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

(19) World Intellectual Property Organization
International Bureau

(43) International Publication Date
19 October 2017 (19.10.2017)

WIPO | PCT

(10) International Publication Number
WO 2017/178509 A1

(51) International Patent Classification:
C12Q 1/68 (2006.01)

(21) International Application Number:
PCT/EP2017/058735

(22) International Filing Date:
12 April 2017 (12.04.2017)

(25) Filing Language: English

(26) Publication Language: English

(30) Priority Data:
16305430.7 12 April 2016 (12.04.2016) EP

(71) Applicant: **XENTECH** [FR/FR]; PÉPINIÈRE GÉNOPOLE ENTREPRISES, 4 rue Pierre Fontaine, 91000 EVRY (FR).

(72) Inventors: **JUDDE, Jean-Gabriel**; 12 rue Louis Frébault, 94110 ARCUEIL (FR). **CAIRO, Stéfano**; 8 rue André Chermette, 91310 LONGPONT SUR ORGE (FR).

(74) Agent: **CABINET PLASSERAUD**; 66 rue de la Chaussée d'Antin, 75440 PARIS CEDEX 09 (FR).

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

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

Published:

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

— *with sequence listing part of description (Rule 5.2(a))*

WO 2017/178509 A1

(54) Title: METHODS FOR PREDICTING SENSIBILITY TO TREATMENT WITH PARP INHIBITORS IN CANCEROUS PATIENTS

(57) Abstract: The present invention relates to a method for predicting the sensibility of a patient afflicted by a cancer to a PARP inhibitor, comprising measuring the GSI in a biological sample of said patient. The invention also relates to a product containing an anti-cancer agent and a PARP inhibitor as a combined preparation for simultaneous, separate or sequential use in cancer therapy, preferably triple negative breast cancer therapy.

Methods for predicting sensibility to treatment with PARP inhibitors in cancerous patients

FIELD OF THE INVENTION

The present invention relates to a molecular diagnostic test useful for predicting the antitumor effectiveness of a PARP inhibitor administered to a patient afflicted by a cancer, preferably a triple-negative breast cancer patient. The invention includes the use of gene expression classifiers for prediction of tumor responsiveness to PARP inhibitors and for selection of triple-negative breast cancer patients for treatment with PARP inhibitors.

The present invention also relates to a product containing a DNA damage-inducing anti-cancer agent, preferably cyclophosphamide, and a PARP inhibitor as a combined preparation for simultaneous, separate or sequential use in cancer therapy, preferably triple-negative breast cancer therapy.

BACKGROUND OF THE INVENTION

Currently, there is a lack of accurate diagnostic tools allowing physicians to precisely identify cancer patients who will benefit from a given therapy. Cancer therapy is often inefficient because methods are lacking for accurately predicting which treatment would be the most effective for a particular cancer's physiology. This results in relatively poor response rates and increased cancer morbidity and death. Furthermore, patients often needlessly undergo ineffective, toxic drug therapy.

Triple negative breast cancer (TNBC) comprises 15-20% of all breast cancers. TNBCs are often more undifferentiated, carry an increased risk of distant metastasis, tend to relapse early and have been associated with a short post-recurrence survival. TNBCs lack hormonal receptors and Her2 overexpression and are therefore not candidate for anti-estrogen or Herceptin® therapy (Linn SC. and Van't Veer L. Clinical relevance of the triple-negative breast cancer concept: genetic basis and clinical utility of the concept. Eur. J Cancer 45, Suppl 1: 11-26, 2009). Today's conventional treatment of TNBC patients is thus based on combinations of cytotoxic drugs, including

anthracyclins, cyclophosphamide, 5-fluorouracil and taxanes, as well as local radiation therapy and surgery. In addition to TNBCs, aggressive or advanced breast cancers of the hormone receptor-positive and Her2-overexpressing subtypes also receive cytotoxic chemotherapy in combination with anti-estrogen or anti-Her2 therapies.

First-line preoperative (neoadjuvant) chemotherapy is often proposed to patients with advanced/infiltrating breast carcinoma. Following neoadjuvant chemotherapy, pathological complete response (pCR) is defined as no microscopic evidence of residual cancer in the breast and regional lymph nodes at the time of surgical resection. Presently, pCR is the best surrogate marker for favorable long-term outcome in breast cancer (BC) patients, corresponding to a 3-year overall survival (OS) of 90-100%. However, a major problem today is that over 70% of patients receiving preoperative chemotherapy do not achieve pCR and have residual disease at the time of surgical resection. This group of patients has a poor outcome, with frequent emergence of metastatic disease and a 3-year OS of only 60-70% whether or not receiving adjuvant chemotherapy or hormonotherapy following surgery. Patients in whom metastatic disease develops have a very poor prognosis, with a median survival of approximately 1 year. No effective standard-of-care therapy exists for patients with metastatic triple-negative breast cancer. TNBC is therefore a disease with a high unmet medical need.

TNBC shares clinical and pathological features with hereditary BRCA1/2-related breast cancers. In TNBCs, besides mutation-associated BRCA inactivation occurring by loss of heterozygosity (LOH) in about 15% of the cases, frequent dysregulation in the homologous-recombination (HR)–dependent DNA-repair pathway, has been attributed to a number of mechanisms, including BRCA1-promoter methylation and other defects in HR pathways, providing a strong rationale for developing new agents that exploit DNA-repair defects in these cancers. Poly (adenosine diphosphate–ribose) polymerase 1 (PARP1) and PARP2, two important regulators of the DNA base-excision–repair pathway, have emerged as therapeutic targets for TNBC. Indeed, a subset of TNBC patients are responsive to PARP inhibitors, a class of novel therapeutic agents that inhibits the function of several PARP family members, including PARP1 and PARP2. Pathogenic BRCA1/2 mutations, and other genomic markers such as high scores of loss of heterozygosity (LOH), large-scale transition (LST) and allelic imbalance (AI) rearrangements in tumor cells, also called genomic scars, have been proposed as

molecular markers to identify tumors with homologous recombination deficiency (HRD) that are more likely to be sensitive to PARP inhibitors (Abkevich V. et al. Pattern of genomic loss of heterozygosity predict homologous recombination repair defects in epithelial ovarian cancer. Br J Cancer (2012) 107: 1776-1782; Myriad Genetics Inc. US Patent Application US 2014/0363521 A1, Methods and materials for assessing homologous recombination deficiency; Marquard AM. et al. Pan-cancer analysis of genomic scar signatures associated with homologous recombination deficiency suggests novel indications for existing cancer drugs. Biomarker Research (2015) 3:9). However, these tests lack specificity as less than half of TNBC patients harboring BRCA-mutated or HRD-positive tumors are responsive to PARP inhibitors, and a number of TNBC patients without BRCA1/2 mutations are responsive to PARP inhibitors (Maxwell KN. And Domshek SM. Cancer treatment according to *BRCA1* and *BRCA2* mutations. Nat Rev Clin Onc, 9, 520-528, 2012. PMID 22825375; Watkins JA. Et al. Genomic scars as biomarkers of homologous recombination deficiency and drug response in breast and ovarian cancers. Breast Cancer Res. 16, 211-222, 2014. PMID 25093514).

Thus, treatment with PARP inhibitors benefits a subset of TNBC and other cancer patients, but the medical use of this class of drugs is hampered by the lack of a test that can identify this subset of responsive patients with enough specificity. Developing such a test, improving over existing methods is therefore of great interest.

SUMMARY OF THE INVENTION

The inventors surprisingly discovered a set of genes related to genomic stress response and designated herein as GSS (for Genomic Stress Signature), whose level of expression in a tumor sample, designated herein as GSI (for Genomic Stress Index), allows predicting the responsiveness of TNBC patients to treatment with a PARP inhibitor. They have thus developed a method for measuring the GSI in a biological sample of a patient afflicted by a cancer, preferably TNBC, and using the GSI to predict the sensitivity of the patient to a PARP inhibitor.

Said prediction is sensitive and reliable, independently of the PARP inhibitor used within the class of PARP inhibitors possessing PARP DNA-trapping activity, such as niraparib or olaparib.

The present invention provides a method for predicting the responsiveness of a cancer patient, preferably a TNBC patient, to treatment with a PARP inhibitor, comprising (1) measuring the expression of at least TDRD7, DDX60, IFIT2, ABCA1 and DDX58 genes, expressed in a tumor sample of the patient, so as to obtain an expression profile; and further (3) comparing the obtained expression profile with cut-off value(s),

wherein if said profile is greater than cut-off value(s), then the cancer patient is likely to be sensitive to a PARP inhibitor treatment.

Preferably, the present invention provides a method for predicting the responsiveness of a cancer patient, preferably a TNBC patient, to treatment with a PARP inhibitor, comprising, or consisting essentially of, (1) measuring the expression of a collection of gene product markers expressed in a tumor sample of the patient; (2) determining a score, preferably the GSI, in the tumor sample based at least on the measurements made in (1), and further (3) comparing the obtained score with cut-off value,

wherein if said score is greater than cut-off value, then the cancer patient is likely to be sensitive to a PARP inhibitor treatment.

Preferably, the method comprises, or consists essentially of, (1) measuring the expression of a collection of gene product markers expressed in the tumor sample; (2) determining the GSI in the tumor sample based at least in part on the measurements made in (1); and (3) comparing the obtained score with cut-off value,

wherein if the GSI is greater than cut-off value, then the cancer patient is likely to be sensitive to a PARP inhibitor treatment.

The present invention also provides a method for identifying a molecular subtype of TNBC tumor demonstrating sensitivity to PARP inhibitors, comprising (1) measuring the expression of a collection of gene product markers expressed in a tumor sample of a TNBC patient, so as to obtain an expression profile; and further (3) comparing the obtained expression profile with cut-off value(s),

wherein if said profile is greater than cut-off value(s), then the TNBC patient is likely to be sensitive to a PARP inhibitor treatment.

The present invention also provides a method for identifying a molecular subtype of TNBC tumor demonstrating sensitivity to PARP inhibitors, comprising, or consisting essentially of, (1) measuring the expression of a collection of gene product markers expressed in a tumor sample of a TNBC patient; (2) determining a score, preferably the GSI, in the tumor sample based at least on the measurements made in (1); and (3) comparing the obtained score with cut-off value,

wherein if the score is greater than cut-off value, then the cancer patient is likely to be sensitive to a PARP inhibitor treatment.

Preferably, the method comprises, or consists essentially of, (1) measuring the expression of a collection of gene product markers expressed in the tumor sample; (2) determining the GSI in the tumor sample based at least in part on the measurements made in (1); and (3) if the GSI is greater than a threshold value, then the tumor is likely to be sensitive to PARP inhibitors.

As shown in the examples, combining assessment of the GSI according to the invention with existing HRD genomic scoring methods provides significantly improved specificity over each method used alone in the identification of PARP inhibitor-sensitive tumors. Thus, preferably, the present invention provides a method for predicting the responsiveness of a cancer patient, preferably a TNBC patient, to treatment with a PARP inhibitor, or for identifying a molecular subtype of TNBC demonstrating sensitivity to PARP inhibitors, comprising combining the GSI evaluation method of the invention with determination of a HRD genomic signature.

Preferably, the method for predicting the responsiveness of a cancer patient, preferably a TNBC patient, to treatment with a PARP inhibitor, according to the invention comprises, or consists essentially of, (1) measuring the expression of a collection of gene product markers expressed in a tumor sample of said cancer patient; (2) determining the GSI in the tumor sample based at least in part on the measurements made in (1); (3) measuring the HRD score in a tumor sample of said cancer patient; and (4) if the GSI and the HRD score obtained in (2) and (3) respectively are both greater

than their respective threshold values, then the cancer patient is likely to be sensitive to a PARP inhibitor treatment.

Variability of GSI values between individual tumors is not limited to a single cancer type like TNBC. Therefore, the GSI evaluation method according to the invention may be used to predict responsiveness to PARP inhibitors across different cancer types in different tissues. In a preferred embodiment of the invention, these genes or gene products are useful for evaluating breast cancer tumors, preferably TNBC tumors.

Moreover, as shown in the examples, it was discovered that combining PARP inhibitors with genotoxic chemotherapy, preferably cyclophosphamide, resulted in higher antitumor efficacy than each treatment used alone only in tumors over-expressing the gene markers predicting sensitivity to PARP inhibitors (eg. classified as GSI-positive, or as both GSI-positive and HRD-positive) and showing partial or complete response to genotoxic chemotherapy.

Therefore, in another aspect, the present invention provides a method of predicting potentiation of a treatment consisting of administering a PARP inhibitor simultaneously with or subsequently to an active chemotherapy containing a range of genotoxic drugs that directly or indirectly affect DNA damage, e.g. neoadjuvant 5-fluorouracil, anthracycline and cyclophosphamide-based regimens such as AC (Adriamycin/cyclophosphamide), FEC (5-fluorouracil/epirubicin/cyclophosphamide) and FAC (5-fluorouracil/Adriamycin/cyclophosphamide), or platinum salts. The method for predicting potentiation of a treatment consisting of administering a PARP inhibitor simultaneously with or subsequently to an active chemotherapy in a cancer patient, comprises, or consists essentially of, (1) assessing the GSI in a tumor sample of said patient; and (2) if the GSI is greater than a threshold value, then the cancer patient is likely to have an increased response to said treatment compared to each treatment used alone. Preferably said method comprises, or consists essentially of, (1) assessing both the GSI and the HRD score in a tumor sample of said patient; and (2) if the GSI and the HRD score are both greater than their respective threshold values, then the cancer patient is likely to have an increased response to a treatment combining a PARP inhibitor with a genotoxic chemotherapy compared to each treatment alone.

The invention also relates to guiding conventional treatment of patients or selecting patients for clinical trials where novel drugs of the classes that directly or indirectly affect DNA damage and/or DNA damage repair, such as PARP inhibitors, are used alone or in combination with genotoxic chemotherapy.

Also provided is a product containing an anti-cancer agent, particularly cyclophosphamide, and a PARP inhibitor, as a combined preparation for simultaneous, separate or sequential use in cancer therapy, preferably triple negative breast cancer therapy.

DETAILED DESCRIPTION OF THE INVENTION

As used herein, the term "PARP inhibitor" or "PARPi" refers to compounds which inhibit signalling through PARP, have PARP DNA-trapping activity, or which inhibit the expression of the PARP genes. An "inhibitor of gene expression" refers to a natural or synthetic compound that has a biological effect to inhibit or significantly reduce the expression of said gene. Consequently an inhibitor of PARP gene expression refers to a natural or synthetic compound that has a biological effect to inhibit or significantly reduce the expression of the targeted PARP. The inhibitors of PARP gene expression include, but are not limited to, antisense oligonucleotides, siRNAs, shRNAs, ribozymes and DNAzymes.

PARP are a family of at least 17 enzymes which encode for poly-(ADP-ribose)-polymerases. These polymerases, among which the most abundant is PARP1, are involved in DNA repair, cell cycle checkpoints and chromatin remodelling, among other known or unknown functions. DNA repair mechanisms are complex, are related to single strand and/or double strand breaks, and involve multiple pathways:

- repair by direct reversion;
- mismatch repair (MMR);
- nucleotide excision repair (NER);
- base excision repair (BER);

- homologous recombination repair (HRR); and
- non-homologous end-joining (NHEJ).

PARP enzymes play a role in single strand lesion repair via the BER mechanism, but also in other DNA repair pathways such as HRR or NHEJ (Beck C. et al. Poly(ADP-ribose) polymerases in double-strand break repair: focus on PARP1, PARP2 and PARP3. Exp Cell Res (2014) 329: 18-25).

DNA repair pathways, which are BRCA-dependent homologous recombination and PARP pathway, are complementary. If one of the channels is deficient, such as by mutation of BRCA, and if the other pathway is blocked by a PARP inhibitor, it results in cell death by apoptosis, while the cell remains viable if one pathway is deficient. This double blocking is called synthetic lethality (Farmer H. Targeting the DNA repair defect in BRCA mutant cells as a therapeutic strategy. Nature (2005) 434:917-21. Kaelin W.G. The concept of synthetic lethality in the context of anticancer therapy, Nat. Rev. Cancer (2005) 5: 689–698).

Preferably, PARP inhibitors are compounds which inhibit signalling through PARP. PARP inhibitors include PARP1 and/or PARP2 inhibitors. Among them, one can quote nicotinamide and analogs, AZD-2281 (also called olaparib), AG014699 (also called rucaparib), ABT-888 (also called veliparib), BMN-673 (also called talazoparib), MK-4827 (also called niraparib) or BGB-290. Preferably, the PARP inhibitor is chosen from those possessing a DNA trapping activity, such as olaparib, rucaparib, talazoparib and niraparib (Murai J. et al. Trapping of PARP1 and PARP2 by clinical PARP inhibitors. Cancer Res (2012) 72: 5588-5599; Murai J. et al. Stereospecific PARP trapping by BMN673 and comparison with olaparib and rucaparib. Mol Cancer Ther (2014) 13: 433-443).

As used herein, the term “cancer” refers to any type of cancer. Preferably, said cancer is chosen from breast cancer, more preferably triple-negative breast cancer, ovarian cancer, lung carcinoma, colorectal cancer, prostate cancer, pancreatic cancer and melanoma.

As used herein, the term “triple-negative breast cancer” or “TNBC” refers to any breast cancer that does not express the genes for estrogen receptor (ER), progesterone receptor (PR) and Her2/neu. Preferably, the cancer is triple-negative breast cancer.

As used herein, the term “patient” denotes a mammal, such as a rodent, a feline, a canine, and a primate. Preferably, a patient according to the invention is a human. Preferably, said patient is not mutated on BRCA2 gene.

As used herein, the term “tumor sample” of a patient refers to any biological sample comprising cancer cells of said patient. Preferably, it is a blood sample, a saliva sample, a urine sample or a tumor biopsy. More preferably it is a tumor biopsy.

In the context of the invention, the term "treating" or "treatment", as used herein, means reversing, alleviating, inhibiting the progress of, or preventing the disorder or condition to which such term applies, or reversing, alleviating, inhibiting the progress of, or preventing one or more symptoms of the disorder or condition to which such term applies.

As used herein, the expression responsiveness, sensibility or sensitivity to PARP inhibitors or chemotherapeutic agents means a measurable decrease in tumor load or a shorter time to progression induced by the treatment. The decrease can be partial, eg. decrease of measurable tumor load, or complete, eg. total loss of measurable tumor load. Response can be assessed using different classification methods, such as overall survival, progression free survival, radiological response, as defined by RECIST, complete response, partial response, or stable disease.

The terms “chemotherapy” or “chemotherapeutic treatment” refer to the treatment of cancer (cancerous cells) with one or more cytotoxic anti-neoplastic drugs (chemotherapeutic agents) as part of a standardized regimen. Chemotherapy may be given with a curative intent or it may aim to prolong life or to palliate symptoms. It is often used in conjunction with other cancer treatments, such as radiation therapy, surgery, and/or hyperthermia therapy. Traditional chemotherapeutic agents act by

killing cells that divide rapidly, one of the main properties of most cancer cells. This means that chemotherapy also harms cells that divide rapidly under normal circumstances: cells in the bone marrow, digestive tract, and hair follicles. This results in the most common side-effects of chemotherapy: myelosuppression (decreased production of blood cells, hence also immunosuppression), mucositis (inflammation of the lining of the digestive tract), and alopecia (hair loss).

Some newer anticancer drugs (for example various monoclonal antibodies such as monoclonal antibodies against EGF receptor or VEGF, like bevacizumab, cetuximab and panitumumab, and also bortezomib – also called PS341, and sold by Millenium Pharmaceuticals under the name Velcade) are not indiscriminately cytotoxic, but rather target proteins that are abnormally expressed in cancer cells and that are essential for their growth. Such treatments are often referred to as "targeted therapy" (as distinct from classic chemotherapy) and are often used alongside traditional chemotherapeutic agents in antineoplastic treatment regimens.

Types of classic chemotherapeutic drugs to which the terms "chemotherapeutic drugs", "anti-cancer agents" and "chemotherapy" as used herein refer are:

Alkylating agents: Alkylating agents are the oldest group of chemotherapeutics in use today. They are so named because of their ability to alkylate many molecules, including proteins, RNA and DNA. This ability to bind covalently to DNA or RNA via their alkyl group is the primary cause for their anti-cancer effects. This leads to a form of programmed cell death called apoptosis. Alkylating agents will work at any point in the cell cycle and thus are known as cell cycle-independent drugs. For this reason the effect on the cell is dose dependent; the fraction of cells that die is directly proportional to the dose of drug. The subtypes of alkylating agents are the nitrogen mustards, nitrosoureas, tetrazines, aziridines, cisplatins and derivatives, and non-classical alkylating agents. Nitrogen mustards include mechlorethamine, cyclophosphamide, melphalan, chlorambucil, ifosfamide and busulfan. Nitrosoureas include N-Nitroso-N-methylurea (MNU), carmustine (BCNU), lomustine (CCNU) and semustine (MeCCNU), fotemustine and streptozotocin. Tetrazines include dacarbazine, mitozolomide and temozolomide. Aziridines include thiotepa, mytomycin and diaziquone (AZQ). Cisplatin and derivatives include cisplatin, carboplatin and oxaliplatin. They impair cell function by forming covalent bonds with the amino, carboxyl, sulfhydryl, and

phosphate groups in biologically important molecules. Non-classical alkylating agents include procarbazine and hexamethylmelamine. Mafosfamide is an oxazaphosphorine (cyclophosphamide-like) alkylating agent under investigation as a chemotherapeutic drug.

Anti-metabolites: The terms "anti-metabolites" and "DNA synthesis and transcription inhibitors" as used herein have an interchangeable meaning and define are a group of molecules that impede DNA and RNA synthesis. Many of them have a similar structure to the building blocks of DNA and RNA. Anti-metabolites resemble either nucleobases or nucleosides, but have altered chemical groups. These drugs exert their effect by either blocking the enzymes required for DNA synthesis or becoming incorporated into DNA or RNA. By inhibiting the enzymes involved in DNA synthesis, they prevent mitosis because the DNA cannot duplicate itself. Also, after misincorporation of the molecules into DNA, DNA damage can occur and programmed cell death (apoptosis) is induced. Unlike alkylating agents, anti-metabolites are cell cycle dependent. This means that they only work during a specific part of the cell cycle, in this case S-phase (the DNA synthesis phase). For this reason, at a certain dose, the effect plateaus and proportionally no more cell death occurs with increased doses. Subtypes of the anti-metabolites are the anti-folates, fluoropyrimidines, deoxynucleoside analogues and thiopurines. The anti-folates include methotrexate and pemetrexed. The fluoropyrimidines include fluorouracil and capecitabine. Fluorouracil is a nucleobase analogue that is metabolised in cells to form at least two active products; 5-fluourouridine monophosphate (FUMP) and 5-fluoro-2'-deoxyuridine 5'-phosphate (fdUMP). FUMP becomes incorporated into RNA and fdUMP inhibits the enzyme thymidylate synthase; both of which lead to cell death. Capecitabine is a prodrug of 5-fluorouracil that is broken down in cells to produce the active drug. The deoxynucleoside analogues include cytarabine, gemcitabine, decitabine, vidaza, fludarabine, nelarabine, cladribine, clofarabine and pentostatin. The thiopurines include thioguanine and mercaptopurine

Anti-microtubule agents: Anti-microtubule agents are plant-derived chemicals that block cell division by preventing microtubule function. Vinca alkaloids and taxanes are the two main groups of anti-microtubule agents. The vinca alkaloids prevent the formation of the microtubules, whereas the taxanes prevent the microtubule

disassembly. By doing so, they prevent the cancer cells from completing mitosis. Following this, cell cycle arrest occurs, which induces programed cell death (apoptosis). Vinca alkaloids are derived from the Madagascar periwinkle, Catharanthusroseus. Taxanes are natural and semi-synthetic drugs. The first drug of their class, paclitaxel, was originally extracted from the Pacific Yew tree, Taxusbrevifolia. Now this drug and another in this class, docetaxel, are produced semi-synthetically from a chemical found in the bark of another Yew tree, Taxusbaccata. These drugs promote microtubule stability, preventing their disassembly. Docetaxel exerts its effect during S-phase.

Topoisomerase inhibitors: Topoisomerase inhibitors are drugs that affect the activity of two enzymes; topoisomerase I and topoisomerase II. When the DNA double stranded helix is unwound, during DNA replication or translation for example, the adjacent unopened DNA winds tighter (supercoils), like opening the middle of a twisted rope. The stress caused by this effect is in part aided by the topoisomerase enzymes. They produce single or double strand breaks into DNA, reducing the tension in the DNA strand. This allows the normal unwinding of DNA to occur during replication or translation. Inhibition of topoisomerase I or II interferes with both of these processes. Two topoisomerase I inhibitors, irinotecan and topotecan, are semi-synthetically derived from camptothecin, which is obtained from the Chinese ornamental tree Camptothecaacuminata. Drugs that target topoisomerase II can be divided into two groups. The topoisomerase II poisons cause increased levels enzymes bound to DNA. This prevents DNA replication and translation, causes DNA strand breaks, and leads to programmed cell death (apoptosis). These agents include etoposide, doxorubicin, mitoxantrone and teniposide. The second group, catalytic inhibitors, is drugs that block the activity of topoisomerase II, and therefore prevent DNA synthesis and translation because the DNA cannot unwind properly. This group includes novobiocin, merbarone, and aclarubicin.

Cytotoxic antibiotics: The cytotoxic antibiotics are a varied group of drugs that have various mechanisms of action. The group includes the anthracyclines and other drugs including actinomycin, bleomycin, plicamycin and mitomycin. Doxorubicin and daunorubicin were the first two anthracyclines, and were obtained from the bacterium Streptomyces peucetius. Derivatives of these compounds include epirubicin and idarubicin. Other clinically used drugs in the anthracyline group are pirarubicin,

aclarubicin and mitoxantrone. The mechanisms of anthracyclines include DNA intercalation (molecules insert between the two strands of DNA), generation of highly reactive free radicals that damage intercellular molecules and topoisomerase inhibition. Actinomycin is a complex molecule that intercalates DNA and prevents RNA synthesis. Bleomycin, a glycopeptide isolated from Streptomyces verticillus, also intercalates DNA, but produces free radicals that damage DNA. This occurs when bleomycin binds to a metal ion, becomes chemically reduced and reacts with oxygen. Mitomycin is a cytotoxic antibiotic with the ability to alkylate DNA.

Combination chemotherapy involves treating a patient with a number of different drugs simultaneously. The drugs differ in their mechanism and side effects. The biggest advantage is minimizing the chances of resistance developing to any one agent. Also, the drugs can often be used at lower doses, reducing toxicity. A prominent example is the combination of doxorubicin and cyclophosphamide (A/C).

Preferably, the anti-cancer agent is an alkylating agent, more preferably cyclophosphamide, optionally in combination with an anthracycline such as doxorubicin or epirubicin.

The term "biomarker" or “marker” is widespread in the art and may broadly denote a biological molecule and/or a detectable portion thereof (e.g. a nucleic acid, a peptide or a lipid such as a glycolipid) whose qualitative and/or quantitative evaluation in an individual is predictive or informative (e.g., predictive, diagnostic and/or prognostic) with respect to one or more aspects of the individual's phenotype and/or genotype, such as, for example, with respect to the status of the individual. Typically, the biomarker is predictive or informative with respect to the outcome for chemotherapeutic treatment of a cancer in an individual. A biomarker is expressed (“expression of the biomarker”) if the biomarker is detectable with methods known in the art. Therefore expression of biomarkers encompasses not only expression at nucleic acid level (DNA and/or RNA) and protein level but also expression (presence) of other biological structures on or in the cells such as glycolipids or the activity of a protein.

The above definitions are applicable to the whole application, and particularly to the following description.

The present invention provides a method for predicting the responsiveness of a cancer patient, preferably a TNBC patient, to treatment with a PARP inhibitor, comprising (1) measuring the expression of at least TDRD7, DDX60, IFIT2, ABCA1 and DDX58 genes, expressed in a tumor sample of the patient, so as to obtain an expression profile; and further (3) comparing the obtained expression profile with cut-off value(s),

wherein if said profile is greater than cut-off value(s), then the cancer patient is likely to be sensitive to a PARP inhibitor treatment.

As used herein, the term "expression profile" refers to quantitative expressions of at least said genes TDRD7, DDX60, IFIT2, ABCA1 and DDX58, in a tumor sample. The expression profile is a repository of the expression level data that can be used to compare the expression levels of different genes, in whatever units are chosen. The term "profile" is also intended to encompass manipulations of the expression level data derived from a cell, tissue or individual. For example, once relative expression levels are determined for a given set of genes, the relative expression levels for that cell, tissue or individual can be compared to a standard to determine if expression levels are higher or lower relative to the same genes in a standard. Standards can include any data deemed by one of skilled in the art to be relevant for comparison, for example determined threshold value or expression profile of a positive and/or negative control.

As used herein, the expression "comparing the expression profile" in all its grammatical forms, refers to the evaluation of the quantitative difference in expressions of said genes. Typically, the person skilled in the art may compare the levels of expression of the genes to cut-off values.

Typically, a "control value" or "threshold value" or "cut-off value" can be determined experimentally, empirically, or theoretically. A threshold value can also be arbitrarily selected based upon the existing experimental and/or clinical conditions, as would be recognized by person skilled in the art. Preferably, the person skilled in the art may compare the expression profile of the genes of interest according to the invention with threshold value(s).

For each gene to be compared to a threshold value, the skilled person in the art will compare the level of expression of said gene to a threshold value.

Preferably, the present invention relates to a method for predicting the responsiveness of a cancer patient, preferably a TNBC patient, to treatment with a PARP inhibitor, comprising measuring a GSI (Genomic Stress Index), as defined below, in a tumor sample of said patient.

Specifically, said method comprises: (1) measuring the expression of genes expressed in a tumor sample of the patient, said genes being at least TDRD7, DDX60, IFIT2, ABCA1 and DDX58; (2) determining the GSI in the tumor sample based at least on the measurements made in (1), and further (3) comparing the obtained the GSI with a cut-off value,

wherein if said GSI is greater than said cut-off value, then the cancer patient is likely to be sensitive to a PARP inhibitor treatment.

The GSI is correlated to the sensibility of a given patient afflicted by a cancer to PARP inhibitor treatment.

When said GSI is higher than a threshold value, then one can conclude that the tumor of the patient is sensitive to PARP inhibitor treatment. Thus, the treatment will lead to a partial or complete regression of said tumor.

To the contrary, when the GSI is lower than a threshold value, then one can conclude that the tumor of the patient is not sensitive (resistant) to PARP inhibitor treatment. Thus, even if the treatment is administered, the tumor will continue its progression.

The method of the invention is applicable, on one hand, in case of a treatment with a PARP inhibitor as a monotherapy, as well as, on the other hand, in case of a treatment with a PARP inhibitor in combination with a chemotherapeutic agent, preferably a genotoxic, more preferably cyclophosphamide.

Thus, according to an embodiment of the invention, the patient has not undergone any treatment of said cancer. In such a case, the method of the invention predicts the sensibility to PARP inhibitor treatment of said patient, and said PARP inhibitor may be administered as monotherapy.

According to another embodiment, the patient has already been treated for said cancer, preferably by genotoxic chemotherapy, and more preferably by cyclophosphamide. In such a case, the method of the invention predicts the sensibility to PARP inhibitor treatment of said treated patient, and said PARP inhibitor may be administered in combination with a chemotherapeutic agent.

According to this embodiment, the purpose of the method is to predict sensibility of the treated patient to PARP inhibitor treatment given in combination with genotoxic chemotherapy, and thus, to select patients in which the addition of a PARP inhibitor to genotoxic chemotherapy, preferably cyclophosphamide, will result in increased antitumor efficacy and prevention of metastasis and/or relapse as compared to genotoxic chemotherapy used alone.

Indeed, according to this embodiment, a method for preventing metastasis and/relapse of a cancer, preferably triple negative breast cancer, in a patient already treated by chemotherapy is also provided. This method comprises, or consists essentially of the following steps:

a) a first step of measuring the expression of at least TDRD7, DDX60, IFIT2, ABCA1 and DDX58 genes, expressed in a tumor sample of the patient, so as to obtain an expression profile,

b) optionally determining the GSI, based at least on the measurements made in a), and

c) comparing the obtained expression profile or the GSI with cut-off value(s), wherein if the patient has an expression profile or GSI greater than a threshold value and shows responsiveness (eg. partial or complete tumor regression) to genotoxic chemotherapy, preferably cyclophosphamide, then said patient is treated with a PARP inhibitor simultaneously or sequentially with genotoxic chemotherapy.

The present invention provides a method for predicting the responsiveness of a cancer patient, preferably a TNBC patient, to treatment with a PARP inhibitor, comprising (1) measuring the expression of at least one gene chosen from TDRD7, DDX60, IFIT2, ABCA1, APOL6, B2M, BST2, C1R, C1S, C3, CD68, CLDN1, DDIT3, DDR2,

DDX58, DTX3L, HERC5, HERC6, HLA-E, IFI27, IFI35, IFI6, IFI44L, IFI44, TLR2, TLR3, TRIM21, TRIM22, TSPAN1, IFIH1, IFIT1, IFITM1, IRF9, C19orf66, USP18, SAMD9, SAMD9L, SAMHD1, SELM, SOD2, SP100, SP110, STAT1, STAT2, CCL5, MUC1, MUC15, MX1, PLSCR1, OAS3, IFIT3, IFIT5, OAS1, OAS2, OASL, PARP12, PARP14, PARP9, LAMP3, RSAD2, UBE2L6 and ZNFX1 genes, expressed in a tumor sample of the patient, so as to obtain an expression profile; and further (3) comparing the obtained expression profile with cut-off value(s),

wherein if said profile is greater than cut-off value(s), then the cancer patient is likely to be sensitive to a PARP inhibitor treatment.

In the present application, the genes correspond to the following:

Gene	Definition (NCBI)
ABCA1 (SEQ ID NO:1)	gi|224589821:c107690527-107543283 Homo sapiens chromosome 9, GRCh37.p9 Primary Assembly
ZNFX1 (SEQ ID NO:2)	gi|224589812:c47894756-47862437 Homo sapiens chromosome 20, GRCh37.p9 Primary Assembly
APOL6 (SEQ ID NO:3)	gi|224589814:36044424-36064456 Homo sapiens chromosome 22, GRCh37.p9 Primary Assembly
B2M (SEQ ID NO:4)	gi|224589806:45003685-45010357 Homo sapiens chromosome 15, GRCh37.p9 Primary Assembly
BST2 (SEQ ID NO:5)	gi|224589810:c17516384-17513755 Homo sapiens chromosome 19, GRCh37.p9 Primary Assembly
C19orf66 (SEQ ID NO:6)	gi|224589810:10196806-10203928 Homo sapiens chromosome 19, GRCh37.p9 Primary Assembly
C1R (SEQ ID NO:7)	gi|224589803:c7189412-7187513 Homo sapiens chromosome 12, GRCh37.p9 Primary Assembly
C1S (SEQ ID NO:8)	gi|394726921:262259-272615 Homo sapiens chromosome 12 genomic patch of type FIX, GRCh37.p9 PATCHES HG344_PATCH

C3 (SEQ ID NO:9)	gi|224589810:c6720662-6677846 Homo sapiens chromosome 19, GRCh37.p9 Primary Assembly
CCL5 (SEQ ID NO:10)	gi|224589808:c34207377-34198495 Homo sapiens chromosome 17, GRCh37.p9 Primary Assembly
CD68 (SEQ ID NO:11)	gi|224589808:7482805-7485429 Homo sapiens chromosome 17, GRCh37.p9 Primary Assembly
CLDN1 (SEQ ID NO:12)	gi|224589815:c190040235-190023490 Homo sapiens chromosome 3, GRCh37.p9 Primary Assembly
DDIT3 (SEQ ID NO:13)	gi|224589803:c57914300-57910371 Homo sapiens chromosome 12, GRCh37.p9 Primary Assembly
DDR2 (SEQ ID NO:14)	gi|224589800:162602228-162750255 Homo sapiens chromosome 1, GRCh37.p9 Primary Assembly
DDX58 (SEQ ID NO:15)	gi|224589821:c32526322-32455300 Homo sapiens chromosome 9, GRCh37.p9 Primary Assembly
DDX60 (SEQ ID NO:16)	gi|224589816:c169239958-169137442 Homo sapiens chromosome 4, GRCh37.p9 Primary Assembly
DTX3L (SEQ ID NO:17)	gi|224589815:122283185-122294050 Homo sapiens chromosome 3, GRCh37.p9 Primary Assembly
HERC5 (SEQ ID NO:18)	gi|224589816:89378268-89427321 Homo sapiens chromosome 4, GRCh37.p9 Primary Assembly
HERC6 (SEQ ID NO:19)	gi|224589816:89299891-89364249 Homo sapiens chromosome 4, GRCh37.p9 Primary Assembly
HLA-E (SEQ ID NO:20)	gi|224589818:30457183-30461982 Homo sapiens chromosome 6, GRCh37.p9 Primary Assembly

IFI27 (SEQ ID NO:21)	gi|224589805:94577079-94583033 Homo sapiens chromosome 14, GRCh37.p9 Primary Assembly
IFI35 (SEQ ID NO:22)	gi|224589808:41158742-41166476 Homo sapiens chromosome 17, GRCh37.p9 Primary Assembly
IFI44 (SEQ ID NO:23)	gi|224589800:79115477-79129763 Homo sapiens chromosome 1, GRCh37.p9 Primary Assembly
IFI44L (SEQ ID NO:24)	gi|224589800:79086088-79111830 Homo sapiens chromosome 1, GRCh37.p9 Primary Assembly
IFI6 (SEQ ID NO:25)	gi|224589800:c27998724-27992572 Homo sapiens chromosome 1, GRCh37.p9 Primary Assembly
IFIH1 (SEQ ID NO:26)	gi|224589811:c163175218-163123589 Homo sapiens chromosome 2, GRCh37.p9 Primary Assembly
IFIT1 (SEQ ID NO:27)	gi|224589801:91152322-91163745 Homo sapiens chromosome 10, GRCh37.p9 Primary Assembly
IFIT2 (SEQ ID NO:28)	gi|224589801:91061706-91069033 Homo sapiens chromosome 10, GRCh37.p9 Primary Assembly
IFIT3 (SEQ ID NO:29)	gi|224589801:91087602-91100725 Homo sapiens chromosome 10, GRCh37.p9 Primary Assembly
IFIT5 (SEQ ID NO:30)	gi|224589801:91174325-91180759 Homo sapiens chromosome 10, GRCh37.p9 Primary Assembly
IFITM1 (SEQ ID NO:31)	gi|224589802:313991-315272 Homo sapiens chromosome 11, GRCh37.p9 Primary Assembly
IRF9 (SEQ ID NO:32)	gi|224589805:24630422-24635774 Homo sapiens chromosome 14, GRCh37.p9 Primary Assembly

LAMP3 (SEQ ID NO:33)	gi|224589815:c182880667-182840003 Homo sapiens chromosome 3, GRCh37.p9 Primary Assembly
MUC1 (SEQ ID NO:34)	gi|224589800:c155162706-155158300 Homo sapiens chromosome 1, GRCh37.p9 Primary Assembly
MUC15 (SEQ ID NO:35)	gi|224589802:c26593815-26580579 Homo sapiens chromosome 11, GRCh37.p9 Primary Assembly
MX1 (SEQ ID NO:36)	gi|224589813:42792520-42831141 Homo sapiens chromosome 21, GRCh37.p9 Primary Assembly
OAS1 (SEQ ID NO:37)	gi|224589803:113344739-113357712 Homo sapiens chromosome 12, GRCh37.p9 Primary Assembly
OAS2 (SEQ ID NO:38)	gi|224589803:113416274-113449528 Homo sapiens chromosome 12, GRCh37.p9 Primary Assembly
OAS3 (SEQ ID NO:39)	gi|224589803:113376249-113411054 Homo sapiens chromosome 12, GRCh37.p9 Primary Assembly
OASL (SEQ ID NO:40)	gi|224589803:c121477045-121458095 Homo sapiens chromosome 12, GRCh37.p9 Primary Assembly
PARP12 (SEQ ID NO:41)	gi|224589819:c139763521-139723549 Homo sapiens chromosome 7, GRCh37.p9 Primary Assembly
PARP14 (SEQ ID NO:42)	gi|224589815:122399672-122449687 Homo sapiens chromosome 3, GRCh37.p9 Primary Assembly
PARP9 (SEQ ID NO:43)	gi|224589815:c122283523-122246757 Homo sapiens chromosome 3, GRCh37.p9 Primary Assembly
PLSCR1 (SEQ ID NO:44)	gi|224589815:c146262628-146232967 Homo sapiens chromosome 3, GRCh37.p9 Primary Assembly

RSAD2 (SEQ ID NO:45)	gi|224589811:7017796-7038363 Homo sapiens chromosome 2, GRCh37.p9 Primary Assembly
SAMD9 (SEQ ID NO:46)	gi|224589819:c92747336-92728826 Homo sapiens chromosome 7, GRCh37.p9 Primary Assembly
SAMD9L (SEQ ID NO:47)	gi|224589819:c92777680-92759368 Homo sapiens chromosome 7, GRCh37.p9 Primary Assembly
SAMHD1 (SEQ ID NO:48)	gi|224589812:c35580246-35520227 Homo sapiens chromosome 20, GRCh37.p9 Primary Assembly
SELM (SEQ ID NO:49)	gi|224589814:c31503551-31500763 Homo sapiens chromosome 22, GRCh37.p9 Primary Assembly
SOD2 (SEQ ID NO:50)	gi|224589818:c160114353-160100148 Homo sapiens chromosome 6, GRCh37.p9 Primary Assembly
SP100 (SEQ ID NO:51)	gi|224589811:231280871-231410317 Homo sapiens chromosome 2, GRCh37.p9 Primary Assembly
SP110 (SEQ ID NO:52)	gi|224589811:c231090444-231033634 Homo sapiens chromosome 2, GRCh37.p9 Primary Assembly
STAT1 (SEQ ID NO:53)	gi|224589811:c191878976-191833762 Homo sapiens chromosome 2, GRCh37.p9 Primary Assembly
STAT2 (SEQ ID NO:54)	gi|224589803:c56754037-56735381 Homo sapiens chromosome 12, GRCh37.p9 Primary Assembly
TDRD7 (SEQ ID NO:55)	gi|224589821:100174302-100258407 Homo sapiens chromosome 9, GRCh37.p9 Primary Assembly
TLR2 (SEQ ID NO:56)	gi|224589816:154605441-154627243 Homo sapiens chromosome 4, GRCh37.p9 Primary Assembly

TLR3 (SEQ ID NO:57)	gi|224589816:186990309-187006252 Homo sapiens chromosome 4, GRCh37.p9 Primary Assembly
TRIM21 (SEQ ID NO:58)	gi|224589802:c4414926-4406127 Homo sapiens chromosome 11, GRCh37.p9 Primary Assembly
TRIM22 (SEQ ID NO:59)	gi|224589802:5710817-5732093 Homo sapiens chromosome 11, GRCh37.p9 Primary Assembly
TSPAN1 (SEQ ID NO:60)	gi|224589800:46640749-46651634 Homo sapiens chromosome 1, GRCh37.p9 Primary Assembly
UBE2L6 (SEQ ID NO:61)	gi|224589802:c57335803-57319128 Homo sapiens chromosome 11, GRCh37.p9 Primary Assembly
USP18 (SEQ ID NO:62)	gi|224589814:18632758-18660164 Homo sapiens chromosome 22, GRCh37.p9 Primary Assembly

By "GSI" or "Genomic Stress Index" according to the invention, it is meant a score obtained thanks to the expressions of at least TDRD7, DDX60, IFIT2, ABCA1 and DDX58 genes.

Preferably, said method comprises: (1) measuring the expression of genes expressed in a tumor sample of the patient, said genes being at least TDRD7, DDX60, IFIT2, ABCA1 and DDX58; (2) determining a score, preferably the GSI, in the tumor sample based at least on the measurements made in (1), and further (3) comparing the obtained the score, preferably the GSI, with a cut-off value,

wherein if said score is greater than cut-off value, then the cancer patient is likely to be sensitive to a PARP inhibitor treatment.

Step (2) is indeed optional; it may be replaced by any statistical method known in the art.

The expression profile may be defined in combination with corresponding scalar weights on the real scale with varying magnitude, which are further combined through linear or non-linear, algebraic, trigonometric or correlative means into a single scalar

value via an algebraic, statistical learning, Bayesian, regression, or similar algorithms which together with a mathematically derived decision function on the scalar value provide a predictive model by which expression profiles from samples may be resolved into discrete classes of responder or non-responder, resistant or non-resistant, to a specified drug or drug class. Such predictive models are developed by learning weights and the decision threshold, optimized for sensitivity, specificity, negative and positive predictive values, hazard ratio or any combination thereof, under cross-validation, bootstrapping or similar sampling techniques, from a set of representative expression profiles from historical patient samples with known drug response and/or resistance.

In one embodiment, the expression profile is used to form a weighted sum of the genes signals, where individual weights can be positive or negative. The resulting sum ("decisive function") is compared with a pre-determined cut-off value. The comparison with the cut-off value may be used to diagnose, or predict a clinical condition or outcome.

As described above, one of ordinary skill in the art will appreciate that the genes included in the classifier provided in the table above will carry unequal weights in a classifier for responsiveness or resistance to a PARP inhibitor. Therefore, while few sequences may be used to diagnose or predict an outcome such as responsiveness to therapeutic agent, the specificity and sensitivity or diagnosis or prediction accuracy may increase using more sequences. As used herein, the term "weight" refers to the relative importance of an item in a statistical calculation. The weight of each gene may be determined on a data set of patient samples using analytical methods known in the art.

In one embodiment, the relative expression levels of said genes in a tumor sample are measured to form the expression profile. The expression profile of the set of genes from a patient tumor sample is summarized in the form of a compound decision score and compared to a score threshold that is mathematically derived from a training set of patient data. The score threshold separates a patient group based on different characteristics such as, but not limited to, responsiveness/non-responsiveness to treatment. The patient training set data is preferably derived from tumor tissue samples

having been characterized by prognosis, likelihood of recurrence, long term survival, clinical outcome, treatment response, diagnosis, tumor classification, or personalized genomics profile. Expression profiles, and corresponding decision scores from patient samples may be correlated with the characteristics of patient samples in the training set that are on the same side of the mathematically derived score decision threshold. The threshold of the linear classifier scalar output is optimized to maximize the sum of sensitivity and specificity under cross-validation as observed within the training dataset.

In one embodiment, the expression profile of a patient tumor sample is evaluated by a linear classifier. As used herein, a linear classifier refers to a weighted sum of the individual genes intensities into a compound decision score ("decision function"). The decision score is then compared to a pre-defined cut-off score, corresponding to a certain set-point in terms of sensitivity and specificity which indicates if a sample is above the score threshold (decision function positive) or below (decision function negative).

Effectively, this means that the data space, i.e. the set of all possible combinations of gene (biomarker) expression values, is split into two mutually exclusive halves corresponding to different clinical classifications or predictions, e.g. one corresponding to responsiveness to a therapeutic agent and the other to resistance. In the context of the overall classifier, relative over-expression of a certain biomarker can either increase the decision score (positive weight) or reduce it (negative weight) and thus contribute to an overall decision of, for example, responsiveness or resistance to a therapeutic agent.

The term "area under the curve" or "AUC" refers to the area under the curve of a receiver operating characteristic (ROC) curve, both of which are well known in the art. AUC measures are useful for comparing the accuracy of a classifier across the complete data range. Classifiers with a greater AUC have a greater capacity to classify unknowns correctly between two groups of interest (e.g., tumor samples and normal or control samples). ROC curves are useful for plotting the performance of a particular feature (e.g., any of the biomarkers described herein and/or any item of additional biomedical information) in distinguishing between two populations (e.g., individuals responding and not responding to a therapeutic agent). Typically, the feature data across the entire

population (e.g., the cases and controls) are sorted in ascending order based on the value of a single feature. Then, for each value for that feature, the true positive and false positive rates for the data are calculated. The true positive rate is determined by counting the number of cases above the value for that feature and then dividing by the total number of cases. The false positive rate is determined by counting the number of controls above the value for that feature and then dividing by the total number of controls. Although this definition refers to scenarios in which a feature is elevated in cases compared to controls, this definition also applies to scenarios in which a feature is lower in cases compared to the controls (in such a scenario, samples below the value for that feature would be counted). ROC curves can be generated for a single feature as well as for other single outputs, for example, a combination of two or more features can be mathematically combined (e.g., added, subtracted, multiplied, etc.) to provide a single sum value, and this single sum value can be plotted in a ROC curve. Additionally, any combination of multiple features, in which the combination derives a single output value, can be plotted in a ROC curve. These combinations of features may comprise a test. The ROC curve is the plot of the true positive rate (sensitivity) of a test against the false positive rate (1-specificity) of the test.

The interpretation of this quantity, i.e. the cut-off threshold responsiveness or resistance to a therapeutic agent, is derived in the development phase ("training") from a set of patients with known outcome. The corresponding weights and the responsiveness/resistance cut-off threshold for the decision score are fixed a priori from training data by methods known to those skilled in the art. In a preferred embodiment of the present method, Partial Least Squares Discriminant Analysis (PLS-DA) is used for determining the weights. (L. Stahle, S. Wold, J. Chemom. 1 (1987) 185-196; D. V. Nguyen, D. M. Rocke, Bioinformatics 18 (2002) 39-50).

Different methods can be used to convert quantitative data measured on these biomarkers into a prognosis or other predictive use. These methods include, but not limited to methods from the fields of pattern recognition (Duda et al. Pattern Classification, 2.sup.nd ed., John Wiley, New York 2001), machine learning (Scholkopf et al. Learning with Kernels, MIT Press, Cambridge 2002, Bishop, Neural Networks for

Pattern Recognition, Clarendon Press, Oxford 1995), statistics (Hastie et al. The Elements of Statistical Learning, Springer, New York 2001), bioinformatics (Dudoit et al., 2002, J. Am. Statist. Assoc. 97:77-87, Tibshirani et al., 2002, Proc. Natl. Acad. Sci. USA 99:6567-6572) or chemometrics (Vandeginste, et al., Handbook of Chemometrics and Qualimetrics, Part B, Elsevier, Amsterdam 1998).

Preferably, step (2) comprises determining the GSI in the tumor sample based on the measurements made in (1), and may be performed as explained in the examples. Typically, it is calculated with the z-scores for each gene expression level for at least TDRD7, DDX60, IFIT2, ABCA1 and DDX58 genes. Said z-score can be calculated from the following formula:

$$z = (X - \mu) / \sigma$$

where z is the z-score, X is the value of the element, μ is the mean of the expression level of one gene, and σ is the standard deviation.

At the end of step (2), the GSI is obtained for the given tumor sample.
Then, if said GSI is higher than a threshold value, then one can conclude that the tumor of the patient is sensitive to PARP inhibitor treatment. Thus, the treatment will lead to a partial or complete regression of said tumor.
To the contrary, if said GSI is lower than a threshold value, then one can conclude that the tumor of the patient is not sensitive to PARP inhibitor treatment. Thus, even if the treatment is administered, the tumor will continue its progression.

The minimal 5 genes necessary for the present invention are TDRD7, DDX60, IFIT2, ABCA1 and DDX58.

Moreover, there may be additional relevant genes.
At least one of the following genes may be further used: APOL6, B2M, BST2, C1R, C1S, C3, CD68, CLDN1, DDIT3, DDR2, DTX3L, HERC5, HERC6, HLA-E, IFI27, IFI35, IFI6, IFI44L, IFI44, TLR2, TLR3, TRIM21, TRIM22, TSPAN1, IFIH1, IFIT1, IFITM1, IRF9, C19orf66, USP18, SAMD9, SAMD9L, SAMHD1, SELM, SOD2, SP100, SP110, STAT1, STAT2, CCL5, MUC1, MUC15, MX1, PLSCR1, OAS3,

IFIT3, IFIT5, OAS1, OAS2, OASL, PARP12, PARP14, PARP9, LAMP3, RSAD2, UBE2L6 and/or ZNFX1.

Preferably, at least one of the following genes may be further used: STAT2, IFI44L, DDR2, IFI44, TLR3, MX1, IFIT1, C19orf66, USP18, SAMD9L, SAMHD1, OASL, CCL5, MUC1, PLSCR1, OAS3, B2M, IFIT3, IFIT5, OAS1, LAMP3, IFITM1, TRIM22, RSAD2 and/or UBE2L6.

Preferably, the additional genes are chosen from STAT2, IFI44L, DDR2, IFI44 and TLR3. Preferably, the additional genes are at least STAT2, IFI44L, DDR2, IFI44 and TLR3.

Preferably, the additional genes are chosen from MX1, IFIT1, C19orf66, USP18, SAMD9L, SAMHD1, OASL, CCL5, MUC1 and PLSCR1. Preferably, the additional genes are at least MX1, IFIT1, C19orf66, USP18, SAMD9L, SAMHD1, OASL, CCL5, MUC1 and PLSCR1.

Preferably, the additional genes are chosen from OAS3, B2M, IFIT3, IFIT5, OAS1, LAMP3, IFITM1, TRIM22, RSAD2 and UBE2L6. Preferably, the additional genes are at least OAS3, B2M, IFIT3, IFIT5, OAS1, LAMP3, IFITM1, TRIM22, RSAD2 and UBE2L6.

Preferably, all the following genes are used for said calculation: STAT2, IFI44L, DDR2, IFI44, TLR3, MX1, IFIT1, C19orf66, USP18, SAMD9L, SAMHD1, OASL, CCL5, MUC1, PLSCR1, OAS3, B2M, IFIT3, IFIT5, OAS1, LAMP3, IFITM1, TRIM22, RSAD2 and UBE2L6.

As used herein, the term "gene expression level" or "level of expression of a gene" refers to an amount or a concentration of a transcription product, for instance mRNA, or of a translation product, for instance a protein or polypeptide. Typically, a level of mRNA expression can be expressed in units such as transcripts per cell or nanograms per microgram of tissue. A level of a polypeptide can be expressed as nanograms per microgram of tissue or nanograms per milliliter of a culture medium, for example. Alternatively, relative units can be employed to describe a gene expression level.

As used herein, the expression of "measuring the level of expression of a gene" encompasses the step of measuring the quantity of a transcription product, preferably mRNA obtained through transcription of said gene, and/or the step of measuring the quantity of translation product, preferably the protein obtained through translation of said gene. Preferably, the step of measuring the expression of a gene refers to the step of measuring the quantity of mRNA obtained through transcription of said gene.

Typically, the step of measuring the gene expression levels may be performed according to the routine techniques, well known of the person skilled in the art.

More preferably, the measurement comprises contacting the cancer cells of the biological sample with selective reagents such as probes, primers, ligands or antibodies, and thereby detecting the presence of nucleic acids or proteins of interest originally in the sample.

In a preferred embodiment, the expression may be measured by measuring the level of mRNA.

Methods for measuring the level of mRNA are well known in the art. For example the nucleic acid contained in the samples (e.g., isolated cancer cells prepared from the patient, like those included in biopsies) is first extracted according to standard methods, for example using lytic enzymes or chemical solutions or extracted by nucleic-acid-binding resins following the manufacturer's instructions. The extracted mRNA is then detected by hybridization (e. g., Northern blot analysis) and/or amplification (e.g., RT-PCR). In a preferred embodiment, the expression of the relevant genes is measured by RT-PCR, preferably quantitative or semi-quantitative RT-PCR, even more preferably real-time quantitative or semi-quantitative RT-PCR.

Other methods of amplification include ligase chain reaction (LCR), transcription-mediated amplification (TMA), strand displacement amplification (SDA) and nucleic acid sequence based amplification (NASBA).

Nucleic acids having at least 10 nucleotides and exhibiting sequence complementarity or homology to the mRNA of interest herein find utility as hybridization probes or amplification primers. It is understood that such nucleic acids need not be identical, but are typically at least about 80% identical to the homologous region of comparable size,

more preferably 85% identical and even more preferably 90-95% identical. In certain embodiments, it will be advantageous to use nucleic acids in combination with appropriate means, such as a detectable label, for detecting hybridization. A wide variety of appropriate indicators are known in the art including, fluorescent, radioactive, enzymatic or other ligands (e. g. avidin/biotin).

Probes typically comprise single-stranded nucleic acids of between 10 to 1000 nucleotides in length, for instance of between 10 and 800, more preferably of between 15 and 700, typically of between 20 and 500. Primers typically are shorter single-stranded nucleic acids, of between 10 to 25 nucleotides in length, designed to perfectly or almost perfectly match a nucleic acid of interest, to be amplified. The probes and primers are "specific" to the nucleic acids they hybridize to, i.e. they preferably hybridize under high stringency hybridization conditions (corresponding to the highest melting temperature Tm, e.g., 50 % formamide, 5x or 6x SCC. SCC is a 0.15 M NaCl, 0.015 M Na-citrate).

The nucleic acid primers or probes used in the above amplification and detection method may be assembled as a kit. Such a kit includes consensus primers and molecular probes. A preferred kit also includes the components necessary to determine if amplification has occurred. The kit may also include, for example, PCR buffers and enzymes; positive control sequences, reaction control primers; and instructions for amplifying and detecting the specific sequences.

Preferably, the tumor samples are archived formalin fixed paraffin-embedded (FFPE) biopsy material, as well as fresh/frozen (FF) tissue, and are used for assay of all transcripts. The expression level may be determined using RNA obtained from FFPE tissue, fresh frozen tissue or fresh tissue that has been stored in solutions such as RNAlater.

The gene expression levels of the GSI may also be measured by using standard immunodiagnostic techniques, including immunoassays such as competition, direct reaction, or sandwich type assays. In such embodiments, cancers cells are purified from the isolated tumor sample. Such assays include, but are not limited to, microarray; qPCR; agglutination tests; enzyme-labelled and mediated immunoassays, such as

ELISAs; biotin/avidin type assays; radioimmunoassays; immunoelectrophoresis; immunoprecipitation.

When the method of the invention is performed with the minimal 5 genes (TDRD7, DDX60, IFIT2, ABCA1 and DDX58), then the threshold may be comprised between 1.81 and 1.87, preferably is around 1.83.

When the method of the invention is performed with the 10 genes (TDRD7, DDX60, IFIT2, ABCA1, DDX58, STAT2, IFI44L, DDR2, IFI44 and TLR3), then the threshold may be comprised between 3.40 and 4.30, preferably is around 3.66.

When the method of the invention is performed with the 20 genes (TDRD7, DDX60, IFIT2, ABCA1, DDX58, STAT2, IFI44L, DDR2, IFI44, TLR3, MX1, IFIT1, C19orf66, USP18, SAMD9L, SAMHD1, OASL, CCL5, MUC1 and PLSCR1), then the threshold may be comprised between 3.40 and 4.30, preferably is around 3.66.

When the method of the invention is performed with the 30 genes (TDRD7, DDX60, IFIT2, ABCA1, DDX58, STAT2, IFI44L, DDR2, IFI44, TLR3, MX1, IFIT1, C19orf66, USP18, SAMD9L, SAMHD1, OASL, CCL5, MUC1, PLSCR1, OAS3, B2M, IFIT3, IFIT5, OAS1, LAMP3, IFITM1, TRIM22, RSAD2 and UBE2L6), then the threshold may be comprised between 7.50 and 11.40, preferably is around 8.66.

Said method for predicting the sensibility of a patient afflicted by a cancer to a PARP inhibitor according to the invention may be combined with the HRD assay developed by Myriad (Myriad Genetics Inc. US Patent Application US 2014/0363521). Indeed, as shown in the examples, the combination of the HRD assay and the GSI assay of the invention shows a better prediction than each method alone, with a lower rate of false positives.

The HRD assay comprises, or consists essentially of, (a) detecting, in a sample or DNA derived therefrom, chromosomal aberrations regions (CA Regions) in at least one pair of human chromosomes or DNA derived therefrom; and (b) determining the number, size (e.g., length), and/or character of said CA Regions.

As used herein, "chromosomal aberration" or "CA" means a somatic change in a cell's chromosomal DNA that falls into at least one of three overlapping categories: LOH (loss of heterozygosity), TAI (telomeric allelic imbalance), or LST (large scale transition). Polymorphic loci within the human genome (e.g., single nucleotide polymorphisms (SNPs)) are generally heterozygous within an individual's germline since that individual typically receives one copy from the biological father and one copy from the biological mother. Somatically, however, this heterozygosity can change (via mutation) to homozygosity. This change from heterozygosity to homozygosity is called loss of heterozygosity (LOH). LOH may result from several mechanisms. For example, in some cases, a locus of one chromosome can be deleted in a somatic cell. The locus that remains present on the other chromosome (the other non-sex chromosome for males) is an LOH locus as there is only one copy (instead of two copies) of that locus present within the genome of the affected cells. This type of LOH event results in a copy number reduction. In other cases, a locus of one chromosome (e.g., one non-sex chromosome for males) in a somatic cell can be replaced with a copy of that locus from the other chromosome, thereby eliminating any heterozygosity that may have been present within the replaced locus. In such cases, the locus that remains present on each chromosome is an LOH locus and can be referred to as a copy neutral LOH locus. LOH and its use in determining HRD is described in detail in WO2011/160063.

A broader class of chromosomal aberration, which encompasses LOH, is allelic imbalance. Allelic imbalance occurs when the relative copy number (i.e., copy proportion) at a particular locus in somatic cells differs from the germline. For example, if the germline has one copy of allele A and one copy of allele B at a particular locus, and a somatic cell has two copies of A and one copy of B, there is allelic imbalance at the locus because the copy proportion of the somatic cell (2:1) differs from the germline (1:1). LOH is an example of allelic imbalance since the somatic cell has a copy proportion (1:0 or 2:0) that differs from the germline (1:1). But allelic imbalance encompasses more types of chromosomal aberration, e.g., 2:1 germline going to 1:1 somatic; 1:0 germline going to 1:1 somatic; 1:1 germline going to 2:1 somatic, etc. Analysis of regions of allelic imbalance encompassing the telomeres of chromosomes is particularly useful in the invention. Thus, a "telomeric allelic imbalance region" or "TAI Region" is defined as a region with allelic imbalance that (a) extends to one of the

subtelomeres and (b) does not cross the centromere. TAI and its use in determining HRD is described in detail in WO2012/027224.

A class of chromosomal aberrations that is broader still, which encompasses LOH and TAI, is referred to herein as large scale transition ("LST"). LST refers to any somatic copy number transition (i.e., breakpoint) along the length of a chromosome where it is between two regions of at least some minimum length (e.g., at least 3, 4, 5, 6, 7, 8 9, 10, 11 12, 13, 14, 15, 16, 17, 18, 19 or 20 or more megabases) after filtering out regions shorter than some maximum length (e.g., 0.1, 0.2, 0.3, 0.4, 0.5, 0.6, 0.7, 0.8, 0.9, 1, 1.5, 2, 2.5, 3, 3.5, 4 or more megabases).

Thus, preferably, the method for predicting the responsiveness of a cancer patient to treatment with a PARP inhibitor, according to the invention, is used in combination with a method comprising (a) detecting, in a tumor sample of the patient (which may be the same or different from the tumor sample used in the method of the invention), chromosomal aberrations regions in at least one pair of human chromosomes or DNA derived therefrom; and (b) determining the number, size, and/or character of said chromosomal aberrations regions.

The present invention also relates to a product containing a genotoxic anti-cancer agent, preferably cyclophosphamide and a PARP inhibitor as a combined preparation for simultaneous, separate or sequential use in cancer therapy, preferably triple-negative breast cancer therapy. Preferably, said combined preparation is administered to TNBC patients having a positive GSI.

The present invention also relates to a PARP inhibitor for use for potentiating the effects of chemotherapy in cancer patients, preferably in TNBC patients, having a positive GSI.

Indeed, as shown in example 2, in TNBC patients having a positive GSI, the use of a PARP inhibitor potentiates the therapeutic effects of chemotherapy. Particularly, said chemotherapy is cyclophosphamide, optionally in combination with doxorubicin. By "potentiation", it is meant that the effects of chemotherapy are increased by the PARP inhibitor. This contributes to inhibiting tumor regrowth, and/or to induce partial or complete tumor regression.

The present invention will now be illustrated by the following examples and figures.

Figures:

Figure 1: Example of tumor response to genotoxic chemotherapy.
Groups of 8-10 mice with subcutaneously growing tumors (HBCx-7, HBCx-23, HBCx-17 or HBCx-6) were treated with saline vehicle (Control) or with cyclophosphamide at 100 mg/kg by ip route at D0 (Treated). Graphs represent the evolution of the mean tumor volume in mm3 (y axis) over time in days (x axis) for each group.

Figure 2: Comparative analysis of GSI, HRD-score and BRCA status classifiers, alone or combined, as predictors of niraparib efficacy.

Figure 3: Effect on tumor growth of cyclophosphamide and PARP inhibitor treatment in the human breast tumor xenograft HBCx-6.
Cyclophosphamide was administered i.p once at 100 mg/kg at day 0. PARP1 inhibitor niraparib was administered at 75 mg/kg i.p qdx28 from day 15 to day 35. PARP1 inhibitor olaparib was administered at 100 mg/kg i.p qdx28 from day 15 to day 35. Data are expressed as median tumor volume (mm^3).

Figure 4: Effect on tumor growth of cyclophosphamide and PARP inhibitor treatment in the human breast tumor xenograft HBCx-14.
Cyclophosphamide was administered i.p once at 100 mg/kg at day 0. PARP1 inhibitor niraparib was administered at 75 mg/kg i.p qdx28 from day 15 to day 35. PARP1 inhibitor olaparib was administered at 100 mg/kg i.p qdx28 from day 15 to day 35. Data are expressed as median tumor volume (mm^3).

Figure 5: Effect on tumor growth of cyclophosphamide and PARP inhibitor treatment in the human breast tumor xenograft HBCx-1.
Cyclophosphamide was administered i.p once at 100 mg/kg at day 0. PARP1 inhibitor niraparib was administered at 75 mg/kg i.p qdx28 from day 15 to day 35. PARP1

inhibitor olaparib was administered at 100 mg/kg i.p qdx28 from day 15 to day 35. Data are expressed as median tumor volume (mm^3).

Figure 6: Effect on tumor growth of cyclophosphamide and PARP inhibitor treatment in the human breast tumor xenograft HBCx-8.

Cyclophosphamide was administered i.p once at 100 mg/kg at day 0. PARP1 inhibitor niraparib was administered at 75 mg/kg i.p qdx28 from day 15 to day 28. PARP1 inhibitor olaparib was administered at 100 mg/kg i.p qdx28 from day 15 to day 28. Data are expressed as median tumor volume (mm^3).

Figure 7: Effect on tumor growth of cyclophosphamide and PARP inhibitor treatment in the human breast tumor xenograft HBCx-33.

Cyclophosphamide was administered i.p once at 100 mg/kg at day 0. PARP1 inhibitor niraparib was administered at 75 mg/kg i.p qdx28 from day 15 to day 28. PARP1 inhibitor olaparib was administered at 100 mg/kg i.p qdx28 from day 15 to day 28. Data are expressed as median tumor volume (mm^3).

EXAMPLES

The following examples use the materials & methods described below:

MATERIALS & METHODS

Animals and establishment of tumor xenografts

Tumor xenografts were generated by direct subcutaneous grafting into immunodeficient mice of human tumor surgical samples with informed written consent of the patients and maintained by serial transplantation. The tumor xenografts have been studied for histology, cytogenetics, genetic and other biological markers, and for their response to a number of anticancer agents, alone and in combination. These studies have shown that tumor xenografts are biologically similar to the patient's tumors from which they derive, in term of both molecular characteristics and response to therapy. They are thus

clinically relevant tumor models, as opposed to xenografts produced from tumor cell lines that have been obtained by in vitro culture and often passaged for many years (Fiebig, H.H. et al. Eur J Cancer 40, 802-820, 2004; Marangoni E., Vincent-Salomon A., Auger N. et al. Clin Cancer Res. 2007 Jul 1;13:3989-98).

***In vivo* studies**

Chemotherapeutic drugs were administered by intraperitoneal or per os route as indicated according to the following doses and schedules: adriamycin, 2 mg/kg, q3wk (Doxorubicin®, Teva Pharmaceuticals, France) and cyclophosphamide, 100 mg/kg, q3wk (Endoxan®, Baxter, France). PARP inhibitor olaparib (AZD2281, Sequoia Research Products, UK) at 50 or 100 mg/kg (po; qd). PARP inhibitor niraparib (MK-4827, Tesaro, US) at 50 or 75 mg/kg (po; qd). Breast tumor xenografts were transplanted subcutaneously into 5-week old female Swiss nude mice as previously described (Legrier et al, Activation of IFN/STAT signaling predicts response to chemotherapy in estrogen receptor-negative breast cancer. Br J Cancer (2016) 114: 177-187). When tumors reached a volume of 60-250 mm3, mice were individually identified and randomly assigned to the control or treated groups (8 to 10 mice per group). Tumor volume was evaluated by measuring two perpendicular diameters of the tumor, with a caliper, biweekly during the treatment period and once a week during the follow-up period. The formula TV (mm3) = [length (mm) x width (mm)^2]/2 was used, where the length and the width were the longest and the shortest diameters of the tumor, respectively. All animals were observed daily for clinical symptoms and weighted biweekly during the treatment period and the follow-up period. Mice were ethically sacrificed when the tumor volume reached 2000 mm3. Response to treatment was evaluated as follows:

Complete regression (CR): disappearance of a measurable tumor for at least two consecutive measurements

Partial regression (PR): decrease in tumor volume for at least two consecutive measurements

Stabilization (S): stable tumor volume for at least two consecutive measurements

Progression (P): tumor volume increase

Time to progression (TTP): time between beginning of treatment and progression

Median TTP: time since beginning of treatment at which half of the mice in a treated group show tumor progression.

Microarray analyses of gene expression

Gene expression data of were obtained by running total RNA extracted from 28 TNBC PDXs on Affymetrix U133.2 plus gene chips. Data were normalized by using the PLIER algorithm, available in the Affymetrix Expression Console.

Construction of the genomic stress signature-based classifier

The genomic stress signature used for PDX classification was identified as described in previous studies (US Patent Application 13/463,129 Markers for Cancer Prognosis and Therapy and Methods of use, May 3, 2012 to Judde JG et al.; Legrier et al, Activation of IFN/STAT signaling predicts response to chemotherapy in estrogen receptor-negative breast cancer. Br J Cancer (2016) 114: 177-187). Probesets corresponding to the 62 genes with the most significant p value selected from this list were identified in the microarray. Probesets containing suffix "x" were removed as unique hybridization is not guaranteed for these probesets, and when multiple probesets were assigned to the same gene a mean value was calculated and used in the analysis. Gene intensity in the dataset was expressed as $\log_2$ values. For each gene, z-score normalized expression value was calculated by using the following formula: $z=(x-\mu)/\sigma$, where x is the individual intensity value, μ is the mean of the population and σ is the standard deviation of the population. For each gene, a mean z-score was calculated for the niraparib-sensitive and niraparib-resistant group of PDXs, and the ratio between the two mean values was used to rank genes. In the analysis, the sum of gene expression values from the top 5, 10, 20 and 30 genes with decreasing responder/non-responder ratio were used to calculate a score for each PDX. As 30% of models show sensitivity to niraparib, we assigned to the responder group PDXs whose GSI was above the 70^{th} percentile (cut-off). The robustness of the predictive method was assessed by comparison with the tumor responses observed in vivo.

EXAMPLE 1: Assay for predicting breast cancer sensitivity to PARP inhibitors

Identification of a gene expression signature as indicator of tumor genomic stress

The previous studies on the effects of genotoxic chemotherapy in a series of breast cancer PDXs showed that treatment with cyclophosphamide or the combination of adriamycine and cyclophosphamide (AC) induced the shared overexpression of a collection of genes, including several interferon-regulated genes, in tumors that were responsive to chemotherapy (US Patent Application 13/463,129 Markers for Cancer Prognosis and Therapy and Methods of use, May 3, 2012 to Judde JG et al.; Legrier et al, Activation of IFN/STAT signaling predicts response to chemotherapy in estrogen receptor-negative breast cancer. Br J Cancer (2016) 114: 177-187). From this collection, a list of 62 genes with the most significant p value (below 0.0007658) was selected and herein designated as genomic stress signature (GSS) and shown in Table 1.

p value	False Discovery Rate	Residual treated tumors (R)	Untreated Tumors (T)	R/T ratio	Unique id	Gene symbol
< 1e-07	< 1e-07	178.85	27.18	6.580206	2343473	IFI44L
< 1e-07	< 1e-07	80.05	16.03	4.993762	3257192	IFIT2
0.0000001	0.000734	403.76	100.68	4.01033	2707876	LAMP3
0.0000002	0.0011	212.51	33.6	6.324702	3432514	OAS2
0.0000003	0.0011	277.61	63.18	4.393954	2692060	PARP9
0.0000003	0.0011	240.23	108.53	2.213489	3753860	CCL5
0.0000009	0.00283	211.69	29.08	7.279574	3257246	IFIT1
0.0000013	0.00358	97.22	51.14	1.901056	3474831	OASL
0.0000023	0.00563	235.59	142.23	1.656402	3457752	STAT2
0.0000057	0.0105	263.69	54.71	4.819777	3432438	OAS1
0.0000073	0.0124	196.79	110.7	1.777687	3529701	IRF9
0.000011	0.0161	135.9	43.55	3.120551	2735362	HERC6
0.0000122	0.0164	422.79	224.49	1.883336	3373962	UBE2L6
0.0000142	0.0164	79.96	21.06	3.796771	3854454	BST2
0.0000152	0.0164	442.99	101.22	4.376507	3922100	MX1
0.0000153	0.0164	71.53	15.46	4.626779	3257204	IFIT3
0.0000156	0.0164	68.48	17.69	3.871114	2343511	IFI44
0.0000177	0.0172	29.97	11.54	2.597054	2792800	DDX60
0.0000182	0.0172	78.21	37.78	2.070143	2531377	SP100
0.0000188	0.0172	3473.08	987.69	3.516367	2403261	IFI6
0.0000227	0.0192	148.26	93.58	1.584313	2982319	SOD2
0.0000236	0.0192	545.25	235.96	2.310773	2592268	STAT1
0.0000244	0.0192	69.7	27.57	2.52811	2468351	RSAD2

0.0000268	0.0203	42.01	22.21	1.89149	3257268	IFIT5
0.000031	0.0221	117.25	69.15	1.695589	3442475	C1R
0.0000311	0.0221	218.31	85.96	2.53967	3432467	OAS3
0.0000377	0.0251	137.72	39.21	3.512369	2639054	PARP14
0.000058	0.0365	188.48	100.69	1.871884	2699726	PLSCR1
0.000063	0.0385	17.37	10.59	1.640227	2754937	TLR3
0.0000651	0.0387	200.5	71.73	2.795204	3203086	DDX58
0.0001084	0.0508	38.02	22.83	1.665353	2334602	TSPAN1
0.0001248	0.0561	62.68	23.75	2.639158	3904691	SAMHD1
0.000151	0.0639	394.28	237.34	1.661246	2437118	MUC1
0.0001587	0.0647	38.12	20.44	1.864971	3360142	TRIM21
0.0001979	0.0739	185.15	120.14	1.541119	3820310	C19orf66
0.0002115	0.0739	139.45	51.89	2.687416	2638962	DTX3L
0.0003197	0.101	120.06	28.39	4.228954	3061438	SAMD9
0.0003941	0.114	45.62	26.09	1.748563	3218528	ABCA1
0.0004272	0.118	156.29	89.89	1.738681	3908831	ZNFX1
0.0004412	0.118	40.47	25.81	1.567997	3722338	IFI35
0.0005033	0.126	1027	680.33	1.509562	3957679	SELM
0.0006154	0.145	217.85	75.96	2.867957	3549575	IFI27
0.0006971	0.157	2731.27	598.59	4.562839	3315675	IFITM1
0.0007578	0.162	178.8	60.54	2.953419	3366903	MUC15
0.0007658	0.162	280.29	155.71	1.800077	3936550	USP18
0.0007726	0.162	100.08	25.97	3.853677	3061456	SAMD9L
0.0012513	0.203	99.84	57.8	1.727336	3181193	TDRD7
0.0012551	0.203	324.66	101.85	3.187629	3848039	C3
0.0012646	0.203	285.67	139.47	2.048254	3075932	PARP12
0.0014747	0.215	68.5	38.15	1.795544	2603051	SP110
0.0016593	0.225	71.48	46.42	1.539854	2748346	TLR2
0.0017661	0.231	113.96	53.75	2.120186	2364231	DDR2
0.0019932	0.246	107.16	71.1	1.507173	3458587	DDIT3
0.0024306	0.258	33.36	21.21	1.572843	2735409	HERC5
0.0027085	0.269	26.81	16.58	1.617008	3403168	C1S
0.0029163	0.283	35.91	15.65	2.294569	3318443	TRIM22
0.0030881	0.291	77.02	38.12	2.020462	2584207	IFIH1
0.0033792	0.304	680.79	244.76	2.781459	2710599	CLDN1
0.0043472	0.344	877.3	273.99	3.201942	3592023	B2M
0.0044266	0.344	287.86	160.23	1.796543	3708858	CD68
0.0045448	0.345	28.25	17.97	1.572065	3944243	APOL6
0.004861	0.355	382.3	250.73	1.524748	2901620	HLA-E

Table 1. Genomic Stress Signature (GSS): 62 genes with the lowest p-values, overexpressed in residual tumor foci from TNBC PDXs treated with AC compared to their untreated counterparts, analyzed by GeneChip Human Exon ST Array (Affymetrix).

The overexpression of GSS genes in tumor cells following genotoxic treatment is associated with DNA damage and repair, activation of the DNA-damage checkpoints. Overexpression of GSS genes is transient and reverts to normal levels upon tumor regrowth (Legrier et al, Activation of IFN/STAT signaling predicts response to chemotherapy in estrogen receptor-negative breast cancer. Br J Cancer (2016) 114: 177-187). Replicative/genomic stress is common in tumors and may have various tumor-specific sources that have yet to be fully identified (Luo J. et al. Principles of cancer therapy: oncogene and non-oncogene addiction. Cell (2009) 136: 823-837; Dobbelstein M. and Sorensen CS. Exploiting replicative stress to treat cancer. Nat. Review Drug Discovery (2015) 14: 405-423). As the inventors observed that about 20-30% of the collection of over 100 PDX of diverse human tumor types shows constitutive overexpression of GSS genes, they hypothesized that it could reflect a state of chronic replicative/genomic stress associated to persistent DNA damage in these tumors, reminiscent of the GSS activation induced by genotoxics.

PARP inhibitors are a family of compounds that block PARP-mediated DNA repair and work particularly well in tumors with impaired homologous recombination (HR)-mediated DNA repair such as those with inactivated BRCA genes. So far, the most accepted biomarker to predict tumor sensitivity to PARP inhibitor is mutation of BRCA1/BRCA2 genes. Another candidate biomarker to identify PARP inhibitor-sensitive tumors is the HRD genomic scar assay, in which the detection of genomic rearrangements signals a tumor with homologous recombination deficiency (HRD). The HRD assay identifies the majority of BRCA-mutated tumors, but also other tumors with putative alterations in other DNA repair pathways.

However, these tests lack specificity as less than half of BRCA-mutated or HRD-positive TNBC or ovarian cancer patients are in fact responsive to PARP inhibitors (Maxwell KN. And Domshek SM. Cancer treatment according to *BRCA1* and *BRCA2* mutations. Nat Rev Clin Onc, 9, 520-528, 2012. PMID 22825375; Watkins JA. Et al. Genomic scars as biomarkers of homologous recombination deficiency and drug response in breast and ovarian cancers. Breast Cancer Res. 16, 211-222, 2014. PMID 25093514).

The inventors hypothesized that a significant fraction of tumors scoring HRD positive (by BRCA mutation or genomic scarring assay) may have reverted back to a phenotype of HR competency, making them refractory to PARP inhibition. In this scenario, they questioned if the GSS that they identified in tumors undergoing DNA damage upon treatment with genotoxic agents might be a feature of HRD-positive tumors in which HR deficiency has not been compensated and is still effective, resulting in PARP inhibitor sensitivity. To evaluate the ability of the GSS to identify HRD tumors with effective HR deficiency that would be responsive to PARP inhibitors, the inventors tested the efficacy of niraparib (provided by Tesaro) and of olaparib in a series of TNBC PDXs. The predictive value of the GSS was compared to that of BRCA mutation and of HRD status.

Identification of tumor classes

In total, 28 TNBC PDXs were tested for their response to niraparib. Several PDXs showed very similar response to olaparib, another PARP inhibitor, thus indicating that in the tested experimental conditions the two drugs efficacy is very similar. Tumor response to the standard of care combination adryamicin/cyclophosphamide (AC) was also evaluated for 27 out of 28 PDXs. The results obtained are shown in Table 2.

Sample Name	HRD-score	20-gene GSI	Response Niraparib*	Response Olaparib**	Response AC	mutated BRCA genes
HBCx-6	72	34.27	CR	CR	CR	
HBCx-10	57	-10.16	PR	CR	CR	BRCA2
HBCx-14	53	17.78	CR	CR	CR	
HBCx-15	68	7.36	CR	CR	CR	
HBCx-17	69	-1.36	S	S	PR	BRCA2
T168	54	7.71	CR		CR	BRCA1
T298	66	-10.15	S		PR	
T330	65	10.51	CR		CR	BRCA1
HBCx-1	50	-9.63	P		S	
HBCx-2	36	-3.07	P		P	
HBCx-7	5	0.32	P		P	
HBCx-8	65	-9.01	P		S	BRCA1
HBCx-9	55	-11.13	P	P	P	
HBCx-11	73	-10.36	P		S	BRCA1
HBCx-12B	78	-9.14	P		P	

HBCx-16	57	-14.14	P		P	
HBCx-23	69	-9.77	P		S	
HBCx-24	55	14.35	P		P	
HBCx-27	27	0.67	P		P	
HBCx-28	63	13.34	P		S	BRCA1
HBCx-30	21	-7.27	P		P	
HBCx-31	25	-8.46	P		P	
HBCx-33	32	1.51	P		S	
HBCx-39	32	-1.00	P		P	
T174	31	2.85	P		P	
T180R	32	-13.30	P		CR	
T311R	25	14.16	P			
T381	30	3.10	P		CR	

Table 2: Description of the PDX cohort analyzed. PDXs labeled in red are considered as responders. Legend: GSS: genomic stress signature; R: responder; NR: non-responder; CR: complete regression; PR: partial regression; S: stabilization; P: progression; NCR: non-complete regression.

** Administered daily at 50-75mg/Kg; ** administered daily at 75-100 mg/Kg. Tumors are considered as HRD-positive when HRD-score is >42. Tumors with Genomic Stress Index (GSI) >4.38 were predicted to be sensitive to PARP inhibitors.*

Tumor responses were evaluated according to the criteria described in the Materials and Methods section, an example of each response type is provided in Figure 1.

Generation of a Genomic Stress Index (GSI) predictive of breast cancer sensitivity to PARP inhibitors

To explore the association between GSS-related gene expression and niraparib sensitivity, expression of GSS-related genes from U133.2 plus gene expression microarray (Affymetrix) underwent z-score normalization and mean z-score value was calculated for each gene in each TNBC PDX. Genes were ranked according to the mean z-score ratio between responder and non-responder group as shown in Table 3.

Gene Symbol	Responders to Niraparib								Non-Responders to Niraparib																	mean R	mean NR	R/NR ratio
	HBCx-6	HBCx-10	HBCx-14	HBCx-15	HBCx-17	T168	T298	T330	HBCx-1	HBCx-2	HBCx7	HBCx-8	HBCx-9	HBCx-11	HBCx-12B	HBCx-16	HBCx-23	HBCx-24	HBCx-27	HBCx-28	HBCx-30	HBCx-31	HBCx-33	HBCx-39	T174	T180R	T311R	T381			
TDRD7	1,9	-0,9	2,1	1,4	1,2	0,8	-0,3	-1,1	-0,8	0,4	0,8	-0,2	-1,1	1,0	-0,3	-1,0	-0,8	0,2	0,6	-1,3	-1,7	-1,2	0,3	-0,7	-0,1	0,5	-0,1	0,3	0,6	-0,3	0,9
DDX60	2,2	-0,8	1,1	0,2	0,0	0,8	0,1	0,5	-2,0	1,4	0,1	-0,8	-0,5	-1,0	0,9	-1,3	-1,5	0,8	0,0	0,8	-0,1	-0,8	-0,4	0,0	-0,1	-1,7	1,2	0,8	0,5	-0,2	0,7
IFIT2	2,2	-0,7	0,5	-0,1	-0,3	1,3	-1,1	2,3	-1,3	-0,5	0,0	-1,2	-1,0	-1,2	-0,3	-0,6	-0,1	0,9	-0,3	1,2	0,0	0,4	-0,1	0,7	0,8	-1,2	0,6	-1,2	0,5	-0,2	0,7
ABCA1	-0,2	0,4	-0,2	1,6	1,1	-0,5	0,7	0,7	-0,3	-0,7	2,2	-0,8	-0,5	1,2	-2,0	-1,4	0,8	-0,8	0,3	1,4	-0,6	-0,4	-0,6	-0,7	0,5	0,1	0,7	-1,6	0,4	-0,2	0,6

| | | |

Table 3. Z-score normalized values and mean responder (R)/non-responder (NR) ratio used to rank genes associated to response to niraparib.

To generate a Genomic Stress Index, the sum of the mean values of the top 5, 10, 20 or 30 genes of Table 3 in term of R/NR ratio value was calculated for each PDX, and the values obtained were used to rank the models.

The top 5 genes were: TDRD7, DDX60, IFIT2, ABCA1 and DDX58.

The top 10 genes were: TDRD7, DDX60, IFIT2, ABCA1, DDX58, STAT2, IFI44L, DDR2, IFI44 and TLR3.

The top 20 genes were: TDRD7, DDX60, IFIT2, ABCA1, DDX58, STAT2, IFI44L, DDR2, IFI44, TLR3, MX1, IFIT1, C19orf66, USP18, SAMD9L, SAMHD1, OASL, CCL5, MUC1 and PLSCR1.

The top 30 genes were: TDRD7, DDX60, IFIT2, ABCA1, DDX58, STAT2, IFI44L, DDR2, IFI44, TLR3, MX1, IFIT1, C19orf66, USP18, SAMD9L, SAMHD1, OASL, CCL5, MUC1, PLSCR1, OAS3, B2M, IFIT3, IFIT5, OAS1, LAMP3, IFITM1, TRIM22, RSAD2 and UBE2L6.

As overall 8 of the 28 PDXs analyzed (28.6%) showed sensitivity to niraparib, the inventors tested the predictive value of the GSI by classifying samples with a GSI above the 70th percentile as putative responders to PARP inhibitors, and those with a GSI below the 70th percentile as putative non-responders. The classifications obtained and corresponding responses observed are presented in Table 4 for each of the 5-gene, 10-gene, 20-gene and 30-gene GSI classifiers.

PDX name	GSI Top 5
HBCx-6	9,59
HBCx-14	3,90
T311R	3,51
T168	3,34
HBCx-28	3,02
T330	2,96
HBCx7	2,30
HBCx-17	1,88
HBCx-24	1,81
HBCx-15	1,80
T174	0,72
HBCx-27	0,26

PDX name	GSI Top 10
HBCx-6	16,49
HBCx-14	7,77
T311R	6,59
HBCx-28	6,05
HBCx-15	5,22
HBCx-24	5,10
T330	4,99
HBCx-17	4,35
T168	3,36
HBCx-27	2,02
T174	1,98
HBCx-33	0,50

PDX name	GSI Top 20
HBCx-6	34,27
HBCx-14	17,78
HBCx-24	14,35
T311R	14,16
HBCx-28	13,34
T330	10,51
T168	7,71
HBCx-15	7,36
T381	3,10
T174	2,85
HBCx-33	1,51
HBCx-27	0,67

PDX name	GSI Top 30
HBCx-6	52,44
HBCx-14	25,46
T311R	25,32
HBCx-24	23,51
HBCx-28	22,04
T330	13,81
HBCx-15	12,04
T168	11,45
HBCx7	7,47
T174	6,51
T381	3,05
HBCx-27	1,83

HBCx-33	0,23	HBCx7	-0,17	HBCx7	0,32	HBCx-2	-4,23
HBCx-2	-0,52	HBCx-39	-0,58	HBCx-39	-1,00	HBCx-30	-5,63
T298	-0,79	T381	-0,88	HBCx-17	-1,36	HBCx-17	-5,82
HBCx-11	-0,79	HBCx-2	-1,16	HBCx-2	-3,07	HBCx-33	-6,15
HBCx-39	-0,96	HBCx-23	-2,38	HBCx-30	-7,27	HBCx-39	-6,47
T381	-1,23	HBCx-11	-2,87	HBCx-31	-8,46	HBCx-9	-9,90
HBCx-31	-1,52	HBCx-30	-3,04	HBCx-8	-9,01	HBCx-8	-10,25
HBCx-12B	-1,92	T298	-3,26	HBCx-12B	-9,14	HBCx-31	-12,64
HBCx-30	-2,50	HBCx-10	-4,09	HBCx-1	-9,63	HBCx-12B	-14,14
HBCx-23	-2,51	HBCx-12B	-5,17	HBCx-23	-9,77	HBCx-23	-15,29
HBCx-10	-2,56	HBCx-1	-5,17	T298	-10,15	HBCx-1	-15,49
HBCx-8	-2,81	HBCx-9	-5,73	HBCx-10	-10,16	HBCx-11	-17,39
T180R	-3,11	HBCx-8	-6,03	HBCx-11	-10,36	HBCx-10	-17,66
HBCx-9	-4,37	T180R	-6,15	HBCx-9	-11,13	HBCx-16	-18,79
HBCx-1	-4,40	HBCx-31	-6,76	T180R	-13,30	T298	-22,17
HBCx-16	-5,32	HBCx-16	-10,99	HBCx-16	-14,14	T180R	-22,89
70th percentile cut-off value	1,83	70th percentile cut-off value	3,66	70th percentile cut-off value	4,38	70th percentile cut-off value	8,66

Table 4. GSI calculated by increasing number of genes listed in Table 3. PDXs with GSI above the cut-off value indicated by the 70th percentile are predicted as sensitive to PARP inhibitors; PDXs that showed sensitivity to niraparib are labeled in green: dark green: complete or partial tumor regression; light green: tumor stabilization.

Of the 8 PDXs classified as responders to niraparib by the GSI approach using different gene lists, 5 showed responsiveness, whereas only 3 of the 20 models predicted to be resistant to treatment showed response to niraparib. In light of these results, the inventors decided to compare the GSI classifier performance according to the invention with that of BRCA mutation status, which is the only biomarker currently approved as a companion diagnostic for olaparib administration in the clinic (Kim G. FDA Approval Summary: Olaparib Monotherapy in Patients with Deleterious Germline BRCA-Mutated Advanced Ovarian Cancer Treated with Three or More Lines of Chemotherapy. Cin Cancer Res (2015) 21: 4257-4261), and with that of Myriad's HRD score, which is currently being tested as a companion diagnostic test for PARP inhibitors in clinical trials (Myriad Genetics Inc. US Patent Application US 2014/0363521 A1, Methods and materials for assessing homologous recombination deficiency). The results of the comparative analysis are shown in Figure 2.

This analysis shows that GSI performs better than Myriad's HRD score and BRCA status in predicting niraparib sensitivity (37.5% versus 52.9% and 42.9% of false positive, respectively), and despite few responders were predicted as resistant (0 with HRD-score versus 3 with GSI) GSI correctly predicted as non-responder 17/20 PDX models versus 11/20 predicted by the HRD-score (85% and 55% respectively). Overall, only 6/28 PDXs were misclassified when using GSI versus 9/28 PDXs misclassified when using the HRD-score. Combining the GSI and the HRD scores performs significantly better than each test alone, with a decreased rate of false-positives (28.6% for GSI+HRD versus 37.5% and 52.9% for GSI and HRD respectively) and overall misclassification is reduced to only 5/28 PDXs (17.6% for GSI+HRD versus 21.4% and 32.1% for GSI and HRD respectively).

The inventors noticed that of the 3 PDXs erroneously predicted as resistant to niraparib by GSI, two harbor BRCA2 mutations. Since BRCA1 and BRCA2 act by different mechanisms in the DNA damage response (DDR) pathway, it is possible that mutation of BRCA2 would not generate or would not be compatible with chronic genomic stress in tumors. Therefore, the GSS-related genes would not be overexpressed in a BRCA2-mutated background. When the inventors generate a classifier that identifies responder tumors as those that present either elevated expression of GSS-related genes or BRCA2 mutation, they correctly predict 7/8 PDXs (87.5%) as responders and 17/20 PDX (85%) as non-responders (Figure 2).

EXAMPLE 2: Biomarker for selecting TNBC patients likely to benefit from combination of genotoxic chemotherapy and PARP inhibitors

Preclinical evidence indicates that PARP inhibitors have therapeutic potential in combination with genotoxic chemotherapy. However, although definitive clinical trial results have not been reported, the toxicity associated with such combinations has generally imposed dose reduction of the chemotherapy, possibly explaining why the clinical activity observed with the combinations has in general not been superior to that of the corresponding single agents given at full dose (Lupo B. and Trusolino L.

Inhibition of poly(ADP-ribosyl)ation in cancer: Old and new paradigms revisited. Biochimica et Biophysica Acta (2014) 1: 201-215; Scott CL. Et al. Poly(ADP-Ribose) Polymerase inhibitors: recent advances and future development. J Clin Oncology (2015) 33:1399-1406). In light of the inventors' previous observations, the inventors hypothesized that activation of the GSS in cancer cells occurring in chemo-sensitive tumors treated with genotoxic drugs may signal the engagement of a DNA damage response associated with PARP dependency, that would result in sensitization of cancer cells to PARP inhibition and at least additive effects of genotoxic drug/PARP inhibitor combination therapy.

The inventors therefore tested if PARP inhibitors could potentiate the antitumor effect of cyclophosphamide-based chemotherapy (C) in several of the tested TNBC models. Treatments were given at full doses, with PARP inhibitor treatment being initiated 14 days after a single active dose of C. Out of 5 tested TNBC models that are responsive to C, two responded to the sequential combination treatment with PARP inhibitors, with a significant increase in TTP over C alone (HBCx-6 and HBCx-14; Figs. 3 & 4 and Table 5). Three other tumors did not respond at all to combination of PARP inhibitor and cyclophosphamide, with no change in TTP over each agent alone (HBCx-1, HBCx-8 and HBCx-33; Figs. 5, 6, 7 and Table 5).

	median TTP values (days)				Classifier	
Tumor	Control	C	C + niraparib	C + olaparib	GSI (20 gene)	HRD
HBCx-6	0	35	>228*	>228*	34.3	72
HBCx-14	0	31	63	77	17.8	53
HBCx-1	0	14	14	14	-9.6	50
HBCx-8	0	21	21	21	-9.0	65
HBCx-33	0	29	25	29	1.5	32

*Table 5: Median TTP values from experiments shown in Figures 6 to 10, and GSI and HRD scores of the corresponding tumor models. Groups of 8-10 mice received the indicated treatments. C: cyclophosphamide. *>228: means that less than half of the mice had their tumor progressing 228 days after treatment start (3/5 tumor-free mice in the C+niraparib group and 8/8 tumor-free mice in the C/olaparib group). In red: positive GSI and HRD scores.*

Surprisingly, these data show that DNA damage and activation of GSS genes induced by cyclophosphamide is not able to sensitize GSI-negative PARP inhibitor-resistant tumors to PARP inhibitors. The combination of cyclophosphamide with PARP

inhibitors is active only in tumors that are GSI-positive and sensitive to single-agent PARP inhibitor (HBCx-6 and HBCx-14; Table 5 and Table 2).

The main implication of these findings is that a combination of PARP inhibitor and C-based chemotherapy will only benefit the subset of TNBC patients whose tumor is sensitive to both C-based chemotherapy and PARP inhibitors.

The GSI assay is therefore useful to identify TNBC patients who are more likely to benefit from PARP inhibitors given in combination with cyclophosphamide-based chemotherapy, either in the neoadjuvant setting, or as maintenance therapy following completion of neoadjuvant chemotherapy.

CLAIMS

1. A method for predicting the responsiveness of a cancer patient to treatment with a PARP inhibitor, comprising: (1) measuring the expression of at least TDRD7, DDX60, IFIT2, ABCA1 and DDX58 genes, expressed in a tumor sample of the patient, so as to obtain an expression profile; and further (3) comparing the obtained expression profile with cut-off value(s),
   wherein if said profile is greater than cut-off value(s), then the cancer patient is likely to be sensitive to a PARP inhibitor treatment.

2. A method according to claim 1, wherein comprising (1) measuring the expression of at least TDRD7, DDX60, IFIT2, ABCA1 and DDX58 genes, expressed in a tumor sample of the patient, so as to obtain an expression profile; (2) determining a score in the tumor sample based at least on the measurements made in (1), and further (3) comparing the obtained score with cut-off value,
   wherein if said score is greater than cut-off value, then the cancer patient is likely to be sensitive to a PARP inhibitor treatment.

3. A method according to claim 1 or 2, wherein said patient has not undergone any treatment of said cancer.

4. A method according to any one of claims 1 to 3, wherein said patient has already been treated for said cancer, preferably by chemotherapy.

5. A method according to any one of claims 1 to 2 or 4, wherein said patient has already been treated for said cancer by cyclophosphamide.

6. A method according to any one of claims 1 to 5, wherein said patient is not mutated on BRCA2 gene.

7. A method according to any one of claims 1 to 6, wherein said cancer is chosen from breast cancer, ovarian cancer, lung cancer, colorectal cancer, prostate cancer,

pancreatic cancer and melanoma, preferably said cancer is triple negative breast cancer.

8. A method according to any one of claims 1 to 7, wherein step (1) further comprises measuring the expression of at least one of the following genes: APOL6, B2M, BST2, C1R, C1S, C3, CD68, CLDN1, DDIT3, DDR2, DTX3L, HERC5, HERC6, HLA-E, IFI27, IFI35, IFI6, IFI44L, IFI44, TLR2, TLR3, TRIM21, TRIM22, TSPAN1, IFIH1, IFIT1, IFITM1, IRF9, C19orf66, USP18, SAMD9, SAMD9L, SAMHD1, SELM, SOD2, SP100, SP110, STAT1, STAT2, CCL5, MUC1, MUC15, MX1, PLSCR1, OAS3, IFIT3, IFIT5, OAS1, OAS2, OASL, PARP12, PARP14, PARP9, LAMP3, RSAD2, UBE2L6 and ZNFX1.

9. A method according to any one of claims 1 to 8, wherein step (1) further comprises measuring the expression of at least one of the following genes: STAT2, IFI44L, DDR2, IFI44, TLR3, MX1, IFIT1, C19orf66, USP18, SAMD9L, SAMHD1, OASL, CCL5, MUC1, PLSCR1, OAS3, B2M, IFIT3, IFIT5, OAS1, LAMP3, IFITM1, TRIM22, RSAD2 and UBE2L6.

10. A method according to any one of claims 1 to 9, wherein step (1) further comprises measuring the expressions of the additional genes STAT2, IFI44L, DDR2, IFI44 and TLR3.

11. A method according to any one of claims 1 to 10, wherein step (1) further comprises measuring the expressions of the additional genes MX1, IFIT1, C19orf66, USP18, SAMD9L, SAMHD1, OASL, CCL5, MUC1 and PLSCR1.

12. A method according to any one of claims 1 to 11, wherein step (1) further comprises measuring the expressions of the additional genes OAS3, B2M, IFIT3, IFIT5, OAS1, LAMP3, IFITM1, TRIM22, RSAD2 and UBE2L6.

13. Product containing an anti-cancer agent, preferably cyclophosphamide, and a PARP inhibitor as a combined preparation for simultaneous, separate or sequential use in cancer therapy, preferably triple negative breast cancer therapy.

14. Method according to any one of claims 1 to 12 or product according to claim 13, wherein said PARP inhibitor is chosen from olaparib, rucaparib, talazoparib and niraparib.

15. Method according to any one of claims 1 to 12 or 14, wherein it is used in combination with a method comprising (a) detecting, in a tumor sample of said patient, chromosomal aberrations regions in at least one pair of human chromosomes or DNA derived therefrom; and (b) determining the number, size, and/or character of said chromosomal aberrations regions.

Figure 1

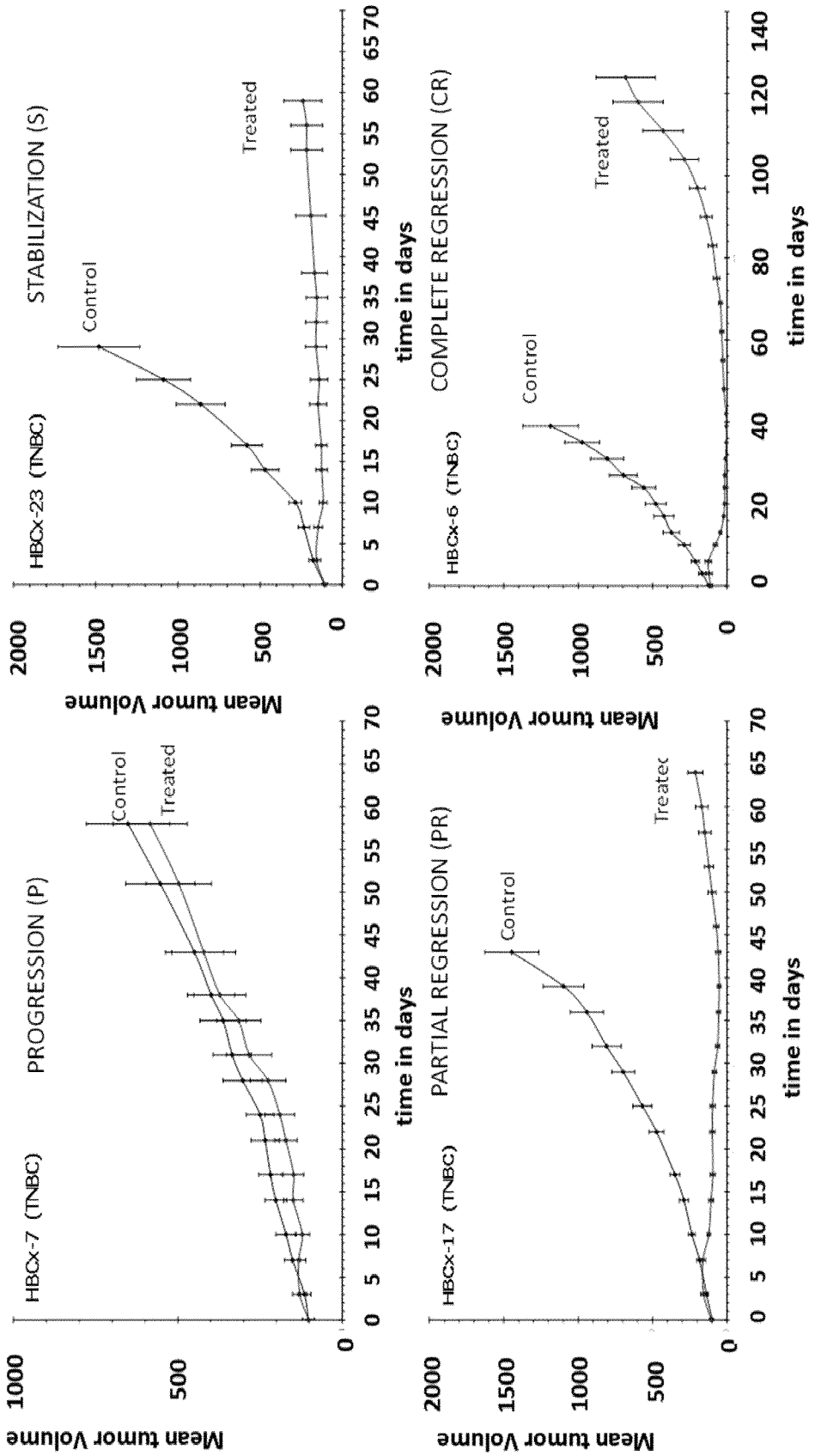

HBCx-7 (TNBC)
PROGRESSION (P)
Control
Treated
Mean tumor Volume
time in days
HBCx-23 (TNBC)
STABILIZATION (S)
Control
Treated
Mean tumor Volume
time in days
HBCx-17 (TNBC)
PARTIAL REGRESSION (PR)
Control
Treated
Mean tumor Volume
time in days
HBCx-6 (TNBC)
COMPLETE REGRESSION (CR)
Control
Treated
Mean tumor Volume
time in days

Figure 2

20-gene GSI

	Predicted R	Predicted NR		Results
R	5	3	8	37.5% false positives
NR	3	17	20	15% false negative
	8	20		

HRD score

	Predicted R	Predicted NR		Results
R	8	0	8	52.9% false positive
NR	9	11	20	0% false negative
	17	11		

BRCA status

	Predicted R	Predicted NR		Results
R	5	3	8	42.9% false positive
NR	3	17	20	19% false negative
	7	21		

GSI+HRD double positive

	Predicted R	Predicted NR		Results
R	5	3	8	28.6% false positive
NR	2	18	20	14.3% false negative
	7	21		

GSI+BRCA double positive

	Predicted R	Predicted NR		Results
R	3	5	8	25% false positive
NR	1	19	20	20.8% false negative
	4	24		

BRCA+HRD double positive

	Predicted R	Predicted NR		Results
R	4	4	8	42,9% false positive
NR	3	17	20	19% false negative
	7	21		

GSI or BRCA2 mutated

	Predicted R	Predicted NR		Results
R	7	1	8	30% false positive
NR	3	17	20	5,56% false negative
	10	18		

Figure 3

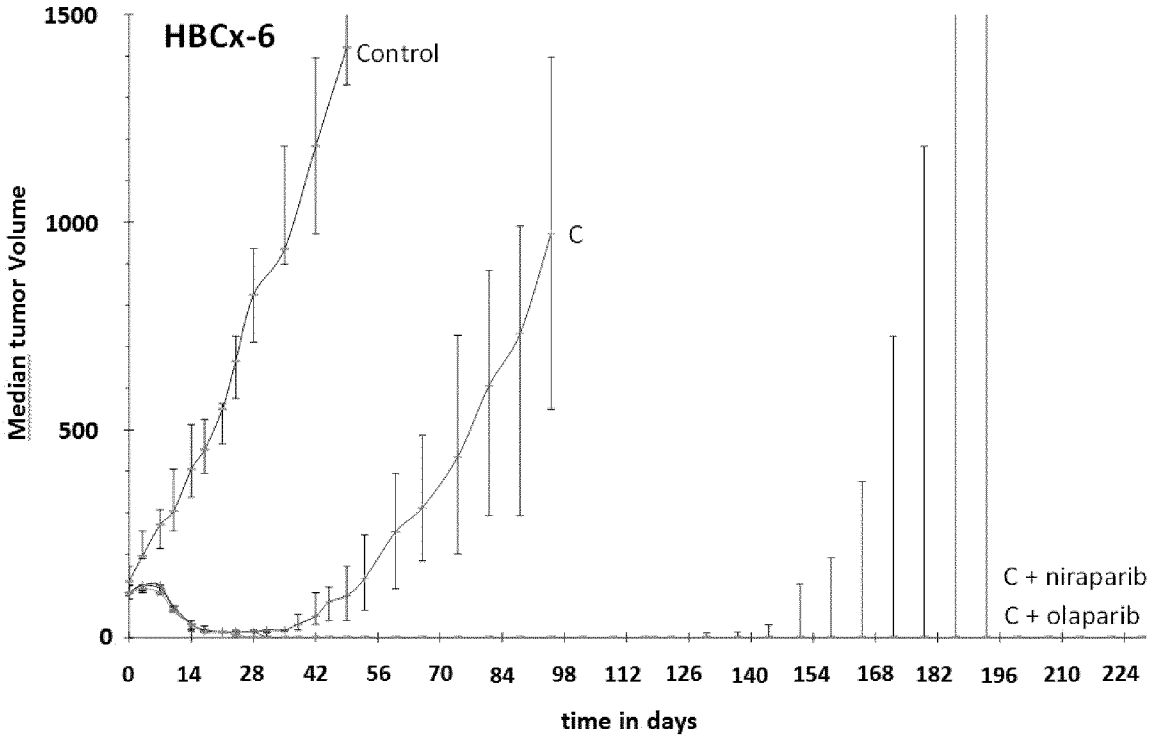

HBCx-6
Control
C
C + niraparib
C + olaparib
Median tumor Volume
1500
1000
500
0
0
14
28
42
56
70
84
98
112
126
140
154
168
182
196
210
224
time in days

Figure 4

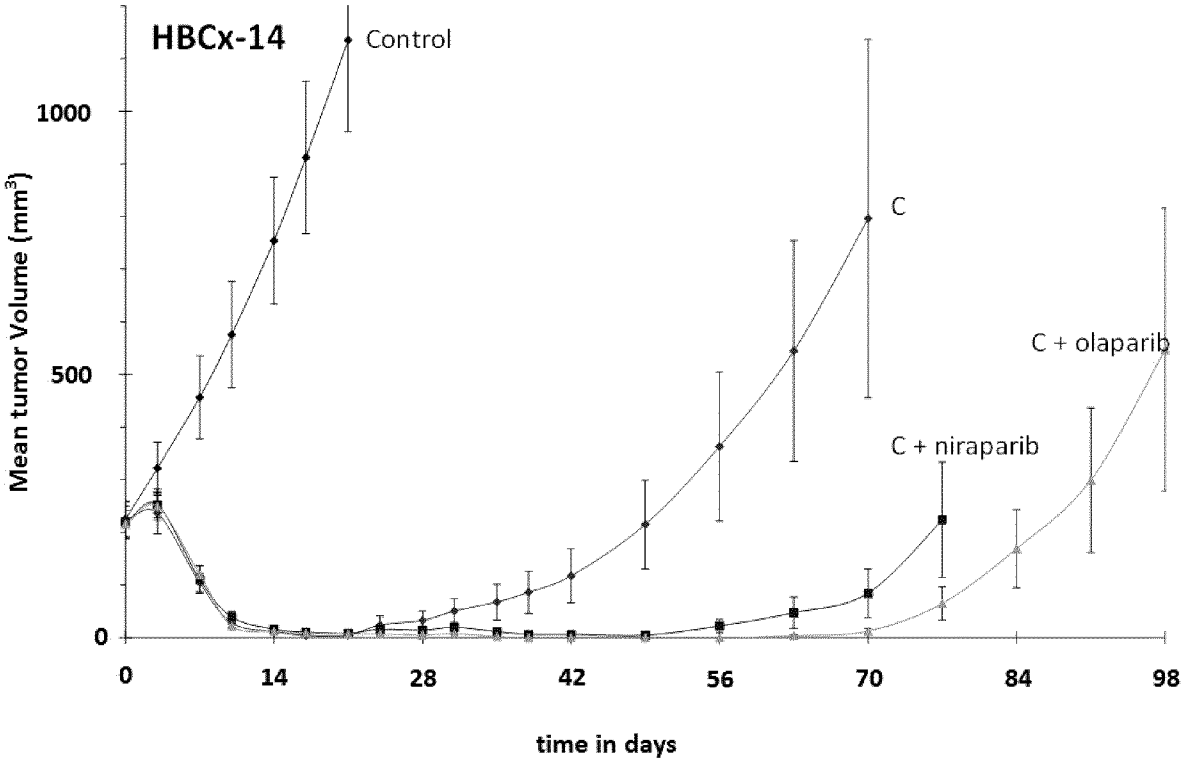

HBCx-14
Control
C
C + olaparib
C + niraparib
Mean tumor Volume (mm3)
1000
500
0
0
14
28
42
56
70
84
98
time in days

Figure 5

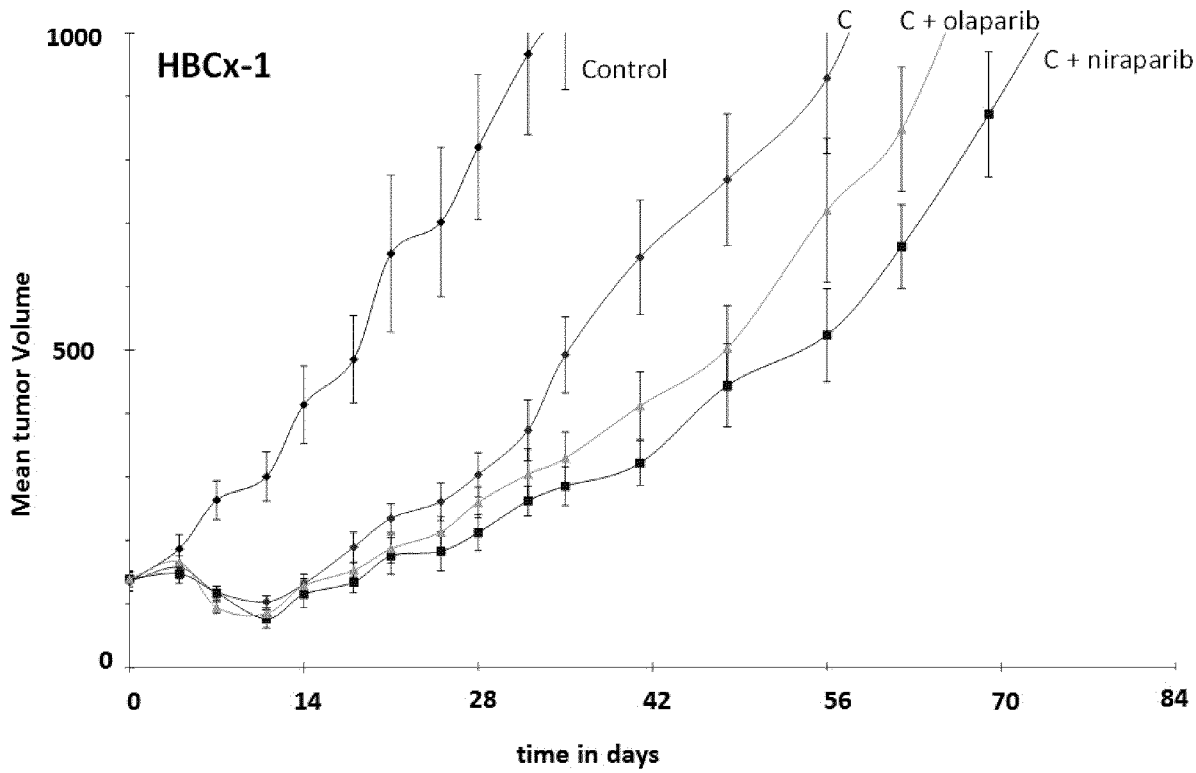

HBCx-1
Control
C
C + olaparib
C + niraparib
Mean tumor Volume
1000
500
0
0
14
28
42
56
70
84
time in days

Figure 6

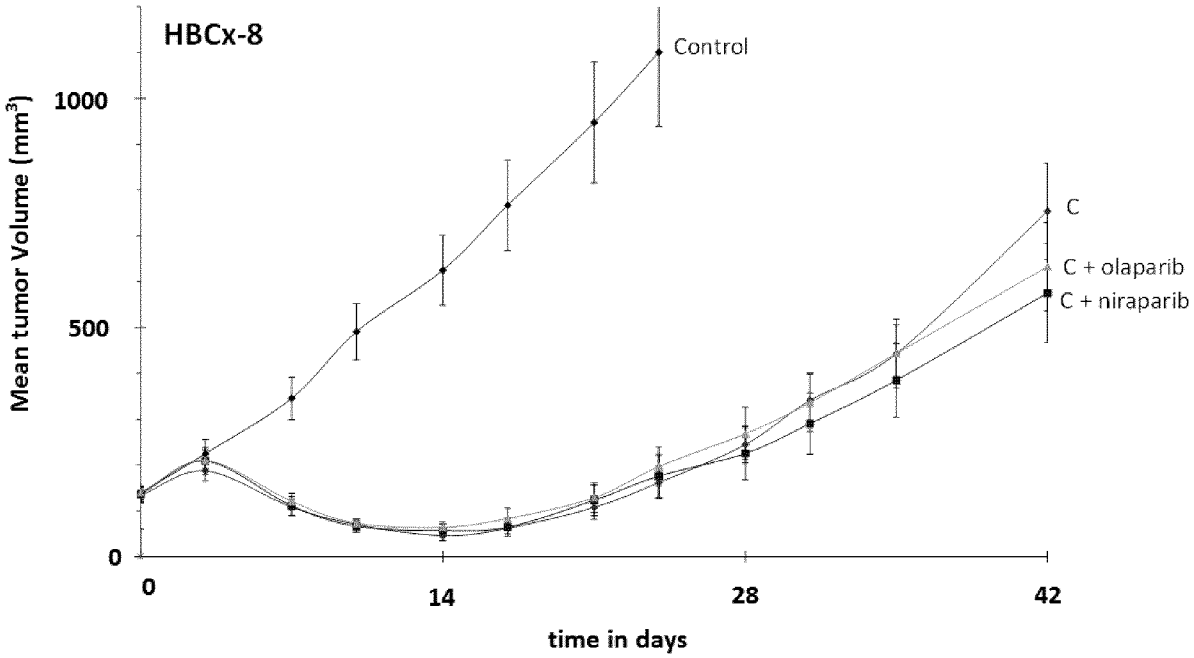
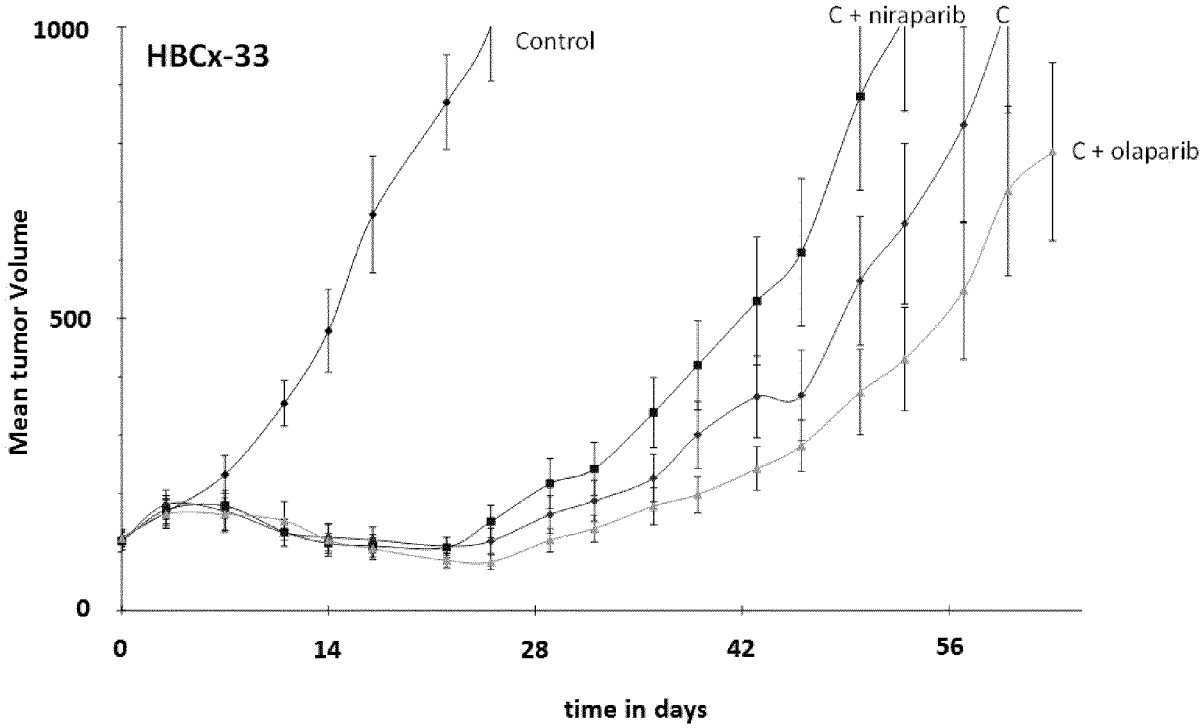

HBCx-8
Control
C
C + olaparib
C + niraparib
Mean tumor Volume (mm3)
1000
500
0
0
14
28
42
time in days

Figure 7


HBCx-33
Control
C + niraparib
C
C + olaparib
Mean tumor Volume
1000
500
0
0
14
28
42
56
time in days

INTERNATIONAL SEARCH REPORT

International application No
PCT/EP2017/058735

A. CLASSIFICATION OF SUBJECT MATTER
INV. C12Q1/68
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

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, 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	WO 2016/025958 A1 (MYRIAD GENETICS INC [US]; ABKEVICH VICTOR [US]; TIMMS KIRSTEN [US]; GU) 18 February 2016 (2016-02-18)	1-15
X	WO 2016/012630 A1 (INSERM INST NAT DE LA SANTÉ ET DE LA RECH MÉDICALE [FR]; UNIV MONTPELL) 28 January 2016 (2016-01-28) page 25	13
X	WO 2008/030887 A2 (BIPAR SCIENCES INC [US]; OSSOVSKAYA VALERIA [US]; BURNIER JOHN [US]; S) 13 March 2008 (2008-03-13) paragraph [0186] - paragraph [0194]	13
A	WO 2014/205105 A1 (UNIV CALIFORNIA [US]) 24 December 2014 (2014-12-24)	1-15

-/--

[X] Further documents are listed in the continuation of Box C.

[X] 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	Date of mailing of the international search report
30 May 2017	08/06/2017

Name and mailing address of the ISA/	Authorized officer
European Patent Office, P.B. 5818 Patentlaan 2 NL - 2280 HV Rijswijk Tel. (+31-70) 340-2040, Fax: (+31-70) 340-3016	Cornelis, Karen

1

INTERNATIONAL SEARCH REPORT

International application No
PCT/EP2017/058735

C(Continuation). DOCUMENTS CONSIDERED TO BE RELEVANT

Category*	Citation of document, with indication, where appropriate, of the relevant passages	Relevant to claim No.
A	US 2014/186468 A1 (GONZALO SUSANA [US] ET AL) 3 July 2014 (2014-07-03)	1-15
A	US 2015/344968 A1 (JOHNSON NEIL [US]) 3 December 2015 (2015-12-03)	1-15
A	MARIE-EMMANUELLE LEGRIER ET AL: "Activation of IFN/STAT1 signalling predicts response to chemotherapy in oestrogen receptor-negative breast cancer", BRITISH JOURNAL OF CANCER, vol. 114, no. 2, 22 December 2015 (2015-12-22), pages 177-187, XP055303474, GB ISSN: 0007-0920, DOI: 10.1038/bjc.2015.398 cited in the application	1-15
X	EP 2 944 961 A1 (XENTECH [FR]) 18 November 2015 (2015-11-18) cited in the application the whole document	1-12,14, 15

1

INTERNATIONAL SEARCH REPORT

Information on patent family members

International application No
PCT/EP2017/058735

Patent document cited in search report	Publication date	Patent family member(s)	Publication date
WO 2016025958 A1	18-02-2016	AU 2015301390 A1	02-02-2017
		CA 2958801 A1	18-02-2016
		EP 3180447 A1	21-06-2017
		WO 2016025958 A1	18-02-2016
WO 2016012630 A1	28-01-2016	EP 3172338 A1	31-05-2017
		WO 2016012630 A1	28-01-2016
WO 2008030887 A2	13-03-2008	AU 2007292302 A1	13-03-2008
		CA 2662335 A1	13-03-2008
		EP 2059802 A2	20-05-2009
		US 2008076778 A1	27-03-2008
		WO 2008030887 A2	13-03-2008
WO 2014205105 A1	24-12-2014	US 2016138114 A1	19-05-2016
		WO 2014205105 A1	24-12-2014
US 2014186468 A1	03-07-2014	NONE	
US 2015344968 A1	03-12-2015	NONE	
EP 2944961 A1	18-11-2015	CA 2835179 A1	15-11-2012
		EP 2705370 A2	12-03-2014
		EP 2944961 A1	18-11-2015
		US 2013042333 A1	14-02-2013
		WO 2012153187 A2	15-11-2012