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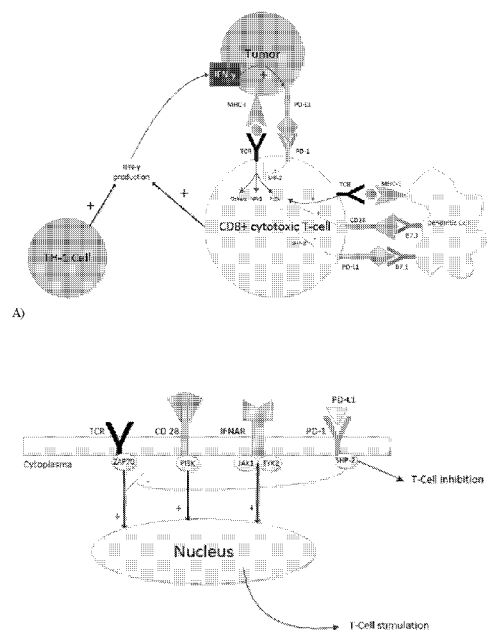
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(54) Title: MARKERS FOR IDENTIFYING PATIENT CLASSES AND USE THEREOF

(57) Abstract: The present invention relates to a method for genotyping a patient suffering from cancer for having a predisposition to a deviant biological response associated with immune checkpoint inhibitor therapy which comprises the administration of an immune checkpoint inhibitor that inhibits PD-1-mediated and/or CTLA-4-mediated signaling in T-cells of said patient, said genotyping method comprising comparing the germline sequence of a PD-1 axis protein gene or the sequence of its RNA in a sample from said patient with the germline sequence of the wild-type gene or the sequence of its RNA, wherein an alteration in the germline sequence of the PD-1 axis protein gene or the sequence of its RNA of the patient indicates a genotype having said predisposition to a deviant biological response.



B)
Figure 1



Title: Markers for identifying patient classes and use thereof.

FIELD OF THE INVENTION

5 This invention generally relates to molecular genetics, particularly to the identification of biomarkers or genetic variants that are associated with diseases, in particular germline polymorphisms, and methods of using the identified variants. The invention further relates to methods of treatment using patient stratification, and to diagnostic methods
10 to evaluate a patient's clinical or genetic signature for guiding treatment decisions.

BACKGROUND OF THE INVENTION

Cancer immunotherapy has changed the focus in cancer
15 treatment from killing of rapidly dividing cells by non-discriminatory chemotherapeutics to targeted therapies using monoclonal antibodies that target specific cell wall receptors in cancer cells. In general, tumors are able to escape from an effective immune response by exploiting phenotypical and functional changes in tumor cells as well as stromal cells that compromise
20 infiltration, migration and local activation of anti-tumor T lymphocytes. Monoclonal immunoglobulin (Ig) antibodies directed against human cell surface receptor PD-1, such as nivolumab and pembrolizumab, counteract this mechanism by inhibiting the immune checkpoint programmed cell death protein 1 (PD-1), which leads to a suppressive effect on T-cell
25 inactivation. Likewise, monoclonal antibodies directed against human cell surface receptor CTLA-4, such as ipilimumab, counteract this mechanism by inhibiting this specific immune checkpoint.

The goal of immunotherapy is to elicit or enhance antitumor immune responses. Cancer immunotherapy has the potential to induce the
30 inherent capacity of the immune system to adapt to mutational tumor

changes. Though cancer immunotherapy approaches have been pursued for decades and have been successful in some cases (e.g. IL-2 in melanoma), checkpoint inhibition appears more promising than the use of interferons, and, in particular, PD-1/PD-L1 blockade through the use of immune
5 checkpoint inhibitors, is the first strategy that is poised to impact the outcome in patients suffering from cancer on a broader scale.

Nivolumab is a human monoclonal antibody that blocks the interaction between PD-1, PD-L1 and PD-L2. Binding of these ligands to the PD-1 receptor found on T cells, inhibits T cell proliferation and cytokine
10 production. Upregulation of the PD-1 ligands occurs in some tumors and signaling through this pathway can contribute to inhibition of active T-cell immune surveillance of tumors. Nivolumab binds to the PD-1 receptor and blocks its interaction with PD-L1 and PD-L2, releasing PD-1 pathway-mediated inhibition of the immune response, including the anti-tumor
15 immune response, resulting in decreased tumor growth.

Both nivolumab and pembrolizumab are currently approved for the treatment of melanoma, non-small cell lung cancer (NSCLC) and, in the case of nivolumab, also for renal cell carcinoma. Moreover, approval for other indications is expected in the near future.

20 One problem associated with the application of anti-PD-1 blocking agents is that the treatment can be accompanied by severe toxicities such as dermatitis, hypothyroidism, colitis and pneumonitis. These auto-immune-related adverse events can compromise treatment outcome. Tools to identify patients who are at risk to develop such toxicities are key to come to a more
25 personalized treatment approach. These toxicities are considered cancer-independent toxicities and are induced upon administration of an inhibitor of PD-1 mediated signaling.

The present inventors have now discovered that certain germline genetic aberrations are associated with systemic toxicity during
30 immunotherapeutic treatment with immune checkpoint inhibitors aimed at

releasing PD-1 pathway-mediated inhibition in cancer cells, hereinafter referred to as immune checkpoint inhibitors.

It is an aim of the present invention to reduce toxicity from immune checkpoint inhibitors. It is another aim of this invention to be able to select patients suffering from cancer eligible for cancer immune checkpoint inhibitor immunotherapy. In general, it is an aim of this invention to improve the treatment of patients suffering from cancer eligible for cancer immune checkpoint inhibitor immunotherapy, by providing a more personalized assessment of the risk of suffering from side effects of immune checkpoint inhibitor administration, such as a predisposition to compound-induced adverse events (toxicity). This allows *inter alia* for new clinical situations that stratify and/or type patients according to a predisposition to adverse events associated with (future) treatment with an inhibitor of PD-1-mediated signaling. According to this stratification, it is now possible to assign a specific anti-cancer therapy and set dosage regimens not by treating all patients as one, but instead according to predictions made on the basis on patient predisposition to such adverse events.

SUMMARY OF THE INVENTION

In a first aspect, the present invention provides a method for genotyping a patient suffering from cancer for having a predisposition to a deviant biological response associated with, or resulting from, immune checkpoint inhibitor therapy which therapy comprises the administration of an immune checkpoint inhibitor that inhibits PD-1-mediated and/or CTLA-4-mediated signaling in T-cells of said patient, said method for genotyping comprising comparing the germline sequence of a PD-1 axis protein gene or the sequence of its RNA in a sample from said patient with the germline sequence of the wild-type gene or the sequence of its RNA, wherein an alteration in the germline sequence of the PD-1 axis protein gene or the

sequence of its RNA of the patient indicates a genotype having said predisposition to a deviant biological response.

In a preferred embodiment of a method of the present invention the method comprises the steps of:

- 5 a) providing a nucleic acid sample from the patient;
- b) analyzing said nucleic acid sample for the presence or absence of a polymorphic site indicating an alteration in the germline sequence in a nucleic acid sequence of the PD-1 axis protein gene or the sequence of its RNA, by performing, or having performed, one or more assays configured to
10 detect said polymorphic site in a PD-1 axis protein gene which assay generates an assay result indicative of the presence or absence of a polymorphic site (for instance by introducing the nucleic acid sample obtained from the patient into an assay instrument which (i) contacts all or a portion of the nucleic acid in said sample with a binding reagent which
15 specifically binds to nucleic acid in the locus of said polymorphic site, and (ii) generates an assay result indicative of binding of the binding reagent in the locus of said polymorphic site, preferably said assay comprising a DNA hybridization or DNA amplification technique); and optionally
- c) correlating, or having correlated, the assay result to the genotype of
20 the patient by using the assay result to assign the patient to a predetermined subpopulation of individuals having a known predisposition to a deviant biological response associated with immune checkpoint inhibitor therapy; and optionally
- d) treating, or having treated, the patient based on the predetermined
25 subpopulation of individuals to which the patient is assigned, wherein the treatment comprises one or more of continuing immune checkpoint inhibitor administration, discontinuing immune checkpoint inhibitor administration, modifying the dosage regimen of immune checkpoint inhibitor administration, and using an alternative therapy to immune checkpoint

inhibitor therapy selected from chemotherapy, immunotherapy, radiation therapy, and hormonal therapy.

In another aspect, the present invention provides a method of treating a patient suffering from cancer eligible for immune checkpoint inhibitor therapy which comprises the administration of an immune checkpoint inhibitor that inhibits PD-1-mediated and/or CTLA-4-mediated signaling in T-cells of said patient, said method comprising the steps of:

5 a) genotyping said patient suffering from cancer for having a predisposition to a deviant biological response associated with immune checkpoint inhibitor therapy by performing a method according to claim 1 or 2, and

b1) commencing or continuing administration of an immune checkpoint inhibitor to said patient using a standard dosage regime in case the patient does not have a predisposition to a deviant biological response, 15 or

b2) discontinuing immune checkpoint inhibitor administration, modifying (e.g. reducing or increasing) the dosage regimen of immune checkpoint inhibitor administration, and/or administering a standard of care therapeutic other than an immune checkpoint inhibitor to treat said patient, in case the patient has a predisposition to a deviant biological response. 20

In a preferred embodiment of a method of the present invention said deviant biological response is an altered therapeutic effect, preferably an increased or reduced therapeutic efficacy of the immune checkpoint inhibitor. 25

In a preferred embodiment of a method of the present invention said deviant biological response is an adverse event associated with (i.e. resulting from) immune checkpoint inhibitor therapy.

In a preferred embodiment of a method of the present invention wherein said deviant biological response is an adverse event associated with 30

immune checkpoint inhibitor therapy said adverse event is selected from the group consisting of diarrhea, nausea, pruritus, rash acneiform, rash maculopapular, erythroderma, papulopustular rash, toxic epidermal necrolysis, anorexia, fatigue, hyperkalemia, hypokalemia, hyponatremia, hypomagnesemia, hypercalcemia, hypocalcemia, decreased platelet count, decreased white blood cell count, decreased lymphocyte count, anemia, increased alanine aminotransferase, increased alkaline phosphatase, increased aspartate aminotransferase, increased lipase, increased serum amylase, increased creatinine, hypertension, upper respiratory infection, pneumonitis, interstitial lung disease, dyspnea, cough, mucositis oral, dry mouth, vomiting, colitis, abdominal pain, constipation, infusion related reaction, dry skin, skin hypopigmentation, alopecia, headache, dizziness, peripheral motor neuropathy, peripheral sensory neuropathy, dry eye, blurred vision, myalgia, arthralgia, bone pain, back pain, pain in extremity, non-cardiac chest pain, jaw pain, vertebral pain, polymyalgia rheumatic, guillain-barre syndrome, demyelination, fever, edema (any anatomical site), weight loss, hyperthyroidism, hypothyroidism, hyperglycemia, hypermagnesemia, hypernatremia, increased blood bilirubin, decreased neutrophil count, anaphylaxis, sinus tachycardia, lung infection, bronchial infection, pleural effusion, pancreatitis, hepatic infection, nephritis, chronic kidney disease, uveitis, urticaria, erythema multiforme, psoriasis, rosacea, vasculitis, arthritis, cardiac chest pain, adrenal insufficiency, autoimmune disorder (hypopituitarism, hypophysitis, thyreoditis), acidosis, dehydration, ventricular arrhythmia, atrial fibrillation, gastritis, duodenal ulcer, and cholestasis, preferably selected from the group consisting of, diarrhea, pruritus, fatigue, anemia, increased aspartate aminotransferase, pneumonitis, interstitial lung disease, dyspnea, colitis, constipation, dry skin, peripheral motor neuropathy, peripheral sensory neuropathy, fever, anaphylaxis and hepatic infection.

In a preferred embodiment of a method of the present invention said PD-1 axis protein gene is a gene encoding a protein selected from the group consisting of PD-1, SHP-1, SHP-2, ZAP70, IFN- γ , PD-L1, CXCL9, CXCL10, IRF7, AKT1, AKT2, preferably wherein said PD-1 axis protein
5 gene is selected from the group consisting of *PDCD1*, *PTPN11*, *ZAP70*, *IFNG*, *CD274*, *CXCL9*, *CXCL10*, *IRF7*, *AKT1*, and *AKT2*.

In a preferred embodiment of a method of the present invention said alteration in the germline sequence is indicated by the presence of a polymorphic site selected from the group of consisting of SNPs Rs2227981,
10 Rs2227982, Rs11568821, Rs41386349, Rs36084323, Rs10204525, and 6867 C/G in *PDCD1*; Rs2301756, Rs12423190, Rs11066301, Rs41279090, Rs12301915, Rs11066320, Rs11066322, and Rs3741983 in *PTPN11*; Rs17695937, Rs13420683, Rs62157588, and Rs2278699 in *ZAP70*; Rs2430561, Rs2069718, Rs2069705, and Rs1861494 in *IFNG*; Rs4143815,
15 Rs1411262, Rs822339, Rs2282055, Rs2297136, and 8923 A/C in *CD274*; Rs10336, and 77147452 G/A in *CXCL9*; Rs8878, Rs3921, and Rs1439490 in *CXCL10*; Rs1131665, and Rs1061501 in *IRF7*; Rs2494752, Rs2498801, Rs1130214, Rs2494732, Rs2498804, Rs1130233, and Rs3803304 in *AKT1*; and Rs7254617 in *AKT2*, preferably said polymorphic site is SNP rs2301756
20 in *PTPN11*.

In a preferred embodiment of a method of the present invention, said immune checkpoint inhibitor is an anti-PD-1/PD-L1 mAb, preferably an anti-PD-1/PD-L1 mAb selected from the group consisting of nivolumab, pembrolizumab, JS001, TSR-042, pidilizumab (CT-011, MDV9300), AMP-
25 224, REGN2810, JNJ-63723283, PDR001, BGB-A317, SHR-1210, MEDI068, atezolizumab, durvalumab, BMS-936559, LY3300054; avelumab, KN035, CA-170 and SHR-1210, more preferably selected from nivolumab and pembrolizumab, most preferably nivolumab.

In a preferred embodiment of a method of the present invention,
30 the presence of the germline alteration is indicative of a genotype having a

predisposition to suffer from adverse events associated with immune checkpoint inhibitor therapy and/ or improved therapeutic efficacy of the immune checkpoint inhibitor.

In yet another aspect, the present invention provides an immune
5 checkpoint inhibitor as defined herein, for use in the treatment of cancer in
a patient, wherein the treatment aims to avoid a deviant biological response
associated with immune checkpoint inhibitor therapy in said patient, and
wherein said patient does not have an alteration in the germline sequence of
a PD-1 axis protein gene or the sequence of its RNA, as determinable by a
10 method of the present invention for genotyping. In the same manner, the
invention provides a use of an immune checkpoint inhibitor as defined
herein in the manufacture of a medicament for the treatment of cancer in a
patient, wherein the treatment aims to avoid a deviant biological response
associated with immune checkpoint inhibitor therapy in said patient, and
15 wherein said patient does not have an alteration in the germline sequence of
a PD-1 axis protein gene or the sequence of its RNA, as determinable by a
method of the present invention for genotyping.

In yet another aspect, the present invention provides an immune
checkpoint inhibitor as defined herein, for use in the treatment of cancer in
20 a patient, wherein the treatment aims to avoid a deviant biological response
associated with immune checkpoint inhibitor therapy in said patient,
wherein said patient has an alteration in the germline sequence of a PD-1
axis protein gene or the sequence of its RNA, as determinable by a method
of the present invention for genotyping, preferably wherein said treatment
25 involves a modified dosage regimen of less than 0.1-2.5 mg/kg body weight
every 2-4 weeks, or more than 3, preferably between 3.5-5 mg/kg body
weight every one or two weeks of the immune checkpoint inhibitor. In the
same manner, the invention provides a use of an immune checkpoint
inhibitor as defined herein in the manufacture of a medicament for the
30 treatment of cancer in a patient, wherein the treatment aims to avoid a

deviant biological response associated with immune checkpoint inhibitor therapy in said patient, wherein said patient has an alteration in the germline sequence of a PD-1 axis protein gene or the sequence of its RNA, as determinable by a method of the present invention for genotyping,
5 preferably wherein said treatment involves a modified dosage regimen of less than 0.1-2.5 mg/kg body weight every 2-4 weeks, or more than 3, preferably between 3.5-5 mg/kg body weight every one or two weeks of the immune checkpoint inhibitor.

In yet another aspect, the present invention provides a kit of
10 parts, comprising at least one pair of single-stranded PCR primers for amplifying a sequence of a gene, an expression product, or part thereof, wherein said gene is selected from the group consisting of *PDCD1*, *PTPN6*, *PTPN11*, *ZAP70*, *IFNG*, *CD274*, *CXCL9*, *CXCL10*, *IRF7*, *AKT1*, and *AKT2*; and wherein amplified gene, product or part includes the position or locus of
15 the SNPs as defined in claim 8; wherein said kit optionally further comprising a labelled oligonucleotide probe for detecting said SNP, wherein said kit optionally further comprises instructions for performing detection of said SNP. Preferably, said kit is for use in a method of the present invention for genotyping.

20 In another aspect, the invention provides a use of a kit of parts of the invention in genotyping a patient suffering from cancer for a predisposition to a deviant biological response associated with immune checkpoint inhibitor therapy.

In yet another aspect, the present invention provides a standard
25 of care therapeutic for use in the treatment of cancer in a patient, wherein the patient suffers from a deviant biological response associated with immune checkpoint inhibitor therapy in said patient, wherein said patient has an alteration in the germline sequence of a PD-1 axis protein gene or the sequence of its RNA, as determinable by a method of the present
30 invention for genotyping. In the same manner, the invention provides a use

of a standard of care therapeutic in the manufacture of a medicament for the treatment of cancer in a patient, wherein the patient suffers from a deviant biological response associated with immune checkpoint inhibitor therapy in said patient, wherein said patient has an alteration in the
5 germline sequence of a PD-1 axis protein gene or the sequence of its RNA, as determinable by a method of the present invention for genotyping.

In some embodiments of the above aspects, the tissue, blood, serum or plasma sample is from a patient with a cancer selected from carcinoma, melanoma, lymphoma, blastoma, sarcoma, germ cell tumors, and
10 leukemia or lymphoid malignancies. Some non-limiting examples of cancers include squamous cell cancer (e.g., epithelial squamous cell cancer), lung cancer including small-cell lung cancer, non-small cell lung cancer, adenocarcinoma of the lung and squamous carcinoma of the lung, esophageal cancer, prostate cancer, cancer of the peritoneum, hepatocellular
15 cancer, gastric or stomach cancer including gastrointestinal cancer, neuroendocrine cancer, pancreatic cancer, glioblastoma, cervical cancer, ovarian cancer, liver cancer, bladder cancer, cancer of the urinary tract, hepatoma, breast cancer, colon cancer, rectal cancer, colorectal cancer, endometrial or uterine carcinoma, salivary gland carcinoma, kidney or renal
20 cancer, prostate cancer, vulval cancer, thyroid cancer, hepatic carcinoma, anal carcinoma, melanoma, multiple myeloma and B-cell lymphoma, brain, as well as head and neck cancer, and associated metastases. Preferably, the tissue sample is from a patient with a relapsed or refractory cancer.

In another aspect, the disclosure provides a reagent kit for an
25 assay for classification of a patient for cancer therapy, such as eligibility for immune checkpoint inhibitor therapy, or for predicting predisposition to suffer from toxicity from such therapy, comprising a container comprising at least one oligonucleotide primer specific for a PD-1 axis gene mutation and instructions relating to detecting mutations in a PD-1 axis gene.

The disclosure provides capability for improving stratification of patients for immune checkpoint inhibitor therapy. The assessment of biomarker levels with the methods disclosed herein also allows tracking of individual patient response to the therapy using a readily obtainable patient sample. The methods have particular utility for treatment of NSCLC, solid tumor cancers, and lymphoma patients with immune checkpoint inhibitors, for example nivolumab, pembrolizumab, and fragments thereof.

The disclosure also relates to other aspects and embodiments that will become apparent in light of the following detailed description.

BRIEF DESCRIPTION OF THE DRAWINGS

FIG. 1, panel a), shows a schematic presentation of tumor-related mechanisms of immune system inhibition. PD-1 inhibits the activation of T-cells by suppressing the recognition of antigens through major histocompatibility complex (MHC). Tumor cells can activate PD-1 when PD-L1 is expressed on their surface. PD-L1 expression is stimulated by interferon-gamma (IFN- γ). In an inflammatory state, IFN- γ is produced, therefore IFN- γ acts as a regulator of the immune response. TCR=T-cell receptor; NFkB=nuclear factor kappa-light-chain-enhancer of activated B-cells; PI3K=Phosphoinositide 3-kinase. Panel b) of Figure 1 shows a schematic presentation of the PD-1 pathway. The T-cell receptor (TCR), CD 28 receptor and IFN- α receptor (IFNAR) stimulate the T-cell. PD-1 partly inhibits T-cell activation by activating SHP-2, SHP-2 then inhibits the function of ZAP70, an important protein in the signaling pathway of the TCR. PI3K=phosphoinositide 3-kinase; JAK1=Janus kinase 1; TYK2=Tyrosine kinase 2.

DETAILED DESCRIPTION OF THE INVENTION

The present inventors found that variants of genes involved in the PD-1 pathway were associated with the occurrence of PD-1-inhibitor-

induced auto-immune related toxicities. Not everyone with a predisposing genotype develops an auto-immune phenotype, but the PD-1 axis activity is believed to be reduced in these subjects. When treated with PD-(L)1 inhibiting drugs, these (asymptomatic) carriers of an aberrant genotype are more prone to develop immune related adverse events than patients with a fully functional PD-1 axis. Hence, patients that harbor germline genetic polymorphisms in the PD-1 axis are more prone to auto-immunity and experience more toxicity from immune checkpoint inhibition therapy than other patients.

DEFINITIONS

As used herein, an “antibody” refers to a protein consisting of one or more polypeptides substantially encoded by immunoglobulin genes or fragments of immunoglobulin genes. This term encompasses polyclonal antibodies, monoclonal antibodies, and fragments thereof, as well as molecules engineered from immunoglobulin gene sequences. The recognized immunoglobulin genes include the kappa, lambda, alpha, gamma, delta, epsilon and mu constant region genes, as well as myriad immunoglobulin variable region genes. Light chains are classified as either kappa or lambda. Heavy chains are classified as gamma, mu, alpha, delta, or epsilon, which in turn define the immunoglobulin classes, IgG, IgM, IgA, IgD and IgE, respectively.

A typical immunoglobulin (antibody) structural unit is known to comprise a tetramer. Each tetramer is composed of two identical pairs of polypeptide chains, each pair having one “light” (about 25 kD) and one “heavy” chain (about 50-70 kD). The N-terminus of each chain defines a variable region of about 100 to 110 or more amino acids primarily responsible for antigen recognition. The terms “variable light chain (VL)” and “variable heavy chain (VH)” refer to these light and heavy chains respectively.

Antibodies exist as intact immunoglobulins or as a number of well-characterized fragments produced by digestion with various peptidases. Thus, for example, pepsin digests an antibody below the disulfide linkages in the hinge region to produce F(ab')₂, a dimer of Fab which itself is a light chain joined to VH—CH₁ by a disulfide bond. The F(ab')₂ may be reduced under mild conditions to break the disulfide linkage in the hinge region thereby converting the (Fab')₂ dimer into a Fab' monomer. The Fab' monomer is essentially a Fab with part of the hinge region (see, Fundamental Immunology, W. E. Paul, ed., Raven Press, N.Y. (1993), for a more detailed description of other antibody fragments). While various antibody fragments are defined in terms of the digestion of an intact antibody, one of skill will appreciate that such Fab' fragments may be synthesized de novo either chemically or by utilizing recombinant DNA methodology.

Thus, the term “antibody,” as used herein also includes antibody fragments either produced by the modification of whole antibodies or synthesized de novo using recombinant DNA methodologies. Useful antibodies include single chain antibodies (antibodies that exist as a single polypeptide chain), more preferably single chain Fv antibodies (sFv or scFv), in which a variable heavy and a variable light chain are joined together (directly or through a peptide linker) to form a continuous polypeptide. The single chain Fv antibody is a covalently linked VH-VL heterodimer which may be expressed from a nucleic acid including VH- and VL-encoding sequences either joined directly or joined by a peptide-encoding linker. Huston, et al. (1988) Proc. Nat. Acad. Sci. USA, 85: 5879-5883. While the VH and VL are connected to each as a single polypeptide chain, the VH and VL domains associate non-covalently. The scFv antibodies and a number of other structures converting the naturally aggregated, but chemically separated, light and heavy polypeptide chains from an antibody V region into a molecule that folds into a three dimensional structure substantially

similar to the structure of an antigen-binding site are known to those of skill in the art (see, e.g., U.S. Pat. Nos. 5,091,513, 5,132,405, and 4,956,778).

Preferred antibodies are human or humanized antibodies that block activation of PD-1 and bind to one of (i) PD-1, (ii) PD-L1 or (iii) PD-L2.

5 The term “subject”, as used herein, refers to the group of mammals, preferably humans. The terms “patient” and “subject” are used interchangeably herein. The subject is more preferably a human patient suffering from cancer. The subject is in certain embodiments preferable a female patient suffering from cancer.

10 The term “administering”, as used herein, refers to the physical introduction of a therapeutic agent to a subject, using any of the various methods and delivery systems known to those skilled in the art. Preferred routes of administration for antibodies as mentioned herein include intravenous, intramuscular, subcutaneous, intraperitoneal, spinal or other
15 parenteral routes of administration, for example by injection or infusion. The term “parenteral administration” as used herein means modes of administration other than enteral and topical administration, usually by injection, and includes, without limitation, intravenous, intramuscular, intraarterial, intrathecal, intralymphatic, intralesional, intracapsular,
20 intraorbital, intracardiac, intradermal, intraperitoneal, transtracheal, subcutaneous, subcuticular, intraarticular, subcapsular, subarachnoid, intraspinal, epidural and intrasternal injection and infusion, as well as in vivo electroporation. Alternatively, antibodies can be administered via a non-parenteral route, such as a topical, epidermal or mucosal route of
25 administration, for example, intranasally, orally, vaginally, rectally, sublingually or topically. Administering can also be performed, for example, once, a plurality of times, and/or over one or more extended periods.

 As used herein, a “immune checkpoint inhibitor”, may refer to either or both of an inhibitor of PD-1-mediated signaling and an inhibitor of
30 CTLA-4-mediated signaling, and includes reference to a therapeutic

compound of any type, including small molecule-, antibody-, antisense-, small interfering RNA-, or microRNA-based compounds, that inhibits or antagonizes PD-1-mediated and/or CTLA-4-mediated signaling, such as by binding to PD-1 and/or to CTLA-4. The inhibitor is preferably an antibody.

- 5 The inhibitor preferably exerts inhibition of PD-1-mediated and/or CTLA-4-mediated signaling at the cell surface-level, such as by interfering with the formation of an activated PD-1 signaling complex and/or the formation of an activated CTLA-4 signaling complex, respectively.

The term “inhibitor of PD-1-mediated signaling” preferably refers
10 to an inhibitor that blocks, reduces or antagonizes activation of PD-1, for instance by binding to PD-1 or one of its ligands PD-L1 or PD-L2. Such an inhibitory effect provides for activation of T-cells and cell-mediated immune responses against tumor cells. The methods are useful with any known or hereafter developed immune checkpoint inhibitor targeting PD-1, PD-L1
15 and/or PD-L2. One immune checkpoint inhibitor targeting PD-1 is nivolumab, which binds to the PD-1 receptor and blocks PD-L1 from binding to PD-1, allowing the T cell to work. The terms “PD-(L)1 inhibitor” and “inhibitor of PD-1-mediated signaling” can be used interchangeable.

The term “inhibitor of CTLA-4-mediated signaling” preferably
20 refers to an inhibitor that blocks, reduces or antagonizes activation of CTLA-4, for instance by binding to CTLA-4 or one of its ligands CD80 or CD86. Such an inhibitory effect provides for activation of T-cells and cell-mediated immune responses against tumor cells. The methods are useful with any known or hereafter developed immune checkpoint inhibitor
25 targeting CTLA-4. One CTLA-4 inhibitor is ipilimumab, which binds to the CTLA-4 receptor and blocks the formation of an active signaling complex, allowing the T cell to work. Another example of a TCLA-4 inhibitor is tremelimumab. The terms “CTLA-4 inhibitor” and “inhibitor of CTLA-4-mediated signaling” can be used interchangeable.

The term “immune checkpoint”, as used herein, refers to molecules, preferably proteins, that are negative regulators of the immune system. In other words, the term refers to inhibitory immune checkpoint molecules, including PD-1 and CTLA-4. Inhibitors of such (inhibitory) checkpoints enhance the proliferation, migration, persistence and/or cytotoxic activity of T cells in a subject and, in particular, the tumor-infiltrating capacity of T cells. Active agents that inhibit, block or antagonize signaling through inhibitory immune checkpoint molecules are referred to as immune checkpoint inhibitors herein.

The term “single nucleotide polymorphism (SNP)” refers to a change of a single nucleotide with a polynucleotide, including within an allele. This can include the replacement of one nucleotide by another, as well as deletion or insertion of a single nucleotide. Most typically, SNPs are biallelic markers although tri- and tetra-allelic markers can also exist. By way of non-limiting example, a nucleic acid molecule comprising SNP A\C may include a C or A at the polymorphic position. For combinations of SNPs, the term “haplotype” can be used, e.g. the genotype of the SNPs in a single DNA strand that are linked, or in linkage disequilibrium, to one another. The term “biological response”, as used herein in the context of the present invention, refers to a particular biological effect in a particular patient or class of patients in response to a medical treatment.

The term “biological effect” in the context of drug treatment, as used herein, includes both a therapeutic effect and a side effect.

The term “therapeutic effect”, as used herein, includes all desirable and beneficial consequences of a medical treatment of any kind of an agent or drug aimed at curing a disease and restoring a patient’s health irrespective of whether the result was expected, unexpected, or even an unintended consequence of the treatment, while excluding all the undesirable and harmful/adverse effects or side effects.

The terms "adverse effect" or "side effect", refer to harmful and undesired consequences of a medical treatment of any kind or an agent aimed at curing a disease and restoring a patient's health, and include adverse events as defined herein.

5 The term "adverse event", as used herein, refers to any unfavourable and/or unintended sign, symptom, or disease associated with the administration of, or treatment with, an inhibitor of PD-1-mediated signaling. Preferably, the adverse event is an immune-, more preferably an auto-immune-, related adverse event. Preferably, the adverse events
10 described herein are ≥ 3 grade adverse events as listed in the CTCAE v4.03 of 14 June 2010.

In some embodiments, the methods according to the invention provide for selection of patients eligible for therapy with human or humanized monoclonal antibodies and fragments thereof.

15 "Therapeutic" as used herein relates broadly to any agent or treatment that can inhibit, slow, or halt progression or proliferation of a cancer cell, cause apoptosis of a cancer cell, induce remission of disease, or provide prophylaxis of or reduce the number and/or severity of symptoms associated with cancer (i.e., provides a degree of clinical benefit to a patient
20 suffering from cancer).

The term a "PD-1 axis gene", as used herein, refers to any gene encoding a protein involved in the PD-1 signaling pathway. In particular, PD-1 axis genes include *PDCD1*, *PTPN11*, *ZAP70*, *IFNG*, *CD274*, *CXCL9*, *CXCL10*, *IRF7*, *AKT1* and *AKT2*, which genes encode the proteins PD-1,
25 SHP2, ZAP70, and IFN- γ , PD-L1, CXCL9, CXCL10, IRF7, AKT1 and AKT2, respectively.

The term "targeted agents" as used herein in the context of cancer treatment refers to other agents than the immune checkpoint inhibitors as defined herein, and refer to alternative therapies. Targeted cancer therapies
30 are based on the use of drugs or other substances that block the growth and

spread of cancer by interfering with specific molecules ("molecular targets") that are involved in the growth, progression, and spread of cancer. Many different therapies are known to one of skill in the art.

5 *Method of Classifying a Patient suffering from cancer for a Therapeutic Regimen*

In a general sense, the disclosure relates to a method of classifying a patient having cancer as a candidate for treatment with a therapeutic. The method includes detecting at least one biomarker in a
10 sample from a patient having cancer and using the presence or absence of the marker to classify the patient as a good candidate for therapy, or as one likely to suffer from adverse effects.

A good candidate for therapy can be a patient that is less likely to suffer from immune checkpoint inhibitor induced toxicity.

15 The method may be for classifying a patient having cancer as a candidate for therapy with an immune checkpoint inhibitor or combination therapy thereof. The method may comprise providing a tissue, blood, serum or plasma sample from a patient having cancer, determining the presence of at least one biomarker in the tissue, blood, serum or plasma sample, and
20 classifying the patient as a candidate for therapy with an immune checkpoint inhibitor when the tissue, blood, serum or plasma sample indicates the absence of the germline polymorphism in the patient.

The method can be used for targeted cancer therapy. Cancer therapy based on immune checkpoint inhibition, as described herein for
25 immune checkpoint inhibitors, has received market authorization for a wide array of cancer indications. The application of such a form of therapy is thus not limited to a particular cancer. The methods described herein are useful for therapy selection for patients having non-small cell lung cancer or other cancers, such as therapy with a therapeutic such as an immune checkpoint
30 inhibitor. The method can be used as companion assays for immune

checkpoint inhibitor therapy, given either as monotherapy or as part of combination therapy with another therapy or therapeutic, such as conventional chemotherapy or radiation therapy. The method can be performed in relation to any cancer type. Other examples of such cancers

5 can include carcinoma, melanoma, lymphoma, blastoma, sarcoma, germ cell tumors, and leukemia or lymphoid malignancies, such as, for example squamous cell cancer (e.g., epithelial squamous cell cancer), lung cancer including small-cell lung cancer, non-small cell lung cancer, adenocarcinoma of the lung and squamous carcinoma of the lung, esophageal cancer,

10 prostate cancer, cancer of the peritoneum, hepatocellular cancer, gastric or stomach cancer including gastrointestinal cancer, neuroendocrine cancer, pancreatic cancer, glioblastoma, cervical cancer, ovarian cancer, liver cancer, bladder cancer, cancer of the urinary tract, hepatoma, breast cancer, colon cancer, rectal cancer, colorectal cancer, endometrial or uterine

15 carcinoma, salivary gland carcinoma, kidney or renal cancer, prostate cancer, vulval cancer, thyroid cancer, hepatic carcinoma, anal carcinoma, melanoma, multiple myeloma and B-cell lymphoma, brain, as well as head and neck cancer, and associated metastases.

Preferably, the cancer in a subject is selected from the group

20 formed by Hodgkin lymphoma (B-Cell Hodgkin lymphoma); lung cancer including small-cell lung cancer or non-small cell lung cancer such as squamous non-small cell lung cancer, melanoma including metastatic melanoma, renal cell cancer including (metastatic) renal cell carcinoma, and, alternatively or in addition, ovarian cancer, anal cancer, bile duct

25 cancer (cholangiocarcinoma), glioblastoma multiforme (GBM), vaginal cancer, fallopian tube cancer, esophageal cancer, follicular lymphoma, cutaneous T-cell lymphoma, sarcomas such as soft tissue sarcoma, nasopharyngeal cancer, skin cancer, chronic lymphocytic leukemia (CLL) such as relapsed or refractory chronic lymphocytic leukemia, cervical cancer,

30 acute myelocytic leukemia (AML), myelocytic leukemia (CML), pancreatic

cancer, gastric cancer, bladder cancer, breast cancer such as metastatic breast cancer, diffuse large B-cell lymphoma, solid tumors, endometrial cancer, B-Cell Non-Hodgkin lymphoma, colon cancer, gastroesophageal (GE) junction carcinomas, head and neck cancer such as head and neck squamous cell carcinoma, hepatocellular carcinoma, malignant pleural mesothelioma, colorectal cancer such as metastatic colorectal cancer, prostate cancer such as metastatic hormone refractory (castration resistant, androgen-independent) prostate cancer, pancreatic ductal adenocarcinoma, peripheral T-cell lymphomas (PTCL), peritoneal cancer, glioblastoma multiforme (GBM) such as recurrent GBM, multiple myeloma such as relapsed multiple myeloma, transitional cell cancer (urothelial cell cancer), transitional cell carcinoma and vulvar cancer. More preferably, the cancer is lung cancer, even more preferably non-small cell lung cancer.

The method may be used for classifying a patient having cancer as a candidate for second-line therapy with a therapeutic such as an immune checkpoint inhibitor. In some embodiments, the patient is selected based on lack of success of one or more prior therapies. The method can be used to assess the viability and provide a prognostic evaluation of whether a patient having a cancer that has been resistant to prior therapies may be successfully treated with a therapy comprising an immune checkpoint inhibitor. The method can be used for targeted cancer therapy selection for patients having a refractory, recurrent, or relapsed cancer such as, for example, non-small cell lung cancer, such as therapy with immune checkpoint inhibitors. The method can be used as companion assays for immune checkpoint inhibitor therapy, given either as monotherapy or as part of combination therapy with another therapy or therapeutic, such as conventional chemotherapy or radiation therapy. The method can be performed in relation to cancer type in patients having a germline polymorphism as defined herein. Other examples of such cancers can include carcinoma, melanoma, lymphoma, blastoma, sarcoma, germ cell

tumors, and leukemia or lymphoid malignancies, such as, for example squamous cell cancer (e.g., epithelial squamous cell cancer), lung cancer including small-cell lung cancer, non-small cell lung cancer, adenocarcinoma of the lung and squamous carcinoma of the lung, esophageal cancer, prostate cancer, cancer of the peritoneum, hepatocellular cancer, gastric or stomach cancer including gastrointestinal cancer, neuroendocrine cancer, pancreatic cancer, glioblastoma, cervical cancer, ovarian cancer, liver cancer, bladder cancer, cancer of the urinary tract, hepatoma, breast cancer, colon cancer, rectal cancer, colorectal cancer, endometrial or uterine carcinoma, salivary gland carcinoma, kidney or renal cancer, prostate cancer, vulval cancer, thyroid cancer, hepatic carcinoma, anal carcinoma, melanoma, multiple myeloma and B-cell lymphoma, brain, as well as head and neck cancer, and associated metastases.

The dosage of the immune checkpoint inhibitor can vary based on any variety of factors known in the art (e.g., patient health, age, weight, gender, cancer type, stage/progression of cancer, etc.), and can be readily determined by one of skill. In some embodiments the immune checkpoint inhibitor can be administered to a patient in an amount of 10 mg/day-500 mg/day, about 10 mg/day to about 200 mg/day (e.g., 10, 20, 30, 40, 50, 60, 70, 80, 90, 100, 110, 120, 130, 140, 150, 160, 170, 180, 190, or 200 mg/day), 100 mg/day to about 200 mg/day, or about 200 mg/day to about 500 mg/day (e.g., 200, 210, 220, 230, 240, 250, 260, 270, 280, 290, 300, 310, 320, 330, 340, 350, 360, 370, 380, 390, 400, 410, 420, 430, 440, 450, 460, 470, 480, 490, or 500 up to 2500 mg/day, inclusive of any single or multi-dose daily administration regimen that falls within that total daily dose range. The exact dose may depend on the specific inhibitor selected for therapy) In some embodiments, the dose is from 150-325 mg/day. In the case of nivolumab, the dosage regime is preferably about 3 mg/kg bodyweight/2 weeks.

Preferably, the immune checkpoint inhibitor is for intravenous administration in the form of an injectable solution. The inhibitor of PD-1-mediated signaling is an antibody or a small molecule. Preferably, the inhibitor is selected from the group formed by, or consisting of, nivolumab
5 (originators Medarex and Ono Pharmaceutical.; CAS number 946414-94-4); pembrolizumab (originators Merck & Co and The Leukemia & Lymphoma Society; CAS Number 1374853-91-4); JS001 (originator Shanghai Junshi Biosciences); TSR-042 (originator AnaptysBio; developer Tesaro, Inc.); Pidilizumab (CT-011, MDV9300; originator CureTech); AMP-224 (originator
10 Amplimmune); REGN2810 (originator Regeneron Pharmaceutical); JNJ-63723283 (originator Janssen Research & Development); PDR001 (originator Novartis); BGB-A317 (originator BeiGene); SHR-1210 (originator Jiangsu Hengrui Medicine Co); MEDI068 or AMP-514 (Astra Zeneca/Medimmune); Atezolizumab (originators Genentech and University
15 Medical Center Groningen; CAS number 1380723-44-3); Durvalumab (originator MedImmune); BMS-936559 (alias MDX-1105; originator Medarex); LY3300054 (originator Eli Lilly); Avelumab (alias MSB0010718C); originators EMD Serono and Merck KGaA); KN035 (originator Alphamab); CA-170 (originator Aurigene Discovery
20 Technologies) and/or SHR-1210 (originator Jiangsu Hengrui Medicine Co.). More preferably, the inhibitor is an antibody selected from the group formed by, or consisting of, nivolumab, pembrolizumab, JS001, TSR-042, Pidilizumab (CT-011, MDV9300), AMP-224, REGN2810, JNJ-63723283, PDR001, BGB-A317, SHR-1210, MEDI068, Atezolizumab, Durvalumab,
25 BMS-936559, LY3300054, Avelumab, KN035, or SHR-1210. A preferred small molecule is CA-170.

More preferably, the inhibitor of PD-1-mediated signaling is an inhibitor that binds to and blocks the activation of PD-1, and is preferably selected from the group formed by nivolumab, pembrolizumab, JS001, TSR-
30 042, Pidilizumab (CT-011, MDV9300), AMP-224, REGN2810, JNJ-

63723283, PDR001, BGB-A317, SHR-1210 and MEDI068. Most preferably, the inhibitor is nivolumab.

Alternatively, the immune checkpoint inhibitor blocks the activation of PD-1 by binding to PD-L1, and is preferably selected from the group formed by, or consisting of, Atezolizumab, Durvalumab, BMS-936559, LY3300054; Avelumab, KN035, CA-170 and/or SHR-1210.

Treatment with immune checkpoint inhibitors may not be started, dosages of ongoing or planned treatment with immune checkpoint inhibitors may be reduced, or treatment with immune checkpoint inhibitors may be halted when the predisposition to adverse events associated with administration of an inhibitor of PD-1-mediated signaling is detected or identified in a subject.

Preferably, the adverse event associated with administration of an inhibitor of PD-1-mediated signaling is selected from the group formed by, or consisting of diarrhea, nausea, pruritus, rash acneiform, rash maculopapular, erythroderma, papulopustular rash, toxic epidermal necrolysis, anorexia, fatigue, hyperkalemia, hypokalemia, hyponatremia, hypomagnesemia, hypercalcemia, hypocalcemia, decreased platelet count, decreased white blood cell count, decreased lymphocyte count, anemia, increased alanine aminotransferase, increased alkaline phosphatase, increased aspartate aminotransferase, increased lipase, increased serum amylase, increased creatinine, hypertension, upper respiratory infection, pneumonitis, interstitial lung disease, dyspnea, cough, mucositis oral, dry mouth, vomiting, colitis, abdominal pain, constipation, infusion related reaction, dry skin, skin hypopigmentation, alopecia, headache, dizziness, peripheral motor neuropathy, peripheral sensory neuropathy, dry eye, blurred vision, myalgia, arthralgia, bone pain, back pain, pain in extremity, non-cardiac chest pain, jaw pain, vertebral pain, polymyalgia rheumatic, guillain-barre syndrome, demyelination, fever, edema (any anatomical site), weight loss, hyperthyroidism, hypothyroidism, hyperglycemia,

hypermagnesemia, hypernatremia, increased blood bilirubin, decreased neutrophil count, anaphylaxis, sinus tachycardia, lung infection, bronchial infection, pleural effusion, pancreatitis, hepatic infection, nephritis, chronic kidney disease, uveitis, urticaria, erythema multiforme, psoriasis, rosacea, vasculitis, arthritis, cardiac chest pain, adrenal insufficiency, autoimmune disorder (hypopituitarism, hypophysitis, thyroiditis), acidosis, dehydration, ventricular arrhythmia, atrial fibrillation, gastritis, duodenal ulcer, and cholestasis.

More preferably, the adverse event is selected from the group formed by, or consisting of, diarrhea, pruritus, fatigue, anemia, increased aspartate aminotransferase, pneumonitis, interstitial lung disease, dyspnea, colitis, constipation, dry skin, peripheral motor neuropathy, peripheral sensory neuropathy, fever, anaphylaxis and hepatic infection.

Preferably, the adverse events listed correspond to the adverse events listed in the Common Terminology Criteria for Adverse Events (CTCAE) v4.03 of 14 June 2010. Preferably, the adverse events listed herein are ≥ 3 grade adverse events as listed in the CTCAE v4.03. The practitioner skilled in the field of cancer therapy knows how to score these adverse events and their corresponding grade, and can for that matter rely on guidance in the CTCAE.

Interestingly, and unexpectedly, it was established that female patients suffering from cancer have an increased risk of, or predisposition to, developing liver toxicity (i.e. elevated liver enzymes, preferably increased alanine aminotransferase, increased alkaline phosphatase, and/or increased aspartate aminotransferase) upon administration of an immune checkpoint inhibitor, compared to their male counterparts.

The predisposition to suffer from adverse events as referred to herein, is detected or identified in a subject when detecting a germline polymorphism in a PD-1 axis gene, wherein said polymorphism is selected

from the group consisting of the alterations set forth in Tables 1 and 2 in a human.

The method of detecting a germline polymorphism in a PD-1 axis gene may suitably comprise analyzing a sequence of a PD-1 axis gene or PD-1 axis gene-RNA from a human sample or analyzing a sequence of a PD-1 axis gene cDNA made from RNA from said human sample, using assays for detection of germline polymorphisms in genes or sequences well known in the art. The invention thus also relates to a method of detecting a germline polymorphism in a PD-1 axis gene, comprising the steps of: a) providing a nucleic acid sample of a patient suffering from a cancer; and detecting in said sample the presence or absence of a germline polymorphism in a PD-1 axis gene or gene product. Preferably, the germline polymorphism in a PD-1 axis gene or gene product is a polymorphic site as referred to herein.

The assays can identify a germline polymorphism in a PD-1 axis gene in the form of detection of the presence of an SNP in a DNA sequence isolated from a human sample, an SNP in an RNA sequence isolated from a human sample, or an SNP in a cDNA sequence prepared from RNA isolated from a human sample.

The detection of the germline polymorphism may be used for predicting therapy response, for monitoring patient response to a therapeutic regimen such as immune checkpoint inhibitor therapy, or for predicting in a subject a predisposition to suffer from adverse events as referred to herein in response to an immune checkpoint inhibitor therapy.

Hence, in one aspect, the present invention provides a method for predicting response to immune checkpoint inhibitor therapy of a patient suffering from cancer, comprising genotyping a subject suffering from cancer by comparing the germline sequence of a PD-1 axis protein gene or the sequence of its RNA in a sample from said patient with the germline sequence of the wild-type gene or the sequence of its RNA, and correlating

the genotype obtained with the therapy response-profile of a reference patient having a known genotype.

For instance, the presence of an alteration in the germline sequence of the PD-1 axis protein gene or the sequence of its RNA of the patient, may indicate a genotype that has a positive response-profile to immune checkpoint inhibitor therapy, or may indicate a genotype that has a negative response-profile to immune checkpoint inhibitor therapy,.

The term “positive response” as used herein, refers to a response profile in a patient showing one or more of a reduction in tumor volume and/or inhibition of tumor growth after treatment with the immune checkpoint inhibitor, the absence of immune-related adverse events, and the responsiveness to lower dosage regimens.

Preferably, with regard to an improved, increased or decreased therapeutic efficacy of the immune checkpoint inhibitor as described herein, a control value for the purpose of comparison is employed, such as a control therapeutic efficacy achieved by administration of an immune checkpoint inhibitor in a therapeutically effective dose to a patient suffering from cancer, said patient (i) not having an alteration in the germline sequence of a PD-1 axis protein gene or the sequence of its RNA as compared to the germline sequence of the wild-type gene or the sequence of its RNA; or (ii) having an alteration in the germline sequence of a PD-1 axis protein gene or the sequence of its RNA as compared to the germline sequence of the wild-type gene or the sequence of its RNA.

Assays for response prediction are run before start of therapy and patients showing presence of germline polymorphism may be indicated as being eligible to receive alternate immune checkpoint inhibitor therapy, for instance in the form of a reduced dosage regimen or chemotherapy.

Alternatively, assays for response prediction are run before start of therapy and patients showing presence of germline polymorphism may be indicated as being ineligible to receive immune checkpoint inhibitor therapy

due to an increased risk of suffering from adverse events as referred to herein.

For monitoring or for confirming patient response, the assay may be performed prior to, during or after the initiation of therapy to establish or
5 confirm the presence of the germline polymorphism in the tissue, blood, serum or plasma sample.

The presence or absence of the germline polymorphism in a PD-1 axis gene or gene product can indicate that the therapy is likely being effective and can be continued or if the patient may not be responding to
10 therapy.

The presence or absence of the germline polymorphism in a PD-1 axis gene or gene product can indicate that the therapy is likely to be toxic and should be discontinued or not started, or replaced by alternative therapies such as by administration of standard-of-care therapeutics, or
15 moderated in dosage regime, be continued or if the patient may not be responding to therapy.

Standard-of-care therapeutics

Certain methods described herein comprise the administration of a
20 standard-of-care therapeutic to a subject. As used herein, a "standard-of-care therapeutic" is a drug, or combination of drugs, that is considered by medical practitioners as appropriate, accepted, and/or widely used for a certain type of patient, disease or clinical circumstance. Standard-of-care therapies for different types of cancer are well known by persons of skill in
25 the art. For example, the National Comprehensive Cancer Network (NCCN) publishes the NCCN Clinical Practice Guidelines in Oncology (NCCN GUIDELINES) that provide detailed up-to-date information on standard-of-care therapies for a wide variety of cancers.

For example, standard-of-care therapy for patients suffering from
30 NSCLC include, other than inhibitors of PD-1-mediated signaling, surgery,

radiotherapy and chemotherapy or so called targeted agents directed against certain mutations. In general, for patients with stage I or II disease, surgical resection provides the best chance for cure, with chemotherapy increasingly being used both pre-operatively and post-operatively.

- 5 Radiotherapy can also be used as adjuvant therapy for patients with resectable NSCLC, the primary local treatment, or as palliative therapy for patients with incurable NSCLC. Patients with Stage IV disease benefit from chemotherapy or targeted agents in case of the presence of a drugable target.

10

Detection of Germline polymorphisms

- The method determines the presence or absence of a germline polymorphism. The presence or absence may be detected relative to the sequence of the wild-type gene. The presence or absence can be determined
15 in a number of different ways, but is preferably determined by using PCR detection.

- Genomic markers can be identified by any technique such as, for example, comparative genomic hybridization (CGH), sequence analysis, or by single nucleotide polymorphism arrays (genotyping microarrays) of
20 nucleic acids extracted from a sample of a subject, including techniques such as qPCR or in situ hybridization. Nucleic acid assay methods for detection of chromosomal DNA sequences or mutations may include: (i) in situ hybridization assays, (ii) microarray hybridization assays, and (iii) polymerase chain reaction (PCR) or other amplification assays. Assays using
25 synthetic analogs of nucleic acids, such as peptide nucleic acids, in any of these formats can also be used.

- Hybridization assays may include the use of detectably labeled nucleic acid-based probes, such as deoxyribonucleic acid (DNA) probes or protein nucleic acid (PNA) probes, or unlabeled primers which are
30 designed/selected to hybridize to the specific designed chromosomal target.

The unlabeled primers are used in amplification assays, such as by polymerase chain reaction (PCR), in which after primer binding, a polymerase amplifies the target nucleic acid sequence for subsequent detection. The detection probes used in PCR or other amplification assays are preferably fluorescent, and still more preferably, detection probes useful in “real-time PCR”. Fluorescent labels are also preferred for use in situ hybridization but other detectable labels commonly used in hybridization techniques, e.g., enzymatic, chromogenic and isotopic labels, can also be used. Useful probe labeling techniques are described in Molecular Cytogenetics: Protocols and Applications, Y.-S. Fan, Ed., Chap. 2, “Labeling Fluorescence In Situ Hybridization Probes for Genomic Targets”, L. Morrison et al., p. 21-40, Humana Press, © 2002, incorporated herein by reference. In detection of the genomic markers by microarray analysis, these probe labeling techniques are applied to label a chromosomal DNA extract from a patient sample, which is then hybridized to the microarray.

In situ hybridization can be used to detect the presence of the germline polymorphism at a genomic locus. Probes for use in the in situ hybridization methods of the invention fall into two broad groups: chromosome enumeration probes, i.e., probes that hybridize to a chromosomal region, usually a repeat sequence region, and indicate the presence or absence of an entire chromosome, and locus specific probes, i.e., probes that hybridize to a specific locus on a chromosome and detect the presence or absence of a specific locus. It is preferred to use a locus specific probe that can detect changes of the unique chromosomal DNA sequences at the interrogated locus. Methods for use of unique sequence probes for in situ hybridization are described in U.S. Pat. No. 5,447,841, incorporated herein by reference.

In situ hybridization probes employ directly labeled fluorescent probes, such as described in U.S. Pat. No. 5,491,224, incorporated herein by reference. U.S. Pat. No. 5,491,224 also describes simultaneous FISH assays

using more than one fluorescently labeled probe. Use of a pair of fluorescent probes, for example, one for the marker (germline polymorphism) and one for the centromere of a chromosome on which it is located.

Useful locus specific probes can be of any desired length and
5 produced in any manner and will generally contain sequences to hybridize to a chromosomal DNA target sequence of about 10,000 to about 1,000,000 bases long. Preferably the probe will hybridize to a target stretch of chromosomal DNA at the target locus of at least 100,000 bases long to about 500,000 bases long, and will also include unlabeled blocking nucleic acid in
10 the probe mix, as disclosed in U.S. Pat. No. 5,756,696, herein incorporated by reference, to avoid non-specific binding of the probe. It is also possible to use unlabeled, synthesized oligomeric nucleic acid or peptide nucleic acid as the blocking nucleic acid or as the centromeric probe. For targeting the particular gene locus, it is preferred that the probes include nucleic acid
15 sequences that span the gene and thus hybridize to both sides of the entire genomic coding locus of the gene. The probes can be produced starting with human DNA containing clones such as Bacterial Artificial Chromosomes (BAC's) or the like. BAC libraries for the human genome are available from Invitrogen and can be investigated for identification of useful clones. The
20 University of California Santa Cruz Genome Browser can be used to identify DNA sequences in the target locus. These DNA sequences can then be used to identify useful clones contained in commercially available or academic libraries. The clones can then be labeled by conventional nick translation methods and tested as in situ hybridization probes.

25 Examples of fluorophores that can be used in the in situ hybridization methods described herein are: 7-amino-4-methylcoumarin-3-acetic acid (AMCA), Texas Red™ (Molecular Probes, Inc., Eugene, Oreg.); 5-(and -6)-carboxy-X-rhodamine, lissamine rhodamine B, 5-(and -6)-carboxyfluorescein; fluorescein-5-isothiocyanate (FITC); 7-
30 diethylaminocoumarin-3-carboxylic acid, tetramethyl-rhodamine-5-(and -6)-

isothiocyanate; 5-(and -6)-carboxytetramethylrhodamine; 7-hydroxy-coumarin-3-carboxylic acid; 6-[fluorescein 5-(and -6)-carboxamido]hexanoic acid; N-(4,4-difluoro-5,7-dimethyl-4-bora-3a,4a diaza-3-indacenepropionic acid; eosin-5-isothiocyanate; erythrosine-5-isothiocyanate; 5-(and -6)-
5 carboxyrhodamine 6G; and Cascade™ blue acetylazide (Molecular Probes).

Probes can be viewed with a fluorescence microscope and an appropriate filter for each fluorophore, or by using dual or triple band-pass filter sets to observe multiple fluorophores. See, e.g., U.S. Pat. No. 5,776,688 to Bittner, et al., which is incorporated herein by reference. Any suitable
10 microscopic imaging method can be used to visualize the hybridized probes, including automated digital imaging systems, such as those available from MetaSystems, Zeiss, Bioview, or Applied Imaging. Alternatively, techniques such as flow cytometry can be used to examine the hybridization pattern of the chromosomal probes.

15 The germline polymorphisms can also be determined by PCR. In this method, chromosomal DNA is extracted from a body sample, and is then amplified by PCR using a pair of primers specific to at least one germline polymorphism or SNP, or by multiplex PCR, using multiple pairs of primers. Any primer sequence for the germline polymorphism can be
20 used, as long as it is capable of specifically detecting the underlying mutation. The presence or absence of the germline polymorphism is then determined by comparison of the PCR signal to a reference amplification standard. Microarray analysis can also be used. The chromosomal DNA after extraction is labeled for hybridization to a microarray comprising a
25 substrate having multiple immobilized unlabeled nucleic acid probes arrayed at probe densities up to several million probes per square centimeter of substrate surface. Multiple microarray formats exist and any of these can be used, including microarrays based on BAC's and on oligonucleotides, such as those available from Agilent Technologies (Palo
30 Alto, Calif.), and Affymetrix (Santa Clara, Calif.). When using a

oligonucleotide microarray to detect germline polymorphisms, it is preferred to use a microarray that has probe sequences specific for the germline polymorphisms and probe sequences that do not hybridize to the germline polymorphisms as controls.

5 In stead of DNA, RNA (including mRNA) can be used in methods described herein, wherein RNA may optionally first be reverse-transcribed into cDNA, prior to analysis for the presence of germline polymorphisms therein, or wherein the RNA is analyzed directly for the presence of germline polymorphisms as indicated herein.

10 A method for detecting or genotyping of the invention analyzes the presence of an SNP in a PD-1 axis gene. A PD-1 axis gene is preferably selected from the group formed by, or consisting of, PDCD1, PTPN11, ZAP70 and IFNG, CD274, CXCL9, CXCL10, IRF7, AKT1 and AKT2. The SNP is preferably selected from Table 1 (association with adverse events) or
15 Table 2 (association with therapeutic efficacy). From Table 2, the SNP is preferably a SNP selected from the group consisting of Rs11568821, Rs41386349, Rs36084323, Rs10204525, 6867 C/G, Rs2301756, Rs12423190, Rs11066301, Rs41279090, Rs12301915, Rs11066320, Rs11066322, Rs3741983, Rs17695937, Rs62157588, Rs2278699, Rs2430561, Rs2069705,
20 Rs1861494, Rs4143815, Rs1411262, Rs822339, Rs2282055, Rs2297136, 8923 A/C, Rs10336, 77147452 G/A, Rs8878, Rs3921, Rs1439490, Rs1131665, Rs1061501, Rs2494752, Rs2498801, Rs1130214, Rs2494732, Rs2498804, Rs1130233, Rs3803304 and Rs7254617.

 Preferably, a method for detecting or genotyping of the invention
25 analyzes the presence of at least one SNP in the human gene *PTPN11* (NCBI Gene Accession Number NG_007459.1), said at least one SNP selected from the group consisting of rs2301756, rs12423190, rs11066301, rs41279090, rs12301915, rs11066320, rs11066322, rs3741983. More preferably, the SNP in PTPN11 is rs2301756, which represents an A>G
30 polymorphism at nucleotide position 39241 of PTPN11. Alternatively, the

SNP rs2301756 in PTPN11 can be defined as an A>G polymorphism at nucleotide/contig position 75217720 of human chromosome 12 (NCBI chromosome 12 with Accession Number NT_029419.13) of the human genome Assembly identified as GRCh38.p2.

5 The skilled person is well aware of the specific germline alterations associated with the various SNPs indicated by specific rs numbers (rsids). The rs number is an accession number used by researchers and databases to refer to specific SNPs. It stands for reference SNP cluster ID. For instance, for the SNP rs12301915 data can be found at
10 https://www.ncbi.nlm.nih.gov/projects/SNP/snp_ref.cgi?rs=rs12301915.
NCBI database references, as used herein, refer to build 148 of 24 June 2016.

Samples

15 The method includes collecting samples from a subject for assessment of germline polymorphisms. Samples containing nucleic acid are routinely obtained from subjects. Such material is any biological matter from which nucleic acid can be prepared. The method can use a patient tissue sample of any type or on a derivative thereof, including peripheral
20 blood, serum or plasma fraction from peripheral blood, tumor or suspected tumor tissues (including fresh frozen and fixed or paraffin embedded tissue), cell isolates such as circulating epithelial cells separated or identified in a blood sample, lymph node tissue, bone marrow and fine needle aspirates. The sample suitable for use in the method can comprise any tissue type or
25 cell isolates from any tissue type, including a peripheral blood sample, a tumor tissue or a suspected tumor tissue, a thin layer cytological sample, a fine needle aspirate sample, a bone marrow sample, a lymph node sample, a urine sample, an ascites sample, a lavage sample, an esophageal brushing sample, a bladder or lung wash sample, a spinal fluid sample, a brain fluid
30 sample, a ductal aspirate sample, a nipple discharge sample, a pleural

effusion sample, a fresh frozen tissue sample, a paraffin embedded tissue sample or an extract or processed sample produced from any of a peripheral blood sample, a serum or plasma fraction of a peripheral blood sample, a tumor tissue or a suspected tumor tissue, a thin layer cytological sample, a fine needle aspirate sample, a bone marrow sample, a lymph node sample, a urine sample, an ascites sample, a lavage sample, an esophageal brushing sample, a bladder or lung wash sample, a spinal fluid sample, a brain fluid sample, a ductal aspirate sample, a nipple discharge sample, a pleural effusion sample, a fresh frozen tissue sample or a paraffin embedded tissue sample. For example, a patient peripheral blood sample can be initially processed to extract an epithelial cell population, a plasma fraction or a serum fraction, and this extract, plasma fraction or serum fraction can then be assayed. A microdissection of the tissue sample to obtain a cellular sample enriched with suspected tumor cells can also be used. The preferred tissue sample for use herein is whole blood.

Preferably, genotyping involves amplification of a subject's nucleic acid, obtained from said sample, using the polymerase chain reaction (PCR). Use of PCR for the amplification of nucleic acids is well known in the art (*see, e.g.*, Mullis et al. (Eds.), *The Polymerase Chain Reaction*, Birkhäuser, Boston, (1994)). Preferably, when analyzing or genotyping for said SNP, in the step of PCR amplification, an allele-specific, preferably minor groove binding, labeled probe, such as flurorescent VIC or VAM labeled probe, is employed for detecting the SNP. The tissue sample can be processed by any desirable method for performing nucleic acid-based assays. PCR based assays can be performed on a TaqMan 7500 System (Life Technologies) using Abgene Absolute QPCR ROX mix for amplification, and using locus-specific primers for amplification of the target region and allele-specific probes for detection of the SNPs.

Kits

In another aspect, the disclosure provides kits for the measurement of germline polymorphisms that comprise containers containing at least one specific primer, labeled probe, or antibody specific for binding and detecting at least one of the germline polymorphisms in a sample. These kits may also include containers with other associated reagents for the assay. In some embodiments, a kit comprises containers containing a nucleic acid probe for binding to a germline polymorphism and at least one calibrator composition. The kit can further comprise components necessary for detecting a detectable label (e.g., an enzyme or a substrate). The kit can also contain a control sample or a series of control samples which can be assayed and compared to the test sample. Each component of the kit can be enclosed within an individual container and all of the various containers can be within a single package, along with instructions for interpreting the results of the assays performed using the kit.

Table 1: List of SNPs in PD-1 axis genes associated with adverse events upon administration of immune checkpoint inhibitor.

Protein	Gene	SNP	MAF
PD-1	PDCD1		
		Rs11568821	0.0409
		Rs41386349	0.1486
		Rs36084323	0.1516
		6867 C/G	
SHP-2	PTPN11	Rs2301756	0.3734
		Rs12423190	0.1262
		Rs11066301	0.1344
		Rs41279090	0.0365
		Rs12301915	0.0429
		Rs11066320	0.1344
		Rs11066322	0.4826
		Rs3741983	0.4882

ZAP70	ZAP70	Rs17695937	0.1488
		Rs62157588	0.3229
		Rs2278699	0.1488
IFN-γ	IFNG	Rs2430561	0.2802
		Rs1861494	0.2258
PD-L1	CD274	Rs4143815	0.2819
		Rs1411262	0.3852
		Rs822339	0.2752
		Rs2282055	0.2969
		Rs2297136	0.3315
		8923 A/C	
CXCL9	CXCL9	Rs10336	0.2997
		77147452 G/A	
CXCL10	CXCL10	Rs8878	0.3085
		Rs3921	0.3075
		Rs1439490	/
IRF7	IRF7	Rs1131665	0.2756
		Rs1061501	0.1833
AKT1	AKT1	Rs2494752	0.3299
		Rs2498801	0.4900
		Rs1130214	0.2402
		Rs2494732	0.4259
		Rs2498804	0.4305
		Rs1130233	0.3225
		Rs3803304	0.2214
AKT2	AKT2	Rs7254617	0.2001

Table 2: List of SNPs in PD-1 axis genes associated with therapeutic efficacy of treatment with an immune checkpoint inhibitor.

Protein	Gene	SNP	MAF
PD-1	PDCD1	Rs2227981	0.3512
		Rs2227982	0.1368
		Rs11568821	0.0409
		Rs41386349	0.1486
		Rs36084323	0.1516
		Rs10204525	0.3514
		6867 C/G	
SHP-2	PTPN11	Rs2301756	0.3734
		Rs12423190	0.1262
		Rs11066301	0.1344
		Rs41279090	0.0365
		Rs12301915	0.0429
		Rs11066320	0.1344
		Rs11066322	0.4826
ZAP70	ZAP70	Rs3741983	0.4882
		Rs17695937	0.1488
		Rs13420683	0.3664
		Rs62157588	0.3229
IFN-γ	IFNG	Rs2278699	0.1488
		Rs2430561	0.2802
		Rs2069718	0.3832
		Rs2069705	0.4778
PD-L1	CD274	Rs1861494	0.2258
		Rs4143815	0.2819
		Rs1411262	0.3852
		Rs822339	0.2752
CXCL9	CXCL9	Rs2282055	0.2969
		Rs2297136	0.3315
		8923 A/C	
		Rs10336	0.2997
CXCL10	CXCL10	77147452 G/A	
		Rs8878	0.3085
		Rs3921	0.3075
IRF7	IRF7	Rs1439490	/
		Rs1131665	0.2756
AKT1	AKT1	Rs1061501	0.1833
		Rs2494752	0.3299
		Rs2498801	0.4900
		Rs1130214	0.2402
		Rs2494732	0.4259

		Rs2498804	0.4305
		Rs1130233	0.3225
		Rs3803304	0.2214
AKT2	AKT2	Rs7254617	0.2001

5 MAF=minor allele frequency

For the purpose of clarity and a concise description, features are described herein as part of the same or separate aspects and preferred embodiments thereof, however, it will be appreciated that the scope of the invention may include embodiments having combinations of all or some of the features described.

The invention will now be illustrated by the following examples, which are provided by way of illustration and not of limitation and it will be understood that many variations in the methods described and the amounts indicated can be made without departing from the spirit of the invention and the scope of the claims.

The content of the documents referred to herein is incorporated by reference.

20 EXAMPLES

Example 1

Study design

We retrospectively collected data from NSCLC patients who started nivolumab treatment at two large Dutch hospitals (the Erasmus MC Cancer Institute, Rotterdam, and at the Amphia Hospital, Breda) between July 26th 2013 and April 19th 2016. Clinical data was collected until May 19th 2016. Patients from whom whole blood for DNA analysis was prospectively collected were included in the study (local ethics board study number MEC 02-1002).

Patient characteristics were collected from the hospitals electronic patient record systems and included demographic and clinical information (e.g. age at start of treatment, gender, ethnicity, WHO performance status at start of treatment, previous anti-tumor treatments, treatment interruptions, NSCLC subtype, toxicities and time to progression of disease). WHO performance status was determined by judgement of the clinician at the nearest point before start of nivolumab treatment, and was regarded as missing if this point was more than one month before treatment start. Concomitant use of oral or intravenous corticosteroids was also recorded and regarded as a surrogate for serious adverse events, since these drugs are used to resolve immune related toxicities.

Adverse events were registered from start of the treatment until end of follow-up according to the National Cancer Institute Common Terminology Criteria for Adverse Events (NCI-CTCAE) v4.0. Hepatitis was based on the judgement of the clinician. An adverse event was considered pre-existent if it was present in the same or higher degree before treatment start.

Selection of SNPs

We selected 7 SNPs in the *PDCD1*, *PTPN11*, *ZAP70* and *IFNG* genes for analysis (see Table 3 for details). SNPs with a reported minor allele frequency (MAF) above 10% were included.

Table 3. Investigated single nucleotide polymorphisms.

Gene	Protein	rs-number	Variant	Assay ID	WT	HET	HVAR	Undetermined	MAF	HWE*
<i>PDCD1</i>	PD-1	rs2227981	804T>C	c_57931286_20	39	78	43	1	50%	0.76
		rs2227982	644C>T	c_57931287_10	160	1	0	0	0%	0.97
		rs10204525	*889G>A	c_172862_10	131	28	2	0	10%	0.72
<i>PTPN11</i>	SHP-2	rs2301756	333-	c_2978562_20	131	28	2	0	10%	0.72
			223A>G							
<i>ZAP70</i>	ZAP70	rs13420683	-21-	c_1278468_10	83	55	17	6	28%	0.10
			4127C>A							
<i>IFNG</i>	IFN- γ	rs2069718	367-	c_15799728_10	54	70	36	2	44%	0.17
			895T>C							
		rs2069705	-1616C>T	c_15944115_20	75	61	25	0	34%	0.04

Abbreviations: WT, wildtype; HET, heterozygous; HVAR, homozygous variant; MAF, minor allele frequency; HWE, Hardy Weinberg equilibrium

* If <0.05 not consistent with HWE

DNA isolation

Four hundred microliters of whole-blood specimens were collected in EDTA tubes and DNA was extracted in a final elution volume of 200 μ L using the MagNAPure Compact instrument (Roche Diagnostics GmbH, Mannheim, Germany) and the Nucleic Acid Isolation Kit I (Roche Diagnostics GmbH).

Taqman genotyping

Genotyping was done using predesigned DME Taqman allelic discrimination assays on the Life Technologies Taqman 7500 system (Applied Biosystems, Life Technologies Europe BV, Bleiswijk, the Netherlands; Table 3). Each assay consisted of two allele-specific minor groove binding (MGB) probes, labeled with the fluorescent dyes VIC and FAM. Polymerase chain reactions (PCR) were performed in a reaction volume of 10 μ l, containing assay-specific primers, allele-specific Taqman MGB probes (Applied Biosystems), Abgene Absolute QPCR ROx Mix (Thermo Scientific, Life Technologies Europe BV, Bleiswijk, The Netherlands) and genomic DNA (20 ng). The thermal profile consisted of 40 cycles of denaturation at 95 °C for 20 seconds, annealing at 92 °C for 3 seconds and extension at 60 °C for 30 seconds. Genotypes were scored by measuring allele-specific fluorescence using the 7500 software v2.3 for allelic discrimination (Applied Biosystems).

Statistics

Distribution of genotypes was tested for Hardy-Weinberg equilibrium (HWE) using the Chi-square test (Table 3). Since *IFNG* -1616C>T was not in HWE, this SNP was excluded from further analyses. SNPs with a MAF <1% in our cohort were also excluded from further analyses, which was the case for *PDCD1* 644C>T. Linkage disequilibrium (LD) analyses were performed using SNAP.¹⁸ Because none of the analyzed

SNP pairs met our preset criteria for LD ($R^2 > 0.8$), all SNPs were analyzed individually. For every SNP, the best fitting model (that is, the model resulting in the best association) was selected from four, i.e. a dominant, recessive, additive and multiplicative model.¹⁹ The dominant and recessive model were used to test associations between SNPs with toxicity, steroid use and temporary or definitive treatment discontinuation due to toxicity using the Chi-square test or, in case one of the observed numbers was ≤ 10 or one of the expected numbers was < 5 , with Fisher's exact test. The additive and multiplicative models were used to test the SNPs in logistic regression as ordinal and linear variables, respectively. If a SNP was associated with the toxicity with $p < 0.1$ and the associated adverse event occurred in approximately 30 or more patients, this SNP was entered in a multivariable logistic regression model corrected for age and gender. SPSS software v21 (SPSS, Chicago, IL, USA) was used for the above-mentioned analyses. A two-sided $p < 0.05$ was regarded as significant. This was an exploratory study and no correction for multiple testing was applied.

Results

Patient characteristics

Blood samples for DNA-analysis were available for 161 patients, of whom 65% (n=105) was male, mean age at start was 65 years and 60% (n=96) had a WHO performance status of 1 at start. More baseline characteristics are depicted in Table 4. All patients were treated with the standard dose of nivolumab at 3 mg/kg Q2W, which could not be changed during treatment. Median absolute starting dose was 222 mg (IQR 189-258 mg) and median duration of follow-up was 98 days (IQR 53-169 days).

Table 4. Patient characteristics at baseline

	Number of patients (n=161)
Gender	
Male	105 (65%)
Female	56 (35%)
Age at start (years)	
Mean ($\pm$ SD)	65 ($\pm$ 8.4)
WHO performance status	
0	29 (18%)
1	96 (60%)
2	4 (3%)
3	1 (1%)
Unknown	31 (19%)
Primary lung tumor	
Adenocarcinoma	92 (57%)
Squamous cell carcinoma	51 (32%)
Great cell carcinoma	16 (10%)
Unspecified	2 (1%)
Number of pre-treatment lines*	
1	97 (60%)
2	46 (29%)
>2	17 (11%)
Unknown	1 (1%)
Ethnicity	
Caucasian	155 (96%)
Eastern	1 (1%)
Unknown	5 (3%)

* All patients had prior treatment with a platinum containing regimen

Adverse events

Thirty-one patients (19%) had drug-related grade ≥ 3 toxicities. When analyzing all-grade toxicity that was not pre-existent, hypothyroidism or hyperthyroidism (n=65; 40%), elevated liver enzymes (n=54; 34%),
 5 elevated creatinine (n=28; 16%) and skin toxicity (n=28; 16%) occurred most frequently (Table 5). These drug-related grade ≥ 3 toxicities encompassed diarrhea, pruritus, fatigue, anemia, increased aspartate aminotransferase, pneumonitis, interstitial lung disease, dyspnea, colitis, constipation, dry
 10 skin, peripheral motor neuropathy, peripheral sensory neuropathy, fever, anaphylaxis and hepatic infection. Fifty-eight patients (36%) required oral or intravenous steroids during nivolumab treatment. Twenty patients (12%) temporarily interrupted nivolumab treatment because of toxicities, whereas five patients (3%) had to stop treatment definitively because of toxicities.

15 **Table 5.** Frequencies of toxicity endpoints corrected for pre-existence

	Number of patients N=161
Any grade toxicity	
Diarrhea	8 (5%)
Skin toxicity	28 (16%)
Elevated liver enzymes	54 (34%)
Hepatitis	7 (4%)
Creatinine elevation	28 (16%)
Hypothyroidism or hyperthyroidism	65 (40%)
Pneumonitis/Interstitial lung disease	8 (5%)
Neuropathy	19 (12%)
Colitis	5 (3%)
Rheumatological	12 (7%)
Hypophysitis	1 (1%)
Any grade 3 or higher toxicity	31 (19%)

Immune related grade 3 or higher toxicity	11 (7%)
Steroid use	58 (36%)
Treatment stop caused by toxicity	
Temporary interruption	20 (12%)
Definitive discontinuation	5 (3%)

Treatment details

At data cut-off, 85 patients (53%) were still receiving nivolumab. A total of 35 patients (22%) stopped because of clinical deterioration, 33 patients (20%) stopped after progressive disease as assessed by radiological imaging, five patients (3%) stopped because of treatment-related toxicities and three patients (2%) stopped because of other reasons, i.e. due to patients' request (n=2) or a non-measurable lesion after one treatment cycle (n=1). Eight patients (5%) were lost-to-follow up.

Association of SNPs with toxicity during nivolumab treatment

For all outcomes that occurred in a sufficient amount of patients for multivariable analysis and with an association of $p < 0.1$ in relation to the concerning SNP in univariable analysis, results are described in Table 6.

Since diarrhea, hepatitis, pneumonitis, neuropathy, colitis, rheumatological toxicity, immune related grade ≥ 3 toxicity and temporary and definitive treatment discontinuation caused by toxicity occurred in insufficient patients for reliable multivariable analysis, these outcomes were excluded from analysis. Skin toxicity, elevated liver enzymes, any grade ≥ 3 adverse events and steroid use were studied in multivariable analysis. Patients with a polymorphism at *PTPN11* 333-223 (rs2301756) had increased odds for developing any grade ≥ 3 adverse event per mutant G-allele (OR 2.4; CI 1.0 - 5.7; $p=0.045$). At least one variant allele at the same position was associated with increased odds for skin toxicity (OR 2.7; CI 1.0 - 7.0; $p=0.048$) and for elevated liver enzymes (OR 2.9; CI 1.2 - 7.1; $p=0.018$). The strong

relationship between steroid use and *IFNG* 367-895 T>C in univariable analysis did not hold in multivariable analysis (OR 0.5; CI 0.2-1.1; $p=0.093$). Furthermore, female patients had an increased risk in developing elevated liver enzymes when compared with male subjects (OR 2.4; CI 1.1 - 5.1; $p=0.025$).

Table 6. Association between SNPs with $p < 0.1$ in the univariable analysis and endpoints.

Endpoint	Factor	Genotype	Univariable		Multivariable	
			OR (95% CI)	p-value	OR (95% CI)	p-value
Skin toxicity	Age				0.993 (0.945-1.043)	0.775
	Gender (f. vs. m.)				1.263 (0.510-3.125)	0.614
	<i>PTPN11</i> 333-223A>G	GG + AG vs. AA	2.436 (0.970-6.117)	0.065†	2.650 (1.009-6.956)	0.048
Elevated liver enzymes (any grade)	Age				0.972 (0.931-1.014)	0.187
	Gender (f. vs. m.)				2.387 (1.114-5.113)	0.025
	<i>PTPN11</i> 333-223A>G	GG + AG vs. AA	2.256 (1.005-5.067)	0.045	2.922 (1.200-7.115)	0.018
	<i>ZAP70</i> -21-4127C>A	CC + CA vs. AA	4.211 (1.292-13.723)	0.016†	2.643 (0.885-7.890)	0.082
Grade ≥ 3 toxicity	Age				0.999 (0.952-1.048)	0.966
	Gender (f. vs. m.)				1.814 (0.771-4.270)	0.173
	<i>PTPN11</i> 333-223A>G	AA $\rightarrow$ AG $\rightarrow$ GG*	2.050 (0.915-4.595)	0.081	2.415 (1.021-5.715)	0.045
Steroid use	Age				1.003 (0.963-1.044)	0.895
	Gender (f. vs. m.)				1.126 (0.556-2.279)	0.742
	<i>IFNG</i> 367-895T>C	TT + TC vs. CC	0.454 (0.191-1.079)	0.076†	0.468 (0.193-1.134)	0.093

Abbreviations: OR, odds ratio; CI, confidence interval; f. vs. m., females versus males.

*Additive model was used. † Fisher's exact test was used. Chi-square-test was used for all other univariable analyses.

Discussion

In this study, the SNP *PTPN11* 333-223A>G (rs2301756) was associated with nivolumab toxicity. At least one variant allele at this locus was associated with increased odds for any grade ≥ 3 adverse event, for skin toxicity and for liver enzyme elevation. Earlier, the variant allele has already been documented to be associated with an increased risk for gastric atrophy after *H. Pylori* infection, possibly caused by chronic inflammation,¹⁷ and with susceptibility for ulcerative colitis in the Japanese population.²⁰ These findings support the notion that genetic variants in the SHP-2 gene (*PTPN11*) prevent T cell suppression and do result in stronger immune reactions, in particular when there is additional inhibition of PD-1 function via nivolumab.

Contradictory to our findings regarding *PTPN11*, SNPs in *PDCD1* and *IFNG*, which mutually affect each other's expression were not associated with outcomes. The investigated SNP in *ZAP70* was neither associated with toxicity, possibly due to the influence of other adjacent pathways on *ZAP70*. Interestingly, our results also show that female patients have an increased odds in experiencing liver enzyme elevation during nivolumab treatment.

Besides toxicity, another primary question would be to explore if SNPs are also associated with survival. Because median survival was not reached in our study population, we were not able yet to look at progression free or overall survival. Although germline genetics are a logical biomarker for general systemic effects, such as adverse events, and although (local) antitumor effects are also influenced by somatic mutations and mutational load,²¹ prior treatment and possibly even the microbiome,²² the current lack of predictive biomarkers for response necessitates the community to look beyond intratumoral factors, such as PD-L1 expression or mutational load. Once our data have matured, we will perform the survival analyses in our cohort. On a larger scale, it would be interesting to have the survival (and

toxicity) data from the KEYNOTE and CheckMate trials tested for its association with germline genetic polymorphisms.

Given the explorative design of this study and given the retrospective data collection, probably resulting in a slightly different low-grade toxicity profile than reported previously,²³ the current results should be interpreted cautiously and require validation in a similar cohort. Furthermore, some endpoints, e.g. colitis, occurred too infrequent to be assessed in multivariable analysis, which requires about ten patients per assessed variable. The current genotyping effort should not be considered comprehensive and even though it is likely that other factors may contribute to the occurrence of adverse events, e.g. other ‘omics’, other genes or even other SNPs in the investigated genes, the association we found between the SNP in *PTPN11* and various adverse events – most importantly any grade ≥ 3 events – provides a rationale for further research into the role of SNPs in the PD-1 pathway, and SHP-2 in particular.

Conclusions

This study, adds another candidate to the potential biomarker spectrum for nivolumab treatment outcome. In case our findings are validated, a single nucleotide polymorphism in *PTPN11* (rs2301756) is associated with the occurrence of adverse events during nivolumab treatment. This implies that research in biological understandings of adverse events should not only focus on intratumoral characteristics, but also on germline genetic variations.

The data gathered from our studies demonstrate that the markers are predictive for toxicity in first and second line therapy using immune checkpoint inhibitors, such as nivolumab and the related compound pembrolizumab, and provide useful information for classification of patients as likely or unlikely to suffer from immune checkpoint inhibitor-induced toxicity and selection of likely or unlikely candidates for therapeutic

intervention with such compounds. These germline polymorphisms can be incorporated into existing and future clinical trials such as, for example, in patients with NSCLC and will allow for further validation and refinement of their utility as markers for determining therapy eligibility of patients as well as predictive markers for suffering from toxic side effects associated with treatment with immune checkpoint inhibitor compounds such as nivolumab.

Example 2: Association of single nucleotide polymorphism with efficacy in nivolumab-treated NSCLC patients.

Introduction

Proper patient selection for PD-1 checkpoint inhibitors is crucial given its limited efficacy in the majority of patients. In Example 1, it is shown that a single nucleotide polymorphism in the auto-immunity (AI) related *PTPN11* gene (rs2301756) is associated with increased toxicity on nivolumab administration. The objective of the current analysis was to assess whether an SNP in *PTPN11* is correlated with treatment efficacy of nivolumab in NSCLC patients.

Methods

The association between rs2301756 and nivolumab treatment efficacy was evaluated in 161 advanced NSCLC patients, after being treated with standard of care chemotherapy. Efficacy measures included early progressive disease (PD; ≤ 90 days after start of nivolumab therapy), tumor response according to Response Evaluation Criteria In Solid Tumors (RECIST) v1.1, progression free survival (PFS) and overall survival (OS). The model resulting in the best association between a SNP and an endpoint, being either a dominant, recessive, multiplicative or additive model, was selected. An SNP was analyzed in multivariable logistic regression models if

it showed a p-value <0.1 in univariable analysis, and was corrected for age and gender. PFS and OS analysis was done using the Kaplan-Meier method.

Results

- 5 Overall, 54 patients showed a Partial Response (PR) or Complete Response (CR) according to RECIST. Patients with at least one variant allele in *PTPN11* had a higher response rate compared to wild-type (50% vs 30%; OR 2.4; 95% CI 1.0 – 5.5; $p = 0.042$).

10 Conclusion

- Our results show that the SNP in *PTPN11* (rs2301756) predisposes for a higher response rate to nivolumab therapy. Together with the earlier finding that this SNP is associated with grade ≥ 3 toxicity, this may indicate that SHP-2 activity influences treatment outcome of anti-PD-1 therapy in
- 15 terms of both toxicity and anti-tumor effects. SNPs in *PTPN11* might thus be considered to be included as a biomarker in routine analysis of NSCLC patients to be treated with nivolumab.

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Claims

1. A method for genotyping a patient suffering from cancer for having a predisposition to a deviant biological response associated with immune checkpoint inhibitor therapy which therapy comprises the administration of an immune checkpoint inhibitor that inhibits PD-1-mediated and/or CTLA-4-mediated signaling in T-cells of said patient, said method for genotyping comprising comparing the germline sequence of a PD-1 axis protein gene or the sequence of its RNA in a sample from said patient with the germline sequence of the wild-type gene or the sequence of its RNA, wherein an alteration in the germline sequence of the PD-1 axis protein gene or the sequence of its RNA of the patient indicates a genotype having said predisposition to a deviant biological response.
2. Method according to claim 1, wherein said deviant biological response is an altered therapeutic effect, preferably an increased or reduced therapeutic efficacy of the immune checkpoint inhibitor.
3. Method according to claim 1, wherein said deviant biological response is an adverse event associated with immune checkpoint inhibitor therapy.
4. Method according to claim 3, wherein said adverse event is selected from the group consisting of diarrhea, nausea, pruritus, rash acneiform, rash maculo-papular, erythroderma, papulopustular rash, toxic epidermal necrolysis, anorexia, fatigue, hyperkalemia, hypokalemia, hyponatremia, hypomagnesemia, hypercalcemia, hypocalcemia, decreased platelet count, decreased white blood cell count, decreased lymphocyte count, anemia, increased alanine aminotransferase, increased alkaline phosphatase, increased aspartate aminotransferase, increased lipase,

increased serum amylase, increased creatinine, hypertension, upper respiratory infection, pneumonitis, interstitial lung disease, dyspnea, cough, mucositis oral, dry mouth, vomiting, colitis, abdominal pain, constipation, infusion related reaction, dry skin, skin hypopigmentation, alopecia,

5 headache, dizziness, peripheral motor neuropathy, peripheral sensory neuropathy, dry eye, blurred vision, myalgia, arthralgia, bone pain, back pain, pain in extremity, non-cardiac chest pain, jaw pain, vertebral pain, polymyalgia rheumatic, guillain-barre syndrome, demyelination, fever, edema (any anatomical site), weight loss, hyperthyroidism, hypothyroidism,

10 hyperglycemia, hypermagnesemia, hypernatremia, increased blood bilirubin, decreased neutrophil count, anaphylaxis, sinus tachycardia, lung infection, bronchial infection, pleural effusion, pancreatitis, hepatic infection, nephritis, chronic kidney disease, uveitis, urticaria, erythema multiforme, psoriasis, rosacea, vasculitis, arthritis, cardiac chest pain,

15 adrenal insufficiency, autoimmune disorder (hypopituitarism, hypophysitis, thyreoditis), acidosis, dehydration, ventricular arrhythmia, atrial fibrillation, gastritis, duodenal ulcer, and cholestasis, preferably selected from the group consisting of, diarrhea, pruritus, fatigue, anemia, increased aspartate aminotransferase, pneumonitis, interstitial lung disease, dyspnea,

20 colitis, constipation, dry skin, peripheral motor neuropathy, peripheral sensory neuropathy, fever, anaphylaxis and hepatic infection.

5. Method according to any one of the preceding claims, wherein said PD-1 axis protein gene is a gene encoding a protein selected from the group

25 consisting of PD-1, SHP-2, ZAP70, IFN- γ , PD-L1, CXCL9, CXCL10, IRF7, AKT1, AKT2, preferably wherein said PD-1 axis protein gene is selected from the group consisting of *PDCD1*, *PTPN11*, *ZAP70*, *IFNG*, *CD274*, *CXCL9*, *CXCL10*, *IRF7*, *AKT1*, and *AKT2*.

6. Method according to any one of the preceding claims, wherein said alteration in the germline sequence is indicated by the presence of a polymorphic site selected from the group of consisting of SNPs Rs2227981, Rs2227982, Rs11568821, Rs41386349, Rs36084323, Rs10204525, and 6867
5 C/G in *PDCD1*; Rs2301756, Rs12423190, Rs11066301, Rs41279090, Rs12301915, Rs11066320, Rs11066322, and Rs3741983 in *PTPN11*; Rs17695937, Rs13420683, Rs62157588, and Rs2278699 in *ZAP70*; Rs2430561, Rs2069718, Rs2069705, and Rs1861494 in *IFNG*; Rs4143815, Rs1411262, Rs822339, Rs2282055, Rs2297136, and 8923 A/C in *CD274*;
10 Rs10336, and 77147452 G/A in *CXCL9*; Rs8878, Rs3921, and Rs1439490 in *CXCL10*; Rs1131665, and Rs1061501 in *IRF7*; Rs2494752, Rs2498801, Rs1130214, Rs2494732, Rs2498804, Rs1130233, and Rs3803304 in *AKT1*; and Rs7254617 in *AKT2*, preferably said polymorphic site is SNP rs2301756 in *PTPN11*.

15

7. Method according to any one of the preceding claims, wherein said immune checkpoint inhibitor is an anti-PD-1/PD-L1 mAb or anti-CTLA-4 mAB, preferably an anti-PD-1/PD-L1 mAb selected from the group consisting of nivolumab, pembrolizumab, JS001, TSR-042, pidilizumab (CT-
20 011, MDV9300), AMP-224, REGN2810, JNJ-63723283, PDR001, BGB-A317, SHR-1210, MEDI068, atezolizumab, durvalumab, BMS-936559, LY3300054; avelumab, KN035, CA-170 and SHR-1210, more preferably selected from nivolumab and pembrolizumab, most preferably nivolumab, preferably an anti-CTLA-4 mAb selected from the group consisting of ipilimumab, ONC
25 392 and tremelimumab.

8. Method according to any one of the preceding claims, wherein the presence of the germline alteration is indicative of a genotype having a predisposition to suffer from adverse events associated with immune

checkpoint inhibitor therapy and/ or improved therapeutic efficacy of the immune checkpoint inhibitor.

9. Method according to claim 6, wherein said polymorphic site is
5 SNP rs2301756 in *PTPN11*.

10. Method according to claim 9, wherein said deviant biological response is an increased therapeutic efficacy of the immune checkpoint inhibitor or an adverse event associated with immune checkpoint inhibitor
10 therapy.

11. Method according to claim 10, wherein said immune checkpoint inhibitor is nivolumab.

15 12. An immune checkpoint inhibitor as defined in any one of the preceding claims, for use in the treatment of cancer in a patient, wherein the treatment aims to avoid a deviant biological response associated with immune checkpoint inhibitor therapy in said patient, and wherein said patient does not have an alteration in the germline sequence of a PD-1 axis
20 protein gene or the sequence of its RNA, as determinable by a method of any one of claims 1-11.

13. An immune checkpoint inhibitor as defined in any one of the preceding claims, for use in the treatment of cancer in a patient, wherein
25 the treatment aims to avoid a deviant biological response associated with immune checkpoint inhibitor therapy in said patient, wherein said patient has an alteration in the germline sequence of a PD-1 axis protein gene or the sequence of its RNA, as determinable by a method of any one of claims 1-11, preferably wherein said treatment involves a modified dosage regimen
30 of less than 0.1-2.5 mg/kg body weight every 2-4 weeks, or more than 3,

preferably between 3.5-5 mg/kg body weight every one or two weeks of the immune checkpoint inhibitor.

14. Use of a kit of parts in a method according to any one of claims 1-
5 11, said kit of parts comprising at least one pair of single-stranded PCR
primers for amplifying a sequence of a gene, an expression product, or part
thereof, wherein said gene is selected from the group consisting of *PDCD1*,
PTPN11, *ZAP70*, *IFNG*, *CD274*, *CXCL9*, *CXCL10*, *IRF7*, *AKT1*, and *AKT2*;
and wherein amplified gene, product or part includes the position or locus of
10 the SNPs as defined in claim 6; wherein said kit optionally further
comprising a labelled oligonucleotide probe for detecting said SNP, wherein
said kit optionally further comprises instructions for performing detection of
said SNP.

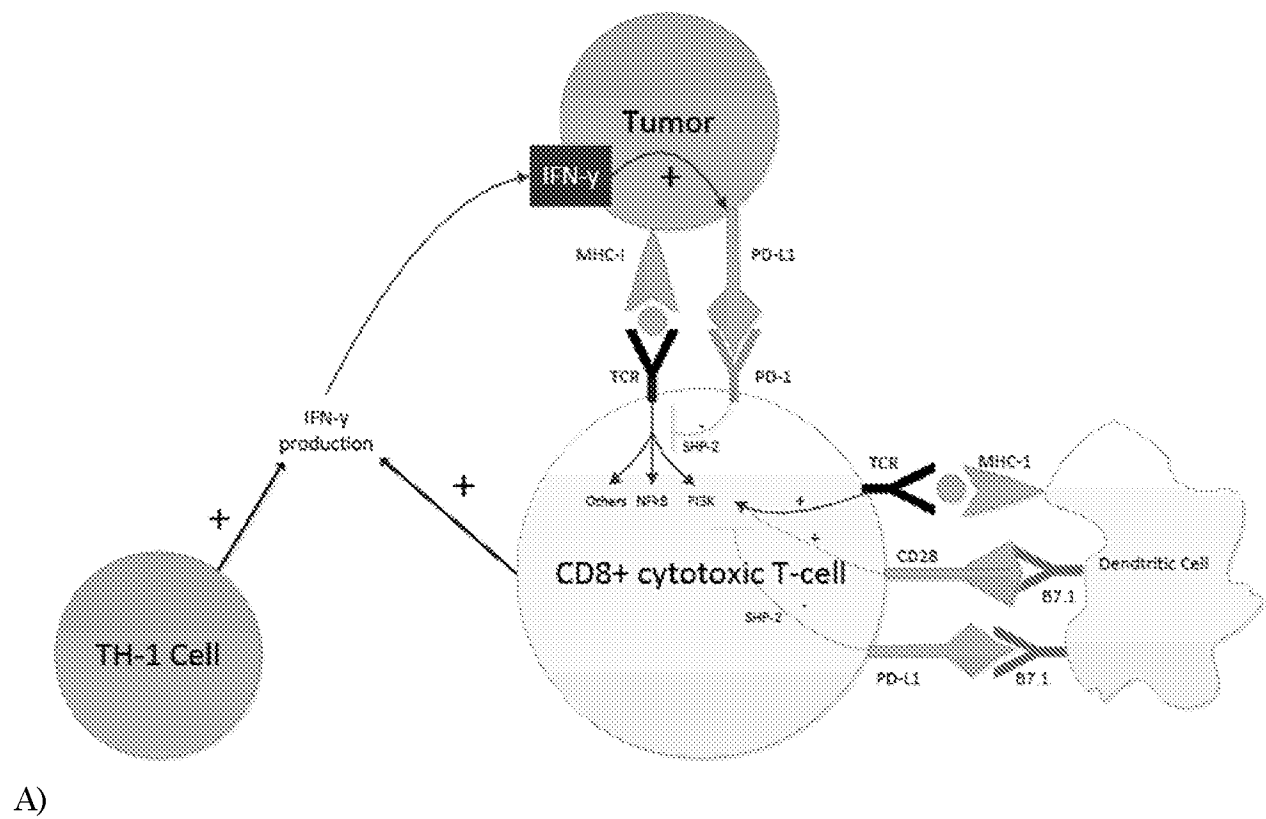
15 15. Standard of care therapeutic for use in the treatment of cancer in
a patient, wherein the patient suffers from a deviant biological response
associated with immune checkpoint inhibitor therapy in said patient,
wherein said patient has an alteration in the germline sequence of a PD-1
axis protein gene or the sequence of its RNA, as determinable by a method
20 of any one of claims 1-11.

16. A method of treating a patient suffering from cancer and eligible
for immune checkpoint inhibitor therapy which therapy comprises the
administration of an immune checkpoint inhibitor that inhibits PD-1-
25 mediated and/or CTLA-4-mediated signaling in T-cells of said patient, said
method comprising the steps of:

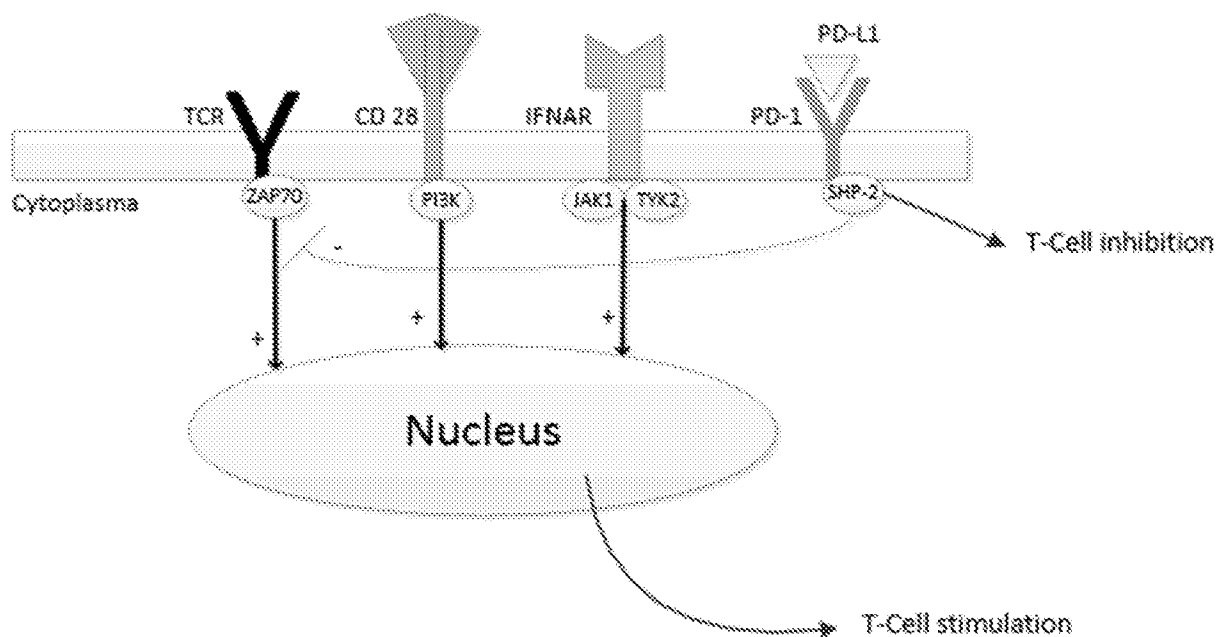
a) genotyping said patient suffering from cancer for a predisposition
to a deviant biological response associated with immune checkpoint
inhibitor therapy by performing a method according to any one of claims 1-
30 11; and

b1) commencing or continuing administration of an immune checkpoint inhibitor to said patient using a standard dosage regime in case the patient does not have a predisposition to a deviant biological response, or

- 5 b2) discontinuing immune checkpoint inhibitor administration, modifying (e.g. reducing or increasing) the dosage regimen of immune checkpoint inhibitor administration, and/or administering a standard of care therapeutic other than an immune checkpoint inhibitor to treat said patient, in case the patient has a predisposition to a deviant biological
- 10 response.



A)



B)

Figure 1

INTERNATIONAL SEARCH REPORT

International application No
PCT/NL2017/050710

A. CLASSIFICATION OF SUBJECT MATTER
INV. C12Q1/68 A61K39/395
ADD.

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

B. FIELDS SEARCHED

Minimum documentation searched (classification system followed by classification symbols)
C12Q A61K

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

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

EPO-Internal, BIOSIS, CAB Data, Sequence Search, WPI Data

C. DOCUMENTS CONSIDERED TO BE RELEVANT

Category*	Citation of document, with indication, where appropriate, of the relevant passages	Relevant to claim No.
A	NIELSEN C ET AL: "Association of a putative regulatory polymorphism in the PD-1 gene with susceptibility to type 1 diabetes", TISSUE ANTIGENS, vol. 62, no. 6, 1 December 2003 (2003-12-01), pages 492-497, XP002991620, MUNKSGAARD, COPENHAGEN, DK ISSN: 0001-2815, DOI: 10.1046/J.1399-0039.2003.00136.X the whole document ----- -/-	1-13,15, 16



Further documents are listed in the continuation of Box C.



See patent family annex.

* Special categories of cited documents :

"A" document defining the general state of the art which is not considered to be of particular relevance

"E" earlier application or patent but published on or after the international filing date

"L" document which may throw doubts on priority claim(s) or which is cited to establish the publication date of another citation or other special reason (as specified)

"O" document referring to an oral disclosure, use, exhibition or other means

"P" document published prior to the international filing date but later than the priority date claimed

"T" later document published after the international filing date or priority date and not in conflict with the application but cited to understand the principle or theory underlying the invention

"X" document of particular relevance; the claimed invention cannot be considered novel or cannot be considered to involve an inventive step when the document is taken alone

"Y" document of particular relevance; the claimed invention cannot be considered to involve an inventive step when the document is combined with one or more other such documents, such combination being obvious to a person skilled in the art

"&" document member of the same patent family

Date of the actual completion of the international search

18 January 2018

Date of mailing of the international search report

26/03/2018

Name and mailing address of the ISA/

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Authorized officer

Hornig, Horst

INTERNATIONAL SEARCH REPORT

International application No

PCT/NL2017/050710

C(Continuation). DOCUMENTS CONSIDERED TO BE RELEVANT		
Category*	Citation of document, with indication, where appropriate, of the relevant passages	Relevant to claim No.
A	PROKUNINA L ET AL: "A regulatory polymorphism in PDCD1 is associated with susceptibility to systemic lupus erythematosus in humans", NATURE GENETICS, vol. 32, no. 4, 20 December 2002 (2002-12-20), pages 666-669, XP002243477, NATURE PUBLISHING GROUP, NEW YORK, US ISSN: 1061-4036, DOI: 10.1038/NG1020 the whole document	1-13,15, 16
A	WO 03/022875 A2 (EVERYGENE AB [SE]; ALARCON-RIQUELME MARTA EUGENIA [SE]; PROKUNINA LUDM) 20 March 2003 (2003-03-20) the whole document	1-13,15, 16
A	WO 2016/040313 A2 (DANA FARBER CANCER INST INC [US]) 17 March 2016 (2016-03-17) the whole document	1-13,15, 16
A	WO 2005/089080 A2 (ALARCON-RIQUELME MARTA E [SE]; PROKUNINA LUDMILA [SE]; EVERYGENE AB [S]) 29 September 2005 (2005-09-29) the whole document	1-13,15, 16
A	EP 0 670 369 A2 (ONO PHARMACEUTICAL CO [JP]; HONJO TASUKU [JP]) 6 September 1995 (1995-09-06) the whole document	1-13,15, 16
A	ISHIDA Y ET AL: "INDUCED EXPRESSION OF PD-1, A NOVEL MEMBER OF THE IMMUNOGLOBULIN GENE SUPERFAMILY, UPON PROGRAMMED CELL DEATH", EMBO JOURNAL, vol. 11, no. 11, 1 January 1992 (1992-01-01), pages 3887-3895, XP002070368, OXFORD UNIVERSITY PRESS, SURREY, GB ISSN: 0261-4189 the whole document	1-13,15, 16
A	BU XIA ET AL: "Learning from PD-1 Resistance: New Combination Strategies.", TRENDS IN MOLECULAR MEDICINE, vol. 22, no. 6, June 2016 (2016-06), pages 448-451, XP002771339, ISSN: 1471-499X the whole document	1-13,15, 16
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INTERNATIONAL SEARCH REPORT

International application No

PCT/NL2017/050710

C(Continuation). DOCUMENTS CONSIDERED TO BE RELEVANT

Category*	Citation of document, with indication, where appropriate, of the relevant passages	Relevant to claim No.
A	O'DONNELL JAKE S ET AL: "Acquired resistance to anti-PD1 therapy: checkmate to checkpoint blockade?", GENOME MEDICINE, vol. 8, no. 1, 111, 25 October 2016 (2016-10-25), pages 1-3, XP002771340, ISSN: 1756-994X, DOI: 10.1186/s13073-016-0365-1 the whole document	1-13,15, 16
A	PITT JONATHAN M ET AL: "Resistance Mechanisms to Immune-Checkpoint Blockade in Cancer: Tumor-Intrinsic and -Extrinsic Factors.", IMMUNITY 21 JUN 2016, vol. 44, no. 6, 21 June 2016 (2016-06-21), pages 1255-1269, XP002771341, ISSN: 1097-4180 the whole document	1-13,15, 16
A	TOPALIAN SUZANNE L ET AL: "Mechanism-driven biomarkers to guide immune checkpoint blockade in cancer therapy.", NATURE REVIEWS. CANCER MAY 2016, vol. 16, no. 5, 15 April 2016 (2016-04-15), pages 275-287, XP002771342, ISSN: 1474-1768 the whole document	1-13,15, 16
A	HE JIABEI ET AL: "Development of PD-1/PD-L1 Pathway in Tumor Immune Microenvironment and Treatment for Non-Small Cell Lung Cancer.", SCIENTIFIC REPORTS, vol. 5, 13110, 17 August 2015 (2015-08-17), pages 1-9, XP002771343, ISSN: 2045-2322, DOI: 10.1038/srep13110 the whole document	1-13,15, 16
A	NOBUYUKI HAMAJIMA ET AL: "Genetic factors involved in the development of Helicobacter pylori-related gastric cancer", CANCER SCIENCE, vol. 97, no. 11, 1 November 2006 (2006-11-01), pages 1129-1138, XP055442097, JP ISSN: 1347-9032, DOI: 10.1111/j.1349-7006.2006.00290.x cited in the application the whole document	1-13,15, 16

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INTERNATIONAL SEARCH REPORT

International application No

PCT/NL2017/050710

C(Continuation). DOCUMENTS CONSIDERED TO BE RELEVANT		
Category*	Citation of document, with indication, where appropriate, of the relevant passages	Relevant to claim No.
A	YUKIKO NARUMI ET AL: "Polymorphisms of PTPN11 Coding SHP-2 as Biomarkers for Ulcerative Colitis Susceptibility in the Japanese Population", JOURNAL OF CLINICAL IMMUNOLOGY, KLUWER ACADEMIC PUBLISHERS-PLENUM PUBLISHERS, NE, vol. 29, no. 3, 22 January 2009 (2009-01-22), pages 303-310, XP019680640, ISSN: 1573-2592 cited in the application the whole document	1-13,15, 16
A,P	----- GAILLARD STÉPHANIE L ET AL: "The role of immune checkpoint inhibition in the treatment of ovarian cancer.", GYNECOLOGIC ONCOLOGY RESEARCH AND PRACTICE, vol. 3, 11, 24 November 2016 (2016-11-24), pages 1-14, XP002771344, DOI: 10.1186/s40661-016-0033-6 the whole document	1-13,15, 16
A,P	----- IWAI YOSHIKO ET AL: "Cancer immunotherapies targeting the PD-1 signaling pathway.", JOURNAL OF BIOMEDICAL SCIENCE, vol. 24, no. 1, 26, 4 April 2017 (2017-04-04), pages 1-11, XP002771345, ISSN: 1423-0127, DOI: 10.1186/s12929-017-0329-9 the whole document	1-13,15, 16
A,P	----- JAREDR LOWE ET AL: "Genetic risk analysis of a patient with fulminant autoimmune type 1 diabetes mellitus secondary to combination ipilimumab and nivolumab immunotherapy", JOURNAL FOR IMMUNOTHERAPY OF CANCER, BIOMED CENTRAL LTD, LONDON, UK, vol. 4, no. 1, 20 December 2016 (2016-12-20), pages 1-8, XP021241416, DOI: 10.1186/S40425-016-0196-Z the whole document	1-13,15, 16
X,P	----- TAKASHI NOMIZO ET AL: "Clinical Impact of Single Nucleotide Polymorphism in PD-L1 on Response to Nivolumab for Advanced Non-Small-Cell Lung Cancer Patients", SCIENTIFIC REPORTS, vol. 7, 23 March 2017 (2017-03-23), page 45124, XP055442142, DOI: 10.1038/srep45124 the whole document	1-11

INTERNATIONAL SEARCH REPORT

International application No.
PCT/NL2017/050710

Box No. II Observations where certain claims were found unsearchable (Continuation of item 2 of first sheet)

This international search report has not been established in respect of certain claims under Article 17(2)(a) for the following reasons:

1. ☐ Claims Nos.:
because they relate to subject matter not required to be searched by this Authority, namely:
2. ☐ Claims Nos.:
because they relate to parts of the international application that do not comply with the prescribed requirements to such an extent that no meaningful international search can be carried out, specifically:
3. ☐ Claims Nos.:
because they are dependent claims and are not drafted in accordance with the second and third sentences of Rule 6.4(a).

Box No. III Observations where unity of invention is lacking (Continuation of item 3 of first sheet)

This International Searching Authority found multiple inventions in this international application, as follows:

see additional sheet

1. ☐ As all required additional search fees were timely paid by the applicant, this international search report covers all searchable claims.
2. ☐ As all searchable claims could be searched without effort justifying an additional fees, this Authority did not invite payment of additional fees.
3. ☐ As only some of the required additional search fees were timely paid by the applicant, this international search report covers only those claims for which fees were paid, specifically claims Nos.:
4. ☒ No required additional search fees were timely paid by the applicant. Consequently, this international search report is restricted to the invention first mentioned in the claims; it is covered by claims Nos.:

1-13, 15, 16

Remark on Protest

- ☐ The additional search fees were accompanied by the applicant's protest and, where applicable, the payment of a protest fee.
- ☐ The additional search fees were accompanied by the applicant's protest but the applicable protest fee was not paid within the time limit specified in the invitation.
- ☐ No protest accompanied the payment of additional search fees.

INTERNATIONAL SEARCH REPORT

Information on patent family members

International application No

PCT/NL2017/050710

Patent document cited in search report	Publication date	Patent family member(s)	Publication date
WO 03022875	A2	20-03-2003	AU 2002362273 A1 24-03-2003
		EP 1425421 A2 09-06-2004	
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		CN 1113518 A 20-12-1995	
		EP 0670369 A2 06-09-1995	
		JP H07291996 A 07-11-1995	
		JP 2003230395 A 19-08-2003	
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		US 5629204 A 13-05-1997	
		US 5698520 A 16-12-1997	

FURTHER INFORMATION CONTINUED FROM PCT/ISA/ 210

This International Searching Authority found multiple (groups of) inventions in this international application, as follows:

1. claims: 1-13, 15, 16

A method for genotyping a patient suffering from cancer for having a predisposition to a deviant biological response associated with immune checkpoint inhibitor therapy [which therapy comprises the administration of an immune checkpoint inhibitor that inhibits PD-1-mediated and/or CTLA 4-mediated signaling in T-cells of said patient], said method for genotyping comprising comparing the germline sequence of a PD-1 axis protein gene or the sequence of its RNA in a sample from said patient with the germline sequence of the wild-type gene or the sequence of its RNA, wherein an alteration in the germline sequence of the PD-1 axis protein gene or the sequence of its RNA of the patient indicates a genotype having said predisposition to a deviant biological response. An immune checkpoint inhibitor as defined in any one of the preceding claims, for use in the treatment of cancer in a patient, wherein the treatment aims to avoid a deviant biological response associated with immune checkpoint inhibitor therapy in said patient, and wherein said patient does not have and/or has an alteration in the germline sequence of a PD-1 axis protein gene or the sequence of its RNA. A standard of care therapeutic for use in the treatment of cancer in a patient, wherein the patient suffers from a deviant biological response associated with immune checkpoint inhibitor therapy in said patient, wherein said patient has an alteration in the germline sequence of a PD-1 axis protein gene or the sequence of its RNA. A method of treating a patient suffering from cancer and eligible for immune checkpoint inhibitor therapy which therapy comprises the administration of an immune checkpoint inhibitor that inhibits PD-1-mediated and/or CTLA-4-mediated signaling in T-cells of said patient.

2. claim: 14(partially)

A kit of parts, comprising at least one pair of single-stranded PCR primers for amplifying a sequence of a gene, an expression product, or part thereof, wherein said gene is selected from the group consisting of PDCD1; and wherein amplified gene, product or part includes the position or locus of the SNPs Rs2227981, Rs2227982, Rs11568821, Rs41386349, Rs36084323, Rs10204525, and 6867 C/G in PDCD1; wherein said kit optionally further comprising a labelled oligonucleotide probe for detecting said SNP, wherein said kit optionally further comprises instructions for performing detection of said SNP. Said kit in a method for genotyping;

3. claim: 14(partially)

FURTHER INFORMATION CONTINUED FROM PCT/ISA/ 210

Idem as invention 2 but limited to PTPN11 and Rs2301756, Rs12423190, Rs11066301, Rs41279090, Rs12301915, Rs11066320, Rs11066322, and Rs3741983 in PTPN11;

4. claim: 14(partially)

Idem as invention 2 but limited to ZAP70 and Rs17695937, Rs13420683, Rs62157588, and R2278699 in ZAP70;

5. claim: 14(partially)

Idem as invention 2 but limited to IFNG and Rs2430561, Rs2069718, Rs2069705, and Rs1861494 in IFNG;

6. claim: 14(partially)

Idem as invention 2 but limited to CD274 and Rs4143815, Rs1411262, Rs822339, Rs2282055, Rs2297136, and 8923 A/C in CD274;

7. claim: 14(partially)

Idem as invention 2 but limited to CXCL9 and Rs10336, and 77147452 G/A in CXCL9;

8. claim: 14(partially)

Idem as invention 2 but limited to CXCL10 and Rs8878, Rs3921, and Rs1439490 in CXCL10;

9. claim: 14(partially)

Idem as invention 2 but limited to IRF7 and Rs1131665, and Rs1061501 in IRF7;

10. claim: 14(partially)

Idem as invention 2 but limited to AKT1 and Rs2494752, Rs2498801, Rs1130214, Rs2494732, Rs2498804, Rs1130233, and Rs3803304 in AKT1;
