11	113845982	113845982	32	0.4375	NA	NA
HTR5A	3'UTR		A	G		7	154876204	154876204	230	0.186956522	NA	NA
ICAM1	Silent	p.A378A	T	C		19	10395287	10395287	88	0.738636364	NA	NA
IFI27L1	3'UTR		T	G		14	94568966	94568966	72	0.361111111	NA	NA
IFNA7	Silent	p.A2A	A	G		9	21202159	21202159	174	0.264367816	NA	NA
IFT27	Silent	p.L86L	A	G		22	37160054	37160054	147	0.333333333	NA	NA
IFT52	Silent	p.E279E	A	G		20	42252599	42252599	483	0.258799172	NA	NA
IGF1R	Silent	p.T28T	T	G		15	99192894	99192894	106	0.58490566	NA	NA
IGF2	Silent	p.R73R	T	G		11	2154834	2154834	73	0.068493151	NA	NA
IGFN1	Silent	p.P3658P	T	C		1	201196197	201196197	76	0.263157895	NA	NA
IGHA1	RNA		T	A		14	106173602	106173602	211	0.142180095	NA	NA
IGHA1	RNA		A	C		14	106173601	106173601	207	0.144927536	NA	NA
IGHV1-24	RNA		A	C		14	106733563	106733563	388	0.020618557	NA	NA
IGHV1OR15-9	RNA		T	G		15	20170044	20170044	381	0.440944882	NA	NA
IGHV3OR16-9	RNA		T	A		16	32077421	32077421	1430	0.151748252	NA	NA
IGHV4-28	RNA		C	G		14	106780800	106780800	180	0.2	NA	NA
IGKV2D-	RNA		T	C		2	89986980	89986980	335	0.244776119	NA	NA
IGKV3-15	RNA		T	G		2	89384814	89384814	242	0.516528926	NA	NA
IGKV3D-	RNA		A	T		2	90154141	90154141	245	0.187755102	NA	NA
IL12A	Intron		C	T		3	159706987	159706987	44	0.545454545	NA	NA
IL31RA	Silent	p.L539L	A	G		5	55209336	55209336	262	0.267175573	NA	NA
IL36G	Intron		-	G		2	113737442	113737442	30	0.233333333	NA	NA
IL37	Silent	p.V10V	A	G		2	113670619	113670619	205	0.209756098	NA	NA
IL6R	Silent	p.T33T	A	C		16	27353470	27353470	97	0.762886598	NA	NA
IQCA1	5'UTR		T	C		2	237416030	237416030	35	0.171428571	NA	NA
IQCA1	Intron		-	CA		2	237285861	237285862	122	0.37704918	NA	NA
ITGA2B	Intron		T	C		17	42452202	42452202	27	0.518518519	NA	NA
ITGA8	Silent	p.Q916Q	T	C		10	15600091	15600091	381	0.194225722	NA	NA
ITGAL	Intron		-	G		16	30528095	30528095	8	0.625	NA	NA
ITK	Silent	p.R193R	A	C		5	156649954	156649954	258	0.763565891	NA	NA
ITPR1	Intron		-	TTTG	rs78487649|rs58112934|rs112871347	3	4715262	4715273	7	0.428571429	NA	NA
IVL	Silent	p.L123L	A	G	rs141684742	1	152882642	152882642	268	0.164179104	NA	NA
JUP	Intron		T	G		17	39785318	39785318	22	0.272727273	NA	NA
KANSL2	Silent	p.L444L	A	G		12	49062984	49062984	15	0.333333333	NA	NA

Figure 11 (cont.)

Gene	Variant_Classification	Alteration	Tumor_allele	Ref._allele	dbSNP_RS	Chr. #	Start_pos.	End_pos.	Coverage	Allelic_fraction	# of Probes	Segment_Mean (Log2[Copy
KCNA7	Silent	p.T175T	T	C		19	49575318	49575318	51	0.333333333	NA	NA
KCNH1	Silent	p.V883V	C	G		1	210856863	210856863	90	0.355555556	NA	NA
KCNIP3	Silent	p.F235F	T	C		2	96049024	96049024	59	0.338983051	NA	NA
KCNJ5	5'UTR		T	G		11	128781082	128781082	17	0.647058824	NA	NA
KCNK1	3'UTR		C	G		1	233807386	233807386	40	0.225	NA	NA
KCNK13	Silent	p.I156I	A	C		14	90650588	90650588	256	0.43359375	NA	NA
KCNN4	5'UTR		T	A		19	44285072	44285072	29	0.827586207	NA	NA
KCTD8	Silent	p.G200G	T	G		4	44449841	44449841	21	0.380952381	NA	NA
KEL	Silent	p.I672I	A	G		7	142639542	142639542	32	0.1875	NA	NA
KIAA0753	Silent	p.Q639Q	T	C		17	6510503	6510503	446	0.746636771	NA	NA
KIAA1549	3'UTR		T	C		11	33620508	33620508	64	0.78125	NA	NA
KIAA1755	Silent	p.R575R	A	G		20	36867952	36867952	172	0.360465116	NA	NA
KIAA1958	3'UTR		T	G		9	115422474	115422474	22	0.454545455	NA	NA
KIF19	Silent	p.L800L	T	G		17	72350392	72350392	132	0.310606061	NA	NA
KIF21A	Silent	p.P1619P	A	T		12	39695359	39695359	396	0.717171717	NA	NA
KIF21B	Silent	p.R1371R	A	C		1	200948671	200948671	151	0.774834437	NA	NA
KIF26A	Silent	p.A1055A	T	C		14	104642707	104642707	34	0.411764706	NA	NA
KIN	Silent	p.I264I	A	G		10	7811185	7811185	511	0.225048924	NA	NA
KLC4	5'UTR		T	C		6	43027909	43027909	243	0.333333333	NA	NA
KRT19P2	RNA		A	C		12	95228684	95228684	9	0.888888889	NA	NA
KRTAP26-1	Silent	p.C163C	A	G		21	31691865	31691865	433	0.697459584	NA	NA
L3MBTL1	Silent	p.R567R	T	G		20	42164842	42164842	48	0.270833333	NA	NA
LAMB1	3'UTR		T	C		7	107599997	107599997	57	0.245614035	NA	NA
LAMC2	Intron		-	T	rs60707856{rs79122995{rs11347730	1	183155716	183155716	8	0.375	NA	NA
LAMP3	3'UTR		C	T		3	182841843	182841843	252	0.793650794	NA	NA
LAPTM5	Silent	p.T138T	G	C		1	31211883	31211883	30	0.466666667	NA	NA
LARP1B	3'UTR		T	G		4	129100490	129100490	35	0.657142857	NA	NA
LARP6	Silent	p.L200L	A	C		15	71125267	71125267	121	0.570247934	NA	NA
LARP6	3'UTR		G	T		15	71124340	71124340	156	0.275641026	NA	NA
LBP	Silent	p.L93L	C	G		20	36979253	36979253	157	0.49044586	NA	NA
LBR	Silent	p.F511F	A	G		1	225592359	225592359	308	0.399350649	NA	NA
LCN15	3'UTR		T	G		9	139656259	139656259	16	0.5625	NA	NA
LECT2	3'UTR		T	G		5	135282816	135282816	8	0.875	NA	NA
LILRA1	3'UTR		T	G		19	55112468	55112468	11	0.636363636	NA	NA
LILRA4	Silent	p.L15L	A	C		19	54850162	54850162	83	0.78313253	NA	NA
LINC00347	RNA		A	-		9	98619290	98619291	6	0.333333333	NA	NA
LIPN	Intron		T	-		10	90521878	90521879	8	0.375	NA	NA
LMTK3	3'UTR		T	G		19	48988945	48988945	12	0.916666667	NA	NA
LPA	Silent	p.T1547T	T	G		6	160978594	160978594	186	0.032258065	NA	NA
LRIT3	Silent	p.K286K	G	A		4	110791312	110791312	203	0.443349754	NA	NA
LRP1B	Silent	p.R2138R	A	T		2	141459303	141459303	338	0.49408284	NA	NA
LRP1B	Intron		-	A		2	141110468	141110468	212	0.132075472	NA	NA
LRP2	Silent	p.H1672H	G	A		2	170093003	170093003	206	0.40776699	NA	NA
LRP2	Silent	p.V744V	A	G		2	170127502	170127502	226	0.300884956	NA	NA
LRRC27	Silent	p.P32AP	A	G		10	134169378	134169378	14	0.428571429	NA	NA
LRRC37A16P	RNA		A	G		17	66126443	66126443	1044	0.012452107	NA	NA
LRRIQ3	3'UTR		A	C		1	74492473	74492473	70	0.342857143	NA	NA
LRRIQ3	Silent	p.K91K	C	T		1	74648522	74648522	136	0.330882353	NA	NA
LRRIQ3	Silent	p.F274F	A	G		1	74575123	74575123	87	0.275862069	NA	NA
LTB4R	Silent	p.T188T	T	G		14	24785421	24785421	192	0.276041667	NA	NA
MAD1L1	Intron		-	C		7	2041831	2041831	106	0.349056604	NA	NA
MAGEB6	Silent	p.P59P	A	C		X	26212170	26212170	155	0.890322581	NA	NA
MAGEC2	Silent	p.F9F	A	G		X	141291747	141291747	206	0.359223301	NA	NA
MAGI2	Silent	p.R860R	G	T		7	77797251	77797251	531	0.011299435	NA	NA
MAN2A2	Silent	p.R374R	T	C		15	91450651	91450651	181	0.254143646	NA	NA
MAPK14	3'UTR		T	G		6	36076270	36076270	91	0.571428571	NA	NA
MAPK6	Silent	p.R130R	C	A		15	52339045	52339045	150	0.213333333	NA	NA
MAPT	Intron		T	-	rs114790373	17	44069147	44069148	6	0.333333333	NA	NA
MAST4	Silent	p.P2313P	T	A		5	66461937	66461937	25	0.72	NA	NA

Figure 11 (cont.)

Gene	Variant_Classification	Alteration	Tumor_allele	Ref._allele	dbSNP_RS	Chr. #	Start_pos.	End_pos.	Coverage	Allelic_fraction	# of Probes	Segment_Mean (Log2[Copy
MAST4	5'UTR		T	G		5	65892457	65892457	43	0.720930233	NA	NA
M821D1	Silent	p.A54A	A	G		6	74161743	74161743	6	0.666666667	NA	NA
MCF2	Silent	p.R15R	T	C		X	138724633	138724633	385	0.820779221	NA	NA
MED16	3'UTR		A	G		5	6372534	6372534	103	0.135339806	NA	NA
MED23	5'UTR		A	C		6	131949232	131949232	173	0.398843931	NA	NA
MEF2A	5'UTR		A	G		15	100173219	100173219	10	0.4	NA	NA
MEF2B	Silent	p.L215L	A	C		19	19257581	19257581	43	0.76744186	NA	NA
MEOX2	3'UTR		G	T		7	15651900	15651900	32	0.46875	NA	NA
METTL12	Silent	p.F226F	T	C		11	62434478	62434478	230	0.317391304	NA	NA
MEX3D	Silent	p.P267P	A	C		19	1556717	1556717	93	0.709677419	NA	NA
MICALCL	3'UTR		A	C		11	12380122	12380122	73	0.342465753	NA	NA
MINPP1	5'UTR		T	A		10	89264638	89264638	30	0.466666667	NA	NA
MIR297	RNA		A	G		4	111781739	111781739	247	0.20242915	NA	NA
MIR506	RNA		A	C		X	146312277	146312277	172	0.470930233	NA	NA
MRX	Silent	p.T281T	T	A		10	27964479	27964479	339	0.448377581	NA	NA
MLLT10	Silent	p.S443S	T	C		10	21962556	21962556	1051	0.434823977	NA	NA
MLLT3	Silent	p.E188S	A	G		9	20414280	20414280	488	0.014344262	NA	NA
MLLT3	Silent	p.S171S	A	G	rs10964556	9	20414331	20414331	279	0.032258065	NA	NA
MMAA	Intron		A	-		4	146572056	146572057	16	0.5	NA	NA
MMRN2	Silent	p.A201A	T	G		10	88704071	88704071	278	0.428057554	NA	NA
MS4A10	3'UTR		A	T		11	60567454	60567454	107	0.308411215	NA	NA
MS4A8	Intron		A	G		11	60476404	60476404	8	0.625	NA	NA
MST1	Silent	p.P333P	A	T		3	49723763	49723763	55	0.163636364	NA	NA
MSX2	Silent	p.H243H	C	T		5	174156511	174156511	142	0.281690141	NA	NA
MTUP	RNA		T	C		16	56670257	56670257	63	0.571428571	NA	NA
MTAP	5'UTR		T	C		9	21802690	21802690	47	0.276595745	NA	NA
MTHFD2P	RNA		G	C		3	95374629	95374629	13	0.307692308	NA	NA
MTOR	Intron		-	A		1	11227303	11227303	8	0.375	NA	NA
MTSS1	5'UTR		A	G		8	125740217	125740217	64	0.296875	NA	NA
MUC13	Silent	p.S470S	G	C	rs151214305	3	124627120	124627120	226	0.748362832	NA	NA
MUC16	Silent	p.L11731L	A	G		19	9086622	9086622	203	0.379310345	NA	NA
MUC16	Intron		A	-		19	9003464	9003465	144	0.798611111	NA	NA
MUC6	Silent	p.V1462V	A	G		11	1018415	1018415	624	0.193910256	NA	NA
MUC7	Silent	p.S205S	T	C		4	71347076	71347076	528	0.410984848	NA	NA
MUL1	3'UTR		C	G		1	20827118	20827118	164	0.256097561	NA	NA
MUM1L1	Silent	p.L266L	A	G		X	105450223	105450223	19	0.789473684	NA	NA
MYH11	Intron		A	G		16	15808734	15808734	167	0.365269461	NA	NA
MYH15	Silent	p.V1278V	T	C		3	108139990	108139990	434	0.493087558	NA	NA
MYH7	Silent	p.R1820R	T	C		14	23884303	23884303	366	0.319672131	NA	NA
MYH8	Silent	p.R1347R	A	C		17	10301898	10301898	296	0.746621622	NA	NA
MYL6	3'UTR		T	C		12	56555034	56555034	29	0.413793103	NA	NA
MYO1B	Silent	p.P1656P	G	C		13	109793594	109793594	66	0.924242424	NA	NA
MYO1G	Silent	p.L19L	C	A		13	109318328	109318328	127	0.787401575	NA	NA
MYO7B	Silent	p.T495T	A	C		2	128341838	128341838	156	0.294871795	NA	NA
NAB2	Intron		-	G		12	57487096	57487096	16	0.6875	NA	NA
NALCN	Intron		AAAAT	-	rs149478126	13	102028867	102028868	6	0.333333333	NA	NA
NBPF18P	RNA		G	C		1	151993658	151993658	409	0.466992665	NA	NA
NDC80	Silent	p.L352L	G	A		18	2595455	2595455	195	0.179487179	NA	NA
NDUFA10	Silent	p.L44L	G	T		2	240961701	240961701	250	0.28	NA	NA
NEU1	Silent	p.F154F	A	G		6	31829118	31829118	116	0.293103448	NA	NA
NFE2L3	Silent	p.F562F	T	C		7	26225004	26225004	520	0.207692308	NA	NA
NFIC	5'UTR		A	C		19	3462988	3462988	12	0.75	NA	NA
NFIL3	5'UTR		T	C		9	94173074	94173074	116	0.232758621	NA	NA
NGB	Silent	p.R16R	T	G		14	77737253	77737253	47	0.510638298	NA	NA
NGEF	Intron		T	-		2	233756330	233756331	17	0.352941176	NA	NA
NIPAL4	Silent	p.L143L	A	C		5	156890307	156890307	99	0.717171717	NA	NA
NIPBL	Silent	p.I410I	T	A		5	36976239	36976239	321	0.426791277	NA	NA
NIPBL	Silent	p.L1989L	T	C		5	37036583	37036583	41	0.268292683	NA	NA
NIT1	5'UTR		A	G		1	161087949	161087949	206	0.257864078	NA	NA
NLE1	Intron		C	-	rs34884628	17	33463313	33463314	781	0.758002561	NA	NA
NLRC4	Silent	p.L203L	T	G		2	32476324	32476324	202	0.544554455	NA	NA

Figure 11 (cont.)

Gene	Variant_Classification	Alteration	Tumor_allele	Ref_allele	dbSNP_RS	Chr. #	Start_pos.	End_pos.	Coverage	Allelic_fraction	#_of_Probes	Segment_Mean (Log2[Copy
NLRC5	Silent	p.L1798L	A	G		16	57115082	57115082	9	0.333333333	NA	NA
NLRP8	3'UTR		G	C		19	56499379	56499379	18	0.833333333	NA	NA
NMT1	3'UTR		T	A		17	43183069	43183069	128	0.578125	NA	NA
NOS1	Intron		-	G		12	117748409	117749409	39	0.589743359	NA	NA
NOTCH3N	3'UTR		A	G		1	145282138	145282138	34	0.117647059	NA	NA
NPR1	Intron		-	C		1	153661329	153661329	54	0.462962963	NA	NA
NPTX2	Silent	p.L174L	T	G		7	98249050	98249050	151	0.245033113	NA	NA
NR2F2	Silent	p.N120N	T	C		15	96875694	96875694	120	0.216666667	NA	NA
NR4A2	Silent	p.V329V	A	G		2	157184923	157184923	231	0.277056277	NA	NA
NR6A1	Silent	p.Q298Q	T	C		9	127298342	127298342	211	0.293838863	NA	NA
NRG1	Intron		A	G		8	32585601	32585601	193	0.735751295	NA	NA
NRG4	Intron		-	A	rs34120380|rs11341073|rs146303179	15	76248134	76248134	8	0.375	NA	NA
NRXN1	Intron		A	-		2	50645594	50645595	19	0.368421053	NA	NA
NRXN3	5'UTR		A	C		14	79111652	79111652	105	0.361904762	NA	NA
NRXN3	Intron		T	-		14	79276717	79276718	136	0.051470588	NA	NA
NTN1	Silent	p.L213L	T	C		17	8926329	8926329	24	0.666666667	NA	NA
NTN5	Intron		-	CATCAT	rs71179032	19	49173236	49173241	6	0.333333333	NA	NA
NTSR2	3'UTR		-	C	rs150611318	2	11798571	11798571	281	0.249110032	NA	NA
NUF2	Silent	p.F85F	T	C		1	163298074	163298074	254	0.306299213	NA	NA
NUP210L	Intron		-	T		1	153994528	153994528	108	0.074074074	NA	NA
OBSCN	Intron		C	-		1	228402387	228402388	15	0.4	NA	NA
OCA2	Silent	p.V488V	A	G		15	28228524	28228524	159	0.376100629	NA	NA
OLFM3	5'UTR		A	C		1	102312583	102312583	75	0.4	NA	NA
OLFM3	Silent	p.V386V	A	G		1	102270013	102270013	309	0.430420712	NA	NA
OLFML2B	Silent	p.G883G	G	C		1	161953675	161953675	935	0.306542056	NA	NA
OR10G9	Silent	p.T145T	A	C		11	123894154	123894154	273	0.542124542	NA	NA
OR10J1	Silent	p.L341L	C	G		1	159409971	159409971	503	0.365205368	NA	NA
OR11G2	Silent	p.P167P	G	T		14	20665995	20665995	337	0.228486647	NA	NA
OR1C1	Silent	p.L27L	G	C		1	247921628	247921628	61	0.278688525	NA	NA
OR2AE1	Silent	p.D87S	A	G	rs142920412	7	99474456	99474456	153	0.477124183	NA	NA
OR2AK2	Silent	p.F50F	C	T		1	248128783	248128783	441	0.331065759	NA	NA
OR2L13	Silent	p.T76T	A	C	rs139483421	1	248262905	248262905	422	0.438388626	NA	NA
OR2L13	Intron		T	G		1	248153619	248153619	173	0.531791908	NA	NA
OR2M2	Silent	p.G308G	G	C		1	248344211	248344211	577	0.284228769	NA	NA
OR2W3	Silent	p.I26I	A	C		1	248058966	248058966	254	0.405511811	NA	NA
OR4A15	Silent	p.L317L	A	G		11	55136310	55136310	462	0.277056277	NA	NA
OR4C16	Silent	p.L15L	A	G		11	55339648	55339648	255	0.039215686	NA	NA
OR4K2	Silent	p.L144L	G	C		14	20344858	20344858	1082	0.347504621	NA	NA
OR4M2	Silent	p.P129P	A	C		15	22368962	22368962	347	0.432276657	NA	NA
OR52A1	Silent	p.V286V	A	G		11	5172742	5172742	212	0.349056604	NA	NA
OR52B2	Silent	p.L132L	T	C		11	6191161	6191161	101	0.059405941	NA	NA
OR56A4	Silent	p.L310L	A	C		11	6023449	6023449	68	0.75	NA	NA
OR5AR1	Silent	p.L86L	G	A		11	56431419	56431419	652	0.208588957	NA	NA
OR5D16	Silent	p.I247I	T	G		11	55606968	55606968	283	0.568904594	NA	NA
OR5W2	Silent	p.G233G	A	C		11	55681360	55681360	167	0.041916168	NA	NA
OR6F1	Silent	p.A155A	C	G		1	247875593	247875593	305	0.262295082	NA	NA
OR6K2	Silent	p.P159P	A	G		1	158660978	158660978	271	0.520295203	NA	NA
OR8A1	Silent	p.L49L	T	C		11	124440111	124440111	208	0.288461538	NA	NA
OR8D1	Silent	p.S74S	A	G		11	124180441	124180441	151	0.245033113	NA	NA
ORC1	Silent	p.S850S	A	G		1	52838889	52838889	343	0.381924198	NA	NA
OSBPL6	Silent	p.L423L	A	C		2	179226524	179226524	155	0.477419355	NA	NA
OSR1	3'UTR		A	C		2	19551940	19551940	39	0.461538462	NA	NA
PAK3	3'UTR		C	A		X	110463699	110463699	93	0.849056604	NA	NA
PALD1	Intron		GGA	-	rs149934174|rs108741659	10	72286140	72286141	7	0.428571429	NA	NA
PAPPA2	Silent	p.D1078D	T	C		1	176668723	176668723	783	0.026755682	NA	NA

Figure 11 (cont.)

Gene	Variant_Classification	Alteration	Tumor_allele	Ref_allele	dbSNP_RS	Chr. #	Start_pos.	End_pos.	Coverage	Allelic_fraction	# of Probes	Segment_Mean (Log2[Copy
PARK7	Silent	p.G157G	T	G		1	8045015	8045015	243	0.2551440833	NA	NA
PCDH17	3'UTR		A	G		13	58299520	58299520	33	0.6060606806	NA	NA
PCDHA2	Silent	p.S689S	T	A		5	140176616	140176616	360	0.727777778	NA	NA
PCDHA5	Silent	p.L810L	C	T		5	140203788	140203788	190	0.026315789	NA	NA
PCDHB6	Silent	p.F793F	T	C		5	140532217	140532217	98	0.37755102	NA	NA
PCDHGA1	Silent	p.P256P	C	G		5	140711019	140711019	188	0.739361702	NA	NA
PCED1A	Silent	p.L143L	A	G		20	2819504	2819504	297	0.444444444	NA	NA
PCED1B	Silent	p.L379L	A	G		12	47629883	47629983	196	0.714285714	NA	NA
PCGF5	Silent	p.E76E	A	G		10	93008280	93008280	329	0.32218845	NA	NA
PCLO	Silent	p.L3685L	T	C		7	82578849	82578849	583	0.044596913	NA	NA
PCLO	Silent	p.I5117I	A	G		7	82387969	82387969	315	0.231746032	NA	NA
PCM1	Intron		T	-		8	17826852	17826953	6	0.333333333	NA	NA
PCNXL2	Silent	p.A1461A	A	G		1	233161114	233161114	220	0.384279476	NA	NA
PDC13	Silent	p.L329L	C	G		2	101188070	101188070	227	0.123348018	NA	NA
PDE10A	Intron		-	A		6	165990045	165990045	52	0.673076923	NA	NA
PDE11A	Intron		-	TA		2	178862151	178862152	274	0.518248175	NA	NA
PDE4A	Intron		T	G		19	10572525	10572525	83	0.78313253	NA	NA
PDE4B	3'UTR		T	G		1	66723123	66723123	234	0.363247863	NA	NA
PDE4DIP	3'UTR		T	G		1	144852340	144852340	50	0.14	NA	NA
PER3	5'UTR		A	G		1	7844835	7844935	41	0.365853659	NA	NA
PEX12	3'UTR		T	C		17	33902679	33902679	24	0.125	NA	NA
PHACTR3	Silent	p.P128P	A	C		20	58330262	58330262	122	0.418032787	NA	NA
PI16	5'UTR		A	G		6	36922507	36922507	125	0.184	NA	NA
PIK3C2B	Intron		A	G		1	204436141	204436141	21	0.238095238	NA	NA
PKD1L1	Intron		-	C		7	47884509	47884509	25	0.36	NA	NA
PKD1L2	RNA		A	C		16	81142852	81142852	45	0.266666667	NA	NA
PKHD1	Silent	p.L2263L	C	T		6	51771032	51771032	158	0.772151899	NA	NA
PKHD1	Intron		-	A		6	51777468	51777468	19	0.578947368	NA	NA
PKHD1L1	Silent	p.L1542L	C	G		8	110455966	110455966	90	0.666666667	NA	NA
PKNOX1	Silent	p.L427L	C	G	rs140174291	21	44450181	44450181	74	0.648648649	NA	NA
PKP2	Intron		-	G	rs12228780	12	32994261	32994261	21	0.6190047619	NA	NA
PLA2G4D	Silent	p.L305L	A	C		15	42373719	42373719	278	0.561151079	NA	NA
PLA2G5	3'UTR		T	C		1	20417193	20417193	302	0.311258278	NA	NA
PLCL1	3'UTR		T	C		2	199011738	199011738	30	0.366666667	NA	NA
PLXNC1	Silent	p.G522G	T	C	rs139278274	12	94620456	94620456	213	0.018779343	NA	NA
PNLIPRP1	Intron		G	A		10	118354104	118354104	46	0.260869565	NA	NA
PNN	3'UTR		T	C		14	39651197	39651197	28	0.571428571	NA	NA
POFUT2	Silent	p.Q93Q	T	C		21	46705696	46705696	197	0.365482234	NA	NA
POGK	Silent	p.G430G	A	G		1	166819106	166819106	242	0.144628099	NA	NA
POLR3B	Intron		-	T		12	106804914	106804914	7	0.428571429	NA	NA
POTEF	3'UTR		A	G		2	130831618	130831618	22	0.227272727	NA	NA
POTEH	3'UTR		A	C		22	16256527	16256527	86	0.127906977	NA	NA
POU2F2	Silent	p.L174L	A	C		19	42600375	42600375	29	0.827586207	NA	NA
PPARGC1	Silent	p.L409L	T	G		5	149212863	149212863	128	0.7890625	NA	NA
PPM1G	Intron		C	T		2	27609215	27609215	122	0.31147541	NA	NA
PPP2R2C	5'UTR		T	G		4	6474028	6474028	134	0.835820896	NA	NA
PRDM9	Silent	p.E124E	T	C		5	23521152	23521152	237	0.206751055	NA	NA
PREX1	Silent	p.S247S	C	T		14	30108066	30108066	386	0.018134715	NA	NA
PRKD3	Intron		-	T		2	37481336	37481336	175	0.04	NA	NA
PRKRIR	3'UTR		G	T		11	76061898	76061898	113	0.608695652	NA	NA
PRPF40A	Silent	p.R9R	T	C		2	153573927	153573927	173	0.25433526	NA	NA
PRSS23	3'UTR		T	G		11	86519942	86519942	51	0.568627451	NA	NA
PRTFDC1	Intron		-	T	rs35562409	10	25226485	25226485	6	0.333333333	NA	NA
PSENEN	3'UTR		T	C		19	36237827	36237827	13	0.692307692	NA	NA
PTBP3	Intron		-	AG		9	114994246	114994247	8	0.75	NA	NA
PTCD1	Silent	p.T481T	A	C		7	99022682	99022682	135	0.518518519	NA	NA
PTGER2	Silent	p.L80L	T	C	rs146696779	14	52781506	52781506	137	0.262773723	NA	NA
PTPN14	5'UTR		G	C		1	214706124	214706124	61	0.295081967	NA	NA
PTPN3	Intron		T	C		9	112145688	112145688	441	0.297052154	NA	NA

Figure 11 (cont.)

Gene	Variant_Classification	Alteration	Tumor_allele	Ref_allele	dbSNP_RS	Chr. #	Start_pos.	End_pos.	Coverage	Allelic_fraction	# of Probes	Segment_Mean (Log2[Copy
PTPN4	Silent	p.A293A	G	A	rs142256215	2	120677695	120677695	320	0.303125	NA	NA
PTPN4	Intron		-	CATATTCTAAAT		2	120725610	120725621	118	0.381355932	NA	NA
PTPN5	Silent	p.T12ST	A	C		11	18764893	18764893	178	0.735955056	NA	NA
PTPN6	Intron		C	G		3	62110052	62110052	69	0.31884058	NA	NA
PTPN9	Silent	p.T86T	A	C		2	220158768	220159768	43	0.302325581	NA	NA
PTPRU	3'UTR		T	G		1	29652210	29652210	136	0.301470588	NA	NA
PUM1	Intron		-	A		1	31422845	31422845	21	0.523809524	NA	NA
PUS10	Silent	p.C276C	A	G		2	61188644	61188644	775	0.007741935	NA	NA
PYDC1	3'UTR		G	C		16	31228079	31228079	180	0.372222222	NA	NA
PYGB	Intron		T	C		20	25269046	25269046	295	0.225531915	NA	NA
PYROXD1	3'UTR		T	A		12	21623298	21623298	20	0.15	NA	NA
PYY	3'UTR		A	C		17	42080174	42080174	18	0.333333333	NA	NA
RAB27B	Silent	p.F81F	T	C		18	52551567	52551567	139	0.654676259	NA	NA
RAB3IL1	Silent	p.R99R	T	C		11	61675100	61675100	101	0.168316832	NA	NA
RAB3IL1	Silent	p.R246R	G	T		11	61672276	61672276	24	0.291666667	NA	NA
RAB6C	5'UTR		T	C		2	130737622	130737622	125	0.224	NA	NA
RAC1	5'UTR		T	C		7	6414352	6414352	15	0.266666667	NA	NA
RALGPS2	5'UTR		T	G		1	178745824	178745824	65	0.492307692	NA	NA
RANBP3L	Silent	p.A463A	T	G		5	36249765	36249765	168	0.857142857	NA	NA
RAP1GAP	Silent	p.A156A	A	C		17	2867338	2867338	155	0.780645161	NA	NA
RAPGEF6	Intron		-	T		5	130800138	130800138	8	0.375	NA	NA
RASAL2	Silent	p.L1194L	A	G		1	178436493	178436493	228	0.175438596	NA	NA
RBBP8	Intron		-	G		18	20569384	20569384	30	0.4	NA	NA
RBM53	Silent	p.L62L	T	G		3	29476347	29476347	258	0.333333333	NA	NA
RECQL4	Silent	p.R715R	T	C		8	145739010	145739010	39	0.333333333	NA	NA
REG1B	Silent	p.S25S	C	G		2	79314046	79314046	327	0.486238532	NA	NA
REG1P	RNA		T	C		2	79362882	79362882	22	0.318181818	NA	NA
REG3G	3'UTR		A	C		2	79255517	79255517	39	0.333333333	NA	NA
RETNLB	3'UTR		T	C		3	108474519	108474519	47	0.234042553	NA	NA
RFPL3	3'UTR		T	A		22	32756949	32756949	19	0.736842105	NA	NA
RFX3	Silent	p.L598L	T	C		9	3257011	3257011	507	0.481262327	NA	NA
RGPD1	Silent	p.S18S	T	C		2	87141026	87141026	32	0.1875	NA	NA
RGS5	3'UTR		A	C		1	163117018	163117018	35	0.685714286	NA	NA
RHBDD2	Silent	p.P147P	T	C		7	75517436	75517436	259	0.243243243	NA	NA
RHD	Intron		T	G		1	25633316	25633316	127	0.362204724	NA	NA
RHOXF1	Intron		-	C		X	119249273	119249273	38	0.921052632	NA	NA
RINT1	Silent	p.K101K	G	A		7	105182884	105182884	257	0.525291829	NA	NA
RIPK4	Silent	p.G629G	T	G		21	43161610	43161610	121	0.041322314	NA	NA
RLF	Intron		T	A		1	40697046	40697046	24	0.333333333	NA	NA
RNF213	3'UTR		T	G		17	78293371	78293371	28	0.178571429	NA	NA
ROBO1	Intron		T	C		3	78763714	78763714	55	0.327272727	NA	NA
ROBO1	5'UTR		A	C		3	79639086	79639086	147	0.380952381	NA	NA
ROPN1	Silent	p.I198I	T	G		3	123688047	123688047	302	0.51986755	NA	NA
RPL	Silent	p.Y41Y	T	C		8	95533649	95533649	272	0.426470588	NA	NA
RP11-113D6.10	3'UTR		A	C		11	18232046	18232046	139	0.748201439	NA	NA
RP11-156P1.3	RNA		G	T	rs1056072	17	45128671	45128671	221	0.022624434	NA	NA
RP11-193H5.1	RNA		T	G		1	238090519	238090519	268	0.455223881	NA	NA
RP11-19N8.2	RNA		T	G		16	33013825	33013825	452	0.168716814	NA	NA
RP11-576I22.2	RNA		A	G		1	170501262	170501262	176	0.147727273	NA	NA
RP11-78J7.5	3'UTR		A	T		12	56151222	56151222	33	0.787878788	NA	NA
RP11-798G7.5	RNA		T	A		17	43581034	43581034	23	0.173913043	NA	NA
RP11-933H2.4	RNA		T	G		3	131081108	131081108	16	0.9375	NA	NA
RPL31P11	RNA		G	A		1	161654688	161654688	173	0.306358382	NA	NA
RPL34	3'UTR		A	G		4	109546386	109546386	84	0.214285714	NA	NA

Figure 11 (cont.)

Gene	Variant_Classification	Alteration	Tumor_allele	Ref_allele	dbSNP_RS	Chr. #	Start_pos.	End_pos.	Coverage	Allelic_fraction	# of Probes	Segment_Mean (Log2[Copy
RPN2	3'UTR		A	G		20	35869734	35869734	248	0.089672131	NA	NA
RSBN1	Silent	p.G465C	A	G		1	114320413	114320413	191	0.387434555	NA	NA
RSF1	5'UTR		T	C		11	77413602	77413602	54	0.611111111	NA	NA
RTL1	Silent	p.R1328I	T	G		14	101350732	101350732	41	0.609756098	NA	NA
RTL1	Silent	p.R522R	A	G		14	101349560	101349560	23	0.652173913	NA	NA
RTN4RL1	Silent	p.R221R	A	G		17	1840453	1840453	43	0.488372093	NA	NA
RTP1	Silent	p.T247T	T	G		3	186917807	186917807	282	0.287234043	NA	NA
RXFP3	Silent	p.R198R	T	G		5	33937439	33937439	206	0.854368932	NA	NA
RYR2	Silent	p.R3604R	T	G		1	237890473	237890473	139	0.395683453	NA	NA
RYR2	Silent	p.S1567S	C	G		1	237774079	237774079	51	0.490196078	NA	NA
RYR2	Silent	p.R1414R	A	C		1	237755118	237755118	97	0.268041237	NA	NA
RYR2	Silent	p.L3470L	A	G		1	237880584	237880584	130	0.238461538	NA	NA
S100A13	Intron		-	G		1	153603190	153603190	111	0.324324324	NA	NA
SIRPA2	Silent	p.F89F	A	G		1	158533942	158533942	555	0.369369369	NA	NA
SACS	5'UTR		A	G		13	23985501	23985501	35	0.314285714	NA	NA
SAGE1	Intron		T	G		X	134994710	134994710	40	0.825	NA	NA
SARM1	Silent	p.R501R	A	C		17	26712269	26712269	11	0.363636364	NA	NA
SBF2	Intron		A	C		11	9802106	9802106	51	0.68627451	NA	NA
SCARF1	5'UTR		T	C		17	1549022	1549022	17	0.411764706	NA	NA
SCD5	5'UTR		T	G		4	83719696	83719696	43	0.465116279	NA	NA
SCIN	Silent	p.L512L	G	C		7	12680097	12680097	136	0.198529412	NA	NA
SCLY	Intron		C	G		2	239002660	239002660	11	0.454545455	NA	NA
SCN10A	Silent	p.I1158I	A	G	rs145568435	3	38763782	38763782	136	0.433823529	NA	NA
SCN2A	Silent	p.S1680S	A	C		2	166245356	166245356	541	0.292051756	NA	NA
SCN7A	Silent	p.K1364K	T	C		2	167263047	167263047	256	0.265625	NA	NA
SDCCAG3	Silent	p.P218P	G	C		9	139301693	139301693	57	0.421052632	NA	NA
SDK1	Silent	p.A909A	T	A		7	4091278	4091278	268	0.447761194	NA	NA
SDK1	Intron		-	C		7	4261010	4261010	36	0.444444444	NA	NA
SDK2	Intron		T	C		17	71354122	71354122	177	0.254237288	NA	NA
SEC24D	Silent	p.V845V	T	C		4	119654032	119654032	383	0.365535248	NA	NA
SELT	Silent	p.*48*	A	G		3	150340180	150340180	10	0.4	NA	NA
2-Sep	Intron		-	A		2	242274537	242274537	224	0.348214286	NA	NA
SERPINA1	Silent	p.P85P	C	G		14	94756796	94756796	106	0.20754717	NA	NA
SERPINA1	Silent	p.P21P	T	G		14	94915049	94915049	270	0.318518519	NA	NA
SERPINA1	RNA		A	G		14	95107957	95107957	53	0.358490566	NA	NA
SERPINB1	Silent	p.N6N	T	C		18	61585252	61585252	97	0.597938144	NA	NA
SETBP1	Silent	p.P637P	A	G		18	42531216	42531216	100	0.62	NA	NA
SETD3	Silent	p.D160D	T	C		21	37418120	37418120	238	0.303418803	NA	NA
SETD5	Silent	p.S1322S	T	G		3	9517706	9517706	72	0.291666667	NA	NA
SH2D3C	3'UTR		G	C		9	130500983	130500983	10	0.4	NA	NA
SH3BGRL	3'UTR		C	G		1	26807695	26807695	28	0.392857143	NA	NA
SH3GL2	Silent	p.V127V	A	C		9	17787427	17787427	341	0.26686217	NA	NA
SHANK1	Intron		-	C		19	51205687	51205687	28	0.785714286	NA	NA
SHISA3	Silent	p.I111I	A	C		4	42403084	42403084	741	0.032388664	NA	NA
SHROOM	3'UTR		A	G		X	50339617	50339617	31	0.451612903	NA	NA
SI	Silent	p.L1811L	T	C		3	164697201	164697201	120	0.166666667	NA	NA
SIDT2	Intron		A	G		11	117066474	117066474	283	0.604240283	NA	NA
SIGLEC1	3'UTR		T	G		20	3669109	3669109	5	0.6	NA	NA
SIGLEC10	3'UTR		G	A		19	51914246	51914246	41	0.073170732	NA	NA
SIGLEC10	3'UTR		A	G		19	51914248	51914248	41	0.097560976	NA	NA
SIGLEC8	5'UTR		C	G		19	52034939	52034939	10	0.5	NA	NA
SIX3	Silent	p.P488P	A	G		19	46269753	46269753	6	0.833333333	NA	NA
SKP2	Silent	p.V195V	T	G		5	36168463	36168463	975	0.862564103	NA	NA
SLAMF7	Intron		-	G		1	160718329	160718329	118	0.296610169	NA	NA
SLAMF9	Silent	p.L21L	A	G		1	159923427	159923427	246	0.195121951	NA	NA
SLC16A7	Intron		-	G		12	59990039	59990039	6	0.333333333	NA	NA
SLC22A16	Silent	p.I365I	A	G		6	110763535	110763535	239	0.430962343	NA	NA
SLC22A16	Silent	p.A76A	T	G		6	110778046	110778046	236	0.783898305	NA	NA
SLC22A23	Intron		A	G		6	3283961	3283961	19	0.315789474	NA	NA
SLC22A8	Silent	p.T373T	T	G		11	62762111	62762111	161	0.51552795	NA	NA
SLC25A46	3'UTR		G	A		5	110097556	110097556	12	0.666666667	NA	NA
SLC26A11	Silent	p.F299F	T	C		17	78210887	78210887	134	0.313432836	NA	NA
SLC26A5	Intron		-	C		7	103048283	103048283	226	0.566371681	NA	NA
SLC2A8	Intron		-	A		6	35928441	35928441	13	0.307692308	NA	NA

Figure 11 (cont.)

Gene	Variant_Classification	Alteration	Tumor_allele	Ref_allele	dbSNP_RS	Chr. #	Start_pos.	End_pos.	Coverage	Allelic_fraction	# of Probes	Segment_Mean (Log2[Copy
SLC2A4	3'UTR		T	C		17	7190047	7190047	55	0.290909091	NA	NA
SLC2A4	Silent	p.A358A	T	G		17	7188460	7188460	12	0.75	NA	NA
SLC35A4	Silent	p.L302L	T	C		5	139947660	139947660	107	0.392523364	NA	NA
SLC39A7	Silent	p.V317V	C	G		6	33170697	33170697	635	0.785826772	NA	NA
SLC3A2	5'UTR		A	C		11	62623599	62623599	33	0.333333333	NA	NA
SLC4A10	Intron		-	T		2	162735960	162735960	8	0.625	NA	NA
SLC4A3	Silent	p.T1175T	T	G		2	220505588	220505588	86	0.523255814	NA	NA
SLC5A2	Silent	p.I285I	T	C		16	31499050	31499050	83	0.108433735	NA	NA
SLC6A10P	RNA		T	A		16	32890611	32890611	93	0.451612903	NA	NA
SLC6A13	Silent	p.L75L	C	G		12	352957	352957	52	0.634615385	NA	NA
SLIT2	Silent	p.D783D	T	C		4	20550114	20550114	125	0.784	NA	NA
SLIT2	Silent	p.I1418I	A	C		4	20619182	20619182	80	0.8	NA	NA
SMG1	Silent	p.L553L	A	G		16	18887677	18887677	43	0.348837209	NA	NA
SMR3B	3'UTR		C	G		4	71255615	71255615	352	0.423295455	NA	NA
SMURF1	Intron		A	-	rs11452990|rs34738644|rs67048171|rs151337137	7	98654600	98654601	6	0.333333333	NA	NA
SNHG14	RNA		G	C		15	25448455	25448455	393	0.384402036	NA	NA
SNHG14	RNA		A	C		15	25417799	25417799	528	0.382272727	NA	NA
SNHG14	RNA		T	G		15	25446535	25446535	514	0.392996109	NA	NA
SNHG14	RNA		G	C		15	25463884	25463884	392	0.321428571	NA	NA
SNRPN	5'UTR		A	C		15	25219593	25219593	188	0.324468085	NA	NA
SNTG1	Silent	p.A379A	A	C		8	51617258	51617258	144	0.784722222	NA	NA
SNTN	5'UTR		T	G		3	63638352	63638352	85	0.435294118	NA	NA
SORCS1	Intron		-	G		10	108459206	108459206	31	0.387096774	NA	NA
SOX11	5'UTR		T	G		2	5832825	5832825	25	0.6	NA	NA
SP140	3'UTR		T	C		2	231177530	231177530	15	0.2	NA	NA
SPATA18	Intron		-	C		4	52942848	52942848	64	0.375	NA	NA
SPATA31	Silent	p.A35A	A	G		9	43630597	43630597	154	0.831168831	NA	NA
SPATA31	Silent	p.I25I	T	C		9	84603808	84603808	300	0.236666667	NA	NA
SPATA31 D5P	RNA		T	C	rs117202352	9	84530910	84530910	57	0.175438596	NA	NA
SPATA31 D5P	RNA		T	A		9	84530909	84530909	57	0.175438596	NA	NA
SPATA7	Intron		T	G		14	88882973	88882973	62	0.467741935	NA	NA
SPATA8	3'UTR		C	G		15	97328420	97328420	126	0.222222222	NA	NA
SPEG	Silent	p.G894G	C	G		2	220327085	220327085	15	0.6	NA	NA
SPG11	Silent	p.V1607V	A	G		15	44907578	44907578	231	0.225108225	NA	NA
SPG11	Silent	p.K1282K	T	C		15	44890875	44890875	305	0.278688525	NA	NA
SPOCK1	3'UTR		T	C		5	136314284	136314284	108	0.037037037	NA	NA
SPRR2F	3'UTR		G	C		1	153084847	153084847	45	0.377777778	NA	NA
SPRR4	3'UTR		A	C		1	152944638	152944638	98	0.357142857	NA	NA
SPTA1	Silent	p.E2366E	T	C		1	158582643	158582643	270	0.485185185	NA	NA
SRM	Silent	p.I83I	A	G		1	11119321	11119321	232	0.267241379	NA	NA
SRP19	3'UTR		C	G		5	112203278	112203278	22	0.5	NA	NA
SRRM4	5'UTR		T	C		12	119419588	119419588	13	0.461538462	NA	NA
SSPO	RNA		T	A		7	149519098	149519098	27	0.185185185	NA	NA
SSPO	RNA		T	G		7	149480039	149480039	60	0.55	NA	NA
SSPO	RNA		A	G		7	149523787	149523787	53	0.264150943	NA	NA
SSPO	RNA		T	G		7	149525822	149525822	23	0.260869565	NA	NA
SSTR5	Silent	p.A163A	T	C		16	1129357	1129357	27	0.703703704	NA	NA
STAC3	Intron		T	-		12	57639158	57639159	8	0.375	NA	NA
STARD3	Silent	p.G77G	A	C		17	37813272	37813272	202	0.222772277	NA	NA
STARD3	Intron		C	G		17	37814604	37814604	49	0.163265306	NA	NA
STEAP2-AS1	RNA		T	C		7	89753434	89753434	124	0.217741935	NA	NA
STX3C	Intron		A	C		10	134038676	134038676	20	0.6	NA	NA
STON1	5'UTR		G	T		2	48822609	48822609	4	1	NA	NA
STON1-GTF2A1L	Silent	p.T831T	C	T		2	48872249	48872249	155	0.038709677	NA	NA
STRC	Intron		A	C		15	43901551	43901551	260	0.261538462	NA	NA
STRIP1	Intron		T	-		1	110589207	110589208	71	0.408450704	NA	NA
SUGP2	Intron		A	G		19	19105353	19105353	108	0.37037037	NA	NA

Figure 11 (cont.)

Gene	Variant_Classification	Alteration	Tumor_allele	Ref_allele	dbSNP_RS	Chr. #	Start_pos.	End_pos.	Coverage	Allelic_fraction	# of Probes	Segment_Mean (Log2[Copy
SUGP2	3'UTR		C	G		19	19105144	19105144	209	0.388421053	NA	NA
SUPT20H	Intron		-	A		13	37607341	37607341	9	0.333333333	NA	NA
SUV39H2	Silent	p.V124V	T	C		10	14939219	14939219	666	0.3996997	NA	NA
SUZ12P	RNA		G	C		17	29086514	29086514	21	0.333333333	NA	NA
SYNE1	Intron		-	T	rs4331993	6	152793572	152793572	133	0.067669173	NA	NA
SYNRG	3'UTR		A	G		17	35879033	35879033	499	0.288577154	NA	NA
SYT13	Silent	p.R42R	A	C		11	45307633	45307633	18	0.611111111	NA	NA
SYT2	Silent	p.D117D	A	G	rs150392142	1	202572241	202572241	159	0.383647799	NA	NA
TAARB	RNA		C	G		6	132859813	132859813	203	0.773399015	NA	NA
TACSTD2	3'UTR		T	G		1	59041843	59041843	53	0.358490566	NA	NA
TAF1B	Intron		G	C		2	10053529	10053529	16	0.375	NA	NA
TAF1L	Silent	p.P1270P	T	G		9	32831768	32831768	337	0.290801187	NA	NA
TAF2	5'UTR		T	C		8	120845002	120845002	4	0.5	NA	NA
TAL1	3'UTR		T	G		1	47685247	47685247	11	0.363636364	NA	NA
TBC1D15	Intron		A	G		12	72243688	72243688	89	0.404494382	NA	NA
TBR1	Silent	p.I512T	A	G		2	162280225	162280225	26	0.230769231	NA	NA
TBX3	Intron		T	C		12	115118610	115118610	85	0.458823529	NA	NA
TBX4	Silent	p.R195R	T	G		17	59556023	59556023	323	0.247678019	NA	NA
TBXAS1	Silent	p.I300I	T	C		7	139661795	139661795	146	0.280821918	NA	NA
TBXAS1	Intron		-	GAG	rs13246458	7	139565405	139565407	7	0.428571429	NA	NA
TCF7L1	Intron		G	-	rs142307693	2	85490442	85490443	11	0.363636364	NA	NA
TCF7L1	Intron		-	C		2	85534899	85534899	64	0.484375	NA	NA
TCL6	RNA		A	G		14	96129929	96129929	181	0.270718232	NA	NA
TDP1	Silent	p.A515A	T	A		14	90485663	90485663	103	0.524271845	NA	NA
TEKT2	Intron		T	G		1	36551411	36551411	30	0.333333333	NA	NA
TENM2	Silent	p.G2584G	A	C		5	167689605	167689605	18	0.722222222	NA	NA
TENM3	Silent	p.T580T	A	C		4	183601796	183601796	80	0.5125	NA	NA
TEX101	Silent	p.T166T	A	C		19	43922082	43922082	248	0.75	NA	NA
TEX14	Intron		-	C		17	56665215	56665215	326	0.294478528	NA	NA
TEX14	Intron		-	G		17	56665220	56665220	301	0.03654485	NA	NA
TEX15	Silent	p.R1672R	T	C		8	30703318	30703318	183	0.371584699	NA	NA
TFAP2C	Silent	p.T191T	G	A		20	55206899	55206899	212	0.358490566	NA	NA
TFCP2L1	Silent	p.R231R	T	G		2	122000012	122000012	498	0.449799197	NA	NA
THSD1	Silent	p.F386F	A	G		13	52952788	52952788	163	0.061349693	NA	NA
THSD4	Intron		C	G		15	71634045	71634045	333	0.228228228	NA	NA
TIPIN	Intron		G	C		15	66639658	66639658	108	0.20754717	NA	NA
TLN2	Silent	p.K1532K	A	G		15	63047850	63047850	87	0.275862069	NA	NA
TMEM12	RNA		T	G		7	75617786	75617786	277	0.288808664	NA	NA
TMEM14	3'UTR		A	T		6	10756758	10756758	198	0.050505051	NA	NA
TMEM15	3'UTR		TGTGT	-	rs3832273|rs	4	83406549	83406550	8	0.375	NA	NA
0C			G		113689314|rs							
					72548147|rs1							
					44382768							
TMEM17	3'UTR		C	G		2	39944471	39944471	63	0.444444444	NA	NA
TMEM23	Silent	p.L263L	A	C		16	75575316	75575316	68	0.397058824	NA	NA
TMSB4X	Intron		-	C		X	12894886	12894886	281	0.024864983	NA	NA
TNFRSF8	Intron		-	C		1	12169776	12169776	48	0.3125	NA	NA
TNFSF18	5'UTR		T	G		3	172241258	172241258	16	0.3125	NA	NA
TNMD	5'UTR		G	C		X	99839955	99839955	15	0.8	NA	NA
TOMM5	5'UTR		T	C		9	37592594	37592594	48	0.375	NA	NA
TPST2	Silent	p.A33A	T	C		22	26937498	26937498	47	0.361702128	NA	NA
TRAC	RNA		C	G		14	23020762	23020762	430	0.306976744	NA	NA
TRAV2	RNA		T	A		14	22180564	22180564	363	0.289256198	NA	NA
TRAV22	RNA		G	C		14	22539225	22539225	197	0.522842639	NA	NA
TRAV8-3	RNA		A	C		14	22321029	22321029	143	0.293706294	NA	NA
TRBV4-2	RNA		-	G		7	142045535	142045535	689	0.325108853	NA	NA
TRBV5-1	RNA		G	C		7	142021317	142021317	209	0.215311005	NA	NA
TRBV6-5	RNA		A	G		7	142180574	142180574	803	0.251556663	NA	NA
TRGV5	RNA		A	G		7	38389468	38389468	83	0.566265506	NA	NA
TRGV5	RNA		T	C		7	38389152	38389152	179	0.82122905	NA	NA

Figure 11 (cont.)

Gene	Variant_Classification	Alteration	Tumor_allele	Ref_allele	dbSNP_RS	Chr. #	Start_pos.	End_pos.	Coverage	Allelic_fraction	# of Probes	Segment_Mean (Log2[Copy
TRIM13	Silent	p.F23F	A	G		1	228594194	228594194	120	0.191666667	NA	NA
TRIM14	Silent	p.L111L	T	C		9	100862417	100862417	378	0.296296296	NA	NA
TRIM2	Silent	p.R562R	T	G		4	154237055	154237055	128	0.5234375	NA	NA
TRIM43	Silent	p.F517F	T	C		5	180662373	180662373	158	0.158227848	NA	NA
TRIM49	Intron		-	C		11	89531953	89531953	6	0.333333333	NA	NA
TRIM51	Silent	p.A240A	G	A		11	55655720	55655720	150	0.506666667	NA	NA
TRIM53B	RNA		-	TG		11	55065457	55065458	131	0.175572519	NA	NA
TRIM69	Intron		-	C		15	45047653	45047653	38	0.5	NA	NA
TRIP12	Intron		A	C		2	230672363	230672363	166	0.277108434	NA	NA
TRPC4AP	3'UTR		A	C		20	33590901	33590901	187	0.342245989	NA	NA
TRPM3	3'UTR		C	T		9	73398890	73398890	25	0.4	NA	NA
TRPM6	Intron		-	G		9	77425867	77425867	48	0.458333333	NA	NA
TRPV6	Silent	p.L337L	A	G		7	142573334	142573334	400	0.205	NA	NA
TRPV6	Intron		T	G		7	142575248	142575248	45	0.622222222	NA	NA
TSGA10	Silent	p.R683R	T	G		2	99634688	99634688	212	0.283018868	NA	NA
TSHZ2	5'UTR		-	GAG		20	51589235	51589237	8	0.375	NA	NA
TSHZ3	Silent	p.P810P	T	G		19	31768269	31768269	173	0.74566474	NA	NA
TSKU	Silent	p.F128F	T	C		11	76507044	76507044	101	0.247524752	NA	NA
TSPY3	3'UTR		-	G		Y	9237769	9237769	122	0.360655738	NA	NA
TTC17	Silent	p.A3A	T	G		11	43380513	43380513	39	0.307692308	NA	NA
TTC27	Intron		-	T	rs34708901|rs72458829|rs66518618|rs8830236	2	32892037	32892037	7	0.428571429	NA	NA
TTC39B	Intron		A	C		9	15186853	15186853	200	0.535	NA	NA
TTN	Silent	p.K16300K	T	C		2	179478341	179479341	142	0.464788732	NA	NA
TTN	Silent	p.P22378P	T	G		2	179444880	179444880	336	0.261904762	NA	NA
TTN	Silent	p.I23734I	T	G		2	179439657	179439657	84	0.535714286	NA	NA
TUBA1B	3'UTR		A	G		12	49521710	49521710	153	0.75483871	NA	NA
TUBBP7	RNA		G	A		16	90162462	90162462	306	0.026143791	NA	NA
TUBBP5	RNA		A	G	rs142836124	9	140370116	140370116	229	0.026200873	NA	NA
TYR	Silent	p.A381A	A	C		11	88961097	88961097	304	0.519736842	NA	NA
UBE2O	3'UTR		A	G		17	74386945	74386945	75	0.28	NA	NA
UBQLN1	5'UTR		T	C		9	86322597	86322597	11	0.454545455	NA	NA
UBR4	Silent	p.V5100V	A	G		1	19404494	19404494	154	0.220779221	NA	NA
UBR4	Silent	p.Q4828Q	T	C		1	19414376	19414376	253	0.249337255	NA	NA
UGGT2	Silent	p.V1338V	T	C		13	96506724	96506724	100	0.29	NA	NA
UGT1A6	Intron		T	C		2	234652352	234652352	423	0.491725768	NA	NA
UGT2B4	Silent	p.L309L	A	G		4	70355234	70355234	1099	0.198300283	NA	NA
UGT3A2	Silent	p.V201V	T	C		5	36049231	36049231	283	0.190812721	NA	NA
ULK3	Intron		A	C		15	75132720	75132720	25	0.56	NA	NA
UMPS	3'UTR		A	G		3	124462963	124462963	130	0.269230769	NA	NA
UNC45B	Intron		T	-		17	33507806	33507807	10	0.6	NA	NA
UNC5C	Intron		-	T		4	96222706	96222706	235	0.340425532	NA	NA
Unknown	IGR		A	C		15	21937054	21937054	208	0.201923077	NA	NA
Unknown	IGR		T	G		7	72617471	72617471	260	0.507692308	NA	NA
Unknown	IGR		T	C		8	81471871	81471871	114	0.184210526	NA	NA
Unknown	IGR		T	G		10	31001600	31001600	190	0.452631579	NA	NA
Unknown	IGR		C	G		7	29771660	29771660	209	0.162679426	NA	NA
Unknown	IGR		A	G		7	65228451	65228451	40	0.15	NA	NA
Unknown	IGR		T	G		7	48964300	48964300	225	0.337777778	NA	NA
Unknown	IGR		T	G		7	72307446	72307446	13	0.538461538	NA	NA
Unknown	IGR		C	G		14	106346859	106346859	66	0.53030303	NA	NA
Unknown	IGR		A	C		3	94890759	94890759	59	0.525423729	NA	NA
Unknown	IGR		A	C		1	179557532	179557532	195	0.420512821	NA	NA
Unknown	IGR		T	C		GL000	118429	118429	329	0.164133739	NA	NA
Unknown	IGR		T	A		7	72333442	72333442	213	0.253521127	NA	NA
Unknown	IGR		C	G		9	90744625	90744625	530	0.309433962	NA	NA
Unknown	IGR		C	T		13	75812429	75812429	461	0.802603037	NA	NA
Unknown	IGR		A	C		3	21447508	21447508	12	0.416666667	NA	NA
Unknown	IGR		T	G		14	106107274	106107274	71	0.281690141	NA	NA
Unknown	IGR		G	C		10	15059281	15059281	161	0.385093168	NA	NA
Unknown	IGR		A	C		3	21447723	21447723	35	0.314285714	NA	NA

Figure 11 (cont.)

Gene	Variant_Classification	Alteration	Tumor_allele	Ref_allele	dbSNP_RS	Chr. #	Start_pos.	End_pos.	Coverage	Allelic_fraction	# of Probes	Segment_Mean (Log2[Copy
Unknown	IGR		T	G		1	211381097	211381097	73	0.767123288	NA	NA
Unknown	IGR		A	C		GL000	2502	2502	70	0.071428571	NA	NA
Unknown	IGR		C	T		GL000	56470	56470	48	0.104166667	NA	NA
Unknown	IGR		A	C		12	9585461	9585461	9	0.888888889	NA	NA
Unknown	IGR		T	G		3	21447868	21447868	9	0.666666667	NA	NA
Unknown	IGR		T	C		11	124095903	124095903	886	0.182844244	NA	NA
Unknown	IGR		A	C		2	1322021B0	1322021B0	167	0.347305389	NA	NA
Unknown	IGR		C	T	rs1062001	15	85056021	85056021	23	0.217391304	NA	NA
Unknown	IGR		T	C		GL000	56412	56412	96	0.052083333	NA	NA
Unknown	IGR		A	C		11	68671290	68671290	140	0.721428571	NA	NA
Unknown	IGR		T	-	rs78389842	1	3816880	3816891	6	0.333333333	NA	NA
Unknown	IGR		-	G		1	148853871	148853871	293	0.023890785	NA	NA
Unknown	IGR		-	C		1	231611812	231611812	41	0.634146341	NA	NA
Unknown	IGR		-	T		1	247452241	247452241	6	0.333333333	NA	NA
Unknown	IGR		-	T		2	16420855	16420855	6	0.333333333	NA	NA
Unknown	IGR		A	-		2	95403095	95403096	6	0.333333333	NA	NA
Unknown	IGR		-	GTG		2	129098917	129098919	13	0.307692308	NA	NA
Unknown	IGR		-	T	rs66735058|rs147114139|rs141065844|rs75865194	2	133018715	133018715	13	0.307692308	NA	NA
Unknown	IGR		-	TTC		2	173020739	173020741	66	0.424242424	NA	NA
Unknown	IGR		-	C		3	90251431	90251431	29	0.344827586	NA	NA
Unknown	IGR		-	AGG	rs113290454	3	94851158	94851160	6	0.333333333	NA	NA
Unknown	IGR		-	G		5	150948605	150948605	17	1	NA	NA
Unknown	IGR		-	G		6	3941688	3941688	51	0.823529412	NA	NA
Unknown	IGR		-	A		6	27839594	27839594	190	0.757894737	NA	NA
Unknown	IGR		-	G		6	112677751	112677751	132	0.75	NA	NA
Unknown	IGR		-	C		7	38982960	38982960	6	0.333333333	NA	NA
Unknown	IGR		-	G		7	47694723	47694723	15	0.6	NA	NA
Unknown	IGR		-	C		7	54487295	54487295	391	0.043478261	NA	NA
Unknown	IGR		-	G		7	64043268	64043268	9	0.444444444	NA	NA
Unknown	IGR		-	C		8	8093417	8093417	66	0.742424242	NA	NA
Unknown	IGR		-	A		8	41370103	41370103	20	0.8	NA	NA
Unknown	IGR		T	-		9	28888976	28888977	160	0.45625	NA	NA
Unknown	IGR		-	G		9	84676626	84676626	292	0.414383562	NA	NA
Unknown	IGR		AG	-	rs34650012|rs149015582	9	133253899	133253900	6	0.333333333	NA	NA
Unknown	IGR		-	C		10	38897825	38897825	7	0.571428571	NA	NA
Unknown	IGR		-	C		11	38469528	38469528	9	0.555555556	NA	NA
Unknown	IGR		T	-		12	127829061	127829062	58	0.644067797	NA	NA
Unknown	IGR		-	G		13	19538294	19538294	19	0.947368421	NA	NA
Unknown	IGR		-	C	rs71433543	13	19782781	19782781	38	0.789473684	NA	NA
Unknown	IGR		-	AGG		13	54570458	54570458	11	0.454545455	NA	NA
Unknown	IGR		-	G		13	89630903	89630903	139	0.776978417	NA	NA
Unknown	IGR		-	C		14	19171333	19171333	8	0.5	NA	NA
Unknown	IGR		-	G		14	20374147	20374147	45	0.177777778	NA	NA
Unknown	IGR		-	G		15	81677344	81677344	35	0.6	NA	NA
Unknown	IGR		-	C		16	1061207	1061207	8	0.5	NA	NA
Unknown	IGR		-	A		16	32178603	32178603	75	0.106666667	NA	NA
Unknown	IGR		-	G		16	53392874	53392874	7	0.428571429	NA	NA
Unknown	IGR		-	T		16	53396351	53396351	185	0.162162963	NA	NA

Figure 11 (cont.)

Gene	Variant_Classification	Alteration	Tumor_allele	Ref_allele	dbSNP_RS	Chr. #	Start_pos.	End_pos.	Coverage	Allelic_fraction	# of Probes	Segment_Mean (Log2[Copy
Unknown	IGR		A	-	rs67578665;rs113176304;rs150922015	16	88372769	88372770	11	0.545454545	NA	NA
Unknown	IGR		-	A	rs11080529	18	11644890	11644890	16	0.3125	NA	NA
Unknown	IGR		TT	-		19	54169771	54169772	13	0.692307692	NA	NA
Unknown	IGR		-	AGA		22	49153040	49153042	7	0.428571429	NA	NA
Unknown	IGR		-	CG		X	44335858	44335859	27	0.666666667	NA	NA
Unknown	IGR		C	-		X	141293675	141293676	6	0.333333333	NA	NA
Unknown	IGR		-	C		X	143344142	143344142	9	0.555555556	NA	NA
Unknown	IGR		AG	-		Y	28780698	28780699	18	0.333333333	NA	NA
UQCRFS1	3'UTR		A	T		19	29698359	29698359	78	0.192307692	NA	NA
USE1	Silent	p.S4S	A	G		19	17326226	17326226	7	0.571428571	NA	NA
USH1C	Silent	p.T285T	T	G		11	17544779	17544779	158	0.734177215	NA	NA
USH2A	Silent	p.T513T	T	G		1	216496827	216496827	365	0.345205479	NA	NA
USH2A	Silent	p.V3050V	T	G		1	216017744	216017744	255	0.388235294	NA	NA
USP48	Intron		A	G		1	22080997	22080997	40	0.175	NA	NA
USP50	5'UTR		T	C	rs115914158	15	50838790	50838790	73	0.095890411	NA	NA
USP8	Silent	p.L1104L	G	C		15	50791240	50791240	217	0.184331797	NA	NA
UTP6	Intron		-	A		17	30216089	30216089	11	0.363636364	NA	NA
VAP8	Silent	p.R174K	A	G		20	57016088	57016088	185	0.259459459	NA	NA
VNN1	3'UTR		T	G		6	133004209	133004209	32	0.8125	NA	NA
VPS48	Intron		-	T	rs76661872	18	61078577	61078577	7	0.428571429	NA	NA
WDFY3	Intron		T	-		4	85716021	85716022	210	0.40952381	NA	NA
WDR25	3'UTR		T	C		14	100996511	100996511	10	0.4	NA	NA
WDR49	Silent	p.I513I	T	G		3	167245817	167245817	89	0.528089888	NA	NA
WDR62	Silent	p.L204L	T	C		19	36558258	36558258	149	0.275167785	NA	NA
WDR86	Silent	p.R235R	A	C		7	151092883	151092883	18	0.444444444	NA	NA
WDR89	3'UTR		T	C		14	64065429	64065429	34	0.382352941	NA	NA
WIPF1	Silent	p.R249R	T	G		2	175436788	175436788	53	0.528301887	NA	NA
XKR4	Intron		A	G		8	56362739	56362739	33	0.696969697	NA	NA
XKR6	Silent	p.V10V	A	C		8	11058819	11058819	53	0.716981132	NA	NA
XPO5	Silent	p.V300V	A	G		16	28167592	28167592	148	0.304054054	NA	NA
YLPM1	Intron		-	T	rs79860790	14	75287731	75287731	7	0.428571429	NA	NA
YME1L1	Intron		-	A		10	27410387	27410387	514	0.017509728	NA	NA
ZBTB4	Silent	p.L847L	C	G		17	7365760	7365760	68	0.811594203	NA	NA
ZC3H18	Silent	p.Q258Q	T	C		16	88664671	88664671	192	0.244791667	NA	NA
ZDHHC11	Silent	p.V174V	A	C		5	843821	843821	78	0.294871795	NA	NA
ZEB2	5'UTR		C	G		2	145274980	145274980	125	0.576	NA	NA
ZFHX4	Silent	p.I3609I	A	C		8	77776777	77776777	44	0.818181818	NA	NA
ZIC3	3'UTR		T	C		3	147131342	147131342	120	0.491666667	NA	NA
ZMPSTE2	5'UTR		A	G		1	40723852	40723852	135	0.481481481	NA	NA
ZMYM1	3'UTR		T	A		1	35580895	35580895	61	0.459016393	NA	NA
ZNF100	5'UTR		A	G		19	21950339	21950339	116	0.336206897	NA	NA
ZNF100	Intron		T	C		19	21934820	21934820	9	0.333333333	NA	NA
ZNF175	Intron		-	C		19	52085029	52085029	78	0.243589744	NA	NA
ZNF208	Silent	p.P804P	C	G		19	22155424	22155424	187	0.508021390	NA	NA
ZNF275	Silent	p.L196L	C	G		X	152612731	152612731	214	0.411214953	NA	NA
ZNF311	Silent	p.G365G	T	C		6	28963684	28963684	132	0.363636364	NA	NA
ZNF341	Intron		TG	-		20	32377235	32377236	42	0.285714286	NA	NA
ZNF519	Silent	p.L305L	T	C		18	14106224	14106224	113	0.336283186	NA	NA
ZNF536	3'UTR		T	G		19	31048080	31048080	3078	0.07179987	NA	NA
ZNF567	Silent	p.F188F	T	C		19	37210283	37210283	106	0.745283019	NA	NA
ZNF655	3'UTR		A	G		7	99171254	99171254	97	0.216494845	NA	NA
ZNF658	Silent	p.E823E	T	C		9	40772806	40772806	728	0.325549451	NA	NA
ZNF676	3'UTR		T	G		19	22362126	22362126	10	0.6	NA	NA
ZNF746	Silent	p.V124V	T	C		7	149191114	149191114	316	0.553797468	NA	NA
ZNF761	RNA		T	G		19	53945107	53945107	19	0.736842105	NA	NA
ZNF775	3'UTR		T	G		7	150095284	150095284	6	0.833333333	NA	NA
ZNF785	Silent	p.I245I	A	G		16	30594364	30594364	106	0.20754717	NA	NA

Figure 11 (cont.)

Gene	Variant_Classification	Alteration	Tumor_allele	Ref_allele	dbSNP_RS	Chr. #	Start_pos.	End_pos.	Coverage	Allelic_fraction	#_of_Probes	Segment_Mean (Log2)Copy
ZNF800	Intron		A	G		7	127013219	127013219	25	0.24	NA	NA
ZNF823	5'UTR		A	C		19	11849730	11849730	15	0.8	NA	NA
ZNF831	Silent	p.T1052T	G	C		20	57769230	57769230	59	0.254237288	NA	NA
ZSWIM3	Silent	p.K208K	A	G	rs148971026	20	44505824	44505824	200	0.215	NA	NA