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(54) Title: GENE SIGNATURE FOR IMMUNE THERAPIES IN CANCER

(57) Abstract: An immune response subtype of cancer is associated with DNA damage which allows subjects to be stratified for particular therapies including immune therapies which may be combined with DNA damage therapeutics. A method for predicting responsiveness to an antagonist of an inhibitory immune checkpoint and/or an agonist of a stimulatory immune checkpoint comprises determining the expression level of at least one gene selected from Table 2B, 2A or 1 in a sample from the subject. The determined expression level is used to predict responsiveness to an antagonist of an inhibitory immune checkpoint and/or an agonist of a stimulatory immune checkpoint.

GENE SIGNATURE FOR IMMUNE THERAPIES IN CANCER

FIELD OF THE INVENTION

The present invention relates to a molecular diagnostic test useful for diagnosing cancers from different anatomical sites that includes the use of an immune response subtype associated with DNA damage. The invention includes the use of a 44-gene classification model to identify this immune response associated with DNA damage repair deficiency molecular subtype. One application is the stratification of response to, and selection of patients for therapeutic drug classes, including antagonists of an inhibitory immune checkpoint and/or agonists of a stimulatory immune checkpoint. Another application is the stratification of cancer patients into those that respond and those that do not respond to antagonists of an inhibitory immune checkpoint and/or agonists of a stimulatory immune checkpoint. The present invention provides a test that can guide conventional therapy selection as well as selecting patient groups for enrichment strategies during clinical trial evaluation of novel therapeutics. Cancer subtypes with activation of the innate immune pathway STING/TBK1/IRF3 can be identified from fresh/frozen (FF) or formalin fixed paraffin embedded (FFPE) patient samples.

BACKGROUND

The biopharmaceutical industry continuously pursues new drug treatment options that are more effective, more specific or have fewer adverse side effects than currently administered drugs. Novel or alternate drug therapies are constantly being developed because genetic variability within the human population results in substantial differences in the effectiveness of many drugs. Therefore, although a wide variety of drug therapeutic options are currently available, more drug therapies are always needed in the event that a patient fails to benefit.

Traditionally, the treatment paradigm used by physicians has been to prescribe a first-line drug therapy that results in the highest success rate possible for treating a disease. Alternative drug therapies are then prescribed if the first is ineffective. This treatment paradigm is clearly not the optimal method for certain diseases. For example, in diseases such as cancer, the first treatment is often the most important and offers the best opportunity for successful therapy, so there exists a heightened need to choose an initial drug that will be the most effective against that particular patient's disease.

It is anticipated that there will be 207,090 new female breast cancer diagnoses in the US this year and 39,840 female breast cancer related deaths (American Cancer Society: Cancer Facts and Figures 2010). Standard chemotherapy typically includes direct DNA damaging agents such as anthracyclines and alkylating agents as well as anti-metabolites and anti-microtubule agents.

Ovarian cancer is the leading cause of death among all gynecological cancers in western countries. This high death rate is due to the diagnosis at an advanced stage in most patients. Epithelial ovarian cancer (EOC) constitutes 90% of ovarian malignancies and is classified into distinct histologic categories including serous, mucinous, endometrioid, clear cell, transitional, mixed, and undifferentiated subtypes. There is increasing evidence that these histologies arise from different etiologies. The current standard treatment for ovarian cancer is debulking surgery and standard platinum taxane - based

cytotoxic chemotherapy. However, not all patients respond to this, and of those that do, approximately 70% will experience a recurrence. Specific targeted therapies for ovarian cancer based on histological or molecular classification have not yet reached the marketplace. Similarly for other types of cancer, there is still no accurate way of selecting appropriate cytotoxic chemotherapeutic agents.

The advent of microarrays and molecular genomics has the potential for a significant impact on the diagnostic capability and prognostic classification of disease, which may aid in the prediction of the response of an individual patient to a defined therapeutic regimen. Microarrays provide for the analysis of large amounts of genomic information, thereby providing a genomic fingerprint of an individual. There is much enthusiasm that this is one of the molecular technologies that will provide the necessary tools for custom-made drug treatment regimens.

Currently, healthcare professionals have limited options to help them identify cancer patients who will benefit from chemotherapeutic agents. Identification of the optimal first-line drug has been difficult because methods are not available for accurately predicting which drug treatment would be the most effective for a particular patient's cancer. This results in relatively poor single agent response rates and increased cancer morbidity and death. Furthermore, patients often needlessly undergo ineffective, and often times toxic drug therapy.

Molecular markers have been used to select appropriate treatments in many cancer types. For example, breast tumors that do not express the estrogen and progesterone hormone receptors as well as the HER2 growth factor receptor, called "triple negative", appear to be responsive to PARP-1 inhibitor therapy (Linn, S. C., and Van 't Veer, L., J. Eur J Cancer *45 Suppl 1*, 11-26 (2009); O'Shaughnessy, J., et al. N Engl J Med *364*, 205-214 (2011). Recent studies indicate that the triple negative status of a breast tumor may indicate responsiveness to combination therapy including PARP-1 inhibitors, but may not be sufficient to indicate responsiveness to individual PARP-1 inhibitors.(O'Shaughnessy et al., 2011).

Furthermore, there have been other studies that have attempted to identify gene classifiers associated with molecular subtypes to indicate responsiveness of chemotherapeutic agents (Farmer*et al.*Nat Med *15*, 68-74 (2009); Konstantinopoulos, P. A., *et al.*, J Clin Oncol *28*, 3555-3561 (2010)). WO2012/037378 describes a molecular diagnostic test for cancer and is incorporated herein by reference.

SUMMARY OF THE INVENTION

The invention is defined in the claims. Medical uses of the relevant therapeutic agents are contemplated in addition to therapeutic methods. In some embodiments, according to all aspects of the invention, the immune checkpoint is not the PD1/PDL1 (referred to interchangeably as PD-1 and PD-L1 respectively throughout the disclosure) checkpoint. In some embodiments, according to all aspects of the invention, the antagonist of an inhibitory immune checkpoint is not pembrolizumab.

The invention is based on the elucidation of the mechanism of immune response associated with DNA damage repair deficient (DDRD) tumours. DNA damage repair deficient (DDRD) tumours activate the immune pathway STING/TBK1/IRF3 resulting in the production of chemokines. Thus, the invention is in part directed to methods of using a collection of gene expression markers in cancer such that when some or all of the transcripts are over or under-expressed, they identify a subtype of cancer that displays an innate immune response which is associated with a deficiency in DNA damage repair. Designation of

this subtype can be considered as a diagnostic test as it is not related to any specific drug but rather describes the biology of the cancer in a manner that has utility in screening and selecting appropriate cancer therapies. The immune response associated with DNA damage does not, however, result in an active T cell anti-tumour response, due to the expression of immune inhibitory molecules associated with T cell exhaustion and anergy, such as IDO1 or PDL1 (CD274). Accordingly, the invention also provides methods for indicating responsiveness or resistance to therapies including antagonists of an inhibitory immune checkpoint and/or agonists of a stimulatory immune checkpoint, optionally in combination with DNA-damage therapeutic agents. In different aspects, this gene or gene product list may form the basis of a single parameter or a multiparametric predictive test that could be delivered using methods known in the art such as microarray, nucleic acid amplification (e.g. Q-PCR), sequencing (including next generation sequencing and RNAseq), immunohistochemistry, ELISA or other technologies that can quantify mRNA or protein expression.

In addition, the biological pathway described herein is a feature of cancer itself, similar to grade and stage, and as such, is not limited to a single cancer disease type. Therefore, the collection of genes or gene products may be used to predict responsiveness of cancer therapeutics across different cancer types in different tissues. In one embodiment of the invention, these genes or gene products are useful for evaluating both breast and ovarian cancer tumors.

The invention described herein is not limited to any one drug; it can be used to identify responders and non-responders to any of a range of drugs that represent antagonists of an inhibitory immune checkpoint and/or agonists of a stimulatory immune checkpoint. Examples are provided herein. Such drugs may be administered in combination with drugs that directly or indirectly affect DNA damage and/or DNA damage repair e.g. neoadjuvant 5-fluorouracil, anthracycline and cyclophosphamide based regimens such as FEC (5-fluorouracil/epirubicin/cyclophosphamide) and FAC (5-fluorouracil/Adriamycin/cyclophosphamide).

The present invention relates to prediction of response to drugs using different classifications of response, such as overall survival, progression free survival, radiological response, as defined by RECIST, complete response, partial response, stable disease and serological markers such as, but not limited to, PSA, CEA, CA125, CA15-3 and CA19-9. In another aspect, the present invention relates to the identification of an innate immune response associated with a DNA damage response deficiency (DDRD) molecular subtype in cancer. This molecular subtype can, inter alia, be detected by the use of two different gene classifiers – one comprising of 40 genes and the other comprising of 44 genes. The DDRD classifier was first defined by a classifier consisting of 53 probesets on the Almac Breast Disease Specific Array (DSA™). So as to validate the functional relevance of this classifier in the context of its ability to predict response to DNA-damaging containing chemotherapy regimens, the classifier needed to be re-defined at a gene level. This would facilitate evaluation of the DDRD classifier using microarray data from independent datasets that were profiled on microarray platforms other than the Almac Breast DSA™. In order to facilitate defining the classifier at a gene level, the genes to which the Almac Breast DSA™ probesets map to needed to be defined. This involved the utilization of publicly available genome browser databases such as Ensembl and NCBI Reference Sequence. Results are provided only for the 44-gene DDRD classifier model, as this model supersedes that of the 40-gene DDRD classifier model. These results demonstrate that the classifier model is an effective and significant predictor of response to chemotherapy regimens that contain DNA damaging therapeutics.

The identification of the subtype by both the 40-gene classifier model and the 44-gene classifier model can be used to predict response to, and select patients for, cancer therapeutic drug classes, in particular antagonists of an inhibitory immune checkpoint and/or agonists of a stimulatory immune checkpoint optionally in combination with DNA damage causing agents and DNA repair targeted therapies.

In another aspect, the present invention relates to kits for conventional diagnostic uses listed above such as qPCR, microarray, sequencing (e.g. RNAseq) and immunoassays such as immunohistochemistry, ELISA, Western blot and the like. Such kits include appropriate reagents and directions to assay the expression of the genes or gene products and quantify mRNA or protein expression.

The invention also provides methods for identifying DNA damage response-deficient (DDRD) human tumors having an increased immune response. It is likely that this invention can be used to identify patients that are sensitive to and respond to, or are resistant to and do not respond to, drugs that influence immune checkpoints, such as antagonists of an inhibitory immune checkpoint and/or agonists of a stimulatory immune checkpoint. These drugs may be combined with drugs that damage DNA directly, damage DNA indirectly or inhibit normal DNA damage signaling and/or repair processes.

The invention also relates to guiding conventional treatment of patients. The invention also relates to selecting patients for clinical trials where novel drugs of the classes that agonise or antagonize specific immune checkpoints.

The present invention and methods accommodate the use of archived formalin fixed paraffin-embedded (FFPE) biopsy material, as well as fresh/frozen (FF) tissue, for assay of all transcripts in the invention, and are therefore compatible with the most widely available type of biopsy material. 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®.

BRIEF DESCRIPTION OF DRAWINGS

FIG. 1 provides a diagram representing the hierarchical analysis of ER-negative (A) and ER-positive (B) BRCA1/2 mutant and sporadic wildtype control breast samples. Probeset cluster groups are annotated on the right-hand side and pathway analysis of each probeset cluster group is annotated on the left-hand side of each image. The legend for each image indicates a sample's mutational status as well as the signature group each sample was assigned to for classifier generation.

FIG. 2 provides a diagram of box plots comparing the AUC performance of each classification model under 10 repeats of 5-fold cross validation for (A) the combined sample set, (B) the ER-negative sample set and (C) the ER-positive sample set. (D) Sensitivity plus specificity plot of the cross validation predictions used to select threshold. The maximum sensitivity plus specificity is 1.682 with a corresponding signature score of ~0.37.

FIG. 3 provides a diagram of a ROC curve of the classification performance for predicting BRCA status using the 44-gene classifier model, estimated by cross validation. The AUC is ~0.68 following application the classifier model. The 95% confidence limits have been estimated from bootstrap with 1000 iterations.

FIG. 4 provides a diagram of a ROC curve of the classification performance of the 44-gene classifier model in a combined analysis of three independent datasets: FEC, FAC1 and FAC2 (Bonnefoi et al., 2007; Iwamoto *et al.*, J Natl Cancer Inst 103, 264-272 (2011); Lee, J. K., et al. Clin Cancer Res *16*, 711-718 (2010) for predicting response to anthracycline-based chemotherapy. The AUC is ~0.78 following application of the classifier model. The 95% confidence limits have been estimated from bootstrap with 1000 iterations.

FIG. 5 provides a diagram of a ROC curve of the classification performance of the 44-gene classifier model in a combined analysis of three independent datasets for response in T/FAC treated samples(Hess*et al.*, J Clin Oncol *24*, 4236-4244 (2006); Lee et al., 2010; Tabchy, A., *et al.*Clin Cancer Res *16*, 5351-5361 (2010). The AUC is ~0.61 following application of the classifier model respectively. The 95% confidence limits were determined using 1000 bootstrap iterations.

FIG. 6 provides a diagram of a ROC curve of the classification performance of the 44-gene classifier model within 259 serous ovarian cancer samples for response in platinum and taxol treated samples from the in-house Almac Diagnostics ovarian dataset. The AUC is ~0.68 following application of the classifier model. The 95% confidence limits were determined using 1000 bootstrap iterations.

FIG. 7 provides a histogram representation of the 44-gene DDRD classifier scores in bone marrow samples taken from healthy donors and patients with Fanconi Anaemia mutations. The AUC is 0.90 following application of the classifier model. The 95% confidence limits were determined using 1000 bootstrap iterations.

FIG. 8 provides a figure correlating the 44-gene classifier model with therapeutic response in BRCA1 mutant and wildtype cell-lines. (A) Western blot analysis confirming increased expression of BRCA1 in the HCC1937-BR cells compared with the HCC1937-EV cells. (B) Mean 44-gene model (DDRD) classifier score (±SEM) within the control vector-only transfected HCC1937 (HCC1937-EV) and HCC1937 with returned exogenous expression of BRCA1 (HCC1937-BR) cell-lines. Histogram representation of cell-viability of HCC1937 parental and HCC1937-BR cells under constant exposure to a range of concentrations of PARP inhibitor KU0058948 (C) and cisplatin (D).

FIG. 9 provides a table and images showing that DDRD Tumours are Associated with Lymphocytic Infiltration.

FIG. 10 shows a DDRD subtype, a type I interferon picture.

FIG. 11 provides a graph showing that DNA damage induces Expression of Chemokines (and other DDRD assay genes). Statistical significance of the data is indicated with * signifying a p value of <0.05, ** a p value of <0.01 and *** a p value of <0.001.

FIG. 12 provides a graph and images showing that correction of DNA Repair Defect Reduces Expression of Chemokines (and other DDRD assay genes).

FIG. 13 shows that DDRD Positive Cells release chemokines into conditioned medium that attract lymphocytes.

FIG. 14 provides graphs showing that DDRD Gene Expression is Induced by DNA Damaging Agents.

FIG. 15 provides graphs showing that expression of DDRD Signature Genes is Cell Cycle Regulated.

FIG. 16 provides a graph showing that DDRD gene expression is Independent of Classic DNA Damage Sensors ATM, ATR & DNAPK.

FIG. 17 shows that the STING activated innate Immune Pathway is related to DDRD Signature Genes.

FIG. 18 provides images and a graph showing that the cytosolic DNA sensor is activated by DNA damage and is required for DDRD signalling.

FIG. 19 provides images showing that S Phase DNA Damage Increases Cytoplasmic DNA.

FIG. 20 provides a table and images showing that DDRD+ shows significant levels of PD-L1 in breast cancer samples.

FIG. 21 provides graphs showing that PDL1 positive tumours have active DDRD signalling.

FIG. 22 provides a graph showing that PDL1 expression is increased by co-culture with lymphocytes, specifically in DDRD+ models.

FIG. 23 provides a graph and images showing that PDL1 Expression is Increased by DNA damage. Statistical significance of the data is indicated with * signifying a p value of <0.05, ** a p value of <0.01 and *** a p value of <0.001.

FIG. 24 provides an image showing that alternative immune checkpoint target IDO1 expression is increased by genomic instability.

FIG 25 provides a graph showing that IDO1 expression is increased by co-culture with lymphocytes, specifically in DDRD+ models

FIG. 26 provides a graph showing that DDRD+ cells are protected from lymphocyte mediated cytotoxicity.

FIG. 27 provides graphs and an image showing that IFN-γ drives PDL-1 expression in DDRD+ and protects against PBMC mediated cytotoxicity.

FIG. 28 provides a graph showing that a blocking antibody to PDL-1 reverses resistance to PBMC mediated cytotoxicity in DDRD+ cells alone.

FIG. 29 shows that DDRD identifies MSI colorectal samples.

FIG. 30 illustrates a model of a DNA damage pathway.

FIG. 31: Kaplan Meier illustrating the difference in local recurrence survival rates for DDRD positive and DDRD negative patients that were treated with an immune based therapy (immune checkpoint modulator such as Ipilimumab or pembrolizumab) and/or a DNA damaging agent. HR = 0.39 [95% CI: 0.18-0.84], p = 0.0008.

FIG. 32: Kaplan Meier illustrating the difference in distant recurrence survival rates for DDRD positive and DDRD negative patients that were treated with an immune based therapy (immune checkpoint modulator such as Ipilimumab or pembrolizumab) and/or a DNA damaging agent. HR = 0.44 [95% CI: 0.19-0.99], p = 0.0095.

FIG. 33: Kaplan Meier illustrating the difference in overall survival rates for DDRD positive and DDRD negative patients that were treated with an immune based therapy (immune checkpoint modulator such as Ipilimumab or pembrolizumab) and/or a DNA damaging agent. HR = 0.31 [95% CI: 0.12-0.81], p = 0.0006.

DETAILED DESCRIPTION OF THE INVENTION

The invention provides a method for predicting responsiveness to a modulator of an immune checkpoint, such as an antagonist of an inhibitory immune checkpoint and/or an agonist of a stimulatory immune checkpoint, comprising: determining the expression level of at least one gene selected from Table 2B, 2A or 1 in a sample from the subject wherein the determined expression level is used to predict responsiveness to an antagonist of an inhibitory immune checkpoint and/or an agonist of a stimulatory immune checkpoint. In any of the methods of the invention, the expression level of one or more additional genes (i.e. genes other than those provided in Table 2B, 2A or 1) may also be determined and used to predict responsiveness to an antagonist of an inhibitory immune checkpoint and/or an agonist of a stimulatory immune checkpoint.

In the methods an increased expression level of the at least one gene may predict responsiveness to a modulator of an immune checkpoint, such as an antagonist of an inhibitory immune checkpoint and/or an agonist of a stimulatory immune checkpoint.

The methods may comprise determining the expression level of at least 2 of the genes and the determined expression levels may be used to generate a combined test score, wherein a positive combined test score (generally above threshold, but may be equal to or above threshold) predicts responsiveness to an antagonist of an inhibitory immune checkpoint and/or an agonist of a stimulatory immune checkpoint.

The methods may comprise: deriving a combined test score that captures the expression levels; providing a threshold score comprising information correlating the combined test score and responsiveness; and comparing the combined test score to the threshold score; wherein responsiveness is predicted when the combined test score exceeds the threshold score.

The methods may comprise determining the expression level of at least 6 genes, at least 7 genes, at least 8 genes, at least 9 genes, at least 10 genes, at least 11 genes, at least 12 genes, at least 13 genes, at least 14 genes, at least 15 genes, at least 16 genes, at least 17 genes, at least 18 genes, at least 19 genes, at least 20 genes, at least 21 genes, at least 22 genes, at least 23 genes, at least 24 genes, at least 25 genes, at least 26 genes, at least 27 genes, at least 28 genes, at least 29 genes, at least 30 genes, at least 31 genes, at least 32 genes, at least 33 genes, at least 34 genes, at least 35 genes, at least 36 genes, at least 37 genes, at least 38 genes, at least 39 genes, at least 40 genes, at least 41 genes, at least 42 genes, or at least 43 genes, selected from CXCL10, MX1, IDO1, IFI44L, CD2, GBP5, PRAME, ITGAL, LRP4, APOL3, CDR1, FYB, TSPAN7, RAC2, KLHDC7B, GRB14, AC138128.1, KIF26A, CD274, CD109, ETV7, MFAP5, OLFM4, PI15, FOSB, FAM19A5, NLRC5, PRICKLE1, EGR1, CLDN10, ADAMTS4, SP140L, ANXA1, RSAD2, ESR1, IKZF3, OR2I1P, EGFR, NAT1, LATS2, CYP2B6, PTPRC, PPP1R1A, and AL137218.1.

The methods may comprise determining the expression level of at least 1 gene selected from CD2, ITGAL, PTPRC, CXCL10 and IDO1, together with at least 1 further gene, at least 2 further genes, at least 3 further genes, at least 4 further genes, at least 5 further genes, at least 6 further genes, at least 7 further genes, at least 8 further genes, at least 9 further genes, at least 10 further genes, at least 11 further genes, at least 12 further genes, at least 13 further genes, at least 14 further genes, at least 15 further genes, at least 16 further genes, at least 17 further genes, at least 18 further genes, at least 19 further genes, at least 20 further genes, at least 21 further genes, at least 22 further genes, at least 23 further genes, at least 24 further genes, at least 25 further genes, at least 26 further genes, at least 27 further genes, at least 28 further genes, at least 29 further genes, at least 30 further genes, at least 31 further genes, at least 32 further genes, at least 33 further genes, at least 34 further genes, at least 35 further genes, at least 36 further genes, at least 37 further genes, or at least 38 further genes, selected from MX1, IFI44L, GBP5, PRAME, LRP4, APOL3, CDR1, FYB, TSPAN7, RAC2, KLHDC7B, GRB14, AC138128.1, KIF26A, CD274, CD109, ETV7, MFAP5, OLFM4, PI15, FOSB, FAM19A5, NLRC5, PRICKLE1, EGR1, CLDN10, ADAMTS4, SP140L, ANXA1, RSAD2, ESR1, IKZF3, OR2I1P, EGFR, NAT1, LATS2, CYP2B6, PPP1R1A, and AL137218.1. Preferably, the methods comprise determining the expression level of at least 1 gene selected from CD2, ITGAL, PTPRC, CXCL10 and IDO1, together with each of MX1, IFI44L, GBP5, PRAME, LRP4, APOL3, CDR1, FYB, TSPAN7, RAC2, KLHDC7B, GRB14, AC138128.1, KIF26A, CD274, CD109, ETV7, MFAP5, OLFM4, PI15, FOSB, FAM19A5, NLRC5,

PRICKLE1, EGR1, CLDN10, ADAMTS4, SP140L, ANXA1, RSAD2, ESR1, IKZF3, OR2I1P, EGFR, NAT1, LATS2, CYP2B6, PPP1R1A, and AL137218.1.

The methods may comprise determining the expression level of at least 2 genes selected from CD2, ITGAL, PTPRC, CXCL10 and IDO1, together with at least 1 further gene, at least 2 further genes, at least 3 further genes, at least 4 further genes, at least 5 further genes, at least 6 further genes, at least 7 further genes, at least 8 further genes, at least 9 further genes, at least 10 further genes, at least 11 further genes, at least 12 further genes, at least 13 further genes, at least 14 further genes, at least 15 further genes, at least 16 further genes, at least 17 further genes, at least 18 further genes, at least 19 further genes, at least 20 further genes, at least 21 further genes, at least 22 further genes, at least 23 further genes, at least 24 further genes, at least 25 further genes, at least 26 further genes, at least 27 further genes, at least 28 further genes, at least 29 further genes, at least 30 further genes, at least 31 further genes, at least 32 further genes, at least 33 further genes, at least 34 further genes, at least 35 further genes, at least 36 further genes, at least 37 further genes, or at least 38 further genes, selected from MX1, IFI44L, GBP5, PRAME, LRP4, APOL3, CDR1, FYB, TSPAN7, RAC2, KLHDC7B, GRB14, AC138128.1, KIF26A, CD274, CD109, ETV7, MFAP5, OLFM4, PI15, FOSB, FAM19A5, NLRC5, PRICKLE1, EGR1, CLDN10, ADAMTS4, SP140L, ANXA1, RSAD2, ESR1, IKZF3, OR2I1P, EGFR, NAT1, LATS2, CYP2B6, PPP1R1A, and AL137218.1. Preferably, the methods comprise determining the expression level of at least 2 genes selected from CD2, ITGAL, PTPRC, CXCL10 and IDO1, together with each of MX1, IFI44L, GBP5, PRAME, LRP4, APOL3, CDR1, FYB, TSPAN7, RAC2, KLHDC7B, GRB14, AC138128.1, KIF26A, CD274, CD109, ETV7, MFAP5, OLFM4, PI15, FOSB, FAM19A5, NLRC5, PRICKLE1, EGR1, CLDN10, ADAMTS4, SP140L, ANXA1, RSAD2, ESR1, IKZF3, OR2I1P, EGFR, NAT1, LATS2, CYP2B6, PPP1R1A, and AL137218.1.

The methods may comprise determining the expression level of at least 3 genes selected from CD2, ITGAL, PTPRC, CXCL10 and IDO1, together with at least 1 further gene, at least 2 further genes, at least 3 further genes, at least 4 further genes, at least 5 further genes, at least 6 further genes, at least 7 further genes, at least 8 further genes, at least 9 further genes, at least 10 further genes, at least 11 further genes, at least 12 further genes, at least 13 further genes, at least 14 further genes, at least 15 further genes, at least 16 further genes, at least 17 further genes, at least 18 further genes, at least 19 further genes, at least 20 further genes, at least 21 further genes, at least 22 further genes, at least 23 further genes, at least 24 further genes, at least 25 further genes, at least 26 further genes, at least 27 further genes, at least 28 further genes, at least 29 further genes, at least 30 further genes, at least 31 further genes, at least 32 further genes, at least 33 further genes, at least 34 further genes, at least 35 further genes, at least 36 further genes, at least 37 further genes, or at least 38 further genes, selected from MX1, IFI44L, GBP5, PRAME, LRP4, APOL3, CDR1, FYB, TSPAN7, RAC2, KLHDC7B, GRB14, AC138128.1, KIF26A, CD274, CD109, ETV7, MFAP5, OLFM4, PI15, FOSB, FAM19A5, NLRC5, PRICKLE1, EGR1, CLDN10, ADAMTS4, SP140L, ANXA1, RSAD2, ESR1, IKZF3, OR2I1P, EGFR, NAT1, LATS2, CYP2B6, PPP1R1A, and AL137218.1. Preferably, the methods comprise determining the expression level of at least 3 genes selected from CD2, ITGAL, PTPRC, CXCL10 and IDO1, together with each of MX1, IFI44L, GBP5, PRAME, LRP4, APOL3, CDR1, FYB, TSPAN7, RAC2, KLHDC7B, GRB14, AC138128.1, KIF26A, CD274, CD109, ETV7, MFAP5, OLFM4, PI15, FOSB, FAM19A5, NLRC5,

PRICKLE1, EGR1, CLDN10, ADAMTS4, SP140L, ANXA1, RSAD2, ESR1, IKZF3, OR2I1P, EGFR, NAT1, LATS2, CYP2B6, PPP1R1A, and AL137218.1.

The methods may comprise determining the expression level of at least 4 genes selected from CD2, ITGAL, PTPRC, CXCL10 and IDO1, together with at least 1 further gene, at least 2 further genes, at least 3 further genes, at least 4 further genes, at least 5 further genes, at least 6 further genes, at least 7 further genes, at least 8 further genes, at least 9 further genes, at least 10 further genes, at least 11 further genes, at least 12 further genes, at least 13 further genes, at least 14 further genes, at least 15 further genes, at least 16 further genes, at least 17 further genes, at least 18 further genes, at least 19 further genes, at least 20 further genes, at least 21 further genes, at least 22 further genes, at least 23 further genes, at least 24 further genes, at least 25 further genes, at least 26 further genes, at least 27 further genes, at least 28 further genes, at least 29 further genes, at least 30 further genes, at least 31 further genes, at least 32 further genes, at least 33 further genes, at least 34 further genes, at least 35 further genes, at least 36 further genes, at least 37 further genes, or at least 38 further genes, selected from MX1, IFI44L, GBP5, PRAME, LRP4, APOL3, CDR1, FYB, TSPAN7, RAC2, KLHDC7B, GRB14, AC138128.1, KIF26A, CD274, CD109, ETV7, MFAP5, OLFM4, PI15, FOSB, FAM19A5, NLRC5, PRICKLE1, EGR1, CLDN10, ADAMTS4, SP140L, ANXA1, RSAD2, ESR1, IKZF3, OR2I1P, EGFR, NAT1, LATS2, CYP2B6, PPP1R1A, and AL137218.1. Preferably, the methods comprise determining the expression level of at least 4 genes selected from CD2, ITGAL, PTPRC, CXCL10 and IDO1, together with each of MX1, IFI44L, GBP5, PRAME, LRP4, APOL3, CDR1, FYB, TSPAN7, RAC2, KLHDC7B, GRB14, AC138128.1, KIF26A, CD274, CD109, ETV7, MFAP5, OLFM4, PI15, FOSB, FAM19A5, NLRC5, PRICKLE1, EGR1, CLDN10, ADAMTS4, SP140L, ANXA1, RSAD2, ESR1, IKZF3, OR2I1P, EGFR, NAT1, LATS2, CYP2B6, PPP1R1A, and AL137218.1.

The methods may comprise determining the expression level of each of CD2, ITGAL, PTPRC, CXCL10 and IDO1, together with at least 1 further gene, at least 2 further genes, at least 3 further genes, at least 4 further genes, at least 5 further genes, at least 6 further genes, at least 7 further genes, at least 8 further genes, at least 9 further genes, at least 10 further genes, at least 11 further genes, at least 12 further genes, at least 13 further genes, at least 14 further genes, at least 15 further genes, at least 16 further genes, at least 17 further genes, at least 18 further genes, at least 19 further genes, at least 20 further genes, at least 21 further genes, at least 22 further genes, at least 23 further genes, at least 24 further genes, at least 25 further genes, at least 26 further genes, at least 27 further genes, at least 28 further genes, at least 29 further genes, at least 30 further genes, at least 31 further genes, at least 32 further genes, at least 33 further genes, at least 34 further genes, at least 35 further genes, at least 36 further genes, at least 37 further genes, or at least 38 further genes, selected from MX1, IFI44L, GBP5, PRAME, LRP4, APOL3, CDR1, FYB, TSPAN7, RAC2, KLHDC7B, GRB14, AC138128.1, KIF26A, CD274, CD109, ETV7, MFAP5, OLFM4, PI15, FOSB, FAM19A5, NLRC5, PRICKLE1, EGR1, CLDN10, ADAMTS4, SP140L, ANXA1, RSAD2, ESR1, IKZF3, OR2I1P, EGFR, NAT1, LATS2, CYP2B6, PPP1R1A, and AL137218.1.

The methods may comprise determining the expression level of at least 12 genes selected from Table 1.

The methods may comprise determining the expression level of at least 1 gene selected from CD2, ITGAL, PTPRC, CXCL10, IDO1, CD3D, HLA-DPB1, CXCL9, CCL5, STAT1, IL2RG, CD3E, IRF1, IKZF3 and IGJ together with at least one further gene selected from (the remaining genes in) Table 1 or together with at least one further gene from the (remaining genes in) Table 2B (the 44 gene panel).

The methods may comprise determining the expression level of each of:

- CXCL10;
- CXCL10 and MX1;
- CXCL10, IDO1 and MX1;
- CXCL10, IDO1, IFI44L and MX1;
- CD2, CXCL10, IDO1, IFI44L and MX1;
- CD2, CXCL10, GBP5, IDO1, IFI44L and MX1;
- CD2, CXCL10, GBP5, IDO1, IFI44L, MX1 and PRAME;
- CD2, CXCL10, GBP5, IDO1, IFI44L, ITGAL, MX1 and PRAME;
- CD2, CXCL10, GBP5, IDO1, IFI44L, ITGAL, LRP4, MX1 and PRAME;
- APOL3, CD2, CXCL10, GBP5, IDO1, IFI44L, ITGAL, LRP4, MX1 and PRAME;
- APOL3, CD2, CDR1, CXCL10, GBP5, IDO1, IFI44L, ITGAL, LRP4, MX1 and PRAME;
- APOL3, CD2, CDR1, CXCL10, FYB, GBP5, IDO1, IFI44L, ITGAL, LRP4, MX1 and PRAME;
- APOL3, CD2, CDR1, CXCL10, FYB, GBP5, IDO1, IFI44L, ITGAL, LRP4, MX1, PRAME and TSPAN7;
- APOL3, CD2, CDR1, CXCL10, FYB, GBP5, IDO1, IFI44L, ITGAL, LRP4, MX1, PRAME, RAC2 and TSPAN7;
- APOL3, CD2, CDR1, CXCL10, FYB, GBP5, IDO1, IFI44L, ITGAL, KLHDC7B, LRP4, MX1, PRAME, RAC2 and TSPAN7;
- APOL3, CD2, CDR1, CXCL10, FYB, GBP5, GRB14, IDO1, IFI44L, ITGAL, KLHDC7B, LRP4, MX1, PRAME, RAC2 and TSPAN7;
- AC138128.1, APOL3, CD2, CDR1, CXCL10, FYB, GBP5, GRB14, IDO1, IFI44L, ITGAL, KLHDC7B, LRP4, MX1, PRAME, RAC2 and TSPAN7;
- AC138128.1, APOL3, CD2, CDR1, CXCL10, FYB, GBP5, GRB14, IDO1, IFI44L, ITGAL, KIF26A, KLHDC7B, LRP4, MX1, PRAME, RAC2 and TSPAN7;
- AC138128.1, APOL3, CD2, CD274, CDR1, CXCL10, FYB, GBP5, GRB14, IDO1, IFI44L, ITGAL, KIF26A, KLHDC7B, LRP4, MX1, PRAME, RAC2 and TSPAN7;
- AC138128.1, APOL3, CD109, CD2, CD274, CDR1, CXCL10, FYB, GBP5, GRB14, IDO1, IFI44L, ITGAL, KIF26A, KLHDC7B, LRP4, MX1, PRAME, RAC2 and TSPAN7;
- AC138128.1, APOL3, CD109, CD2, CD274, CDR1, CXCL10, ETV7, FYB, GBP5, GRB14, IDO1, IFI44L, ITGAL, KIF26A, KLHDC7B, LRP4, MX1, PRAME, RAC2 and TSPAN7;
- AC138128.1, APOL3, CD109, CD2, CD274, CDR1, CXCL10, ETV7, FYB, GBP5, GRB14, IDO1, IFI44L, ITGAL, KIF26A, KLHDC7B, LRP4, MFAP5, MX1, PRAME, RAC2 and TSPAN7;
- AC138128.1, APOL3, CD109, CD2, CD274, CDR1, CXCL10, ETV7, FYB, GBP5, GRB14, IDO1, IFI44L, ITGAL, KIF26A, KLHDC7B, LRP4, MFAP5, MX1, OLFM4, PRAME, RAC2 and TSPAN7;
- AC138128.1, APOL3, CD109, CD2, CD274, CDR1, CXCL10, ETV7, FYB, GBP5, GRB14, IDO1,

IFI44L, ITGAL, KIF26A, KLHDC7B, LRP4, MFAP5, MX1, OLFM4, PI15, PRAME, RAC2 and TSPAN7;

- AC138128.1, APOL3, CD109, CD2, CD274, CDR1, CXCL10, ETV7, FOSB, FYB, GBP5, GRB14, IDO1, IFI44L, ITGAL, KIF26A, KLHDC7B, LRP4, MFAP5, MX1, OLFM4, PI15, PRAME, RAC2 and TSPAN7;
- AC138128.1, APOL3, CD109, CD2, CD274, CDR1, CXCL10, ETV7, FAM19A5, FOSB, FYB, GBP5, GRB14, IDO1, IFI44L, ITGAL, KIF26A, KLHDC7B, LRP4, MFAP5, MX1, OLFM4, PI15, PRAME, RAC2 and TSPAN7;
- AC138128.1, APOL3, CD109, CD2, CD274, CDR1, CXCL10, ETV7, FAM19A5, FOSB, FYB, GBP5, GRB14, IDO1, IFI44L, ITGAL, KIF26A, KLHDC7B, LRP4, MFAP5, MX1, NLRC5, OLFM4, PI15, PRAME, RAC2 and TSPAN7;
- AC138128.1, APOL3, CD109, CD2, CD274, CDR1, CXCL10, ETV7, FAM19A5, FOSB, FYB, GBP5, GRB14, IDO1, IFI44L, ITGAL, KIF26A, KLHDC7B, LRP4, MFAP5, MX1, NLRC5, OLFM4, PI15, PRAME, PRICKLE1, RAC2 and TSPAN7;
- AC138128.1, APOL3, CD109, CD2, CD274, CDR1, CXCL10, EGR1, ETV7, FAM19A5, FOSB, FYB, GBP5, GRB14, IDO1, IFI44L, ITGAL, KIF26A, KLHDC7B, LRP4, MFAP5, MX1, NLRC5, OLFM4, PI15, PRAME, PRICKLE1, RAC2 and TSPAN7;
- AC138128.1, APOL3, CD109, CD2, CD274, CDR1, CLDN10, CXCL10, EGR1, ETV7, FAM19A5, FOSB, FYB, GBP5, GRB14, IDO1, IFI44L, ITGAL, KIF26A, KLHDC7B, LRP4, MFAP5, MX1, NLRC5, OLFM4, PI15, PRAME, PRICKLE1, RAC2 and TSPAN7;
- AC138128.1, ADAMTS4, APOL3, CD109, CD2, CD274, CDR1, CLDN10, CXCL10, EGR1, ETV7, FAM19A5, FOSB, FYB, GBP5, GRB14, IDO1, IFI44L, ITGAL, KIF26A, KLHDC7B, LRP4, MFAP5, MX1, NLRC5, OLFM4, PI15, PRAME, PRICKLE1, RAC2 and TSPAN7;
- AC138128.1, ADAMTS4, APOL3, CD109, CD2, CD274, CDR1, CLDN10, CXCL10, EGR1, ETV7, FAM19A5, FOSB, FYB, GBP5, GRB14, IDO1, IFI44L, ITGAL, KIF26A, KLHDC7B, LRP4, MFAP5, MX1, NLRC5, OLFM4, PI15, PRAME, PRICKLE1, RAC2, SP140L and TSPAN7;
- AC138128.1, ADAMTS4, ANXA1, APOL3, CD109, CD2, CD274, CDR1, CLDN10, CXCL10, EGR1, ETV7, FAM19A5, FOSB, FYB, GBP5, GRB14, IDO1, IFI44L, ITGAL, KIF26A, KLHDC7B, LRP4, MFAP5, MX1, NLRC5, OLFM4, PI15, PRAME, PRICKLE1, RAC2, SP140L and TSPAN7;
- AC138128.1, ADAMTS4, ANXA1, APOL3, CD109, CD2, CD274, CDR1, CLDN10, CXCL10, EGR1, ETV7, FAM19A5, FOSB, FYB, GBP5, GRB14, IDO1, IFI44L, ITGAL, KIF26A, KLHDC7B, LRP4, MFAP5, MX1, NLRC5, OLFM4, PI15, PRAME, PRICKLE1, RAC2, RSAD2, SP140L and TSPAN7;
- AC138128.1, ADAMTS4, ANXA1, APOL3, CD109, CD2, CD274, CDR1, CLDN10, CXCL10, EGR1, ESR1, ETV7, FAM19A5, FOSB, FYB, GBP5, GRB14, IDO1, IFI44L, ITGAL, KIF26A, KLHDC7B, LRP4, MFAP5, MX1, NLRC5, OLFM4, PI15, PRAME, PRICKLE1, RAC2, RSAD2, SP140L and TSPAN7;
- AC138128.1, ADAMTS4, ANXA1, APOL3, CD109, CD2, CD274, CDR1, CLDN10, CXCL10, EGR1, ESR1, ETV7, FAM19A5, FOSB, FYB, GBP5, GRB14, IDO1, IFI44L, IKZF3, ITGAL, KIF26A, KLHDC7B, LRP4, MFAP5, MX1, NLRC5, OLFM4, PI15, PRAME, PRICKLE1, RAC2, RSAD2, SP140L and TSPAN7;

- AC138128.1, ADAMTS4, ANXA1, APOL3, CD109, CD2, CD274, CDR1, CLDN10, CXCL10, EGR1, ESR1, ETV7, FAM19A5, FOSB, FYB, GBP5, GRB14, IDO1, IFI44L, IKZF3, ITGAL, KIF26A, KLHDC7B, LRP4, MFAP5, MX1, NLRC5, OLFM4, OR2I1P, PI15, PRAME, PRICKLE1, RAC2, RSAD2, SP140L and TSPAN7;
- AC138128.1, ADAMTS4, ANXA1, APOL3, CD109, CD2, CD274, CDR1, CLDN10, CXCL10, EGFR, EGR1, ESR1, ETV7, FAM19A5, FOSB, FYB, GBP5, GRB14, IDO1, IFI44L, IKZF3, ITGAL, KIF26A, KLHDC7B, LRP4, MFAP5, MX1, NLRC5, OLFM4, OR2I1P, PI15, PRAME, PRICKLE1, RAC2, RSAD2, SP140L and TSPAN7;
- AC138128.1, ADAMTS4, ANXA1, APOL3, CD109, CD2, CD274, CDR1, CLDN10, CXCL10, EGFR, EGR1, ESR1, ETV7, FAM19A5, FOSB, FYB, GBP5, GRB14, IDO1, IFI44L, IKZF3, ITGAL, KIF26A, KLHDC7B, LRP4, MFAP5, MX1, NAT1, NLRC5, OLFM4, OR2I1P, PI15, PRAME, PRICKLE1, RAC2, RSAD2, SP140L and TSPAN7;
- AC138128.1, ADAMTS4, ANXA1, APOL3, CD109, CD2, CD274, CDR1, CLDN10, CXCL10, EGFR, EGR1, ESR1, ETV7, FAM19A5, FOSB, FYB, GBP5, GRB14, IDO1, IFI44L, IKZF3, ITGAL, KIF26A, KLHDC7B, LATS2, LRP4, MFAP5, MX1, NAT1, NLRC5, OLFM4, OR2I1P, PI15, PRAME, PRICKLE1, RAC2, RSAD2, SP140 and TSPAN7;
- AC138128.1, ADAMTS4, ANXA1, APOL3, CD109, CD2, CD274, CDR1, CLDN10, CXCL10, CYP2B6, EGFR, EGR1, ESR1, ETV7, FAM19A5, FOSB, FYB, GBP5, GRB14, IDO1, IFI44L, IKZF3, ITGAL, KIF26A, KLHDC7B, LATS2, LRP4, MFAP5, MX1, NAT1, NLRC5, OLFM4, OR2I1P, PI15, PRAME, PRICKLE1, RAC2, RSAD2, SP140L and TSPAN7;
- AC138128.1, ADAMTS4, ANXA1, APOL3, CD109, CD2, CD274, CDR1, CLDN10, CXCL10, CYP2B6, EGFR, EGR1, ESR1, ETV7, FAM19A5, FOSB, FYB, GBP5, GRB14, IDO1, IFI44L, IKZF3, ITGAL, KIF26A, KLHDC7B, LATS2, LRP4, MFAP5, MX1, NAT1, NLRC5, OLFM4, OR2I1P, PI15, PRAME, PRICKLE1, PTPRC, RAC2, RSAD2, SP140L and TSPAN7;
- AC138128.1, ADAMTS4, ANXA1, APOL3, CD109, CD2, CD274, CDR1, CLDN10, CXCL10, CYP2B6, EGFR, EGR1, ESR1, ETV7, FAM19A5, FOSB, FYB, GBP5, GRB14, IDO1, IFI44L, IKZF3, ITGAL, KIF26A, KLHDC7B, LATS2, LRP4, MFAP5, MX1, NAT1, NLRC5, OLFM4, OR2I1P, PI15, PPP1R1A, PRAME, PRICKLE1, PTPRC, RAC2, RSAD2, SP140L and TSPAN7; or
- CXCL10, MX1, IDO1, IFI44L, CD2, GBP5, PRAME, ITGAL, LRP4, APOL3, CDR1, FYB, TSPAN7, RAC2, KLHDC7B, GRB14, AC138128.1, KIF26A, CD274, CD109, ETV7, MFAP5, OLFM4, PI15, FOSB, FAM19A5, NLRC5, PRICKLE1, EGR1, CLDN10, ADAMTS4, SP140L, ANXA1, RSAD2, ESR1, IKZF3, OR2I1P, EGFR, NAT1, LATS2, CYP2B6, PTPRC, PPP1R1A, and AL137218.1.

In the methods the weight values for each gene may be as set out in Table 2B or the weight and/or bias values for each gene may be as set out in any one of Tables 3-45.

The methods may comprise determining the expression level of at least one, up to all, of CCL5, CXCL9 and CXCL10 together with at least one further gene selected from (the remaining genes in) Table 1 or together with at least one further gene from the (remaining genes in) Table 2B (the 44 gene panel).

The invention provides a method for predicting responsiveness to a modulator of an immune checkpoint, such as an antagonist of an inhibitory immune checkpoint and/or an agonist of a stimulatory immune checkpoint in combination with a DNA damage therapeutic agent comprising: determining the expression level of at least one gene selected from Table 2B, 2A or 1 in a sample from the subject wherein the determined expression level is used to predict responsiveness to a modulator of an immune checkpoint, such as an antagonist of an inhibitory immune checkpoint and/or an agonist of a stimulatory immune checkpoint in combination with a DNA damage therapeutic agent. The determined expression level can be used to predict responsiveness to the simultaneous, separate or sequential administration (or use) of a modulator of an immune checkpoint, such as an antagonist of an inhibitory immune checkpoint and/or an agonist of a stimulatory immune checkpoint in combination with a DNA damage therapeutic agent

In the methods an increased expression level of the at least one gene may predict responsiveness to a modulator of an immune checkpoint, such as an antagonist of an inhibitory immune checkpoint and/or an agonist of a stimulatory immune checkpoint in combination with a DNA damage therapeutic agent.

The methods may comprise determining the expression level of at least 2 of the genes and the determined expression levels may be used to generate a combined test score, wherein a positive combined test score (generally above threshold, but may be equal to or above threshold) predicts responsiveness to a modulator of an immune checkpoint, such as an antagonist of an inhibitory immune checkpoint and/or an agonist of a stimulatory immune checkpoint in combination with a DNA damage therapeutic agent.

The methods for predicting responsiveness to a modulator of an immune checkpoint, such as an antagonist of an inhibitory immune checkpoint and/or an agonist of a stimulatory immune checkpoint in combination with a DNA damage therapeutic agent may comprise determining the expression level of any of the genes or sets of genes described herein.

The invention provides a method for identifying a cancer that can be effectively treated with a modulator of an immune checkpoint, such as an antagonist of an inhibitory immune checkpoint and/or an agonist of a stimulatory immune checkpoint comprising:
determining the expression level of at least one gene selected from Table 2B, 2A or 1 in a sample from the subject wherein the determined expression level is used to identify a cancer that can be effectively treated with a modulator of an immune checkpoint, such as an antagonist of an inhibitory immune checkpoint and/or an agonist of a stimulatory immune checkpoint.

In the methods an increased expression level of the at least one gene may identify a cancer that can be effectively treated with a modulator of an immune checkpoint, such as an antagonist of an inhibitory immune checkpoint and/or an agonist of a stimulatory immune checkpoint.

The methods may comprise determining the expression level of at least 2 genes and the determined expression levels may be used to generate a combined test score, wherein a positive combined test score (generally above threshold, but may be equal to or above threshold) identifies a cancer that can be

effectively treated with a modulator of an immune checkpoint, such as an antagonist of an inhibitory immune checkpoint and/or an agonist of a stimulatory immune checkpoint.

The methods may comprise: deriving a combined test score that captures the expression levels; providing a threshold score comprising information correlating the combined test score and responsiveness; and comparing the combined test score to the threshold score; wherein a cancer that can be effectively treated is identified when the combined test score exceeds the threshold score.

The methods for identifying a cancer that can be effectively treated with a modulator of an immune checkpoint, such as an antagonist of an inhibitory immune checkpoint and/or an agonist of a stimulatory immune checkpoint may comprise determining the expression level of any of the genes or sets of genes described herein.

The invention provides a method for identifying a cancer that can be effectively treated with a modulator of an immune checkpoint, such as an antagonist of an inhibitory immune checkpoint and/or an agonist of a stimulatory immune checkpoint in combination with a DNA damage therapeutic agent comprising: determining the expression level of at least one gene selected from Table 2B, 2A or 1 in a sample from the subject wherein the determined expression level is used to identify a cancer that can be effectively treated with a modulator of an immune checkpoint, such as an antagonist of an inhibitory immune checkpoint and/or an agonist of a stimulatory immune checkpoint in combination with a DNA damage therapeutic agent. The determined expression level can be used to identify a cancer that can be effectively treated with the simultaneous, separate or sequential administration (or use) of a modulator of an immune checkpoint, such as an antagonist of an inhibitory immune checkpoint and/or an agonist of a stimulatory immune checkpoint in combination with a DNA damage therapeutic agent.

In the methods an increased expression level of the at least one gene may identify a cancer that can be effectively treated with a modulator of an immune checkpoint, such as an antagonist of an inhibitory immune checkpoint and/or an agonist of a stimulatory immune checkpoint in combination with a DNA damage therapeutic agent.

The methods may comprise determining the expression level of at least 2 of the genes and the determined expression levels may be used to generate a combined test score, wherein a positive combined test score (generally above threshold, but may be equal to or above threshold) identifies a cancer that can be effectively treated with a modulator of an immune checkpoint, such as an antagonist of an inhibitory immune checkpoint and/or an agonist of a stimulatory immune checkpoint in combination with a DNA damage therapeutic agent.

The methods for identifying a cancer that can be effectively treated with a modulator of an immune checkpoint, such as an antagonist of an inhibitory immune checkpoint and/or an agonist of a stimulatory immune checkpoint in combination with a DNA damage therapeutic agent may comprise determining the expression level of any of the genes or sets of genes described herein.

The invention provides a method for selecting treatment for a cancer comprising: determining the expression level of at least one gene selected from Table 2B, 2A or 1 in a sample from the subject wherein the determined expression level is used to select a modulator of an immune checkpoint, such as an antagonist of an inhibitory immune checkpoint and/or an agonist of a stimulatory immune checkpoint for use in treatment of the cancer.

In the methods an increased expression level of the at least one gene is used to select a modulator of an immune checkpoint, such as an antagonist of an inhibitory immune checkpoint and/or an agonist of a stimulatory immune checkpoint for use in treatment of the cancer.

The methods may comprise determining the expression level of at least 2 of the genes and the determined expression levels may be used to generate a combined test score, wherein a positive combined test score (generally above threshold, but may be equal to or above threshold) is used to select a modulator of an immune checkpoint, such as an antagonist of an inhibitory immune checkpoint and/or an agonist of a stimulatory immune checkpoint for use in treatment of the cancer.

The methods may further comprise treating the cancer using the selected antagonist and/or agonist.

The methods may comprise: deriving a combined test score that captures the expression levels; providing a threshold score comprising information correlating the combined test score and responsiveness; and comparing the combined test score to the threshold score; wherein a modulator of an immune checkpoint, such as an antagonist of an inhibitory immune checkpoint and/or an agonist of a stimulatory immune checkpoint is selected for use when the combined test score exceeds the threshold score.

The methods for selecting treatment for a cancer may comprise determining the expression level of any of the genes or sets of genes described herein.

The invention provides a method for selecting treatment for a cancer comprising: determining the expression level of at least one gene selected from 2B, 2A or 1 in a sample from the subject wherein the determined expression level is used to select a modulator of an immune checkpoint, such as an antagonist of an inhibitory immune checkpoint and/or an agonist of a stimulatory immune checkpoint, in combination with a DNA damage therapeutic agent, for use in treatment of the cancer. The determined expression level can be used to select a modulator of an immune checkpoint, such as an antagonist of an inhibitory immune checkpoint and/or an agonist of a stimulatory immune checkpoint, in combination with a DNA damage therapeutic agent, for simultaneous, separate or sequential use in treatment of the cancer.

In the methods an increased expression level of the at least one gene may be used to select a modulator of an immune checkpoint, such as an antagonist of an inhibitory immune checkpoint and/or an agonist of a stimulatory immune checkpoint, in combination with a DNA damage therapeutic agent, for use in treatment of the cancer.

The methods may comprise determining the expression level of at least 2 of the genes and the determined expression levels may be used to generate a combined test score, wherein a positive combined test score (generally above threshold, but may be equal to or above threshold) is used to select a modulator of an immune checkpoint, such as an antagonist of an inhibitory immune checkpoint and/or an agonist of a stimulatory immune checkpoint, in combination with a DNA damage therapeutic agent, for use in treatment of the cancer.

The methods may comprise treating the cancer using the selected modulator, such as antagonist and/or agonist, in combination with a DNA damage therapeutic agent.

The methods may comprise: deriving a combined test score that captures the expression levels; providing a threshold score comprising information correlating the combined test score and responsiveness; and comparing the combined test score to the threshold score; wherein a modulator of an immune checkpoint, such as an antagonist of an inhibitory immune checkpoint and/or an agonist of a stimulatory immune checkpoint in combination with a DNA damage therapeutic agent is selected for use when the combined test score exceeds the threshold score.

In the methods the combined test score (or "signature score") may be derived according to the formula:

$$SignatureScore = \sum_i w_i \times (ge_i - b_i) + k$$

Where w_i is a weight for each gene, b_i is a gene-specific bias, ge_i is the gene expression after pre-processing, and *k* is a constant offset.

The combined test score may be derived using the expression level(s) of any of the genes or groups of genes described herein. The combined test score may be derived using the expression level of one or more additional genes.

The invention provides a method of treating cancer comprising administration of a modulator of an immune checkpoint, such as an antagonist of an inhibitory immune checkpoint and/or an agonist of a stimulatory immune checkpoint to a subject, characterised in that a sample from the subject, prior to administration, displays a positive combined test score derived from the determined expression levels of at least 2 genes from Table 2B, 2A or 1 or an increased level of expression of at least 1 gene from Table 2B, 2A or 1.

The invention provides a method of treating cancer comprising administration of a modulator of an immune checkpoint, such as an antagonist of an inhibitory immune checkpoint and/or an agonist of a stimulatory immune checkpoint, in combination with a DNA damage therapeutic agent, to a subject, characterised in that a sample from the subject, prior to administration, displays a positive combined test score derived from the determined expression levels of at least 2 genes from Table 2B, 2A or 1 or an increased level of expression of at least 1 gene from Table 2B, 2A or 1. The a modulator of an immune checkpoint, such as antagonist of an inhibitory immune checkpoint and/or the agonist of a stimulatory

immune checkpoint, and the DNA damage therapeutic agent can be administered simultaneously, separately or sequentially to the subject,

The methods of treating cancer may comprise determining the expression level of any of the genes or sets of genes described herein.

The invention provides a modulator of an immune checkpoint, such as an antagonist of an inhibitory immune checkpoint and/or an agonist of a stimulatory immune checkpoint for use in the treatment of cancer in a subject wherein, prior to administration of the antagonist and/or agonist, a sample from the subject displays a positive combined test score derived from the determined expression levels of at least 2 genes from Table 2B, 2A or 1 or an increased level of expression of at least 1 gene from Table 2B, 2A or 1.

The invention provides a modulator of an immune checkpoint, such as an antagonist of an inhibitory immune checkpoint and/or an agonist of a stimulatory immune checkpoint for use in the treatment of cancer in a subject wherein, prior to administration of the antagonist and/or agonist, a sample from the subject displays a positive combined test score derived from the determined expression levels of at least 2 genes from Table 2B, 2A or 1 or an increased level of expression of at least 1 gene from Table 2B, 2A or 1, and wherein the antagonist and/or agonist is administered in combination with a DNA damage therapeutic agent. The modulator of an immune checkpoint, such as antagonist of an inhibitory immune checkpoint and/or the agonist of a stimulatory immune checkpoint, and the DNA damage therapeutic agent can be administered simultaneously, separately or sequentially to the subject,

The invention provides a modulator of an immune checkpoint, such as an antagonist of an inhibitory immune checkpoint in combination with a DNA damage therapeutic agent and/or an agonist of a stimulatory immune checkpoint in combination with a DNA damage therapeutic agent for use in the treatment of cancer in a subject wherein, prior to administration of the antagonist and/or agonist and DNA damage therapeutic agent, a sample from the subject displays a positive combined test score derived from the determined expression levels of at least 2 genes from Table 2B, 2A or 1 or an increased level of expression of at least 1 gene from Table 2B, 2A or 1. The a modulator of an immune checkpoint, such as antagonist of an inhibitory immune checkpoint and/or the agonist of a stimulatory immune checkpoint, and the DNA damage therapeutic agent can be for simultaneous, separate or sequential use in the treatment of cancer in the subject,

The genes for which the expression level is determined may be any of the genes or sets of genes described herein.

The subject may be selected for treatment according to any of the methods described herein.

The sample may comprise cancer cells. The sample may be a tissue sample e.g. a fixed and embedded tissue sample.

The cancer may be selected from leukemia, brain cancer, prostate cancer, liver cancer, ovarian cancer, stomach cancer, colorectal cancer, throat cancer, breast cancer, skin cancer, melanoma, lung cancer, sarcoma, cervical cancer, testicular cancer, bladder cancer, endocrine cancer, endometrial cancer, esophageal cancer, glioma, lymphoma, neuroblastoma, osteosarcoma, pancreatic cancer, pituitary cancer, renal cancer or head and neck cancer.

The inhibitory immune checkpoint may be a regulatory pathway, or a molecule in such a pathway, that inhibits an immune response. The inhibitory immune checkpoint may be a polypeptide expressed by B-cells and/or T-cells. The inhibitory immune checkpoint may be an inhibitory receptor. The inhibitory immune checkpoint may be a membrane receptor. Preferably, the inhibitory immune checkpoint is an inhibitory membrane receptor. The ligand of the inhibitory immune checkpoint may be membrane bound or soluble.

The inhibitory immune checkpoint may be selected from A2AR, B7-H3 (CD276), B7-H4 (VTCN1), BTLA (CD272), CTLA-4 (CD152), IDO, KIR, LAG3, PD-1/PD-L1, TIM-3 and VISTA. In some embodiments, the inhibitory immune checkpoint is not PD-1/PD-L1. In some embodiments, the immune checkpoint is IDO.

The antagonist of an inhibitory immune checkpoint may amplify an antigen-specific B-cell and/or T-cell response. The antagonist of an inhibitory immune checkpoint may inhibit the interaction between an inhibitory receptor and its ligand. The antagonist of an inhibitory immune checkpoint may be selected from an antibody and an inhibitory nucleic acid molecule.

The antibody may be of monoclonal or polyclonal origin. Fragments and derivative antibodies may also be utilised, to include without limitation Fab fragments, ScFv, single domain antibodies, nanoantibodies, heavy chain antibodies, aptamers etc. which retain peptide-specific binding function and these are included in the definition of "antibody". Such antibodies are useful in the practice of the invention. Methods for generating specific antibodies are known to those skilled in the art. Antibodies may be of human or non-human origin (e.g. rodent, such as rat or mouse) and be humanized etc. according to known techniques (Jones *et al.*, Nature (1986) May 29-Jun. 4;321(6069):522-5; Roguska *et al.*, Protein Engineering, 1996, 9(10):895-904; and Studnicka *et al.,* Humanizing Mouse Antibody Frameworks While Preserving 3–D Structure. Protein Engineering, 1994, Vol.7, pg 805).

The inhibitory nucleic acid molecule may be single stranded or double stranded. Examples of inhibitory nucleic acid molecules include antisense nucleic acid, RNAi, siRNA, shRNA, miRNA, shmiRNA, or derivatives or pre-cursors thereof.

The antagonist of an inhibitory immune checkpoint may be selected from MGA271 (targets B7-H3), ipilimumab (Yervoy - targets CTLA-4), indoximod (targets IDO pathway), NLG919 (targets IDO pathway), lirilumab (targets KIR), IMP321 (targets LAG3), BMS-986016 (targets LAG3), CT-011 (PD-1 blockade), nivolumab/BMS-936558 (PD-1 blockade) , BMS-936559 (PDL1 blockade) and pembrolizumab (Keytruda – targets PD-1). Preferably, the antagonist is not pembrolizumab. Further antagonists include MGB453 (targets TIM-3), LAG525 (targets LAG-3) and PDR001 (PD1 Blockade).

The stimulatory immune checkpoint may be a regulatory pathway, or a molecule in such a pathway, that activates an immune response. The stimulatory immune checkpoint may be a polypeptide expressed by B-cells and/or T-cells. The stimulatory immune checkpoint may be a membrane receptor. The stimulatory immune checkpoint may be a co-stimulatory receptor. The co-stimulatory receptor may be a T-cell co-stimulatory receptor or a B-cell co-stimulatory receptor. The ligand of the stimulatory immune checkpoint may be membrane bound or soluble.

The stimulatory immune checkpoint may be selected from CD27, CD28, CD40, CD122, CD137, OX40, GITR and ICOS.

The agonist of a stimulatory immune checkpoint may amplify an antigen-specific B-cell and/or T-cell response. The agonist of a stimulatory immune checkpoint may increase the interaction between a co-stimulatory receptor and its ligand. The agonist of a stimulatory immune checkpoint may comprise a ligand molecule that binds to a (co-)stimulatory receptor. The agonist of a stimulatory immune checkpoint may be selected from an antibody (as described herein), a lipocalin and a cytokine.

The lipocalin may be a molecule that incorporates a lipocalin, or a fragment or derivative of a lipocalin. Such molecules which retain the function of acting as an agonist of a stimulatory immune checkpoint are included in the definition of "lipocalin".

The cytokine may be a molecule that incorporates a cytokine, or a fragment or derivative of a cytokine. Such molecules which retain the function of acting as an agonist of a stimulatory immune checkpoint are included in the definition of "cytokine".

The agonist of a stimulatory immune checkpoint may be selected from CDX-1127 (agonist of CD27), NKTR-214 (agonist of CD122), BMS-663513 (agonist of CD137), TRX518 (agonist of GITR), CP-870893 (CD40 agonist), MEDI0562, MEDI6469 and MEDI6383 (OX40 agonists).

The DNA damage therapeutic agent may be selected from a DNA damaging agent, a DNA repair targeted therapy, an inhibitor of DNA damage signalling, an inhibitor of DNA damage induced cell cycle arrest and an inhibitor of a process indirectly leading to DNA damage.

The DNA damaging agent may be selected from an alkylating agent, a topoisomerase inhibitor and radiation. The alkylating agent may be selected from a platinum containing agent, cyclophosphamide and busulphan. The platinum containing agent may be selected from cisplatin, carboplatin and oxaliplatin. The topoisomerase inhibitor may be selected from a topoisomerase I inhibitor and a topoisomerase II inhibitor. The topoisomerase I inhibitor may be selected from irinotecan and topotecan. The topisomerase II inhibitor may be selected from etoposide and an anthracycline. The anthracycline may be selected from doxorubicin and epirubicin. The radiation may be ionising radiation

The DNA repair targeted therapy may be selected from an inhibitor of Non-homologous end-joining, an inhibitor of homologous recombination, an inhibitors of nucleotide excision repair, an inhibitor of base excision repair and an inhibitor of the Fanconi anemia pathway. The inhibitor of Non-homologous end-joining may be selected from a DNA-PK inhibitor, Nu7441 and NU7026. The inhibitor of base excision repair may be selected from a PARP inhibitor, AG014699, AZD2281, ABT-888, MK4827, BSI-201, INO-1001, TRC-102, an APEX 1 inhibitor, an APEX 2 inhibitor and a Ligase III inhibitor.

The inhibitor of DNA damage signalling may be selected from an ATM inhibitor, a CHK 1 inhibitor and a CHK 2 inhibitor. The ATM inhibitor may be selected from CP466722 and KU-55933. The CHK 1 inhibitor may be selected from XL-844, UCN-01, AZD7762 and PF00477736. The CHK 2 inhibitor may be selected from XL-844, AZD7762 and PF00477736.

The inhibitor of DNA damage induced cell cycle arrest may be selected from a Wee1 kinase inhibitor and a CDC25a, b or c inhibitor.

The inhibitor of a process indirectly leading to DNA damage may be selected from a histone deacetylase inhibitor and a heat shock protein inhibitor.

The heat shock protein inhibitor may be selected from geldanamycin and AUY922.

Unless defined otherwise, technical and scientific terms used herein have the same meaning as commonly understood by one of ordinary skill in the art to which this invention belongs. Although any methods, devices, and materials similar or equivalent to those described herein can be used in the practice or testing of the invention, the preferred methods, devices and materials are now described.

All publications, published patent documents, and patent applications cited in this application are indicative of the level of skill in the art(s) to which the application pertains. All publications, published patent documents, and patent applications cited herein are hereby incorporated by reference to the same extent as though each individual publication, published patent document, or patent application was specifically and individually indicated as being incorporated by reference.

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

A major goal of current research efforts in cancer is to increase the efficacy of perioperative systemic therapy in patients by incorporating molecular parameters into clinical therapeutic decisions. Pharmacogenetics/genomics is the study of genetic/genomic factors involved in an individual’s response to a foreign compound or drug. Agents or modulators which have a stimulatory or inhibitory effect on expression of a marker of the invention can be administered to individuals to treat (prophylactically or therapeutically) cancer in a patient. It is ideal to also consider the pharmacogenomics of the individual in conjunction with such treatment. Differences in metabolism of therapeutics may possibly lead to severe toxicity or therapeutic failure by altering the relationship between dose and blood concentration of the pharmacologically active drug. Thus, understanding the pharmacogenomics of an individual permits the selection of effective agents (e.g., drugs) for prophylactic or therapeutic treatments. Such

pharmacogenomics can further be used to determine appropriate dosages and therapeutic regimens. Accordingly, the level of expression of a marker of the invention in an individual can be determined to thereby select appropriate agent(s) for therapeutic or prophylactic treatment of the individual.

The invention is directed to a unique collection of gene or gene product markers (hereinafter referred to as "biomarkers") expressed in a cancer tissue. In different aspects, this biomarker list may form the basis of a single parameter or multiparametric predictive test that could be delivered using methods known in the art such as microarray, Q-PCR, sequencing (e.g. RNA seq), immunohistochemistry, ELISA or other technologies that can quantify mRNA or protein expression.

The present invention also relates to kits and methods that are useful for prognosis following cytotoxic chemotherapy or selection of specific treatments for cancer. Methods are provided such that when some or all of the transcripts are over or under-expressed, the expression profile indicates responsiveness or resistance to immune checkpoint therapies, such as antagonists of an inhibitory immune checkpoint and/or agonists of a stimulatory immune checkpoint. These kits and methods employ gene or gene product markers that are differentially expressed in tumors of patients with cancer. In one embodiment of the invention, the expression profiles of these biomarkers are correlated with clinical outcome (response or survival) in archival tissue samples under a statistical method or a correlation model to create a database or model correlating expression profile with responsiveness to one or more immune checkpoint therapies, such as antagonists of an inhibitory immune checkpoint and/or agonists of a stimulatory immune checkpoint, optionally in combination with DNA-damage therapeutic agents. The predictive model may then be used to predict the responsiveness in a patient whose responsiveness to the immune checkpoint therapies, such as antagonists of an inhibitory immune checkpoint and/or agonists of a stimulatory immune checkpoint is unknown. In many other embodiments, a patient population can be divided into at least two classes based on patients' clinical outcome, prognosis, or responsiveness to immune checkpoint therapies, such as antagonists of an inhibitory immune checkpoint and/or agonists of a stimulatory immune checkpoint, and the biomarkers are substantially correlated with a class distinction between these classes of patients. The biological pathways described herein are common to cancer as a disease, similar to grade and stage, and as such, the classifiers and methods are not limited to a single cancer disease type.

Predictive Marker Panels/Expression Classifiers

A unique collection of biomarkers as a genetic classifier expressed in a cancer tissue is provided that is useful in determining responsiveness or resistance to therapeutic agents, such as immune checkpoint therapies, such as antagonists of an inhibitory immune checkpoint and/or agonists of a stimulatory immune checkpoint, used to treat cancer. Such a collection may be termed a "marker panel", "expression classifier", or "classifier".

Some biomarkers useful in the present methods are identified in Table 1. These biomarkers are identified as having predictive value to determine a patient response to a therapeutic agent, or lack thereof. Their expression correlates with the response to an agent, and more specifically, immune checkpoint therapies, such as antagonists of an inhibitory immune checkpoint and/or agonists of a stimulatory immune checkpoint, optionally in combination with a DNA-damage therapeutic agent. By examining the expression of a collection of the identified biomarkers in a tumor, it is possible to

determine which therapeutic agent or combination of agents will be most likely to reduce the growth rate of a cancer, and in some embodiments, breast or ovarian cancer cells. By examining a collection of identified transcript gene or gene product markers, it is also possible to determine which therapeutic agent or combination of agents will be the least likely to reduce the growth rate of a cancer. By examining the expression of a collection of biomarkers, it is therefore possible to eliminate ineffective or inappropriate therapeutic agents. Importantly, in certain embodiments, these determinations can be made on a patient-by-patient basis or on an agent-by-agent basis. Thus, one can determine whether or not a particular therapeutic regimen is likely to benefit a particular patient or type of patient, and/or whether a particular regimen should be continued.

Table 1A

Sense genes (166)		Antisense of known genes (24)		
Gene Symbol	EntrezGene ID	Almac Gene ID	Almac Gene symbol	SEQ ID NO:
ABCA12	26154		N/A	
ALDH3B2	222		N/A	
APOBEC3G	60489		N/A	
APOC1	341		N/A	
APOL6	80830		N/A	
ARHGAP9	64333		N/A	
BAMBI	25805		N/A	
BIK	638		N/A	
BIRC3	330	AS1_BIRC3	Hs127799.0C7n9_at	1
BTN3A3	10384		N/A	
C12orf48	55010		N/A	
C17orf28	283987		N/A	
C1orf162	128346		N/A	
C1orf64	149563		N/A	
C1QA	712		N/A	
C21orf70	85395		N/A	
C22orf32	91689		N/A	
C6orf211	79624		N/A	
CACNG4	27092		N/A	
CCDC69	26112		N/A	
CCL5	6352		N/A	
CCNB2	9133		N/A	
CCND1	595		N/A	
CCR7	1236		N/A	
CD163	9332		N/A	
CD2	914		N/A	
CD22	933		N/A	
CD24	100133941		N/A	
CD274	29126		N/A	
CD3D	915		N/A	
CD3E	916		N/A	
CD52	1043		N/A	
CD53	963		N/A	
CD79A	973		N/A	
CDH1	999		N/A	
CDKN3	1033		N/A	
CECR1	51816		N/A	
CHEK1	1111		N/A	
CKMT1B	1159		N/A	
CMPK2	129607		N/A	
CNTNAP2	26047		N/A	

COX16	51241		N/A	
CRIP1	1396		N/A	
CXCL10	3627		N/A	
CXCL9	4283		N/A	
CYBB	1536		N/A	
CYP2B6	1555		N/A	
DDX58	23586		N/A	
DDX60L	91351		N/A	
ERBB2	2064		N/A	
ETV7	51513		N/A	
FADS2	9415		N/A	
FAM26F	441168		N/A	
FAM46C	54855		N/A	
FASN	2194		N/A	
FBP1	2203		N/A	
FBXO2	26232		N/A	
FKBP4	2288		N/A	
FLJ40330	645784		N/A	
FYB	2533		N/A	
GBP1	2633		N/A	
GBP4	115361		N/A	
GBP5	115362	AS1_GBP5	BRMX.5143C1n2_at	2
GIMAP4	55303		N/A	
GLRX	2745		N/A	
GLUL	2752		N/A	
GVIN1	387751		N/A	
H2AFJ	55766		N/A	
HGD	3081		N/A	
HIST1H2BK	85236		N/A	
HIST3H2A	92815		N/A	
HLA-DOA	3111		N/A	
HLA-DPB1	3115		N/A	
HMGB2	3148		N/A	
HMGB3	3149		N/A	
HSP90AA1	3320		N/A	
IDO1	3620		N/A	
IFI27	3429		N/A	
IFI44	10561		N/A	
IFI44L	10964	AS1_IFI44L	BRSA.1606C1n4_at	3
IFI6	2537		N/A	
IFIH1	64135		N/A	
IGJ	3512	AS1_IGJ	BRIH.1231C2n2_at	4
IKZF1	10320		N/A	
IL10RA	3587		N/A	
IL2RG	3561		N/A	
IL7R	3575		N/A	
IMPAD1	54928		N/A	
IQGAP3	128239	AS1_IQGAP3	BRAD.30779_s_at	5
IRF1	3659		N/A	
ISG15	9636		N/A	
ITGAL	3683		N/A	
KIAA1467	57613		N/A	
KIF20A	10112		N/A	
KITLG	4254		N/A	
KLRK1	22914		N/A	
KRT19	3880		N/A	
LAIR1	3903		N/A	
LCP1	3936		N/A	
LOC100289702	100289702		N/A	
LOC100294459	100294459	AS1_LOC100294459	BRSA.396C1n2_at	6

LOC150519	150519		N/A	
LOC439949	439949		N/A	
LYZ	4069		N/A	
MAL2	114569		N/A	
MGC29506	51237		N/A	
MIAT	440823		N/A	
MS4A1	931		N/A	
MX1	4599	AS1_MX1	BRMX.2948C3n7_at	7
NAPSB	256236		N/A	
NCKAP1L	3071		N/A	
NEK2	4751		N/A	
NLRC3	197358		N/A	
NLRC5	84166		N/A	
NPNT	255743		N/A	
NQO1	1728		N/A	
OAS2	4939		N/A	
OAS3	4940		N/A	
PAQR4	124222		N/A	
PARP14	54625		N/A	
PARP9	83666		N/A	
PIK3CG	5294		N/A	
PIM2	11040		N/A	
PLEK	5341		N/A	
POU2AF1	5450		N/A	
PP14571	100130449		N/A	
PPP2R2C	5522		N/A	
PSMB9	5698		N/A	
PTPRC	5788		N/A	
RAC2	5880		N/A	
RAMP1	10267		N/A	
RARA	5914		N/A	
RASSF7	8045		N/A	
RSAD2	91543		N/A	
RTP4	64108		N/A	
SAMD9	54809		N/A	
SAMD9L	219285		N/A	
SASH3	54440		N/A	
SCD	6319		N/A	
SELL	6402		N/A	
SIX1	6495	AS1_SIX1	Hs539969.0C4n3_at	8
SLAMF7	57823		N/A	
SLC12A2	6558		N/A	
SLC9A3R1	9368	AS1_SLC9A3R1	Hs396783.3C1n4_at	9
SPOCK2	9806		N/A	
SQLE	6713		N/A	
ST20	400410		N/A	
ST6GALNAC2	10610		N/A	
STAT1	6772	AS1_STAT1	BRMX.13670C1n2_at	10
STRA13	201254		N/A	
SUSD4	55061		N/A	
SYT12	91683		N/A	
TAP1	6890		N/A	
TBC1D10C	374403		N/A	
TNFRSF13B	23495		N/A	
TNFSF10	8743		N/A	
TOB1	10140	AS1_TOB1	BRAD.30243_at	11
TOM1L1	10040		N/A	
TRIM22	10346		N/A	
UBD	10537	AS1_UBD	BRMX.941C2n2_at	12
UBE2T	29089		N/A	

UCK2	7371		N/A	
USP18	11274		N/A	
VNN2	8875		N/A	
XAF1	54739		N/A	
ZWINT	11130		N/A	
		AS1_C1QC	BRMX.4154C1n3_s_at	13
		AS1_C2orf14	BRAD.39498_at	14
		AS1_EPSTI1	BRAD.34868_s_at	15
		AS1_GALNT6	5505575.0C1n42_at	16
		AS1_HIST1H4H	BREM.1442_at	17
		AS1_HIST2H4B	BRHP.827_s_at	18
		AS2_HIST2H4B	BRRS.18322_s_at	19
		AS3_HIST2H4B	BRRS.18792_s_at	20
		AS1_KIAA1244	Hs632609.0C1n37_at	21
		AS1_LOC100287927	Hs449575.0C1n22_at	22
		AS1_LOC100291682	BRAD.18827_s_at	23
		AS1_LOC100293679	BREM.2466_s_at	24

Table 1B

Novel genes	
Gene symbol	**SEQ ID NO:**
BRAD.2605_at	25
BRAD.33618_at	26
BRAD.36579_s_at	27
BRAD1_5440961_s_at	28
BRAD1_66786229_s_at	29
BREM.2104_at	30
BRAG_AK097020.1_at	31
BRAD.20415_at	32
BRAD.29668_at	33
BRAD.30228_at	34
BRAD.34830_at	35
BRAD.37011_s_at	36
BRAD.37762_at	37
BRAD.40217_at	38
BRAD1_4307876_at	39
BREM.2505_at	40
Hs149363.0CB4n5_s_at	41
Hs172587.9C1n9_at	42
Hs271955.16C1n9_at	43
Hs368433.18C1n6_at	44
Hs435736.0C1n27_s_at	45
Hs493096.15C1n6_at	46
Hs493096.2C1n15_s_at	47
Hs592929.0CB2n8_at	48
Hs79953.0C1n23_at	49
BRMX.2377C1n3_at	50

All or a portion of the biomarkers recited in Table 1 may be used in a predictive biomarker panel. For example, biomarker panels selected from the biomarkers in Table 1 can be generated using the methods provided herein and can comprise between one, and all of the biomarkers set forth in Table 1 and each and every combination in between (e.g., four selected biomarkers, 16 selected biomarkers, 74 selected biomarkers, etc.). In some embodiments, the predictive biomarker set comprises at least 5, 10, 20, 40, 60, 100, 150, 200, or 300 or more biomarkers. In other embodiments, the predictive biomarker set comprises no more than 5, 10, 20, 40, 60, 100, 150, 200, 300, 400, 500, 600 or 700 biomarkers. In some

embodiments, the predictive biomarker set includes a plurality of biomarkers listed in Table 1. In some embodiments the predictive biomarker set includes at least about 1%, about 5%, about 10%, about 20%, about 30%, about 40%, about 50%, about 60%, about 70%, about 80%, about 90%, about 95%, about 96%, about 97%, about 98%, or about 99% of the biomarkers listed in Table 1. Selected predictive biomarker sets can be assembled from the predictive biomarkers provided using methods described herein and analogous methods known in the art. In one embodiment, the biomarker panel contains all 203 biomarkers in Table 1. In another embodiment, the biomarker panel contains 40 or 44 biomarkers in Table 1 or 2.

Predictive biomarker sets 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, including biomarker membership, 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 biomarkers are used to form a weighted sum of their signals, where individual weights can be positive or negative. The resulting sum ("decisive function") is compared with a pre-determined reference point or value. The comparison with the reference point or 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 biomarkers included in the classifier provided in Table 1 will carry unequal weights in a classifier for responsiveness or resistance to a therapeutic agent. Therefore, while as few as one sequence 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 biomarker in a gene expression classifier may be determined on a data set of patient samples using analytical methods known in the art.

In one embodiment the biomarker panel is directed to the 40 biomarkers detailed in Table 2A with corresponding ranks and weights detailed in the table or alternative rankings and weightings, depending, for example, on the disease setting. In another embodiment, the biomarker panel is directed to the 44 biomarkers detailed in Table 2B with corresponding ranks and weights detailed in the table or alternative rankings and weightings, depending, for example, on the disease setting. Tables 2A and 2B rank the biomarkers in order of decreasing weight in the classifier, defined as the rank of the average weight in the compound decision score function measured under cross-validation. Table 2C present the probe sets that represent the genes in Table 2A and 2B with reference to their sequence ID numbers. Table 2D presents the antisense probe sequences that were present on the array for the genes in the signatures.

Table 2A
Gene IDs and EntrezGene IDs for 40-gene DDRD classifier model with associated ranking and weightings

DDRD classifier 40 gene model			
Rank	**Genes Symbol**	**EntrezGene ID**	**Weights**
1	GBP5	115362	0.022389581
2	CXCL10	3627	0.021941734
3	IDO1	3620	0.020991115
4	MX1	4599	0.020098675
5	IFI44L	10964	0.018204957
6	CD2	914	0.018080661
7	PRAME	23532	0.016850837
8	ITGAL	3683	0.016783359
9	LRP4	4038	-0.015129969
10	SP140L	93349	0.014646025
11	APOL3	80833	0.014407174
12	FOSB	2354	-0.014310521
13	CDR1	1038	-0.014209848
14	RSAD2	91543	0.014177132
15	TSPAN7	7102	-0.014111562
16	RAC2	5880	0.014093627
17	FYB	2533	0.01400475
18	KLHDC7B	113730	0.013298413
19	GRB14	2888	0.013031204
20	KIF26A	26153	-0.012942351
21	CD274	29126	0.012651964
22	CD109	135228	-0.012239425
23	ETV7	51513	0.011787297
24	MFAP5	8076	-0.011480443
25	OLFM4	10562	-0.011130113
26	PI15	51050	-0.010904326
27	FAM19A5	25817	-0.010500936
28	NLRC5	84166	0.009593449
29	EGR1	1958	-0.008947963
30	ANXA1	301	-0.008373991
31	CLDN10	9071	-0.008165127
32	ADAMTS4	9507	-0.008109892
33	ESR1	2099	0.007524594
34	PTPRC	5788	0.007258669
35	EGFR	1956	-0.007176203
36	NAT1	9	0.006165534
37	LATS2	26524	-0.005951091
38	CYP2B6	1555	0.005838391
39	PPP1R1A	5502	-0.003898835
40	TERF1P1	348567	0.002706847

Table 2B
Gene IDs and EntrezGene IDs for 44-gene DDRD classifier model with associated ranking and weightings

DDRD Classifier - 44 Gene Model (NA:genomic sequence)			
Rank	**Gene symbol**	**EntrezGene ID**	**Weight**
1	CXCL10	3627	0.023

2	MX1	4599	0.0226
3	IDO1	3620	0.0221
4	IFI44L	10964	0.0191
5	CD2	914	0.019
6	GBP5	115362	0.0181
7	PRAME	23532	0.0177
8	ITGAL	3683	0.0176
9	LRP4	4038	-0.0159
10	APOL3	80833	0.0151
11	CDR1	1038	-0.0149
12	FYB	2533	-0.0149
13	TSPAN7	7102	0.0148
14	RAC2	5880	-0.0148
15	KLHDC7B	113730	0.014
16	GRB14	2888	0.0137
17	AC138128.1	N/A	-0.0136
18	KIF26A	26153	-0.0136
19	CD274	29126	0.0133
20	CD109	135228	-0.0129
21	ETV7	51513	0.0124
22	MFAP5	8076	-0.0121
23	OLFM4	10562	-0.0117
24	PI15	51050	-0.0115
25	FOSB	2354	-0.0111
26	FAM19A5	25817	0.0101
27	NLRC5	84166	-0.011
28	PRICKLE1	144165	-0.0089
29	EGR1	1958	-0.0086
30	CLDN10	9071	-0.0086
31	ADAMTS4	9507	-0.0085
32	SP140L	93349	0.0084
33	ANXA1	301	-0.0082
34	RSAD2	91543	0.0081
35	ESR1	2099	0.0079
36	IKZF3	22806	0.0073
37	OR2I1P	442197	0.007
38	EGFR	1956	-0.0066
39	NAT1	9	0.0065
40	LATS2	26524	-0.0063
41	CYP2B6	1555	0.0061

42	PTPRC	5788	0.0051
43	PPP1R1A	5502	-0.0041
44	AL137218.1	N/A	-0.0017

Table 2C
Probe set IDs and SEQ Numbers for genes contained in 40- and 44-gene signature

Probe set IDs and SEQ Numbers for genes contained in 40 and 44 gene signature		
Gene Symbol	**Probe Set ID**	**SEQ ID NO.**
FYB	BRAD.10849_at	83
CLDN10	BRAD.10890_at	84
PPP1R1A	BRAD.11026_at	85
PI15	BRAD.12809_at	86
MFAP5	BRAD.14326_s_at	87
ESR1	BRAD.15436_s_at	88
FYB	BRAD.15833_s_at	89
ESR1	BRAD.19080_s_at	90
TERF1P1	BRAD.2707_at	91
PRICKLE1	BRAD.27716_s_at	92
LATS2	BRAD.28628_s_at	93
IKZF3	BRAD.28643_at	94
MX1	BRAD.28663_s_at	95
CD274	BRAD.29038_at	96
FAM19A5	BRAD.30917_at	97
LATS2	BRAD.31470_at	98
EGFR	BRAD.32716_at	99
EGFR	BRAD.33042_at	100
EGFR	BRAD.33341_at	101
ANXA1	BRAD.33405_at	102
EGFR	BRAD.33431_at	103
KLHDC7B	BRAD.35695_at	104
IKZF3	BRAD.35710_at	105
PTPRC	BRAD.37907_at	106
TERF1P1	BRAD.40353_at	107
EGFR	BRAD.40654_s_at	108
FYB	BRAD.4701_at	109
PTPRC	BRAD.5967_at	110
EGFR	BRAD.7701_at	111
ESR1	BREM.1048_at	112
EGFR	BREM.1129_at	113
NAT1	BREM.1226_at	114
FOSB	BREM.1262_at	115
OR2I1P	BREM.130_at	116
ADAMTS4	BREM.1689_s_at	117
CYP2B6	BREM.2334_at	118
EGFR	BREM.2382_at	119
ETV7	BREM.532_at	120
ANXA1	BRHP.106_s_at	121
ESR1	BRIH.10647C1n2_at	122
EGFR	BRIH.1453C1n2_at	123
EGR1	BRIH.1518C1n4_at	124
ANXA1	BRIH.2770C3n31_at	125

NAT1	BRIH.365C1n2_at	126
IFI44L	BRIH.5410C1n7_at	127
MX1	BRIH.5478C1n2_s_at	128
ESR1	BRIH.5650C1n2_at	129
CD109	BRIH.5952C1n2_s_at	130
CXCL10	BRIH.7359C1n3_s_at	131
FYB	BRIHRC.10930C1n2_s_at	132
AC138128.1	BRMX.13731C1n18_at	133
TERF1P1	BRMX.25436C1n2_at	134
GBP5	BRMX.25712C1n2_at	135
EGR1	BRMX.3079C1n3_at	136
EGR1	BRMX.3079C2n3_at	137
ESR1	BRPD.10690C1n5_at	138
FYB	BRPD.4019C1n3_s_at	139
GBP5	BRPD.5301C1n2_s_at	140
NLRC5	BRRS.12588_at	141
GBP5	BRRS.13369_s_at	142
RSAD2	BRRS.13576_at	143
PTPRC	BRRS.13647_at	144
PTPRC	BRRS.13648_s_at	145
CD109	BRRS.13767_at	146
SP140L	BRRS.13859_at	147
KLHDC7B	BRRS.13881_at	148
APOL3	BRRS.14465_s_at	149
PRICKLE1	BRRS.15053_at	150
CLDN10	BRRS.16228_s_at	151
EGFR	BRRS.16746_s_at	152
EGFR	BRRS.16747_at	153
PRAME	BRRS.16948_s_at	154
TERF1P1	BRRS.17863_s_at	155
TERF1P1	BRRS.17909_s_at	156
AL137218.1	BRRS.18137_at	157
KIF26A	BRRS.18652_s_at	158
FYB	BRRS.2573_s_at	159
CXCL10	BRRS.2644_at	160
CD2	BRRS.2783_s_at	161
EGR1	BRRS.2935_at	162
IDO1	BRRS.3099_at	163
ITGAL	BRRS.3131_at	164
LRP4	BRRS.3220_at	165
MX1	BRRS.3319_at	166
MX1	BRRS.3319_s_at	167
RAC2	BRRS.3645_s_at	168
MFAP5	BRRS.4126_s_at	169
NAT1	BRRS.455_at	170
CDR1	BRRS.4562_at	171
ANXA1	BRRS.487_s_at	172
GRB14	BRRS.4891_s_at	173
TSPAN7	BRRS.4996_at	174
CYP2B6	BRRS.524_s_at	175
ADAMTS4	BRRS.5356_at	176
EGFR	BRRS.5451_at	177
OLFM4	BRRS.6371_at	178
FOSB	BRRS.6611_at	179
PPP1R1A	BRRS.6619_at	180
PPP1R1A	BRRS.6619-22_at	181
IFI44L	BRRS.6684_at	182
CD274	BRRS.7616_at	183
LATS2	BRRS.7901_at	184
ESR1	BRRS.81_at	185

ESR1	BRRS.81-22_at	186
FAM19A5	BRRS.8480_s_at	187
PI15	BRRS.8711_at	188
ETV7	BRRS.8900_s_at	189
EGR1	BRSA.1686C1n5_at	190
RAC2	BRSA.8072C1n2_s_at	191
SP140L	Hs369056.20C1n2_at	192
EGFR	Hs488293.0CB1n69_at	193
ANXA1	Hs494173.0CB4n15_at	194
GBP5	Hs513726.0C2n39_s_at	195
TERF1P1	Hs514006.0C1n8_at	196
TERF1P1	Hs522202.0C1n6_at	197
PRICKLE1	Hs524348.0CB1n97_at	198
PRICKLE1	Hs524348.2C1n5_s_at	199
NLRC5	Hs528836.0C1n3_s_at	200
TERF1P1	Hs591893.1C1n4_s_at	201
RSAD2	Hs7155.0CB1n102_at	202

Table 2D
Almac IDs and Almac Gene symbol and SEQ ID numbers
for antisense probe sets in 40-gene signature

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(D) Almac IDs and Almac Gene symbol and SEQ ID numbers for antisense probe sets in 40 gene signature				
Gene Symbol	EntrezGene ID (40)	Almac Gene ID (32)	Almac Gene symbol	SEQ ID NO:
ADAMTS4	9507			
ANXA1	301			
ANXA1	301	AS1_ANXA1	BRAD.33405_at	51
APOL3	80833			
CD109	135228			
CD2	914			
CD274	29126			
CD274	29126	AS1_CD274	Hs584242.2C1n64_at	52
CDR1	1038			
CDR1	1038	AS1_CDR1	BRRS1RC_NM_004065_at	53
CLDN10	9071			
CLDN10	9071	AS1_CLDN10	BRRS.8182_at	54
CXCL10	3627			
CXCL10	3627	AS1_CXCL10	BRMX.13815C1n5_at	55
CYP2B6	1555			
EGFR	1956			
EGFR	1956	AS1_EGFR	BRMX.2637C1n26_at	56
EGFR	1956	AS2_EGFR	BRAD.36737_at	57
EGFR	1956	AS3_EGFR	BRAD.3853_at	58
EGFR	1956	AS4_EGFR	BRAD1_19760734_at	59
EGR1	1958			
EGR1	1958	AS1_EGR1	BRMX.2797C4n2_at	60
ESR1	2099			
ESR1	2099	AS1_ESR1	BRMX.10399C1n5_at	61
ESR1	2099	AS2_ESR1	BRMX.8912C1n3_at	62
ETV7	51513			
FAM19A5	25817			
FOSB	2354			
FOSB	2354	AS1_FOSB	BRMX.13731C1n18_at	63
FYB	2533			
FYB	2533	AS1_FYB	BRAD.25947_at	64
GBP5	115362			
GBP5	115362	AS1_GBP5	BRMX.5143C1n2(2)_at	65
GRB14	2888			
IDO1	3620			
IFI44L	10964			
IFI44L	10964	AS1_IFI44L	Hs633116.0C1n30_at	66
IFI44L	10964	AS2_IFI44L	BRSA.1606C1n4(2)_at	67
ITGAL	3683			
ITGAL	3683	AS1_ITGAL	BRAD.41047_at	68
ITGAL	3683	AS2_ITGAL	BRAD.4420_at	69
KIF26A	26153			
KLHDC7B	113730			
KLHDC7B	113730	AS1_KLHDC7B	Hs137007.0C1n9_at	70
LATS2	26524			
LATS2	26524	AS1_LATS2	BRSA.18050C1n3_at	71
LRP4	4038			
MFAP5	8076			
MX1	4599			
MX1	4599	AS1_MX1	BRMX.2948C3n7(2)_at	72

MX1	4599	AS2_MX1	Hs43047.0C4n40_at	73
MX1	4599	AS2_MX1	Hs926.1C10n7_at	74
NAT1	9			
NLRC5	84166			
NLRC5	84166	AS1_NLRC5	Hs528836.0CB6n98_s_at	75
OLFM4	10562			
OLFM4	10562	AS1_OLFM4	BRMX.7284C1n6_at	76
PI15	51050			
PI15	51050	AS1_PI15	BRAD1_19751014_at	77
PPP1R1A	5502			
PRAME	23532			
PTPRC	5788			
RAC2	5880			
RAC2	5880	AS1_RAC2	BRMX.13502C1n6_at	78
RSAD2	91543			
SP140L	93349			
SP140L	93349	AS1_SP140L	BRMX.1111C4n3_at	79
SP140L	93349	AS2_SP140L	Hs369056.9C26n3_at	80
TERF1P1	348567			
TERF1P1	348567	AS1_TERF1P1	BRMX.24432C1n2_at	81
TERF1P1	348567	AS2_TERF1P1	BRRS.17773_at	82
TSPAN7	7102			

In different embodiments, subsets of the biomarkers listed in Table 2A and Table 2B may be used in the methods described herein. These subsets include but are not limited to biomarkers ranked 1-2, 1-3, 1-4, 1-5, 1-10, 1-20, 1-30, 1-40, 1-44, 6-10, 11-15, 16-20, 21-25, 26-30, 31-35, 36-40, 36-44, 11-20, 21-30, 31-40, and 31-44 in Table 2A or Table 2B. In one aspect, therapeutic responsiveness is predicted in an individual by conducting an assay on a biological sample from the individual and detecting biomarker values that each correspond to at least one of the biomarkers GBP5, CXCL10, IDO1 and MX1 and at least N additional biomarkers selected from the list of biomarkers in Table 2B, wherein N equals 1, 2, 3, 4, 5, 6, 7, 8, 9, 10, 11, 12, 13, 14, 15, 16, 17, 18, 19, 20, 21, 22, 23, 24, 25, 26, 27, 28, 29, 30, 31, 32, 33, 34, 35, or 36. As used herein, the term “biomarker” can refer to a gene, an mRNA, cDNA, an antisense transcript, a miRNA, a polypeptide, a protein, a protein fragment, or any other nucleic acid sequence or polypeptide sequence that indicates either gene expression levels or protein production levels. In some embodiments, when referring to a biomarker of CXCL10, IDO1, CD2, GBP5, PRAME, ITGAL, LRP4, APOL3, CDR1, FYB, TSPAN7, RAC2, KLHDC7B, GRB14, AC138128.1, KIF26A, CD274, ETV7, MFAP5, OLFM4, PI15, FOSB, FAM19A5, NLRC5, PRICKLE1, EGR1, CLDN10, ADAMTS4, SP140L, ANXA1, RSAD2, ESR1, IKZF3, OR2I1P, EGFR, NAT1, LATS2, CYP2B6, PTPRC, PPP1R1A, or AL137218.1, the biomarker comprises an mRNA of CXCL10, IDO1, CD2, GBP5, PRAME, ITGAL, LRP4, APOL3, CDR1, FYB, TSPAN7, RAC2, KLHDC7B, GRB14, AC138128.1, KIF26A, CD274, ETV7, MFAP5, OLFM4, PI15, FOSB, FAM19A5, NLRC5, PRICKLE1, EGR1, CLDN10, ADAMTS4, SP140L, ANXA1, RSAD2, ESR1, IKZF3, OR2I1P, EGFR, NAT1, LATS2, CYP2B6, PTPRC, PPP1R1A, or AL137218.1, respectively. In further or other embodiments, when referring to a biomarker of MX1, GBP5, IFI44L, BIRC3, IGJ, IQGAP3, LOC100294459, SIX1, SLC9A3R1, STAT1, TOB1, UBD, C1QC, C2orf14, EPSTI, GALNT6, HIST1H4H, HIST2H4B, KIAA1244, LOC100287927, LOC100291682, or LOC100293679, the biomarker comprises an antisense transcript of MX1, IFI44L, GBP5, BIRC3, IGJ, IQGAP3, LOC100294459, SIX1, SLC9A3R1, STAT1, TOB1, UBD, C1QC, C2orf14, EPSTI, GALNT6, HIST1H4H, HIST2H4B, KIAA1244, LOC100287927, LOC100291682, or LOC100293679, respectively.

In a further aspect, therapeutic responsiveness is predicted, or a cancer diagnosis is indicated, in an individual by conducting an assay on a biological sample from the individual and detecting biomarker values that each correspond to the biomarkers GBP5, CXCL10, IDO1 and MX1 and one of at least N additional biomarkers selected from the list of biomarkers in Table 2B, wherein N equals 1, 2, 3, 4, 5, 6, 7, 8, 9, 10, 11, 12, 13, 14, 15, 16, 17, 18, 19, 20, 21, 22, 23, 24, 25, 26, 27, 28, 29, 30, 31, 32, 33, 34, 35, or 36. In a further aspect, therapeutic responsiveness is predicted, or a cancer diagnosis is indicated, in an individual by conducting an assay on a biological sample from the individual and detecting biomarker values that each correspond to the biomarker GBP5 and one of at least N additional biomarkers selected from the list of biomarkers in Table 2B, wherein N equals 1, 2, 3, 4, 5, 6, 7, 8, 9, 10, 11, 12, 13, 14, 15, 16, 17, 18, 29, 20, 21, 22, 23, 24, 25, 26, 27, 28, 29, 30, 31, 32, 33, 34, 35, 36, 37, 38 or 39. In a further aspect, therapeutic responsiveness is predicted, or a cancer diagnosis is indicated, in an individual by conducting an assay on a biological sample from the individual and detecting biomarker values that each correspond to the biomarker CXCL10 and one of at least N additional biomarkers selected from the list of biomarkers in Table 2B, wherein N equals 1, 2, 3, 4, 5, 6, 7, 8, 9, 10, 11, 12, 13, 14, 15, 16, 17, 18, 29, 20, 21, 22, 23, 24, 25, 26, 27, 28, 29, 30, 31, 32, 33, 34, 35, 36, 37, 38 or 39. In a further aspect, therapeutic responsiveness is predicted, or a cancer diagnosis is indicated, in an individual by conducting an assay on a biological sample from the individual and detecting biomarker values that each correspond to the biomarker IDO1 and one of at least N additional biomarkers selected from the list of biomarkers in Table 2B, wherein N equals 1, 2, 3, 4, 5, 6, 7, 8, 9, 10, 11, 12, 13, 14, 15, 16, 17, 18, 29, 20, 21, 22, 23, 24, 25, 26, 27, 28, 29, 30, 31, 32, 33, 34, 35, 36, 37, 38 or 39. In a further aspect, therapeutic responsiveness is predicted, or a cancer diagnosis is indicated, in an individual by conducting an assay on a biological sample from the individual and detecting biomarker values that each correspond to the biomarker MX-1 and one of at least N additional biomarkers selected from the list of biomarkers in Table 2B, wherein N equals 1, 2, 3, 4, 5, 6, 7, 8, 9, 10, 11, 12, 13, 14, 15, 16, 17, 18, 29, 20, 21, 22, 23, 24, 25, 26, 27, 28, 29, 30, 31, 32, 33, 34, 35, 36, 37, 38 or 39.

In a further aspect, therapeutic responsiveness is predicted, or a cancer diagnosis is indicated, in an individual by conducting an assay on a biological sample from the individual and detecting biomarker values that each correspond to at least two of the biomarkers CXCL10, MX1, IDO1 and IFI44L and at least N additional biomarkers selected from the list of biomarkers in Table 2B, wherein N equals 1, 2, 3, 4, 5, 6, 7, 8, 9, 10, 11, 12, 13, 14, 15, 16, 17, 18, 19, 20, 21, 22, 23, 24, 25, 26, 27, 28, 29, 30, 31, 32, 33, 34, 35, 36, 37, 38, 39, or 40. In a further aspect, therapeutic responsiveness is predicted, or a cancer diagnosis is indicated, in an individual by conducting an assay on a biological sample from the individual and detecting biomarker values that each correspond to the biomarkers CXCL10, MX1, IDO1 and IFI44L and one of at least N additional biomarkers selected from the list of biomarkers in Table 2B, wherein N equals 1, 2, 3, 4, 5, 6, 7, 8, 9, 10, 11, 12, 13, 14, 15, 16, 17, 18, 19, 20, 21, 22, 23, 24, 25, 26, 27, 28, 29, 30, 31, 32, 33, 34, 35, 36, 37, 38, 39, or 40. In a further aspect, therapeutic responsiveness is predicted, or a cancer diagnosis is indicated, in an individual by conducting an assay on a biological sample from the individual and detecting biomarker values that each correspond to the biomarker CXCL10 and one of at least N additional biomarkers selected from the list of biomarkers in Table 2B, wherein N equals 1, 2, 3, 4, 5, 6, 7, 8, 9, 10, 11, 12, 13, 14, 15, 16, 17, 18, 29, 20, 21, 22, 23, 24, 25, 26, 27, 28, 29, 30, 31, 32, 33, 34, 35, 36, 37, 38, 39, 40, 41, 42 or 43. In a further aspect, therapeutic responsiveness is predicted, or a cancer diagnosis is indicated, in an individual by conducting an assay on a biological sample from

the individual and detecting biomarker values that each correspond to the biomarker MX1 and one of at least N additional biomarkers selected from the list of biomarkers in Table 2B, wherein N equals 1, 2, 3, 4, 5, 6, 7, 8, 9, 10, 11, 12, 13, 14, 15, 16, 17, 18, 29, 20, 21, 22, 23, 24, 25, 26, 27, 28, 29, 30, 31, 32, 33, 34, 35, 36, 37, 38, 39, 40, 41, 42 or 43. In a further aspect, therapeutic responsiveness is predicted, or a cancer diagnosis is indicated, in an individual by conducting an assay on a biological sample from the individual and detecting biomarker values that each correspond to the biomarker IDO1 and one of at least N additional biomarkers selected from the list of biomarkers in Table 2B, wherein N equals 1, 2, 3, 4, 5, 6, 7, 8, 9, 10, 11, 12, 13, 14, 15, 16, 17, 18, 29, 20, 21, 22, 23, 24, 25, 26, 27, 28, 29, 30, 31, 32, 33, 34, 35, 36, 37, 38, 39, 40, 41, 42 or 43. In a further aspect, therapeutic responsiveness is predicted, or a cancer diagnosis is indicated, in an individual by conducting an assay on a biological sample from the individual and detecting biomarker values that each correspond to the biomarker IFI44L and one of at least N additional biomarkers selected from the list of biomarkers in Table 2B, wherein N equals 1, 2, 3, 4, 5, 6, 7, 8, 9, 10, 11, 12, 13, 14, 15, 16, 17, 18, 29, 20, 21, 22, 23, 24, 25, 26, 27, 28, 29, 30, 31, 32, 33, 34, 35, 36, 37, 38, 39, 40, 41, 42 or 43.

In other embodiments, the probes listed in Table 2C (SEQ ID NOs:83-202), or subsets thereof, may be used in the methods described herein. These subsets include but are not limited to a subset of SEQ ID NOs corresponding to one or more of GBP5, CXCL10, IDO1, MX1, IF144l, CD2, PRAME, ITGAL, LRP4, and APOL3. In other embodiments, the probes correspond to all of the biomarkers CXCL10, MX1, IDO1, IF144L, CD2, GBP5, PRAME, ITGAL, LRP4, APOL3, CDR1, FYB, TSPAN7, RAC2, KLHDC7B, GRB14, AC138128.1, KIF26A, CD274, CD109, ETV7, MFAP5, OLFM4, PI15, FOSB, FAM19A5, NLRC5, PRICKLE1, EGR1, CLDN10, ADAMTS4, SP140L, ANXA1, RSAD2, ESR1, IKZF3, OR2l1P, EGFR, NAT1, LATS2, CYP2B6, PTPRC, PPP1R1A, and AL137218.1. It should be understood that each subset can include multiple probes directed to the same biomarker. For example, the probes represented by SEQ ID NOs: 135, 140, 142 and 195 are all directed to GBP5. Accordingly, a subset containing probes directed or corresponding to GBP5 includes one or more of SEQ ID NOs: 135, 140, 142 and 195. A subset containing probes directed to or corresponding to CXCL10 includes one or more of SEQ ID NOs: 131 and 160.

In other embodiments, specific nucleic acid amplification assays (e.g. PCR, such as qPCR) may be used to determine the expression level of one or more of the genes or sets of genes described herein. The expression level(s) of one or more of the genes may be determined using primers (primer pairs) and/or probes that hybridize with the sequence of the one or more genes. Exemplary primer pairs and probes are provided in Table 2E for each of the genes of the 44-gene DDRD classifier model. The primer pairs and/or probes provided for each gene may be used alone or two or more of the primer pairs and/or probes may be used in combination in accordance with any of the sets of genes described herein. For example, the primer pairs and/or probes provided in Table 2E may be used to determine the expression level of any of the gene signatures provided in Tables 3-45. Exemplary PCR assays are summarized in Table 2E for each of the genes of the 44-gene DDRD classifier model. The PCR assay provided for each gene may be used alone or two or more of the assays may be used in combination in accordance with any of the sets of genes described herein. For example, the PCR assays provided in the table may be used to determine the expression level of any of the gene signatures provided in Tables 3-45.

Table 2E – PCR assays designed for each of the 44 genes listed in Table 2B

GenBank ID	Gene Symbol	Assay ID	Forward Primer ID	Forward Primer SEQ ID NO	Forward Primer ABI Tm (ºC)	Reverse Primer ID	Reverse Primer SEQ ID NO	Reverse Primer ABI Tm (ºC)	Probe ID	Probe SEQ ID NO	Probe ABI Tm (ºC)
NM_002164	IDO1	IDO_A1	IDO_F1	203	56.80	IDO_R1	204	56.30	IDO_P1	205	62.00
NM_001767	CD2	CD2_A1	CD2_F1	206	58.20	CD2_R1	207	57.80	CD2_P1	208	64.90
NM_052942	GBP5	GBP5_A1	GBP5_F1	209	57.00	GBP5_R1	210	58.10	GBP5_P1	211	64.30
NM_206953	PRAME	PRAME_A1	PRAME_F1	212	58.40	PRAME_R1	213	58.40	PRAME_P1	214	64.00
NM_002209	ITGAL	ITGAL_A1	ITGAL_F1	215	59.20	ITGAL_R1	216	58.30	ITGAL_P1	217	66.10
NM_002334	LRP4	LRP4_A1	LRP4_F1	218	57.20	LRP4_R1	219	58.50	LRP4_P1	220	63.80
NM_145640	APOL3	APOL3_A1	APOL3_F1	221	57.10	APOL3_R1	222	57.90	APOL3_P1	223	63.10
NM_004065	CDR1	CDR1_A1	CDR1_F1	224	58.30	CDR1_R1	225	57.90	CDR1_P1	226	63.80
NM_001465	FYB	FYB_A1	FYB_F1	227	59.10	FYB_R1	228	59.20	FYB_P1	229	64.70
NM_004615	TSPAN7	TSPAN7_A1	TSPAN7_F1	230	58.00	TSPAN7_R1	231	57.40	TSPAN7_P1	232	63.20
NM_002872	RAC2	RAC2_A1	RAC2_F1	233	59.10	RAC2_R1	234	58.70	RAC2_P1	235	64.20

NM_138433	KLHDC7B	KLHDC7B_A1	KLHDC7B_F1	236	59.40	KLHDC7B_R1	237	59.70	KLHDC7B_P1	238	66.50
NM_004490	GRB14	GRB14_A1	GRB14_F1	239	57.70	GRB14_R1	240	57.30	GRB14_P1	241	62.70
NM_015656	KIF26A	KIF26A_A1	KIF26A_F1	242	58.40	KIF26A_R1	243	59.40	KIF26A_P1	244	66.80
NM_014143	CD274	CD274_A1	CD274_F1	245	58.60	CD274_R1	246	57.50	CD274_P1	247	65.80
NM_133493	CD109	CD109_A1	CD109_F1	248	59.60	CD109_R1	249	58.70	CD109_P1	250	67.10
NM_016135	ETV7	ETV7_A1	ETV7_F1	251	58.90	ETV7_R1	252	59.20	ETV7_P1	253	65.70
NM_003480	MFAP5	MFAP5_A1	MFAP5_F1	254	58.80	MFAP5_R1	255	59.40	MFAP5_P1	256	64.90
NM_006418	OLFM4	OLFM4_A1	OLFM4_F1	257	57.70	OLFM4_R1	258	58.60	OLFM4_P1	259	63.60
NM_015886	PI15	PI15_A1	PI15_F1	260	58.20	PI15_R1	261	58.60	PI15_P1	262	64.20
NM_006732	FOSB	FOSB_A1	FOSB_F1	263	59.30	FOSB_R1	264	59.40	FOSB_P1	265	66.80
NM_001565	CXCL10	CXCL10_A1	CXCL10_F1	266	63.87	CXCL10_R1	267	63.23	CXCL10_P1	268	72.36
NM_001144925.2	MX1	MX1_A1	MX1_F1	269	61.41	MX1_R1	270	61.01	MX1_P1	271	72.48
NM_006820.3	IFI44L	IFI44L_A1	IFI44L_F1	272	65.75	IFI44L_R1	273	65.12	IFI44L_P1	274	69.81
NM_001166049.1	AC138128.1	AC138128.1_A1	AC138128.1_F1	275	60.48	AC138128.1_R1	276	64.46	AC138128.1_P1	277	72.44

NM_001082967.2	FAM19A5	FAM19A5_A1	FAM19A5_F1	278	64.30	FAM19A5_R1	279	61.45	FAM19A5_P1	280	73.11
NM_032206.4	NLRC5	NLRC5_A1	NLRC5_F1	281	62.55	NLRC5_R1	282	62.69	NLRC5_P1	283	69.77
NM_001144881.1	PRICKLE1	PRICKLE1_A1	PRICKLE1_F1	284	68.01	PRICKLE1_R1	285	65.53	PRICKLE1_P1	286	70.66
NM_001964.2	EGR1	EGR1_A1	EGR1_F1	287	61.27	EGR1_R1	288	61.27	EGR1_P1	289	68.60
NM_001160100.1	CLDN10	CLDN10_A1	CLDN10_F1	290	68.19	CLDN10_R1	291	62.50	CLDN10_P1	292	68.19
NM_005099.4	ADAMTS4	ADAMTS4_A1	ADAMTS4_F1	293	63.69	ADAMTS4_R1	294	63.82	ADAMTS4_P1	295	71.38
NM_001308162.1	SP140L	SP140L_A1	SP140L_F1	296	60.61	SP140L_R1	297	62.67	SP140L_P1	298	72.17
NM_000700.2	ANXA1	ANXA_A1	ANXA_F1	299	64.80	ANXA_R1	300	64.81	ANXA_P1	301	72.90
NM_080657.4	RSAD2	RSAD2_A1	RSAD2_F1	302	63.44	RSAD2_R1	303	63.00	RSAD2_P1	304	70.95
NM_000125.3	ESR1	ESR1_A1	ESR1_F1	305	61.54	ESR1_R1	306	64.65	ESR1_P1	307	70.06
NM_001257408.1	IKZF3	IKZF3_A1	IKZF3_F1	308	62.37	IKZF3_R1	309	64.92	IKZF3_P1	310	69.53
NT_167248.2	OR2I1P	OR2I1P_A1	OR2I1P_F1	353	N/A	OR2I1P_R1	354	58.13	OR2I1P_P1	355	67.43
NM_005228.3	EGFR	EGFR_A1	EGFR_F1	311	62.20	EGFR_R1	312	62.13	EGFR_P1	313	70.85

NM_000662.7	NAT1	NAT1_A1	NAT1_F1	314	60.92	NAT1_R1	315	62.70	NAT1_P1	316	70.40
NM_014572.2	LATS2	LATS2_A1	LATS2_F1	317	60.44	LATS2_R1	318	60.54	LATS2_P1	319	71.63
NM_000767.4	CYP2B6	CYP2B6_A1	CYP2B6_F1	320	64.52	CYP2B6_R1	321	62.64	CYB2B6_P1	322	73.80
NM_001267798	PTPRC	PTPRC_A1	PTPRC_F1	323	62.95	PTPRC_R1	324	62.81	PTPRC_P1	325	70.74
NM_006741.3	PPP1R1A	PPP1R1A_A1	PPP1R1A_F1	326	62.77	PPP1R1A_R1	327	66.34	PPP1R1A_P1	328	69.55
NR_003366.2	AL137218.1	AL137218.1_A1	AL137218.1_F1	356	57.17	AL137218.1_R1	357	56.40	AL137218.1_P1	358	67.23

It should be noted that the complement of each sequence described herein may be employed as appropriate (e.g. for designing hybridizing probes and/or primers, including primer pairs).

Additional gene signatures representing selections of the 44 gene signature are described herein and are applicable to all aspects of the invention. The additional gene signatures are set forth in Tables 3-45, together with suitable weight and bias scores that may be adopted when calculating the final signature score (as further described herein). The k value for each signature can be set once the threshold for defining a positive signature score has been determined, as would be readily appreciated by the skilled person. Similarly, the rankings for each gene in the signature can readily be determined by reviewing the weightings attributed to each gene (where a larger weight indicates a higher ranking in the signature - see Tables 2A and 2B for the rank order in respect of the 40 and 44 gene signatures, respectively).

Whilst Tables 3-45 provide an exemplary weight and bias for each gene in each signature, it will be appreciated that the gene signatures provided by these tables are not limited to the particular weights and biases given. Weight values may indicate the directionality of expression that is measured to indicate a positive signature score according to the invention. Thus, a positive weight indicates that an increase in gene expression contributes to a positive signature score/identification of DDRD biology and vice versa.

Suitable probes and probesets to investigate the expression of the genes included in Tables 3-45 are provided in Table 2C and Table 2D. In addition, suitable PCR assays to investigate the expression of the genes included in Tables 3-45 are provided in Table 2E.

Table 3 – One gene signature

Gene Names	Weight	Bias
CXCL10	0.137044	2.03931

Table 4 – Two gene signature

Gene Names	Weight	Bias
CXCL10	0.081638	2.03931
MX1	0.080192	3.43549

Table 5 – Three gene signature

Gene Names	Weight	Bias
CXCL10	0.058512	2.03931
IDO1	0.055977	0.725702
MX1	0.057475	3.43549

Table 6 – Four gene signature

Gene Names	Weight	Bias
CXCL10	0.048331	2.03931
IDO1	0.046238	0.725702
IFI44L	0.0401	1.17581
MX1	0.047475	3.43549

Table 7 – Five gene signature

Gene Names	Weight	Bias
CD2	0.034275	4.09036
CXCL10	0.041595	2.03931
IDO1	0.039792	0.725702
IFI44L	0.034511	1.17581
MX1	0.040858	3.43549

Table 8 – Six gene signature

Gene Names	Weight	Bias
CD2	0.030041	4.09036
CXCL10	0.036456	2.03931
GBP5	0.028552	1.39771
IDO1	0.034877	0.725702
IFI44L	0.030247	1.17581
MX1	0.03581	3.43549

Table 9 – Seven gene signature

Gene Names	Weight	Bias
CD2	0.025059	4.09036
CXCL10	0.03041	2.03931
GBP5	0.023817	1.39771
IDO1	0.029093	0.725702
IFI44L	0.025231	1.17581
MX1	0.029872	3.43549
PRAME	0.023355	2.2499

Table 10 – Eight gene signature

Gene Names	Weight	Bias

CD2	0.02446	4.09036
CXCL10	0.029683	2.03931
GBP5	0.023247	1.39771
IDO1	0.028397	0.725702
IFI44L	0.024628	1.17581
ITGAL	0.022705	3.21615
MX1	0.029157	3.43549
PRAME	0.022796	2.2499

Table 11 – Nine gene signature

Gene Names	Weight	Bias
CD2	0.023997	4.09036
CXCL10	0.029122	2.03931
GBP5	0.022807	1.39771
IDO1	0.02786	0.725702
IFI44L	0.024162	1.17581
ITGAL	0.022275	3.21615
LRP4	-0.02008	0.306454
MX1	0.028606	3.43549
PRAME	0.022365	2.2499

Table 12 – Ten gene signature

Gene Names	Weight	Bias
APOL3	0.017969	2.20356
CD2	0.02255	4.09036
CXCL10	0.027366	2.03931
GBP5	0.021432	1.39771
IDO1	0.02618	0.725702
IFI44L	0.022705	1.17581
ITGAL	0.020932	3.21615
LRP4	-0.01887	0.306454
MX1	0.026881	3.43549
PRAME	0.021017	2.2499

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Table 13 – Eleven gene signature

Gene Names	Weight	Bias
APOL3	0.018313	2.20356
CD2	0.022983	4.09036

CDR1	-0.01806	4.79794
CXCL10	0.027891	2.03931
GBP5	0.021844	1.39771
IDO1	0.026683	0.725702
IFI44L	0.023141	1.17581
ITGAL	0.021334	3.21615
LRP4	-0.01923	0.306454
MX1	0.027397	3.43549
PRAME	0.02142	2.2499

Table 14 – Twelve gene signature

Gene Names	Weight	Bias
APOL3	0.017235	2.20356
CD2	0.021629	4.09036
CDR1	-0.017	4.79794
CXCL10	0.026248	2.03931
FYB	0.016949	1.56179
GBP5	0.020557	1.39771
IDO1	0.025111	0.725702
IFI44L	0.021778	1.17581
ITGAL	0.020077	3.21615
LRP4	-0.0181	0.306454
MX1	0.025783	3.43549
PRAME	0.020158	2.2499

Table 15 – Thirteen gene signature

Gene Names	Weight	Bias
APOL3	0.017102	2.20356
CD2	0.021463	4.09036
CDR1	-0.01687	4.79794
CXCL10	0.026046	2.03931
FYB	0.016819	1.56179
GBP5	0.020399	1.39771
IDO1	0.024918	0.725702
IFI44L	0.02161	1.17581
ITGAL	0.019923	3.21615
LRP4	-0.01796	0.306454
MX1	0.025585	3.43549
PRAME	0.020003	2.2499

TSPAN7	-0.01675	1.65843

Table 16 – Fourteen gene signature

Gene Names	weight	bias
APOL3	0.016213	2.20356
CD2	0.020347	4.09036
CDR1	-0.01599	4.79794
CXCL10	0.024692	2.03931
FYB	0.015945	1.56179
GBP5	0.019338	1.39771
IDO1	0.023622	0.725702
IFI44L	0.020487	1.17581
ITGAL	0.018887	3.21615
LRP4	-0.01703	0.306454
MX1	0.024255	3.43549
PRAME	0.018963	2.2499
RAC2	0.01586	3.03644
TSPAN7	-0.01588	1.65843

Table 17 – Fifteen gene signature

Gene Names	Weight	Bias
APOL3	0.015496	2.20356
CD2	0.019447	4.09036
CDR1	-0.01528	4.79794
CXCL10	0.023599	2.03931
FYB	0.015239	1.56179
GBP5	0.018482	1.39771
IDO1	0.022577	0.725702
IFI44L	0.01958	1.17581
ITGAL	0.018051	3.21615
KLHDC7B	0.014303	1.43954
LRP4	-0.01627	0.306454
MX1	0.023181	3.43549
PRAME	0.018124	2.2499
RAC2	0.015158	3.03644
TSPAN7	-0.01518	1.65843

Table 18 – Sixteen gene signature

Gene	Weight	Bias

Names		
APOL3	0.016001	2.20356
CD2	0.020081	4.09036
CDR1	-0.01578	4.79794
CXCL10	0.024369	2.03931
FYB	0.015736	1.56179
GBP5	0.019085	1.39771
GRB14	0.014473	0.269629
IDO1	0.023313	0.725702
IFI44L	0.020219	1.17581
ITGAL	0.01864	3.21615
KLHDC7B	0.014769	1.43954
LRP4	-0.0168	0.306454
MX1	0.023937	3.43549
PRAME	0.018715	2.2499
RAC2	0.015653	3.03644
TSPAN7	-0.01567	1.65843

Table 19 – Seventeen gene signature

Gene Names	Weight	Bias
AC138128.1	-0.01406	1.4071
APOL3	0.015604	2.20356
CD2	0.019583	4.09036
CDR1	-0.01539	4.79794
CXCL10	0.023765	2.03931
FYB	0.015346	1.56179
GBP5	0.018612	1.39771
GRB14	0.014114	0.269629
IDO1	0.022735	0.725702
IFI44L	0.019718	1.17581
ITGAL	0.018178	3.21615
KLHDC7B	0.014403	1.43954
LRP4	-0.01639	0.306454
MX1	0.023344	3.43549
PRAME	0.018251	2.2499
RAC2	0.015265	3.03644
TSPAN7	-0.01528	1.65843

Table 20 – Eighteen gene signature

Gene	Weight	Bias

Names		
AC138128.1	-0.01401	1.4071
APOL3	0.015556	2.20356
CD2	0.019522	4.09036
CDR1	-0.01534	4.79794
CXCL10	0.023691	2.03931
FYB	0.015298	1.56179
GBP5	0.018554	1.39771
GRB14	0.01407	0.269629
IDO1	0.022665	0.725702
IFI44L	0.019656	1.17581
ITGAL	0.018121	3.21615
KIF26A	-0.01397	2.05036
KLHDC7B	0.014359	1.43954
LRP4	-0.01634	0.306454
MX1	0.023271	3.43549
PRAME	0.018194	2.2499
RAC2	0.015217	3.03644
TSPAN7	-0.01524	1.65843

Table 21 – Nineteen gene signature

Gene Names	Weight	Bias
AC138128.1	-0.01338	1.4071
APOL3	0.014853	2.20356
CD2	0.01864	4.09036
CD274	0.013043	1.37297
CDR1	-0.01465	4.79794
CXCL10	0.02262	2.03931
FYB	0.014607	1.56179
GBP5	0.017716	1.39771
GRB14	0.013434	0.269629
IDO1	0.02164	0.725702
IFI44L	0.018768	1.17581
ITGAL	0.017302	3.21615
KIF26A	-0.01334	2.05036
KLHDC7B	0.01371	1.43954
LRP4	-0.0156	0.306454
MX1	0.022219	3.43549
PRAME	0.017372	2.2499

RAC2	0.014529	3.03644
TSPAN7	-0.01455	1.65843

Table 22 – Twenty gene signature

Gene Names	Weight	Bias
AC138128.1	-0.0137	1.4071
APOL3	0.015205	2.20356
CD109	-0.01292	0.947671
CD2	0.019081	4.09036
CD274	0.013352	1.37297
CDR1	-0.015	4.79794
CXCL10	0.023156	2.03931
FYB	0.014953	1.56179
GBP5	0.018135	1.39771
GRB14	0.013752	0.269629
IDO1	0.022153	0.725702
IFI44L	0.019212	1.17581
ITGAL	0.017712	3.21615
KIF26A	-0.01366	2.05036
KLHDC7B	0.014034	1.43954
LRP4	-0.01597	0.306454
MX1	0.022746	3.43549
PRAME	0.017783	2.2499
RAC2	0.014874	3.03644
TSPAN7	-0.01489	1.65843

Table 23 – Twenty one gene signature

Gene Names	Weight	Bias
AC138128.1	-0.01327	1.4071
APOL3	0.014725	2.20356
CD109	-0.01251	0.947671
CD2	0.018479	4.09036
CD274	0.012931	1.37297
CDR1	-0.01452	4.79794
CXCL10	0.022425	2.03931
ETV7	0.012047	1.46783
FYB	0.014481	1.56179
GBP5	0.017563	1.39771
GRB14	0.013318	0.269629

IDO1	0.021453	0.725702
IFI44L	0.018606	1.17581
ITGAL	0.017153	3.21615
KIF26A	-0.01323	2.05036
KLHDC7B	0.013591	1.43954
LRP4	-0.01546	0.306454
MX1	0.022028	3.43549
PRAME	0.017222	2.2499
RAC2	0.014404	3.03644
TSPAN7	-0.01442	1.65843

Table 24 – Twenty two gene signature

Gene Names	Weight	Bias
AC138128.1	-0.01326	1.4071
APOL3	0.014714	2.20356
CD109	-0.0125	0.947671
CD2	0.018466	4.09036
CD274	0.012921	1.37297
CDR1	-0.01451	4.79794
CXCL10	0.022409	2.03931
ETV7	0.012038	1.46783
FYB	0.014471	1.56179
GBP5	0.01755	1.39771
GRB14	0.013309	0.269629
IDO1	0.021438	0.725702
IFI44L	0.018593	1.17581
ITGAL	0.017141	3.21615
KIF26A	-0.01322	2.05036
KLHDC7B	0.013582	1.43954
LRP4	-0.01545	0.306454
MFAP5	-0.01172	2.69918
MX1	0.022012	3.43549
PRAME	0.01721	2.2499
RAC2	0.014394	3.03644
TSPAN7	-0.01441	1.65843

Table 25 – Twenty three gene signature

Gene Names	Weight	Bias
AC138128.1	-0.01361	1.4071

APOL3	0.015108	2.20356
CD109	-0.01284	0.947671
CD2	0.018961	4.09036
CD274	0.013268	1.37297
CDR1	-0.0149	4.79794
CXCL10	0.02301	2.03931
ETV7	0.012361	1.46783
FYB	0.014858	1.56179
GBP5	0.018021	1.39771
GRB14	0.013666	0.269629
IDO1	0.022013	0.725702
IFI44L	0.019091	1.17581
ITGAL	0.0176	3.21615
KIF26A	-0.01357	2.05036
KLHDC7B	0.013946	1.43954
LRP4	-0.01587	0.306454
MFAP5	-0.01204	2.69918
MX1	0.022602	3.43549
OLFM4	-0.01167	0.636684
PRAME	0.017671	2.2499
RAC2	0.01478	3.03644
TSPAN7	-0.0148	1.65843

Table 26 – Twenty four gene signature

Gene Names	Weight	Bias
AC138128.1	-0.01365	1.4071
APOL3	0.015148	2.20356
CD109	-0.01287	0.947671
CD2	0.01901	4.09036
CD274	0.013302	1.37297
CDR1	-0.01494	4.79794
CXCL10	0.023069	2.03931
ETV7	0.012393	1.46783
FYB	0.014897	1.56179
GBP5	0.018068	1.39771
GRB14	0.013701	0.269629
IDO1	0.02207	0.725702
IFI44L	0.019141	1.17581
ITGAL	0.017646	3.21615

KIF26A	-0.01361	2.05036
KLHDC7B	0.013982	1.43954
LRP4	-0.01591	0.306454
MFAP5	-0.01207	2.69918
MX1	0.022661	3.43549
OLFM4	-0.0117	0.636684
PI15	-0.01146	0.335476
PRAME	0.017717	2.2499
RAC2	0.014818	3.03644
TSPAN7	-0.01484	1.65843

Table 27 – Twenty five gene signature

Gene Names	Weight	Bias
AC138128.1	-0.01342	1.4071
APOL3	0.014899	2.20356
CD109	-0.01266	0.947671
CD2	0.018698	4.09036
CD274	0.013084	1.37297
CDR1	-0.0147	4.79794
CXCL10	0.022691	2.03931
ETV7	0.01219	1.46783
FOSB	-0.01093	1.85886
FYB	0.014653	1.56179
GBP5	0.017771	1.39771
GRB14	0.013476	0.269629
IDO1	0.021708	0.725702
IFI44L	0.018827	1.17581
ITGAL	0.017357	3.21615
KIF26A	-0.01338	2.05036
KLHDC7B	0.013753	1.43954
LRP4	-0.01565	0.306454
MFAP5	-0.01187	2.69918
MX1	0.022289	3.43549
OLFM4	-0.01151	0.636684
PI15	-0.01128	0.335476
PRAME	0.017426	2.2499
RAC2	0.014575	3.03644
TSPAN7	-0.01459	1.65843

Table 28 – Twenty six gene signature

Gene Names	Weight	Bias
AC138128.1	-0.01339	1.4071
APOL3	0.014858	2.20356
CD109	-0.01262	0.947671
CD2	0.018647	4.09036
CD274	0.013048	1.37297
CDR1	-0.01465	4.79794
CXCL10	0.022629	2.03931
ETV7	0.012157	1.46783
FAM19A5	-0.01083	0.413683
FOSB	-0.0109	1.85886
FYB	0.014613	1.56179
GBP5	0.017723	1.39771
GRB14	0.013439	0.269629
IDO1	0.021649	0.725702
IFI44L	0.018775	1.17581
ITGAL	0.017309	3.21615
KIF26A	-0.01335	2.05036
KLHDC7B	0.013715	1.43954
LRP4	-0.0156	0.306454
MFAP5	-0.01184	2.69918
MX1	0.022228	3.43549
OLFM4	-0.01148	0.636684
PI15	-0.01125	0.335476
PRAME	0.017379	2.2499
RAC2	0.014535	3.03644
TSPAN7	-0.01455	1.65843

Table 29 – Twenty seven gene signature

Gene Names	Weight	Bias
AC138128.1	-0.01316	1.4071
APOL3	0.014603	2.20356
CD109	-0.01241	0.947671
CD2	0.018326	4.09036
CD274	0.012824	1.37297
CDR1	-0.0144	4.79794
CXCL10	0.022239	2.03931
ETV7	0.011947	1.46783
FAM19A5	-0.01064	0.413683

FOSB	-0.01071	1.85886
FYB	0.014361	1.56179
GBP5	0.017417	1.39771
GRB14	0.013208	0.269629
IDO1	0.021276	0.725702
IFI44L	0.018452	1.17581
ITGAL	0.017011	3.21615
KIF26A	-0.01312	2.05036
KLHDC7B	0.013479	1.43954
LRP4	-0.01534	0.306454
MFAP5	-0.01164	2.69918
MX1	0.021845	3.43549
NLRC5	0.009724	2.26863
OLFM4	-0.01128	0.636684
PI15	-0.01105	0.335476
PRAME	0.017079	2.2499
RAC2	0.014285	3.03644
TSPAN7	-0.0143	1.65843

Table 30 – Twenty eight gene signature

Gene Names	Weight	Bias
AC138128.1	-0.01326	1.4071
APOL3	0.014712	2.20356
CD109	-0.0125	0.947671
CD2	0.018464	4.09036
CD274	0.01292	1.37297
CDR1	-0.01451	4.79794
CXCL10	0.022407	2.03931
ETV7	0.012037	1.46783
FAM19A5	-0.01072	0.413683
FOSB	-0.01079	1.85886
FYB	0.014469	1.56179
GBP5	0.017548	1.39771
GRB14	0.013307	0.269629
IDO1	0.021436	0.725702
IFI44L	0.018591	1.17581
ITGAL	0.017139	3.21615
KIF26A	-0.01322	2.05036
KLHDC7B	0.01358	1.43954

LRP4	-0.01545	0.306454
MFAP5	-0.01172	2.69918
MX1	0.02201	3.43549
NLRC5	0.009797	2.26863
OLFM4	-0.01137	0.636684
PI15	-0.01114	0.335476
PRAME	0.017208	2.2499
PRICKLE1	-0.00864	1.77018
RAC2	0.014392	3.03644
TSPAN7	-0.01441	1.65843

Table 31 – Twenty nine gene signature

Gene Names	Weight	Bias
AC138128.1	-0.01307	1.4071
APOL3	0.014506	2.20356
CD109	-0.01232	0.947671
CD2	0.018204	4.09036
CD274	0.012739	1.37297
CDR1	-0.01431	4.79794
CXCL10	0.022092	2.03931
EGR1	-0.00827	2.18651
ETV7	0.011868	1.46783
FAM19A5	-0.01057	0.413683
FOSB	-0.01064	1.85886
FYB	0.014266	1.56179
GBP5	0.017302	1.39771
GRB14	0.01312	0.269629
IDO1	0.021135	0.725702
IFI44L	0.01833	1.17581
ITGAL	0.016898	3.21615
KIF26A	-0.01303	2.05036
KLHDC7B	0.013389	1.43954
LRP4	-0.01523	0.306454
MFAP5	-0.01156	2.69918
MX1	0.021701	3.43549
NLRC5	0.009659	2.26863
OLFM4	-0.01121	0.636684
PI15	-0.01098	0.335476
PRAME	0.016966	2.2499

PRICKLE1	-0.00852	1.77018
RAC2	0.01419	3.03644
TSPAN7	-0.01421	1.65843

Table 32 – Thirty gene signature

Gene Names	Weight	Bias
AC138128.1	-0.01326	1.4071
APOL3	0.014722	2.20356
CD109	-0.01251	0.947671
CD2	0.018476	4.09036
CD274	0.012928	1.37297
CDR1	-0.01452	4.79794
CLDN10	-0.00834	-0.34464
CXCL10	0.022421	2.03931
EGR1	-0.00839	2.18651
ETV7	0.012045	1.46783
FAM19A5	-0.01073	0.413683
FOSB	-0.0108	1.85886
FYB	0.014478	1.56179
GBP5	0.01756	1.39771
GRB14	0.013316	0.269629
IDO1	0.02145	0.725702
IFI44L	0.018603	1.17581
ITGAL	0.01715	3.21615
KIF26A	-0.01323	2.05036
KLHDC7B	0.013589	1.43954
LRP4	-0.01546	0.306454
MFAP5	-0.01173	2.69918
MX1	0.022024	3.43549
NLRC5	0.009803	2.26863
OLFM4	-0.01137	0.636684
PI15	-0.01114	0.335476
PRAME	0.017219	2.2499
PRICKLE1	-0.00864	1.77018
RAC2	0.014402	3.03644
TSPAN7	-0.01442	1.65843

Table 33 – Thirty one gene signature

Gene Names	Weight	Bias

AC138128.1	-0.01339	1.4071
ADAMTS4	-0.00837	1.95693
APOL3	0.014864	2.20356
CD109	-0.01263	0.947671
CD2	0.018654	4.09036
CD274	0.013053	1.37297
CDR1	-0.01466	4.79794
CLDN10	-0.00842	-0.34464
CXCL10	0.022638	2.03931
EGR1	-0.00847	2.18651
ETV7	0.012161	1.46783
FAM19A5	-0.01083	0.413683
FOSB	-0.0109	1.85886
FYB	0.014618	1.56179
GBP5	0.017729	1.39771
GRB14	0.013444	0.269629
IDO1	0.021657	0.725702
IFI44L	0.018782	1.17581
ITGAL	0.017316	3.21615
KIF26A	-0.01335	2.05036
KLHDC7B	0.01372	1.43954
LRP4	-0.01561	0.306454
MFAP5	-0.01184	2.69918
MX1	0.022236	3.43549
NLRC5	0.009898	2.26863
OLFM4	-0.01148	0.636684
PI15	-0.01125	0.335476
PRAME	0.017385	2.2499
PRICKLE1	-0.00873	1.77018
RAC2	0.014541	3.03644
TSPAN7	-0.01456	1.65843

Table 34 – Thirty two gene signature

Gene Names	Weight	Bias
AC138128.1	-0.01332	1.4071
ADAMTS4	-0.00832	1.95693
APOL3	0.014789	2.20356
CD109	-0.01256	0.947671
CD2	0.01856	4.09036

CD274	0.012987	1.37297
CDR1	-0.01459	4.79794
CLDN10	-0.00838	-0.34464
CXCL10	0.022523	2.03931
EGR1	-0.00843	2.18651
ETV7	0.0121	1.46783
FAM19A5	-0.01078	0.413683
FOSB	-0.01085	1.85886
FYB	0.014544	1.56179
GBP5	0.01764	1.39771
GRB14	0.013377	0.269629
IDO1	0.021548	0.725702
IFI44L	0.018688	1.17581
ITGAL	0.017228	3.21615
KIF26A	-0.01329	2.05036
KLHDC7B	0.013651	1.43954
LRP4	-0.01553	0.306454
MFAP5	-0.01178	2.69918
MX1	0.022124	3.43549
NLRC5	0.009848	2.26863
OLFM4	-0.01143	0.636684
PI15	-0.01119	0.335476
PRAME	0.017298	2.2499
PRICKLE1	-0.00868	1.77018
RAC2	0.014467	3.03644
SP140L	0.00825	0.550538
TSPAN7	-0.01449	1.65843

Table 35 – Thirty three gene signature

Gene Names	Weight	Bias
AC138128.1	-0.01348	1.4071
ADAMTS4	-0.00842	1.95693
ANXA1	-0.0081	2.00146
APOL3	0.014961	2.20356
CD109	-0.01271	0.947671
CD2	0.018776	4.09036
CD274	0.013138	1.37297
CDR1	-0.01476	4.79794
CLDN10	-0.00848	-0.34464

CXCL10	0.022785	2.03931
EGR1	-0.00853	2.18651
ETV7	0.01224	1.46783
FAM19A5	-0.0109	0.413683
FOSB	-0.01097	1.85886
FYB	0.014713	1.56179
GBP5	0.017845	1.39771
GRB14	0.013532	0.269629
IDO1	0.021798	0.725702
IFI44L	0.018905	1.17581
ITGAL	0.017428	3.21615
KIF26A	-0.01344	2.05036
KLHDC7B	0.01381	1.43954
LRP4	-0.01571	0.306454
MFAP5	-0.01192	2.69918
MX1	0.022381	3.43549
NLRC5	0.009962	2.26863
OLFM4	-0.01156	0.636684
PI15	-0.01132	0.335476
PRAME	0.017498	2.2499
PRICKLE1	-0.00878	1.77018
RAC2	0.014635	3.03644
SP140L	0.008345	0.550538
TSPAN7	-0.01465	1.65843

Table 36 – Thirty four gene signature

Gene Names	Weight	Bias
AC138128.1	-0.01334	1.4071
ADAMTS4	-0.00834	1.95693
ANXA1	-0.00802	2.00146
APOL3	0.014812	2.20356
CD109	-0.01258	0.947671
CD2	0.018589	4.09036
CD274	0.013007	1.37297
CDR1	-0.01461	4.79794
CLDN10	-0.00839	-0.34464
CXCL10	0.022558	2.03931
EGR1	-0.00844	2.18651
ETV7	0.012118	1.46783

FAM19A5	-0.0108	0.413683
FOSB	-0.01086	1.85886
FYB	0.014567	1.56179
GBP5	0.017667	1.39771
GRB14	0.013397	0.269629
IDO1	0.021581	0.725702
IFI44L	0.018716	1.17581
ITGAL	0.017255	3.21615
KIF26A	-0.01331	2.05036
KLHDC7B	0.013672	1.43954
LRP4	-0.01556	0.306454
MFAP5	-0.0118	2.69918
MX1	0.022159	3.43549
NLRC5	0.009863	2.26863
OLFM4	-0.01144	0.636684
PI15	-0.01121	0.335476
PRAME	0.017324	2.2499
PRICKLE1	-0.0087	1.77018
RAC2	0.01449	3.03644
RSAD2	0.007894	1.44894
SP140L	0.008262	0.550538
TSPAN7	-0.01451	1.65843

Table 37 – Thirty five gene signature

Gene Names	Weight	Bias
AC138128.1	-0.0137	1.4071
ADAMTS4	-0.00856	1.95693
ANXA1	-0.00823	2.00146
APOL3	0.015208	2.20356
CD109	-0.01292	0.947671
CD2	0.019085	4.09036
CD274	0.013355	1.37297
CDR1	-0.015	4.79794
CLDN10	-0.00862	-0.34464
CXCL10	0.023161	2.03931
EGR1	-0.00867	2.18651
ESR1	0.007943	0.851213
ETV7	0.012442	1.46783
FAM19A5	-0.01108	0.413683

FOSB	-0.01115	1.85886
FYB	0.014956	1.56179
GBP5	0.018139	1.39771
GRB14	0.013755	0.269629
IDO1	0.022157	0.725702
IFI44L	0.019216	1.17581
ITGAL	0.017716	3.21615
KIF26A	-0.01366	2.05036
KLHDC7B	0.014037	1.43954
LRP4	-0.01597	0.306454
MFAP5	-0.01212	2.69918
MX1	0.022751	3.43549
NLRC5	0.010127	2.26863
OLFM4	-0.01175	0.636684
PI15	-0.01151	0.335476
PRAME	0.017787	2.2499
PRICKLE1	-0.00893	1.77018
RAC2	0.014877	3.03644
RSAD2	0.008105	1.44894
SP140L	0.008483	0.550538
TSPAN7	-0.0149	1.65843

Table 38 – Thirty six gene signature

Gene Names	Weight	Bias
AC138128.1	-0.01359	1.4071
ADAMTS4	-0.00849	1.95693
ANXA1	-0.00816	2.00146
APOL3	0.015081	2.20356
CD109	-0.01281	0.947671
CD2	0.018926	4.09036
CD274	0.013244	1.37297
CDR1	-0.01487	4.79794
CLDN10	-0.00855	-0.34464
CXCL10	0.022968	2.03931
EGR1	-0.0086	2.18651
ESR1	0.007876	0.851213
ETV7	0.012338	1.46783
FAM19A5	-0.01099	0.413683
FOSB	-0.01106	1.85886

FYB	0.014831	1.56179
GBP5	0.017988	1.39771
GRB14	0.01364	0.269629
IDO1	0.021973	0.725702
IFI44L	0.019056	1.17581
IKZF3	0.007318	-0.58991
ITGAL	0.017568	3.21615
KIF26A	-0.01355	2.05036
KLHDC7B	0.01392	1.43954
LRP4	-0.01584	0.306454
MFAP5	-0.01202	2.69918
MX1	0.022561	3.43549
NLRC5	0.010042	2.26863
OLFM4	-0.01165	0.636684
PI15	-0.01141	0.335476
PRAME	0.017639	2.2499
PRICKLE1	-0.00885	1.77018
RAC2	0.014753	3.03644
RSAD2	0.008038	1.44894
SP140L	0.008412	0.550538
TSPAN7	-0.01477	1.65843

Table 39 – Thirty seven gene signature

Gene Names	Weight	Bias
AC138128.1	-0.01342	1.4071
ADAMTS4	-0.00838	1.95693
ANXA1	-0.00806	2.00146
APOL3	0.014896	2.20356
CD109	-0.01265	0.947671
CD2	0.018694	4.09036
CD274	0.013081	1.37297
CDR1	-0.01469	4.79794
CLDN10	-0.00844	-0.34464
CXCL10	0.022686	2.03931
EGR1	-0.00849	2.18651
ESR1	0.00778	0.851213
ETV7	0.012187	1.46783
FAM19A5	-0.01086	0.413683
FOSB	-0.01092	1.85886

FYB	0.014649	1.56179
GBP5	0.017767	1.39771
GRB14	0.013473	0.269629
IDO1	0.021703	0.725702
IFI44L	0.018823	1.17581
IKZF3	0.007228	-0.58991
ITGAL	0.017353	3.21615
KIF26A	-0.01338	2.05036
KLHDC7B	0.01375	1.43954
LRP4	-0.01564	0.306454
MFAP5	-0.01187	2.69918
MX1	0.022284	3.43549
NLRC5	0.009919	2.26863
OLFM4	-0.01151	0.636684
OR2I1P	0.00685	-1.30235
PI15	-0.01127	0.335476
PRAME	0.017422	2.2499
PRICKLE1	-0.00875	1.77018
RAC2	0.014572	3.03644
RSAD2	0.007939	1.44894
SP140L	0.008309	0.550538
TSPAN7	-0.01459	1.65843

Table 40 – Thirty eight gene signature

Gene Names	Weight	Bias
AC138128.1	-0.01345	1.4071
ADAMTS4	-0.0084	1.95693
ANXA1	-0.00808	2.00146
APOL3	0.014924	2.20356
CD109	-0.01268	0.947671
CD2	0.01873	4.09036
CD274	0.013106	1.37297
CDR1	-0.01472	4.79794
CLDN10	-0.00846	-0.34464
CXCL10	0.022729	2.03931
EGFR	-0.00649	-0.17669
EGR1	-0.00851	2.18651
ESR1	0.007795	0.851213
ETV7	0.01221	1.46783

FAM19A5	-0.01088	0.413683
FOSB	-0.01095	1.85886
FYB	0.014677	1.56179
GBP5	0.017801	1.39771
GRB14	0.013499	0.269629
IDO1	0.021745	0.725702
IFI44L	0.018858	1.17581
IKZF3	0.007242	-0.58991
ITGAL	0.017386	3.21615
KIF26A	-0.01341	2.05036
KLHDC7B	0.013776	1.43954
LRP4	-0.01567	0.306454
MFAP5	-0.01189	2.69918
MX1	0.022327	3.43549
NLRC5	0.009938	2.26863
OLFM4	-0.01153	0.636684
OR2I1P	0.006863	-1.30235
PI15	-0.0113	0.335476
PRAME	0.017456	2.2499
PRICKLE1	-0.00876	1.77018
RAC2	0.0146	3.03644
RSAD2	0.007954	1.44894
SP140L	0.008325	0.550538
TSPAN7	-0.01462	1.65843

Table 41 – Thirty nine gene signature

Gene Names	Weight	Bias
AC138128.1	-0.01356	1.4071
ADAMTS4	-0.00847	1.95693
ANXA1	-0.00815	2.00146
APOL3	0.015054	2.20356
CD109	-0.01279	0.947671
CD2	0.018892	4.09036
CD274	0.01322	1.37297
CDR1	-0.01485	4.79794
CLDN10	-0.00853	-0.34464
CXCL10	0.022926	2.03931
EGFR	-0.00654	-0.17669
EGR1	-0.00858	2.18651

ESR1	0.007862	0.851213
ETV7	0.012316	1.46783
FAM19A5	-0.01097	0.413683
FOSB	-0.01104	1.85886
FYB	0.014805	1.56179
GBP5	0.017955	1.39771
GRB14	0.013616	0.269629
IDO1	0.021933	0.725702
IFI44L	0.019022	1.17581
IKZF3	0.007305	-0.58991
ITGAL	0.017536	3.21615
KIF26A	-0.01352	2.05036
KLHDC7B	0.013895	1.43954
LRP4	-0.01581	0.306454
MFAP5	-0.012	2.69918
MX1	0.02252	3.43549
NAT1	0.006442	-0.79732
NLRC5	0.010024	2.26863
OLFM4	-0.01163	0.636684
OR2I1P	0.006922	-1.30235
PI15	-0.01139	0.335476
PRAME	0.017607	2.2499
PRICKLE1	-0.00884	1.77018
RAC2	0.014726	3.03644
RSAD2	0.008023	1.44894
SP140L	0.008397	0.550538
TSPAN7	-0.01474	1.65843

Table 42 – Forty gene signature

Gene Names	Weight	Bias
AC138128.1	-0.01357	1.4071
ADAMTS4	-0.00848	1.95693
ANXA1	-0.00815	2.00146
APOL3	0.015057	2.20356
CD109	-0.01279	0.947671
CD2	0.018896	4.09036
CD274	0.013223	1.37297
CDR1	-0.01485	4.79794
CLDN10	-0.00853	-0.34464

CXCL10	0.022931	2.03931
EGFR	-0.00654	-0.17669
EGR1	-0.00858	2.18651
ESR1	0.007864	0.851213
ETV7	0.012319	1.46783
FAM19A5	-0.01097	0.413683
FOSB	-0.01104	1.85886
FYB	0.014808	1.56179
GBP5	0.017959	1.39771
GRB14	0.013619	0.269629
IDO1	0.021938	0.725702
IFI44L	0.019026	1.17581
IKZF3	0.007306	-0.58991
ITGAL	0.01754	3.21615
KIF26A	-0.01353	2.05036
KLHDC7B	0.013898	1.43954
LATS2	-0.00622	0.486251
LRP4	-0.01581	0.306454
MFAP5	-0.012	2.69918
MX1	0.022525	3.43549
NAT1	0.006444	-0.79732
NLRC5	0.010026	2.26863
OLFM4	-0.01163	0.636684
OR2I1P	0.006924	-1.30235
PI15	-0.0114	0.335476
PRAME	0.017611	2.2499
PRICKLE1	-0.00884	1.77018
RAC2	0.014729	3.03644
RSAD2	0.008025	1.44894
SP140L	0.008399	0.550538
TSPAN7	-0.01475	1.65843

Table 43 – Forty one gene signature

Gene Names	Weight	Bias
AC138128.1	-0.01374	1.4071
ADAMTS4	-0.00859	1.95693
ANXA1	-0.00826	2.00146
APOL3	0.015253	2.20356
CD109	-0.01296	0.947671

CD2	0.019143	4.09036
CD274	0.013395	1.37297
CDR1	-0.01504	4.79794
CLDN10	-0.00864	-0.34464
CXCL10	0.02323	2.03931
CYP2B6	0.006181	0.921835
EGFR	-0.00663	-0.17669
EGR1	-0.00869	2.18651
ESR1	0.007966	0.851213
ETV7	0.01248	1.46783
FAM19A5	-0.01112	0.413683
FOSB	-0.01119	1.85886
FYB	0.015001	1.56179
GBP5	0.018194	1.39771
GRB14	0.013797	0.269629
IDO1	0.022224	0.725702
IFI44L	0.019274	1.17581
IKZF3	0.007402	-0.58991
ITGAL	0.017769	3.21615
KIF26A	-0.0137	2.05036
KLHDC7B	0.014079	1.43954
LATS2	-0.0063	0.486251
LRP4	-0.01602	0.306454
MFAP5	-0.01215	2.69918
MX1	0.022819	3.43549
NAT1	0.006528	-0.79732
NLRC5	0.010157	2.26863
OLFM4	-0.01178	0.636684
OR2I1P	0.007014	-1.30235
PI15	-0.01154	0.335476
PRAME	0.01784	2.2499
PRICKLE1	-0.00896	1.77018
RAC2	0.014921	3.03644
RSAD2	0.00813	1.44894
SP140L	0.008509	0.550538
TSPAN7	-0.01494	1.65843

Table 44 – Forty two gene signature

Gene Names	Weight	Bias

AC138128.1	-0.01365	1.4071
ADAMTS4	-0.00853	1.95693
ANXA1	-0.0082	2.00146
APOL3	0.015146	2.20356
CD109	-0.01287	0.947671
CD2	0.019008	4.09036
CD274	0.013301	1.37297
CDR1	-0.01494	4.79794
CLDN10	-0.00858	-0.34464
CXCL10	0.023067	2.03931
CYP2B6	0.006138	0.921835
EGFR	-0.00658	-0.17669
EGR1	-0.00863	2.18651
ESR1	0.00791	0.851213
ETV7	0.012392	1.46783
FAM19A5	-0.01104	0.413683
FOSB	-0.01111	1.85886
FYB	0.014895	1.56179
GBP5	0.018065	1.39771
GRB14	0.013699	0.269629
IDO1	0.022067	0.725702
IFI44L	0.019138	1.17581
IKZF3	0.00735	-0.58991
ITGAL	0.017644	3.21615
KIF26A	-0.01361	2.05036
KLHDC7B	0.01398	1.43954
LATS2	-0.00626	0.486251
LRP4	-0.01591	0.306454
MFAP5	-0.01207	2.69918
MX1	0.022658	3.43549
NAT1	0.006482	-0.79732
NLRC5	0.010085	2.26863
OLFM4	-0.0117	0.636684
OR2I1P	0.006965	-1.30235
PI15	-0.01146	0.335476
PRAME	0.017715	2.2499
PRICKLE1	-0.00889	1.77018
PTPRC	0.005152	-1.11824
RAC2	0.014816	3.03644
RSAD2	0.008072	1.44894

SP140L	0.008449	0.550538
TSPAN7	-0.01484	1.65843

Table 45 – Forty three gene signature

Gene Names	Weight	Bias
AC138128.1	-0.01364	1.4071
ADAMTS4	-0.00852	1.95693
ANXA1	-0.0082	2.00146
APOL3	0.015139	2.20356
CD109	-0.01286	0.947671
CD2	0.018999	4.09036
CD274	0.013295	1.37297
CDR1	-0.01493	4.79794
CLDN10	-0.00858	-0.34464
CXCL10	0.023056	2.03931
CYP2B6	0.006135	0.921835
EGFR	-0.00658	-0.17669
EGR1	-0.00863	2.18651
ESR1	0.007907	0.851213
ETV7	0.012386	1.46783
FAM19A5	-0.01103	0.413683
FOSB	-0.0111	1.85886
FYB	0.014889	1.56179
GBP5	0.018057	1.39771
GRB14	0.013693	0.269629
IDO1	0.022057	0.725702
IFI44L	0.01913	1.17581
IKZF3	0.007346	-0.58991
ITGAL	0.017636	3.21615
KIF26A	-0.0136	2.05036
KLHDC7B	0.013974	1.43954
LATS2	-0.00625	0.486251
LRP4	-0.0159	0.306454
MFAP5	-0.01206	2.69918
MX1	0.022648	3.43549
NAT1	0.006479	-0.79732
NLRC5	0.010081	2.26863
OLFM4	-0.0117	0.636684
OR2I1P	0.006962	-1.30235

PI15	-0.01146	0.335476
PPP1R1A	-0.0041	1.76371
PRAME	0.017707	2.2499
PRICKLE1	-0.00889	1.77018
PTPRC	0.00515	-1.11824
RAC2	0.01481	3.03644
RSAD2	0.008069	1.44894
SP140L	0.008445	0.550538
TSPAN7	-0.01483	1.65843

Measuring Gene Expression Using Classifier Models

A variety of methods have been utilized in an attempt to identify biomarkers and diagnose disease. For protein-based markers, these include two-dimensional electrophoresis, mass spectrometry, and immunoassay methods. For nucleic acid markers, these include mRNA expression profiles, microRNA profiles, FISH, serial analysis of gene expression (SAGE), methylation profiles, and large-scale gene expression arrays.

When a biomarker indicates or is a sign of an abnormal process, disease or other condition in an individual, that biomarker is generally described as being either over-expressed or under-expressed as compared to an expression level or value of the biomarker that indicates or is a sign of a normal process, an absence of a disease or other condition in an individual. "Up-regulation", "up-regulated", "over-expression", "over-expressed", and any variations thereof are used interchangeably to refer to a value or level of a biomarker in a biological sample that is greater than a value or level (or range of values or levels) of the biomarker that is typically detected in similar biological samples from healthy or normal individuals. The terms may also refer to a value or level of a biomarker in a biological sample that is greater than a value or level (or range of values or levels) of the biomarker that may be detected at a different stage of a particular disease.

"Down-regulation", "down-regulated", "under-expression", "under-expressed", and any variations thereof are used interchangeably to refer to a value or level of a biomarker in a biological sample that is less than a value or level (or range of values or levels) of the biomarker that is typically detected in similar biological samples from healthy or normal individuals. The terms may also refer to a value or level of a biomarker in a biological sample that is less than a value or level (or range of values or levels) of the biomarker that may be detected at a different stage of a particular disease.

Further, a biomarker that is either over-expressed or under-expressed can also be referred to as being "differentially expressed" or as having a "differential level" or "differential value" as compared to a "normal" expression level or value of the biomarker that indicates or is a sign of a normal process or an absence of a disease or other condition in an individual. Thus, "differential expression" of a biomarker can also be referred to as a variation from a "normal" expression level of the biomarker.

The terms "differential biomarker expression" and "differential expression" are used interchangeably to refer to a biomarker whose expression is activated to a higher or lower level in a

subject suffering from a specific disease, relative to its expression in a normal subject, or relative to its expression in a patient that responds differently to a particular therapy or has a different prognosis. The terms also include biomarkers whose expression is activated to a higher or lower level at different stages of the same disease. It is also understood that a differentially expressed biomarker may be either activated or inhibited at the nucleic acid level or protein level, or may be subject to alternative splicing to result in a different polypeptide product. Such differences may be evidenced by a variety of changes including mRNA levels, miRNA levels, antisense transcript levels, or protein surface expression, secretion or other partitioning of a polypeptide. Differential biomarker expression may include a comparison of expression between two or more genes or their gene products; or a comparison of the ratios of the expression between two or more genes or their gene products; or even a comparison of two differently processed products of the same gene, which differ between normal subjects and subjects suffering from a disease; or between various stages of the same disease. Differential expression includes both quantitative, as well as qualitative, differences in the temporal or cellular expression pattern in a biomarker among, for example, normal and diseased cells, or among cells which have undergone different disease events or disease stages.

In certain embodiments, the expression profile obtained is a genomic or nucleic acid expression profile, where the amount or level of one or more nucleic acids in the sample is determined. In these embodiments, the sample that is assayed to generate the expression profile employed in the diagnostic or prognostic methods is one that is a nucleic acid sample. The nucleic acid sample includes a population of nucleic acids that includes the expression information of the phenotype determinative biomarkers of the cell or tissue being analyzed. In some embodiments, the nucleic acid may include RNA or DNA nucleic acids, e.g., mRNA, cRNA, cDNA etc., so long as the sample retains the expression information of the host cell or tissue from which it is obtained. The sample may be prepared in a number of different ways, as is known in the art, e.g., by mRNA isolation from a cell, where the isolated mRNA is used as isolated, amplified, or employed to prepare cDNA, cRNA, etc., as is known in the field of differential gene expression. Accordingly, determining the level of mRNA in a sample includes preparing cDNA or cRNA from the mRNA and subsequently measuring the cDNA or cRNA. The sample is typically prepared from a cell or tissue harvested from a subject in need of treatment, e.g., via biopsy of tissue, using standard protocols, where cell types or tissues from which such nucleic acids may be generated include any tissue in which the expression pattern of the to be determined phenotype exists, including, but not limited to, disease cells or tissue, body fluids, etc.

The expression profile may be generated from the initial nucleic acid sample using any convenient protocol. While a variety of different manners of generating expression profiles are known, such as those employed in the field of differential gene expression/biomarker analysis, one representative and convenient type of protocol for generating expression profiles is array-based gene expression profile generation protocols. Such applications are hybridization assays in which a nucleic acid that displays "probe" nucleic acids for each of the genes to be assayed/profiled in the profile to be generated is employed. In these assays, a sample of target nucleic acids is first prepared from the initial nucleic acid sample being assayed, where preparation may include labeling of the target nucleic acids with a label, e.g., a member of a signal producing system. Following target nucleic acid sample preparation, the sample is contacted with the array under hybridization conditions, whereby complexes are formed between target nucleic acids that are complementary to probe sequences attached to the

array surface. The presence of hybridized complexes is then detected, either qualitatively or quantitatively. Specific hybridization technology which may be practiced to generate the expression profiles employed in the subject methods includes the technology described in U.S. Pat. Nos. 5,143,854; 5,288,644; 5,324,633; 5,432,049; 5,470,710; 5,492,806; 5,503,980; 5,510,270; 5,525,464; 5,547,839; 5,580,732; 5,661,028; 5,800,992; the disclosures of which are herein incorporated by reference; as well as WO 95/21265; WO 96/31622; WO 97/10365; WO 97/27317; EP 373 203; and EP 785 280. In these methods, an array of "probe" nucleic acids that includes a probe for each of the biomarkers whose expression is being assayed is contacted with target nucleic acids as described above. Contact is carried out under hybridization conditions, e.g., stringent hybridization conditions as described above, and unbound nucleic acid is then removed. The resultant pattern of hybridized nucleic acids provides information regarding expression for each of the biomarkers that have been probed, where the expression information is in terms of whether or not the gene is expressed and, typically, at what level, where the expression data, i.e., expression profile, may be both qualitative and quantitative.

Creating a Biomarker Expression Classifier

In one embodiment, the relative expression levels of biomarkers in a cancer tissue are measured to form a gene expression profile. The gene expression profile of a set of biomarkers from a patient tissue 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 cancer tissue samples having been characterized by prognosis, likelihood of recurrence, long term survival, clinical outcome, treatment response, diagnosis, cancer 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.

The overall expression data for a given sample is normalized using methods known to those skilled in the art in order to correct for differing amounts of starting material, varying efficiencies of the extraction and amplification reactions, etc. Using a linear classifier on the normalized data to make a diagnostic or prognostic call (e.g. responsiveness or resistance to therapeutic agent) effectively means to split the data space, i.e. all possible combinations of expression values for all genes in the classifier, into two disjoint halves by means of a separating hyperplane. This split is empirically derived on a large set of training examples, for example from patients showing responsiveness or resistance to a therapeutic agent. Without loss of generality, one can assume a certain fixed set of values for all but one biomarker, which would automatically define a threshold value for this remaining biomarker where the decision would change from, for example, responsiveness or resistance to a therapeutic agent. Expression values above this dynamic threshold would then either indicate resistance (for a biomarker with a negative weight) or responsiveness (for a biomarker with a positive weight) to a therapeutic agent. The precise value of this threshold depends on the actual measured expression profile of all other biomarkers within

the classifier, but the general indication of certain biomarkers remains fixed, i.e. high values or "relative over-expression" always contributes to either a responsiveness (genes with a positive weight) or resistance (genes with a negative weights). Therefore, in the context of the overall gene expression classifier, relative expression can indicate if either up- or down-regulation of a certain biomarker is indicative of responsiveness or resistance to a therapeutic agent.

In one embodiment, the biomarker expression profile of a patient tissue sample is evaluated by a linear classifier. As used herein, a linear classifier refers to a weighted sum of the individual biomarker intensities into a compound decision score ("decision function"). The decision score is then compared to a pre-defined cut-off score threshold, 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 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., ovarian cancer 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. Ståhle, S. Wold, J. Chemom. 1 (1987) 185-196; D. V. Nguyen, D.M. Rocke, Bioinformatics 18 (2002) 39-50). Other methods for performing the classification, known to those skilled in the art, may also be with the methods described herein when applied to the transcripts of a cancer classifier.

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, 2nd ed., John Wiley, New York 2001), machine learning (Schölkopf 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).

In a training step, a set of patient samples for both responsiveness/resistance cases are measured and the prediction method is optimised using the inherent information from this training data to optimally predict the training set or a future sample set. In this training step, the used method is trained or parameterised to predict from a specific intensity pattern to a specific predictive call. Suitable transformation or pre-processing steps might be performed with the measured data before it is subjected to the prognostic method or algorithm.

In a preferred embodiment of the invention, a weighted sum of the pre-processed intensity values for each transcript is formed and compared with a threshold value optimised on the training set (Duda et al. Pattern Classification, 2nd ed., John Wiley, New York 2001). The weights can be derived by a multitude of linear classification methods, including but not limited to Partial Least Squares (PLS, (Nguyen et al., 2002, Bioinformatics 18 (2002) 39-50)) or Support Vector Machines (SVM, (Schölkopf et al. Learning with Kernels, MIT Press, Cambridge 2002)).

In another embodiment of the invention, the data is transformed non-linearly before applying a weighted sum as described above. This non-linear transformation might include increasing the dimensionality of the data. The non-linear transformation and weighted summation might also be performed implicitly, e.g. through the use of a kernel function. (Schölkopf et al. Learning with Kernels, MIT Press, Cambridge 2002).

In another embodiment of the invention, a new data sample is compared with two or more class prototypes, being either real measured training samples or artificially created prototypes. This comparison is performed using suitable similarity measures, for example, but not limited to Euclidean distance (Duda et al. Pattern Classification, 2nd ed., John Wiley, New York 2001), correlation coefficient (Van't Veer, et al. 2002, Nature 415:530) etc. A new sample is then assigned to the prognostic group with the closest prototype or the highest number of prototypes in the vicinity.

In another embodiment of the invention, decision trees (Hastie et al., The Elements of Statistical Learning, Springer, New York 2001) or random forests (Breiman, Random Forests, Machine Learning 45:5 2001) are used to make a prognostic call from the measured intensity data for the transcript set or their products.

In another embodiment of the invention neural networks (Bishop, Neural Networks for Pattern Recognition, Clarendon Press, Oxford 1995) are used to make a prognostic call from the measured intensity data for the transcript set or their products.

In another embodiment of the invention, discriminant analysis (Duda et al., Pattern Classification, 2nd ed., John Wiley, New York 2001), comprising but not limited to linear, diagonal linear, quadratic and logistic discriminant analysis, is used to make a prognostic call from the measured intensity data for the transcript set or their products.

In another embodiment of the invention, Prediction Analysis for Microarrays (PAM, (Tibshirani et al., 2002, Proc. Natl. Acad. Sci. USA 99:6567-6572)) is used to make a prognostic call from the measured intensity data for the transcript set or their products.

In another embodiment of the invention, Soft Independent Modelling of Class Analogy (SIMCA, (Wold, 1976, Pattern Recogn. 8:127-139)) is used to make a predictive call from the measured intensity data for the transcript set or their products.

Therapeutic agents

As described above, the methods described herein permit the classification of a patient as responsive or non-responsive to a therapeutic agent that targets tumors with increased immune signaling associated with abnormal DNA repair. In particular, the therapeutic agents may be immune checkpoint therapies, such as antagonists of an inhibitory immune checkpoint and/or agonists of a stimulatory immune checkpoint. In some embodiments, the inhibitory immune checkpoint is selected from A2AR, B7-H3 (CD276), B7-H4 (VTCN1), BTLA (CD272), CTLA-4 (CD152), IDO, KIR, LAG3, PD-1/PD-L1, TIM-3 and VISTA. In some embodiments, the inhibitory immune checkpoint is not PD-1/PD-L1. In some embodiments, the inhibitory immune checkpoint is IDO. In some embodiments, the antagonist of an inhibitory immune checkpoint is selected from an antibody and an inhibitory nucleic acid molecule as defined herein. In some embodiments, the antagonist of an inhibitory immune checkpoint is selected from MGA271 (targets B7-H3), ipilimumab (Yervoy - targets CTLA-4), indoximod (targets IDO pathway), NLG919 (targets IDO pathway), lirilumab (targets KIR), IMP321 (targets LAG3), BMS-986016 (targets LAG3), CT-011 (PD-1 blockade), nivolumab/BMS-936558 (PD-1 blockade) , BMS-936559 (PDL1 blockade) and pembrolizumab (Keytruda – targets PD-1), optionally wherein the antagonist is not pembrolizumab. In some embodiments, the stimulatory immune checkpoint is selected from CD27, CD28, CD40, CD122, CD137, OX40, GITR and ICOS. In some embodiments, the agonist of a stimulatory immune checkpoint is selected from an antibody, a lipocalin and a cytokine, as defined herein. In some embodiments, the agonist of a stimulatory immune checkpoint is selected from CDX-1127 (agonist of CD27), NKTR-214 (agonist of CD122), BMS-663513 (agonist of CD137), TRX518 (agonist of GITR), CP-870893 (CD40 agonist), MEDI0562, MEDI6469 and MEDI6383 (OX40 agonists).

In some embodiments, the immune checkpoint therapies, such as antagonists of an inhibitory immune checkpoint and/or agonists of a stimulatory immune checkpoint may be administered in combination with a “DNA-damage therapeutic agent”. As used herein “DNA- damage therapeutic agent” includes agents known to damage DNA directly, agents that prevent DNA damage repair, agents that inhibit DNA damage signaling, agents that inhibit DNA damage induced cell cycle arrest, and agents that

inhibit processes indirectly leading to DNA damage. Some current such therapeutics used to treat cancer include, but are not limited to, the following DNA-damage therapeutic agents.

1) DNA damaging agents:
    a. Alkylating agents (platinum containing agents such as cisplatin, carboplatin, and oxaliplatin; cyclophosphamide; busulphan).
    b. Topoisomerase I inhibitors (irinotecan; topotecan)
    c. Topisomerase II inhibitors (etoposide;anthracylcines such as doxorubicin and epirubicin)
    d. Ionising radiation

2) DNA repair targeted therapies
    a. Inhibitors of Non-homologous end-joining (DNA-PK inhibitors, Nu7441, NU7026)
    b. Inhibitors of homologous recombination
    c. Inhibitors of nucleotide excision repair
    d. Inhibitors of base excision repair (PARP inhibitors, AG014699, AZD2281, ABT-888, MK4827, BSI-201, INO-1001, TRC-102, APEX 1 inhibitors, APEX 2 inhibitors, Ligase III inhibitors
    e. Inhibitors of the Fanconi anemia pathway

3) Inhibitors of DNA damage signalling
    a. ATM inhibitors (CP466722, KU-55933)
    b. CHK 1 inhibitors (XL-844,UCN-01, AZD7762, PF00477736)
    c. CHK 2 inhibitors (XL-844, AZD7762, PF00477736)

4) Inhibitors of DNA damage induced cell cycle arrest
    a. Wee1 kinase inhibitors
    b. CDC25a, b or c inhibitors

5) Inhibition of processes indirectly leading to DNA damage
    a. Histone deacetylase inhibitors
    b. Heat shock protein inhibitors (geldanamycin, AUY922),

Diseases and Tissue Sources

The predictive classifiers described herein are useful for determining responsiveness or resistance to a therapeutic agent for treating cancer. The biological pathway described herein is a feature of cancer itself, similar to grade and stage, and as such, is not limited to a single cancer disease type. Therefore, the collection of genes or gene products may be used to predict responsiveness of cancer therapeutics across different cancer types in different tissues. In one embodiment, this collection of genes or gene products is useful for evaluating both breast and ovarian cancer tumors.

As used herein, cancer includes, but is not limited to, leukemia, brain cancer, prostate cancer, liver cancer, ovarian cancer, stomach cancer, colorectal cancer, throat cancer, breast cancer, skin cancer, melanoma, lung cancer, sarcoma, cervical cancer, testicular cancer, bladder cancer, endocrine

cancer, endometrial cancer, esophageal cancer, glioma, lymphoma, neuroblastoma, osteosarcoma, pancreatic cancer, pituitary cancer, renal cancer, head and neck cancer and the like.

In one embodiment, the methods described herein refer to cancers that are treated with chemotherapeutic agents of the classes immune checkpoint therapies, such as antagonists of an inhibitory immune checkpoint and/or agonists of a stimulatory immune checkpoint, optionally in combination with DNA damaging agents, DNA repair target therapies, inhibitors of DNA damage signalling, inhibitors of DNA damage induced cell cycle arrest and inhibition of processes indirectly leading to DNA damage, (i.e. “DNA-damage therapeutic agent” as the term is used herein).

"Biological sample", "sample", and "test sample" are used interchangeably herein to refer to any material, biological fluid, tissue, or cell obtained or otherwise derived from an individual. This includes blood (including whole blood, leukocytes, peripheral blood mononuclear cells, buffy coat, plasma, and serum), sputum, tears, mucus, nasal washes, nasal aspirate, breath, urine, semen, saliva, meningeal fluid, amniotic fluid, glandular fluid, lymph fluid, nipple aspirate, bronchial aspirate, synovial fluid, joint aspirate, ascites, cells, a cellular extract, and cerebrospinal fluid. This also includes experimentally separated fractions of all of the preceding. For example, a blood sample can be fractionated into serum or into fractions containing particular types of blood cells, such as red blood cells or white blood cells (leukocytes). If desired, a sample can be a combination of samples from an individual, such as a combination of a tissue and fluid sample. The term "biological sample" also includes materials containing homogenized solid material, such as from a stool sample, a tissue sample, or a tissue biopsy, for example. The term "biological sample" also includes materials derived from a tissue culture or a cell culture. Any suitable methods for obtaining a biological sample can be employed; exemplary methods include, e.g., phlebotomy, swab (e.g., buccal swab), and a fine needle aspirate biopsy procedure. Samples can also be collected, e.g., by micro dissection (e.g., laser capture micro dissection (LCM) or laser micro dissection (LMD)), bladder wash, smear (e.g., a PAP smear), or ductal lavage. A "biological sample" obtained or derived from an individual includes any such sample that has been processed in any suitable manner after being obtained from the individual.

In such cases, the target cells may be tumor cells, for example colon cancer cells or stomach cancer cells. The target cells are derived from any tissue source, including human and animal tissue, such as, but not limited to, a newly obtained sample, a frozen sample, a biopsy sample, a sample of bodily fluid, a blood sample, preserved tissue such as a paraffin-embedded fixed tissue sample (i.e., a tissue block), or cell culture.

Methods and Kits

Kits for Gene Expression Analysis

Reagents, tools, and/or instructions for performing the methods described herein can be provided in a kit. For example, the kit can contain reagents, tools, and instructions for determining an appropriate therapy for a cancer patient. Such a kit can include reagents for collecting a tissue sample from a patient, such as by biopsy, and reagents for processing the tissue. The kit can also include one or more reagents for performing a biomarker expression analysis, such as reagents for performing RT-PCR, qPCR, northern blot, proteomic analysis, or immunohistochemistry to determine expression levels of

biomarkers in a sample of a patient. For example, primers for performing RT-PCR, probes for performing northern blot analyses, and/or antibodies for performing proteomic analysis such as Western blot, immunohistochemistry and ELISA analyses can be included in such kits. Appropriate buffers for the assays can also be included. Detection reagents required for any of these assays can also be included. The appropriate reagents and methods are described in further detail below.

The kits featured herein can also include an instruction sheet describing how to perform the assays for measuring biomarker expression. The instruction sheet can also include instructions for how to determine a reference cohort, including how to determine expression levels of biomarkers in the reference cohort and how to assemble the expression data to establish a reference for comparison to a test patient. The instruction sheet can also include instructions for assaying biomarker expression in a test patient and for comparing the expression level with the expression in the reference cohort to subsequently determine the appropriate chemotherapy for the test patient. Methods for determining the appropriate chemotherapy are described above and can be described in detail in the instruction sheet.

Informational material included in the kits can be descriptive, instructional, marketing or other material that relates to the methods described herein and/or the use of the reagents for the methods described herein. For example, the informational material of the kit can contain contact information, e.g., a physical address, email address, website, or telephone number, where a user of the kit can obtain substantive information about performing a gene expression analysis and interpreting the results, particularly as they apply to a human's likelihood of having a positive response to a specific therapeutic agent.

The kits featured herein can also contain software necessary to infer a patient's likelihood of having a positive response to a specific therapeutic agent from the biomarker expression.

a) Gene expression profiling methods

Measuring mRNA in a biological sample may be used as a surrogate for detection of the level of the corresponding protein in the biological sample. Thus, any of the biomarkers or biomarker panels described herein can also be detected by detecting the appropriate RNA. Methods of gene expression profiling include, but are not limited to, microarray, RT-PCT, qPCR, northern blots, SAGE, mass spectrometry.

mRNA expression levels are measured by reverse transcription quantitative polymerase chain reaction (RT-PCR followed with qPCR). RT-PCR is used to create a cDNA from the mRNA. The cDNA may be used in a qPCR assay to produce fluorescence as the DNA amplification process progresses. By comparison to a standard curve, qPCR can produce an absolute measurement such as number of copies of mRNA per cell. Northern blots, microarrays, Invader assays, and RT-PCR combined with capillary electrophoresis have all been used to measure expression levels of mRNA in a sample. See Gene Expression Profiling: Methods and Protocols, Richard A. Shimkets, editor, Humana Press, 2004.

miRNA molecules are small RNAs that are non-coding but may regulate gene expression. Any of the methods suited to the measurement of mRNA expression levels can also be used for the corresponding miRNA. Recently many laboratories have investigated the use of miRNAs as biomarkers for disease. Many diseases involve widespread transcriptional regulation, and it is not surprising that miRNAs might find a role as biomarkers. The connection between miRNA concentrations and disease is

often even less clear than the connections between protein levels and disease, yet the value of miRNA biomarkers might be substantial. Of course, as with any RNA expressed differentially during disease, the problems facing the development of an in vitro diagnostic product will include the requirement that the miRNAs survive in the diseased cell and are easily extracted for analysis, or that the miRNAs are released into blood or other matrices where they must survive long enough to be measured. Protein biomarkers have similar requirements, although many potential protein biomarkers are secreted intentionally at the site of pathology and function, during disease, in a paracrine fashion. Many potential protein biomarkers are designed to function outside the cells within which those proteins are synthesized.

Gene expression may also be evaluated using mass spectrometry methods. A variety of configurations of mass spectrometers can be used to detect biomarker values. Several types of mass spectrometers are available or can be produced with various configurations. In general, a mass spectrometer has the following major components: a sample inlet, an ion source, a mass analyzer, a detector, a vacuum system, and instrument-control system, and a data system. Difference in the sample inlet, ion source, and mass analyzer generally define the type of instrument and its capabilities. For example, an inlet can be a capillary-column liquid chromatography source or can be a direct probe or stage such as used in matrix-assisted laser desorption. Common ion sources are, for example, electrospray, including nanospray and microspray or matrix-assisted laser desorption. Common mass analyzers include a quadrupole mass filter, ion trap mass analyzer and time-of-flight mass analyzer. Additional mass spectrometry methods are well known in the art (see Burlingame et al., Anal. Chem. 70:647 R-716R (1998); Kinter and Sherman, New York (2000)).

Protein biomarkers and biomarker values can be detected and measured by any of the following: electrospray ionization mass spectrometry (ESI-MS), ESI-MS/MS, ESI-MS/(MS)n, matrix-assisted laser desorption ionization time-of-flight mass spectrometry (MALDI-TOF-MS), surface-enhanced laser desorption/ionization time-of-flight mass spectrometry (SELDI-TOF-MS), desorption/ionization on silicon (DIOS), secondary ion mass spectrometry (SIMS), quadrupole time-of-flight (Q-TOF), tandem time-of-flight (TOF/TOF) technology, called ultraflex III TOF/TOF, atmospheric pressure chemical ionization mass spectrometry (APCI-MS), APCI-MS/MS, APCI-(MS).sup.N, atmospheric pressure photoionization mass spectrometry (APPI-MS), APPI-MS/MS, and APPI-(MS).sup.N, quadrupole mass spectrometry, Fourier transform mass spectrometry (FTMS), quantitative mass spectrometry, and ion trap mass spectrometry.

Sample preparation strategies are used to label and enrich samples before mass spectroscopic characterization of protein biomarkers and determination biomarker values. Labeling methods include but are not limited to isobaric tag for relative and absolute quantitation (iTRAQ) and stable isotope labeling with amino acids in cell culture (SILAC). Capture reagents used to selectively enrich samples for candidate biomarker proteins prior to mass spectroscopic analysis include but are not limited to aptamers, antibodies, nucleic acid probes, chimeras, small molecules, an $F(ab')_2$ fragment, a single chain antibody fragment, an Fv fragment, a single chain Fv fragment, a nucleic acid, a lectin, a ligand-binding receptor, affybodies, nanobodies, ankyrins, domain antibodies, alternative antibody scaffolds (e.g. diabodies etc) imprinted polymers, avimers, peptidomimetics, peptoids, peptide nucleic acids, threose nucleic acid, a hormone receptor, a cytokine receptor, and synthetic receptors, and modifications and fragments of these.

The foregoing assays enable the detection of biomarker values that are useful in methods for

predicting responsiveness of a cancer therapeutic agent, where the methods comprise detecting, in a biological sample from an individual, at least N biomarker values that each correspond to a biomarker selected from the group consisting of the biomarkers provided in Tables 1 or 2, wherein a classification, as described in detail below, using the biomarker values indicates whether the individual will be responsive to a therapeutic agent. While certain of the described predictive biomarkers are useful alone for predicting responsiveness to a therapeutic agent, methods are also described herein for the grouping of multiple subsets of the biomarkers that are each useful as a panel of two or more biomarkers. Thus, various embodiments of the instant application provide combinations comprising N biomarkers, wherein N is at least three biomarkers. It will be appreciated that N can be selected to be any number from any of the above-described ranges, as well as similar, but higher order, ranges. In accordance with any of the methods described herein, biomarker values can be detected and classified individually or they can be detected and classified collectively, as for example in a multiplex assay format.

b) Microarray methods

In one embodiment, the present invention makes use of "oligonucleotide arrays" (also called herein "microarrays"). Microarrays can be employed for analyzing the expression of biomarkers in a cell, and especially for measuring the expression of biomarkers of cancer tissues.

In one embodiment, biomarker arrays are produced by hybridizing detectably labeled polynucleotides representing the mRNA transcripts present in a cell (e.g., fluorescently-labeled cDNA synthesized from total cell mRNA or labeled cRNA) to a microarray. A microarray is a surface with an ordered array of binding (e.g., hybridization) sites for products of many of the genes in the genome of a cell or organism, preferably most or almost all of the genes. Microarrays can be made in a number of ways known in the art. However produced, microarrays share certain characteristics. The arrays are reproducible, allowing multiple copies of a given array to be produced and easily compared with each other. Preferably the microarrays are small, usually smaller than 5 cm^2, and they are made from materials that are stable under binding (e.g., nucleic acid hybridization) conditions. A given binding site or unique set of binding sites in the microarray will specifically bind the product of a single gene in the cell. In a specific embodiment, positionally addressable arrays containing affixed nucleic acids of known sequence at each location are used.

It will be appreciated that when cDNA complementary to the RNA of a cell is made and hybridized to a microarray under suitable hybridization conditions, the level of hybridization to the site in the array corresponding to any particular gene will reflect the prevalence in the cell of mRNA transcribed from that gene/biomarker. For example, when detectably labeled (e.g., with a fluorophore) cDNA or cRNA complementary to the total cellular mRNA is hybridized to a microarray, the site on the array corresponding to a gene (i.e., capable of specifically binding the product of the gene) that is not transcribed in the cell will have little or no signal (e.g., fluorescent signal), and a gene for which the encoded mRNA is prevalent will have a relatively strong signal. Nucleic acid hybridization and wash conditions are chosen so that the probe "specifically binds" or "specifically hybridizes' to a specific array site, i.e., the probe hybridizes, duplexes or binds to a sequence array site with a complementary nucleic acid sequence but does not hybridize to a site with a non-complementary nucleic acid sequence. As used herein, one polynucleotide sequence is considered complementary to another when, if the shorter

of the polynucleotides is less than or equal to 25 bases, there are no mismatches using standard base-pairing rules or, if the shorter of the polynucleotides is longer than 25 bases, there is no more than a 5% mismatch. Preferably, the polynucleotides are perfectly complementary (no mismatches). It can be demonstrated that specific hybridization conditions result in specific hybridization by carrying out a hybridization assay including negative controls using routine experimentation.

Optimal hybridization conditions will depend on the length (e.g., oligomer vs. polynucleotide greater than 200 bases) and type (e.g., RNA, DNA, PNA) of labeled probe and immobilized polynucleotide or oligonucleotide. General parameters for specific (i.e., stringent) hybridization conditions for nucleic acids are described in Sambrook et al., supra, and in Ausubel et al., "Current Protocols in Molecular Biology", Greene Publishing and Wiley-interscience, NY (1987), which is incorporated in its entirety for all purposes. When the cDNA microarrays are used, typical hybridization conditions are hybridization in 5xSSC plus 0.2% SDS at 65C for 4 hours followed by washes at 25℃ in low stringency wash buffer (1xSSC plus 0.2% SDS) followed by 10 minutes at 25℃ in high stringency wash buffer (0.1SSC plus 0.2% SDS) (see Shena *et al.*, Proc. Natl. Acad. Sci. USA, Vol. 93, p. 10614 (1996)). Useful hybridization conditions are also provided in, e.g., Tijessen, Hybridization With Nucleic Acid Probes", Elsevier Science Publishers B.V. (1993) and Kricka, "Nonisotopic DNA Probe Techniques", Academic Press, San Diego, Calif. (1992).

c) Immunoassay methods

Immunoassay methods are based on the reaction of an antibody to its corresponding target or analyte and can detect the analyte in a sample depending on the specific assay format. To improve specificity and sensitivity of an assay method based on immunoreactivity, monoclonal antibodies are often used because of their specific epitope recognition. Polyclonal antibodies have also been successfully used in various immunoassays because of their increased affinity for the target as compared to monoclonal antibodies Immunoassays have been designed for use with a wide range of biological sample matrices Immunoassay formats have been designed to provide qualitative, semi-quantitative, and quantitative results.

Quantitative results may be generated through the use of a standard curve created with known concentrations of the specific analyte to be detected. The response or signal from an unknown sample is plotted onto the standard curve, and a quantity or value corresponding to the target in the unknown sample is established.

Numerous immunoassay formats have been designed. ELISA or EIA can be quantitative for the detection of an analyte/biomarker. This method relies on attachment of a label to either the analyte or the antibody and the label component includes, either directly or indirectly, an enzyme. ELISA tests may be formatted for direct, indirect, competitive, or sandwich detection of the analyte. Other methods rely on labels such as, for example, radioisotopes (I^{125}) or fluorescence. Additional techniques include, for example, agglutination, nephelometry, turbidimetry, Western blot, immunoprecipitation, immunocytochemistry, immunohistochemistry, flow cytometry, Luminex assay, and others (see ImmunoAssay: A Practical Guide, edited by Brian Law, published by Taylor & Francis, Ltd., 2005 edition).

Exemplary assay formats include enzyme-linked immunosorbent assay (ELISA), radioimmunoassay, fluorescent, chemiluminescence, and fluorescence resonance energy transfer

(FRET) or time resolved-FRET (TR-FRET) immunoassays. Examples of procedures for detecting biomarkers include biomarker immunoprecipitation followed by quantitative methods that allow size and peptide level discrimination, such as gel electrophoresis, capillary electrophoresis, planar electrochromatography, and the like.

Methods of detecting and/or quantifying a detectable label or signal generating material depend on the nature of the label. The products of reactions catalyzed by appropriate enzymes (where the detectable label is an enzyme; see above) can be, without limitation, fluorescent, luminescent, or radioactive or they may absorb visible or ultraviolet light. Examples of detectors suitable for detecting such detectable labels include, without limitation, x-ray film, radioactivity counters, scintillation counters, spectrophotometers, colorimeters, fluorometers, luminometers, and densitometers.

Any of the methods for detection can be performed in any format that allows for any suitable preparation, processing, and analysis of the reactions. This can be, for example, in multi-well assay plates (e.g., 96 wells or 384 wells) or using any suitable array or microarray. Stock solutions for various agents can be made manually or robotically, and all subsequent pipetting, diluting, mixing, distribution, washing, incubating, sample readout, data collection and analysis can be done robotically using commercially available analysis software, robotics, and detection instrumentation capable of detecting a detectable label.

d) Sequencing

Gene expression may also be determined using sequencing methods, which include the various next generation sequencing technologies. In specific embodiments RNAseq may be utilized.

Clinical Uses

In some embodiments, methods are provided for identifying and/or selecting a cancer patient who is responsive to a therapeutic regimen. In particular, the methods are directed to identifying or selecting a cancer patient who is responsive to a therapeutic regimen that includes administering immune checkpoint therapies, such as antagonists of an inhibitory immune checkpoint and/or agonists of a stimulatory immune checkpoint, optionally in combination with an agent that directly or indirectly damages DNA. Methods are also provided for identifying a patient who is non-responsive to a therapeutic regimen. These methods typically include determining the level of expression of a collection of predictive markers in a patient's tumor (primary, metastatic or other derivatives from the tumor such as, but not limited to, blood, or components in blood, urine, saliva and other bodily fluids)(e.g., a patient's cancer cells), comparing the level of expression to a reference expression level, and identifying whether expression in the sample includes a pattern or profile of expression of a selected predictive biomarker or biomarker set which corresponds to response or non-response to therapeutic agent.

In some embodiments a method of predicting responsiveness of an individual to immune checkpoint therapies, such as antagonists of an inhibitory immune checkpoint and/or agonists of a stimulatory immune checkpoint, optionally in combination with a DNA-damage therapeutic agent, comprises the following steps: obtaining a test sample from the individual; measuring expression levels of one or more biomarkers in the test sample, wherein the one or more biomarkers are selected from the

group consisting of CXCL10, MX1, IDO1, IF144L, CD2, GBP5, PRAME, ITGAL, LRP4, and APOL3; deriving a test score that captures the expression levels; providing a threshold score comprising information correlating the test score and responsiveness; and comparing the test score to the threshold score; wherein responsiveness is predicted when the test score exceeds the threshold score. One of ordinary skill in the art can determine an appropriate threshold score, and appropriate biomarker weightings, using the teachings provided herein including the teachings of Example 1.

In other embodiments, the method of predicting responsiveness of an individual to immune checkpoint therapies, such as antagonists of an inhibitory immune checkpoint and/or agonists of a stimulatory immune checkpoint, optionally in combination with a DNA-damage therapeutic agent comprises measuring the expression levels of one or more biomarkers in the test sample, wherein the one or more biomarkers are selected from the group consisting of CXCL10, MX1, IDO1, IF144L, CD2, GBP5, PRAME, ITGAL, LRP4, APOL3, CDR1, FYB, TSPAN7, RAC2, KLHDC7B, GRB14, AC138128.1, KIF26A, CD274, CD109, ETV7, MFAP5, OLFM4, PI15, FOSB, FAM19A5, NLRC5, PRICKLE1, EGR1, CLDN10, ADAMTS4, SP140L, ANXA1, RSAD2, ESR1, IKZF3, OR2I1P, EGFR, NAT1, LATS2, CYP2B6, PTPRC, PPP1R1A, and AL137218.1. The method may comprise deriving a test score that captures the expression levels; providing a threshold score comprising information correlating the test score and responsiveness; and comparing the test score to the threshold score; wherein responsiveness is predicted when the test score exceeds the threshold score. Tables 2A and 2B provide exemplary gene signatures (or gene classifiers) wherein the biomarkers consist of 40 or 44 of the gene products listed therein, respectively, and wherein a threshold score is derived from the individual gene product weightings listed therein. In one of these embodiments wherein the biomarkers consist of the 44 gene products listed in Table 2B, and the biomarkers are associated with the weightings provided in Table 2B, a test score that exceeds a threshold score of 0.3681 indicates a likelihood that the individual will be responsive to immune checkpoint therapies, such as antagonists of an inhibitory immune checkpoint and/or agonists of a stimulatory immune checkpoint, optionally in combination with a DNA-damage therapeutic agent.

A cancer is "responsive" to a therapeutic agent if its rate of growth is inhibited as a result of contact with the therapeutic agent, compared to its growth in the absence of contact with the therapeutic agent. Growth of a cancer can be measured in a variety of ways, for instance, the size of a tumor or the expression of tumor markers appropriate for that tumor type may be measured.

A cancer is "non-responsive" to a therapeutic agent if its rate of growth is not inhibited, or inhibited to a very low degree, as a result of contact with the therapeutic agent when compared to its growth in the absence of contact with the therapeutic agent. As stated above, growth of a cancer can be measured in a variety of ways, for instance, the size of a tumor or the expression of tumor markers appropriate for that tumor type may be measured. The quality of being non-responsive to a therapeutic agent is a highly variable one, with different cancers exhibiting different levels of "non-responsiveness" to a given therapeutic agent, under different conditions. Still further, measures of non-responsiveness can be assessed using additional criteria beyond growth size of a tumor, including patient quality of life, degree of metastases, etc.

An application of this test will predict end points including, but not limited to, overall survival, progression free survival, radiological response, as defined by RECIST, complete response, partial

response, stable disease and serological markers such as, but not limited to, PSA, CEA, CA125, CA15-3 and CA19-9.

Alternatively, non-array based methods for detection, quantification and qualification of RNA, DNA or protein within a sample of one or more nucleic acids or their biological derivatives such as encoded proteins may be employed, including quantitative PCR (QPCR), enzyme-linked immunosorbent assay (ELISA) or immunohistochemistry (IHC) and the like.

After obtaining an expression profile from a sample being assayed, the expression profile is compared with a reference or control profile to make a diagnosis regarding the therapy responsive phenotype of the cell or tissue, and therefore host, from which the sample was obtained. The terms "reference" and "control" as used herein in relation to an expression profile mean a standardized pattern of gene or gene product expression or levels of expression of certain biomarkers to be used to interpret the expression classifier of a given patient and assign a prognostic or predictive class. The reference or control expression profile may be a profile that is obtained from a sample known to have the desired phenotype, e.g., responsive phenotype, and therefore may be a positive reference or control profile. In addition, the reference profile may be from a sample known to not have the desired phenotype, and therefore be a negative reference profile.

If quantitative PCR is employed as the method of quantitating the levels of one or more nucleic acids, this method quantifies the PCR product accumulation through measurement of fluorescence released by a dual-labeled fluorogenic probe (i.e. TaqMan® probe).

In certain embodiments, the obtained expression profile is compared to a single reference profile to obtain information regarding the phenotype of the sample being assayed. In yet other embodiments, the obtained expression profile is compared to two or more different reference profiles to obtain more in depth information regarding the phenotype of the assayed sample. For example, the obtained expression profile may be compared to a positive and negative reference profile to obtain confirmed information regarding whether the sample has the phenotype of interest.

The comparison of the obtained expression profile and the one or more reference profiles may be performed using any convenient methodology, where a variety of methodologies are known to those of skill in the array art, e.g., by comparing digital images of the expression profiles, by comparing databases of expression data, etc. Patents describing ways of comparing expression profiles include, but are not limited to, U.S. Pat. Nos. 6,308,170 and 6,228,575, the disclosures of which are herein incorporated by reference. Methods of comparing expression profiles are also described above.

The comparison step results in information regarding how similar or dissimilar the obtained expression profile is to the one or more reference profiles, which similarity information is employed to determine the phenotype of the sample being assayed. For example, similarity with a positive control indicates that the assayed sample has a responsive phenotype similar to the responsive reference sample. Likewise, similarity with a negative control indicates that the assayed sample has a non-responsive phenotype to the non-responsive reference sample.

The level of expression of a biomarker can be further compared to different reference expression levels. For example, a reference expression level can be a predetermined standard reference level of expression in order to evaluate if expression of a biomarker or biomarker set is informative and make an assessment for determining whether the patient is responsive or non-responsive. Additionally, determining the level of expression of a biomarker can be compared to an internal reference marker level

of expression which is measured at the same time as the biomarker in order to make an assessment for determining whether the patient is responsive or non-responsive. For example, expression of a distinct marker panel which is not comprised of biomarkers of the invention, but which is known to demonstrate a constant expression level can be assessed as an internal reference marker level, and the level of the biomarker expression is determined as compared to the reference. In an alternative example, expression of the selected biomarkers in a tissue sample which is a non-tumor sample can be assessed as an internal reference marker level. The level of expression of a biomarker may be determined as having increased expression in certain aspects. The level of expression of a biomarker may be determined as having decreased expression in other aspects. The level of expression may be determined as no informative change in expression as compared to a reference level. In still other aspects, the level of expression is determined against a pre-determined standard expression level as determined by the methods provided herein.

The invention is also related to guiding conventional treatment of patients. Patients in which the diagnostics test reveals that they are responders to the immune checkpoint therapies, such as antagonists of an inhibitory immune checkpoint and/or agonists of a stimulatory immune checkpoint, optionally in combination with, can be administered with that therapy and both patient and oncologist can be confident that the patient will benefit. Patients that are designated non-responders by the diagnostic test can be identified for alternative therapies which are more likely to offer benefit to them.

The invention further relates to selecting patients for clinical trials where novel drugs of the class of immune checkpoint therapies, such as antagonists of an inhibitory immune checkpoint and/or agonists of a stimulatory immune checkpoint, optionally in combination with. Enrichment of trial populations with potential responders will facilitate a more thorough evaluation of that drug under relevant criteria.

The invention still further relates to methods of diagnosing patients as having a cancer with increased innate immune response associated with a DNA damage response deficiency (DDRD). DDRD is defined herein as any condition wherein a cell or cells of the patient have a reduced ability to repair DNA damage, which reduced ability is a causative factor in the development or growth of a tumor. The DDRD diagnosis may be associated with a mutation in the Fanconi anemia/BRCA pathway. The DDRD diagnosis may also be associated with breast cancer or ovarian cancer. These methods of diagnosis comprise the steps of obtaining a test sample from the individual; measuring expression levels of one or more biomarkers in the test sample, wherein the one or more biomarkers are selected from Table 2B, 2A or 1A, including the group consisting of CXCL10, MX1, IDO1, IF144L, CD2, GBP5, PRAME, ITGAL, LRP4, and APOL3; deriving a test score that captures the expression levels; providing a threshold score comprising information correlating the test score and a diagnosis of the cancer; and comparing the test score to the threshold score; wherein the individual is determined to have the cancer when the test score exceeds the threshold score. One of ordinary skill in the art can determine an appropriate threshold score, and appropriate biomarker weightings, using the teachings provided herein including the teachings of Example 1.

In other embodiments, the methods of diagnosing patients as having developing a cancer with increased innate immune response associated with DDRD comprise measuring expression levels of one or more biomarkers in the test sample, wherein the one or more biomarkers are selected from the group consisting of CXCL10, MX1, IDO1, IF144L, CD2, GBP5, PRAME, ITGAL, LRP4, APOL3, CDR1, FYB, TSPAN7, RAC2, KLHDC7B, GRB14, AC138128.1, KIF26A, CD274, CD109, ETV7, MFAP5, OLFM4,

PI15, FOSB, FAM19A5, NLRC5, PRICKLE1, EGR1, CLDN10, ADAMTS4, SP140L, ANXA1, RSAD2, ESR1, IKZF3, OR2I1P, EGFR, NAT1, LATS2, CYP2B6, PTPRC, PPP1R1A, and AL137218.1. The method may comprise deriving a test score that captures the expression levels; providing a threshold score comprising information correlating the test score and a diagnosis of the cancer; and comparing the test score to the threshold score; wherein the individual is determined to have the cancer when the test score exceeds the threshold score. Tables 2A and 2B provide exemplary gene signatures (or gene classifiers) wherein the biomarkers consist of 40 or 44 of the gene products listed therein, respectively, and wherein a threshold score is derived from the individual gene product weightings listed therein. In one of these embodiments wherein the biomarkers consist of the 44 gene products listed in Table 2B, and the biomarkers are associated with the weightings provided in Table 2B, a test score that exceeds a threshold score of 0.3681 indicates a diagnosis of cancer or of being susceptible to developing a cancer.

The invention is also defined in the following numbered clauses:

1. A method for predicting responsiveness to an antagonist of an inhibitory immune checkpoint and/or an agonist of a stimulatory immune checkpoint comprising:
determining the expression level of at least one gene selected from Table 2B, 2A or 1 in a sample from the subject wherein the determined expression level is used to predict responsiveness to an antagonist of an inhibitory immune checkpoint and/or an agonist of a stimulatory immune checkpoint.

2. The method of clause 1 wherein an increased expression level of the at least one gene predicts responsiveness to an antagonist of an inhibitory immune checkpoint and/or an agonist of a stimulatory immune checkpoint.

3. The method of clause 1 or 2 which comprises determining the expression level of at least 2 of the genes and the determined expression levels are used to generate a combined test score, wherein a positive combined test score (generally above threshold, but may be equal to or above threshold) predicts responsiveness to an antagonist of an inhibitory immune checkpoint and/or an agonist of a stimulatory immune checkpoint.

4. The method of any preceding clause which comprises:
(i) deriving a combined test score that captures the expression levels;
(ii) providing a threshold score comprising information correlating the combined test score and responsiveness;
(iii) and comparing the combined test score to the threshold score; wherein responsiveness is predicted when the combined test score exceeds the threshold score.

5. The method of any preceding clause which comprises determining the expression level of at least 6 genes selected from CXCL10, MX1, IDO1, IFI44L, CD2, GBP5, PRAME, ITGAL, LRP4, APOL3, CDR1, FYB, TSPAN7, RAC2, KLHDC7B, GRB14, AC138128.1, KIF26A, CD274, CD109, ETV7, MFAP5, OLFM4, PI15, FOSB, FAM19A5, NLRC5, PRICKLE1, EGR1, CLDN10, ADAMTS4, SP140L, ANXA1, RSAD2, ESR1, IKZF3, OR2I1P, EGFR, NAT1, LATS2, CYP2B6, PTPRC, PPP1R1A, and AL137218.1.

6. The method of any preceding clause which comprises determining the expression level of at least 1 gene selected from CD2, ITGAL, PTPRC, CXCL10 and IDO1, together with at least one further gene selected from MX1, IFI44L, GBP5, PRAME, LRP4, APOL3, CDR1, FYB, TSPAN7, RAC2, KLHDC7B, GRB14, AC138128.1, KIF26A, CD274, CD109, ETV7, MFAP5, OLFM4, PI15, FOSB, FAM19A5, NLRC5, PRICKLE1, EGR1, CLDN10, ADAMTS4, SP140L, ANXA1, RSAD2, ESR1, IKZF3, OR2I1P, EGFR, NAT1, LATS2, CYP2B6, PPP1R1A, and AL137218.1.

7. The method of any preceding clause which comprises determining the expression level of at least 12 genes selected from Table 1.

8. The method of any preceding clause which comprises determining the expression level of at least 1 gene selected from CD2, ITGAL, PTPRC, CXCL10, IDO1, CD3D, HLA-DPB1, CXCL9, CCL5, STAT1, IL2RG, CD3E, IRF1, IKZF3 and IGJ together with at least one further gene selected from (the remaining genes in) Table 1 or together with at least one further gene from the (remaining genes in) Table 2B (the 44 gene panel).

9. The method of any preceding clause which comprises determining the expression level of each of CXCL10, MX1, IDO1, IFI44L, CD2, GBP5, PRAME, ITGAL, LRP4, APOL3, CDR1, FYB, TSPAN7, RAC2, KLHDC7B, GRB14, AC138128.1, KIF26A, CD274, CD109, ETV7, MFAP5, OLFM4, PI15, FOSB, FAM19A5, NLRC5, PRICKLE1, EGR1, CLDN10, ADAMTS4, SP140L, ANXA1, RSAD2, ESR1, IKZF3, OR2I1P, EGFR, NAT1, LATS2, CYP2B6, PTPRC, PPP1R1A, and AL137218.1.

10. The method of any of clauses 1 to 4 which comprises determining the expression level of each of the genes from any one of Tables 4 to 45.

11. The method of any preceding clause wherein the weight values for each gene are as set out in Table 2B, or wherein the weight and/or bias values for each gene are as set out in any one of Tables 3 to 45.

12. The method of any preceding clause which comprises determining the expression level of at least one, up to all, of CCL5, CXCL9 and CXCL10 together with at least one further gene selected from (the remaining genes in) Table 1 or together with at least one further gene from the (remaining genes in) Table 2B (the 44 gene panel).

13. The method of any preceding clause wherein determining the expression level employs at least one primer or primer pair from Table 2E and/or at least one probe from Table 2E.

14. A method for predicting responsiveness to an antagonist of an inhibitory immune checkpoint and/or an agonist of a stimulatory immune checkpoint in combination with a DNA damage therapeutic agent comprising:

determining the expression level of at least one gene selected from Table 2B, 2A or 1 in a sample from the subject wherein the determined expression level is used to predict responsiveness to an antagonist of an inhibitory immune checkpoint and/or an agonist of a stimulatory immune checkpoint in combination with a DNA damage therapeutic agent.

15. The method of clause 14 wherein an increased expression level of the at least one gene predicts responsiveness to an antagonist of an inhibitory immune checkpoint and/or an agonist of a stimulatory immune checkpoint in combination with a DNA damage therapeutic agent.

16. The method of clause 14 or 15 which comprises determining the expression level of at least 2 of the genes and the determined expression levels are used to generate a combined test score, wherein a positive combined test score (generally above threshold, but may be equal to or above threshold) predicts responsiveness to an antagonist of an inhibitory immune checkpoint and/or an agonist of a stimulatory immune checkpoint in combination with a DNA damage therapeutic agent.

17. The method of any of clauses 14 to 16 which comprises:

(i) deriving a combined test score that captures the expression levels;

(ii) providing a threshold score comprising information correlating the combined test score and responsiveness;

(iii) and comparing the combined test score to the threshold score; wherein responsiveness is predicted when the combined test score exceeds the threshold score.

18. The method of any of clauses 14 to 17 which comprises determining the expression level of at least 6 genes selected from CXCL10, MX1, IDO1, IFI44L, CD2, GBP5, PRAME, ITGAL, LRP4, APOL3, CDR1, FYB, TSPAN7, RAC2, KLHDC7B, GRB14, AC138128.1, KIF26A, CD274, CD109, ETV7, MFAP5, OLFM4, PI15, FOSB, FAM19A5, NLRC5, PRICKLE1, EGR1, CLDN10, ADAMTS4, SP140L, ANXA1, RSAD2, ESR1, IKZF3, OR2I1P, EGFR, NAT1, LATS2, CYP2B6, PTPRC, PPP1R1A, and AL137218.1.

19. The method of any of clauses 14 to 18 which comprises determining the expression level of at least 1 gene selected from CD2, ITGAL, PTPRC, CXCL10 and IDO1, together with at least one further gene selected from MX1, IFI44L, GBP5, PRAME, LRP4, APOL3, CDR1, FYB, TSPAN7, RAC2, KLHDC7B, GRB14, AC138128.1, KIF26A, CD274, CD109, ETV7, MFAP5, OLFM4, PI15, FOSB, FAM19A5, NLRC5, PRICKLE1, EGR1, CLDN10, ADAMTS4, SP140L, ANXA1, RSAD2, ESR1, IKZF3, OR2I1P, EGFR, NAT1, LATS2, CYP2B6, PPP1R1A, and AL137218.1.

20. The method of any of clauses 14 to 19 which comprises determining the expression level of at least 12 genes selected from Table 1.

21. The method of any of clauses 14 to 20 which comprises determining the expression level of at least 1 gene selected from CD2, ITGAL, PTPRC, CXCL10, IDO1, CD3D, HLA-DPB1, CXCL9, CCL5, STAT1, IL2RG, CD3E, IRF1, IKZF3 and IGJ together with at least one further gene selected from (the

remaining genes in) Table 1 or together with at least one further gene from the (remaining genes in) Table 2B (the 44 gene panel).

22. The method of any of clauses 14 to 21 which comprises determining the expression level of each of CXCL10, MX1, IDO1, IFI44L, CD2, GBP5, PRAME, ITGAL, LRP4, APOL3, CDR1, FYB, TSPAN7, RAC2, KLHDC7B, GRB14, AC138128.1, KIF26A, CD274, CD109, ETV7, MFAP5, OLFM4, PI15, FOSB, FAM19A5, NLRC5, PRICKLE1, EGR1, CLDN10, ADAMTS4, SP140L, ANXA1, RSAD2, ESR1, IKZF3, OR2I1P, EGFR, NAT1, LATS2, CYP2B6, PTPRC, PPP1R1A, and AL137218.1.

23. The method of any of clauses 14 to 17 which comprises determining the expression level of each of the genes from any one of Tables 4 to 45.

24. The method of any of clauses 14 to 23 wherein the weight values for each gene are as set out in Table 2B, or wherein the weight and/or bias values for each gene are as set out in any one of Tables 3 to 45.

25. The method of any of clauses 14 to 24 which comprises determining the expression level of at least one, up to all, of CCL5, CXCL9 and CXCL10 together with at least one further gene selected from (the remaining genes in) Table 1 or together with at least one further gene from the (remaining genes in) Table 2B (the 44 gene panel).

26. The method of any of clauses 14 to 25 wherein determining the expression level employs at least one primer or primer pair from Table 2E and/or at least one probe from Table 2E.

27. A method for identifying a cancer that can be effectively treated with an antagonist of an inhibitory immune checkpoint and/or an agonist of a stimulatory immune checkpoint comprising: determining the expression level of at least one gene selected from Table 2B, 2A or 1 in a sample from the subject wherein the determined expression level is used to identify a cancer that can be effectively treated with an antagonist of an inhibitory immune checkpoint and/or an agonist of a stimulatory immune checkpoint.

28. The method of clause 27 wherein an increased expression level of the at least one gene identifies a cancer that can be effectively treated with an antagonist of an inhibitory immune checkpoint and/or an agonist of a stimulatory immune checkpoint.

29. The method of clause 27 or 28 which comprises determining the expression level of at least 2 genes and the determined expression levels are used to generate a combined test score, wherein a positive combined test score (generally above threshold, but may be equal to or above threshold) identifies a cancer that can be effectively treated with an antagonist of an inhibitory immune checkpoint and/or an agonist of a stimulatory immune checkpoint.

30. The method of any of clauses 27 to 29 which comprises:

(i) deriving a combined test score that captures the expression levels;

(ii) providing a threshold score comprising information correlating the combined test score and responsiveness;

(iii) and comparing the combined test score to the threshold score; wherein a cancer that can be effectively treated is identified when the combined test score exceeds the threshold score.

31. The method of any of clauses 27 to 30 which comprises determining the expression level of at least 6 genes selected from CXCL10, MX1, IDO1, IFI44L, CD2, GBP5, PRAME, ITGAL, LRP4, APOL3, CDR1, FYB, TSPAN7, RAC2, KLHDC7B, GRB14, AC138128.1, KIF26A, CD274, CD109, ETV7, MFAP5, OLFM4, PI15, FOSB, FAM19A5, NLRC5, PRICKLE1, EGR1, CLDN10, ADAMTS4, SP140L, ANXA1, RSAD2, ESR1, IKZF3, OR2I1P, EGFR, NAT1, LATS2, CYP2B6, PTPRC, PPP1R1A, and AL137218.1.

32. The method of any of clauses 27 to 31 which comprises determining the expression level of at least 1 gene selected from CD2, ITGAL, PTPRC, CXCL10 and IDO1, together with at least one further gene selected from MX1, IFI44L, GBP5, PRAME, LRP4, APOL3, CDR1, FYB, TSPAN7, RAC2, KLHDC7B, GRB14, AC138128.1, KIF26A, CD274, CD109, ETV7, MFAP5, OLFM4, PI15, FOSB, FAM19A5, NLRC5, PRICKLE1, EGR1, CLDN10, ADAMTS4, SP140L, ANXA1, RSAD2, ESR1, IKZF3, OR2I1P, EGFR, NAT1, LATS2, CYP2B6, PPP1R1A, and AL137218.1.

33. The method of any one of clauses 27 to 32 which comprises determining the expression level of at least 12 genes selected from Table 1.

34. The method of any of clauses 27 to 33 which comprises determining the expression level of at least 1 gene selected from CD2, ITGAL, PTPRC, CXCL10, IDO1, CD3D, HLA-DPB1, CXCL9, CCL5, STAT1, IL2RG, CD3E, IRF1, IKZF3 and IGJ together with at least one further gene selected from (the remaining genes in) Table 1 or together with at least one further gene from the (remaining genes in) Table 2B (the 44 gene panel).

35. The method of any of clauses 27 to 34 which comprises determining the expression level of each of CXCL10, MX1, IDO1, IFI44L, CD2, GBP5, PRAME, ITGAL, LRP4, APOL3, CDR1, FYB, TSPAN7, RAC2, KLHDC7B, GRB14, AC138128.1, KIF26A, CD274, CD109, ETV7, MFAP5, OLFM4, PI15, FOSB, FAM19A5, NLRC5, PRICKLE1, EGR1, CLDN10, ADAMTS4, SP140L, ANXA1, RSAD2, ESR1, IKZF3, OR2I1P, EGFR, NAT1, LATS2, CYP2B6, PTPRC, PPP1R1A, and AL137218.1.

36. The method of any of clauses 27 to 30 which comprises determining the expression level of each of the genes from any one of Tables 4 to 45.

37. The method of any of clauses 27 to 36 wherein the weight values for each gene are as set out in Table 2B, or wherein the weight and/or bias values for each gene are as set out in any one of Tables 3 to 45.

38. The method of any of clauses 27 to 37 which comprises determining the expression level of at least one, up to all, of CCL5, CXCL9 and CXCL10 together with at least one further gene selected from (the remaining genes in) Table 1 or together with at least one further gene from the (remaining genes in) Table 2B (the 44 gene panel).

39. The method of any of clauses 27 to 38 wherein determining the expression level employs at least one primer or primer pair from Table 2E and/or at least one probe from Table 2E.

40. A method for identifying a cancer that can be effectively treated with an antagonist of an inhibitory immune checkpoint and/or an agonist of a stimulatory immune checkpoint in combination with a DNA damage therapeutic agent comprising:
determining the expression level of at least one gene selected from Table 2B, 2A or 1 in a sample from the subject wherein the determined expression level is used to identify a cancer that can be effectively treated with an antagonist of an inhibitory immune checkpoint and/or an agonist of a stimulatory immune checkpoint in combination with a DNA damage therapeutic agent.

41. The method of clause 40 wherein an increased expression level of the at least one gene identifies a cancer that can be effectively treated with an antagonist of an inhibitory immune checkpoint and/or an agonist of a stimulatory immune checkpoint in combination with a DNA damage therapeutic agent.

42. The method of clause 40 or 41 which comprises determining the expression level of at least 2 of the genes and the determined expression levels are used to generate a combined test score, wherein a positive combined test score (generally above threshold, but may be equal to or above threshold) identifies a cancer that can be effectively treated with an antagonist of an inhibitory immune checkpoint and/or an agonist of a stimulatory immune checkpoint in combination with a DNA damage therapeutic agent.

43. The method of any of clauses 40 to 42 which comprises:
(i) deriving a combined test score that captures the expression levels;
(ii) providing a threshold score comprising information correlating the combined test score and responsiveness;
(iii) and comparing the combined test score to the threshold score; wherein a cancer that can be effectively treated is identified when the combined test score exceeds the threshold score.

44. The method of any of clauses 40 to 43 which comprises determining the expression level of at least 6 genes selected from CXCL10, MX1, IDO1, IFI44L, CD2, GBP5, PRAME, ITGAL, LRP4, APOL3, CDR1, FYB, TSPAN7, RAC2, KLHDC7B, GRB14, AC138128.1, KIF26A, CD274, CD109, ETV7, MFAP5, OLFM4, PI15, FOSB, FAM19A5, NLRC5, PRICKLE1, EGR1, CLDN10, ADAMTS4, SP140L, ANXA1, RSAD2, ESR1, IKZF3, OR2I1P, EGFR, NAT1, LATS2, CYP2B6, PTPRC, PPP1R1A, and AL137218.1.

45. The method of any of clauses 40 to 44 which comprises determining the expression level of at least 1 gene selected from CD2, ITGAL, PTPRC, CXCL10 and IDO1, together with at least one further gene selected from MX1, IFI44L, GBP5, PRAME, LRP4, APOL3, CDR1, FYB, TSPAN7, RAC2, KLHDC7B, GRB14, AC138128.1, KIF26A, CD274, CD109, ETV7, MFAP5, OLFM4, PI15, FOSB, FAM19A5, NLRC5, PRICKLE1, EGR1, CLDN10, ADAMTS4, SP140L, ANXA1, RSAD2, ESR1, IKZF3, OR2I1P, EGFR, NAT1, LATS2, CYP2B6, PPP1R1A, and AL137218.1.

46. The method of any of clauses 40 to 45 which comprises determining the expression level of at least 12 genes selected from Table 1.

47. The method of any of clauses 40 to 46 which comprises determining the expression level of at least 1 gene selected from CD2, ITGAL, PTPRC, CXCL10, IDO1, CD3D, HLA-DPB1, CXCL9, CCL5, STAT1, IL2RG, CD3E, IRF1, IKZF3 and IGJ together with at least one further gene selected from (the remaining genes in) Table 1 or together with at least one further gene from the (remaining genes in) Table 2B (the 44 gene panel).

48. The method of any of clauses 40 to 47 which comprises determining the expression level of each of CXCL10, MX1, IDO1, IFI44L, CD2, GBP5, PRAME, ITGAL, LRP4, APOL3, CDR1, FYB, TSPAN7, RAC2, KLHDC7B, GRB14, AC138128.1, KIF26A, CD274, CD109, ETV7, MFAP5, OLFM4, PI15, FOSB, FAM19A5, NLRC5, PRICKLE1, EGR1, CLDN10, ADAMTS4, SP140L, ANXA1, RSAD2, ESR1, IKZF3, OR2I1P, EGFR, NAT1, LATS2, CYP2B6, PTPRC, PPP1R1A, and AL137218.1.

49. The method of any of clauses 40 to 43 which comprises determining the expression level of each of the genes from any one of Tables 4 to 45.

50. The method of any of clauses 40 to 49 wherein the weight values for each gene are as set out in Table 2B, or wherein the weight and/or bias values for each gene are as set out in any one of Tables 3 to 45.

51. The method of any of clauses 40 to 50 which comprises determining the expression level of at least one, up to all, of CCL5, CXCL9 and CXCL10 together with at least one further gene selected from (the remaining genes in) Table 1 or together with at least one further gene from the (remaining genes in) Table 2B (the 44 gene panel).

52. The method of any preceding clause wherein determining the expression level employs at least one primer or primer pair from Table 2E and/or at least one probe from Table 2E.

53. A method for selecting treatment for a cancer comprising:
determining the expression level of at least one gene selected from Table 2B, 2A or 1 in a sample from the subject wherein the determined expression level is used to select an antagonist of an inhibitory immune checkpoint and/or an agonist of a stimulatory immune checkpoint for use in treatment of the cancer.

54. The method of clause 53 wherein an increased expression level of the at least one gene is used to select an antagonist of an inhibitory immune checkpoint and/or an agonist of a stimulatory immune checkpoint for use in treatment of the cancer.

55. The method of clause 53 or 54 which comprises determining the expression level of at least 2 of the genes and the determined expression levels are used to generate a combined test score, wherein a positive combined test score (generally above threshold, but may be equal to or above threshold) is used to select an antagonist of an inhibitory immune checkpoint and/or an agonist of a stimulatory immune checkpoint for use in treatment of the cancer.

56. The method of any of clauses 53 to 55 further comprising treating the cancer using the selected antagonist and/or agonist.

57. The method of any of clauses 53 to 56 which comprises:

(i) deriving a combined test score that captures the expression levels;

(ii) providing a threshold score comprising information correlating the combined test score and responsiveness;

(iii) and comparing the combined test score to the threshold score; wherein an antagonist of an inhibitory immune checkpoint and/or an agonist of a stimulatory immune checkpoint is selected for use when the combined test score exceeds the threshold score.

58. The method of any of clauses 53 to 57 which comprises determining the expression level of at least 6 genes selected from CXCL10, MX1, IDO1, IFI44L, CD2, GBP5, PRAME, ITGAL, LRP4, APOL3, CDR1, FYB, TSPAN7, RAC2, KLHDC7B, GRB14, AC138128.1, KIF26A, CD274, CD109, ETV7, MFAP5, OLFM4, PI15, FOSB, FAM19A5, NLRC5, PRICKLE1, EGR1, CLDN10, ADAMTS4, SP140L, ANXA1, RSAD2, ESR1, IKZF3, OR2I1P, EGFR, NAT1, LATS2, CYP2B6, PTPRC, PPP1R1A, and AL137218.1.

59. The method of any of clauses 53 to 58 which comprises determining the expression level of at least 1 gene selected from CD2, ITGAL, PTPRC, CXCL10 and IDO1, together with at least one further gene selected from MX1, IFI44L, GBP5, PRAME, LRP4, APOL3, CDR1, FYB, TSPAN7, RAC2, KLHDC7B, GRB14, AC138128.1, KIF26A, CD274, CD109, ETV7, MFAP5, OLFM4, PI15, FOSB, FAM19A5, NLRC5, PRICKLE1, EGR1, CLDN10, ADAMTS4, SP140L, ANXA1, RSAD2, ESR1, IKZF3, OR2I1P, EGFR, NAT1, LATS2, CYP2B6, PPP1R1A, and AL137218.1.

60. The method of any of clauses 53 to 59 which comprises determining the expression level of at least 12 genes selected from Table 1.

61. The method of any of clauses 53 to 60 which comprises determining the expression level of at least 1 gene selected from CD2, ITGAL, PTPRC, CXCL10, IDO1, CD3D, HLA-DPB1, CXCL9, CCL5, STAT1, IL2RG, CD3E, IRF1, IKZF3 and IGJ together with at least one further gene selected from (the

remaining genes in) Table 1 or together with at least one further gene from the (remaining genes in) Table 2B (the 44 gene panel).

62. The method of any of clauses 53 to 61 which comprises determining the expression level of each of CXCL10, MX1, IDO1, IFI44L, CD2, GBP5, PRAME, ITGAL, LRP4, APOL3, CDR1, FYB, TSPAN7, RAC2, KLHDC7B, GRB14, AC138128.1, KIF26A, CD274, CD109, ETV7, MFAP5, OLFM4, PI15, FOSB, FAM19A5, NLRC5, PRICKLE1, EGR1, CLDN10, ADAMTS4, SP140L, ANXA1, RSAD2, ESR1, IKZF3, OR2I1P, EGFR, NAT1, LATS2, CYP2B6, PTPRC, PPP1R1A, and AL137218.1.

63. The method of any of clauses 53 to 57 which comprises determining the expression level of each of the genes from any one of Tables 4 to 45.

64. The method of any of clauses 53 to 63 wherein the weight values for each gene are as set out in Table 2B, or wherein the weight and/or bias values for each gene are as set out in any one of Tables 3 to 45.

65. The method of any of clauses 53 to 64 which comprises determining the expression level of at least one, up to all, of CCL5, CXCL9 and CXCL10 together with at least one further gene selected from (the remaining genes in) Table 1 or together with at least one further gene from the (remaining genes in) Table 2B (the 44 gene panel).

66. The method of any of clauses 53 to 65 wherein determining the expression level employs at least one primer or primer pair from Table 2E and/or at least one probe from Table 2E.

67. A method for selecting treatment for a cancer comprising:
determining the expression level of at least one gene selected from 2B, 2A or 1 in a sample from the subject wherein the determined expression level is used to select an antagonist of an inhibitory immune checkpoint and/or an agonist of a stimulatory immune checkpoint, in combination with a DNA damage therapeutic agent, for use in treatment of the cancer.

68. The method of clause 67 wherein an increased expression level of the at least one gene is used to select an antagonist of an inhibitory immune checkpoint and/or an agonist of a stimulatory immune checkpoint, in combination with a DNA damage therapeutic agent, for use in treatment of the cancer.

69. The method of clause 67 or 68 which comprises determining the expression level of at least 2 of the genes and the determined expression levels are used to generate a combined test score, wherein a positive combined test score (generally above threshold, but may be equal to or above threshold) is used to select an antagonist of an inhibitory immune checkpoint and/or an agonist of a stimulatory immune checkpoint, in combination with a DNA damage therapeutic agent, for use in treatment of the cancer.

70. The method of any of clauses 67 to 69 further comprising treating the cancer using the selected antagonist and/or agonist, in combination with a DNA damage therapeutic agent.

71 The method of any of clauses 67 to 70 which comprises:

(i) deriving a combined test score that captures the expression levels;

(ii) providing a threshold score comprising information correlating the combined test score and responsiveness;

(iii) and comparing the combined test score to the threshold score; wherein an antagonist of an inhibitory immune checkpoint and/or an agonist of a stimulatory immune checkpoint in combination with a DNA damage therapeutic agent is selected for use when the combined test score exceeds the threshold score.

72. The method of any of clauses 67 to 71 which comprises determining the expression level of at least 6 genes selected from CXCL10, MX1, IDO1, IFI44L, CD2, GBP5, PRAME, ITGAL, LRP4, APOL3, CDR1, FYB, TSPAN7, RAC2, KLHDC7B, GRB14, AC138128.1, KIF26A, CD274, CD109, ETV7, MFAP5, OLFM4, PI15, FOSB, FAM19A5, NLRC5, PRICKLE1, EGR1, CLDN10, ADAMTS4, SP140L, ANXA1, RSAD2, ESR1, IKZF3, OR2I1P, EGFR, NAT1, LATS2, CYP2B6, PTPRC, PPP1R1A, and AL137218.1.

73. The method of any of clauses 67 to 72 which comprises determining the expression level of at least 1 gene selected from CD2, ITGAL, PTPRC, CXCL10 and IDO1, together with at least one further gene selected from MX1, IFI44L, GBP5, PRAME, LRP4, APOL3, CDR1, FYB, TSPAN7, RAC2, KLHDC7B, GRB14, AC138128.1, KIF26A, CD274, CD109, ETV7, MFAP5, OLFM4, PI15, FOSB, FAM19A5, NLRC5, PRICKLE1, EGR1, CLDN10, ADAMTS4, SP140L, ANXA1, RSAD2, ESR1, IKZF3, OR2I1P, EGFR, NAT1, LATS2, CYP2B6, PPP1R1A, and AL137218.1.

74. The method of any of clauses 67 to 73 which comprises determining the expression level of at least 12 genes selected from Table 1.

75. The method of any of clauses 67 to 74 which comprises determining the expression level of at least 1 gene selected from CD2, ITGAL, PTPRC, CXCL10, IDO1, CD3D, HLA-DPB1, CXCL9, CCL5, STAT1, IL2RG, CD3E, IRF1, IKZF3 and IGJ together with at least one further gene selected from (the remaining genes in) Table 1 or together with at least one further gene from the (remaining genes in) Table 2B (the 44 gene panel).

76. The method of any of clauses 67 to 75 which comprises determining the expression level of each of CXCL10, MX1, IDO1, IFI44L, CD2, GBP5, PRAME, ITGAL, LRP4, APOL3, CDR1, FYB, TSPAN7, RAC2, KLHDC7B, GRB14, AC138128.1, KIF26A, CD274, CD109, ETV7, MFAP5, OLFM4, PI15, FOSB, FAM19A5, NLRC5, PRICKLE1, EGR1, CLDN10, ADAMTS4, SP140L, ANXA1, RSAD2, ESR1, IKZF3, OR2I1P, EGFR, NAT1, LATS2, CYP2B6, PTPRC, PPP1R1A, and AL137218.1.

77. The method of any of clauses 67 to 71 which comprises determining the expression level of each of the genes from any one of Tables 4 to 45.

78. The method of any of clauses 67 to 77 wherein the weight values for each gene are as set out in Table 2B, or wherein the weight and/or bias values for each gene are as set out in any one of Tables 3 to 45.

79. The method of any of clauses 67 to 78 which comprises determining the expression level of at least one, up to all, of CCL5, CXCL9 and CXCL10 together with at least one further gene selected from (the remaining genes in) Table 1 or together with at least one further gene from the (remaining genes in) Table 2B (the 44 gene panel).

80. The method of any of clauses 67 to 79 wherein determining the expression level employs at least one primer or primer pair from Table 2E and/or at least one probe from Table 2E.

81. The method of any preceding clause wherein the combined test score (or "signature score") is derived according to the formula:

$$SignatureScore = \sum_i w_i \times (ge_i - b_i) + k$$

Where w_i is a weight for each gene, b_i is a gene-specific bias, ge_i is the gene expression after pre-processing, and k is a constant offset.

82. A method of treating cancer comprising administration of an antagonist of an inhibitory immune checkpoint and/or an agonist of a stimulatory immune checkpoint to a subject, characterised in that a sample from the subject, prior to administration, displays a positive combined test score derived from the determined expression levels of at least 2 genes from Table 2B, 2A or 1 or an increased level of expression of at least 1 gene from Table 2B, 2A or 1.

83. A method of treating cancer comprising administration of an antagonist of an inhibitory immune checkpoint and/or an agonist of a stimulatory immune checkpoint, in combination with a DNA damage therapeutic agent, to a subject, characterised in that a sample from the subject, prior to administration, displays a positive combined test score derived from the determined expression levels of at least 2 genes from Table 2B, 2A or 1 or an increased level of expression of at least 1 gene from Table 2B, 2A or 1.

84. An antagonist of an inhibitory immune checkpoint and/or an agonist of a stimulatory immune checkpoint for use in the treatment of cancer in a subject wherein, prior to administration of the antagonist and/or agonist, a sample from the subject displays a positive combined test score derived from the determined expression levels of at least 2 genes from Table 2B, 2A or 1 or an increased level of expression of at least 1 gene from Table 2B, 2A or 1.

85. An antagonist of an inhibitory immune checkpoint and/or an agonist of a stimulatory immune checkpoint for use in the treatment of cancer in a subject wherein, prior to administration of the antagonist and/or agonist, a sample from the subject displays a positive combined test score derived from the determined expression levels of at least 2 genes from Table 2B, 2A or 1 or an increased level of

expression of at least 1 gene from Table 2B, 2A or 1, and wherein the antagonist and/or agonist is administered in combination with a DNA damage therapeutic agent.

86. An antagonist of an inhibitory immune checkpoint in combination with a DNA damage therapeutic agent and/or an agonist of a stimulatory immune checkpoint in combination with a DNA damage therapeutic agent for use in the treatment of cancer in a subject wherein, prior to administration of the antagonist and/or agonist and DNA damage therapeutic agent, a sample from the subject displays a positive combined test score derived from the determined expression levels of at least 2 genes from Table 2B, 2A or 1 or an increased level of expression of at least 1 gene from Table 2B, 2A or 1.

87. The method of clause 82 or 83, or the antagonist and/or agonist for use of any of clauses 84 to 86, wherein the combined test score (or "signature score") is derived according to the formula:

$$SignatureScore = \sum_i w_i \times (ge_i - b_i) + k$$

Where w_i is a weight for each gene, b_i is a gene-specific bias, ge_i is the gene expression after pre-processing, and k is a constant offset.

88. The method of any of clauses 82, 83 or 87, or the antagonist and/or agonist for use of any of clauses 84 to 87, wherein the combined test score is derived from the determined expression level of at least 6 genes selected from CXCL10, MX1, IDO1, IFI44L, CD2, GBP5, PRAME, ITGAL, LRP4, APOL3, CDR1, FYB, TSPAN7, RAC2, KLHDC7B, GRB14, AC138128.1, KIF26A, CD274, CD109, ETV7, MFAP5, OLFM4, PI15, FOSB, FAM19A5, NLRC5, PRICKLE1, EGR1, CLDN10, ADAMTS4, SP140L, ANXA1, RSAD2, ESR1, IKZF3, OR2I1P, EGFR, NAT1, LATS2, CYP2B6, PTPRC, PPP1R1A, and AL137218.1.

89. The method of any of clauses 82, 83, 87 or 88, or the antagonist and/or agonist for use of any of clauses 84 to 88, wherein the combined test score is derived from the determined expression level of at least 1 gene selected from CD2, ITGAL, PTPRC, CXCL10 and IDO1, together with at least one further gene selected from MX1, IFI44L, GBP5, PRAME, LRP4, APOL3, CDR1, FYB, TSPAN7, RAC2, KLHDC7B, GRB14, AC138128.1, KIF26A, CD274, CD109, ETV7, MFAP5, OLFM4, PI15, FOSB, FAM19A5, NLRC5, PRICKLE1, EGR1, CLDN10, ADAMTS4, SP140L, ANXA1, RSAD2, ESR1, IKZF3, OR2I1P, EGFR, NAT1, LATS2, CYP2B6, PPP1R1A, and AL137218.1.

90. The method of any of clauses 82, 83 or 87 to 89, or the antagonist and/or agonist for use of any of clauses 84 to 89, wherein the combined test score is derived from the determined expression level of at least 12 genes selected from Table 1.

91. The method of any of clauses 82, 83 or 87 to 90, or the antagonist and/or agonist for use of any of clauses 84 to 90, wherein the combined test score is derived from the determined expression level of at least 1 gene selected from CD2, ITGAL, PTPRC, CXCL10, IDO1, CD3D, HLA-DPB1, CXCL9, CCL5, STAT1, IL2RG, CD3E, IRF1, IKZF3 and IGJ together with at least one further gene selected from (the

remaining genes in) Table 1 or together with at least one further gene from the (remaining genes in) Table 2B (the 44 gene panel).

92. The method of any of clauses 82, 83 or 87 to 91, or the antagonist and/or agonist for use of any of clauses 84 to 91, wherein the combined test score is derived from the determined expression level of each of CXCL10, MX1, IDO1, IFI44L, CD2, GBP5, PRAME, ITGAL, LRP4, APOL3, CDR1, FYB, TSPAN7, RAC2, KLHDC7B, GRB14, AC138128.1, KIF26A, CD274, CD109, ETV7, MFAP5, OLFM4, PI15, FOSB, FAM19A5, NLRC5, PRICKLE1, EGR1, CLDN10, ADAMTS4, SP140L, ANXA1, RSAD2, ESR1, IKZF3, OR2I1P, EGFR, NAT1, LATS2, CYP2B6, PTPRC, PPP1R1A, and AL137218.1.

93. The method of any of clauses 82, 83 or 87, or the antagonist and/or agonist for use of any of clauses 84 to 87, wherein the combined test score is derived from the determined expression level of the genes from any one of Tables 4 to 45.

94. The method of any of clauses 82, 83 or 87 to 93, or the antagonist and/or agonist for use of any of clauses 84 to 93, wherein the weight values for each gene are as set out in Table 2B, or wherein the weight and/or bias values for each gene are as set out in any one of Tables 3 to 45.

95. The method of any of clauses 82, 83 or 87 to 94, or the antagonist and/or agonist for use of any of clauses 84 to 94, wherein the combined test score is derived from the determined expression level of at least one, up to all, of CCL5, CXCL9 and CXCL10 together with at least one further gene selected from (the remaining genes in) Table 1 or together with at least one further gene from the (remaining genes in) Table 2B (the 44 gene panel).

96. The method of any of clauses 82, 83 or 87 to 95, or the antagonist and/or agonist for use of any of clauses 84 to 95, wherein the expression levels are determined using at least one primer or primer pair from Table 2E and/or at least one probe from Table 2E.

97. The method of any one of clauses 82, 83 or 87 to 96, or the antagonist and/or agonist for use of any of clauses 84 to 96, wherein the subject is selected for treatment according to a method as described in any one of clauses 1 to 81.

98. The method of any of clauses 1 to 83 or 87 to 97, or the antagonist and/or agonist for use of any of clauses 84 to 97, wherein the sample comprises cancer cells.

99. The method of any of clauses 1 to 83 or 87 to 98, or the antagonist and/or agonist for use of any of clauses 84 to 98, wherein the sample is a tissue sample.

100. The method of clause 99, or the antagonist and/or agonist for use of clause 99, wherein the tissue sample is a fixed and embedded tissue sample.

101. The method of any of clauses 1 to 83 or 87 to 100, or the antagonist and/or agonist for use of any of clauses 84 to 100, wherein the cancer is selected from leukemia, brain cancer, prostate cancer, liver cancer, ovarian cancer, stomach cancer, colorectal cancer, throat cancer, breast cancer, skin cancer, melanoma, lung cancer, sarcoma, cervical cancer, testicular cancer, bladder cancer, endocrine cancer, endometrial cancer, esophageal cancer, glioma, lymphoma, neuroblastoma, osteosarcoma, pancreatic cancer, pituitary cancer, renal cancer or head and neck cancer.

102. The method of any of clauses 1 to 83 or 87 to 101, or the antagonist and/or agonist for use of any of clauses 84 to 101, wherein the inhibitory immune checkpoint is selected from A2AR, B7-H3 (CD276), B7-H4 (VTCN1), BTLA (CD272), CTLA-4 (CD152), IDO, KIR, LAG3, PD-1/PD-L1, TIM-3 and VISTA, optionally wherein the inhibitory immune checkpoint is not PD-1/PD-L1.

103. The method of any of clauses 1 to 83 or 87 to 102, or the antagonist and/or agonist for use of any of clauses 84 to 102, wherein the antagonist of an inhibitory immune checkpoint is selected from an antibody and an inhibitory nucleic acid molecule.

104. The method of any of clauses 1 to 83 or 87 to 103, or the antagonist and/or agonist for use of any of clauses 84 to 103, wherein the antagonist of an inhibitory immune checkpoint is selected from MGA271 (targets B7-H3), ipilimumab (Yervoy - targets CTLA-4), indoximod (targets IDO pathway), NLG919 (targets IDO pathway), lirilumab (targets KIR), IMP321 (targets LAG3), BMS-986016 (targets LAG3), CT-011 (PD-1 blockade), nivolumab/BMS-936558 (PD-1 blockade) , BMS-936559 (PDL1 blockade) and pembrolizumab (Keytruda – targets PD-1), optionally wherein the antagonist is not pembrolizumab; and/or wherein the antagonist of an inhibitory immune checkpoint is selected from MGB453 (targets TIM-3), LAG525 (targets LAG-3) and PDR001 (PD1 Blockade).

105. The method of any of clauses 1 to 83 or 87 to 104, or the antagonist and/or agonist for use of any of clauses 84 to 104, wherein the stimulatory immune checkpoint is selected from CD27, CD28, CD40, CD122, CD137, OX40, GITR and ICOS.

106. The method of any of clauses 1 to 83 or 87 to 105, or the antagonist and/or agonist for use of any of clauses 84 to 105, wherein the agonist of a stimulatory immune checkpoint is selected from an antibody, a lipocalin and a cytokine.

107. The method of any of clauses 1 to 83 or 87 to 106, or the antagonist and/or agonist for use of any of clauses 84 to 106, wherein the agonist of a stimulatory immune checkpoint is selected from CDX-1127 (agonist of CD27), NKTR-214 (agonist of CD122), BMS-663513 (agonist of CD137), TRX518 (agonist of GITR), CP-870893 (CD40 agonist), MEDI0562, MEDI6469 and MEDI6383 (OX40 agonists).

108. The method of any of clauses 1 to 83 or 87 to 107, or the antagonist and/or agonist for use of any of clauses 84 to 107, wherein the DNA damage therapeutic agent is selected from a DNA damaging agent, a DNA repair targeted therapy, an inhibitor of DNA damage signalling, an inhibitor of DNA damage induced cell cycle arrest and an inhibitor of a process indirectly leading to DNA damage.

109. The method of clause 108, or the antagonist and/or agonist for use of clause 108, wherein the DNA damaging agent is selected from an alkylating agent, a topoisomerase inhibitor and radiation.

110. The method of clause 109, or the antagonist and/or agonist for use of clause 109, wherein the alkylating agent is selected from a platinum containing agent, cyclophosphamide and busulphan.

111. The method of clause 110, or the antagonist and/or agonist for use of clause 110, wherein the platinum containing agent is selected from cisplatin, carboplatin and oxaliplatin.

112. The method of clause 109, or the antagonist and/or agonist for use of clause 109, wherein the topoisomerase inhibitor is selected from a topoisomerase I inhibitor and a topoisomerase II inhibitor.

113. The method of clause 112, or the antagonist and/or agonist for use of clause 112, wherein the topoisomerase I inhibitor is selected from irinotecan and topotecan.

114. The method of clause 112, or the antagonist and/or agonist for use of clause 112, wherein the topisomerase II inhibitor is selected from etoposide and an anthracycline.

115. The method of clause 114, or the antagonist and/or agonist for use of clause 114, wherein the anthracycline is selected from doxorubicin and epirubicin.

116. The method of clause 109, or the antagonist and/or agonist for use of clause 109, wherein the radiation is ionising radiation

117. The method of any of clauses 108 to 116, or the antagonist and/or agonist for use of any of clauses 108 to 116, wherein the DNA repair targeted therapy is selected from an inhibitor of Non-homologous end-joining, an inhibitor of homologous recombination, an inhibitors of nucleotide excision repair, an inhibitor of base excision repair and an inhibitor of the Fanconi anemia pathway.

118. The method of clause 117, or the antagonist and/or agonist for use of clause 117, wherein the inhibitor of Non-homologous end-joining is selected from a DNA-PK inhibitor, Nu7441 and NU7026.

119. The method of clause 117, or the antagonist and/or agonist for use of clause 117, wherein the inhibitor of base excision repair is selected from a PARP inhibitor, AG014699, AZD2281, ABT-888, MK4827, BSI-201, INO-1001, TRC-102, an APEX 1 inhibitor, an APEX 2 inhibitor and a Ligase III inhibitor.

120. The method of any of clauses 108 to 119, or the antagonist and/or agonist for use of any of clauses 108 to 119, wherein the inhibitor of DNA damage signalling is selected from an ATM inhibitor, a CHK 1 inhibitor and a CHK 2 inhibitor.

121. The method of clause 120, or the antagonist and/or agonist for use of clause 120, wherein the ATM inhibitor is selected from CP466722 and KU-55933.

122. The method of clause 120, or the antagonist and/or agonist for use of clause 120, wherein the CHK 1 inhibitor is selected from XL-844, UCN-01, AZD7762 and PF00477736.

123. The method of clause 120, or the antagonist and/or agonist for use of clause 120, wherein the CHK 2 inhibitor is selected from XL-844, AZD7762 and PF00477736.

124. The method of any of clauses 108 to 123, or the antagonist and/or agonist for use of any of clauses 108 to 123, wherein the inhibitor of DNA damage induced cell cycle arrest is selected from a Wee1 kinase inhibitor and a CDC25a, b or c inhibitor.

125. The method of any of clauses 108 to 124, or the antagonist and/or agonist for use of any of clauses 108 to 124, wherein the inhibitor of a process indirectly leading to DNA damage is selected from a histone deacetylase inhibitor and a heat shock protein inhibitor.

126. The method of clause 125, or the antagonist and/or agonist for use of clause 125, wherein the heat shock protein inhibitor is selected from geldanamycin and AUY922.

127. A method as described herein with reference to the accompanying figures.

The following examples are offered by way of illustration and not by way of limitation.

EXAMPLES

Example 1

Tissue processing, hierarchical clustering, subtype identification and classifier development

Tumor Material

The genes determined to be useful in the present methods (Table 2) were identified from gene expression analysis of a cohort of 107 macrodissected breast tumor FFPE tissue samples sourced from the Mayo Clinic Rochester. Ethical approval for this study was obtained from the Institutional Review Board and the Office of Research Ethics Northern Ireland.

This cohort of samples can be further described as follows:

- 47 samples were wild-type for BRCA1 and BRCA2 i.e. expressed biologically functional BRCA1 and BRCA2 proteins. These samples shall henceforth be referred to as sporadic controls.
- 31 samples were BRCA1 mutant i.e. did not express biologically functional BRCA1 protein.
- 29 samples were BRCA2 mutant i.e. did not express biologically functional BRCA2 protein.

Gene Expression Profiling

Total RNA was extracted from the macrodissected FFPE tumor samples using the Roche High Pure RNA Paraffin Kit (Roche Diagnostics GmbH, Mannheim, Germany). Total RNA was amplified using the NuGEN WT-Ovation™ FFPE System (NuGEN Technologies Inc., San Carlos, CA, USA). The amplified single-stranded cDNA was then fragemented and biotin labeled using the FL-Ovation™ cDNA Biotin Module V2 (NuGEN Technologies Inc.). It was then hybridized to the Almac Breast Cancer DSA™. The Almac's Breast Cancer DSA™ research tool has been optimised for analysis of FFPE tissue samples, enabling the use of valuable archived tissue banks. The Almac Breast Cancer DSA™ research tool is an innovative microarray platform that represents the transcriptome in both normal and cancerous breast tissues. Consequently, the Breast Cancer DSA™ provides a comprehensive representation of the transcriptome within the breast disease and tissue setting, not available using generic microarray platforms. Arrays were scanned using the Affymentrix Genechip® Scanner 7G (Affymetrix Inc., Santa Clara, CA).

Data Preparation

Quality Control (QC) of profiled samples was carried out using MAS5 pre-processing algorithm. Different technical aspects were addressed: average noise and background homogeneity, percentage of present call (array quality), signal quality, RNA quality and hybridization quality. Distributions and Median Absolute Deviation of corresponding parameters were analyzed and used to identify possible outliers.

Almac's Ovarian Cancer DSA™ contains probes that primarily target the area within 300 nucleotides from the 3' end of a polynucleotide. Therefore standard Affymetrix RNA quality measures were adapted – for housekeeping genes intensities of 3' end probesets along with ratios of 3' end probeset intensity to the average background intensity were used in addition to usual 3'/5' ratios. Hybridization controls were checked to ensure that their intensities and present calls conform to the requirements specified by Affymetrix.

Tumor samples from the BRCA1/2 mutant and sporadic control training set were split into 2 datasets based on the transcript levels of ESR1 (Estrogen receptor 1). mRNA expression level $E_{.avg}$ for each sample was determined by the average expression of all ESR1 probe sets (BRAD.15436_s_at, BRAD.19080_s_at, BREM.1048_at, BRIH.10647C1n2_at, BRIH.5650C1n2_at, BRPD.10690C1n5_at, BRRS.81_at and BRRS.81-22_at). The mRNA median expression ($E_{.med.all}$) was calculated for all samples. Samples were considered ER positive when $E_{.avg} - E_{.med.all} > 0.5$ and ER negative when $E_{.avg} - E_{.med.all} < 0.5$.

Pre-processing was performed in expression console v1.1 with Robust Multi-array Analysis (RMA) (Irizarry et al., 2003) resulting in 2 data matrices of ER positive and ER negative samples composed of 56 and 51 samples respectively. An additional transformation was performed to remove the variance associated with array quality as described by Alter (Alter et al., 2000).

Feature selection

A combined background & variance filter was applied to each data matrix to identify the most variable probesets. The background filter is based on the selection of probe sets with expression *E* and expression variance var_E above the thresholds defined by background standard deviation σBg (from the Expression Console software) and quantile of the standard normal distribution $z_a$at a specified significance *a* probesets were kept if:

$$E > \log_2((z_a\sigma_{Bg}));\log_2((var_E) > 2\,[\log_2(\sigma_{Bg}) - E - \log_2(\log(2))]$$

where the significance threshold was $a = 6.3.10^{-5}$, see Table 1 for the list of selected probesets and their gene annotations.

Hierarchical clustering analysis

Hierarchical clustering techniques were applied to microarray data from 199 epithelial serous ovarian tumors analysed using the Ovarian Cancer DSA™ (disease specific array) platform (FIG. 1). Raw expression data was preprocessed using the standard Robust Multichip Algorithm (RMA) procedure. Non-biological systematic variance in the data set was identified and removed. Those probesets whose expression levels varied significantly from tumor to tumor were identified. These probesets formed the intrinsic list.

2-D cluster analysis (tumor, probeset) was performed to establish tumor relationships based on the intrinsic list. Hierarchical agglomerative clustering was applied (Pearson correlation distance and Ward's linkage). Optimal partition number was selected using the GAP index (Tibshirani et al., 2002, J. R. Stat. Soc., 63:411-423). All probesets available in the subclusters were mapped to genes names.

Functional analysis of gene clusters

To establish the functional significance of the probeset clusters, probesets were mapped to genes (Entrez gene ID) and an enrichment analysis, based on the hypergeometric function (False Discovery Rate applied (Benjamini and Hochberg, 1995, J. R. Stat. Soc. 57:289:300)), was performed.Over-representation of biological processes and pathways were analysed for each gene group generated by the hierarchical clustering for both ER-positive and ER-negative samples using Metacore™ single experiment analysis workflow from GeneGo®. Antisense probesets were excluded from the analysis. Hypergeometric p-values were assessed for each enriched functional entity class. Functional entity classes with the highest p-values were selected as representative of the group and a general functional category representing these functional entities was assigned to the gene clusters based on significance of representation (i.e. p-value).

Genes in clusters enriched for the IFN/DD general functional terms were grouped into a DNA-damage response-deficiency (DDRD) sample group and used for the classifier generation. The sample clusters from ER-positive and ER-negative datasets represented by the IFN/DD general functional terms

were selected for classification and labelled as DDRD. Those not represented by these functional terms were labelled as non-DDRD.

Classifier development at a probeset level

Following the identification of a class of tumors that form the DDRD subgroup, computational classification of these tumors vs. all the others in the tumor cohort (non-DDRD) was performed, with reference to the functional DDRD gene list (Table 1), to identify a refined gene classification model that classifies the DDRD subgroup.This was evaluated using all combinations of the following options (a total of 18):

- Three sample sets
  - Combined sample set of ER-negative and ER-positive samples (combined sample set)
  - ER-negative samples alone
  - ER-positive samples alone
- Two feature sets
  - Full feature list with 75% variance/intensity filtering and forced inclusion of the DDRD list. Here 75% of the probesets with the lowest combined variance and intensity were removed, based on the average rank of both. When used, the term "VarInt" refers to this option.
  - DDRD list only. When used, the term "List only" refers to this option.
- Three classification algorithms
  - PLS (Partial Least Squares) (de Jong, 1993)
  - SDA (Shrinkage Discriminate Analysis)(Ahdesmaki and Strimmer, 2010)
  - DSDA (Diagonal SDA)(Ahdesmaki and Strimmer, 2010)

The AUC was used to assess the performance of the different models. Iterative Feature Elimination (IFE) was implemented throughout the development of each model, where the maximum AUC was the main criteria in selecting an optimal number of features over cross validation. In cases where there was no visible AUC difference across features, the minimum feature length was selected.

Classifier development at a gene level

To facilitate validation of the classifier across multiple array platforms, the selected probeset classifier was regenerated at the gene level. A redevelopment of the probeset classifier at a gene level required two separate steps:

1. The expression intensities of the unique genes in the probeset classifier were estimated from the median of the probesets mapping to each gene, excluding anti-sense probesets.
2. The classifier parameters used for classification were re-estimated

A threshold was chosen based on the maximum sensitivity and specificity over all cross validation predictions.

Similarly the gene level defined expression intensities for the 10 top genes (or any number of features present in current 44 gene signature) could be used to re-develop the classifier based on only these 10 genes (or any number of features present in current 44 gene signature) by re-estimating classification parameters in cross-validation in the training data set as well as to re-establish the threshold by assessing and maximising the sensitivity and specificity obtained from all cross-validation predictions. The methodology would be similar to the method used when working from a larger feature set (described above) except there will be no feature selection involved: the features will remain the same but will be assigned new weights.

Calculating classifier scores for validation data sets

Public Datasets

The datasets used in for this analysis are namely: FAC1 [GEO accession number GSE20271, (Tabchy et al., 2010)], FAC2 [GEO accession number GSE22093, (Iwamoto et al., 2011)], FEC [GEO accession number GSE6861, (Bonnefoi et al., 2007)], T/FAC1 [http://bioinformatics.mdanderson.org/pubdata.html, (Hess et al., 2006)], T/FAC2 [GEO accession number GSE16716, (Lee et al., 2010)] and T/FAC3 [GEO accession number GSE20271, (Tabchy et al., 2010)]. It must be noted that there is an overlap in 31 samples between the FAC1 and FAC2 datasets. These samples were removed from the FAC2 dataset and as such were only included once in the combined analysis of the FAC1, FAC2 and FEC datasets. In addition, sample GSM508092 was removed from FAC1 as it is a metastatic lymph node sample.

All datasets were pre-processed using RMA (Irizarry et al., 2003). For each validation set, the probesets that map to the classifier genes were determined, excluding anti-sense probesets (if applicable). Annotation for Affymetrix X3P and U133A arrays are available from the Affymetrix website. The median intensity over all probesets mapping to each gene in the classifier was calculated, resulting in a gene intensity matrix. The classifier was then applied to this data matrix to produce a classifier score/prediction for each sample.

Calculating performance metrics

To calculate NPV and PPV, the prevalence of each end point (BRCA status/Response) was estimated using the proportions of each class in the corresponding data set.

Univariate and Multivariate analysis

Univariate and multivariate analysis was carried out to assess respectively the association between the DDRD classifier and response, and to determine if the association, if any, was independent to known clinical predictors. The p-values presented Table 47, for univariate analysis were calculated using logistic regression in MATLAB. For the multivariate analysis we used step-wise logistic regression (Dupont, 2009), where the p-values represent the log-likelihood of the variable. The log-likelihood is a measure of the importance of the variable's fit to the model, thus highlighting it's independence as a predictor relative to the other predictors. In both univariate and multivariate analysis, a p-value < 0.05

was used as the criterion for significance. Furthermore, samples with unknown clinical factors were excluded in this assessment.

Results

Selection of samples for classifier generation

The objective of this study was to characterize at a transcriptomic level a set of genes that would be capable of determining responsiveness or resistance of a pathogenic cell to DNA-damage therapeutic agents. With this in mind, those samples within the Almac breast cancer dataset that best represented this biology were to be selected and compared to the remaining samples for classifier generation (see next section). It was decided that the samples from sample cluster two within the ER-ve sample set were the most relevant samples for this selection as these showed the greatest proportion of BRCA mutant samples (64%) and they exhibited the most dominant biology (IFN/immune response). From within the ER+ve sample set, the samples from sample cluster two and three were selected as these sample clusters had 73% and 67% BRCA mutant tumors respectively. In addition, the most dominant biology within these clusters was related to cell cycle, DNA damage response and IFN/immune response. Immune signaling and cell-cycle pathways have been reported to be modulated in response to DNA-damage (Jackson, S. P., and Bartek, J., Nature 461, 1071-1078 (2009); Rodier, F., *et al.*, Nat Cell Biol *11*, 973-979 (2009); Xu, Y.,Nat Rev Immunol6, 261-270 (2006), andthese subgroups were combined to form a putative DDRD subgroup. Those samples within cluster two of the ER-ve sample set (described below) and clusters two and three of the ER+ve sample set (described below) were class labelled DDRD (DNA damage response deficient) (see FIG. 1A) whilst the samples within sample clusters one and three of the ER-ve sample set and sample clusters one, four, five and six of the ER+ve sample set were class labeled non-DDRD(see FIG. 1B).

ER-ve sample set: Within the ER-ve sample set, the hierarchical cluster analysis defined three sample clusters and six probeset cluster groups. Probeset cluster three was identified as the most significant biology within the ER-ve sample set and was enriched for interferon and immune response signaling.

ER+ve sample set: Within the ER+ve sample set, the hierarchical analysis defined six sample groups and six probeset cluster groups. Probeset cluster five was identified as the most significant biology within the ER+ve sample set and was enriched for extracellular matrix remodeling. The next most significant probeset cluster within the ER+ve sample set is probeset cluster six and again was enriched for interferon and immune response signaling.

Development and validation of the DDRD classifier model

Following the identification of a class of tumors, that form the DDRD subgroup, computational classification of these tumors vs. all others in the tumor cohort with reference to the functional DDRD (IFN/DNA damage) gene list was performed to identify a refined gene classification model, which classifies the DDRD subgroup.

The classification pipeline was used to derive a model using the set of combined ER-ve and ER+ve breast cancer samples. The classification pipeline has been developed in accordance with commonly accepted good practice [MAQC Consortium, Nat Biotechnol 2010]. The process will, in parallel: 1) derive gene classification models from empirical data; and 2) assess the classification performance of the models, both under cross-validation. The performance and success of the classifier generation depends on a number of parameters that can be varied, for instance the choice of classification method or probe set filtering. Taking this into account, two feature sets were evaluated (i) the full feature list with 75% variance/intensity filtering (with forced inclusion of the DDRD (IFN/DNA damage) list, Table 1) and (ii) the DDRD (IFN/DNA damage) list only; and three classification algorithms were evaluated, namely PLS (Partial Least Squares); SDA (Shrinkage Discriminate Analysis) and DSDA (Diagonal SDA). Iterative Feature Elimination (IFE) was used throughout model development, which is an iterative procedure removing a fraction of the worst-ranked features at each iteration; stopping when only a minimum number of features remain. The Area under the Receiver Operating Characteristics Curve (AUC-ROC), denoted AUC, was used to assess the classification performance, as this measure is independent of cut-off between groups and prevalence rates in the data. It is also one of the recognized measurements of choice for classification performance. As such, the best number of features for each model was chosen based on the average AUC under cross-validation.

A cross comparison of the models was made, by first selecting the best number of features for each model based on the highest average AUC, and then using box-plots to visualize the performance for each model. This is demonstrated in FIG. 2. From left to right, the first three plots represent the PLS, SDA and DSDA classifiers respectively that were developed using an initial filtering of probe sets to remove 75% with the lowest average variance and intensity (forcing the inclusion of the gene list). The next three plots respectively represent the PLS, SDA and DSDA classifiers developed using the DDRD (IFN/DNA damage) list only.

From FIG. 2, it is clear that the 'PLS VarInt' classification model, comprising 53 probe sets, is the highest performing model, with a significantly higher AUC than the majority of the other 5 models. This model was then taken forward to the next phase for validation on independent external data sets, to assess the ability of the DDRD classification scores to stratify patients with respect to response and prognosis.

A non-orthodox approach to validating the classification model was taken, due to the fact that the validation data sets where either public or internal data with different array platforms. Commonly used approaches are not designed to be applicable to alternative array platforms, and as such a phased approach for classification model development and independent validation was followed:

1. Phase I - Model generation at the probe set level, selecting the best model under cross validation for classifying the DDRD subgroup (described previously)

2. Phase II - Transformation of the probe set level classification model to a gene level classification model

3. Phase III - Validation of re-developed gene classification model using external data sets

Having selected a candidate model to progress to the validation stage, this model needed to be re-built at the gene level (Phase II). This involved mapping the probe sets in the classification model to the gene level and recalculating the weights for each gene. The 53 probe sets in the selected model mapped to 40 genes listed in Table 2A and subsequently mapped to 44 genes listed in Table 2B when the accuracy of the annotation pipeline was improved through further analysis.

In the re-development of the gene classification model, to ensure that all information relating to the gene is used, the median intensity of all probe sets associated with each gene (Table 2C) is used as the gene expression value. This was calculated for all samples, resulting in a gene expression data matrix, as opposed to a probe set expression data matrix that was used in Phase I for model development and selection. To stabilize the intensities across different batches, the median of all probe sets for each sample was subtracted from the corresponding intensity of each gene for that sample.

New weights were calculated for each gene using PLS regression, resulting in the final gene classifier models (40-gene and 44-gene classifier models) that may be used for validation on external data sets from different array platforms (Phase III).

In Phase III, the validation of the classifier using data sets that may be from other array platforms, the following steps were taken:

1. The probe sets that map to the genes in the classifier are determined, excluding anti-sense probe sets (if applicable)
2. The median intensity over all probe sets relating to each gene in the classifier is calculated resulting in a reduced gene intensity matrix
    a. If no probe sets exist for the gene on the particular array platform, the observed average from the training data will be used as a replacement
3. The median value of all probe sets for each sample is calculated and subtracted from the reduced gene intensity matrix
4. The value for each gene is multiplied by the "weight" of that gene in the signature.
5. The values obtained in point 4 for each of the genes in the signature are added together to produce a signature score for that sample.
6. The classifier produces a score for each sample, which can then be used to stratify patients from say, more likely to respond to less likely to respond.

Example 2

In silico validation of the 44-gene DDRD classifier model

The performance of the 44-gene DDRD classifier model was validated by the Area Under the ROC (Receiver Operator Characteristic) Curve (AUC) within the original Almac breast dataset and three independent datasets. The AUC is a statistic calculated on the observed disease scale and is a measure of the efficacy of prediction of a phenotype using a classifier model (Wray et. al., PLoS Genetics Vol 6, 1-9). An AUC of 0.5 is typical of a random classifier, and an AUC of 1.0 would represent perfect separation of classes. Therefore, in order to determine if the 44-gene DDRD classifier model is capable of predicting response to, and selecting patients for, standard breast and ovarian cancer therapeutic drug classes, including DNA damage causing agents and DNA repair targeted therapies, the hypothesis is that the

AUCs following application within these datasets should be above 0.5 with the lowest confidence interval also above 0.5.

Assessment of 44-gene classifier model's ability to separate BRCA mutant from sporadic tumors

The classifier scores for predicting DDRD status were utilized to assess the ability of the model to separate BRCA mutant samples from sporadic samples. This analysis was performed to assess the relationships between the classifier model and BRCA mutation status. BRCA mutant tumors display a high degree of genomic instability due to a deficiency in DNA damage response by virtue of the loss of functional BRCA1/2. As such, the hypothesis is that the DDRD classifier models should be able to separate BRCA mutant samples from BRCA wildtype sporadic samples.

FIG. 3 shows that the 44-gene classifier models separate the BRCA mutants from the sporadic samples with an AUC of ~0.68, where the lower confidence interval is ~0.56 for both models (Table 46A); showing that the performance is significantly better than a random classifier. As such, this analysis confirms that the 44-gene DDRD classifier model is capable of identifying samples with high genomic instability due to an inability to repair DNA damage.

Application of classifier model to independent microarray clinical datasets

Independent breast microarray clinical datasets

(1) Assessment of the 44-gene DDRD classifier model's predictive power to DNA-damaging chemotherapy

To assess the ability of the 44-gene DDRD classifier model to predict response to DNA-damaging chemotherapeutics, it was applied to data combined from three publicly available datasets. In each study, breast cancer patients were treated with neoadjuvant 5-fluorouracil, anthracycline, and cyclophosphamide-based regimens, drugs that directly damage DNA. The first (Tabchy *et al.*, 2010) and second (Iwamoto *et al.*, 2011) datasets had response data for 87 and 50 ER-positive and ER-negative primary breast tumor samples respectively following neoadjuvant treatment with fluorouracil, doxorubicin and cyclophosphamide (FAC). The third dataset (Bonnefoi *et al.*, Lancet Oncol *8*, 1071-1078(2007)) had response data for 66 ER-negative primary breast tumor samples following neoadjuvant 5-fluorouracil, epirubicin and cyclophosphamide (FEC) treatment. Each study used pathological complete response (pCR) or residual disease (RD) as endpoints. As each dataset was relatively small, the data was combined to increase the power of the analysis.

The analysis revealed that that the 44-gene DDRD classifier model was significantly associated with response to anthracycline-based chemotherapy (relative risk (RR) = 4.13, CI = 1.94-9.87; AUC = 0.78, CI = 0.70-0.85, P = 0.001; Table 46B, FIG. 4). The negative predictive value (NPV) of the classifier was considerably higher than the positive predictive value (PPV) (0.90 versus 0.44, Table 46B), indicating that DDRD-negative tumors were unlikely to respond to DNA-damaging chemotherapy.

Stepwise logistic regression was used to determine the ability of the 44-gene DDRD classifier model to predict response in the combined datasets when adjusting for clinical variables (Table 47). The

44-gene DDRD classifier model was determined to be the most significant clinical variable in univariate analysis. Multivariate analysis confirmed that the 44-gene DDRD classifier model's predictive value was independent of stage, grade and notably ER status.

Negativity for estrogen, progesterone and HER2 receptors has been suggested as a biomarker of abnormal DDR and thus response to DNA-damaging and DNA repair targeted therapies (Foulkes et al., 2010). However, this approach excludes the 20% of BRCA1 and the 40% of BRCA2 mutant tumors that are reported to be ER-positive (Foulkes et al., 2004; Tung et al., 2010). In contrast, by virtue of the analysis approach we adopted, the 44-gene DDRD classifier detects the DDRD subgroup in both ER-positive and ER-negative tumors, as validated by the multivariate analysis of the 44-gene DDRD classifier's predictive value within the combined analysis of FEC and FAC datasets, demonstrating its independence from ER status. Clinically, this is an important aspect of the translational application of the DDRD classifier as it suggests it can be applied to all breast cancer patients, irrespective of ER status, to determine their predicted responsiveness to DNA-damaging therapeutics.

(2) Assessment of 44-gene DDRD classifier model's predictive power to taxane-containing chemotherapy regimens

The ability of the 44-gene DDRD classifier model to predict response to chemotherapy regimens that contained non-DNA-damaging agents such as taxanes was assessed. Data was combined from 3 datasets with response data following neoadjuvant treatment with paclitaxel and FAC (T/FAC) for 321 primary breast cancer patients, where response was defined as pCR (Hess et al., 2006; Lee et al., 2010; Tabchy et al., 2010). Whilst the 44-gene DDRD classifier model was both associated with response (AUC = 0.61, CI = ~0.52-0.69, Table 46B, FIG. 5), this performance was significantly reduced compared to that within the FAC/FEC only treated samples. In addition, multivariate analysis indicated the DDRD classifier was not independent from other clinical parameters (P = 0.21) in its ability to predict response to T/FAC (Table 47). This suggests that the subgroup detected by the DDRD classifier is more sensitive to DNA-damaging only regimens rather than regimens also containing anti-microtubule agents.

Independent ovarian microarray clinical datasets

It was decided to explore the performance of the 44-gene DDRD classifier model in another disease area. As such, the performance of the classifier models was assessed within a set of 259 FFPE primary ovarian cancer samples with serous histology. These samples were from patients that received either adjuvant platinum treatment or adjuvant platinum and taxane treatment and were profiled on the Ovarian cancer DSA™. Response data was determined by RESIST and/or the serum marker CA125 levels. Applying the 44-gene DDRD classifier model to these samples proved to separate the responders from the non-responders significantly, with an AUC of ~0.68 and a lower confidence limit of approx 0.59 (FIG. 6). The 44-gene DDRD classifier model detects dysfunction of the Fanconi Anemia/BRCA pathway.

The Fanconi anemia/BRCA (FA/BRCA) pathway, which includes BRCA1 and BRCA2, plays an integral role in DNA repair and can be lost in breast cancer either due to mutation or epigenetic silencing (Kennedy and D'Andrea, 2006). It was therefore determined if the 44-gene DDRD classifier model could detect abrogation of members of this pathway in addition to BRCA1 and BRCA2. A public dataset was

identified with microarray data generated from the bone marrow of 21 FA patients carrying a range of mutations in the FA/BRCA pathway and 11 healthy controls with a functional FA/BRCA pathway (Vanderwerf, S. M., *et al.*, Blood *114*, 5290-5298 (2009). The 44-gene DDRD classifier model significantly distinguished between the FA/BRCA mutant and normal samples with an AUC of 0.90 (CI = 0.76-1.00, P<0.001, FIG. 7), demonstrating a strong correlation between the DDRD classifier and dysfunction of the FA/BRCA pathway through multiple mechanisms.

Summary of *in silico* validation of 44-gene DDRD classifier model

The *in silico* validation of the 44-gene DDRD classifier model has shown the following:

(a) The 44-gene DDRD classifier model is able to significantly separate BRCA mutant breast tumor samples from wildtype BRCA (sporadic) breast tumor samples. This implies that the DDRD classifier model is capable of detecting biology related to tumors with a high level of genomic instability, such as BRCA mutant tumors. These tumors typically respond better to DNA damaging chemotherapeutic regimens.

(b) The 44-gene DDRD classifier model is able to significantly separate defined responders (those that demonstrated pCR) from the non-responders (those that did not demonstrate pCR) in a combination of three independent breast datasets following neoadjuvant treatment with FAC and FEC (Bonnefoi et al., 2007; Iwamoto et al., 2011; Tabchy et al., 2010) and T/FAC (Hess et al., 2006; Lee et al., 2010; Tabchy et al., 2010). The 44-gene DDRD classifier model was found to be independent of other clinical factors and the most significant independent predictor of response in the FAC/FEC combined analysis. These studies were carried out using fresh frozen (FF) samples and using two different microarray platforms, namely the Affymetrix X3P microarray and the Affymetrix U133A microarray. These results validate the performance of the 44-gene DDRD classifier model within independent breast datasets utilizing a different sample material (FF instead of FFPE) and utilizing microarray data from two different microarray platforms.

(c) The 44-gene DDRD classifier model is able to significantly separate responders from non-responders within an independent Almac ovarian dataset following adjuvant treatment with platinum or platinum/taxane based therapy. This data was generated using FFPE samples profiled upon the Almac Ovarian DSA™.

(d) The 44-gene DDRD classifier model is able to significantly distinguish between FA/BRCA mutant and normal samples using bone marrow tissue samples, demonstrating a strong correlation between the DDRD classifier and dysfunction of the FA/BRCA pathway through multiple mechanisms.

In summary, the DDRD classifier model has been independently validated and demonstrated robustness in performance across three different disease areas (breast, ovarian and FA), demonstrated ability to separate responders from non-responders to four different chemotherapeutic regimens (FAC, FEC, T/FAC and platinum/taxane) in two different sample types (FFPE and FF) utilizing data from four different microarray platforms (Almac Breast DSA™ and Almac Ovarian DSA™, Affymetrix X3P

microarray and Affymetrix U133A microarray). It has been demonstrated that the DDRD is an independent predictor of response to DNA-damage therapeutic agents and can predict mutations in the FA/BRCA pathways. This plasticity and repeatability of performance implies that the biology identified within the DDRD subgroup identified via the 44-gene classifier model is significantly and robustly related to predicting response to DNA damage causing agents and as such supports the claim of this invention which is to identify a subtype that can be used to predict response to, and select patients for, standard breast and ovarian cancer therapeutic drug classes, including drugs that damage DNA directly, damage DNA indirectly or inhibit normal DNA damage signaling and/or repair processes.

Table 46:
Performance metrics and independence assessment of the
44-gene DDRD classifier model in breast datasets

Numbers in brackets denote the 95% confidence limits from +/- 2SD from cross-validation (A) or bootstrapping with 1000 repeats (B). AUC=Area Under the Receiver Operating Characteristics Curve; ACC=Accuracy; SENS=Sensitivity; SPEC=Specificity; PPV=Positive Predictive value; NPV=Negative Predictive Value; RR=Relative Risk, pCR=pathological complete response, RD=residual disease.

(A) Prediction of BRCA mutation status using the 44-gene DDRD classifier model										
Data set	**No.**	**Treat-ment**	**Clinical Outcome**	**AUC (CI)**	**ACC (CI)**	**SENS (CI)**	**SPEC (CI)**	**PPV (CI)**	**NPV (CI)**	**RR (CI)**
Train-ing	107	N/A	BRCA mutant V wildtype	0.68 (0.56-0.78)	0.70 (0.57-0.76)	0.58 (0.48-0.65)	0.79 (0.64-0.86)	0.78 (0.63-0.85)	0.60 (0.49-0.65)	1.93 (1.23-2.55)
(B) Prediction of pCR using 44-gene DDRD classifier model										
Data set	**No.**	**Treat-ment**	**Clinical Outcome**	**AUC (CI)**	**ACC (CI)**	**SENS (CI)**	**SPEC (CI)**	**PPV (CI)**	**NPV (CI)**	**RR (CI)**
FAC1 FAC2 and FEC	203	FEC and FAC	pCR V RD	0.78 (0.70-0.85)	0.76 (0.64-0.83)	0.82 (0.69-0.92)	0.58 (0.52-0.62)	0.44 (0.36-0.48)	0.90 (0.81-0.95)	4.13 (1.94-9.87)
T/ FAC	321	T/FAC	pCR V RD	0.61 (0.53-0.69)	0.53 (0.43-0.62)	0.49 (0.38-0.60)	0.67 (0.64-0.70)	0.29 (0.22-0.35)	0.83 (0.80-0.87)	1.72 (1.05-2.65

Table 47
Univariate and Multivariate Analysis of the 44-gene DDRD classifier model

Comparison of the 44-gene DDRD classifier model to standard pathological parameters in independent validation sets. The predictive value of the DDRD classifier model as well as significant clinical parameters were evaluated in a univariate and multivariate analysis using logistic regression models with *p*-values coming from a log-likelihood test.

Univariate and Multivariate Analysis of the 44-gene DDRD classifier model		
FAC1, FAC2 and FEC	**Univariate**	**Multivariate**
Variable	**P value**	**P value**
DDRD classifier	0.0000	0.0014
ER	0.0004	0.0249
Stage	0.0459	0.0492
Grade	0.0100	0.0468
T/FAC	**Univariate**	**Multivariate**
Variable	**P value**	**P value**
DDRD classifier	0.0129	0.2100
ER	0.0000	0.0000
Stage	0.3626	0.0359
Grade	0.0000	0.0115

Example 3

In vitro validation of the 44-gene DDRD classifier model

In order to assess the biology underlying the genes contained within the 44-gene classifier model, a number of studies were carried out *in vitro* using a panel of breast cell-lines.

Methods

Maintenance of cell-lines

The HCC1937 parental, HCC1937-EV and HCC1937-BR cell-lines were kindly donated by Professor Paul Harkin from Queen's University College Belfast (QUB). The cell-lines were routinely maintained in RPMI-1640 medium supplemented with 50 U penicillin/ml, 50µg streptomycin/ml, 2mM glutamine, 1mM Sodium Pyruvate and 20% (v/v) fetal bovine serum (FBS). The HCC1937-EV and HCC937-BR cell-lines also required 0.2ml/mg geneticin. Cell-lines were cultured at 37°C with a humidified atmosphere of 5% CO_2.

Clonogenic assays – determination of PARP-1 inhibitor sensitivity

For measurement of sensitivity to PARP-1 inhibitor (KU0058948), exponentially growing cells were seeded into 6-well plates. Twenty-four hours following seeding the cells were exposed to medium containing increasing doses of drug. Cell medium was replenished every 4-5 days. After 12-14 days the cells were fixed in methanol, stained with crystal violet and counted. The percentage survival of control for a given dose was calculated as the plating efficiencies for that dose divided by the plating efficiencies of vehicle-treated cells. Survival curves and half maximal inhibitory concentration (IC_{50}) values were calculated using GraphPad Prism.

Cell viability assay – determination of cisplatin sensitivity

For measurement of sensitivity to cisplatin, exponentially growing cells were seeded into 96-well plates. 24 hours following seeding the cells were exposed to medium containing increasing doses of cisplatin. Cells were incubated in the presence of drug for 96 hours following which time the viability of the cells was assessed using the Promega CellTitre-Glo luminescent cell viability assay. The sensitivity of the cells was calculated as the percentage of vehicle (DMSO) control. Survival curves and half maximal inhibitory concentration (IC_{50}) values were calculated using GraphPad Prism.

Results

The DDRD subgroup can be identified within breast cancer cell-line models

A preclinical model system was used to confirm that the 44-gene DDRD classifier was a measure of abnormal DDR. The HCC1937 breast cancer cell-line is DDRD due to a BRCA1 mutation (Tomlinson et al., 1998). The 44-gene classifier was applied to HCC1937 empty vector control cells (HCC1937-EV) and HCC1937 cells in which BRCA1 functionality was corrected (HCC1937-BR) (FIG. 8A). The DDRD 44-gene classifier score was found to be higher within HCC1937-EV relative to HCC1937-BR cells, with average scores of 0.5111 and 0.1516 respectively (FIG. 8B). Consistent with the DDRD 44-gene classifier scores, the HCC1937 BRCA1 mutant cell-line was more sensitive to the PARP-1 inhibitor KU0058948 (FIG. 8C) and cisplatin (FIG. 8D) relative to the BRCA1 corrected cell-line. These preclinical data suggest that the DDRD 44-gene classifier measures immune signalling in DDRD-positive tumor cells and correlates with response to both a DNA-damaging agent (cisplatin) and a DNA repair targeted agent (PARP-1 inhibitor).

The DDRD 44-gene classifier detects dysfunction of the Fanconi Anemia/BRCA pathway

The Fanconi anemia/BRCA (FA/BRCA) pathway, which includes BRCA1 and BRCA2, plays an integral role in DNA repair and can be lost in breast cancer either due to mutation or epigenetic silencing (Kennedy, R. D., and D'Andrea, A. D., J Clin Oncol *24*, 3799-3808 (2006)). It was determined if the DDRD 44-gene classifier could detect abrogation of members of this pathway in addition to BRCA1 and BRCA2. A public dataset was identified with microarray data generated from the bone marrow of 21 FA patients carrying a range of mutations in the FA/BRCA pathway and 11 healthy controls with a functional FA/BRCA pathway (Vanderwerf et al., 2009). The DDRD 44-gene classifier significantly distinguished between the FA/BRCA mutant and normal samples with an AUC of 0.90 (CI = 0.76-1.00, $P<0.001$), demonstrating a strong correlation between the DDRD classifier and dysfunction of the FA/BRCA pathway through multiple mechanisms.

Conclusion

The DDRD 44-gene classifier score was significantly higher in the BRCA1 mutant, and thus DDRD, HCC1937 breast cancer cell-line relative to an isogenic BRCA1 corrected cell-line. As the 44-gene classifier score correlates with DDR dysfunction within these cells, it demonstrates that the immune signalling detected by the DDRD classifier is intrinsic to the cell and not a function of lymphocytic infiltrate. BRCA1 and BRCA2 represent part of the FA/BRCA DDR network, which contains a number of other proteins that have been reported to be mutant or under-expressed in approximately 33% of breast cancer (Kennedy, R. D., and D'Andrea, A. D., J Clin Oncol *24*, 3799-3808 (2006).As described previously, the DDRD 44-gene classifier significantly separated bone marrow samples from patients with FA mutations from normal controls. This suggests that the DDRD classifier is capable of detecting any abnormality within the pathway rather than specifically BRCA1 or BRCA2 dysfunction. It is possible that the DDRD 44-gene classifier may identify tumors with DDR-deficiency due to other mechanisms such as

PTEN loss, cell-cycle checkpoint dysfunction or increased reactive oxygen species due to metabolic disturbance. Due to constitutive DNA-damage, these tumors are likely to respond to DNA repair targeted therapies such as PARP-1 or CHK1/2 inhibitors.

Example 4

Endogenous and Exogenous DNA damage activates expression of innate immune genes via the cGAS-STING pathway

Methodologies

Immunohistochemistry

All immunohistochemistry was using the Ventana Discovery®-XT Automated Stainer. Immunohistochemical application was performed on 4 µm sections taken from FFPE blocks. Sections for IHC were cut at 4 µm on a rotary microtome, dried at 37 °C overnight and then used for IHC assays. A Tissue MicroArray of a previously described cohort of 191 N0-N1 ER positive and ER negative FFPE breast tumour samples was scored in triplicate for immune expression analysis. CD4 (4B12, M7310, Dako) was diluted 1:50, and CD8 (C8/144B, M7103, Dako) was diluted 1:50 to enable visualisation of immune cell infiltrate. CD274 (PDL1) (Roche, SP142) was diluted 1:40 and an 8 minute amplification step using the OptiView Amplification Kit (Roche). A semi-quantitative scoring system was employed for CD4 and CD8 characterisation. Briefly, a score of 3 indicates strong CD4 or CD8 expression, 2 indicates moderate expression and 1 low or weak expression. If there was absence of CD4 or CD8 expression the score 0 was applied. Scores were determined by two independent observers for both the stromal and intratumoral component of cores visualized on TMA. For CD274 (PDL1) previously published cut offs of >1% and >5% were used for scoring positive cores on TMA. CD274 (PDL1) staining was investigated in both the tumour and stroma of cores.

Reverse siRNA Transfection

siRNA oligonucleotides (MWG Eurofins) were resuspended to a concentration of 100µM according to the manufacturer's instructions. The following sequences were used for siRNA:

STING_a	*5' CAGCGGCUGUAUAUUCUCCUCCCUU 3'*
STING_b	*5' GGUCAUAUUACAUCGGAUAUU 3'*
TBK1_a	*5' GGAAAUAUCAUGCGUGUUAUU 3'*
TBK1_b	*5' UGGUGCAGCUAGAGAAUUAUU 3'*
IRF3_a	*5' CCUCUGAGAACCCACUGAAUU 3'*
IRF3_b	*5' GGACAAUCCCACUCCCUUCUU 3'*
cGAS_a	*5' AGAGAAAUGUUGCAGGAAAUU 3'*
cGAS_b	*5' CAGCUUCUAAGAUGCUGUCAAAGUU 3'*
BRCA1_a	*5' CCUAUCGGAAGAAGGCAAGUU 3'*
BRCA1_b	*5' CAUACAGCUUCAUAAAUAAUU 3*

BRCA2_a *5' GGACACAAUUACAACUAAAUU 3'*

BRCA2_b *5' GGAGGAAUAUCGUAGGUAAUU 3'*

FancD2_a *5' GCAGAUUCAUGAAGAGAAAUU 3'*

FancD2_b *5' GGUUAAAGCACAUUGUAGAUU 3'*

In a 6 well plate, 20 µl of 2 µM siRNA stock was resuspended in 500 µl 1:100 Optimem:Lipofectamine® RNAiMax (Life Technologies), incubated for 5 minutes at room temperature. This was then incubated for 20 minutes at room temperature, during which time cells were trypsinised and counted using a Countess Automated Cell Counter (Life Technologies). Cells were then resuspended in antibiotic free medium to a concentration determined to yield 50% confluency at 24 hours with 1.5ml of cell suspension added to each plate. Media was changed at 24 hours, and drug treatment added at this point if indicated. Cells were then incubated for a further 48 hours before harvesting RNA and protein.

Quantitative real-time PCR (qRT-PCR)

Reverse transcription was performed using the First Strand cDNA synthesis kit (Roche). 500 ng of RNA was reverse transcribed according to manufacturer's instructions. Exon-spanning qPCR primers were designed using Roche Universal Probe Library Assay Design Centre and were used at a concentration of 0.5 µM. The following primer sequences were used:

CXCL10

Forward 5' GGC CAT CAA GAA TTT ACT GAA AGC A 3'

Reverse 5' TCT GTG TGG TCC ATC CTT GGA A 3'

CCL5

Forward 5' TGC CCA CAT CAA GGA GTA TTT 3'

Reverse 5' CTT TCG GGT GAC AAA GAC G 3'

IDO1

Forward 5' CAT CTG CAA ATC GTG ACT AAG 3'

Reverse 5' CAG TCG ACA CAT TAA CCT TCC TTC 3'

PDL1

Forward 5' GGC ATC CAA GAT ACA AAC TCA AAG A 3'

Reverse 5' AGT TCC AAT GCT GGA TTA CGT CT 3'

PUM1 (Housekeeping gene)

Forward 5' CCA GAA AGC TCT TGA GTT TAT TCC 3'

Reverse 5' CAT CTA GTT CCC GAA CCA TCT C 3'

To preform absolute quantification from qPCR, we used a standard curve method. The efficiency of each primer set was derived from the standard curve using the following equation:

$$E = 10^{(-1/slope)}$$

The product of Reverse Transcription was diluted 1:10 in Nuclease Free Water (NFW). Each 10 µl PCR reaction, consisted of 0.5 µl of 10 µM Forward primer, 0.5µl of 10 µM Reverse primer, 5 µl of 2X

LightCycler® 480 SYBR Green I Master mix (Roche), 1.5 µl NFW and 2.5 µl diluted Reverse Transcription product. These 10 µl reactions were pipetted into wells of a LightCycler® 480 multiwell 96 plate (Roche), the plate was then sealed using clear adhesive film (Roche). The plate was placed into the LightCycler® 480 (Roche) and run with the following protocol. (95°C for 10 mins, 45 cycles of; 95°C for 15 secs, 55°C for 30 secs and 72°C for 30 secs,finishing with a melt curve for confirmation of primer specificity. All qPCR data was analysed using the LightCycler® 480 software provided by Roche. For analysis, the Cp value from a technical duplicate was calculated and the relative amount of a gene was calculated Cp value to an in-run standard curve. Each mean value was then normalised to the mean concentration of the housekeeping gene PUM1 within the corresponding sample, by dividing the concentration of the target gene by the concentration of the house keeping gene. Relative expression refers to the gene expression levels that have been normalised to the housekeeping gene and made relative to the associated control samples. From these normalized values, the fold changes for each gene were calculated and the average of three individual fold changes were derived from three independent experimental triplicates. The unpaired, two-tailed students T-test available on GraphPad Prism 5.0 software was used to detect statistical significance.

Western Blotting

Adherent cells formed whole cell lysates suspended in RIPA buffer containing phosphatase and protease inhibitors (Roche Inhibitor cocktails, Germany). Lysates were then spun to eliminate cell debris. Protein was quantified using the BCA Assay (Pierce, Rockford, IL, USA) according to manufacturer's instructions using a plate reader. Equal amounts of protein per sample was prepared in mercaptoethanol protein loading buffer and separated by size using a gradient 4-12% Bolt® Tris-Bis plus polyacrylamide gel (Life Technologies, Thermo Fisher Scientific Inc.) or a gradient 3-8% NuPAGE® Novex® Tris-acetate gel (for BRCA1 only; Life Technologies, Thermo Fisher Scientific Inc.) and transferred to a PVDF 0.45 µm membrane (Immoblion-P, Millipore) by electroblotting. For investigation of PDL1 expression, the membranes were blocked in 3% BSA/TBST and probed overnight with ant-PDL1 antibody (catalogue no. #13684, Cell Signalling, Technology, MA, USA) diluted 1:500 in 3%BSA/TBST. For the investigation of BRCA1 (HPA034966, Sigma Aldrich), Lamin B1 (ab16048, Abcam), cGAS (HPA031700, Sigma Aldrich), Histone H3 (ab1791, Abcam), MHC class I/HLA A/HLA B (ab134189, Abcam), and HLA G (ab52455, Abcam), membranes were blocked in 3% non-fat milk/TBST and probed overnight with antibody diluted 1:1000 in 3% milk/TBST. For the investigation of IDO1 expression (catalogue no. #12006, Cell Signalling Technology), membranes were blocked in 5% BSA/TBST and probed overnight with antibody diluted 1:500 in 5% BSA/TBST. For loading controls, membranes were blocked in 3% Milk/TBST and probed with anti-β-actin (Sigma Aldrich) diluted 1:10,000 in 3% Milk/TBST or Vinculin (sc-73614, Santa Cruz) diluted 1:2000 in 3% Milk/TBST before the appropriate HRP-conjugated secondary was added. Results were then visualized and recorded using Luminata Crescendo Western HRP substrate (Millipore, UK) and an alpha imager.

Invasion Assay

To test the invasive properties of cell secretions, conditioned media was collected from indicated cell lines with and without transfected knockdowns. Cells were seeded and/or treated on day 0, media was changed to Optimem on day 1 and collected on day 3. Media was then centrifuged at 800 g for 5 minutes to remove cellular debris. Invasion assays were performed using Corning® Transwell® polycarbonate membrane 5 µm 24 well cell culture inserts (Sigma, MO, USA). PBMCs were counted, and resuspended in Optimem 0.5% BSA at a density of 5 x 10^6 cells/ml. 100 µl of cell suspension was placed in the top chamber of the transwell plate equating to 5 x 10^5 cells. 600 µl of conditioned media was placed in the bottom chamber and the assay was incubated for 16 hours. After 16 hours, 100 µl of media from the bottom chamber was removed and a CellTiter-Glo® (Promega, PA, USA) assay was performed per manufacturer's instructions. Invaded cell numbers were derived from a standard curve generated with the CellTiter-Glo® assay and samples of cells counted with a countess (Life technologies, Paisley, UK).

Cytotoxicity

The cytotoxic effects of lymphocytes on cancer cells was measured using LIVE/DEAD® Cell-Mediated Cytotoxicity Kit (Life technologies, Paisley, UK.) RKO parental and Fanc G cells were trypsinised, counted and stained with green-fluorescent membrane stain DiOC18 in PBS at a concentration of 2 µl of stain per ml. Cells were incubated with the stain for 20 minutes at 37 ℃ before being seeded into 12 well plates at a density of 1 x 10^5 cells per well and left to adhere overnight. The next day PMBCs were counted and added to RKO cell cultures at the ratios indicated. For 1:1 ratio 1 x 10^5 PBMCs were added, for 5:1 ratio 5 x 10^5 PBMCs were added. The co-cultures were incubated for 4 hours before cells were collected for analysis by flow cytometry. A BD FACSCalibur™ (BD Biosciences, CA, USA) was used for the analysis of samples and Flow Jo software was used for data analysis. Cells were treated with interferon-γ at a concentration of 20 ng/ml for 16 hours. Cells were treated with LEAF purified anti-human CD274 (Clone 29E.2A3) antibody (BioLegend, CA, USA) at a concentration of 100 µg/ml for 16 hours prior to addition of PBMCs.

Small Molecule Inhibitors & Chemotherapeutic agents

For analysis of effects of ATM, ATR and DNAPK on cytokine expression, cells were seeded in six well plates at ~60% confluency. Following incubation overnight, small molecule inhibitors of ATM (Ku60019, Selleck Chem) at a dose of 1 µm, ATR (ETP46464, Selleck Chem) at a dose of 5 µm and DNAPK (Nu7441, Selleck Chem) at a dose of 5 µm were added. At 24 hours, RNA and protein samples were obtained for analysis. For analysis of the effects of DNA damaging agents and paclitaxel on cytokine expression, cells were seeded in six well plates at ~60% confluency. Following incubation overnight, IC50 doses of Cisplatin and Paclitaxel (obtained from fresh Pharmacy stock) and Hydroxyurea (Sigma Aldrich) were added for 24 hours to 48 hours. At the appropriate timepoint, RNA and protein samples were obtained for analysis.

Cell Cycle Analysis

Cells were trypsinised and fixed in 70% ethanol, incubated with RNase A and propidium iodide (PI) and analysed using a BD FACSCalibur™ (BD Biosciences, CA, USA). Data was analysed using Flow Jo software to perform cell cycle analysis.

Immunoprecipitation

Whole cell lysates were prepared and quantified as in the Western Blotting section. For immunoprecipitation, 500 µg of protein was rotated at 4 °C overnight with 2 µg of TBK1 (sc-52957, Santa Cruz Biotechnology) or IRF3 (Catalogue no. #4302, Cell Signalling Technology). Appropriate secondary anti-mouse or anti-rabbit Dynabeads® (Invitrogen) were pre-washed with RIPA buffer and equal amounts added to samples. Following rotation for 2 hours at 4 °C, samples were washed with RIPA, using the Dynamag Magnetic Rack. Samples were then boiled at 95 °C for 15 minutes in NuPAGE LDS sample buffer (Life Technologies) and NuPAGE Reducing Agent (Life Technologies). Equal amounts of reduced samples were separated by size using a gradient 4-12% Bolt® Tris-Bis plus polyacrylamide gel (Life Technologies, Thermo Fisher Scientific Inc). Western blotting procedure was followed as described previously. Membranes were blocked for 1 hour at room temperature in 5% BSA/TBST and probed with either pTBK1 (Ser172) (Catalogue no. #5483, Cell Signalling Technology) or pIRF3 (Ser396) (Catalogue no. #4947, Cell Signalling Technology) overnight at 4 °C. Membranes were then probed with appropriate HRP-conjugated secondary (Anti-rabbit IgG, Catalogue no. #7074, Cell Signalling Technology for pTBK1; Anti-rabbit Light Chain Specific IgG, 211-032-171, Jackson ImmunoResearch Laboratories Inc. for pIRF3). Results were then visualized and recorded using Luminata Crescendo Western HRP substrate (Millipore, UK) and an alpha imager.

Cell Fractionation

Cells were fractionated using Buffer A (10mM Hepes pH7.4; 1.5 nM MgCl2, 10 mM NaCl, 0.1% NP-40, Protease and Phosphatase inhibitors) and Buffer C (10 mM Hepes pH7.4; 1.5 nM MgCl2, 420mM NaCl, 0.1% NP-40, Protease and Phosphatase inhibitors). Cells were cultured at ~70% confluency. Cells were then removed in PBS using a cell scraper and transferred to an Eppendorf. Following centrifugation at 1000 rpm at 4 °C for 5 minutes, cell pellets were resuspended in 350 µl Buffer A. Cells were lysed on ice for twenty minutes, following which samples were centrifuged at 12000 rpm for 2 minutes. Supernatants were removed, and spun a further two times at 12000 rpm for 2 minutes. The supernatant (cytoplasmic fraction) was carefully removed and stored at -80 °C until quantification using the BCA Assay (Pierce, Rockford, IL, USA) according to manufacturer's instructions using a plate reader. The remaining pellet was washed x1 in Buffer A, then centrifuged at 12000 rpm for 2 minutes. The pellet was resuspended in Buffer C, lysed on ice for 10 minutes and sonicated at 20K cycles/second for 30 seconds. Samples were then centrifuged at 12000 rpm for two minutes to remove debris, and supernatant (nuclear fraction) stored at -80 °C until quantification as described above.

Co-immunoprecipitation

Cytoplasmic fractions were prepared as described above. 500 µg of protein was rotated at 4 °C overnight with 2 µg of Histone H3 antibody (ab1791, Abcam) resuspended in Pierce IP Lysis Buffer (Thermo Scientific). Secondary anti-rabbit Dynabeads® (Invitrogen) were pre-washed with Pierce IP Lysis Buffer and equal amounts added to samples. Following rotation for 2 hours at 4 °C, samples were washed with Pierce IP Lysis Buffer, using the Dynamag Magnetic Rack. Samples were then boiled at 95 °C for 15 minutes in NuPAGE LDS sample buffer (LifeTechnologies) and NuPAGE Reducing Agent (Life Technologies). Equal amounts of reduced samples were separated by size using a gradient 4-12% Bolt® Tris-Bis plus polyacrylamide gel (Life Technologies, Thermo Fisher Scientific Inc). Western blotting procedure was followed as described previously. Membranes were blocked for 1 hour at room temperature in 5% BSA/TBST and probed with cGAS antibody (HPA031700, Sigma Aldrich) in 5% BSA/TBST at 4 °C overnight. Membranes were probed with HRP-conjugated secondary (Anti-rabbit IgG, Catalogue no. #7074, Cell Signalling Technology). Results were then visualized and recorded using Luminata Crescendo Western HRP substrate (Millipore, UK) and an alpha imager.

Results

CD4+ and CD8+ T lymphocytes are associated with DDRD assay positive tumours.

As we had observed up-regulation of interferon-related genes including T-cell specific ligands in DDRD tumors , we asked if these were associated with a T cell immune response. The presence of intratumoral CD4+ and CD8+ T lymphocytes have previously been described as prognostic in breast cancer. The presence of intratumoral and stromal CD4+ and CD8+ T lymphocytes was assessed by IHC using a semi-quantitative score between 0-3 whereby a higher score represents a greater number of T lymphocytes present. A total cohort of 191 N0-N1 ER positive and ER negative breast scored as DDRD positive or negative using the DDRD assay. A significant association of both CD4+ and CD8+ intratumoral tumour infiltrating lymphocytes (iTILs) and stromal tumour infiltrating lymphocytes (sTILs) with DDRD positivity was identified *(Fig 9).* This is demonstrated by the increased proportion of tumour sample cores with a greater IHC score within the DDRD positive CD8+ (DDRD pos CD8) and DDRD positive CD4+ (DDRD pos CD4) populations ($p<0.0001$) *(Fig 9A).* The association between CD4+ and CD8+ T-lymphocytes and DDRD positivity was confirmed by the IHC images whereby an increased staining intensity is indicative of a greater presence of iTILs and sTILS within the tumours *(Fig 9B).*

Chemokine production is associated with DNA damage repair deficiency.

CXCL10 is the most discriminating gene in the DDRD assay, and has previously been reported as a prognostic factor in breast cancer[1]. CCL5 (RANTES) was identified as the top differentially expressed gene in DDRD positive ER negative tumours (Table 48). The majority of differentially expressed genes were identified as interferon responsive indicated by an area under curve (AUC) greater than 0.5. This is in keeping with a chemokine rich inflammatory tumour microenvironment *(Fig 10A).* Further interferome analysis of the differentially expressed genes showed that 53.1% of these genes were interferon driven with a predominant association with Type I interferons *(Fig 10B).* The CXCL10/CXCR3 axis has been reported as key for the chemotaxis of CD4+ and CD8+ T lymphocytes to sites of inflammation[2]. CXCL10 and CCL5 overexpression are associated with the presence of CD8+

lymphocytes in melanoma, gastric and colorectal cancers[3-5]. We therefore sought to identify the mechanism of production of these key chemokines, CXCL10 and CCL5, in DNA damage repair deficient tumours.

Table 48 - Differentially Expressed Genes in ER negative DDRD positive tumors (FC>3)

	Gene Symbol	Gene ID	Mean DDRD neg	Mean DDRD pos	p-value	Fold-Change	Fold-Change Description
1	IFI44L	10964	-0.41495	1.37672	6.11E-13	-3.46216	DDRD neg down vs DDRD pos
2	IDO1	3620	-0.260724	1.46939	2.18E-12	-3.31754	DDRD neg down vs DDRD pos
3	GBP5	115362	-0.313374	1.42788	2.96E-12	-3.34325	DDRD neg down vs DDRD pos
4	**CCL5**	6352	-0.460764	1.26706	1.71E-11	-3.31227	DDRD neg down vs DDRD pos
5	ART3	419	-0.40557	1.29577	4.67E-11	-3.25203	DDRD neg down vs DDRD pos
6	DDX60	55601	-0.199854	1.44622	5.19E-11	-3.12981	DDRD neg down vs DDRD pos
7	XAF1	54739	-0.274869	1.39532	5.75E-11	-3.18257	DDRD neg down vs DDRD pos
8	GBP5	115362	-0.33513	1.33699	1.03E-10	-3.18682	DDRD neg down vs DDRD pos
9	GBP5	115362	-0.358814	1.29533	2.59E-10	-3.14737	DDRD neg down vs DDRD pos
10	CD274	29126	-0.397379	1.26159	2.60E-10	-3.15791	DDRD neg down vs DDRD pos
11	GABBR1 /// UBD	2550 /// 10537	-0.372545	1.28096	2.88E-10	-3.14596	DDRD neg down vs DDRD pos
12	PSMB9	5698	-0.301324	1.33198	3.82E-10	-3.10222	DDRD neg down vs DDRD pos
13	TNFSF13B	10673	-0.260167	1.35947	4.29E-10	-3.07297	DDRD neg down vs DDRD pos
14	CCL5	6352	-0.507855	1.13341	5.06E-10	-3.11939	DDRD neg down vs DDRD pos
15	ISG15	9636	-0.30543	1.31872	5.71E-10	-3.0826	DDRD neg down vs DDRD pos
16	---	---	-0.343938	1.28446	6.48E-10	-3.0917	DDRD neg down vs DDRD pos
17	STAT1	6772	-0.388005	1.24196	7.59E-10	-3.09505	DDRD neg down vs DDRD pos
18	---	---	-0.222883	1.36652	9.16E-10	-3.00925	DDRD neg down vs DDRD pos
19	IFI44L	10964	-0.472957	1.14736	1.16E-09	-3.07443	DDRD neg down vs DDRD pos
20	STAT1	6772	-0.331983	1.27178	1.51E-09	-3.03936	DDRD neg down vs DDRD pos
21	PSMB9	5698	-0.312228	1.28678	1.57E-09	-3.02935	DDRD neg down vs DDRD pos
22	STAT1	6772	-0.397685	1.20857	1.83E-09	-3.04461	DDRD neg down vs DDRD pos
23	CD3G	917	-0.543926	1.05165	2.03E-09	-3.02216	DDRD neg down vs DDRD pos
24	STAT1	6772	-0.495509	1.10127	2.44E-09	-3.02466	DDRD neg down vs DDRD pos
25	GBP4	115361	-0.414399	1.18384	2.50E-09	-3.02774	DDRD neg down vs DDRD pos

26	SAMD9L	219285	-0.339994	1.25098	2.51E-09	-3.01252	DDRD neg down vs DDRD pos
27	EPSTI1	94240	-0.356971	1.23204	2.91E-09	-3.00843	DDRD neg down vs DDRD pos
28	PARP14	54625	-0.373931	1.21386	3.24E-09	-3.00589	DDRD neg down vs DDRD pos
29	OAS2	4939	-0.394501	1.1948	3.26E-09	-3.00904	DDRD neg down vs DDRD pos

We next asked if a loss in DNA damage response may result in the observed DDRD assay immune response. We inhibited BRCA1, BRCA2 and FANCD2 function using siRNA knockdown constructs in T47D cells to address the role of intrinsic DNA damage repair deficiency and therein DDRD biology in the chemokine production. CXCL10 and CCL5 were identified as significantly upregulated in response to loss of DNA repair proteins. The increased relative expression of CXCL10 and CCL5 upon inhibiting BRCA1 (using BRCA1_a/b siRNAs), BRCA2 (using BRCA2_c/d siRNAs) and FANCC (FancC_1/2 siRNAs) compared to the control scrambled sequence siRNA (AS) in T47D cells confirmed that DNA damage induced the expression of chemokines *(Fig 11)*. Using isogenic cell lines, HCC1937 EV (DDRD Pos) and HCC1937 + BRCA1 (DDRD Neg); and MDA-436 EV (DDRD Pos) and MDA-436 + BRCA1 (DDRD Neg), we again observed significant upregulation of CXCL10 and CCL5 in the DNA damage repair deficient cells compared to their repair-corrected line. Therefore Figure 12A displays that upon correction of the DNA repair defect through the re-expression of BRCA1, the relative expression of both CXCL10 and CCL5 was significantly reduced *(Fig 12A)*. Western blotting confirms the protein expression of BRCA1 in both the corrected cell line models compared to the empty vector (EV) paired equivalent *(Fig 12B)*. To address if the upregulation of CXCL10 and CCL5 contributed to lymphocytic infiltration, we used a migration assay of activated peripheral blood mononuclear cells (PBMCs) with conditioned media from MDA436-EV and + BRCA1 cells *(Fig 13A & B)*. After co-culture for four hours, we observed a significant increase in the migration of PBMCs to conditioned media from the DNA damage repair deficient line. The MDA436-EV which are DDRD positive (DDRD +ve) displayed a greater fold change in cell invasion compared to the corrected cell line pair expressing BRCA1 which are DDRD negative (DDRD –ve) *(Fig 13C)* ($p<0.001$). Therefore endogenous DNA damage repair deficiency causes chemokine production and the subsequent immune cell infiltration. The MDA436-EV which are DDRD positive (DDRD +ve) displayed a greater fold change in cell invasion compared to the corrected cell line pair expressing BRCA1 which are DDRD negative (DDRD –ve) (Fig 13D) ($p<0.001$). Additionally, siRNA mediated knockdown of CXCL10 and CCL5 reduced PBMC migration, indicating their importance for lymphocytic infiltration ($p<0.05$; Fig 13E).

Chemokine expression is controlled in a cell cycle specific manner.

HeLa, HCC1937 EV and MDA-MB-436 EV cells were treated with IC-50 doses of DNA damaging agents Cisplatin and Hydroxyurea, and the microtubule-stabilising agent Paclitaxel. As demonstrated by the increased relative expression compared to DMSO control, upregulation of CXCL10 and CCL5 expression was stimulated in all cell lines following treatment with cisplatin and hydroxyurea. However, CXCL10 and CCL5 expression was not significantly increased with Paclitaxel treatment in either cell line model *(Fig 14)*. Treatment with cisplatin and hydroxyurea resulted in an increased proportion of cells in

S phase *(Fig 14)*. However, treatment with a further anti-mitotic agent, Nocodazole caused an arrest in the M phase of cell cycle as observed by the reduced mRNA expression of CXCL10 *(Fig 15A)*. The block in M phase was confirmed by the changes in the cell cycle profile shown in Figure 15 *(Fig 15B)*. Together these data support an S-phase specific signal for activation of the immune response to DNA damage.

Chemokine expression is independent of DNA damage sensors ATM, ATR and DNAPK.

The kinases Ataxia Telangiectasia Mutated (ATM), ATM-and RAD3 related (ATR), and DNA-dependent protein kinase catalytic subunit (DNA-PKcs) are activated in response to DNA damage. Activation of ATM has previously been reported to result in the upregulation of immune genes suggesting that ATM may be required for chemokine production in response to DNA damage repair deficiency[6]. We treated DDRD positive cells (MDA-MB-436 EV) with small molecule inhibitors of ATM (Ku60019), ATR (ETP-46464) and DNAPK (Nu7440). No significant decreases in CXCL10 and CCL5 chemokine production were identified upon treatment with ATM inhibitors (ATMi), ATR inhibitors (ATRi) or DNAPK inhibitors (DNAPKi) compared to the DMSO control *(Fig 16)*. However, inhibition of DNAPK (DNAPKi) significantly increased CXCL10 and CCL5 chemokine expression levels compared to the DMSO control *(Fig 16)*. Together these data indicate that these DNA damage response kinases are not required for the interferon response to endogenous DNA damage repair deficiency.

The STING/TBK1/IRF3 pathway is constitutively active in DDRD tumour cells.

Next we performed a transcription factor analysis to identify those that could activate genes upregulated in DDRD tumours. IRFs (Interferon Regulatory Factors) gene targets were significantly enriched within this list. In addition, stimulation of the innate immune pathway STING/TBK1/IRF3 *(Fig 17A)* has been reported as a driver of CXCL10 expression[7]. IRF3 is recognised to be active in response to DNA damaging agents[8], therefore we hypothesised that IRF3 would be active in DDRD positive cells. Supporting this we observed enhanced phosphorylation of IRF3 (pIRF3) from whole cell lysates of BRCA1-deficenct cells MDA-436 EV and HCC1937 EV compared to their BRCA1 corrected isogenic line (MDA-436 +BRCA1 and HCC1937 +BRCA1) *(Fig 17B)*. Similarly, TBK1 was constructively phosphorylation was observed in the repair deficient cells (EV) compared to the repair corrected DDRD negative cell lines (BRCA1) *(Fig 17B)*. Using siRNA mediated knockdown, we inhibited the function of STING (Sting_a/b), TBK1 (TBK1_a/b) and IRF3 (IRF3_a/b) in both MDA-436 and HCC1937 cells. When compared to the control (AS), the knockdown of STING, TBK1 and IRF3 significantly reduced the relative expression of both CXCL10 and CCL5 *(Fig 17C)*.

These data demonstrates the requirement of STING, TBK1 and IRF3 for the immune response to DNA damage response deficiency.

Endogenous or exogenous DNA damage results in an increase in cytosolic DNA

The cytosolic DNA sensor cGAS has been described as the most potent activator of the STING pathway. We therefore asked if it cytosolic DNA was associated with observed immune response to S phase specific DNA damage[9]. Using co-immunoprecipitation (co-IP), we identified cGAS as bound to Histone H3 in the cytosolic fraction of DDRD positive cells MDA-436 EV and HCC1937 *(Fig 18A, top panel of blots).* Binding of double stranded DNA to cGAS results in activation of STING via cGAMP, and immune gene expression. Additionally, in HeLa cells treated with Cisplatin (Cisp) or Hydroxyurea (HU), co-IP showed that cGAS was again bound to Histone H3. The binding of cGAS to Histone H3 was not observed in the DMSO treated control *(Fig 18B, top panel of blots).* Abrogation of cGAS function using siRNA mediated knockdown constructs (cGAS_a/b) in both MDA-436 and HCC1937 cells, resulted in significant reduction in both CXCL10 and CCL5 chemokine relative expression levels in the context of endogenous DDRD, and in response to DNA damaging agents *(Fig 18C).* Therefore, cGAS is required for expression of chemokines from the tumour cell in response to DNA damage.

Cytosolic DNA is present in response to endogenous and exogenous DNA damage.

We probed cytosolic fractions of DDRD positive cells MDA-436-EV and +BRCA1, and HCC1937-EV and +BRCA1 cells for the presence of Histone H3, and found Histone H3 protein expression was increased in the repair deficient lines (EV) *(Fig 19A, top panel of blots).* We also confirmed increased Histone H3 protein levels in response to DNA damage in HeLa cells treated with Cisplatin (Cisp) and Hydroxyurea (HU) compared to DMSO control treatment *(Fig 19B, top panel of blots).* PicoGreen fluorescent staining was used to detect double-stranded DNA (ds-DNA). HeLa cells treated with IC50 doses of DNA damaging agents Cisplatin (HeLa + Cisplatin IC50) and Hydroxyurea (HeLa + Hydroxyurea IC50) revealed increased cytosolic DNA when examined by confocal microscopy. This increase in cytosolic DNA was however not observed in response to treatment with Paclitaxel (HeLa + Paclitaxel IC50) *(Fig 19C).*

DDRD positive tumours are associated with expression of PDL1.

The apparent paradox of upregulation of chemoattractants and subsequent lymphocytic infiltration in DDRD positive tumours is potentially explained by the upregulation of the immune checkpoint target PDL1. This target is known to cause lymphocyte exhaustion and effectively switches off the immune cytotoxic response to the cancer cells. Using the Roche SP142 antibody to PDL1 we performed IHC analysis on the original cohort of breast tumours previously scored for CD4+ and CD8+ T lymphocytic infiltration. Previously reported cut-offs of >1% and >5% were used to define PDL1 positivity, for both infiltrating tumour immune cell and tumour cell PDL1 expression[10] *(Fig 20).* Significant association of PDL1 expression at both the predefined cut offs was identified within DDRD positive tumours displayed by the 46.2% and 21.5% positivity for tumour populations positive for both DDRD (DDRD pos) and PDL1 (PDL1 pos) at >1% and >5% respectively ($p<0.0001$, $p=0.0004$) *(Fig 20A, tumour).* In addition, infiltrating immune cell PDL1 positivity was also associated with DDRD positivity as demonstrated by the 75.4% and 40% positivity for lymphocytes at both >1% and >5% respectively ($p<0.0001$) (Fig 20A, lymphocytes). Immunohistochemistry staining confirms strong PDL1 expression within the tumour, with additional PDL1 expression with lymphocytic infiltration as depicted by the

staining patterns and intensities *(Fig 20B).* In sum, both tumour cell PDL1 positivity and infiltrating immune cell PDL1 expression were significantly associated with DDRD positivity *(Fig 20).*

Additionally, the tumours were analysed based on their DDRD scores which assigns each tumour sample to a DDRD positive or DDRD negative subgroup based on the cut-off values within the defined gene signature. The DDRD scores of the PDL1 positive cohort (PDL1 pos) based on the aggregate tumour and lymphocyte staining using the predefined >1% and >5% cut offs demonstrated significantly higher DDRD scores than PDL1 negative cohort (PDL1 neg) ($p<0.001$) *(Fig 21).* This data suggests that PDL1 protein expression is associated with a positive DDRD assay result and likewise PDL1 positive tumours have active DDRD signalling.

DNA Damage Repair Deficient cell lines are primed to express PDL1 in response to co-culture with PBMCs

MDA-436 EV and MDA-436 + BRCA1 cells (repair corrected) were co-cultured with activated PBMCs. Within the co-culture, PDL1 relative expression levels were significantly upregulated in both repair deficient cells (436 EV + Act) ($p=0.0001$) and BRCA1 repair corrected MDA-436 cells (436 BRCA1 + Act) ($p=0.0359$). Moreover, the increased levels of PDL1 expression was more enhanced in DDRD positive cell models in co-culture (436 EV + Act) compared to the DDRD negative cells (436 BRCA1 + Act) ($p=0.0033$) *(Fig 22).* Therefore, PDL1 expression is increased by co-culture with lymphocytes, specifically in DDRD positive models.

DNA Damage induces expression of PDL1

Treatment of HHC1937 EV, MDA-MB436 EV and HeLa cells treated with the DNA damaging Cisplatin (Cisp) or Hydroxyurea (HU) but not Paclitaxel induce expression of CD274 (PDL1) through Q-PCR analysis (Fig 23A). This effect was confirmed at the protein level through western blot analysis (Fig 23B).

Other potential immune checkpoint targets are activated in response to DNA damage.

To determine the involvement of other potential immune checkpoint targets, we checked the protein expression of the alternative immune checkpoint target IDO1 in MDA-436 and HCC1937 isogenic cell line pairs. Accordingly DDRD positive cells (MDA-436 EV and HCC1937 EV) demonstrated increased IDO1 protein levels in comparison to corrected DDRD negative isogenic pairs (MDA-436 + BRCA1 and HCC1937 + BRCA1) *(Fig 24, top panel of blot).* Furthermore, within co-culture with lymphocytes, IDO1 relative expression levels were significantly upregulated in both repair deficient cells (436 EV + Act) ($p=0.0002$) and BRCA1 repair corrected MDA-436 cells (436 BRCA1 + Act) ($p=0.0660$). Moreover, the increased levels of IDO1 expression was more enhanced in DDRD positive cell models in co-culture (436 EV + Act) compared to the DDRD negative cells (436 BRCA1 + Act) ($p=0.0013$) *(Fig 25).* Therefore, similarly to PDL1, IDO1 expression is also increased by co-culture with lymphocytes, specifically in DDRD positive models.

DDRD+ cells are protected from lymphocyte mediated cytotoxicity.

PBMCs were co-cultured with RKO Parental and RKO FANCG-/- for 4 hours and labelled with 5-(6)-carboxyfluorescein diacetate succinimidyl ester (CFSE) to label the cancer cells, in combination with 7-AAD to label the dead cancer cells and PBMCs. The RKO FANCG-/- at both ratios (FANCG-/- 1:1 and FANCG-/- 5:1) demonstrated reduced lymphocyte mediated toxicity compared to the RKO Parental cells (Parental 1:1 and Parental 5:1), as shown by the lower percentage of cytotoxicity. This reduction in toxicity is consistent with the expression of PDL1 in these cells. It is apparent that DDRD positive cells exhibit protection against lymphocyte mediated toxicity *(Fig 26).* Furthermore pre-treatment of the cancer cells with Interferon-γ (Fanc G IFN 5:1) extends the differential cytotoxicity between the RKO FANCG-/- and RKO Parental cells (p-value <0.05) *(Fig 27A).* In addition, treatment of RKO cells with Interferon-γ significantly increases PDL1 gene expression levels, as demonstrated by the difference in fold change of RKO Par IFN, RKO Fanc C IFN and RKO Fanc G IFN *(Fig 27B).* The enhanced PDL1 levels upon Interferon-γ pre-treatment were confirmed at the protein level by western blotting (RKO Par IFN and RKO Fanc G IFN) *(Fig 27C, top panel).* Taken together these data suggest that DDRD positive cells substantially overexpress PDL1 which protects the DDRD positive cells from lymphocyte mediated cell death.

Blockade of PDL1 function reverses DDRD resistance to lymphocyte mediated cytotoxicity.

To further assess the protective properties against lymphocyte mediated toxicity, a PDL1 blocking antibody was introduced to inhibit PDL1 function. Pre-treatment of RKO Parental cells and RKO FANCG-/- cells with Interferon-γ in combination with the PDL1 blocking antibody prior to cytotoxicity assays using PMBCs resulted in significantly more cytotoxicity in the DDRD positive RKO FANCG-/- . This was demonstrated by the enhanced percentage cytotoxicity of Fanc G treated with both Interferon-γ and PDL1 antibody (Fanc G IFN 5:1 + PDL1 AB) in comparison to Fanc G with Interferon-γ alone (Fanc G IFN 5:1) (p<0.01) *(Fig 28).* Of note, no significant difference in cytotoxicity was observed between the Parental RKO treated with IFN (Par IFN 5:1) and the Parental RKO treated with a combination of IFN and PDL1 antibody (Par IFN 5:1 + PDL1 AB) *(Fig 28).*

The DDRD subtype has upregulation of multiple Immune checkpoint targets in multiple indications.

To assess if other immune checkpoint targets were upregulated and therefore protected DDRD positive tumours from immune mediated cytotoxicity, we performed differential gene expression analysis of two breast cancer datasets[11], a publically available colorectal cancer dataset[12] and a melanoma dataset[13]. In each instance hierarchal clustering using DDRD genes identified from the breast cancer discovery cohort were used to define class labels. A number of additional immune checkpoint targets including PDL1, IDO1, LAG3, HAVCR2 and CTLA4 were upregulated in DDRD positive tumours when compared to DDRD negative tumours *(Table 49).* A number of these immune checkpoint genes have therapeutic targets identified towards them.

Table 49 - DDRD positive tumors have increased expression of multiple immune checkpoint targets

Gene ID	Alias	BC Discovery ER Negative	BC Discovery All samples	BC Validation All samples	CRC (E-GEOD-39582 Marisa)	Melanoma (GSE19293_Augustine)
CD274	PDL1	3.16	1.74	1.90	3.3	4.20
IDO1		3.32	3.04	3.58	4.62	2.82
LAG3		2.89	NA	NA	1.82	2.98
HAVCR2	TIM-3	2.43	2.36	1.68	1.83	2.79
CD80		2.03	NA	NA	NA	1.78
CD86		2.52	1.57	NA	2.26	3.38
CTLA4		2.55	1.50	NA	1.65	3.54
MHC Class 1						
	HLA-DRA	2.38	1.73	1.87	4.25	4.77
	HLA-DPA1	2.25	1.75	1.74	3.91757	4.55

The DDRD biology is significantly enriched in Microsatellite Instable (MSI) colorectal cancers

To date the only known genetic stratification for response to PDL1 inhibition is Microsatellite Instability (MSI)[14], which results from impaired DNA mismatch repair (MMR). We hypothesised that the DDRD biology would represent MSI cancers and could be used as an improved stratification tool. We performed semi-supervised clustering on a public gene expression dataset using an intrinsic DDRD biology derived from the breast cancer analysis (Mayo clinic data, Marisa dataset). This process identified a group of colorectal samples with activation of the DDRD biology and was highly enriched in MSI tumours *(Fig 29A, outlined within the box).* Of this identified group, specifically 80% of the MSI tumours were present within the DDRD positive group as indicated by the percentage of cases with deficient MMR (dMMR) *(Fig 29B).* Separately analysis of a cohort of stage II colorectal cancer samples which we have previously profiled[15] demonstrated that samples with known MSI status (MSI-H) had significantly higher DDRD scores than microsatellite stable (MSS) samples ($p>0.05$) *(Fig 29C).*

Our current model, intrinsic or extrinsic DNA damage causes an accumulation of cytosolic DNA, this leads to activation of the innate immune STING mediated pathway which is responsible for chemokine production resulting in an inflammatory microenvironment in DNA damage repair deficient breast tumors. Expression of PD-L1 is also associated with tumors deficient in DNA damage repair and prevents T cell mediated cytotoxicity (Figure 30).

Discussion

The DDRD molecular subtype represents tumours that have loss of function of the FA/BRCA pathway, the primary response mechanism to DNA damage and stalled DNA replication in the S-phase of the cell cycle. Our new data suggest that in the absence of a functional FA/BRCA pathway or as a result of exogenous S phase DNA damage, there is a mechanism through which an accumulation of cytosolic DNA activates the STING/TBK1/IRF3 innate immune response.

Previous studies have suggested that genomic instability may activate immune signalling through the production of neoantigens [3]. Our model proposes cytosolic DNA as an important immune-stimulating factor in response to DNA damage in the S phase of the cell cycle. This immune signal arises from the epithelial component of the cell and does not require immune recognition of abnormal proteins. Although it is unclear why S-phase DNA damage should result in cytosolic DNA we hypothesize that this may be a by-product of replication fork processing. Indeed there is some evidence that the cell may actively export DNA fragments from the nucleus, possibly to prevent misincorporation into genomic DNA [16]. Normally cytosolic DNA is processed by cytoplasmic DNase II, however it may be that this mechanism is overwhelmed by a failure to respond to endogenous DNA damage or following exogenous DNA damage thereby triggering the cGAS-mediated innate immune response. Indeed, a similar activation of the STING pathway in response to an abnormal accumulation of cytosolic DNA has been observed in the disease Systemic Lupus Erythramatosis (SLE) [17].

Our DDRD gene assay contains 2 immune checkpointing genes that represent therapeutic targets, PD-L1 and IDO1. Inhibition of the PD1/PD-L1 axis has resulted in dramatic responses in a subset of patients with advanced solid tumors including melanoma and non-small cell lung cancer [18]. Importantly, our observation that DDRD positive tumours associate with PD-L1 expression provides a rationale for exploration of immune checkpoint treatments in this molecular subgroup. Using isolated lymphocytes we have demonstrated that blockade of PD-L1 causes significant increase in lymphoctyte mediated toxicity in DDRD positive tumours.

In further support of this approach is the recent report for activity of PD-L1 inhibitors in mismatch repair deficient colorectal cancer (REF ASCO). Mismatch repair proteins have been reported to have a role in the response to S phase replication fork stalling [19] that our study suggests should activate the STING/TBK/IRF3 pathway and upregulate PD-L1 expression. Importantly we have demonstrated that the DDRD assay is sensitive in detecting colorectal MSI tumours.

The S phase specific nature of the immune signal also raises a potentially important issue around combination therapies with immune-checkpoint inhibitors. Interestingly direct activation of the STING pathway using synthetic cyclic dinucleotide molecules has been reported to enhance responses to PD1 antibodies, which is in keeping with our data [20]. Another logical combination may be an S phase specific DNA damaging agents such as cisplatin along with a PD-L1 inhibitor. Anti-microtubule agents, however, may antagonise PD-L1 inhibitors by causing cell cycle arrest in the mitotic phase thereby preventing the STING-mediated immune response. Additionally we also expect that these effects are not specific to PD-L1 as we have demonstrated activation of a number of additional immune checkpoint targets in DDRD positive tumours.

In summary, we have identified the mechanism of immune response in breast tumours deficient in DNA repair. Activation of the innate immune STING mediated pathway is responsible for chemokine production in response to DNA damage *in vitro*, resulting in an inflammatory microenvironment in DNA damage repair deficient breast tumors. Expression of PD-L1 is associated with tumors deficient in DNA damage repair, and we provide a rationale for investigating the role of immune treatments in the context of endogenous or exogenous S-phase DNA damage.

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Example 5

Recursive feature elimination was performed on the 44 gene signature to define subsets of signatures comprising a single gene up to 43 genes.

Samples

The DDRD training set comprising 107 samples with known DDRD status were used for this analysis.

Methods

The DDRD signature of length 44 was used as a starting point for this analysis, where the absolute weight of the 44 genes was considered as a means for ranking the individual genes. The lowest ranked gene, i.e. the gene with the lowest absolute weight, was removed from the signature and the model parameters were re-trained using partial least squares (PLS) regression with the 43 gene expression data against the DDRD class labels. The weighting parameters of the 43 gene signature were used to reduce the signature by one gene as previously described, and this process was repeated until only one gene remained. Leave one out cross validation was used to enable performance estimates to be calculated for each signature length evaluated. The performance of the signature was measured using area under the receiver operating characteristic curve (AUC), which assess the ability of the signature to discriminate between the DDRD positive and DDRD negative samples at each feature length considered. Details of each of the sub-signatures are provided in Table 3-45.

Results

Table 50 shows the AUC performance for predicting the subtype using a minimum of one gene up to 43 genes (see Tables 3-45 for details of the sub signatures). At a minimum of one gene, the AUC performance is significantly greater than 0.5, therefore it is possible to predict the DDRD molecular subgroup significantly better than by chance with a minimum of one gene.

Table 50 - AUC performance for predicting the subtype using sub signatures of 1 to 43 genes

Sub signature size	AUC (leave-one-out CV)
1	0.7694
2	0.7925
3	0.7847
4	0.7866
5	0.7910

6	0.7899
7	0.7746
8	0.7873
9	0.8000
10	0.8026
11	0.8190
12	0.8172
13	0.8276
14	0.8265
15	0.8265
16	0.8377
17	0.8459
19	0.8496
20	0.8642
21	0.8612
22	0.8679
23	0.8813
24	0.8847
25	0.8899
26	0.8948
27	0.8937
28	0.8996
28	0.8526
29	0.9000
30	0.9049
31	0.9101
32	0.9108
33	0.9157
34	0.9149
35	0.9231
36	0.9231
37	0.9216
38	0.9228
39	0.9257
40	0.9269
41	0.9310
42	0.9306
43	0.9317

Example 6

In silico validation of the DDRD assay in a cohort of melanoma patients treated with immune checkpoint modulators and/or DNA-damaging agents

Methods

This study analysed RNAseq gene expression data from a TCGA cohort of 474 patients with skin cutaneous melanoma. Level 3 normalised gene expression data was downloaded from the TCGA data portal and the data matrix reduced to include only the DDRD genes. To remove zero counts in the data matrix, a constant value of 0.01 was added to all gene expression values and the resulting data matrix log transformed (using natural log).

DDRD assay scores were generated (as described in Mulligan et al. 2014) and dichotomized such that 75% of samples (with highest DDRD scores) were classified as DDRD-positive and 25% of samples (with lowest DDRD scores) were classified as DDRD-negative.

Patients who had received an immune based therapy (immune checkpoint modulator such as Ipilimumab or pembrolizumab) and/or a DNA damaging agent were subsequently analysed for differences in survival outcome based on their DDRD classification. Kaplan Meier plots were used to visualise differences in survival probability for DDRD positive vs DDRD negative and the Logrank test used to assess if the survival curves differ significantly. A hazard ratio was also calculated for the DDRD assay to estimate the relative risk of an event occurring in the DDRD positive compared to the DDRD negative group. The endpoints used for this analysis were time to local recurrence, time to distant recurrence, time to death (overall survival).

Results

Figures 31, 32 and 33 respectively are the Kaplan Meier survival graphs illustrating the difference in survival probability by DDRD status, for time to local recurrence (Figure 31), time to distant recurrence (Figure 32) and overall survival time (Figure 33). The resulting analyses of each endpoint demonstrated that, in a cohort treated with an immune based therapy (immune checkpoint modulator such as Ipilimumab or pembrolizumab) and/or a DNA damaging agent, patients in the DDRD positive group have a significantly lower risk of an event occurring after treatment compared to patients in the DDRD negative group:

- Time to local recurrence: HR = 0.39 [95% CI: 0.18-0.84], p = 0.0008
- Time to distant recurrence: HR = 0.44 [95% CI: 0.19-0.99], p = 0.0095
- Time to overall survival: HR = 0.31 [95% CI: 0.12-0.81], p = 0.0006

Summary

This data demonstrates that the DDRD assay identifies a group of Melanoma patients that have significantly improved survival following treatment with an immune based therapy (immune checkpoint modulator such as Ipilimumab or pembrolizumab) and/or a DNA damaging agent which have been licensed for use in Melanoma.

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SEQUENCE LISTING

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ACCATCCTGGCTAACACAGTGAAACCCTGTCTCTACTAAAAAAATACAAAAAATTAGCTTGGCGTGG
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GCGGAGCTTGCAGTGAGCCAAGATCGCGCCACTGCACTCCAGCCCGGGTGACAGAGCAAGACTCC
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BRMX.941C2n2_at (SEQ ID NO:12)
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CAGTACATATTCAATCTGCTGAGAAATGTAGACAATAACTACTATAAGAATCCTAATGCTACAGAAGT
CACTGGCTGCTGGGAAACCGGGGAAAACTTGGCTATGGACGTGGGGGCTTGTGTCGGACTCTGAA
TAAAGAGCAGAATGATTGGCGTCCTACTGAGATACATAGTAAAGGGGGCGAGGGCAGGGAGGAAG
TGGCAAGAATAACATTTGTGAAGATGTCCAGGTGAGAAATAGAGGTTTTAATGCTCAAGATGTTTCCT
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BRMX.4154C1n3_s_at (SEQ ID NO:13)
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CAGCTCCCAAAGGATAAACCAGCAGTTGCTTTATTGGTCTTCAGATGTGGCTGCAAACACTTGAGAC
TGAACTAAGCTTAAAACACGGTACTTAGCAATCGGGTTGCCAGCAAAGCACTGGATGCAAGCCTTG
CCTTCCAGAAGCTTACCAGTCGGGTTGCCAGCAAAGCAGTGGATGCAAGACTTGCCCTCCAGGAGC
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CTACTATAAGAATCCTAATGCTACAGAAGTCACTGGCTGCTGGGAAACCGGGGAAAACTTGGCTATG
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BRAD.39498_at (SEQ ID NO:14)
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BRAD.34868_s_at (SEQ ID NO:15)
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CCATCGTTTTCTTCACTGTCCTCCAAGCCAGCGGGAATGGCAGCTGCCACGCCGCCCTAAAAGCAC
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CAGGCCAGGCCAGCTGCCTCCGCGTGATCAAAGCCACACTCTGGGCTCCAGAGTGGGGATGACAT
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AAAAA

Hs505575.0C1n42_at (SEQ ID NO:16)
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BREM.1442_at (SEQ ID NO:17)
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BRHP.827_s_at (SEQ ID NO:18)
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GTTGAGCTCTGTGCAGTAATCGAGATTGGGAGAATTTGGGCAGCGCGTGAGAAGTGCTAAGCTACT
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BRRS.18322_s_at (SEQ ID NO:19)
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CTCATTCAGTCTGCTTTGGAGTCTACTGAACATCAAGCTTGCTATGAGCAGGATCTTAGAGCTGAGG
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GAGGGCTGTGCTGGTGTAAATGGTGACATATTGAGCTGGGGGGATGCTTTCGGGGTGGGGGGACT
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AGAAAAGATGTTTGTTTTCCATGTCATTACCCCCAGGGGATACCGAATGTGTGGGTAGAAATTTCTC
TGTAGATTAAAAATCAGATTTTTACATGGATTCAACAAAGGAGCGTCACTTGGATTTTTGTTTTCATCC
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BRRS.18792_s_at (SEQ ID NO:20)
GCACGTCTACGGGGCTGGACAGAGTGTGGTTAACCGGGGAACTGGGCAAGCCGGCGCCGAGCCT
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TGCACCCCTGTATACCTGGTCCTGGCAGTAGACAATCTGTTCCATCTGGAAGTGGAGGCGGATCAG
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CT

Hs632609.0C1n37_at (SEQ ID NO:21)
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AGAGTATTAACACTTTAACTCTTAGATCCTTGGAACATAAGGAAGAGAGGCTGGAACAAAAAGGGGT
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GCCAGACACGTGGCTCACACCTCTAATCCCAGCACTTTGGGAGCTGAGCCAGGAGGATTGCTTGAG
TCTAGGAGTTCAAGACCGGTCTGGGCAACATGGTGAAACCCTGTCTCTACAAAAAAATACAAAAATT
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CCTGAGC

Hs449575.0C1n22_at (SEQ ID NO:22)
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AGTAAAGATACAGTTGATTAGGTAGATTTGAATGTTGAATCACTGACATTTCCTGAAGGTAGAGCTAC
AAATTACTTTTTTAAAACCACTAACCCACCCCCACCTTACCTCACTTACTCTTTTTGGCCTTACCACCT
ACTTTAGTCATACCCTATACATGTTACTCAGACCAAATGGCTCTCATAAACAATCTCAGTATATGT

BRAD.18827_s_at (SEQ ID NO:23)
TTAAGAAGGTATGGAAAGAGTCTGGGAGTGACTAAACTATCCAATGTCATTGAAATAAAGCAATGAA
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NNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNGGGACTACAGGCACATAC
CACCACACCTGGCTTCATGTTCCCGGTATTAGTACAATGCCAAAATATTTAAAATTCTTAAAGGTTAA
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BREM.2466_s_at (SEQ ID NO:24)
GCCCGTGCCGCCCCAGCCGCTGCCGCCTGCACCGGACCCGGAGCCGCCATGCCCAAGTGTCCCA
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ACCCTACTGCAACCACCCCTGCTACGCAGCCATGTTTGGGCCTAAAGGCTTTGGGCGGGGCGGAG
CCGAGAGCCACACTTTCAAGTAAACCAGGTGGTGGAGACCCCATCCTTGGCTGCTTGCAGGGCCAC
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AGTAAACCTGAACACTTGGAAAAAAAAAAAAAAAAAA

BRAD.2605_at (SEQ ID NO:25)
CAACCAGGAAGAACCGTACCAGAACCACTCCGGCCGATTCGTCTGCACTGTACCCGGCTACTACTA
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GGTCCGACGCTCCCTGGGCTTCTGTGACACCACCAACAAGGGGCTCTTCCAGGTGGTGTCAGGGG
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TATTGCTTCAGCTGCTGAAGGGAGGGGGCTGGCTCTGAGAGCCCCAGGACTGGCTGCCCCGTGAC
ACATGCTCTAAGAAGCTCGTTTCTTAGACCTCTTCCTGGAATAAACATCTGTGTCTGTGTCTGCTGAA
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AGAGTCA

BRAD.33618_at (SEQ ID NO:26)
GGGTCGACCCTTGCCACTACACTTCTTAAGGCGAGCATCAAAAGCCGGGGAGGTTGATGTTGAACA
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GCAGCAGTATTACACAGGATACACAGAGAATGAAGTATTGGAAGTCATGCAGCACATGGCCAAGAA
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AGGAAGGTCCTAGGCTGCCGTGGGCCCTGGGGATGTGTGCTTCATTGTGCCCTTTTTCTTATTGGT
TTAGAACTCTTGATTTTGTACATAGTCCTCTGGTCTATCTCATGAAACCTCTTCTCAGACCAGTTTTCT
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BRAD.36579_s_at (SEQ ID NO:27)
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CTGCCCTCCTGATGTGCATATCCGTACTTCCATGAGGTGTTTTCTGTGTGCAGAACATTGTCACCTC
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AATCAAGTGATTGGTGTGCCTGGGTCTCACTACAAGCAGCCTATCTGCTTAAGAGACTCTGGAGTTT
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BRAD1_5440961_s_at (SEQ ID NO:28)
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GGGGATGTCCTTTGCACTGACCCTGAACTGCCAGATTGACAAAACCAGCCAGACCATAGGGCTATG
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BRAD1_66786229_s_at (SEQ ID NO:29)
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ACTGCA

BREM.2104_at (SEQ ID NO:30)
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TCCTGATCCGGTACATGTTCCGCCACAAGGGCACCTACCATACCAACGAAGCAAAGGGGGCGGAG
TCGGCAGAGAGCGCGGACGCCGCCATCATGAACAACGACCCCAACTTCACAGAGACCATTGATGAA
AGCAAAAAGGAATGGCTCATTTGAGGGGTGGCTACTTGGCTATGGGATAGGGAGGAGGGAATTACT
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CCTTTTTAATATTTCTTTATAGCTGAGTTTTCCCTTCTGTATCAAAACAAAATAATACAAAAAATGCTTT
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BRAG_AK097020.1_at (SEQ ID NO:31)
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CTTGTAGCTGCTTGTCTCCTACTATACCTGTCTGACACAATATCACCAGAGCAAGCCATAGACAGCC
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CAAATAGCATATATATGACCATGTCTGAAATGTCAGTTCTCTAGCATAATTTGTATTGAAATGAAACCA
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BRAD.20415_at (SEQ ID NO:32)
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BRAD.29668_at (SEQ ID NO:33)
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ACCTCATAAGTTAATTCTAACTTTTATTCTTGAATTTTATTTCATTTCAATAGCTTGTTTCATTTGCACG
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BRAD.30228_at (SEQ ID NO:34)

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CTCCCTCCTCCCGACCCGCCCCCTCGCCCCCACCCCTGTGTGTTTCGCCAGTTAAGCACCTGTGAC
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ATGTT

BRAD.34830_at (SEQ ID NO:35)
TGCTCAGACCAGCTCTTCCGAAAACCAGGCCTTATCTCCAAGACCAGAGATAGTGGGGAGACTTCT
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TTTCTTCACTGTCCTCCAAGCCAGCGGGAATGGCAGCTGCCACGCCGCCCTAAAAGCACACTCATC
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AGGCCAGCTGCCTCCGCGTGATCAAAGCCACACTCTGGGCTCCAGAGTGGGGATGACATGCACTC
AGCTCTTGGCTCCACTGGGATGGGAGGAGAGGACAAGGGAAATGTCAGGGGCGGGGAGGGTGAC
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TGTTCTGCTTTCGATTCGTTAAGAGAGCAACATTTTACCCACACACAGATAAAGTTTTCCCTTGAGGA
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AAAAA

BRAD.37011_s_at (SEQ ID NO:36)
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ATACCTGCCCTGACATGCACACCTCCCCTGCCCCACCAGCCCACTGGCCATCTCCACCCGGAGCTG
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GAGACGTACGTATCAGCGGCCCCTTGACTCTGGGGACTCCGGGTTTGAGATGGACACACTGGTGT
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BRAD.37762_at (SEQ ID NO:37)
GGGCGGTTCTCCAAGCACCCAGCATCCTGCTAGACGCGCCGCGCACCGACGGAGGGGACATGGG
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TTTATTCCAGTGAAACAACAACTGGAACTTCAAGTAACTCCTCCCAGAGTACTTCCAACTCTGGGTTG
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BRAD.40217_at (SEQ ID NO:38)
ACCCTGTGCCAGAAAAGCCTCATTCGTTGTGCTTGAACCCTTGAATGCCACCAGCTGTCATCACTAC
ACAGCCCTCCTAAGAGGCTTCCTGGAGGTTTCGAGATTCAGATGCCCTGGGAGATCCCAGAGTTTC
CTTTCCCTCTTGGCCATATTCTGGTGTCAATGACAAGGAGTACCTTGGCTTTGNCACATGTCAAGGC
TGAAGAAACAGTGTCTCCAACAGAGCTCCTTGTGTTATCTGTTTGTACATGTGCATTTGTACAGTAAT
TGGTGTGACAGTGTTCTTTGTGTGAATTACAGGCAAGAATTGTGGCTGAGCAAGGCACATAGTCTAC
TCAGTCTATTCCTAAGTCCTAACTCCTCCTTGTGGTGTTGGATTTGTAAGGCACTTTATCCCTTTTGT
CTCATGTTTCATCGTAAATGGCATAGGCAGAGATGATACCTAATTCTGCATTTGATTGTCACTTTTTG
TACCTGCATTAATTTAATAAAATATTCTTATTTATTTTGTTANNTNGTANANNANNATGTCCATTTTCTT
GTTTATTTTGTGTTTAATAAAATGTTCAGTTTAACATCCCANNNGAGAAAGTTAAAAAA

BRAD1_4307876_at (SEQ ID NO:39)
CTCCTGGTTCAAAAGCAGCTAAACCAAAAGAAGCCTCCAGACAGCCCTGAGATCACCTAAAAAGCT
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CCTCCTGGTTATGGTACAGATACAAACTGGACTCTCAGGACAAAACGACACCAGCCAAACCAGCAG
CCCCTCAGCATCCAGCAACATAAGCGGAGGCATTTTCCTTTTCTTCGTGGCCAATGCCATAATCCAC
CTCTTCTGCTTCAGTTGAGGTGACACGTCTCAGCCTTAGCCCTGTGCCCCCTGAAACAGCTGCCAC
CATCACTCGCAAGAGAATCCCCTCCATCTTTGGGAGGGGTTGATGCCAGACATCACCAGGTTGTAG

AAGTTGACAGGCAGTGCCATGGGGGCAACAGCCAAAATAGGGGGGTAATGATGTAGGGGCCAAGC
AGTGCCCAGCTGGGGGTCAATAAAGTTACCCTTGTACTTGCAAAAAAAAAAAAAAAAAAA

BREM.2505_at (SEQ ID NO:40)
GCCATCAAGAATTTACTGAAAGCAGTTAGCAAGGAAAGGTCTAAAAGATCTCCTTAAAACCAGAGGG
GAGCAAAATCGATGCAGTGCTTCCAAGGATGGACCACACAGAGGCTGCCTCTCCCATCACTTCCCT
ACATGGAGTATATGTCAAGCCATAATTGTTCTTAGTTTGCAGTTACACTAAAAGGTGACCAATCATGG
TCACCAAATCAGCTGCTACTACTCCTGTAGGAAGGTTAATGTTCATCATCCTAAGCTATTCAGTAATA
ACTCTACCCTGGCACTATAATGTAAGCTCTACTGAGGTGCTATGTTCTTAGTGGATGTTCTGACCCT
GCTTCAAATATTTCCCTCACCTTTCCCATCTTCCAAGGGTATAAGGAATCTTTCTGCTTTGGGGTTTA
TCAGAATTCTCAGAATCTCAAATAACTAAAAGGTATGCAATCAAATCTGCTTTTTAAAGAATGCTCTTT
ACTTCATGGACTTCCACTGCCATCCTCCCAAGGGGCCCAAATTCTTTCAGTGGCTACCTACATACAA
TTCCAAACACATACAGGAAGGTAGAAATATCTGAAAATGTATGTGTAAGTATTCTTATTT

Hs149363.0CB4n5_s_at (SEQ ID NO:41)
GGGAAATCAGTGAATGAAGCCTCCTATGATGGCAAATACAGCTCCTATTGATAGGACATAGTGGAAG
TGGGCTACAACGTAGTACGTGTCGTGTAGTACGATGTCTAGTGATGAGTTTGCTAATACAATGCCAG
TCAGGCCACCTACGGTGAAAAGAAAGATGAATCCTAGGGCTCAGAGCACTGCAGCAGATCATTTCA
TATTGCTTCCGTGGAGTGTGGCGAGTCAGCTAAATGGCAGGGGCAGCAAGATGGTGTTGCAGACC
CAGGTCTTCATTTCTCTGTTGCTCTGGATCTCTGGTGCCTACGGGGACATCGTGATGACCCAGTCTC
CAGACTCCCTGGCTGTGTCTCTGGGCGAGAGGGCCACCATCAAGTGCAAGTCCAGCCAGAGTATTT
TATATAGGTCCAACAACAAGAACTACTTAGCTTGGTACCAGCAGAAAGCAGGACAGCCTCCTAAATT
GTTCATTTACTGGGCATCTACCCGGGAATCCGGGGTCCCTGACCGATT

Hs172587.9C1n9_at (SEQ ID NO:42)
AACGAAAGTCTAGCCTTTCGTACCCGTATATATAAAGACACCCCTGTTCTGATTGGACAAGGCAGCC
TTTCCCCTGCAGCTCGATTGGTGGAGACGCCCACTCCCTGACAGAACATCTCCTGCATGTAGACCA
AATATTAAAACTTTCCTCCGTCCATCTTTAACTGCTGGTGTTTTCAACCCTTTCCCCTCTGTGCCATG
TTTCTAGCTTTTATTTAAAACGTACTTTGGTTTTCCTTGGCAAAATTGTGTCTAGCTACTAGGATGACG
TGTCTTAATTTTTTTTTAAATGTTGGCGCTGAAACTGGCTTTGATCAACGTTTTAAAAAGACGCGCGC
TAGTTGTGATTGGCCAAGTGATTTCTTCTTACCCTCTTAAGTTTAGAAAGGTTAATTTCATATCTTGAT
TTGTCTATTTAAACTTGGAGATATTTTCAATAATTTGTTCCAAATGCACCATGACTATTAACTCATAAG
TAACAATATGAAACCTGATGTTAAGCTACATGAACACATTTAATTTCACCACAATATGACATCCTCATA
TGAAAGCACTCTCTTATCTTTTACAAGTTCAACTGGTATTTGTGTAATCTGCTGT

Hs271955.16C1n9_at (SEQ ID NO:43)
TGCTACCATGCCTGACTAGTTTTTGTATTTTTAGTAGAGACAGGGTTTGACCATATTGGCCAGGTTG
GTCTTGGACTCCTGACAAGTGATCCGCCCTCCTCNNNCNCNCGAAGTGCTAGGGTTACNAGGTGTG
AACCACCATGCCTAACTATCGTTGCTACTTTCTATTGGAAGAGAAGGCAGCCCTGATTTAGTCTGTTT
ACAGTCTGCATTATGTGGAGAATAGAGAGCCATCATAGTCCCTAAAACTTTCCTTGCCAGTTAACCC
AGCAGGACAACCTGTCTTTGTCTCTTGACAACTGTTAACTGAGAACAGGGCCCTTGCTCCTCTAGGT
GTGCACATTAAGGACTTTGCACAGTGTGGATGTAGCTCATGCTGCTCTGCCNTNNAGTACATGCTGC
TTGAATTTTCATCATNANCCTCCACNCCTTNCACCTNCNNGNNAAAAAAAAAGCGTGCAGGAAGTAG
CATTTCAGATCCTTCTCCACCACCTCTGCTTCCCTTCTCCCTTCTTTTCCTCCTTGCAGCATTCCCTT
TAGTACNAGGGAGGGATGGTGGTTGAAAATGGGGGGAATGATGTTGCTCAGAAAAAAAAAAAA

Hs368433.18C1n6_at (SEQ ID NO:44)
ATAATGCTGGAAACAGAAGCACCAAACTGATTGTGCAATTACTCCTTTTGTAGAAGAGGCCAAAATC
CTCCTCCTCCTTCCTTTCTCCTATATTCACTCCTCCAGGATCATAAAGCCTCCCTCTTGTTTATCTGT
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CAACCTTCCCTCGGAAGGAGACAATTCGAGGTGCTGGTACATTTCCCTTGTTTTCTATGTTCTTCTTT
CTAGTGGGTCTCATGTAGAGATAGAGATATTTTTTTGTTTTAGAGATTCCAAAGTATATATTTTTAGTG
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TCCCATAGCAATTTATGGTAAGAACAAGACCCCTTTCCCTCACCACCGAGTCTCGTGGTCTGTGTCT
GTGAACCAGGGCAGGTAATTGTGACACTGCATCTCATAGAACTCTGCCTGCCCAGATTTTTGTGTGC
TCACCTCAATGGGTGAAAAATAAAGTCTGTGTAAACTGTTAAAAAAAAAAAAAAAAAAA

Hs435736.0C1n27_s_at (SEQ ID NO:45)
TCCTCAGACCCAGTAATTCCACCCCTAGGAATCCAGCTTACACACACAAGAAAGAAAAGATAAATGT
ACAAGGTTAGTCACTGCACAGTGAGACAGCAAAAGATTAGAAAGAACCCAAGTGATTATTGATCTGG
GTTTTATTCCTTTATAGCCCAACCATATGATGGAATACTATAATGTTGTAAAAATGGGTTAAGAGTTCT
TTATGAATTGGTGTGGAAACATCGCCAAGATATGAAAGCCAAATGCAGAAAAATATATGTGGTATGC
TATTATCTATGTGAAAAAGACATTACTATTCTCTGGAAGGATAAACACAAATTTGAGAATGGTGGATA

TCTGGGGTGAGAGGTATCCTTTTCACTGTTCTTTAAAAGTTTTGNNATTTTGGTGTTTGCCTATTCAA
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GTTAAAAAAAAAAAAAAAAAAA

Hs493096.15C1n6_at (SEQ ID NO:46)
GCCCATAGTCCCATCTTTTTACAGGCATTTTTTACACCTGGAGCAGCCAGAGGACGCATGCATGGCT
CTTCGGAAGGTAATTTAGGGATCACCCATGTAAGTTTCCTAAGGATTTCTTTAACATGGTTCTTCTGA
TTCAGTCCGGCCAATTAAATCTAAATCCACCCCTGAAAGCCATCTGGTGTGGATAACAAGCCCACAA
ATGAGCAGTCAGCTTTTTGTGCCCTTTAGGGCCTGGGACAACCACGGGATCTAAAAGGGGCTGGAA
CTAGAGGTCTTGAGCTCCTGTTCCTAAAATCATCTTCATCCTATATCTGCAGCCTTCTCCTGCCACG
GCATGCACCCACACATGCGAGCCTCCCGGGTACTGTCATCCTGAATTCTGAGACCATCCAGCACTT
CCTTTAGTTTTGCCCTGGTGCTGTTGACTTTTGTTTACTGAAGAGTGTGCTGGAGGCAGGACAAGGG
ACATGGAAGGCTGCAATTTAAGAGTCTAAAAGGTTTTAGAATCCTGAAGGAGGTTTAACAAGCTGAA
TTGAAGAATAATACCTTTCTCAACTGGAGAGAATTTACATGATTGCATTATTGTTAAAATTAACA

Hs493096.2C1n15_s_at (SEQ ID NO:47)
ATCATTTAGTTGAATCATTATAAGTCTAGGACTGTCTGTAGATGTAAATTTGTTAAGAATTAGGACTCA
AGAGTAGAATTCCTTTAATCCACATAGACTTACAATGGTGCTGTGCACATGGAGCCCCTAAATCATT
GCTGACTGAGTAGATTTCCCAGGGTAAGCCCAAGAAGTTACTCCTAGAAGGGGCTGGTAGGGGAAA
GAGCCAACATCCCACATGCCTGCCCACTTTGGGTCTGGTCCCAAGAAACAAACTCCAGTGGCCTCG
AAAATTTAATATTGCTGTCAGAAGGGCCTCCCCTTCAAAGGAACAGGTCCTGATAGCTCTTGTTATAT
GCAAAGTGGAAAGGTAACGTGACTGTTCTCTGCATTTCCTGCCTTTCAATTGAGTGAAGACAGACAG
ATGATTTATTGGGCATTTCCTAGCCTCCCCTTCACCATAGGAAACCAGACTGAAAAAAAGGTGCAAA
TTTTAAAAAGATGTGTGAGTATCTTGAGGGGGCTGGGGGAGAATTCCTGTGTACCACTAAAGCAAAA
AAAGAAAACTCTCTAACAGCAGGACCTCTGATCTGGAGGCATATTGACCATAAATTTACGCCA

Hs592929.0CB2n8_at (SEQ ID NO:48)
TTTTTCTGAGCAACATCATTCCCCCCATTTTCAACCACCATCCCTCCCTGGTACTAAAGGGAATGCTG
CAAGGAGGAAAAGAAGGGAGAAGGGAAGCAGAGGTGGTGGAGAAGGATCTGAAATGCTACTTCCT
GCACGCTTTTTTTCTTCTTGGAGGTGGAAGGAGTGGAGGATGATGATGAAAATTCAAGCAGCATGTA
CTAGACGGCAGAGCAGCATGAGCTACATCCACACTGTGCAAAGTCCTTAATGTGCACACCTAGAGG
AGCAAGGGCCCTGTTCTCAGTTAACAGTTGTCAAGAGACAAAGACAGGTTGTCCTGCTGGGTTAACT
GGCAAGGAAAGTTTTAGGGACTATGATGGCTCTCTATTCTCCACATAATGCAGACTGTAAACAGACT
AAATCAGGGCTGCCTTCTCTTCCAATAGAAAGTAGCAACGATAGTTAGGCATGGTGGTTCACACCTT
GTAACCCTAGCACTTCGTGGGCAG

Hs79953.0C1n23_at (SEQ ID NO:49)
ATCAGAACAATTTCATGTTATACAAATAACATCAGAAAAATATCTTAAATTATATGGCATATTCTATTGA
TTCATCCACAAATTTATAAGTCCTTACCACCTTTCATTATATTGGTACTAGGCATTATAGTAGTGCTAG
GCACTATAGTAATGCTGGGGTATAAACAAGAATAAAACAAAATAAGTTCCTTATTTCAGGTAACTTAC
AGTATAGGTCAGTGGTTCTTAGCTTGCTTTTTAATTATGAATTCCTTTGAAAGTCTAGTAAAATAATCC
AACACCATTATTCCCCATTGCACATACCCCCAGATGTTTTAGACATATTTTCAATTGCTCCATGGACC
TTAAGAAAACTTGGTTGGTGTGCAGTTTGGTGTATTATGGGTAAGACTGGACCTGGTGTTAGAAAAT
CTGCATTTGAGGCTTTGTTCTGACAGTGTCTAGTGTAAACATGGGCAGACCACTTAAACCTCTCTTTA
GTCTTCTCTGTAGAATGATGATAATACCATCTAATTAGCAGGATTGTTGTTTTATTCAGTGAGACAGC
ATATGTAAATAACTTAGTAAAATAAAAAGCAACGTGTTTATAATGGTAAAAAA

BRMX.2377C1n3_at (SEQ ID NO:50)
TGGGAATCATGAACTCCTTCGTCAACGACATCTTCGAACGCATCGCGGGTGAGGCTTCCCGCCTGG
CGCATTACAACAAGCGCTCGACCATCACCTCCAGGGAGATCCAGACGGCCGTGCGCCTGCTGCTG
CCCGGGGAGTTGGCCAAGCACGCCGTGTCCGAGGGCACCAAGGCCGTCACCAAGTACACCAGCG
CTAAGTAAACTTGCCAAGGAGGGACTTTCTCTGGAATTTCCTGATATGACCAAGAAAGCTTCTTATCA
AAAGAAGCACAATTGCCTTCGGTTACCTCATTATCTACTGCAGAAAAGAAGACGAGAATGCAACCAT
ACCTAGATGGACTTTTCCACAAGCTAAAGCTGGCCTCTTGATCTCATTCAGATTCCAAAGAGAATCAT
TTACAAGTTAATTTCTGTCTCCTTGGTCCATTCCTTCTCTCTAATAATCATTTACTGTTCCTCAAAGAA
TTGTCTACATTACCCATCTCCTCTTTTGCCTCTGAGAAAGAGTATATAAGCTTCTGTACCCCACTGGG
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BRAD.33405_at (SEQ ID NO:51)
GAAAGTGATAATACAGAAAGGTGGGGCTGGTGTAGGGNTNAAGNCAGGATGCTTTGGNANAGCATG
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CNGATGAGNCACTTAGAAGNAGCAATTAGTTAGGCAAAGGGAAGTGAATGTGNAGGAGGAACAAGC

ATTCCAGGCAAGAAGAACACCCTATCGAAAAGCCTGGAAGCAAAACATTAGTGAGGCTACCTTTCAT
AAATTGCTTTCTGTAAGTCATGCCATTGTGTAGTCTTAATTGCTTTCTCTCACCAGGGAAGGTGTGGG
AAGGACTTGTGAAATACATATTCGAGGAAAAACTATGCACAAGGCCGTGCATTTAAAAATAAACTCC
CTAAGGCTGGGGTGAAACCTGCTACGGTCTGCGCAAGTTGACTGTTAATGAATTTGATTCTCAGGTG
TGAGTGATTAAAAGAACACTGATCATGTCATTTTCTTTTTGGTCACTAATTCCCTCCCTCCCTTCTCTT
TCTTTTCTTTTTTCTTTTCTTTTCTTTTTCTTTCTTTCTTCCCGACAGAGAAAGACTCCATCTC

Hs584242.2C1n64_at (SEQ ID NO:52)
TAAGATGTTTAAGTATATCCAACCGTCCCAGACCACATTGGCCTATTTCCTCCTCTTGGCAACACTGC
TCGGGTTTTCCCCTCGCATCATCCTTATGCTATGACACTGGACTAAATTGTAATAATACATTTTCTTGT
TAATCTCCTCATTATACTATGAGCTCCTTGAGGACAGGTACTTTGTCTTGCTCACATCTGTAGATTCA
ATGCCTGGCACAGCGATTGATATTGCAAGGGCACTTAATAAATGGTTTTTGAATAAAAGAATTGCTTA
AAGTAAAATATAGCTGTAAATTGTATTATAAAAGGACAGTGGGTGGCAGTCTGAGGTCTGCTATTTAC
TGGTTTGGGCAAGTTACTTAATCTGTTTGCTTCCTCAGCTGTACGATGGGTAAAATAATAGTGGTTAT
CACAACAGGGTGGTTACAGCGATGAAATGAGATTATGTGTGTAGGCTACCACATAATTGTAAAGCTG
ATATTTAAATGGAACAGATACTGCACAGACACTTGAGGTCTGAGAATAAGATTAGGTCAACCAGAGT
ATTAATGGGTTAAATAAAGGTGACATCCTATGCAACCAACGGTTTGATCTTTATGCT

BRRS1RC_NM_004065_at (SEQ ID NO:53)
GTCTTCCAGTCAGTCAGTGTCTTCCAGAAAAATCTACGTCTTCCACCAAATCCAGGTCTTCCAGTCA
ATCCACATCTTCCGGAAAAAATCCAGGTCTTCCAGCCAATATATGTCTTCCTGAAGATCCACGTCTTC
CAGAAAATCCATGTCTTCCAGAAAATCCATGTCTTCCAGTAACCTCCCAGTCTTCCAGAAAATCCAC
GTCTTCCCAACAATCCAAGTCTTCCGGATAATTTGGGTCTTCCTGAAAATCTACGTCTTCCAAAAAAG
CCATGTCTTCCAGAAAATCCACATCTTCCAATGGCCTCCAGGTCTTCCAGACTATCCATGTCTTCCA
GAAAATCCTTGTCTTCCCTTAAATCTATAGCTTCCAAAAAATCCGGGTCTTCCAGGAAATCCGTGTCT
TCCAGCAAGTCCACGTCTTCCAACAAAGCCATGTCTTCCAGACTATCCATGTCTTCCAGAAAATCCTT
GTCTTCCCTCAAATCCATAGCTTCCGAAAAATCCAGGTCTTCCAGGAAATCCGTGTCTTCCAGCAAA
TCCACGTCTTCCAACAAAGCCATGTCTTCCATCAAATTAATGTCTTCCAGCCTACTTGTG

BRRS.8182_at (SEQ ID NO:54)
AGCATCGTTTATGAAAACAACTAAATATTCACTAATGGTGCCAGTGGAATAAATCAGAGAACATCCCC
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GGCCATGGGCATGTGGATATGGCCATGAATGAGCAAGATCCTCTCTGATCCTGTAGAAGTTAAGTTC
TACCAGATAACTTGCTGCTTCAACAAAAAGATTTACCTTTTTAAATAAATGTTGTAGAATACTTAAAAA
AAACAAACTAGAATTTGCCTGTGTGCAGCCAGTAACATGTCTATTTAACCTGGACACCTTTTGAGGAA
TATTCTCAGATTGCCCCCATGCTGTTTATAAGACATTGTTCCTTATACACCTGTTTATGAATGAAAAGA
AACATAAGGAGTGGGTACAAAGACTTCTATCTATGAATGATTAAAAAGGCTAGAGTACGAATACTTCT
TGAACCTTTGGTACTAAATGCTTTTCATGTTCTATATAAATGTAGAAAACATTTTACAAATCCTGTAAA
TAAACTGTTTATTTTTTATAGAAAGCCAAAAAAAAAAAAAAAAAAAAAAAAAA

BRMX.13815C1n5_at (SEQ ID NO:55)
TCTTTCAACATTTAGATAGTCTTTCTTAATATTTCCAGGAGAGTACCTCATTTTTATTTTGAAAACCATT
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ATTGATACATAGTACTTAATTACATGTTATTCCATGTACACTGAAAACAATATAGGAAATATATACATC
TAAGACTTCTACTTTGTACAGTCTTTCATTAAATAAGAATACTTACACATACATTTTCAGATATTTCTAC
CTTCCTGTATGTGTTTGGAATTGTATGTAGGTAGCCACTGAAAGAATTTGGGCCCCTTGGGAGGATG
GCAGTGGAAGTCCATGAAGTAAAGAGCATTCTTTAAAAAGCAGATTTGATTGCATACCTTTTAGTTAT
TTGAGATTCTGAGAATTCTGATAAACCCCAAAGCAGAAAGATTCCTTAGTACCCTTGGAAGATGGGA
AAGGTGAGGGAAATATTTGAAGCAGGGTCAGAACATCCACTAAGAACATAGCACCTCAGTAGAGCTT
ACATTATAGTGCCAGGGTAGAGTTATTACTGAACCAACTTTTTTGTACAAAGT

BRMX.2637C1n26_at (SEQ ID NO:56)
TCCATCAGGGCACGGTAGAAGTTGGAGTCTGTAGGACTTGGCAAATGCATTCTTTCATCCCCCTGAA
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GGCGACTATCTGCGTCTATCATCCAGCACTTGACCATGATCATGTAGACATCGATGGTACATATGGG
TGGCTGAGGGAGGCGTTCTCCTTTCTCCAGGATGGAGGAGATCTCGCTGGCAGGGATTCCGTCATA
TGGCTTGGATCCAAAGGTCATCAACTCCCAAACGGTCACCCCGTAGCTCCAGACATCACTCTGGTG
GGTATAGATTCTGTGTAAAATTGATTCCAATGCCATCCACTTGATAGGCACTTTGCCTCCTTCTGCAT
GGTATTCTTTCTCTTCCGCACCCAGCAGTTTGGCCAGCCCAAAATCTGTGATCTTGACATGCTGCGG
TGTTTTCACCAGTACGTTCCTGGCTGCCAGGTCGCGGTGCACCAAGCGACGGTCCTCCAAGTAGTT
CATGCCCTTTGCGATCTGCACACACCAGTTGAGCAGGTACTGGGAGCCAATATTGTCTTTGTGCCAA

BRAD.36737_at (SEQ ID NO:57)
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AACTGCATTTTGCACAAAAGTGTTGATTCAACATGAAGTCAAATAATATGTTCTAATGAAACCACACC
TCTCACACACATATCCTTTCTCTCAAACCTCGGTGTTACTCTGGCCAAAAGTCTTAGGTTTCTTGAAG
TGTTTGTGGAAGAGTAGATGGAGTTTTATTTAACATTATCAAGAAATCCAAGCTGCAGACCCCACACA
TA

BRAD.3853_at (SEQ ID NO:58)
AGACTTTTTAGTAGCTTCCAACTACAAAAAAAGAGAAATAATCAATTATGTACTAATCAGACACTTTTA
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TCCTTTTAACATAATGAGTAATATTTGTGTTGGGAATGACTGAGAAGAAATTTCATAATGATGTGAAG
ATCTACCTGTAAATAGTTCCTCTGTCGTATGCTGGTATTTATATTCTAGCATCTCAACAGTGCTGATG
GTCACTCATCTTGGAGTTCCCTGAATTTTTTTTTTTTTTCAAAACTCCTGTAATGTTACATTACCCATA
CTTTTGTTGTTGCTGCTGTTGTTGTTGTTTTGAGACGGAGTGTCGCTCTGTCGCCCAGGCTGGAGTG
CANGTNGNNCCGCGCCCGGCACATGACTGCATACTTTCAAGGAGAGGACTCAGAGCTTTTATTTATT
TAAAGAAACTTGAAAGGAGGAAAGTGGATTAAGAAAAAAAAAA

BRAD1_19760734_at (SEQ ID NO:59)
TTTTTTTTTTTTTTACATAAAGGCATGAATATACAAGGTAATGTCAGCAGCTGTACTCCACTCTTTATT
CGTTGCAAATCTACCTATTTGTTTCCAAAGGATGTCTGCAAATAAATAGGTAACATTGTACAGCTTTC
AACAGTGGATCAGAACATAGATGTCTCTTCTAATTCACAAGTACCAATGGCTCAATTAATTTAAGGGA
CATTTTCTGAGTTGTGTGATTTCACATGTATTTATCGTGTCTAGAAGTGTGCAAACTTTTGTTTCATTT
CTCTCTTAGATTTCTGTAGGAAGAGTTAAAGGATGTGAAGTAGTCATTTTACTTATTCATAACACATTT
TAGGGAAAATTGTGCTGTTGCTGTTGGGGAGAAAGTTAAAGCTATCAACTATAACCTGGACTCCAGT
CCAATTTTTCACATCTGGTTGCTACTTTTAAAAAGGATCATTTTAATTTTTAAATGCAGAATGTGTTGC
ACTTTACCTTTGACATTCCAGGTTTCCTCATGGTCATTTAGAAAAATAAAGCAGGAAATTCTAATGCC
TTAGCATCTACTTTAATAAGATGTTTGCATTTATAAAAATAACAAGAAACTGA

BRMX.2797C4n2_at (SEQ ID NO:60)
TTTAATTTTTTGGAAGGATATACACCACATATCCCATGGGCAATAAAGCGCATTCAATGTGTTTATAA
GCCAAACAGTCACTTTGTTTAAGCAAACACAAGTACAAAGTAAAATAGAACCACAAAATAATGAACTG
CATGTTCATAACATACAAAAATCGCCGCCTACTCAGTAGGTAACTACAACATTCCAACTCCTGAATAT
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TTCTTACATTCTGGAGAACCCGAAGCTNCAGCTCAGCCCCTCTTCCCTTATTTTGCTCCCCAAAGCC
TTCCCCCCCAAATCATCACTCNCCTGCCCCCCTTAAGGGCTAGAGGGTGAGGCATGTCCCTCACAAT
TGGCACATGGTNCAAGGCCATCAGGCAAGGGNGCATTCACACAAAAGGGCACCAGG

BRMX.10399C1n5_at (SEQ ID NO:61)
GAAACAACTGGTAAACACAGTAAGCCCATTTCTGGGCTTTTAGAAAAACATTGCTCTCTTTTCTTTCC
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ATTCTGAACCAAAGGTAAACAGGGTTCCTCCCCATGCCTTGGAGAGCTCCAGTCTGCAGAAAGCTA
ATGAAGCCCTTGAAGCAGTATCTTGTCTTCCATCCACACTTTATTGAAATGCTTTTGAATCTTATTGTG
TTGTAATTACATACTATAGAAAACTCCGCCAACCTCTATTTCAAGGTTTGGGCCCATGACTCTCGCTA
AAACATTTCAGTTCCATTTTCCAGAACATACCATTTCTAAATGCATCTGTGAGGGCCCTCCACAAGTA
TTTTCAGTCCACATTTCAGAAAACTTGAAAGTGACGCAGGTTCCTGACTTAGTTGATGGTGGGTAAA
GGGAATGCCATTATGAGTGGTGGAGGTTGTTTTCTTTTTTCTTGCCATATTCTCAGCATAATATTTGA
AACCTACAAAAGAAGTTTGATAATATAACTGTATATTTTATGCCTGCACTAGTGGAGGA

BRMX.8912C1n3_at (SEQ ID NO:62)
GAGGTAGGAACTGATATTCCCATTGTACAGATGAGAAGACAGATGCTCAGAGAGCTTATTTGTCTGT
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ATTTGGATCAAGTGACTAAATAAATCACACACCACACACATTAAGATACGCCAGAGTGACAGGGACA
TTAAATAAATCAAGTATCCATGAAGTTTGCTGCCTTCCAAATCAGCCCCCTATTCTTTTGCCCTAAGA
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GAATGACTCTAGGAATCCTGTTGAGTGTATGTGTGTGCGTGTTCTTTTCTTTTTCTCTCATGAATATTA
CACTTTTATTAGCCAGCTATACTTGTGTTGATGAAAAAGACAAAATGGAATTTTGTTTTCCTTTAACAA
TCAAGTATGAATGGTCTGCTTACAGGATGTCCCTTCTTGGGGTCCTTGGAGGTAACAAAAGCTCATC
ATTAAACAGGTAGCTATCATTTCTACATGCTTAGTATCACTTCCGATTATCTTATTC

BRMX.13731C1n18_at (SEQ ID NO:63)
GGGCTGAGGGTCCTGAGGAGAGAGAGAGAGGCCACGTGGATGGAGGACTGTCACCCCCTTCTCG
GTTCTGTCACCCCCTTGAGTCTAACTCACTGTTGAGGGGAGGAAGAAGGGGGATGGACGGAAGGG

AGACCGAGGAAAGGCTTTCGGGAGTGGGGACATTATCCCCCCAGAGGTGTGCTGCCCCACCCAGC
TGCACCCCACAATCTGGCCAACTCATTTCACAGTATAAATCACTCCAGCAGGACGGCATCACAGCAG
CCCCTGCTGCCTGAAATCAGAGCGGCCCAACGAGGAAGGCCAGGAGGGTCGGCTGGCAGGGGGC
AGGGTCTTGGGATAACACTGTCATCAGAAACAAGGCTGGGGGCTGATTTCGGGGTGGGGAGCCTT
AGGAGGCCAGAAATTCCAATCAGAGCCAGTTTTTCTGGGAGGGAGTGGCTAGACAGTCAAGGAAGG
ACGTTCACATTTCAAAAGAAGTCGGGTGGGGGGATGAGATTATTCTAGGGGGGCATCGAATTCCCT
TTAAGGGGGGGGCTCACTTCTGCCCAGAGTAAAGAGGATCTCACACCATGGAAATGTGCCAACTTT
TTTGTACAAAGT

BRAD.25947_at (SEQ ID N:64)
CTTCCATTCCTCATGATTTTAGGGTTATCCTCATTCAGATCTACTCTAGTTATAATAGTACTTTAAACA
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TTAGACTTCAATGTGCATACTAAATGCATAACATTCGTATCAAATAATTAACATTTATATACAATTAACA
AATAAGGACAAATTTTATACAAAACTTCTACTACTGCTATAATTTTTGAAAACATTTAACCCACTAGCA
AGAGGTAAGACAGCACTGCCTTTTTAAAAGACAGGTCACTTGAATAGAGAATATAAGATATAACCATA
AGTAGGAGTATAAACAATAATTTTTCTTCTTGTGGAATGTTTTTAAATTTCCTTTCTTATATTATTATTC
TTCCTTAGGTTTTTTTAGACAGGTCATTTCTTCCTGAATGATTTTCCTTTTTCTTTTATTTTTATTTTTTG
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AAATGGAATATTTAATAATTCTTACATCTCACTAATGTAGTCTTCTG

BRMX.5143C1n2(2)_at (SEQ ID NO:65)
AATAATTATAAAGTTTATTTAAATGTTGATTGTCCCAAGGTCTACAGTTTCTTTTCTGTTGTGTCATCA
GTGACAAAGAGTAAAAAAAAGGAAACTCCCATATTTAGCACTTTAGAGTAAAACACATGGATCATCGT
TATTAACAGTCCTCTGGGCGTGCTGGAGCTCACTGAGAAGGCTTCTATTTTGAGCTTGGAATGTTGT
GCTGAGCTGTGCAGCCTGTTCCTGCATCTGTTGTTCCTGCATTTTCTGTTGCTCTGCCAGCCAATTT
TGTTTGGCTATCTCCATTTAACTCACTTGTTCCTGATGGAGTCTCTCCCTCTCCTGCATCATTTGCTC
GTTCTGCCTTTGAATCGCCGCCAACCTTTGCGCTTCAGCCTTTTCAGCTTCTGCTTTCACTTGTGCCT
CTGAGGAGAAAAAGATAATC

Hs633116.0C1n30_at (SEQ ID NO:66)
GTGTCAACATTTATGCTCCTAAAGGATGTTGGGTCAAATGAAATGTTCCTCATTGTTTCTCTCTCTTG
ATCTCTCCTTCACTCCTTCTCTTCCTTGCAGGATCTCCAACTCCTTCATAAGGGCACTCTGTGTTACC
CCTTTAAACAAAATAAAGAAGTCCTACATTCTGCCCAGATTTTTTTCAGGCTCCACCAAAGGGTTGGG
TGAATTATGGCCCAAAAGTTGGTGAGGATGATGGTGAACCTTCAATCACCTTCAGTCTCCCAACCAA
CAATGGTCATGGCTTGTTTTCTCCCTGGATTACATGGAGAAAATCATGCCCTACTTTTTGGACCTGTT
GCTTCTACATTTGTATGGTAACTGTGAAACCATCCTAATGAACAGCAAACATTAACCACTACATAAAA
TGTAGACTTTGAATAAAAACACAGCTAAGTACTAACCAGCTTGCCCTTTAAGCCAATTCCCTGTAGCT
ACTTACAGCACGACTGTTAGCTCCTTTCCTTATAGTTTCTTACTGCCTTAAAGTCACATAGATGTGGT
CACAAGGCACTAACTTCCCTTAGTTATTTCTATAAGATAATATATGTAACGTTGGCA

BRSA.1606C1n4(2)_at (SEQ ID NO:67)
AGTGCAGAGAGGATGAGAATATCCTTCATGGGGTCCAGTTCCAAATCTGAAGCATAATTTCCAACCA
TCAAAATATTGGAAATAGGAATGCCTAGCATTTTATGGACATTCATGACCCGGCTTTGAGAAGTCATA
GATCTACTCATGTTTAAAAAGTTGTCTTGAAGAACCTCACTGCAATCATCCACTTTAGTAAGCAAGGC
CACATATGCTATACCACAGTTTAATACTTCTTTGTGAACTTGCTTCACTTTTGCCAACATTTTAGAGTA
GAGATTGTCAATAGAGTTGATGTCTAAGACATAAGCCACACAGTGAATCCTGTCCTTCAGAGATGGA
GAGGTGATAAAAGTAGAATGCTCAGGTGTAATTGGTTTACGGGAATTAAACTGTTATAAAAACATAAG
GTAACATTCAGAAATCAGAGAGCCTCTGTTTAACCCTTAAAGACACAATTAATGCTTCTAATACTGTA
ACTACTGATCTCCCTCTTTCTCCTCAGCTACTCTTTCCCCAAACAGTAGCACCTCCTCTTTACTTCCT
TTCTCACTGGGGGGCATAATGCCACCAACTTTTTTGTACAAAGTTCCCTTTTTAATG

BRAD.41047_at (SEQ ID NO:68)
TTATCTTATACTAAATTCCAACATGTATCTGAGTTTGCTTCTAGATTTTCTGTTCTGTCCCAGTGGTTG
GATATTTCTTCATACACGTCTATCATACTGTTTTGACTATAGAGGCTTTTCAGTGTCATTTAATATCTG
TGATGGCAATCCCTACTCAAAGCTCTTTGTTTTCAGTGTTCCTGTATTGCTCTTTTGTTAATCCCTTAA
TATAAAAGTAAATAATAACCCAGTTGGCATATTATTTTGATGACATTAAATTGGGGAGAATAGATACT
GTGATTTTTGAAGCTTCCTACAAATATGATATGCTTTTCATTTGTGCAAGTACTTTAGTATAATGTTAA
CTGGTGGTGGTAATGGAGGAAATTCTGTCATGTTCCTTACTTTTAGTTTCCTCTAGCGCTTTCTATTT
TTTTATTTTTTTTCAGATGGAGTCTTGCTCTGTCTTCTATCCAGGCTGAGGCAGGAGGATCACTTGAA
CCCAGTAGTTCAAGGCTGCAGTGAGCTATGGTTACACCACTGCACTCCAGCCTGGGTGACAGAGCA
AGATGCCATCTCTTAAAAAAAAAAAAAAAAA

BRAD.4420_at (SEQ ID NO:69)

GTTAATATCTTTTTCGTTTATTGTCTGTCTCTGAAGGTAGGGACTTTGCCTCATTTACTGCTTTTCAGT
TCTTGGAACAATGCTCGGCACATAGGCAATCAACGAATGTTTGTTGAATAAATGATTTTTTTCTCTGG
AAATTGTCAAAATCTGCATGAGGTGTATCAGGCCAGCCATTGTCAGCCTCAGTTTAGAGGCAAGGAA
ATAGGTTCAGAAAGGTTCAAGGACGTGCTGAAGTCACAGGGCGAGGCAGCAGCAGAGAGCCTGCT
TGTTGAGAGCCAAGTCTTATGGGACTTGCCTCCTTCTCTCCCACTGAGGCTGGGGACACCAGGTGG
CCCAGAGGCATGTGGATACCTCCAGTGGGAGGTTAGGAGAGTGCTACACAGAAACTCTGAGTTCTA
ACACTCTTGGGACCATAAAAAATGGAACAAGTCTGGGCATGGTAACTCACGCCTGTAATCACAGTAT
TTTGAGAGGCTGAGGTGGGAGGATCACTTGTGGCCAGGAGTTCGAGGCTGCAGTGAGCTATGATC
CTGCCACTGTACTCCAGCCTGGGCAACACAGAGAGACCTCACTTCTTTAAAAAAAAAAAAAAAAAA

Hs137007.0C1n9_at (SEQ ID NO:70)
AGGAGAAAGGGAAGTCAAATGTCTCGTCCAAGTCTACACAGCTAAAAAGGGGCAGAACTAGGGTGA
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GAGAGCCCCACAAGGCTGCAGAGGGTGGTGAGCCCGGAGTGGGCCTGGCCTGGTGTGGGCTGGG
GGTATGGGCAGGAGCTGCAGACAGCAGGGCTGCACCAGCGGACCAGTTTCAGAGGCAAGGGTTCT
AGGCCCTTGAGAATCCACAGTGCCAAACAGACCCAGATAGCTACGGGGTTGGTACCTGGGGAGGC
CTTAGGACAGGCAGAAAGTCCCAGAGGCGAGGGCGTTGCCTGGGGACGTTTTTGCTCCCTGTCCT
GCTGACAGAGCATAGGAAGTGTGAATGTTTTCTACCCCCTCCTCTCTCGGCTCAGCAGAGCTCCAG
CGAGCCAAGTCCTTGTCTGTGGAGACGCATCAGTCCCTGGCTCTAGGGAATAGGGAGTCCCACAGA
CAGGGGGGTGTCAGCAAGCTGAGAGGGTCTGTAAGTAGGTACGGAATTGAGTCAGGAAACAGTCT
GGGTGTGGAGTGAG

BRSA.18050C1n3_at (SEQ ID NO:71)
TGCAAAAAGCCAAAAAAAGCAGCTTTTAACATTATATCATTATATCACAATTTTGAAACATGGGNNNN
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NNNNNNNNNNACATTATTGCACAGAGATTTCTCATCAATGTTCTTCAGTTTTTATGTCTTTTCCTAAAT
GTGAATAAGTGCTATGGATAAAATACAAATGTAGAAAATAACAGCAGCATGATTTGTCAAAGTTAATC
CCTATAATTTAGTAAGAAAAAATGGATATAAACAAAATAAGTGCTCTTTCTAAACTGTACTAAATTTTC
AAAAATATTGTTTTAATGCAGTGAAGGTCCTGAAAAGCCTATTGAAAGCGATGCTGAGTCCTGTTTTC
AAAAGTGTCCTGTTTGGGTTTTCTTGGTGAAGAGCAGAATTTCAAGTGAAGTAATCGACGGACTAAT
TTAAAACAAAACAGCCCTCGGCTTCCCTATTGGCCTGTGAGGGCACCGGCTCCGGGACCCTGACCT
GGGAGGCAGCGAGTGGTGGGGGTGCCTGGCCCCCATCTACACGTACACAGGCTGGCCAA

BRMX.2948C3n7(2)_at (SEQ ID NO:72)
GCACGTCTACGGGGCTGGACAGAGTGTGGTTAACCGGGGAACTGGGCAAGCCGGCGCCGAGCCT
GCGTCAGCCGTGCAAGCCGCTCCTTCAGGAACTTCCGCTTGTCGCTGGTGTCGCTCCGCTCCTTCA
GGAGCCAGCTGTAGGTGTCCTTGTCCTGCAGGAGCTGCAGCATGGCCTTCTGAAGCTGCTGGCCG
TACGTCTGGAGCATGAAGAACTGGATGATCAAAGGGATGTGGCTGGAGATGCGCTTGCTGGCCTCC
TGGTGATAGGCCATCAGGTGCTGAAAGATCTCCTCCATGGAAGAGTCTGTTGCCGAGCTGGACTGG
AAAGCCCCAAAATCCCAGGATTTCTTCTTCTTTTCTTCTTCCAGCTCCTTCTCTCTGACCTTCTGCAA
TGCACCCCTGTATACCTGGTCCTGGCAGTAGACAATCTGTTCCATCTGGAAGTGGAGGCGGATCAG
CTTCTCACCTTCTCTCTCTTGTTCTGCTCTAATGTCTTCAATTTTGGACTTGGCGGTTCTGTGGAGGT
TAAAAAACTCTTCAAAATTTTTTATCGCCAACTTTTTTGTACAAAGTTGGCCTTATAAAGAAAGCATTG
CT

Hs43047.0C4n40_at (SEQ ID NO:73)
NNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNN
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NNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNN
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NNNNNCTAAAAAAATATGTACTGCTTATTTTGTTAGCATACTTTTAATTATATTCTTATTCTTTCTACCC
CTCTCAAAATGTATTTTTCCAGCTTGCCATTTAATTGGTAAACAGCTGTAAAGTTCAAACGTGAAATTC
TTAAAGCTCCCTAGAGACATACACAATAACTTCTGTGGCATGGACTTTTCTCGGCATTAAAAAAATCT
AGTACCTCTCTTGGCCAGAACCCCTAATTTTACACTTTATGGTGTTGCGTCGTTTTTCNNNNNNNNN
NNNNNNNNNNNNNNNNNNNTTACTGGCAAGTTTTTCCTCCAAACAGTTTTCTAATCAAGTCTAATAA
GTT

Hs926.1C10n7_at (SEQ ID NO:74)
NNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNGATGAGCCAGGCATGGTGGT
ATGTGCCTTTAGTCCCAGCTATCTGGGAATNNNNNNNNNNNNNNNNNNNNNNNNNTGACGGCAA
GAGCCTGTCTCTGNNNNNNNNNNNNNNNNNNNNNNNNNNNNNTCTGATCAGTTAAATGAATATGGA
AACTTAATCTTGTACCCCTTACCTCCCAAGCATACAGCCACAGTTTACCGTTGGAGGGATCTTTCCA

CGGAGGTAAACAGTGCTGTTTTCTCCAAGTGCCAGAACAAAAACACAACAGCACACACACAATGAGA
TGGTTTGGCTCTGTGTCCCCAACCAAATCTCATCTCAAATTGTGTTTGGCTCTGTGTCCCCAACCAA
ATCTCATCTCAAATTGTGTTTGGCTCTGTGTCCCCATCCAAATCTCATCTCAAATTGTAATCCCCATG
TGTCAAGAGAGCAACCTGGTGGGAGGTGACTAGGTCATGGGGGTGGTTTTTCTCATGCTGCTCTCA
TGATGGTAAGTGAGTTCTCACAGGATCTGATAGTTTAAAAGTGTTTAGGGGCTGGGAGCAGTGGCT
CAT

Hs528836.0CB6n98_s_at (SEQ ID NO:75)
GGGTGAGGACCCACAGCTCTGATGTGGGCGCTTCAGGCCATGGTGGAGCTGAGATTCAGGTTGGC
TTTTCCCCTCAGCTCCCAGCTGGCTGGTGAACCCATCATCATAGCCAAAAGTACTCAGCAGCAGCA
CCTCCAGGTCCAGAGGCACCTCCAGCTGCATGCACACACAATGAATGAAAGACTGCCAGGTGTCCG
AACCCTGGACATGCAGCTTGTTGAGTTGCAGGATGACTCTCTGTTCAGGGTCCAAGGTCTCGTTCCT
GGAATCCAGGTCCGTGTTGGGGAGGAAGAACTTCATCTTGGCGTTCAGCCATTCTGGGTCTTTGGT
GAGCAGCCTCACAAGACAGCTCCACAGGTTCTTGTTGCCGAGCTGGAGGCCAACGGGGTCCATGA
GGAGCCAGCCTTGGTCTCCTCGTTCATGATAGGTGCTCTAGGGTCCCCACGGAGAGGGTCTCATG
GGTGTCTGGGCTATGTGTGCCTTGAGCTGGATTGACAGGTTGTTTCCATAGTGCAGACTCCCTCAG
CGCTCGCGGCTCCTCCGCGCTCTGCACGAAACTGAAAGTAGAAGCCGCCGCCTAGAGCTGCTCCG
CCAGTGCAT

BRMX.7284C1n6_at (SEQ ID NO:76)
TGGCAAGGACATTGTTTTTGTCTAGTGTCTCAAGCTTCTCTACCAAGAGAGTCATATTTCTTATCTCC
ACCTCCAGCTGGTCAACAATTTCTGAGCTTCCACCAAAACTCTCCTTCAGCTGTATGACCAGTTTTTC
CATCTCCTTCACTTCTACCTTGATCAGCTCGAAGTCCAGTTCAGTGTAAGAAATGGTATCCTTCTCCA
TGATGTCAATTCGGACAGTTAGGTTTAACAGTTTCTTTTCATACACACTAATTAATTGGACATATTCCC
TCACTTTAGAAAGTTCTTTCTCAAACTTCTGAGAAAGAACATGAGCTGTGAATTCCAAGCGTTCCACT
CTGTCCACGGGAAAGGTGGTGTCTGGCAGGGAAACAGAGCACTGGCAGGTCCCACGGTCATCCAC
GGAGCCGGTGAAATTGGAAAACAACTGGGACACAGAACCTCCGCTGCCTAAGCTGCGGCTGGAGC
TGGAGCCCGACCTGGAGCTGGAGCTGAAGCTGGAGCTGGAGTCAACACCTGGGAAAGAGCTGAAG
CCGGGGCTGGGAATTGGAGGTCCCACATCCCCCAAATCCCCTGCAGCTTGGCCAAGGAAGCCAA

BRAD1_19751014_at (SEQ ID NO:77)
TCTTTTATTGAAAGAAAAAACAATACAATGGACTTTAAAAAGCTACATTTGTTATGGTTCATAAGGACA
GAGGTTTACACAGGTTTTATATATGTACACACTGACAATACTATATCACAACATCAGAGGCACCATTT
TTGCCACAGAATTAGGTAATGAATAAAACTTCTCCAAATTAATCTGTTTAAAAAATATCTAAAATGGTA
CAGTATATTTGAGGATTATATAAATATGTGAGACATATTTAGATATTTTTTAAAAATAGTGTTTATATAT
ATGCATCACAATCTTCTCTAATTCTCAAAATATTATGGCACCAAAATTCTGTTTGTCAAATAAAACACA
AGATGCTGTAATATGTATCCAAGCACCAGCTTAGCACAGTATTTAATTCTCCCCCAAACTGAAAGACT
GCTAACAGGTACAAACTGAACTGAATATTTCACACAACCATTGAAATAATTTAGGCCCTCAAATTTTTT
TTTTATTAGCTGATTGTTTTTAGAGAAAAAAGAGGGAGCTAAACCATTTACATTAATGTTGCTCTGTGT
GATAGAATCAATCCTAGGGCTCAGAGAAGATATTCCTAGGCACTGGAGA

BRMX.13502C1n6_at (SEQ ID NO:78)
TCAAACTTGAATCNTTTAAATTTATTTTCTGCTTAAGCAGGTTTGAGTTGGGTTTTCTATTTGCAATAG
CAAAAGTCCTGACTGGCAAGGTTTAAAAGTTTGAAGACTCTCACAGGTAAGTGCAGCTCAGGATCCT
GTGAGTGCAGCAGAAAGTCTTAAGAAATGGCAGGGGCTGGTTGAACCCAGATTTTCCATTGGCTGA
GCAGATATCCCCAGAGGCGTAGAAAATTAAATTTGTTTTATGTTGTTCCAAAAGAGGAGAACTGAGG
CCAGAGGAGCACACTTCTGAGACACTCATTTTTGCTGGGTAGAGGAACTCTCTGGGCAAGCAGGAC
CATCGATATTAGAGCAGCTGGCCTCAGGAGGGGAGTAAGAGCCCCATCCCTGAAGGTACACAAGTT
GTGGCAGCAACCATCTGGCCTGCAGTTTCCAGAGGGGAGTCAGGCGTGGGGTGGGACTGGAGTGA
ACGGGTACC

BRMX.1111C4n3_at (SEQ ID NO:79)
TTTTTTCTTCTTTTCCTCTTGGGTTTTCCCAAAGTAGAGTTGTTTGCAATATCCACAGTATCCATTTTG
CCACATGCTTGGTCACTTTCCTTCCTTGCTTCCGGGCTTTCTGGCACTTCTCCTTGTTTAAGACTTAG
TTTGATGTCAGGCCTCTCTTCCCTTTCTTTTCGATCACTTTCTTGGAAAGACAATTTGTCTTGGATTG
CATTTTTGAAGCTTTTATAAATGTGAATTAAATCGGGGTATTCCTGCATGTTGACCTCGCTGAACAGT
GCTTCCAAAACTGACAGGTTAAATGTCTTCTCCAGTTCACTGAGAACATTGTACACCACTCTTTGTAC
AGGGACCAGGTTTCTACAAGAATCTTCAGAATCTTCAAACATTTTATTTGTGATGAGTTCCCGATCGC
GGAGGCCCTCAAGGAATGGAAATGTCTTTTTTATTGCATTTGATATCTCCAGCTTATGTCTTTTGAAG
TGCTTGAATACAGTGTCATAGACAAGTCCCTCATCTACATCCTGGTCTTCCGTGAACAGCCTGGCTC
GGAAGGTCCTACGCCCACGGACTCTCACTGATTGCTAGCACAGCAGTCTGAGCCAA

Hs369056.9C26n3_at (SEQ ID NO:80)

CCTTCCCCATTTCTCACTTTCCACAGGTGGGATGTGGCAGTCCTCATGGAAGACTCTTGAACAAGTG
TCGCAACAGAACAGCTCCCCTCCGTCCCGGCACACCTCACACTCATCCAAGTTTCTCATCTAGAAG
GTAAAACAGTGTCCACGTCACTGGGAATCACAAGATTCAGGAAGGCCACCCCTCTGGGCATCTAGA
ACACACTGCTTATGTGTGAGCCTGTATAGACAGGCATATGCTTCTCCCTGGGATATGAAGGAAAAAT
ATGGCATGGAGATTTCAGAACAAATCCTGGTCTGCAGTGAAGTTCAGGAGGAAGGGGTATATGTCA
GAATAAAAACGTTTTCCTTATAAAACCAGAGATTATGACACAGAAAGCCTAGCAACAAAGCAAGAGG
ATGATCTTATAGGAATCTGAATAATTGTATTATGCTGCAGATAAAACCAGGTTTTGAAGTAAAAGTGT
TAAATCCATTTGTCTATACTACAAATCAACTCATGAAAGGGAGACCCAGAGAATTACATATGATGGAA
TAACCTTCTAAGATATCATCACATCCCATATTCTTGGCCATAAGTTCCCCATGAGTTGAAGACAG

BRMX.24432C1n2_at (SEQ ID NO:81)
GTGGCTGTTGCTGGCCCCACCTCCGCTTATGTCCTTAACATGCCTCAGGTGGTTCATCCCTTTTGGC
ACTCATGGTGCCCCCTGTGGGCTGATACAGGAGTGAGTCTACTGTGAAGGCACTCAGTATAGTGGA
AAAAACAAATATCAACCTCCTGCTTTTTTTCAGTGTAAAAACTATAAGCTCTATGGGAGTTTCTGCAG
ATGGTACCATAATGGCCTGAGGGAGGAGTATCACAGTCACAGAGTATTGGTTCTCTCACTGCATAAG
CCATGGTTTTACCCACCTTCACAGGCTAAAGGTGCTTCATAACCTTGTTCATGTATTGAGGTTCTGTT
GGCTCTTGTAATGGTAATTTCACATGTGGGCAGTTGTTCATATTGATGTTTCTATAGGGGTATGATAG
CTGGAGAGGTCTGCGCCACTGTCTTGCTCTGCCTTGATCANNNNNNNNNNNNAACAAGAATTTGTC
TCCTCCTAGTTTTTCTTTTTCTCTTAACCGACCTAGGTTTAGCCTTTTAATCCTTCTCCCTCCTCTGCT
TCTAATGTCATTGTTTCTTTGTATGCCTATCATATCTACATGCTACATGACCTTCAGCTGG

BRRS.17773_at (SEQ ID NO:82)
AGTTTTAAGGAAAAATTGTATGATTTAAAAGATTATAAAACTTTATTACTGGGCTATTTACACATTTTAA
TTGTTTCTCATAAAATATATAACATTACAATATTTATGGAAGTAGGATATTTTTGTATCATATGTACGAT
GATAATTTATAGGGTATTTTAAATGATGTTTTTTAGCCTCCTTAAGTTTTAAGTGGATCTTGCAAATGA
AAACAAGTATTATTGAGTTTGACATACTCAAATTGCCCAAATATCAGCTGTTTAAACAACCAAGTCAT
CATTGATACTTTAGTAAAGGTTAGTAAATGTCATCAAAGGCTTATTTGCAGTTTACAGTTTTTATTACT
TAGGAGACTTAAGGAGTACCTGCCAGGTTTGTCCATGCTAATGCTACGATTTTGTTTTTGTAGTTCAA
CCATATTTTGTATGGAGATACTTTGAGGCTCTGTAAATTTCTGGTTACTCCTCAGAACCCACTAGATT
TAGCATTTCATGGATGACTTGTGTTTGAACAATTATTACTATAATGGTTGCCAGATGATTATTTTCTTA
TTCTCTTCTTTGTTCTACATGGAGAAATAAAACCAATAAATAAGGGAGA

BRAD.10849_at (SEQ ID NO:83)
GTGCCAATGTGAAGTCTGGATTTTAATTGGCATGTTATTGGGTATCAAGAAAATTAATGCACAAAACC
ACTTATTATCATTTGTTATGAAATCCCAATTATCTTTACAAAGTGTTTAAAGTTTGAACATAGAAAATAA
TCTCTCTGCTTAATTGTTATCTCAGAAGACTACATTAGTGAGATGTAAGAATTATTAAATATTCCATTT
CCGCTTTGGCTACAATTATGAAGAAGTTGAAGGTACTTCTTTTAGACCACCAGTAAATAATCCTCCTT
C

BRAD.10890_at (SEQ ID NO:84)
AATGCTTATGTCTAAAAGAGCTCGCTGGCAAGCTGCCTCTTGAGTTTGTTATAAAAGCGAACTGTTC
ACAAAATGATCCCATCAAGGCCCTCCCATAATTAACACTCAAAACTATTTTTAAAATATGCATTTGAAG
CATCTGTTGATTGTATGGATGTAAGTGTTCTTACATAGTTAGTTATAT

BRAD.11026_at (SEQ ID NO:85)
CTGGGCACCTCTGGGACAGCAAAAAAAACTGCAGAATGCATCCCTAAAACTCACGAGAGAGGCAGT
AAGGAACCCAGCACAAAAGAACCCTCAACCCATATACCACCACTGGATTCCAAGGGAGCCAACTCG
GTCTGAGAGAGGAGGAGGTATCTTGGGATCAAGACTGCAGTTTGGGAATGCATGGACACCGGATTT
GTTTCTTA

BRAD.12809_at (SEQ ID NO:86)
ACCATGTTCATCTTGTCCTCCAAGTTATGGGGGATCTTGTACTGACAATCTGTGTTTTCCAGGAGTTA
CGTCAAACTACCTGTACTGGTTTAAATAAGTTTACCTTTTCCTCCAGGAAATATAATGATTTCTGGGA
ACATGGGCATGTATATATATATATGGAGAGAGAATTTTGCACATATTATACATATTTTGTGCTAATCTT
GTTTTCCTCTTAGTATTCCTTTGTATAAATTAGTGTTTGTCTAGCATGTTTGTTTAATCCTTT

BRAD.14326_s_at (SEQ ID NO:87)
GATGGCTGGTCTGCCCCCTAGGAGACTCCGTCGCTCCAATTACTTCCGACTTCCTCCCTGTGAAAAT
GTGGATTTGCAGAGACCCAATGGTCTGTGATCATTGAAAAAGAGGAAAGAAGAAAAAATGTATGGGT
GAGAGGAAGGAGGATCTCCTTCTTCTCCAACCATTGACAGCTAACCCTTAGACAGTATTTCTTAAAC
CAATCCTTTTGCAATGTCCAGCTTTTACCCCTA

BRAD.15436_s_at (SEQ ID NO:88)

GGCATGGAGCATCTGTACAGCATGAAGTGCAAGAACGTGGTGCCCCTCTATGACCTGCTGCTGGAG
ATGCTGGACGCCCACCGCCTACATGCGCCCACTAGCCGTGGAGGGGCATCCGTGGAGGAGACGG
ACCAAAGCCACTTGGCCACTGCGGGCTCTACTTCATCGCATTCCTTGCAAAAGTATTACATCACGGG
GGAGGCAGAGGGTTTCCCTGCCACAGTCTGAGAG

BRAD.15833_s_at (SEQ ID NO:89)
GAAATTAGAGTCCTATATTCAACTAAAGTTACAACTTCCATAACTTCTAAAAAGTGGGGAACCAGAGA
TCTACAGGTAAAACCTGGTGAATCTCTAGAAGTTATACAAACCACAGATGACACAAAAGTTCTCTGCA
GAAATGAAGAAGGGAAATATGGTTATGTCCTTCGGAGTTACCTAGCGGACAATGATGGAGAGATCTA
TGATGATATTGCTGATGGCTGCATCTATGACAATGACT

BRAD.19080_s_at (SEQ ID NO:90)
TTAGATTTCCAGCTTGTCACCTTCAAGGTTACCTTGTGAATAGGACTTTTTTGAGCTATTTCTATCCA
GTTGACTATGGATTTTGCCTGTTGCTTTGTTTCCACCAACTCTCCCTGAAGATGAGGCGCACAGACA
GACAACTCACAGGCAAGAACAGCCTGGTCCATCTTGAAAGATTCTCAAGACTATTCTCCACAAG

BRAD.2707_at (SEQ ID NO:91)
TGTTTAAAAATGTTGTGGGTACATAGTATGTGTTGTGGGTACATCGTATGTGTTGTGGGTACATAGTA
TNGTGGGGTCCATGAGATGTTTTGATACAGGCATGCAATGTGAAATAAGCACATCATGGGGAATGG
GGTATCCCTCCCCTCAAGCGTTTATCCTTCAAGTTATAAAAAATTCAATTACAGTCTTAGTTATGTCAA
AATGTAC

BRAD.27716_s_at (SEQ ID NO:92)
ACCAGAATTTATGGATGAACTGATTGCTTATATTTTAGTCAGGGTTTATAAATGTAGATGGTCAAATTT
ACATTGCCTAGTGATGGAAAATTCAACTTTTTTTGATTTTTTTTTTCCAATATTAAAAAAGGCTCTGTAT
GCATGGTGGG

BRAD.28628_s_at (SEQ ID NO:93)
AAGATTCCTGTGTACTGGTTTACATTTGTGTGAGTGGCATACTCAAGTCTGCTGTGCCTGTCGTCGT
GACTGTCAGTATTCTCGCTATTTTATAGTCGTGCCATGTTGTTACTCACAGCGCTCTGACATACTTTC
ATGTGGTAGGTTCTTTCTCAGGAACTCAGTTTAACTATTATTTATTGATATATCATTACCTTTGAAAAG
CTTCTACTGGCACAATTTATTAT

BRAD.28643_at (SEQ ID NO:94)
TCTCCTCTCATCTGCATTTCTCAGAAATGCCCTCCCTGCCCAGTGGTGACTTTCCCTCGTCACTCCT
ATGGAGTTCTACCTGGAGCCCAGCCATGTGTGGAACTGTGAAGTTTACTCCTCTGTAAAGATGGTTT
AAAGAAAGTCAGCTTCTGAAATGTAACAATGCTAACCCTTGCTGGAACCCTGTAAGAAATAGCCCTG
CTGATAGTTTTCTAGGTTTATCATGTTTGATTTTTACACTGAAA

BRAD.28663_s_at (SEQ ID NO:95)
GAATTTTTCTCTATTTCCAGCACGCTGATTTGATTTAAAAATGTAATAAGACCAAGAGTTGGAGTAAA
GGGATATTCATTCCATGTTAAAAGTGGCTTCATAGCTACTGACAAATGTCTGAACTATTGTCGTGCCC
TTCAAAACTGGAGTTTTCTAAAATAATCTTATTTTTATACTTGTATGTTCCAGCAATTTAAGATATATAC
CATTGAAAGGGAAAT

BRAD.29038_at (SEQ ID NO:96)
GGCTGAGCAAGGCACATAGTCTACTCAGTCTATTCCTAAGTCCTAACTCCTCCTTGTGGTGTTGGAT
TTGTAAGGCACTTTATCCCTTTTGTCTCATGTTTCATCGTAAATGGCATAGGCAGAGATGATACCTAA
TTCTGCATTTGATTGTCACTTTTTGTACCTGCATTAATTTA

BRAD.30917_at (SEQ ID NO:97)
AACGCAGGCCGCTTTATTCCTCTGTACTTAGATCAACTTGACCGTACTAAAATCCCTTTCTGTTTTAA
CCAGTTAAACATGCCTCTTCTACAGCTCCATTTTTGATAGTTGGATAATCCAGTATCTGCCAAGAGCA
TGTTGGGTCTCCCGTGACTGCTGCCTCATCGATACCCCATTTAGCTCCAGAAAGCAAAGAAAACTCG
AGTAACACTTGTTTGA

BRAD.31470_at (SEQ ID NO:98)
TCATCTCCGTATTCTTCAGCTTCATCCAAAACTGACTTAGAAGCCTCCCTTGACCCTCACCTGACTAT
TCACAGGTTATAGCACTTTATGTTTTTCAGTTCTGTTATTTTAATTGGTGCCTCTGTTTGTGATCTTTA
AGAACATAAAATTCTGGCAAGTAACTATTTGCTA

BRAD.32716_at (SEQ ID NO:99)

CACTTTGCAGCCTTGAGAGGTGCAGAAGAGACACCGAGGGGTTCACCACCAGAGCCACCATTGTCA
GAGAGGCGTCCAGCTGTGTCCACCTGGGACTCTGCCTTCAGGGCTTCTTGCCTGGCTGGGAGCTG
CACAGGCAGACTCCTGGGACGGTGTGCCGACAGCTCTGGGCACCCCCTTCTAGGATCTGATTCCT
GAGGAATCACAATGTGGATTTCACAATCACTTCCAGTGTCTTTTGCCAACCTCTGTGAACAGATGT

BRAD.33042_at (SEQ ID NO:100)
AAGTTTGCACAGTTCTAGACACGATAAATACATGTGAAATCACACAACTCAGAAAATGTCCCTTAAAT
TAATTGAGCCATTGGTACTTGTGAATTAGAAGAGACATCTATGTTCTGATCCACTGTTGAAAGCTGTA
CAATGTTACCTATTTATTTGCAGACATCCTTTGGAAACAAATAGGTAGATTTGCAACAAATAAAGAGT
GGAGTACAGCTGCTGACATTACCTTGTATATTCATGCCTTTATG

BRAD.33341_at (SEQ ID NO:101)
GACTGCACAGCAGCAAGACAGATTGCCATGGAGCATGTTGTGCCCAACTAGGGACAGCGCAGATA
GATTCTGTAATTTGCCTAACAATGTCTATAGGATGATCCCATTTGTCAAAAAAAAAANNGAACTGGGC
TTTATTGATGTCACCTAAATGCACCTAAACTTCTTTTTTGCCCCATGCTCTTCTGTACTCTTGATCTTT
CCCCAAATTTTTAAAAACATGACACTCATTCCCTTATTTTTCCTACTTAG

BRAD.33405_at (SEQ ID NO:102)
TTAATTGCTTTCTCTCACCAGGGAAGGTGTGGGAAGGACTTGTGAAATACATATTCGAGGAAAAACT
ATGCACAAGGCCGTGCATTTAAAAATAAACTCCCTAAGGCTGGGGTGAAACCTGCTACGGTCTGCG
CAAGTTGACTGTTAATGAATTTGATTCTCAGGTGTGAGTGATTAAAAGAACACTGATCATGTCATTTT
CTTTTTGGTCACTAATTCCCTCC

BRAD.33431_at (SEQ ID NO:103)
GTCATCCAGAGTTATAATGGCCCATTATCTAATGGTCAGAGTTTACTTAGGCTTTCACTACTTCCACT
GCCCACTTGAAACAGGGAAAAATATTTTCCCCCCGCGCTGTGAGTGTGCTATTTAGAGCTGACCACA
AGCGGGGGGAAGAGAGGATGGCTCGGATGCTGCATTTCCACTGAGAACACAAGGCTGGCAAAGCT
TGTCTGCTGCCCAGCAAGCACTTCAGGCTCACACCATTTTAGGTTCACTTTAAGTAGTTTCTCAAT

BRAD.35695_at (SEQ ID NO:104)
TGGACAGTGGACGTCTGTCACCCAAGAGAGTTGTGGGAGACAAGATCACAGCTATGAGCACCTCGC
ACGGTGTCCAGGATGCACAGCACAATCCATGATGCGTTTTCTCCCCTTACGCACTTTGAAACCCATG
CTAGAAAAGTGAATACATCTGACTGTGCTCCACTCCAACCTCCAGCCTGGATGTCCCTGTCTGGGC
CCTTTTTCTGTTTTTTATTCTATGTTCAGCACCACTGGCACCAAATACATTT

BRAD.35710_at (SEQ ID NO:105)
TCCATGGCAACAGTCCCAACATGTTTGAGACTTCAGCTAAAGGAATGGATGTATNNNGGNGTGTAGT
CTTCAGTATATCACTGTATTTCCGTAATACTAGACTCNAAGNTATGCNAGATNGNTTATTCCCTTNGT
GAANNNGGAGTTGCTCATTACGTTCTTGAAATATCGCACATCCTGTTGGTTCTTCAAAGGAAGCCTT
TCCACCAGATTAGTGTTCAAGTCTTTGCAGAGGAGACCAACTTTT

BRAD.37907_at (SEQ ID NO:106)
AAGGCTATGCTTTCAATCTCCTACACAAATTTTACATCTGGAATGATCTGAAGGTTCTTCAAAGACAT
TCAAAATTAGGCTTTTTTATGTCCTGTTTTAAGTGAAAATATTTATTCTTCTAAGGGTCCATTTTATTTG
TATTCATTCTTTTGTAAACCTCTTTACATTTCTCTTTACATTTTATTCTTTGCCCAAATCAAAAGTGATT
CCT

BRAD.40353_at (SEQ ID NO:107)
CTTAGCATTAGAACACTCAGTAATCATATGAATTGTGCATTTGTTTGTTTTGCTTAACTCTTTCTGTTT
GTTTATGTTTGGGGTTTTATTGTTGTTGTTTCACTTTTCTCCCATCTCTTCCTGACTTGGTCAAATCCA
AAGGAATNTTCCAAATTGTGGGGAGCAAGGCATCTGAAATGGCTAAAAC

BRAD.40654_s_at (SEQ ID NO:108)
ATGCTATATGCTGTATCCCACCTTTCTCTGAATGTTACATTTTCTCCCCTATCCCAGGCTGCATCTAA
GAAAACTCAAAGGGAATATGCTATCTATCTTTTCCGAGCAATGAAAGCTCTNGGGTTTTTTCCTTGCT
TTTCAGGGCACNATACTTCTCTTTCTTCCTGGTTAGACAGGATAAGTTCTGAGTCCCNTGGTATCATC
AGCTTACTTCTTCTCTGTTAAATATTCACA

BRAD.4701_at (SEQ ID NO:109)
GTGGTCTTCCTCTGAATATTAGCAGAAGTTTCTTATTCAAAGGCCTCCTCCCAGAAGAAGTCAGTGG
GAAGAGATGGCCAGGGGAGGAAGTGGGTTTATTTTCTGTTGCTATTGATAGTCATTGTATTACTAGA
AATGAACTGTTGATGAATAGAATATATTCAGGACAATTTGGTCAATTCCAATGCAAGTACGGAAACTG
AGTTGTCCCAAATTGATGTGACAGTCAGGCTGTTTCATCTTTTTTG

BRAD.5967_at (SEQ ID NO:110)
TATCCTATTACTGTACTTAGTTGGCTATGCTGGCATGTCATTATGGGTAAAAGTTTGATGGATTTATTT
GTGAGTTATTTGGTTATGAAAATCTAGAGATTGAAGTTTTTCATTAGAAAATAACACACATAACAAGTC
TATGATCATTTTGCATTTCTGTAATCACAGAATAGTTCTGCAATATTTCATGTATATTGGAATTGAAGT
TCAATTGAATTTTATCTGTATTTAGTAAAAATTAACTTTAGCTTTGATACTAATGAATAAAGCTGGGTTT

BRAD.7701_at (SEQ ID NO:111)
GGGATTTTGAGCTATCATCTCTGCACATGCTTAGTGAGAAGACTACACAACATTTCTAAGAATCTGAG
ATTTTATATTGTCAGTTAACCACTTTCATTATTCATTCACCTCAGGACATGCAGAAATATTTCAGTCAG
AACTGGGAAACAGAAGGACCTACATTCTGCTGTCACTTATGTGTCAAGAAGCAGATGATCGATGAGG
CAGGTCAGTTGTAAGTGAGTCACATTGTAGCATTAAATTCT

BREM.1048_at (SEQ ID NO:112)
TTGAATAGATCATCAGTGGCCACTGATGTAATTAATCATGTCTATGTAATGAAGCTGCCATAAAAAAC
CCAGGAGGACAGTGTTGAGAGAGCTTCTAGGTTGGTGAACACTTGGGGGTGTCTGGAAGACAGCC
CACCTGGAGAGGACACGGAGGCTCTTCGCACCTTCCCCCATACCTGGCTCTCTCCATCTCTTCATTT
GTCCATCTGTATCTTTTTCATTATATTATCCTTGATAATAAACTGGTAAATATAAGTGTTTCCCTAAGTT
CTATGAGCCACCAT

BREM.1129_at (SEQ ID NO:113)
AGGCCTCTGATTGCACTTGTGTAGGATGAAGCTGGTGGGTGATGGGAACTCAGCACCTCCCCTCAG
GCAGAAAAGAATCATCTGTGGAGCTTCAAAAGAAGGGGCCTGGAGTCTCTGCAGACCAATTCAACC
CAAATCTCGGGGGCTCTTTCATGATTCTAATGGGCAACCAGGGTTGAAACCCTTATTTCTAGGGTCT
TCAGTTGTACAAGACTGTGGGTCTGTACCAGAGCCCCCGTCAGAGTAGAATAAAAGGCTGGGTAGG
GTAGAGATTCCCATGTGCAGTGGAG

BREM.1226_at (SEQ ID NO:114)
ATACGTTTTTCACTTTCTGACCAGGACCATGCCTGTGGAGTAGATGTTGACAAGAAACACTGACCAG
ATCAAAATGTGTCTCAAGGAGAATGGCACAATTTTGTGCAAATGAATCAAGGAAGTCTTATTGCACAA
GAGTATCCTGGAACCCAGTGCAATTGATTTTTTAGAAAAATATATCACATAGGGGAAAAAAACTGGAA
TATGTTGAAGGAGACGTATATAATATTTAGCATCCAGATTGATGACTTCTGCCCTAACTATGCAATG

BREM.1262_at (SEQ ID NO:115)
CGCTTGAACCTGGAAAGTGGACATTGCAGTGAGCTGAGATTGTGCCACTGCACTCCAGCCTGGGCA
ACACAGCGAGACTCTGTCTCAAAAAAAAAAAAAAAAGAAAGAAAAAAAAGAGAAAACTCAGAGATTC
GTGGAGACTGGAACCACGGGTGTGGAGAGAGGGGTTAGTAGAGACCAGATTCTGCAGGTACTATA
ATGACATTCCCAGGCTAAGGAGTTTAGATCTT

BREM.130_at (SEQ ID NO:116)
ATCTACACCCTCAGGAATAAGAAAGTGAAGGGGGCAGCGAGGAGGCTGCTGCGGAGTCTGGGGAG
AGGCCAGGCTGGGCAGTGAGTAGTTGGGGAGGGGAGAAAGTATTAAGCCAGAACCCAAGGATGGA
AATACCCCTTAGTGAGTCAGTTTAGACTTCAGGCTGTTCATTTTTGTATGATAATCTGCAAGATTTGT
CCTAAGGAGTCCAATGGGGGATATGTTTTCCTCCCGTGAGGAAATGTTTAGTTCTTGAGGGAAAAAT
CCCTAAATCCTCTATATA

BREM.1689_s_at (SEQ ID NO:117)
GGGTAGCAAGTTCACCACAGTGTTAATGGGGGTCCCAAGGTATTCTTCCCCCAGGCCTAGGTATAG
GGCTATTACTCCTCTCTGCTCCAGGTGTAGACATACATTTACATT

BREM.2334_at (SEQ ID NO:118)
TGGAGGGTGAAATTCTGATAGACTTGAGGCTTTGAGATGTGGTCCTGGGGTGGAGCAAGACAAGAA
AAGTACTGGAGATTGGGGTTTGAGGAGTCTATGCAATTATTTTTATTTTTAAAAATCTTTGTGGCTAC
ATAGCAGGTGTATATATTTATGTGGTAAGTGAGATATTTCGATACAGACATACAATGTATAATCACAG
GCATACAATGTAGACAGGCATAAAGTGTATAGTCAC

BREM.2382_at (SEQ ID NO:119)
AATGTGAAACTGCTCCATGAACCCCAAAGAATTATGCACATAGATGCGATCATTAAGATGCGAAGCC
ATCGAGTTACCACCTGGCATGCTTAAACTGTAAAGAGTGGGTCAAAGTAAACTGAATTGGAAAATCC
AAAGTTATGCAGAAAAACAATAAAGGAGATAGTAAAAAGGGTTAACGAGCCAGTCCAGGGGAAGCG
AAGAAGACAAAAAGAGTCCTTTTCTGGGCCAAGTTTGATAAATTAGGCCTCCCGACCCTTTGCTCTG
TTGCTTTATCAACTCTACTCGGCAATAACAAT

BREM.532_at (SEQ ID NO:120)
GATTAAGAACAGTTTTTTCAACAAATAGTGTTGGGACAATGGGTGTCCACATGCAAAAGAATAAAGTT
GTCCCCTTACCTTACACCATCTCCAAAAATTAACTCAAAATATGTCAAAGACATAAACGTAAGAGCTA
AAACTGTAAAACTCCTAGAATAAAACATAGGAGTAAATCTTCATGACCTTGGATTAGGCCATTGTGTC
TTAAATATAACACCAAAAGAATAAGTAATAAAAAAATAGATAAATTGAACTCCATCAAAATTAAAAGCC
TTTGTGCTTCATAGGACACCA

BRHP.106_s_at (SEQ ID NO:121)
TCTCAAGCTATGATCAGAAGACTTTAATTATATATTTTCATCCTATAAGCTTAAATAGGAAAGTTTCTT
CAACAGGATTACAGTGTAGCTACCTACATGCTGAAAAATATAGCCTTTAAATCATTTTTATATTATAAC
TCTGTATAATAGAGATAAGTCCATTTTTTAAAAATGTTTTCCCCAAACCATAAAACCCTATACAAGTTG
TTCTAGTAACAATACATGAGAAAGATGTCTATGTAGCTGAAAATAAAATGACGTCACAAGAC

BRIH.10647C1n2_at (SEQ ID NO:122)
TCTTTCTTTTCCAGACAACTTTGAATGGAGAGGAGCAAATTAGTCTTTTGGTTTAATTCTGTCTCAGTT
TGCTTATCTAAAGAAAGGAAAACAGAGTGGCTACACTTGTTTAGAACCATATGCATACTCCAGAGAA
AGATGCTCTATTAATCCAAAAAAATACAGCCACTTGAAACCAGCCAAAGCGAAAGTGTAAGGGACTT
CATGGAAAGGAGGCAGTTCACCAAAGTATTGAGGGGTTTTATATTTTAAACTCCGCCAGTGAATTGA
CGTGTTATGTCACTTAC

BRIH.1453C1n2_at (SEQ ID NO:123)
GAATTTATTGGAGCATGACCACGGAGGATAGTATGAGCCCTAAAAATCCAGACTCTTTCGATACCCA
GGACCAAGCCACAGCAGGTCCTCCATCCCAACAGCCATGCCCGCATTAGCTCTTAGACCCACAGAC
TGGTTTTGCAACGTTTACACCGACTAGCCAGGAAGTACTTCCACCTCGGGCACATTTTGGGAAGTTG
CATTCCTTTGTCTTCAAACTGTGAAGCATTTACAGAAACGCATCCAGCAAGAATATTGTCCCTTTGAG
CAGAAAT

BRIH.1518C1n4_at (SEQ ID NO:124)
TCCCCGGTTACTACCTCTTATCCATCCCCGGCCACCACCTCATACCCATCCCCTGTGCCCACCTCCT
TCTCCTCTCCCGGCTCCTCGACCTACCCATCCCCTGTGCACAGTGGCTTCCCCTCCCCGTCGGTGG
CCACCACGTACTCCTCTGTTCCCCCTGCTTTCCCGGCCCAGGTCAGCAGCTTCCCTTCCTCAGCTG
TCACCAACTCCTTCAGCGCCTCCACAGGGCTTTCGGACATGACAGCAACCTTTTCTCCCAGGACAAT
TGAAATTTGC

BRIH.2770C3n31_at (SEQ ID NO:125)
ATGAAGACTTGGCTGATTCAGATGCCAGGGCCTTGTATGAAGCAGGAGAAAGGAGAAAGGGGACA
GACGTAAACGTGTTCAATACCATCCTTACCACCAGAAGCTATCCACAACTTCGCAGAGTGTTTCAGA
AATACACCAAGTACAGTAAGCATGACATGAACAAAGTTCTGGACCTGGAGTTGAAAGGTGACATTGA
GAAATGCCTCACAGCTATCGTGAAGTGCGCCACAAGCAAACCAGCTTTCTTTGCAGAGAAGCTTCAT
CAAGCCATGAAAGTATGTACCATTCT

BRIH.365C1n2_at (SEQ ID NO:126)
TGCCTTGTGTCTTCCGTTTGACGGAAGAGAATGGATTCTGGTATCTAGACCAAATCAGAAGGGAACA
GTACATTCCAAATGAAGAATTTCTTCATTCTGATCTCCTAGAAGACAGCAAATACCGAAAAATCTACT
CCTTTACTCTTAAGCCTCGAACAATTGAAGATTTTGAGTCTATGAATACATACCTGCAGACATCTCCA
TCATCTGTGTTTACTAGTAATCATTTTGTTCCTT

BRIH.5410C1n7_at (SEQ ID NO:127)
GGTATAGCATATGTGGCCTTGCTTACTAAAGTGGATGATTGCAGTGAGGTTCTTCAAGACAACTTTTT
AAACATGAGTAGATCTATGACTTCTCAAAGCCGGGTCATGAATGTCCATAAAATGCTAGGCATTCCT
ATTTCCAATATTTTGATGGTTGGAAATTATGCTTCAGATTTGGAACTGGACCCCATGAAGGATATTCT
CATCCTCTCTGCACTGAGGCAGATGCTGCGGGCTGCAGATGATTTTTTAGAAGATTTGCCTCTTGAG
GAAACTGGTGCATTT

BRIH.5478C1n2_s_at (SEQ ID NO:128)
TGCTTATCCGTTAGCCGTGGTGATTTAGCAGGAAGCTGTGAGAGCAGTTTGGTTTCTAGCATGAAGA
CAGAGCCCCACCCTCAGATGCACATGAGCTGGCGGGATTGAAAGATGCTGTCTTCGTACTGGGAAA
GGGATTTTCAGCCCTCAGAATCGCTCCACCTTGCAGCTCTCCCCTTCTCTGTATTCCTAGAAACTGA
CACATGCTGAACATCACAGCTTATTTCCTCATT

BRIH.5650C1n2_at (SEQ ID NO:129)
TAGGCACCACATGGGATCCTTGTTCTTCCTCCTTGTAAGCAGTAATTGAAATCAGTTTGGCAGCCTG
GTTTACAGTGACCATGGTGGCTTGTCTCCCGTGCTCTTACCTCACTCTGTTGATGTTGTAAAACCTC

CAGCTAACTTCATGGGGTGGCTGACCCACGTTGCTCATTTATTCATTCAACACATATTCATTGACCAT
CTACTCTATGCCAGGTATTGTTATCAGCACTGGGAATAGATCAGTGAACTATTGATCTATTTGTCTAA

BRIH.5952C1n2_s_at (SEQ ID NO:130)
CTCAGTTCTGGTCCTTCAAGCCTGTATGGTTTGGATTTTCAGTAGGGGACAGTTGATGTGGAGTCAA
TCTCTTTGGTAC

BRIH.7359C1n3_s_at (SEQ ID NO:131)
CTGAGGTGCTATGTTCTTAGTGGATGTTCTGACCCTGCTTCAAATATTTCCCTCACCTTTCCCATCTT
CCAAGGGTATAAGGAATCTTTCTGCTTTGGGGTTTATCAGAATTCTCAGAATCTCAAATAACTAAAAG
GTATGCAATCAAATCTGCTTTTTAAAGAATGCTCTTTACTTCATGGACTTCCACTGCCATCCTCCCAA
GGGGCCCAAATTCTTTCAGTGGCTACCTACATACAATTCCAAACACATACAG

BRIHRC.10930C1n2_s_at (SEQ ID NO:132)
TAACAAATCATCAACTTCCACTGGTCAATATATAGATTTTGGGTGTCTGAGGCCCCAAGATTAGATGC
CACTAATCTCCAAAGATTCCCTCCAA

BRMX.13731C1n18_at (SEQ ID NO:133)
GCAGGGTCTTGGGATAACACTGTCATCAGAAACAAGGCTGGGGGCTGATTTCGGGGTGGGGAGCC
TTAGGAGGCCAGAAATTCCAATCAGAGCCAGTTTTTCTGGGAGGGAGTGGCTAGACAGTCAAGGAA
GGACGTTCACATTTCAAAAGAAGTCGGGTGGGGGGATGAGATTATTCTAGGGGGGCATCGAATTCC
CTTTAAGGGGGGGGCTCACTTCTGCCCAGAGTAAAGAGGATCTCACACCATGGAAAT

BRMX.25436C1n2_at (SEQ ID NO:134)
TAGTTATACTTACACACTCCTCTCATGTTGTCTATGGAGTGGTGGATGCTGCAGGGAGGGTGACATC
CTAGTTAGTCCTAAGAGCCAGACTGCCTGAAGCTCACTATAACAAGTCCTGCCTTGGGGAAGAAGG
AAGTGTGTCTCTGTGAACCTCCCACCTGGGCCGAAAGGGAGGCCACTCTCTCTGCTGCCTCTCCCC
AACCTTGGCCTTCTGTGCTCCTAGTGAACCTCTCACCCCCTGCCTACAGCCTCGAATCTCAGACCAT
GATGACCTCTGGTCACCCTGAATCAGAGCTTT

BRMX.25712C1n2_at (SEQ ID NO:135)
GTAAAATTCCTATGTCAGCACCCTAATGAGACAAATGACATCCTAATTCTTCCCCTTGGCTTGCCAGT
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TATGAGCAGCATGTGGTTATTTATGTATTCACTCTTTTCTCCTACTTCTGTGGTGACCTGGAACAAAT
TCTCTTATGTATGTAAAGATTGGACAGCCCACCTGATTCCGATGTCACTTAGATACACTGTTTTTGTA
TCAGCCTCTTCTCTTAGAAA

BRMX.3079C1n3_at (SEQ ID NO:136)
GATTGTTGGCCAATAGACCTTCCACTCCAGTAGAGAGGGAGGACTTGGCTCTGAGAACCTCCATCT
GACCTAAGAGGAAACCTCCTCTCCTATGGCCATCTCCTCCTCCTGTCCTTTAAGTCCTCTGTGGTTA
CTATATCTCCTTTTCCCTTTCTTACCCTTTCGCTTAGCAATTTCAAT

BRMX.3079C2n3_at (SEQ ID NO:137)
AAGTTCTTTGGGATAGAGGGTGAAGAACTTGGGACATGGGCTGTTTCAGGGCAGCTGAAGTTCAAA
GGGGAATAGGTAATTGGGGGGAAGGGGGGAAGTTGGGGCAGAAAGGGATTGTTGGGCCAATAGG
ACCTTTCCACT

BRPD.10690C1n5_at (SEQ ID NO:138)
AGGATTATACTTCAGTCCCTGCTTTACATTTATTTCTTAAAGAAGCTTCTGGTAAATTAGAGCAATAGC
ATCGGCTTAGTTTAGTGTTGTTCTGTTGGACTAAGGATATCAGTTCTATCCGTATGGTCGGGCCTAA
AGCCTGGGAAATATTTAATGAAGGNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNA
TAACAAATAACAAAACAAAAACCAAGCCATTTCCCTTTATAGTAAGA

BRPD.4019C1n3_s_at (SEQ ID NO:139)
ACAGAAGCCATTGCCTCCCTTGTTTACCTTGGGTCCACCTCCACCAAAACCCAACAGACCACCAAAT
GTTGACCTGACGAAATTCCACAAAACCTCTTCTGGAAACAGTACTAGCAAAGGCCAGACGTCTTACT
CAACAACTTCCCTGCCACCACCTCCACCATCCCATCCGGCCAGCCAACCACCATTGCCAGCATCTC
ACCCATCACAACCACCAGTCCCAAGCCTACCTCCCAGAAACATTAAACCTCCGTTTGAC

BRPD.5301C1n2_s_at (SEQ ID NO:140)
GCACAGCTCAGCACAACATTCCAAGCTCAAAATAGAAGCCTTCTCAGTGAGCTCCAGCACGCCCAG
AGGACTGTTAATAACGATGATCCATGTGTTTTACTCTAAAGTGCTAAATATGGGAGTTTCCTTTTTTTT
ACTCTTTGTCACTGATGACACAACAGAAAAGAAACTGTAGACCTTGGGACAATCAACATTTAAA

BRRS.12588_at (SEQ ID NO:141)
CCTGCCCTGGAAGTAATCTTGCTGTCCTGGAATCTCCTCGGGGATGAGGCAGCTGCCGAGCTGGC
CCAGGTGCTGCCGAAGATGGGCCGGCTGAAGAGAGTGGACCTGGAGAAGAATCAGATCACAGCTT
TGGGGGCCTGGCTCCTGGCTGAAGGACTGGCCCAGGGGTCTAGCATCCAAGTCATCCGCCTCTGG
AATAACCCCATTCCCTGCGACATGGCCCAGCACCTGAAGAGCCAGGAGCCCAGGCTGGACTTTGC
CTTCTTTGACAACCAGCCC

BRRS.13369_s_at (SEQ ID NO:142)
GCACAGCTCAGCACAACATTCCAAGCTCAAAATAGAAGCCTTCTCAGTGAGCTCCAGCACGCCCAG
AGGACTGTTAATAACGATGATCCATGTGTTTTACTCTAAAGTGCTAAATATGGGAGTTTCCTTTTTTTA
CTCTTTGTCACTGATGACACAACAGAAAAGAAACTGTAGACCTTGGGACAATCAACATTTAAA

BRRS.13576_at (SEQ ID NO:143)
GAGAGTTCAACTAAGAAAGGTCACATATGTGAAAGCCCAAGGACACTGTTTGATATACAGCAGGTAT
TCAATCAGTGTTATTTGAAACCAAATCTGAATTTGAAGTTTGAATCTTCTGAGTTGGAATGAATTTTTT
TCTAGCTGAGGGAAACTGTATTTTTCTTTCCCCAAAGAGGAATGTAA

BRRS.13647_at (SEQ ID NO:144)
CTCGATTATTCCCTGTACAATATTTAAAATTTATTGCTTGATACTTTTGACAACAAATTAGGTTTTGTAC
AATTGAACTTAAATAAATGTCATTAAAATAAATAAATGCAATATGTATTAATATTCATTGTATAAAAATA
GAAGAATACAAACATATTTGTTAAATATTTACATATGAAATTTAATATAGCTATTTTTATGGAATTTTTC
ATTGATATGAAAAATATGATATTGCATATGCATAGTTCCCATGTTAAATCCCATTCATAACTTTCATTA
AAGCATTTACTTTGA

BRRS.13648_s_at (SEQ ID NO:145)
GCAAATAAATTCATACATAGTACATACAAAATAAGAGAAAAAATTAAATTGCAGATGGTTAAATATCAC
ATCACTTAACTGATGTTACTGAAAATGTATTTTCCTGCATAATCATATGGTTGACAGTATGCATTAAGA
AGGTAAGTAAAACAATGAAGACAATTTTGATTTAATATGGTAATGCACAATTCCAACTAACGTACATT
CAACAGATCATGAAATTGGGTTATT

BRRS.13767_at (SEQ ID NO:146)
TTGCCTTCTAAATATACTGAAATGATTTAGATATGTGTCAACAATTAATGATCTTTTATTCAATCTAAGA
AATGGTTTAGTTTTTCTCTTTAGCTCTATGGCATTTCACTCAAGTGGACAGGGGAAAAAGTAATTGCC
ATGGGCTCCAAAGAATTTGCTTTATGTTTTTAGCTAT

BRRS.13859_at (SEQ ID NO:147)
CCTGGCCACTCGCAAGACCTTTTATCTGAAAACCAGCCAAGCTTTATTCACGACACACTTCTTCCCTT
CACTCTCCCACTTCTGTGGTCAACTCCCTGCAGAACTCCCAAACTGCCGTTCTTTTCGATAGCTCAC
GATGGTGTATGAGTGTCAATCATCTGACCCTTCTTGGAGTCTCATATTTCGTGGAAC

BRRS.13881_at (SEQ ID NO:148)
CTGAGGACCGGCTGCAGACCTCACTCTGAGTGGCAGGCAGAGAACCAAAGCTGCTTCGCTGCTCT
CCAGGGAGACCCTCCTGGGATGGGCCTGAGAGGCCGGGGCTCAGGGAAGGGGCTGGGATCGGAA
CTTCCTGCTCTTGTTTCTGGACAACTTTCCCCTTCTGCTTTAAAGGTTGTCGATTATT

BRRS.14465_s_at (SEQ ID NO:149)
AGTGTGATGGATCCCCTTTAGGTTATTTAGGGGTATATGTCCCCTGCTTGAACCCTGAAGGCCAGGT
AATGAGCCATGGCCATTGTCCCCAGCTGAGGACCAGGTGTCTCTAAAAACCCAAACATCCTGGAGA
GTATGCGAGAACCTACCAAGAAAAACAGTCTCATTACTCATATACAGCAGGCAAAGAGACAGAAAAT
TAACTGAAAAGCAGTTTAGAGACTGGGGGAGGCCGGATCTCTAGAGCCATCCTG

BRRS.15053_at (SEQ ID NO:150)
GCGTTACAGATGGACGTAGCTGCCTTGGTTTTCCAGTCCTCAAGGGAATACTGAAGATGCTGACTG
AAGGGGATTGGATGTTGATTTTAGAAGATGGAGAACTCCAGCCACCTTTGTAAAGCACTAGTGTTTG
TCATTTATGTAAGTCAGGTCGGCTCAGGTCTTGATAGTCCGTCTTGGTGTGAGGCATGC

BRRS.16228_s_at (SEQ ID NO:151)
CACAGTAATGTCGAAACTAGGCCTTTGAACCAAGGCAGTCTAGGGTAAAATATAGTTTCAAAGTATG
AATAAGAATTGGTATTTGTGTTATCTTTGAGTAAGAAACTGTCCGATATGAATCACAACGTGGGTGAA
TGTAGTATTTTCCTGAAGTGTG

BRRS.16746_s_at (SEQ ID NO:152)

GGCCATGAACATCACCTGCACAGGACGGGGACCAGACAACTGTATCCAGTGTGCCCACTACATTGA
CGGCCCCCACTGCGTCAAGACCTGCCCGGCAGGAGTCATGGGAGAAAACAACACCCTGGTCTGGA
AGTACGCAGACGCCGGCCATGTGTGCCACCTGTGCCATCCAAACTGCACCTACGGG

BRRS.16747_at (SEQ ID NO:153)
ATCACAGGTTTGAGCTGAATTATCACATGAATATAAATGGGAAATCAGTGTTTTAGAGAGAGAACTTT
TCGACATATTTCCTGTTCCCTTGGAATAAAAACA

BRRS.16948_s_at (SEQ ID NO:154)
AGTTTCAGACAAATGTTCAGTGTGAGTGAGGAAAACATGTTCAGTGAGGAAAAAACATTCAGACAAA
TGTTCAGTGAGGAAAAAAAGGGGAAGTTGGGGATAGGCAGATGTTGACTTGAGGAGTTAATGTGAT
CTTTGGGGAGATACATCTTATAGAGTTAGAAATAGAATCTGAATTTCTAAAGGGAGATTCTGGCTTGG
GA

BRRS.17863_s_at (SEQ ID NO:155)
AACTTAAGCTGAATGTGTAATGGATTTGTCTATAGTTTTACATATTTGGAAGCATTTTAAAATAGGTTT
TAATCTTACATAAAATTACTTTTATACTTGTGTTAACATTTTCTTCTGTGCCTTTTGGGTAATTTAATTT
CTGTTATGAATTTCTGGTGCCTATGAGCTAGCTATCACCTACCTGAAAGGTGCTTAGAGGTGAAGGT
ACTGTTTCTAAAAACACATCACTGTGACACCTTTCTATCCTCACATTTTCAAGCTTGCCTCTTTTCT

BRRS.17909_s_at (SEQ ID NO:156)
GTGACTGCTTATGAAGGGTTATTGCTCAGCTAAGTATTTCTGAATGAGTCTTAGGTCTGTTGGCCTTC
AATCTCTACCGAAACCCTGAGAACTTGATGATGCTTTTGTTTTCTGAGAATCGTTTCAGTGTGCTGG

BRRS.18137_at (SEQ ID NO:157)
CATTTGCTGCAACTCTCAGTGGTAAGAATGATTAAGTGCAGCTATAGGAGAATACTTCCATTGGCAT
GCCACCTGCGTAAAACACACAATTTTGTTAAGATATACAATAAAATTATTATGCTAATAGCAAATATTT
TATGTAGCTCACTATGTTCCATGTAGTCTTCTAAGTGCTTCATGTTAGTCCCCAGTTAAACACCTGGT
TTTGGAAGGCTGAG

BRRS.18652_s_at (SEQ ID NO:158)
GTGAGCCTGCCAGCGTTTGCGACGTCCCCGCACGACAGGCTCATACTTTCTGAGGATCGTGCATAG
CATAGGACGTCTGAACCTTTGTACAAATGTGTAGATGACATCTTGCTACAGCTTTTATTTGTGAAT

BRRS.2573_s_at (SEQ ID NO:159)
GTAAATTCAATACAATGTCAGTTTTTAAAAGTCAAAGTTAGATCAAGAGAATATTTCAGAGTTTTGGTT
TACACATCAAGAAACAGACACACATACCTAGGAAAGATTTACACAATAGATAATCATCTT

BRRS.2644_at (SEQ ID NO:160)
ACTGTACAAAGTATAAGTCTTAGATGTATATATTTCCTATATTGTTTTCAGTGTACATGGAATAACATG
TAATTAAGTACTATGTATCAATGAGTAACAGGAAAATTTTAAAAATACAGATAGATATATGCTCTGCAT
GTTACATAAGATAAATGTGCTGAATGGTTTTCAAATAAAAATGAGGTACTCTCCTGGAAATATTAAGA
AAGACTATCTAAATGTTGAAAGA

BRRS.2783_s_at (SEQ ID NO:161)
GAGGACCGAGCACAGAAATCTTAGAGATTTCTTGTCCCCTCTCAGGTCATGTGTAGATGCGATAAAT
CAAGTGATTGGTGTGCCTGGGTCTCACTACAAGCAGCCTATCTGCTTAAGAGACTCTGGAGTTTCTT
ATGTGCCCTGGTGGACACTTGCCCACCATCCTGTGAGTAAAAGTGAA

BRRS.2935_at (SEQ ID NO:162)
TCTGAACTCTCAAAAGTCTATTTTTTTAACTGAAAATGTAAATTTATAAATATATTCAGGAGTTGGAAT
GTTGTAGTTACCTACTGAGTAGGCGGCGATTTTTGTATGTTATGAACATGCAGTTCATTATTTTGTGG
TTCTATTTTACTTTGTACTTGTGTTTGCTTAAACAAAGTGACTGTTTGGCTTATAAACACATTGAATGC
GCTTTATTGCCCATGGGATATGTGGTGTATATCCTTCCAAAAAATTAAAACGAAAATAAAGTAGCTGC
GATTGG

BRRS.3099_at (SEQ ID NO:163)
ATTCCTGTCATTACCCATTGTAACAGAGCCACAAACTAATACTATGCAATGTTTTACCAATAATGCAAT
ACAAAAGACCTCAAAATACCTGTGCATTTCTTGTAGGAAAACAACAAAAGGTAATTATGTGTAATTAT
ACTAGAAGTTTTGTAATCTGTATCTTATC

BRRS.3131_at (SEQ ID NO:164)
CAGGACCCATCACGCCTGTGCAGTGGCCCCCACAGAAAGACTGAGCTCAAGGTGGGAACCACGTC
TGCTAACTTGGAGCCCCAGTGCCAAGCACAGTGCCTGCATGTATTTATCCAATAAATGTGAAATTCT
GTCC

BRRS.3220_at (SEQ ID NO:165)
AAAGTGGCATTTTCTTGATTGGAAAGGGGGAAGGATCTTATTGCACTTGGGCTGTTCAGAATGTAGA
AAGGACATATTTGAGGAAGTATCTATTTGAGCACTGATTTACTCTGTAAAAAGCAAAATCTCTCTGTC
CTAAACTAATGGAAGCGATTCTCCCATGCTCATGTGTAATGGTTTTAACGTTACTCACTGGAGAGATT
GGACTTTCTGGAGTTATTTAACCACTATGTTCAG

BRRS.3319_at (SEQ ID NO:166)
TTTATAATGTCCCTTCACAAACCCAGTGTTTTAGGAGCATGAGTGCCGTGTGTGTGCGTCCTGTCGG
AGCCCTGTCTCCTCTCTCT

BRRS.3319_s_at (SEQ ID NO:167)
CACCCTCAGATGCACATGAGCTGGCGGGATTGAAGGATGCTGTCTTCGTACTGGGAAAGGGATTTT
CAGCCCTCAGAATCGCTCCACCTTGCAGCTCTCCCCTTCTCTGTATTCCTAGAAACTGACACATGCT
GAACATCACAGCTTATTTCCTCATTT

BRRS.3645_s_at (SEQ ID NO:168)
AAATTTAATTTTCTACGCCTCTGGGGATATCTGCTCAGCCAATGGAAAATCTGGGTTCAACCAGCCC
CTGCCATTTCTTAAGACTTTCTGCTGCACTCACAGGATCCTGAGCTGCACTTACCTGTGAGAGTCTT
CAAACTTTTAAACCTTGCCAGTCAGGACTTTTGCTATTGCA

BRRS.4126_s_at (SEQ ID NO:169)
CTACTCCTTACAGTCTCTAGAATTAAATGTACTCATTTAGACAACATATTAAATGCATATTTTAGCCAC
TTTAGAGAAACCTCATAGGCACAGAGTTTCCAAGATTAATTTTAAGAATATCTTCACGAACTTGACCC
TCCTACTCCACATTGCAACATTTCCATCAGACAGCATTTCAATTCCAGTATTAT

BRRS.455_at (SEQ ID NO:170)
GTCATCATATATAATTAAACAGCTTTTTAAAGAAACATAACCACAAACCTTTTCAAATAATAATAATAAT
AATAATAAAAAATGTATTTTAAAGATGGCCTGTGGTTATCTTGGAAATTGGTGATTTATGCTAGAAAG
CTTTTAATGTTGGTTTATTGTTGAATTCCTAGAA

BRRS.4562_at (SEQ ID NO:171)
CATGGATTAGCTGGAAGATCTGTATTTGATGGAAGACCTTGAAATTATTGGAAGACATGGATTTCCT
GGAAGACGTGGATTTTCCTGGAAGATCTGGATTTGGTGGAAGACCAGTAATTGCTGGAAGACTGGA
TTTGCTGGAAGACTTGATTTACTGGAAGACTTGGAGCTTCTTGGAAGACATGGATTGTCCGGAAGAC
ATGGATTGTCTGGAAGATGTGGATTTTCTGGAAGCTCAG

BRRS.487_s_at (SEQ ID NO:172)
GTGGAGGAAACTAAACATTCCCTTGATGGTCTCAAGCTATGATCAGAAGACTTTAATTATATATTTTC
ATCCTATAAGCTTAAATAGGAAAGTTTCTTCAACAGGATTACAGTGTAGCTACCTACATGCTGAAAAA
TATAGCCTTTAAATCATTTTTATATTATAACTCTGTATAATAGAGATAAGTCCATTTTTTAAAAATGTTT
TCCCCAAACCATAAAACCCTATACAAGTTGTTCTAGTAACAATACATGA

BRRS.4891_s_at (SEQ ID NO:173)
TCAATAAGGGCGTTCTTCCTTGCAAGTTGAAACATTATTGTGCTAGGATTGCTCTCTAGACAAGCCA
GAAGTGACTTATTAAACTATTGAAGGAAAAGGACTCAAGAAAAATAATAAAAGACCATAAATAAGGGC
GAAAACATTACCATGTGAAAAGAATGTATTTCACCTGCAAGTTACAAAAAAATAGTTTGTGCATTGCA
AATAAGCAAAGACTTGGATTGACTTTACATTCATC

BRRS.4996_at (SEQ ID NO:174)
AAGCTGTGTTGTTGCTTCTTGTGAAGGCCATGATATTTTGTTTTTCCCCAATTAATTGCTATTGTGTTA
TTTTACTACTTCTCTCTGTATTTTTTCTTGCATTGACATTATAGACATTGAGGACCTCATCCAAACAAT
TTAAAAATGAGTGTGAAGGGGGAACAAGTCAAAATATTTTTAAAAGATCTTCAAAAATAATGCCTCTG
TCTAGCATGCCAACAAGAATGCAT

BRRS.524_s_at (SEQ ID NO:175)
TGCCTGTTGTAGACCACAGTCACACACTGCTGTAGTCTTCCCCAGTCCTCATTCCCAGCTGCCTCTT
CCTACTGCTTCCGTCTATCAAAAAGCCCCCTTGGCCCAGGTTCCCTGAGCTGTGGGATTCTGCACT
GGTGCTTTGGATTCCCTGATATGTTCCTTCAAA

BRRS.5356_at (SEQ ID NO:176)
GTCAGACAGATGTGGTTGCATCCTAACTCCATGTCTCTGAGCATTAGATTTCTCATTTGCCAATAATA
ATACCTCCCTTAGAAGTTTGTTGTGAGGATTAAATAATGTAAATAAAGAACTAGCATAACACTCAAAA
A

BRRS.5451_at (SEQ ID NO:177)
TCTGTGTGTGCCCTGTAACCTGACTGGTTAACAGCAGTCCTTTGTAAACAGTGTTTTAAACTCTCCTA
GTCAATATCCACCCCATCCAATTTATCAAGGAAGAAATGGTTCAGAAAATATTTTCAGCCTACAGTTA
TGTTCAGTCACACACACATACAAAATGTTCCTTTTGCTTTTAAAGTAATTTTTGACTCCCAGATCAGTC
AGAGCCCCTACAGCATTGTTAA

BRRS.6371_at (SEQ ID NO:178)
GTTTAAGCCTGGAACTTGTAAGAAAATGAAAATTTAATTTTTTTTTTCTAGGACGAGCTATAGAAAAGCT
ATTGAGAGTATCTAGTTAATCAGTGCAGTAGTTGGAAACCTTGCTGGTGTATGTGATGTGCTTCTGT
GCTTTTGAATGACTTTATCATCTAGTCTTTGTCTATTTTTCCTTTGATGTTCAAGTCCTAGTCTATAGG
ATTGGCAGTTTAA

BRRS.6611_at (SEQ ID NO:179)
GACTGAGGGATCGTAGATTTTTACAATCTGTATCTTTGACAATTCTGGGTGCGAGTGTGAGAGTGTG
AGCAGGGCTTGCTCCTGCCAACCACAATTCAATGAATCCCCGACCCCCCTACCCCATGCTGTACTT
GTGGTTCTCTTTTTGTATTTTGCATCTGACCCCGGGGGGGCTGGGACAGATTGGCAATGGGCCGTCC
CCTCTCCCCTTGGTTCTGCACTGTTGCCAATAAAAAGCTCTTAA

BRRS.6619_at (SEQ ID NO:180)
GGAGGGAAGGCAAGATTCTTTCCCCCTCCCTGCTGAAGCATGTGGTACAGAGGCAAGAGCAGAGC
CTGAGAAGCGTCAGGTCCCACTTCTGCCATGCAGCTACTATGAGCCCTCGGGGCCTCCTCCTGGG
CCTCAGCTTGCCCAGATACATACCTAAATATATATATATATATATGAGGGAGAACGCCTCACCCAGAT
TTTATCATGCTGGAAAGAGTGTATGTATGTGAAGATGCTTGGTCAACTTGTACCCAGTGAACACACA
AA

BRRS.6619-22_at (SEQ ID NO:181)
GGAGGGAAGGCAAGATTCTTTCCCCCTCCCTGCTGAAGCATGTGGTACAGAGGCAAGAGCAGAGC
CTGAGAAGCGTCAGGTCCCACTTCTGCCATGCAGCTACTATGAGCCCTCGGGGCCTCCTCCTGGG
CCTCAGCTTGCCCAGATACATACCTAAATATATATATATATATATGAGGGAGAACGCCTCACCCAGAT
TTTATCATGCTGGAAAGAGTGTATGTATGTGAAGATGCTTGGTCAACTTGTACCCAGTGAACACACA
AA

BRRS.6684_at (SEQ ID NO:182)
TATTCTTCTATAACACTCTATATAGAGCTATGTGAGTACTAATCACATTGAATAATAGTTATAAAATTAT
TGTATAGACATCTGCTTCTTAAACAGATTGTGAGTTCTTTGAGAAACAGCGTGGATTTTACTTATCTG
TGTATTCACAGAGCTTAGCACAGTGCCTGGTAATGAGCAAGCATACTTGCCATTACTTTTCCTTCCCA

BRRS.7616_at (SEQ ID NO:183)
CCTAATTTGAGGGTCAGTTCCTGCAGAAGTGCCCTTTGCCTCCACTCAATGCCTCAATTTGTTTTCTG
CATGACTGAGAGTCTCAGTGTTGGAACGGGACAGTATTTATGTATGAGTTTTTCCTATTTATTTTGAG
TCTGTGAGGTCTTCTTGTCATGTGAGTGTGGTTGTGAATGATTTCTTTTGAAGATATATTGTAGTAGA
TGTTACAATTTTGTCGCCAAACTAAACTTGCTGCTTAATGATTTGCTCACATCTAGTAAA

BRRS.7901_at (SEQ ID NO:184)
GGACACTTTTGAAAACAGGACTCAGCATCGCTTTCAATAGGCTTTTCAGGACCTTCACTGCATTAAAA
CAATATTTTTAAAAATTTAGTACAGTTTAGAAAGAGCACTTATTTTGTTTATATCCATTTTTTCTTACTA
AATTATAGGGATTAACTTTGACAAATCATGCTGCTGTTATTTTCTACATTTGTATTTTATCCATAGCAC
TTATTCACATTTAGGAAAA

BRRS.81_at (SEQ ID NO:185)
CAGTTTCTGTTCTCTCACAGGTGATAAACAATGCTTTTTGTGCACTACATACTCTTCAGTGTAGAGCT
CTTGTTTTATGGGAAAAGGCTCAAATGCCAAATTGTGTTTGATGGATTAATATGCCCTTTTGCCGATG
CATACTATTACTGATGTGACTCGGTTTTGTCGCAGCTTTGCTTTGTTTAATGAAACACACTTGTAAAC
CTCTTTTGCACTTTGAAAAAGAATCCAGCGGGATGCTCGAGCACCTGTAAACAATTTTCTCAACCTAT
TTG

BRRS.81-22_at (SEQ ID NO:186)

CAGTTTCTGTTCTCTCACAGGTGATAAACAATGCTTTTTGTGCACTACATACTCTTCAGTGTAGAGCT
CTTGTTTTATGGGAAAAGGCTCAAATGCCAAATTGTGTTTGATGGATTAATATGCCCTTTTGCCGATG
CATACTATTACTGATGTGACTCGGTTTTGTCGCAGCTTTGCTTTGTTTAATGAAACACACTTGTAAAC
CTCTTTTGCACTTTGAAAAAGAATCCAGCGGGATGCTCGAGCACCTGTAAACAATTTTCTCAACCTAT
TTG

BRRS.8480_s_at (SEQ ID NO:187)
AGCAAGTGTAGACACCTTCGAGGGCAGAGATCGGGAGATTTAAGATGTTACAGCATATTTTTTTTTC
TTGTTTTACAGTATTCAATTTTGTGTTGATTCAGCTAAATTATGAAA

BRRS.8711_at (SEQ ID NO:188)
GTCTCACATATTTATATAATCCTCAAATATACTGTACCATTTTAGATATTTTTTAAACAGATTAATTTGG
AGAAGTTTTATTCATTACCTAATTCTGTGGCAAAAATGGTGCCTCTGATGTTGTGATATAGTATTGTC
AGTGTGTACATATATAAAACCTGTGTAAACCTCTGTCCTTATGAACCATAACAAATGTAGCTTTTTA

BRRS.8900_s_at (SEQ ID NO:189)
CAGCCCCACCCCTGTAAATGGAATTTACCAGATGAAGGGAATGAAGTCCCTCACTGAGCCTCAGATT
TCCTCACCTGTGAAATGGGCTGAGGCAGGAAATGGGAAAAAGTGTTAGTGCTTCCAGGCGGCACTG
ACAGCCTCAGTAACAATAAAAACAA

BRSA.1686C1n5_at (SEQ ID NO:190)
TCAGCTGCCCTGAAACAGCCCATGTCCCAAGTTCTTCACCTCTATCCAAAGAACTTGATTTGCATGG
ATTTTGGATAAATCATTTCAGTATCATCTCCATCATATGCCTGACCCCTTGCTCCCTTCAATGCTAGA
AAATCGAGTTGGCAAAATGGGGTTTGGGCCCCTCAGAGCCCTGCCCTGCACCCTTGTACAGTGTCT
GTGCCATGGATTTCGTTTTTCTTGGGGTACTCTTGATGTGAAGATAATTTGCA

BRSA.8072C1n2_s_at (SEQ ID NO:191)
GAGTGTCTCAGAAGTGTGCTCCTCTGGCCTCAGTTCTCCTCTTTTGGAACAACATAAAACAAATTTAA
TTTTCTACGCCTCTGGGGATATCTGCTCAGCCAATGGAAAATCTGGGTTCAACCAGCCCCTGCCATT
TCTTAAGACTTTCTGCTCCACTCACAGGATCCTGAGCTGCACTTACCTGTGAGAGTCTTCAAACTTTT
AAACCTTGCCAGTCAGGACTTTTGCTATTGCA

Hs369056.20C1n2_at (SEQ ID NO:192)
GAGGGACGTCAGAAAATCAGTGCATTGTGGAGTCACTTTTCTGATAAAGGGCACATCAGACTGCAAA
TGGTCCAGACAGCCAGATTCAGGACACTGATGAGTTTCTGGGGTCACCATAGCATCCCTGGAGTCA
GCTGCTCTGCAGCCTGAAGGAGGGCTGACAGTGTGGAGTCACTGCTATTACTTAATGAAATTATATA
GAAATTCTATAATGATTATGTAATTGCATAATGAAAACTCTCCATATCAGAGTTCAGAATATCTCCCAA
TTTCCAGTACAGAATATTATCCATAAC

Hs488293.0CB1n69_at (SEQ ID NO:193)
GACAGCAATAACTTCGTTTTAGAAACATTCAAGCAATAGCTTTATAGCTTCAACATATGGTACGTTTTA
ACCTTGAAAGTTTTGCAATGATGAAAGCAGTATTTGTACAAATGAAAAGCAGAATTCTCTTTTATATG
GTTTATACTGTTGATCAGAAATGTTGATTGTGCATTGAGTATTAAAAAATTAGATGTATATTATTCATT
GTTCTTTACTCATGAGTACCTTATAATAATAATAATGTATTCTTTGTTAACAATGCCATGTTGGTACTA
GTTATTAATCATATC

Hs494173.0CB4n15_at (SEQ ID NO:194)
GGCAGGATATTGTAAGCCTTGAAAAAGAATTAGGCAGGATATCGGAAGCCCTGATTAGATTCTATCC
TAAGAGCAACAGAAGATCACTGACAGTGTTTTAAATAGATAGACTAGTTTATTAGATTTGCAGTTTAG
AAGTTCCCTTTTTTTGTAATTATTGGACAGTGTAGAGACCGGATGGTGAGAGATGAGTTAGGAAGTT
GTGACAGCTCTCTATACCTACCGCTAATGTAGAGGATTATTTATTTTCATTTCATTACCATTCGTGT

Hs513726.0C2n39_s_at (SEQ ID NO:195)
GTAATATGTTTATAATCCTTTAGATCTTATAAATATGTGGTATAAGGAATGCCATATAATGTGCCAAAA
ATCTGAGTGCATTTAATTTAATGCTTGCTTATAGTGCTAAAGTTAAATGATCTTAATTCTTTGCAATTAT
ATATGAAAAATGACTGATTTTTCTTAAAATATGTAACTTATATAAATATATCTGTTTGTACAGATTTTAA
CCATAA

Hs514006.0C1n8_at (SEQ ID NO:196)
GTATCCTTGAACTGGAAACCATCCACGATCGAGTATCGAGTCATTCAACACTATCAATTCCTGGGTG
ACTTTTTGAAAAAGTAGTATCTCTTGTTGCAAGAAATGCTCCATCTGTGAGTCCATGTCTCTCACTGG

AATTGGATGGAAGTGGTGAATTTCAGCCAAAGTGGCCAAAGAAATCCTGTTCCTGTGATTCTGACGT
CATCAGCCTCTGCACCTCTGTCTTCCCTTCTGCCACATGTTGCCTGTTCTCCGTGACTTTGGTAAGA

Hs522202.0C1n6_at (SEQ ID NO:197)
GAGAGAGTGATCACGCTGCTGTGCCCACCTATGCGGTAGACCTTGTTCCTGGGTTGGGAGATGTTT
TATGATCAGGGTGCAGTAGAAAGAGCACACTAGTAGCAGTAAAGAGAGGTGACCCTGGCTGCAGTT
CTGCCTCTAACTTCCTGAGTGACCTCAGGCTAGTCACACAGTGACTGCTCCCCACATTTCTTTTTGT
AAGCTGCAAGGATTGAATCAGACAATAGCCTCTAAGTTTCTTCTGAACTCTCATACTCAGGGATGCC
AA

Hs524348.0CB1n97_at (SEQ ID NO:198)
TTCCCTCCCACTAATTTGTTGGCCTTTAACAGCAATTTTGAAAACTGGGTCTTCTGGTTATGTTTTTGT
TTTAAAATCTTTAAATTAGAGGATGCTGTGCCATTGAGTACTTTAAGTTAATATGAGGTTCTGGTTCAA
GGAAAACTTACGTTGGATCTGAACCAATGAGCAGATATTTTGATATGTGCCACTCTTGCATATACATC
TCAGTCCTAACTAAAGGTTCTAGTGGCATCCAGGACCTTTAGGGAGGCATTT

Hs524348.2C1n5_s_at (SEQ ID NO:199)
CACTGCGTCTGGCAATAATGTAACTTTGAAGCTTAAAAATTAATCCCAGTTTGTAGCAATAACAGAAG
ACTATCTACAACGGAAGAAAGAAGCAACTGCCTTACAGTTCTGTAAAGAATTGGCAAGAAAATAAAG
CCTATAGTTGCC

Hs528836.0C1n3_s_at (SEQ ID NO:200)
CCCTTACTTACATACTAGCTTCCAAGGACAGGTGGAGGTAGGGCCAGCCTGGCGGGAGTGGAGAA
GCCCAGTCTGTCCTATGTAAGGGACAAAGCCAGGTCTAATGGTACTGGGTAGGGGGCACTGCCAA
GACAATAAGCTAGGCTACTGGGTCCAGCTACTACTTTGGTGGGATTCAGGTGAGTCTCCATGCACTT
CACATGTTACCCAGTGTTCTTGTTACTTCCAAGGAGAACCAAGAATGGCTCTGTCACACTCGAAGCC
AGGTTTGATC

Hs591893.1C1n4_s_at (SEQ ID NO:201)
CCTCCTTTCTAAATGCAGCGACCTGTGTTCTTCAGCCCTATCCCTTTCTATTCCTCTGACCCCGCCTC
CTTTCTAAATGCAGCGACCTCTGTTCTTCAGCCCTATCCCTTTCTATTCCTCTGACCCCGCCTCCTTT
CTAAATGCAGCGACCTCTG

Hs7155.0CB1n102_at (SEQ ID NO:202)
GGCGTCGGCGCCTAGGGCGAAGTGAGCCAGGGTGCAGTCGGGAAGCTCCAGGACGAAGCGGCGC
GGCGGAGCCATGGCCCCAGCGCAGACCCCGCGCCGCCCGAGCAGCGGCCCCGACAGTGGCCCG
CGCAGGAGCCGGCGGGCGAAGGCCATGGGCGCCTCAGCGACGCCGCCCTCGGCCCCGCCTCGG
AAACGAAACCTGGCGGGAGCCAGGCGCCGGCGGGAAACGAAACCCGGAGGGAGCCAGGCGCCA
GCGGGAAACGAAAGCGAAGCGT

IDO_F1 (SEQ ID NO:203)
AGAGACATCTGTATGCATTCCTG

IDO_R1 (SEQ ID NO:204)
GGTATTTTGAGGTCTTTTGTATTGC

IDO_P1 (SEQ ID NO:205)
ACCCATTGTAACAGAGCCACAAACT

CD2_F1 (SEQ ID NO:206)
TCTCAGGTCATGTGTAGATGCG

CD2_R1 (SEQ ID NO:207)
CTCCAGAGTCTCTTAAGCAGATAGG

CD2_P1 (SEQ ID NO:208)
AGACCCAGGCACACCAATCACTTGA

GBP5_F1 (SEQ ID NO:209)
AACAACAGATGCAGGAACAGG

GBP5_R1 (SEQ ID NO:210)
AGTCCTCTGGGCGTGCTG

GBP5_P1 (SEQ ID NO:211)
CACAGCTCAGCACAACATTCCAAGC

PRAME_F1 (SEQ ID NO:212)
CTGCATACTTGGACACTAAAGCC

PRAME_R1 (SEQ ID NO:213)
ATGTTTTCCTCACTCACACTGAAC

PRAME_P1 (SEQ ID NO:214)
AGCAACAAAGCAGCCACAGTTTCAG

ITGAL_F1 (SEQ ID NO:215)
ACAGAAAGACTGAGCTCAAGGTG

ITGAL_R1 (SEQ ID NO:216)
TGCAGGCACTGTGCTTGG

ITGAL_P1 (SEQ ID NO:217)
AACCACGTCTGCTAACTTGGAGCCC

LRP4_F1 (SEQ ID NO:218)
TGTAAAAAGCAAAATCTCTCTGTCC

LRP4_R1 (SEQ ID NO:219)
AGTCCAATCTCTCCAGTGAGTAAC

LRP4_P1 (SEQ ID NO:220)
AATGGAAGCGATTCTCCCATGCTCA

APOL3_F1 (SEQ ID NO:221)
GACCAGGTGTCTCTAAAAACCC

APOL3_R1 (SEQ ID NO:222)
TTGCCTGCTGTATATGAGTAATGAG

APOL3_P1 (SEQ ID NO:223)
CCTGGAGAGTATGCGAGAACCTACC

CDR1_F1 (SEQ ID NO:224)
GAAGACGTGGATTTTCCTGGAAG

CDR1_R1 (SEQ ID NO:225)
TCCAAGTCTTCCAGTAAATCAAGTC

CDR1_P1 (SEQ ID NO:226)
TCCAGCAAATCCAGTCTTCCAGCAA

FYB_F1 (SEQ ID NO:227)
AACAAATCATCAACTTCCACTGGTC

FYB_R1 (SEQ ID NO:228)
TGGAGGGAATCTTTGGAGATTAGTG

FYB_P1 (SEQ ID NO:229)
TCTAATCTTGGGGCCTCAGACACCC

TSPAN7_F1 (SEQ ID NO:230)
GACATTGAGGACCTCATCCAAAC

TSPAN7_R1 (SEQ ID NO:231)
GACAGAGGCATTACTTTTGAAGATC

TSPAN7_P1 (SEQ ID NO:232)
TTGACTTGTTCCCCCTTCACACTCA

RAC2_F1 (SEQ ID NO:233)
CTCAGTTCTCCTCTTTTGGAACAAC

RAC2_R1 (SEQ ID NO:234)
TTGAACCCAGATTTTCCATTGGC

RAC2_P1 (SEQ ID NO:235)
TCTACGCCTCTGGGGATATCTGCTC

KLHDC7B_F1 (SEQ ID NO:236)
TGGCACTGTGGATTCTCAAGG

KLHDC7B_R1 (SEQ ID NO:237)
CTGGGGGTATGGGCAGGAG

KLHDC7B_P1 (SEQ ID NO:238)
CACCAGCGGACCAGTTTCAGAGGCA

GRB14_F1 (SEQ ID NO:239)
CTAATACAGCTGGTGGAGTTCTATC

GRB14_R1 (SEQ ID NO:240)
AGCAATCCTAGCACAATAATGTTTC

GRB14_P1 (SEQ ID NO:241)
ACTCAATAAGGGCGTTCTTCCTTGC

KIF26A_F1 (SEQ ID NO:242)
AGGAATTTTTACCAAAACCACAAGC

KIF26A_R1 (SEQ ID NO:243)
AACAGAACCTTTACAAAACCCTACC

KIF26A_P1 (SEQ ID NO:244)
AACAGACCACCACGACCAACAACA

CD274_F1 (SEQ ID NO:245)
TTGGTGTGACAGTGTTCTTTGTG

CD274_R1 (SEQ ID NO:246)
AGGAGGAGTTAGGACTTAGGAATAG

CD274_P1 (SEQ ID NO:247)
TGCCTTGCTCAGCCACAATTCTTGC

CD109_F1 (SEQ ID NO:248)
TGTGGATTTGAATGTGTGTACAAGC

CD109_R1 (SEQ ID NO:249)
GGCACCATAAAGCCACTTAATAGG

CD109_P1 (SEQ ID NO:250)
AAGAGCCATGCCACTCCTACCCGG

ETV7_F1 (SEQ ID NO:251)
CCCTCACTGAGCCTCAGATTTC

ETV7_R1 (SEQ ID NO:252)
GCCGCCTGGAAGCACTAAC

ETV7_P1 (SEQ ID NO:253)
TCCCATTTCCTGCCTCAGCCCATTT

MFAP5_F1 (SEQ ID NO:254)
GGCTGGTCTGCCCCCTAG

MFAP5_R1 (SEQ ID NO:255)
ACCATTGGGTCTCTGCAAATCC

MFAP5_P1 (SEQ ID NO:256)
ACTCCGTCGCTCCAATTACTTCCGA

OLFM4_F1 (SEQ ID NO:257)
AGGACGAGCTATAGAAAAGCTATTG

OLFM4_R1 (SEQ ID NO:258)
CATTCAAAAGCACAGAAGCACATC

OLFM4_P1 (SEQ ID NO:259)
CACCAGCAAGGTTTCCAACTACTGC

PI15_F1 (SEQ ID NO:260)
TTTTCCAGGCTAAAGCAAATGAAAG

PI15_R1 (SEQ ID NO:261)
CTATCCTAGCACCATTGTTGCATG

PI15_P1 (SEQ ID NO:262)
TTGCTGGTATCAACACAGCCTGCCA

FOSB_F1 (SEQ ID NO:263)
TGAGTGAGACTGAGGGATCGTAG

FOSB_R1 (SEQ ID NO:264)
GTGGTTGGCAGGAGCAAGC

FOSB_P1 (SEQ ID NO:265)
CACACTCTCACACTCGCACCCAGAA

CXCL10_F1 (SEQ ID NO:266)
ACCAGAGGGGAGCAAAATCGA

CXCL10_R1 (SEQ ID NO:267)
TGCCTCTCCCATCACTTCCC

CXCL10_P1 (SEQ ID NO:268)
CCTCTGTGTGGTCCATCCTTGGAAGCA

MX1_F1 (SEQ ID NO:269)
CAGCACCTGATGGCCTATCAC

MX1_R1 (SEQ ID NO:270)
CAGTTCTTCATGCTCCAGACGTAC

MX1_P1 (SEQ ID NO:271)
CGCATCTCCAGCCACATCCCTTTGA

IFI44L_F1 (SEQ ID NO:272)
CCTCTTGAGGAAACTGGTGCAATTG

IFI44L_R1 (SEQ ID NO:273)
TGATTCTGACATTTGGCCCAGC

IFI44L_P1 (SEQ ID NO:274)
TCTCAAATGCAGGGCTGTAACGCTCTC

AC138128.1_F1 (SEQ ID NO:275)
GCTAGAGCAGGACTTCGTCTCC

AC138128.1_R1 (SEQ ID NO:276)
GAGAAGATCTGGCCTTATGCCCA

AC138128.1_P1 (SEQ ID NO:277)
TCTCTGGAACAGCTCATCGCCGCAT

FAM19A5_F1 (SEQ ID NO:278)
GGAAGGCTGCGACTTGTTAATCAA

FAM19A5_R1 (SEQ ID NO:279)
CTCCTGACAAACACAGCCCC

FAM19A5_P1 (SEQ ID NO:280)
CCGTGGTGGTCTTTATCCTCCCGCC

NLRC5_F1 (SEQ ID NO:281)
GAGAGTGGACCTGGAGAAGAATCAG

NLRC5_R1 (SEQ ID NO:282)
TAGCATCCAAGTCATCCGCCT

NLRC5_P1 (SEQ ID NO:283)
AGTCCTTCAGCCAGGAGCCAGGC

PRICKLE1_F1 (SEQ ID NO:284)
GTTCGGGATTCGATGGATTCTTTGG

PRICKLE1_R1 (SEQ ID NO:285)
CCAAGGCCATCATTGTATTCTCTGC

PRICKLE1_P1 (SEQ ID NO:286)
TCTCCATCCACCGAAGCCCCTGT

EGR1_F1 (SEQ ID NO:287)
GCAGCACCTTCAACCCTCAG

EGR1_R1 (SEQ ID NO:288)
TCTCTGAACAACGAGAAGGTGCT

EGR1_P1 (SEQ ID NO:289)
CCTACGAGCACCTGACCGCAGAGT

CLDN10_F1 (SEQ ID NO:290)
AGCCGCTCTGTTTATTGGATGG

CLDN10_R1 (SEQ ID NO:291)
TCTGACAACAACAAAACACCCAGA

CLDN10_P1 (SEQ ID NO:292)
ACACCACCAATTATGCACAGTGAGGCT

ADAMTS4_F1 (SEQ ID NO:293)
TGGCTCCAAGAAGAAGTTTGACAAG

ADAMTS4_R1 (SEQ ID NO:294)
TCCTTCAGGAAATTCAGGTACGGAT

ADAMTS4_P1 (SEQ ID NO:295)
CCTGACTGCTTGCTGCAACCAGAACC

SP140L_F1 (SEQ ID NO:296)
AGTGGAGGGGTTTGTACAAGACA

SP140L_R1 (SEQ ID NO:297)
CAAATGGGACTTAGACTGGAGGCT

SP140L_P1 (SEQ ID NO:298)
CGCCTCATCTTCCAGAACCACAGGG

ANXA_F1 (SEQ ID NO:299)
CCACAAGCAAACCAGCTTTCTTTG

ANXA_R1 (SEQ ID NO:300)
TGATCAGGATTATGGTTTCCCGTTC

ANXA_P1 (SEQ ID NO:301)
TGGCGAGTTCCAACACCTTTCATGGC

RSAD2_F1 (SEQ ID NO:302)
GGAAGAGGACATGACGGAACAGA

RSAD2_R1 (SEQ ID NO:303)
GTGTTCCAGTGCCTCTTAATTGAGG

RSAD2_P1 (SEQ ID NO:304)
CAAAGCACTAAACCCTGTCCGCTGGAA

ESR1_F1 (SEQ ID NO:305)
CTGCAGCAGCAGCACCAG

ESR1_R1 (SEQ ID NO:306)
CATCAGGCACATGAGTAACAAAGGC

ESR1_P1 (SEQ ID NO:307)
CCCAGCTCCTCCTCATCCTCTCCC

IKZF3_F1 (SEQ ID NO:308)
GCAGAGATGGGAAGTGAAAGAGC

IKZF3_R1 (SEQ ID NO:309)
TCAATGCCTCAGAAATTCATTGGTG

IKZF3_P1 (SEQ ID NO:310)
TGCCACATTGCTTGCTAATCTGTCCAG

EGFR_F1 (SEQ ID NO:311)
GACAGCTTCTTGCAGCGATACAG

EGFR_R1 (SEQ ID NO:312)
CCTTCCTCCCAGTGCCTGA

EGFR_P1 (SEQ ID NO:313)
TCGTCTATGCTGTCCTCAGTCAAGGCG

NAT1_F1 (SEQ ID NO:314)
AGAGCACTTCCTCATAGACCTTGG

NAT1_R1 (SEQ ID NO:315)
TTCAAGCCAGGAAGAAGCAGC

NAT1_P1 (SEQ ID NO:316)
TGCATTCAGTCTAGTTCCTGGTTGCCG

LATS2_F1 (SEQ ID NO:317)
GCAAGATGGGCTACCTGGAC

LATS2_R1 (SEQ ID NO:318)
TTAAGCAGACCTCCCCAGGA

LATS2_P1 (SEQ ID NO:319)
ACCCGCACAATCTGCTCATTCCTCG

CYP2B6_F1 (SEQ ID NO:320)
TCTCCTTAGGGAAGCGGATTTGTC

CYP2B6_R1 (SEQ ID NO:321)
TTCTTCACCACCATCCTCCAGA

CYB2B6_P1 (SEQ ID NO:322)
CATCGCCCGTGCGGAATTGTTCCT

PTPRC_F1 (SEQ ID NO:323)
CTGGCCATCTGCAAGCTGAG

PTPRC_R1 (SEQ ID NO:324)
CAGTTCAGCCTTCAGTTGGTGG

PTPRC_P1 (SEQ ID NO:325)
AGCAAGGAAGCCAATCCAAGTCACCAA

PPP1R1A_F1 (SEQ ID NO:326)
ACCCATATACCACCACTGGATTCC

PPP1R1A_R1 (SEQ ID NO:327)
CAGTTTGGGAATGCATGGACACC

PPP1R1A_P1 (SEQ ID NO:328)
ACCTCCTCCTCTCTCAGACCGAGTTGG

STING_a (SEQ ID:329)
CAGCGGCUGUAUAUUCUCCUCCCUU

STING_b (SEQ ID:330)
GGUCAUAUUACAUCGGAUAUU

TBK1_a (SEQ ID:331)
GGAAAUAUCAUGCGUGUUAUU

TBK1_b (SEQ ID:332)
UGGUGCAGCUAGAGAAUUAUU

IRF3_a (SEQ ID:333)
CCUCUGAGAACCCACUGAAUU

IRF3_b (SEQ ID:334)
GGACAAUCCCACUCCCUUCUU

cGAS_a (SEQ ID:335)
AGAGAAAUGUUGCAGGAAAUU

cGAS_b (SEQ ID:336)
CAGCUUCUAAGAUGCUGUCAAAGUU

BRCA1_a (SEQ ID:337)
CCUAUCGGAAGAAGGCAAGUU

BRCA1_b (SEQ ID:338)
CAUACAGCUUCAUAAAUAAUU

BRCA2_a (SEQ ID:339)
GGACACAAUUACAACUAAAUU

BRCA2_b (SEQ ID:340)
GGAGGAAUAUCGUAGGUAAUU

FancD2_a (SEQ ID:341)
GCAGAUUCAUGAAGAGAAAUU

FancD2_b (SEQ ID:342)
GGUUAAAGCACAUUGUAGAUU

CXCL10 Forward (SEQ ID NO:343)
GGCCATCAAGAATTTACTGAAAGCA

CXCL10 Reverse (SEQ ID NO:344)
TCTGTGTGGTCCATCCTTGGAA

CCL5 Forward (SEQ ID NO:345)
TGCCCACATCAAGGAGTATTT

CCL5 Reverse (SEQ ID NO:346)
CTTTCGGGTGACAAAGACG

IDO1 Forward (SEQ ID NO:347)
CATCTGCAAATCGTGACTAAG

IDO1 Reverse (SEQ ID NO:348)
CAGTCGACACATTAACCTTCCTTC

PDL1 Forward (SEQ ID NO:349)
GGCATCCAAGATACAAACTCAAAGA

PDL1 Reverse (SEQ ID NO:350)
AGTTCCAATGCTGGATTACGTCT

PUM1 (Housekeeping gene) Forward (SEQ ID NO:351)
CCAGAAAGCTCTTGAGTTTATTCC

PUM1 (Housekeeping gene) Reverse (SEQ ID NO:352)
CATCTAGTTCCCGAACCATCTC

OR2I1P_F1 (SEQ ID NO:353)
CTCAACCCGCTCATCTACAC

OR2I1P_R1 (SEQ ID NO:354)
TCCTTGGGTTCTGGCTTAATAC

OR2I1P_P1 (SEQ ID NO:355)
TCGCTGCCCCCTTCACTTTCTTATT

AL137218.1_F1 (SEQ ID NO:356)
TGCTTCATGTTAGTCCCCAG

AL137218.1_R1 (SEQ ID NO:357)
GGGTCTCACTATATTGCTCTGG

AL137218.1_P1 (SEQ ID NO:358)
CCTCAGCCTTCCAAAACCAGGTGT

5

CLAIMS

1. A method for predicting responsiveness to an antagonist of an inhibitory immune checkpoint and/or an agonist of a stimulatory immune checkpoint comprising:
determining the expression level of at least one gene selected from Table 2B, 2A or 1 in a sample from the subject wherein the determined expression level is used to predict responsiveness to an antagonist of an inhibitory immune checkpoint and/or an agonist of a stimulatory immune checkpoint.

2. The method of claim 1 wherein an increased expression level of the at least one gene predicts responsiveness to an antagonist of an inhibitory immune checkpoint and/or an agonist of a stimulatory immune checkpoint.

3. The method of claim 1 or 2 which comprises determining the expression level of at least 2 of the genes and the determined expression levels are used to generate a combined test score, wherein a positive combined test score (generally above threshold, but may be equal to or above threshold) predicts responsiveness to an antagonist of an inhibitory immune checkpoint and/or an agonist of a stimulatory immune checkpoint.

4. The method of any preceding claim which comprises:
(i) deriving a combined test score that captures the expression levels;
(ii) providing a threshold score comprising information correlating the combined test score and responsiveness;
(iii) and comparing the combined test score to the threshold score; wherein responsiveness is predicted when the combined test score exceeds the threshold score.

5. A method for predicting responsiveness to an antagonist of an inhibitory immune checkpoint and/or an agonist of a stimulatory immune checkpoint in combination with a DNA damage therapeutic agent comprising:
determining the expression level of at least one gene selected from Table 2B, 2A or 1 in a sample from the subject wherein the determined expression level is used to predict responsiveness to an antagonist of an inhibitory immune checkpoint and/or an agonist of a stimulatory immune checkpoint in combination with a DNA damage therapeutic agent.

6. The method of claim 5 wherein an increased expression level of the at least one gene predicts responsiveness to an antagonist of an inhibitory immune checkpoint and/or an agonist of a stimulatory immune checkpoint in combination with a DNA damage therapeutic agent.

7. The method of claim 5 or 6 which comprises determining the expression level of at least 2 of the genes and the determined expression levels are used to generate a combined test score, wherein a positive combined test score (generally above threshold, but may be equal to or above threshold) predicts responsiveness to an antagonist of an inhibitory immune checkpoint and/or an agonist of a stimulatory immune checkpoint in combination with a DNA damage therapeutic agent.

8. The method of any of claims 5 to 7 which comprises:

(i) deriving a combined test score that captures the expression levels;

(ii) providing a threshold score comprising information correlating the combined test score and responsiveness;

(iii) and comparing the combined test score to the threshold score; wherein responsiveness is predicted when the combined test score exceeds the threshold score.

9. A method for identifying a cancer that can be effectively treated with an antagonist of an inhibitory immune checkpoint and/or an agonist of a stimulatory immune checkpoint comprising: determining the expression level of at least one gene selected from Table 2B, 2A or 1 in a sample from the subject wherein the determined expression level is used to identify a cancer that can be effectively treated with an antagonist of an inhibitory immune checkpoint and/or an agonist of a stimulatory immune checkpoint.

10. The method of claim 9 wherein an increased expression level of the at least one gene identifies a cancer that can be effectively treated with an antagonist of an inhibitory immune checkpoint and/or an agonist of a stimulatory immune checkpoint.

11. The method of claim 9 or 10 which comprises determining the expression level of at least 2 genes and the determined expression levels are used to generate a combined test score, wherein a positive combined test score (generally above threshold, but may be equal to or above threshold) identifies a cancer that can be effectively treated with an antagonist of an inhibitory immune checkpoint and/or an agonist of a stimulatory immune checkpoint.

12. The method of any of claims 9 to 11 which comprises:

(i) deriving a combined test score that captures the expression levels;

(ii) providing a threshold score comprising information correlating the combined test score and responsiveness;

(iii) and comparing the combined test score to the threshold score; wherein a cancer that can be effectively treated is identified when the combined test score exceeds the threshold score.

13. A method for identifying a cancer that can be effectively treated with an antagonist of an inhibitory immune checkpoint and/or an agonist of a stimulatory immune checkpoint in combination with a DNA damage therapeutic agent comprising:
determining the expression level of at least one gene selected from Table 2B, 2A or 1 in a sample from the subject wherein the determined expression level is used to identify a cancer that can be effectively treated with an antagonist of an inhibitory immune checkpoint and/or an agonist of a stimulatory immune checkpoint in combination with a DNA damage therapeutic agent.

14. The method of claim 13 wherein an increased expression level of the at least one gene identifies a cancer that can be effectively treated with an antagonist of an inhibitory immune checkpoint and/or an agonist of a stimulatory immune checkpoint in combination with a DNA damage therapeutic agent.

15. The method of claim 13 or 14 which comprises determining the expression level of at least 2 of the genes and the determined expression levels are used to generate a combined test score, wherein a positive combined test score (generally above threshold, but may be equal to or above threshold) identifies a cancer that can be effectively treated with an antagonist of an inhibitory immune checkpoint and/or an agonist of a stimulatory immune checkpoint in combination with a DNA damage therapeutic agent.

16. The method of any of claims 13 to 15 which comprises:

(i) deriving a combined test score that captures the expression levels;

(ii) providing a threshold score comprising information correlating the combined test score and responsiveness;

(iii) and comparing the combined test score to the threshold score; wherein a cancer that can be effectively treated is identified when the combined test score exceeds the threshold score.

17. A method for selecting treatment for a cancer comprising:

determining the expression level of at least one gene selected from Table 2B, 2A or 1 in a sample from the subject wherein the determined expression level is used to select an antagonist of an inhibitory immune checkpoint and/or an agonist of a stimulatory immune checkpoint for use in treatment of the cancer.

18. The method of claim 17 wherein an increased expression level of the at least one gene is used to select an antagonist of an inhibitory immune checkpoint and/or an agonist of a stimulatory immune checkpoint for use in treatment of the cancer.

19. The method of claim 17 or 18 which comprises determining the expression level of at least 2 of the genes and the determined expression levels are used to generate a combined test score, wherein a positive combined test score (generally above threshold, but may be equal to or above threshold) is used to select an antagonist of an inhibitory immune checkpoint and/or an agonist of a stimulatory immune checkpoint for use in treatment of the cancer.

20. The method of any of claims 17 to 19 further comprising treating the cancer using the selected antagonist and/or agonist.

21. The method of any of claims 17 to 20 which comprises:

(i) deriving a combined test score that captures the expression levels;

(ii) providing a threshold score comprising information correlating the combined test score and responsiveness;

(iii) and comparing the combined test score to the threshold score; wherein an antagonist of an inhibitory immune checkpoint and/or an agonist of a stimulatory immune checkpoint is selected for use when the combined test score exceeds the threshold score.

22. A method for selecting treatment for a cancer comprising:
determining the expression level of at least one gene selected from 2B, 2A or 1 in a sample from the subject wherein the determined expression level is used to select an antagonist of an inhibitory immune checkpoint and/or an agonist of a stimulatory immune checkpoint, in combination with a DNA damage therapeutic agent, for use in treatment of the cancer.

23. The method of claim 22 wherein an increased expression level of the at least one gene is used to select an antagonist of an inhibitory immune checkpoint and/or an agonist of a stimulatory immune checkpoint, in combination with a DNA damage therapeutic agent, for use in treatment of the cancer.

24. The method of claim 22 or 23 which comprises determining the expression level of at least 2 of the genes and the determined expression levels are used to generate a combined test score, wherein a positive combined test score (generally above threshold, but may be equal to or above threshold) is used to select an antagonist of an inhibitory immune checkpoint and/or an agonist of a stimulatory immune checkpoint, in combination with a DNA damage therapeutic agent, for use in treatment of the cancer.

25. The method of any of claims 22 to 24 further comprising treating the cancer using the selected antagonist and/or agonist, in combination with a DNA damage therapeutic agent.

26. The method of any of claims 22 to 25 which comprises:
(i) deriving a combined test score that captures the expression levels;
(ii) providing a threshold score comprising information correlating the combined test score and responsiveness;
(iii) and comparing the combined test score to the threshold score; wherein an antagonist of an inhibitory immune checkpoint and/or an agonist of a stimulatory immune checkpoint in combination with a DNA damage therapeutic agent is selected for use when the combined test score exceeds the threshold score.

27. The method of any preceding claim which comprises determining the expression level of at least 6 genes selected from CXCL10, MX1, IDO1, IFI44L, CD2, GBP5, PRAME, ITGAL, LRP4, APOL3, CDR1, FYB, TSPAN7, RAC2, KLHDC7B, GRB14, AC138128.1, KIF26A, CD274, CD109, ETV7, MFAP5, OLFM4, PI15, FOSB, FAM19A5, NLRC5, PRICKLE1, EGR1, CLDN10, ADAMTS4, SP140L, ANXA1, RSAD2, ESR1, IKZF3, OR2I1P, EGFR, NAT1, LATS2, CYP2B6, PTPRC, PPP1R1A, and AL137218.1.

28. The method of any preceding claim which comprises determining the expression level of at least 1 gene selected from CD2, ITGAL, PTPRC, CXCL10 and IDO1, together with at least one further gene selected from MX1, IFI44L, GBP5, PRAME, LRP4, APOL3, CDR1, FYB, TSPAN7, RAC2, KLHDC7B, GRB14, AC138128.1, KIF26A, CD274, CD109, ETV7, MFAP5, OLFM4, PI15, FOSB, FAM19A5, NLRC5,

PRICKLE1, EGR1, CLDN10, ADAMTS4, SP140L, ANXA1, RSAD2, ESR1, IKZF3, OR2I1P, EGFR, NAT1, LATS2, CYP2B6, PPP1R1A, and AL137218.1.

29. The method of any preceding claim which comprises determining the expression level of at least 12 genes selected from Table 1.

30. The method of any preceding claim which comprises determining the expression level of at least 1 gene selected from CD2, ITGAL, PTPRC, CXCL10, IDO1, CD3D, HLA-DPB1, CXCL9, CCL5, STAT1, IL2RG, CD3E, IRF1, IKZF3 and IGJ together with at least one further gene selected from (the remaining genes in) Table 1 or together with at least one further gene from the (remaining genes in) Table 2B (the 44 gene panel).

31. The method of any preceding claim which comprises determining the expression level of each of CXCL10, MX1, IDO1, IFI44L, CD2, GBP5, PRAME, ITGAL, LRP4, APOL3, CDR1, FYB, TSPAN7, RAC2, KLHDC7B, GRB14, AC138128.1, KIF26A, CD274, CD109, ETV7, MFAP5, OLFM4, PI15, FOSB, FAM19A5, NLRC5, PRICKLE1, EGR1, CLDN10, ADAMTS4, SP140L, ANXA1, RSAD2, ESR1, IKZF3, OR2I1P, EGFR, NAT1, LATS2, CYP2B6, PTPRC, PPP1R1A, and AL137218.1.

32. The method of any preceding claim which comprises determining the expression level of each of the genes from any one of Tables 4 to 45.

33. The method of any preceding claim wherein the weight values for each gene are as set out in Table 2B, or wherein the weight and/or bias values for each gene are as set out in any one of Tables 3 to 45.

34. The method of any preceding claim which comprises determining the expression level of at least one, up to all, of CCL5, CXCL9 and CXCL10 together with at least one further gene selected from (the remaining genes in) Table 1 or together with at least one further gene from the (remaining genes in) Table 2B (the 44 gene panel).

35. The method of any preceding claim wherein determining the expression level employs at least one primer or primer pair from Table 2E and/or at least one probe from Table 2E.

36. The method of any preceding claim wherein the combined test score (or "signature score") is derived according to the formula:

$$SignatureScore = \sum_i w_i \times (ge_i - b_i) + k$$

Where w_i is a weight for each gene, b_i is a gene-specific bias, ge_i is the gene expression after pre-processing, and k is a constant offset.

37. A method of treating cancer comprising administration of an antagonist of an inhibitory immune checkpoint and/or an agonist of a stimulatory immune checkpoint to a subject, characterised in that a

sample from the subject, prior to administration, displays a positive combined test score derived from the determined expression levels of at least 2 genes from Table 2B, 2A or 1 or an increased level of expression of at least 1 gene from Table 2B, 2A or 1.

38. A method of treating cancer comprising administration of an antagonist of an inhibitory immune checkpoint and/or an agonist of a stimulatory immune checkpoint, in combination with a DNA damage therapeutic agent, to a subject, characterised in that a sample from the subject, prior to administration, displays a positive combined test score derived from the determined expression levels of at least 2 genes from Table 2B, 2A or 1 or an increased level of expression of at least 1 gene from Table 2B, 2A or 1.

39. An antagonist of an inhibitory immune checkpoint and/or an agonist of a stimulatory immune checkpoint for use in the treatment of cancer in a subject wherein, prior to administration of the antagonist and/or agonist, a sample from the subject displays a positive combined test score derived from the determined expression levels of at least 2 genes from Table 2B, 2A or 1 or an increased level of expression of at least 1 gene from Table 2B, 2A or 1.

40. An antagonist of an inhibitory immune checkpoint and/or an agonist of a stimulatory immune checkpoint for use in the treatment of cancer in a subject wherein, prior to administration of the antagonist and/or agonist, a sample from the subject displays a positive combined test score derived from the determined expression levels of at least 2 genes from Table 2B, 2A or 1 or an increased level of expression of at least 1 gene from Table 2B, 2A or 1, and wherein the antagonist and/or agonist is administered in combination with a DNA damage therapeutic agent.

41. An antagonist of an inhibitory immune checkpoint in combination with a DNA damage therapeutic agent and/or an agonist of a stimulatory immune checkpoint in combination with a DNA damage therapeutic agent for use in the treatment of cancer in a subject wherein, prior to administration of the antagonist and/or agonist and DNA damage therapeutic agent, a sample from the subject displays a positive combined test score derived from the determined expression levels of at least 2 genes from Table 2B, 2A or 1 or an increased level of expression of at least 1 gene from Table 2B, 2A or 1.

42. The method of claim 37 or 38, or the antagonist and/or agonist for use of any of claims 39 to 41, wherein the combined test score (or "signature score") is derived according to the formula:

$$SignatureScore = \sum_i w_i \times (ge_i - b_i) + k$$

Where w_i is a weight for each gene, b_i is a gene-specific bias, ge_i is the gene expression after pre-processing, and *k* is a constant offset.

43. The method of any of claims 37, 38 or 42, or the antagonist and/or agonist for use of any of claims 39 to 42, wherein the combined test score is derived from the determined expression level of at least 6 genes selected from CXCL10, MX1, IDO1, IFI44L, CD2, GBP5, PRAME, ITGAL, LRP4, APOL3, CDR1, FYB, TSPAN7, RAC2, KLHDC7B, GRB14, AC138128.1, KIF26A, CD274, CD109, ETV7, MFAP5,

OLFM4, PI15, FOSB, FAM19A5, NLRC5, PRICKLE1, EGR1, CLDN10, ADAMTS4, SP140L, ANXA1, RSAD2, ESR1, IKZF3, OR2I1P, EGFR, NAT1, LATS2, CYP2B6, PTPRC, PPP1R1A, and AL137218.1.

44. The method of any of claims 37, 38, 42 or 43, or the antagonist and/or agonist for use of any of claims 39 to 43, wherein the combined test score is derived from the determined expression level of at least 1 gene selected from CD2, ITGAL, PTPRC, CXCL10 and IDO1, together with at least one further gene selected from MX1, IFI44L, GBP5, PRAME, LRP4, APOL3, CDR1, FYB, TSPAN7, RAC2, KLHDC7B, GRB14, AC138128.1, KIF26A, CD274, CD109, ETV7, MFAP5, OLFM4, PI15, FOSB, FAM19A5, NLRC5, PRICKLE1, EGR1, CLDN10, ADAMTS4, SP140L, ANXA1, RSAD2, ESR1, IKZF3, OR2I1P, EGFR, NAT1, LATS2, CYP2B6, PPP1R1A, and AL137218.1.

45. The method of any of claims 37, 38 or 42 to 44, or the antagonist and/or agonist for use of any of claims 39 to 44, wherein the combined test score is derived from the determined expression level of at least 12 genes selected from Table 1.

46. The method of any of claims 37, 38 or 42 to 45, or the antagonist and/or agonist for use of any of claims 39 to 45, wherein the combined test score is derived from the determined expression level of at least 1 gene selected from CD2, ITGAL, PTPRC, CXCL10, IDO1, CD3D, HLA-DPB1, CXCL9, CCL5, STAT1, IL2RG, CD3E, IRF1, IKZF3 and IGJ together with at least one further gene selected from (the remaining genes in) Table 1 or together with at least one further gene from the (remaining genes in) Table 2B (the 44 gene panel).

47. The method of any of claims 37, 38 or 42 to 46, or the antagonist and/or agonist for use of any of claims 39 to 46, wherein the combined test score is derived from the determined expression level of each of CXCL10, MX1, IDO1, IFI44L, CD2, GBP5, PRAME, ITGAL, LRP4, APOL3, CDR1, FYB, TSPAN7, RAC2, KLHDC7B, GRB14, AC138128.1, KIF26A, CD274, CD109, ETV7, MFAP5, OLFM4, PI15, FOSB, FAM19A5, NLRC5, PRICKLE1, EGR1, CLDN10, ADAMTS4, SP140L, ANXA1, RSAD2, ESR1, IKZF3, OR2I1P, EGFR, NAT1, LATS2, CYP2B6, PTPRC, PPP1R1A, and AL137218.1.

48. The method of any of claims 37, 38 or 42, or the antagonist and/or agonist for use of any of claims 39 to 42, wherein the combined test score is derived from the determined expression level of the genes from any one of Tables 4 to 45.

49. The method of any of claims 37, 38 or 42 to 48, or the antagonist and/or agonist for use of any of claims 39 to 48, wherein the weight values for each gene are as set out in Table 2B, or wherein the weight and/or bias values for each gene are as set out in any one of Tables 3 to 45.

50. The method of any of claims 37, 38 or 42 to 49, or the antagonist and/or agonist for use of any of claims 39 to 49, wherein the combined test score is derived from the determined expression level of at least one, up to all, of CCL5, CXCL9 and CXCL10 together with at least one further gene selected from (the remaining genes in) Table 1 or together with at least one further gene from the (remaining genes in) Table 2B (the 44 gene panel).

51. The method of any of claims 37, 38 or 42 to 50, or the antagonist and/or agonist for use of any of claims 39 to 50, wherein the expression levels are determined using at least one primer or primer pair from Table 2E and/or at least one probe from Table 2E.

52. The method of any one of claims 37, 38 or 42 to 51, or the antagonist and/or agonist for use of any of claims 39 to 51, wherein the subject is selected for treatment according to a method as claimed in any one of claims 1 to 36.

53. The method of any of claims 1 to 38 or 42 to 52, or the antagonist and/or agonist for use of any of claims 39 to 52, wherein the sample comprises cancer cells.

54. The method of any of claims 1 to 38 or 42 to 53, or the antagonist and/or agonist for use of any of claims 39 to 53, wherein the sample is a tissue sample; optionally wherein the tissue sample is a fixed and embedded tissue sample.

55. The method of any of claims 1 to 38 or 42 to 54, or the antagonist and/or agonist for use of any of claims 39 to 54, wherein the cancer is selected from leukemia, brain cancer, prostate cancer, liver cancer, ovarian cancer, stomach cancer, colorectal cancer, throat cancer, breast cancer, skin cancer, melanoma, lung cancer, sarcoma, cervical cancer, testicular cancer, bladder cancer, endocrine cancer, endometrial cancer, esophageal cancer, glioma, lymphoma, neuroblastoma, osteosarcoma, pancreatic cancer, pituitary cancer, renal cancer or head and neck cancer.

56. The method of any of claims 1 to 38 or 42 to 55, or the antagonist and/or agonist for use of any of claims 39 to 55, wherein the inhibitory immune checkpoint is selected from A2AR, B7-H3 (CD276), B7-H4 (VTCN1), BTLA (CD272), CTLA-4 (CD152), IDO, KIR, LAG3, PD-1/PD-L1, TIM-3 and VISTA, optionally wherein the inhibitory immune checkpoint is not PD-1/PD-L1.

57. The method of any of claims 1 to 38 or 42 to 56, or the antagonist and/or agonist for use of any of claims 39 to 56, wherein the antagonist of an inhibitory immune checkpoint is selected from:

(a) an antibody and an inhibitory nucleic acid molecule; and/or

(b) MGA271 (targets B7-H3), ipilimumab (Yervoy - targets CTLA-4), indoximod (targets IDO pathway), NLG919 (targets IDO pathway), lirilumab (targets KIR), IMP321 (targets LAG3), BMS-986016 (targets LAG3), CT-011 (PD-1 blockade), nivolumab/BMS-936558 (PD-1 blockade) , BMS-936559 (PDL1 blockade) and pembrolizumab (Keytruda – targets PD-1), optionally wherein the antagonist is not pembrolizumab; and/or wherein the antagonist of an inhibitory immune checkpoint is selected from MGB453 (targets TIM-3), LAG525 (targets LAG-3) and PDR001 (PD1 Blockade).

58. The method of any of claims 1 to 38 or 42 to 57, or the antagonist and/or agonist for use of any of claims 39 to 57, wherein the stimulatory immune checkpoint is selected from CD27, CD28, CD40, CD122, CD137, OX40, GITR and ICOS.

59. The method of any of claims 1 to 38 or 42 to 58, or the antagonist and/or agonist for use of any of claims 39 to 58, wherein the agonist of a stimulatory immune checkpoint is selected from:

(a) an antibody, a lipocalin and a cytokine; and/or

(b) CDX-1127 (agonist of CD27), NKTR-214 (agonist of CD122), BMS-663513 (agonist of CD137), TRX518 (agonist of GITR), CP-870893 (CD40 agonist), MEDI0562, MEDI6469 and MEDI6383 (OX40 agonists).

60. The method of any of claims 1 to 38 or 42 to 59, or the antagonist and/or agonist for use of any of claims 39 to 59, wherein the DNA damage therapeutic agent is selected from a DNA damaging agent, a DNA repair targeted therapy, an inhibitor of DNA damage signalling, an inhibitor of DNA damage induced cell cycle arrest and an inhibitor of a process indirectly leading to DNA damage; optionally wherein:

(a) the DNA damaging agent is selected from an alkylating agent, a topoisomerase inhibitor and radiation; optionally wherein:

(i) the alkylating agent is selected from a platinum containing agent, cyclophosphamide and busulphan; optionally wherein the platinum containing agent is selected from cisplatin, carboplatin and oxaliplatin;

(ii) the topoisomerase inhibitor is selected from a topoisomerase I inhibitor and a topoisomerase II inhibitor; optionally wherein

(A) the topoisomerase I inhibitor is selected from irinotecan and topotecan; and/or

(B) the topisomerase II inhibitor is selected from etoposide and an anthracycline; optionally wherein the anthracycline is selected from doxorubicin and epirubicin;

(iii) the radiation is ionising radiation; and/or

(b) the DNA repair targeted therapy is selected from an inhibitor of Non-homologous end-joining, an inhibitor of homologous recombination, an inhibitors of nucleotide excision repair, an inhibitor of base excision repair and an inhibitor of the Fanconi anemia pathway; optionally wherein

(i) the inhibitor of Non-homologous end-joining is selected from a DNA-PK inhibitor, Nu7441 and NU7026;

(ii) the inhibitor of base excision repair is selected from a PARP inhibitor, AG014699, AZD2281, ABT-888, MK4827, BSI-201, INO-1001, TRC-102, an APEX 1 inhibitor, an APEX 2 inhibitor and a Ligase III inhibitor;

(iii) the inhibitor of DNA damage signalling is selected from an ATM inhibitor, a CHK 1 inhibitor and a CHK 2 inhibitor; optionally wherein

(A) the ATM inhibitor is selected from CP466722 and KU-55933;

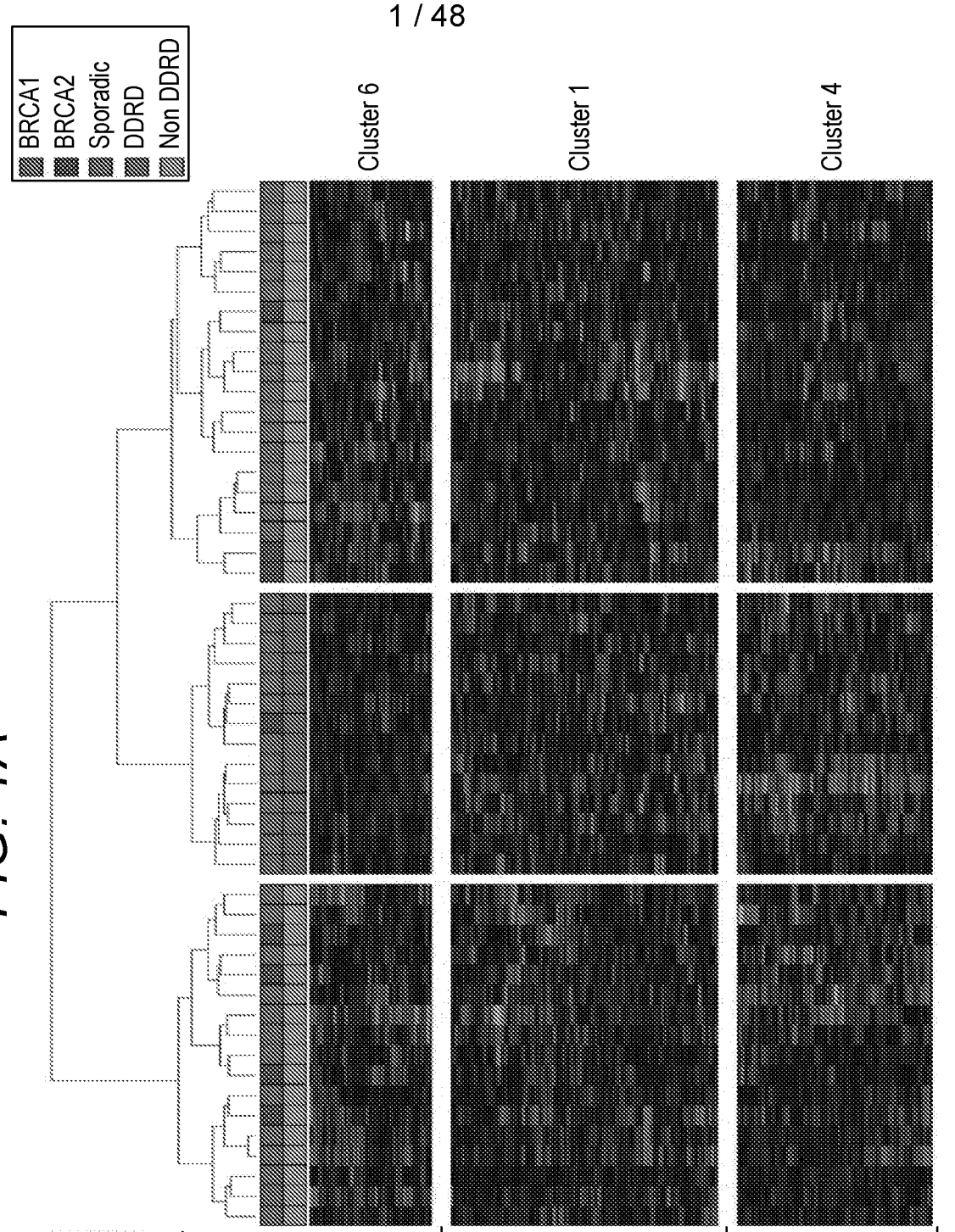
(B) the CHK 1 inhibitor is selected from XL-844, UCN-01, AZD7762 and PF00477736;

(C) the CHK 2 inhibitor is selected from XL-844, AZD7762 and PF00477736; and/or

(c) the inhibitor of DNA damage induced cell cycle arrest is selected from a Wee1 kinase inhibitor and a CDC25a, b or c inhibitor; and/or

(d) the inhibitor of a process indirectly leading to DNA damage is selected from a histone deacetylase inhibitor and a heat shock protein inhibitor; optionally wherein the heat shock protein inhibitor is selected from geldanamycin and AUY922.

FIG. 1A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BRCA2
Sporadic
DDRD
Non DDRD
Colour Key
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Value
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Cluster 1
Cluster 4
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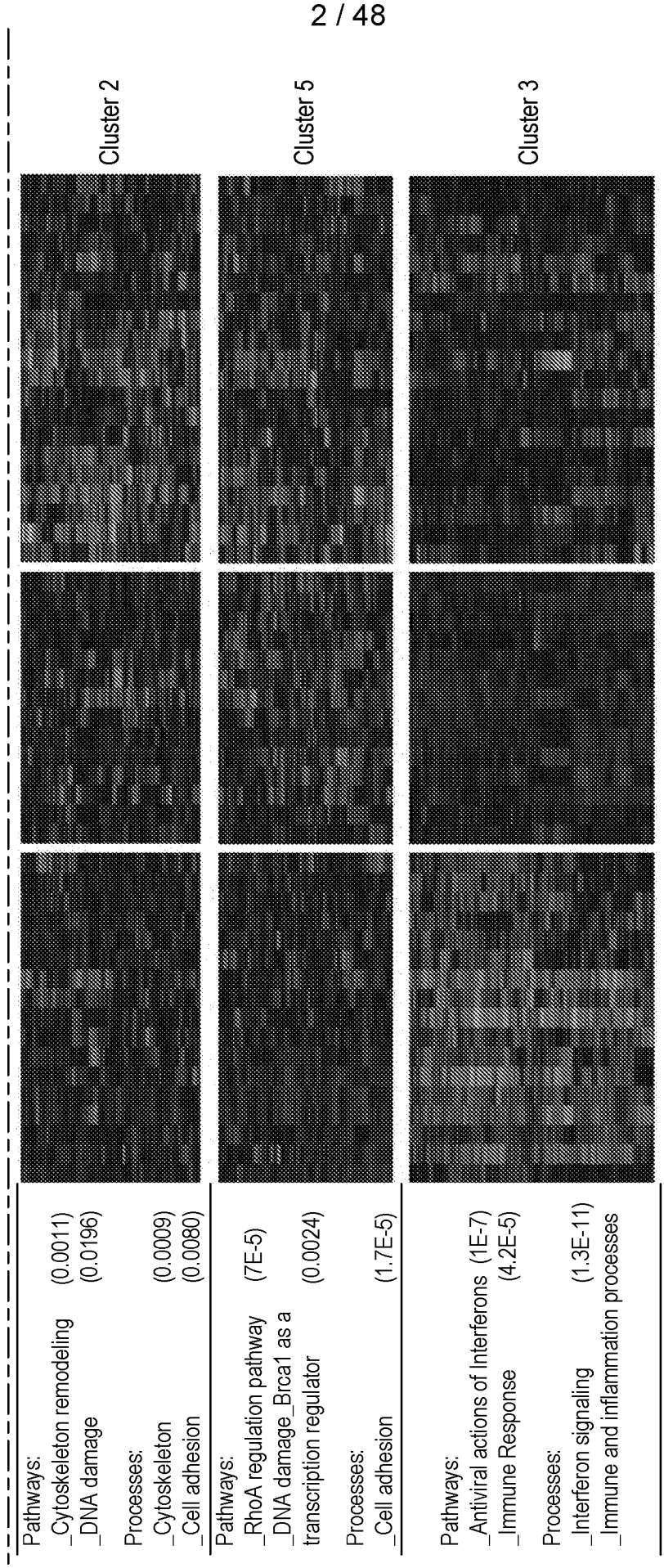
_WNT signaling path Part 2 (0.006)
Processes: Immune processes (0.0007)
Pathways:
_ERBB (0.0003)
_Apoptosis (0.0059)
_ESR1/ESR2 (0.0097)
Processes:
_Reproduction processes (0.0007)
_ECM remodeling (0.0031)
Pathways:
_ECM remodeling (1.3E-6)
_Hypoxia-induced EMT (0.0005)
Processes:
_ECM remodeling (3.8E-9)
_Cell adhesion, development...


Cluster 2
Cluster 5
Cluster 3
Pathways:
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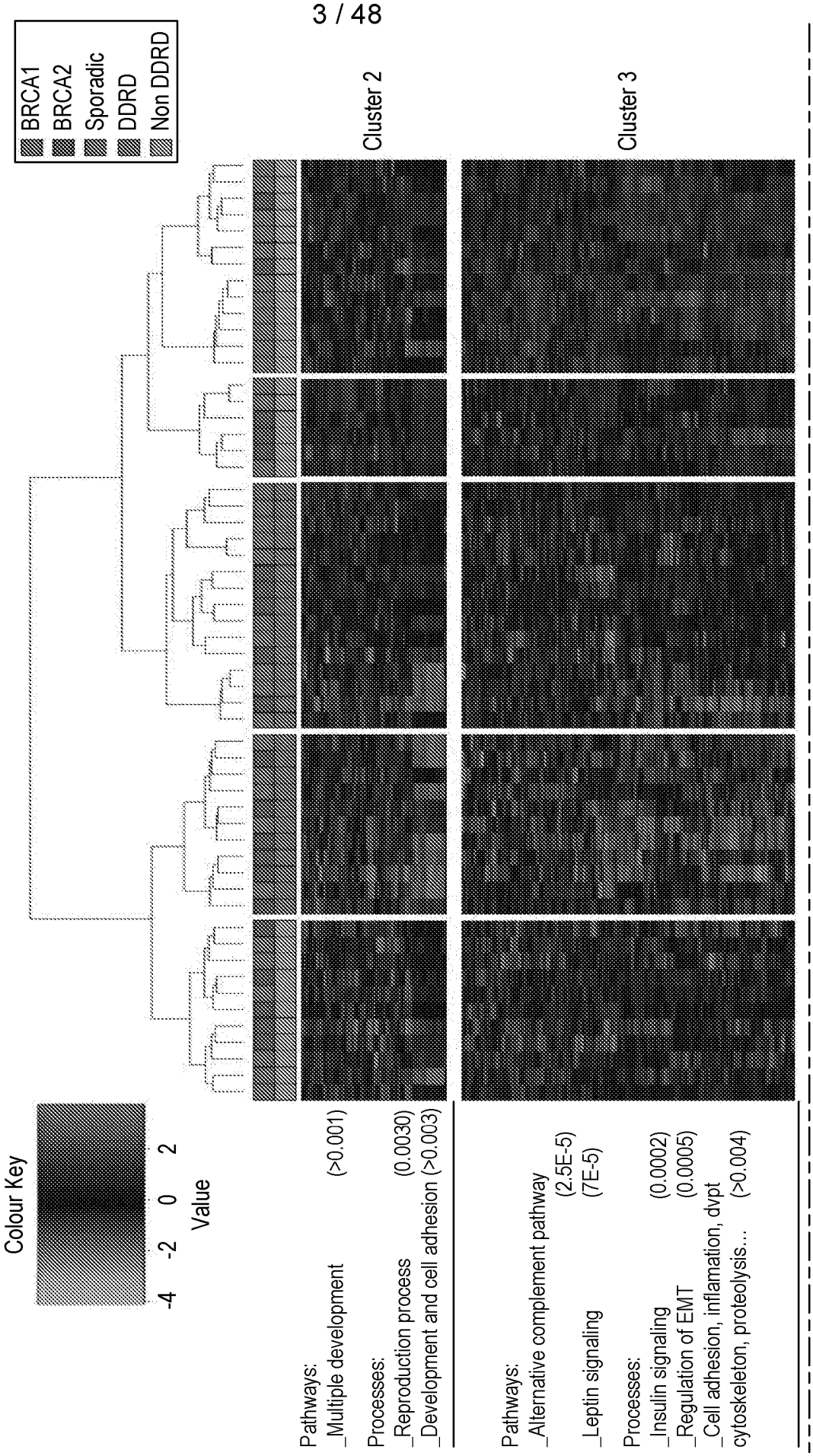
_DNA damage (0.0196)
Processes:
_Cytoskeleton (0.0009)
_Cell adhesion (0.0080)
Pathways:
_RhoA regulation pathway (7E-5)
_DNA damage_Brca1 as a transcription regulator (0.0024)
Processes:
_Cell adhesion (1.7E-5)
Pathways:
_Antiviral actions of Interferons (1E-7)
_Immune Response (4.2E-5)
Processes:
_Interferon signaling (1.3E-11)
_Immune and inflammation processes

FIG. 1A Cont'd

FIG. 1B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BRCA2
Sporadic
DDRD
Non DDRD
Cluster 2
Cluster 3
Colour Key
-4 -2 0 2
Value
Pathways:
_Multiple development (>0.001)
Processes:
_Reproduction process (0.0030)
_Development and cell adhesion (>0.003)
Pathways:
_Alternative complement pathway (2.5E-5)
_Leptin signaling (7E-5)
Processes:
_Insulin signaling (0.0002)
_Regulation of EMT (0.0005)
_Cell adhesion, inflamation, dvpt cytoskeleton, proteolysis... (>0.004)

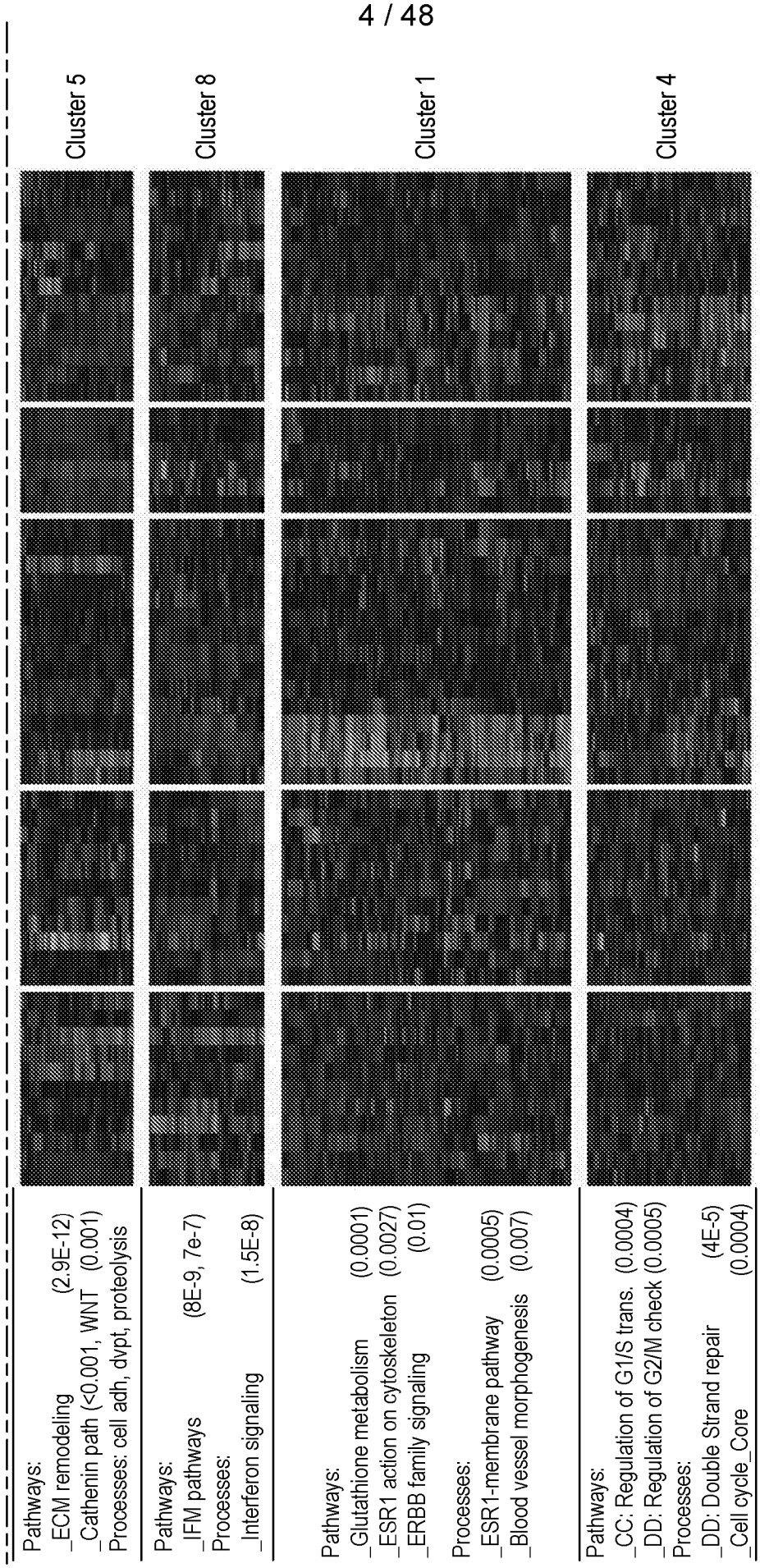

Cluster 5
Pathways:
_ECM remodeling (2.9E-12)
_Cathenin path (<0.001, WNT (0.001)
Processes: cell adh, dvpt, proteolysis
Cluster 8
Pathways:
_IFM pathways (8E-9, 7e-7)
Processes:
_Interferon signaling (1.5E-8)
Cluster 1
Pathways:
_Glutathione metabolism (0.0001)
_ESR1 action on cytoskeleton (0.0027)
_ERBB family signaling (0.01)
Processes:
_ESR1-membrane pathway (0.0005)
_Blood vessel morphogenesis (0.007)
Cluster 4
Pathways:
_CC: Regulation of G1/S trans. (0.0004)
_DD: Regulation of G2/M check (0.0005)
Processes:
_DD: Double Strand repair (4E-5)
_Cell cycle_Core (0.0004)

FIG. 1B Cont'd

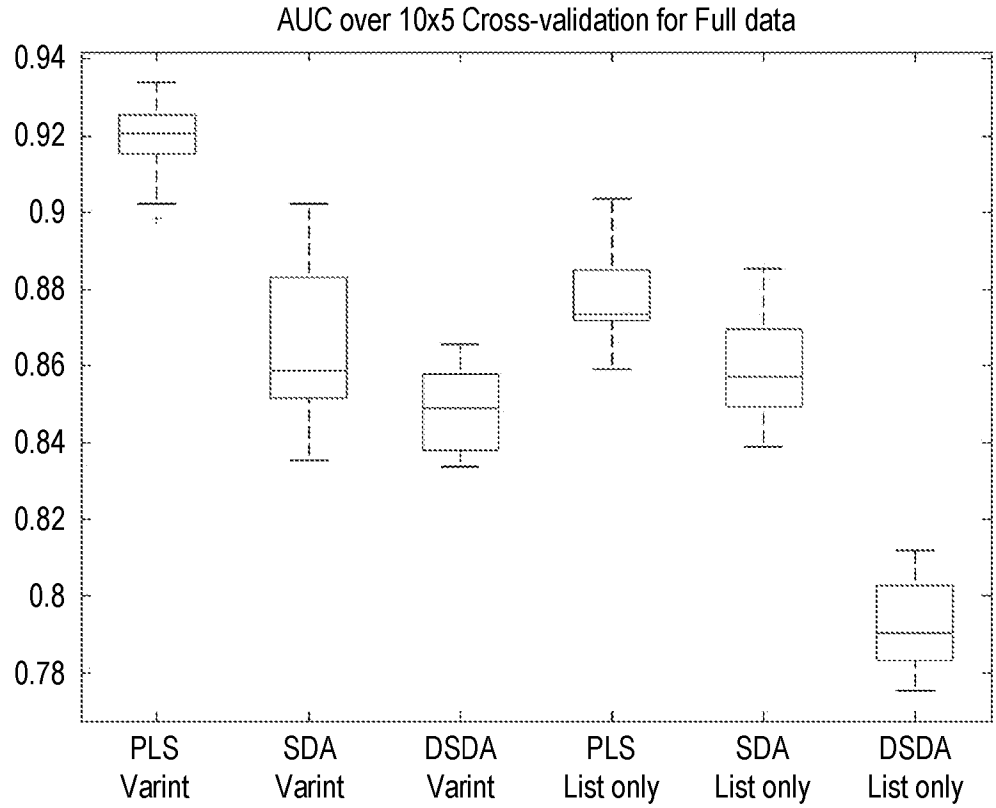

AUC over 10x5 Cross-validation for Full data
0.94
0.92
0.9
0.88
0.86
0.84
0.82
0.8
0.78
PLS Varint
SDA Varint
DSDA Varint
PLS List only
SDA List only
DSDA List only

FIG. 2A

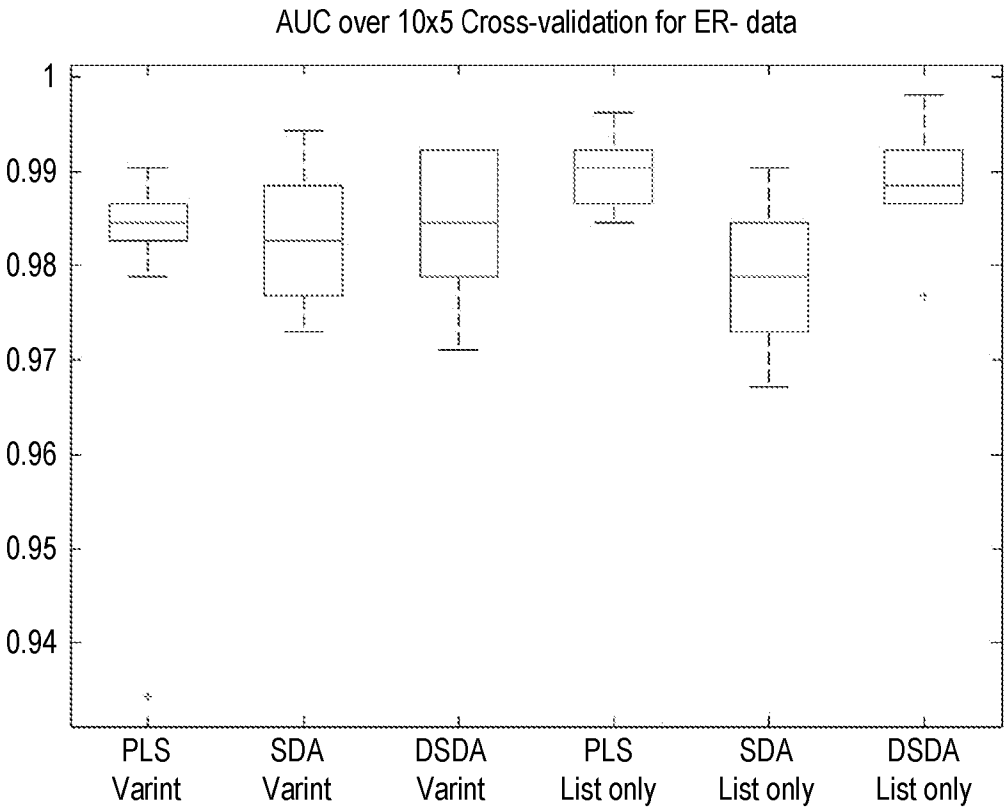

AUC over 10x5 Cross-validation for ER- data
1
0.99
0.98
0.97
0.96
0.95
0.94
PLS Varint
SDA Varint
DSDA Varint
PLS List only
SDA List only
DSDA List only

FIG. 2B

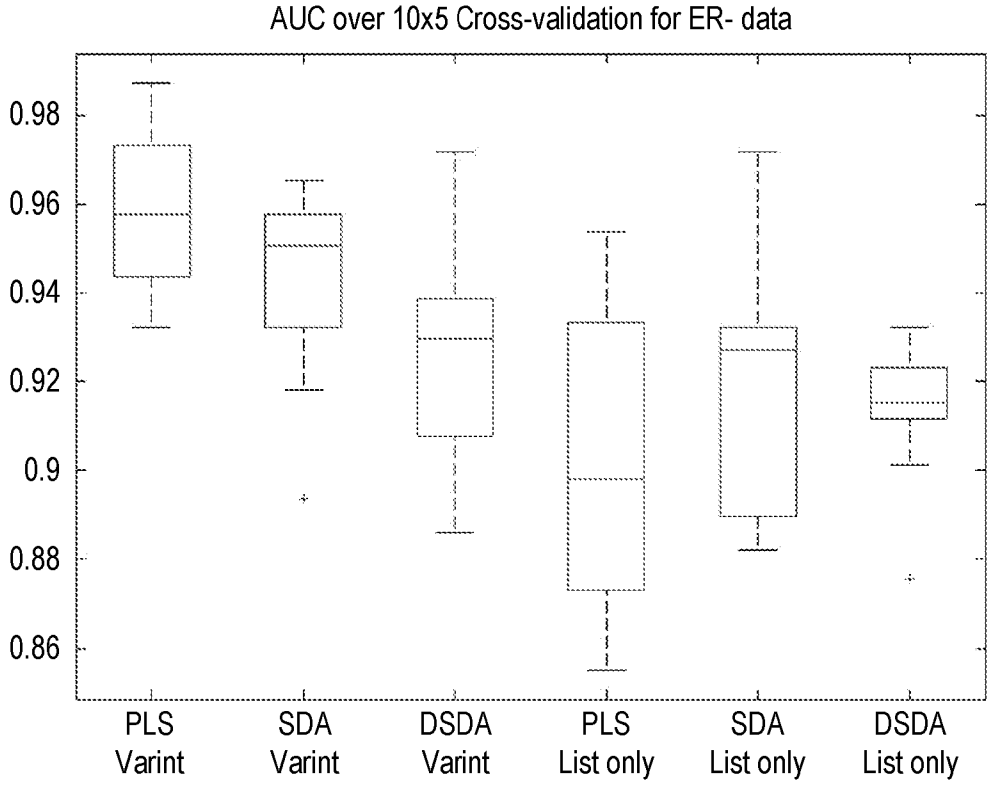

AUC over 10x5 Cross-validation for ER- data
0.98
0.96
0.94
0.92
0.9
0.88
0.86
PLS Varint
SDA Varint
DSDA Varint
PLS List only
SDA List only
DSDA List only

FIG. 2C

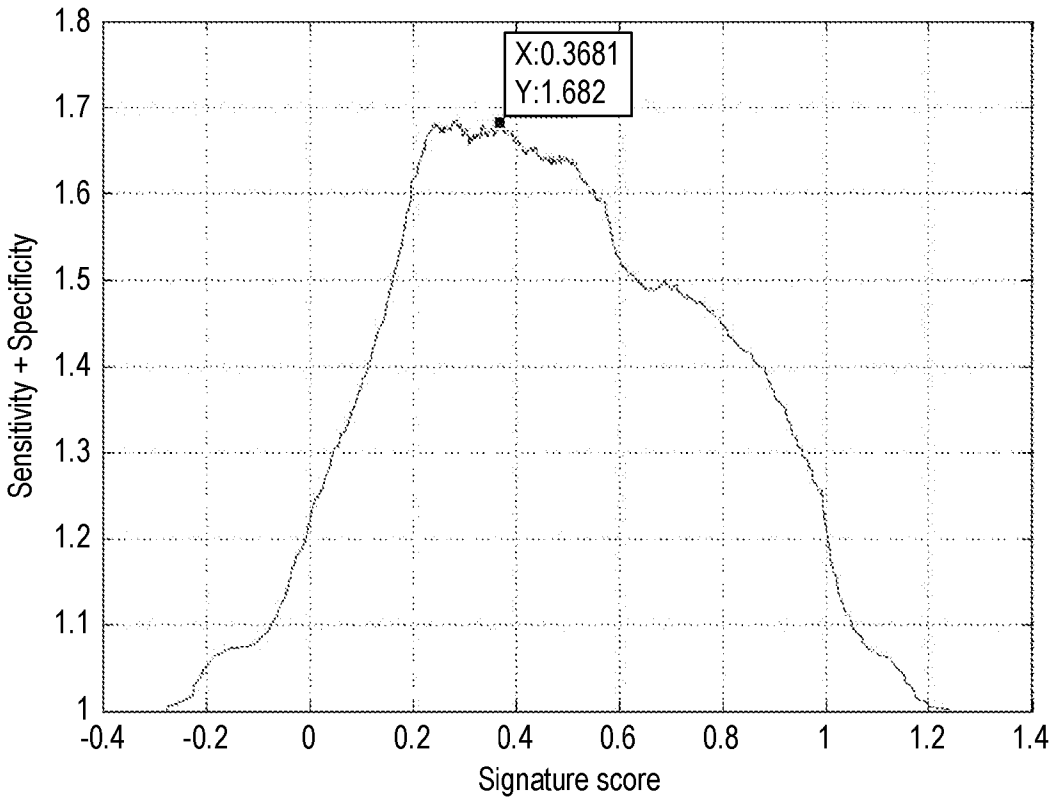

X:0.3681
Y:1.682
Sensitivity + Specificity
1.8
1.7
1.6
1.5
1.4
1.3
1.2
1.1
1
-0.4
-0.2
0
0.2
0.4
0.6
0.8
1
1.2
1.4
Signature score

FIG. 2D

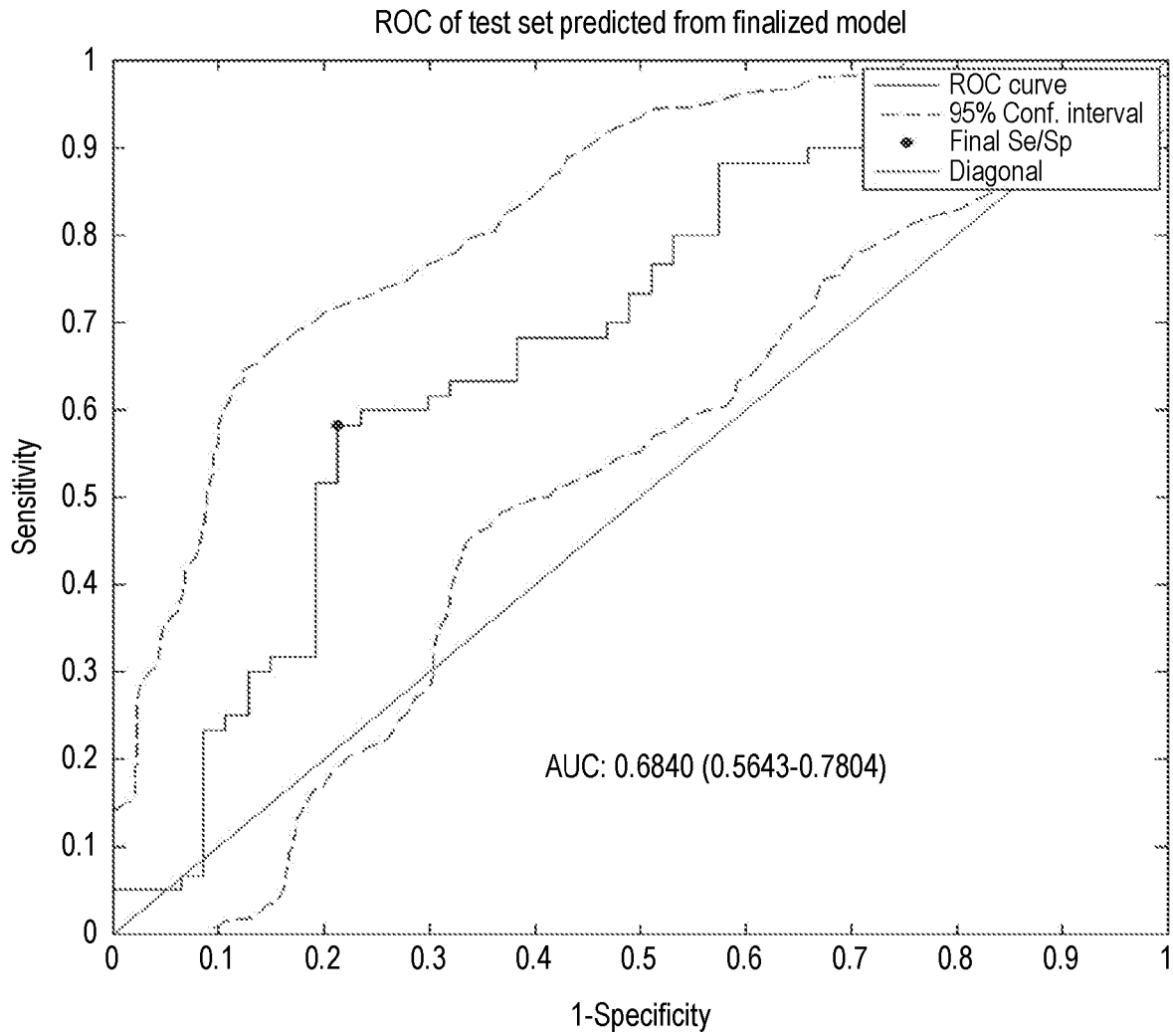

ROC of test set predicted from finalized model
ROC curve
95% Conf. interval
Final Se/Sp
Diagonal
AUC: 0.6840 (0.5643-0.7804)
Sensitivity
1-Specificity
0
0.1
0.2
0.3
0.4
0.5
0.6
0.7
0.8
0.9
1

FIG. 3

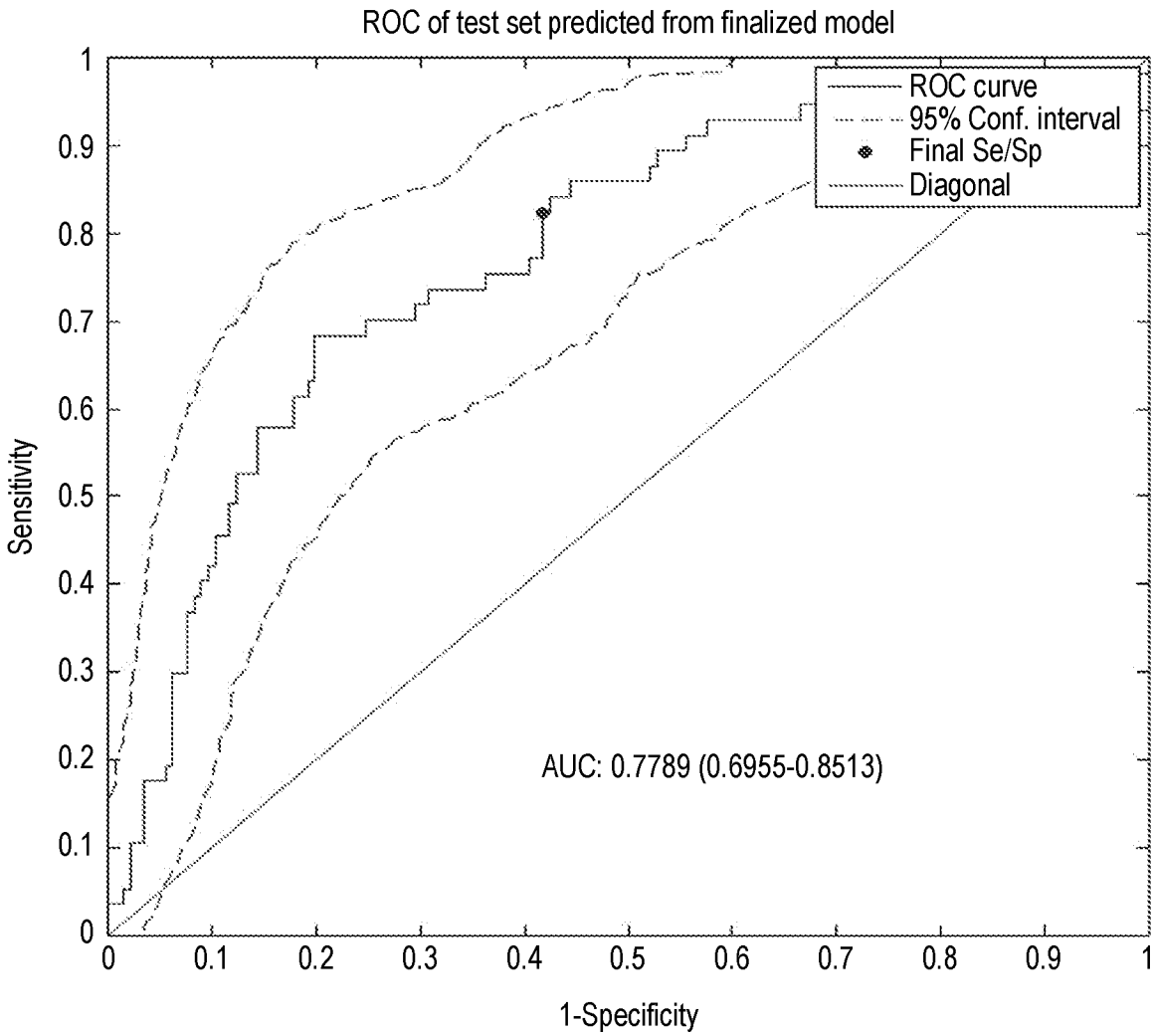

ROC of test set predicted from finalized model
ROC curve
95% Conf. interval
Final Se/Sp
Diagonal
AUC: 0.7789 (0.6955-0.8513)
Sensitivity
1-Specificity
0
0.1
0.2
0.3
0.4
0.5
0.6
0.7
0.8
0.9
1

FIG. 4

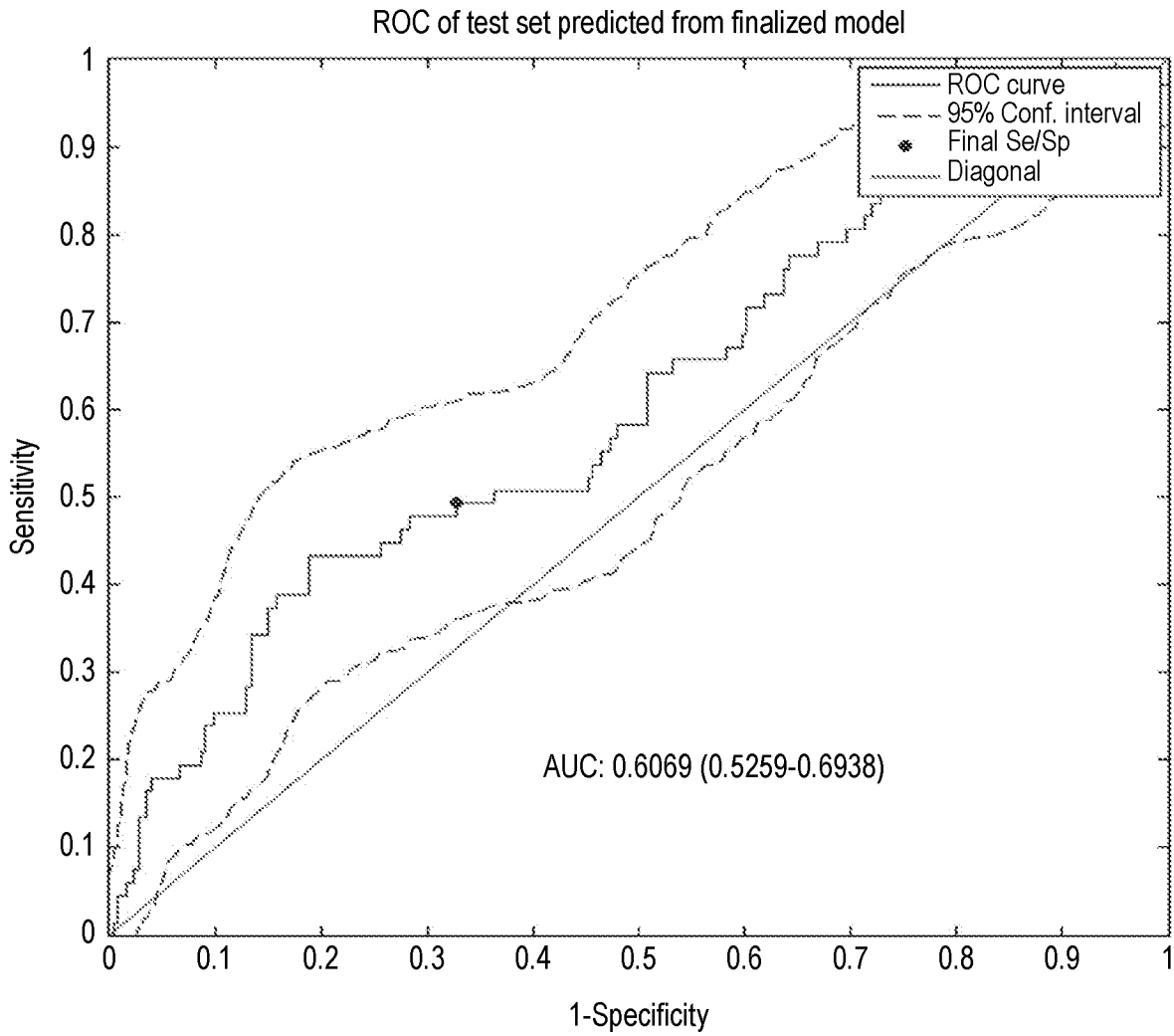

ROC of test set predicted from finalized model
ROC curve
95% Conf. interval
Final Se/Sp
Diagonal
AUC: 0.6069 (0.5259-0.6938)
Sensitivity
1-Specificity
0
0.1
0.2
0.3
0.4
0.5
0.6
0.7
0.8
0.9
1

FIG. 5

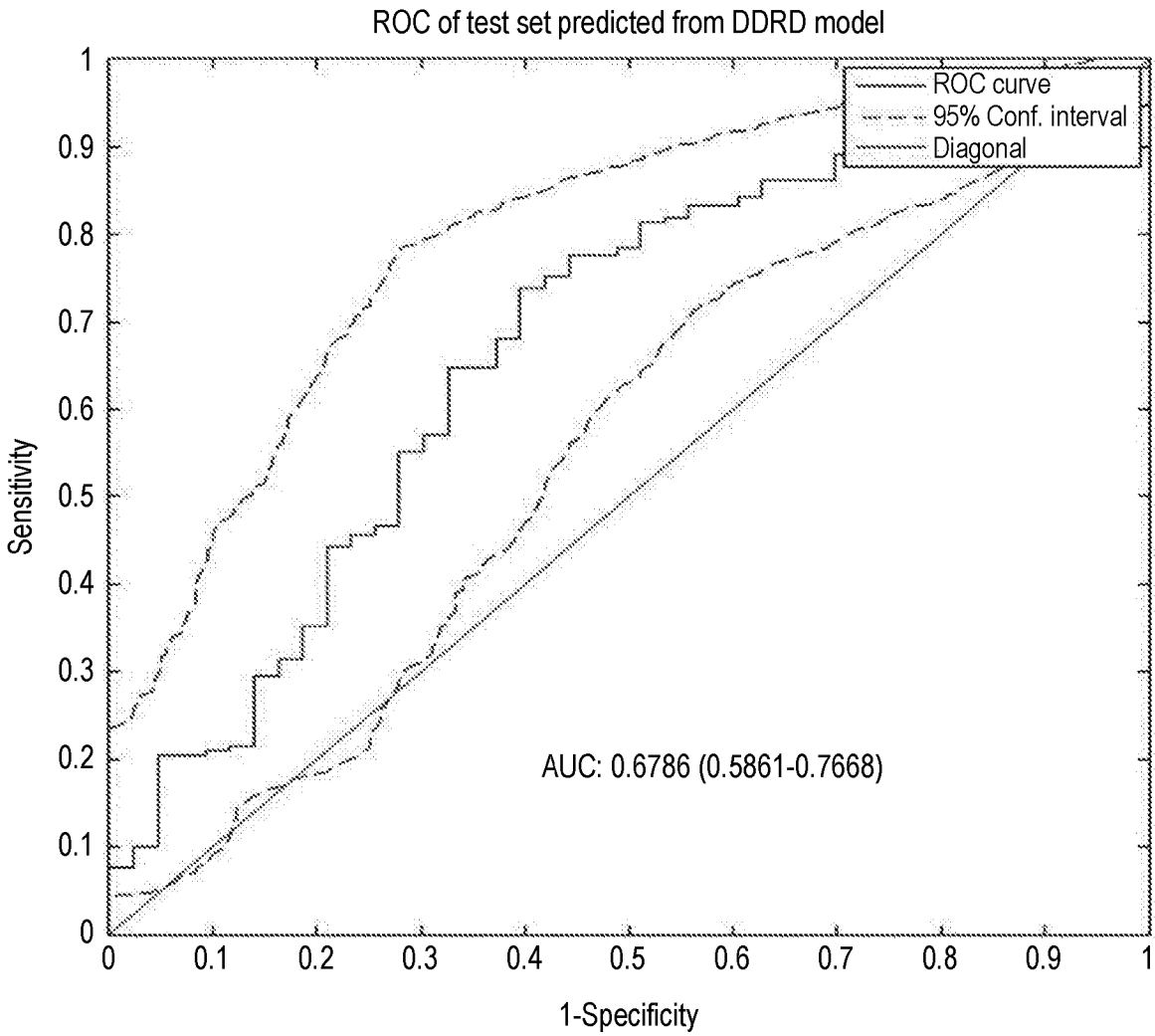

ROC of test set predicted from DDRD model
ROC curve
95% Conf. interval
Diagonal
AUC: 0.6786 (0.5861-0.7668)
Sensitivity
1-Specificity
0
0.1
0.2
0.3
0.4
0.5
0.6
0.7
0.8
0.9
1

FIG. 6

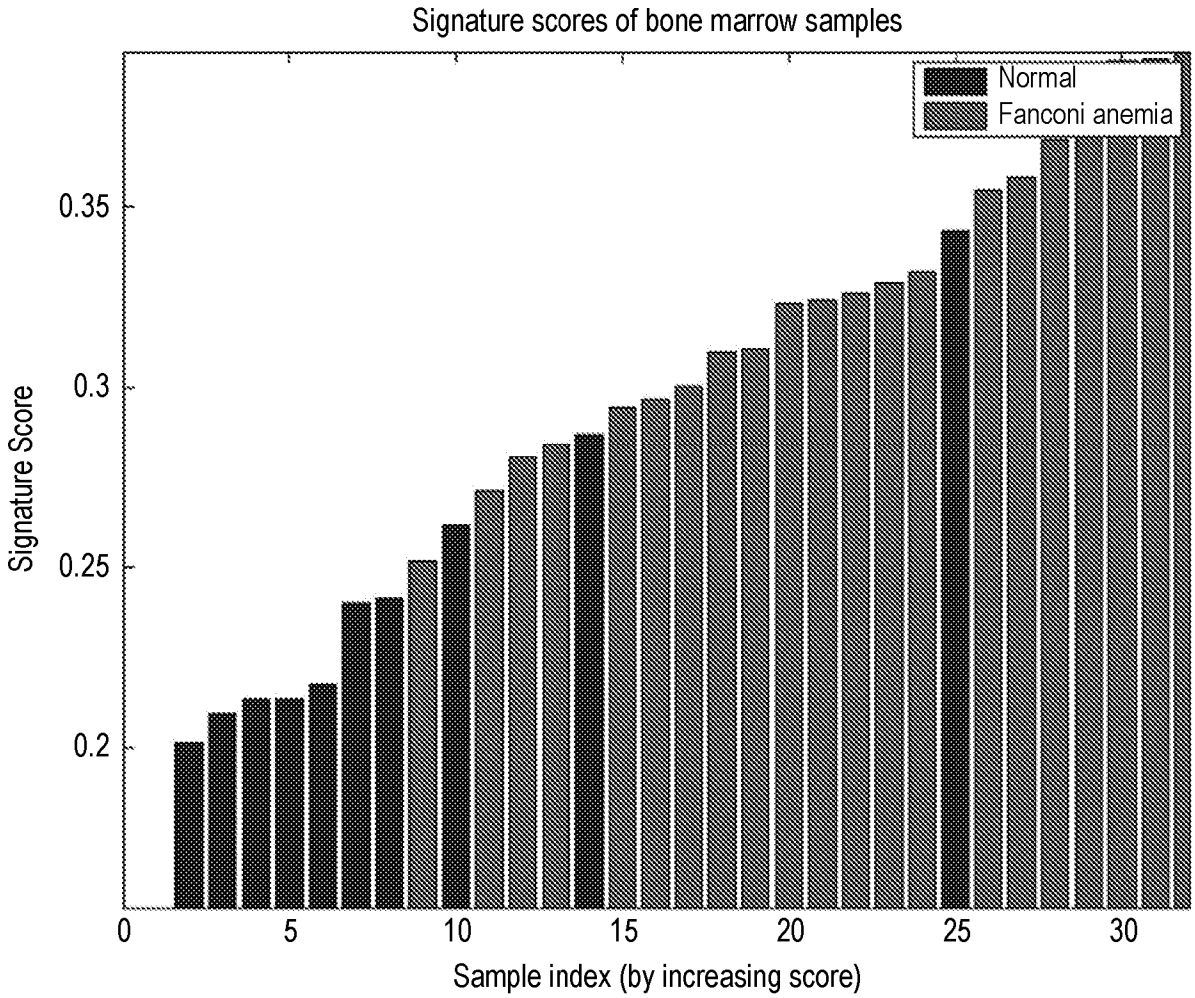

Signature scores of bone marrow samples
Normal
Fanconi anemia
Signature Score
0.35
0.3
0.25
0.2
0
5
10
15
20
25
30
Sample index (by increasing score)

FIG. 7

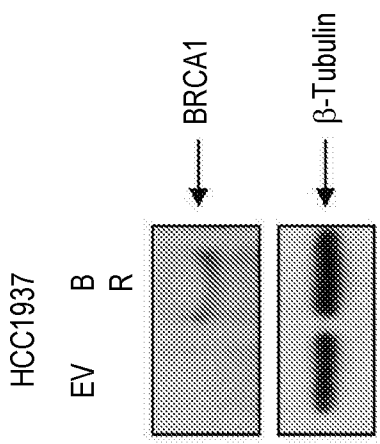


FIG. 8A

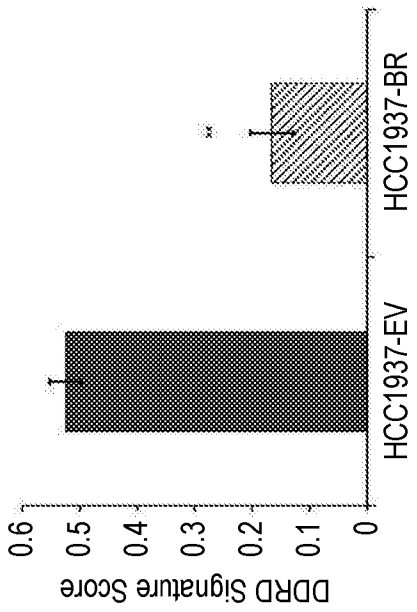


FIG. 8B

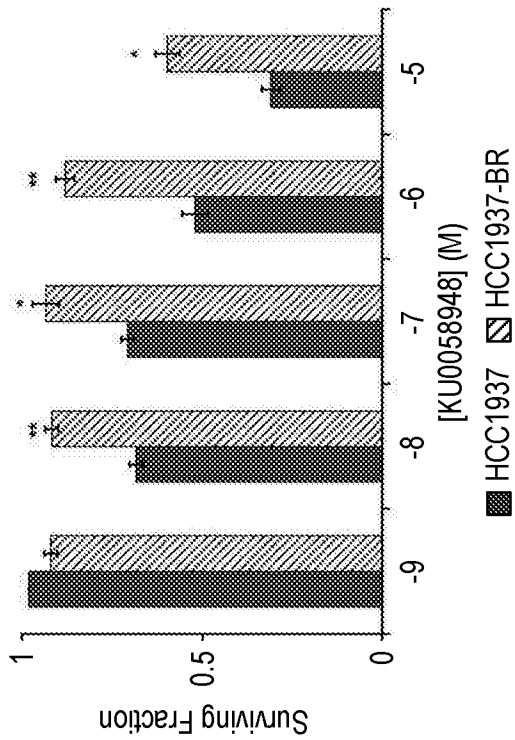


FIG. 8C

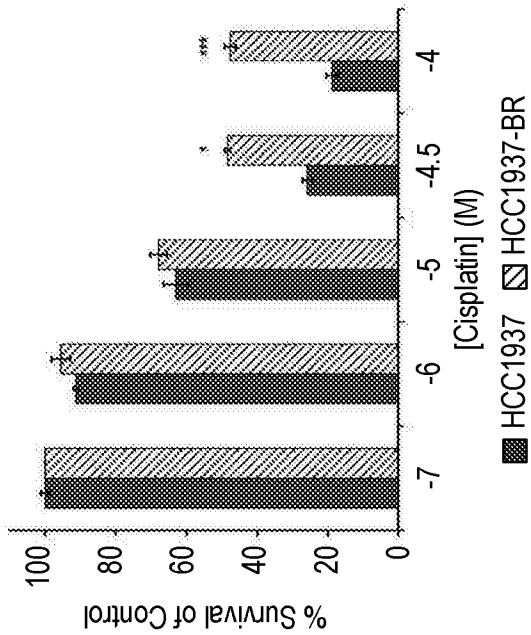


FIG. 8D

Intratumoral Lymphocytic Infiltrate

CD8+ score	*DDRD pos* (n=66)	*DDRD neg* (n=119)	*Fisher's exact test*
3	6 (9.09%)	0 (0%)	P<0.0001
2	29 (43.94%)	19 (15.97%)	
1	26 (39.39%)	70 (58.82%)	
0	5 (7.75%)	30 (25.21%)	

CD4+ score	*DDRD pos* (n=66)	*DDRD neg* (n=121)	*Fisher's exact test*
3	3 (4.54%)	1 (0.82%)	P<0.0001
2	14 (21.21%)	3 (2.48%)	
1	43 (65.15%)	77 (63.64%)	
0	6 (9.09%)	40 (33.06%)	

Stromal Lymphocytic Infiltrate

CD8+ score	*DDRD pos* (n=66)	*DDRD neg* (n=119)	*Fisher's exact test*
3	20 (30.30%)	8 (6.72%)	P<0.0001
2	39 (59.09%)	58 (48.74%)	
1	7 (10.61%)	46 (38.66%)	
0	0 (0%)	7 (5.88%)	

CD4+ score	*DDRD pos* (n=66)	*DDRD neg* (n=121)	*Fisher's exact test*
3	21 (31.82%)	7 (5.79%)	P<0.0001
2	32 (48.48%)	32 (26.45%)	
1	13 (19.70%)	68 (56.20%)	
0	0 (0%)	14 (11.57%)	

FIG. 9A

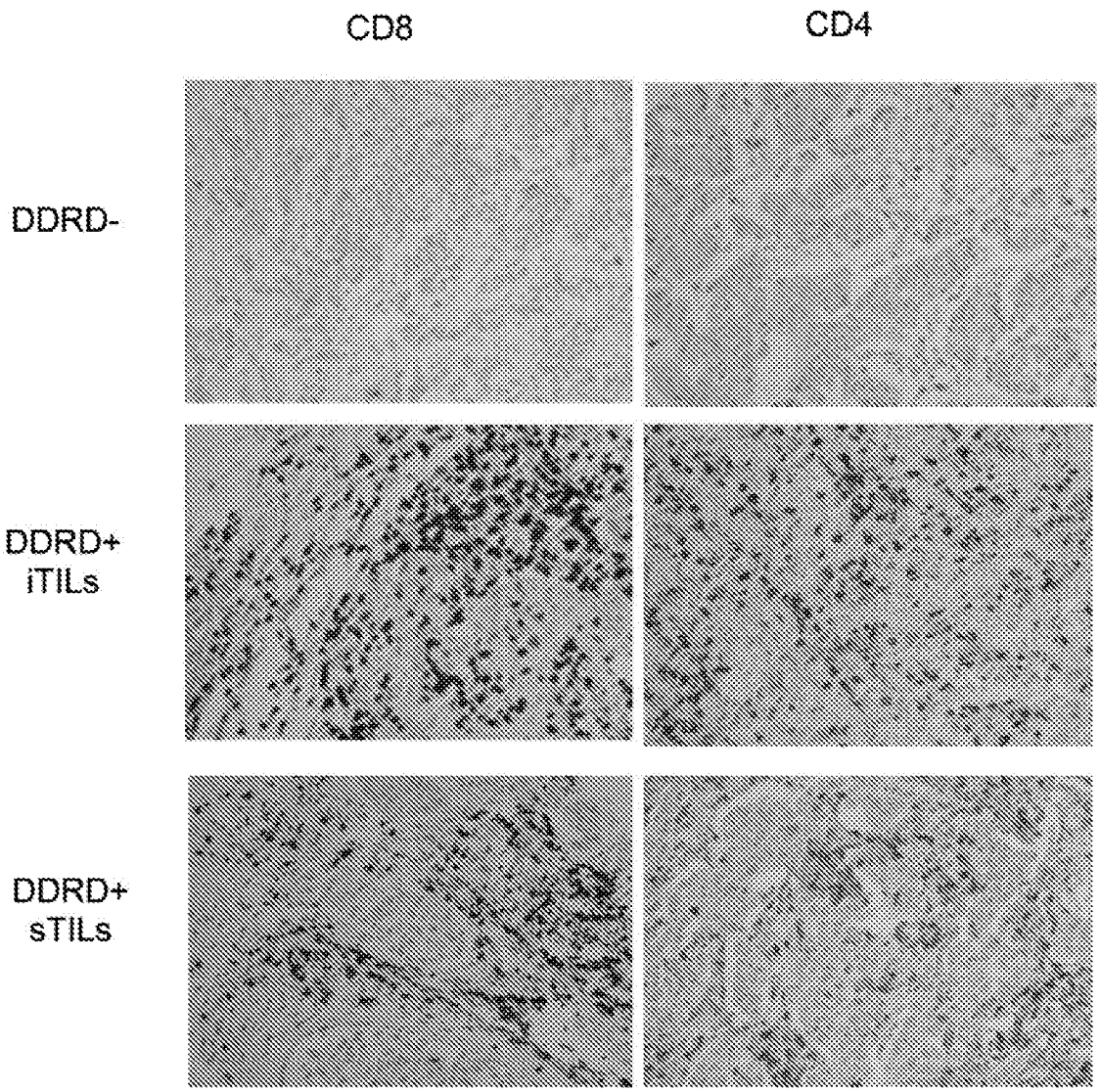


FIG. 9B

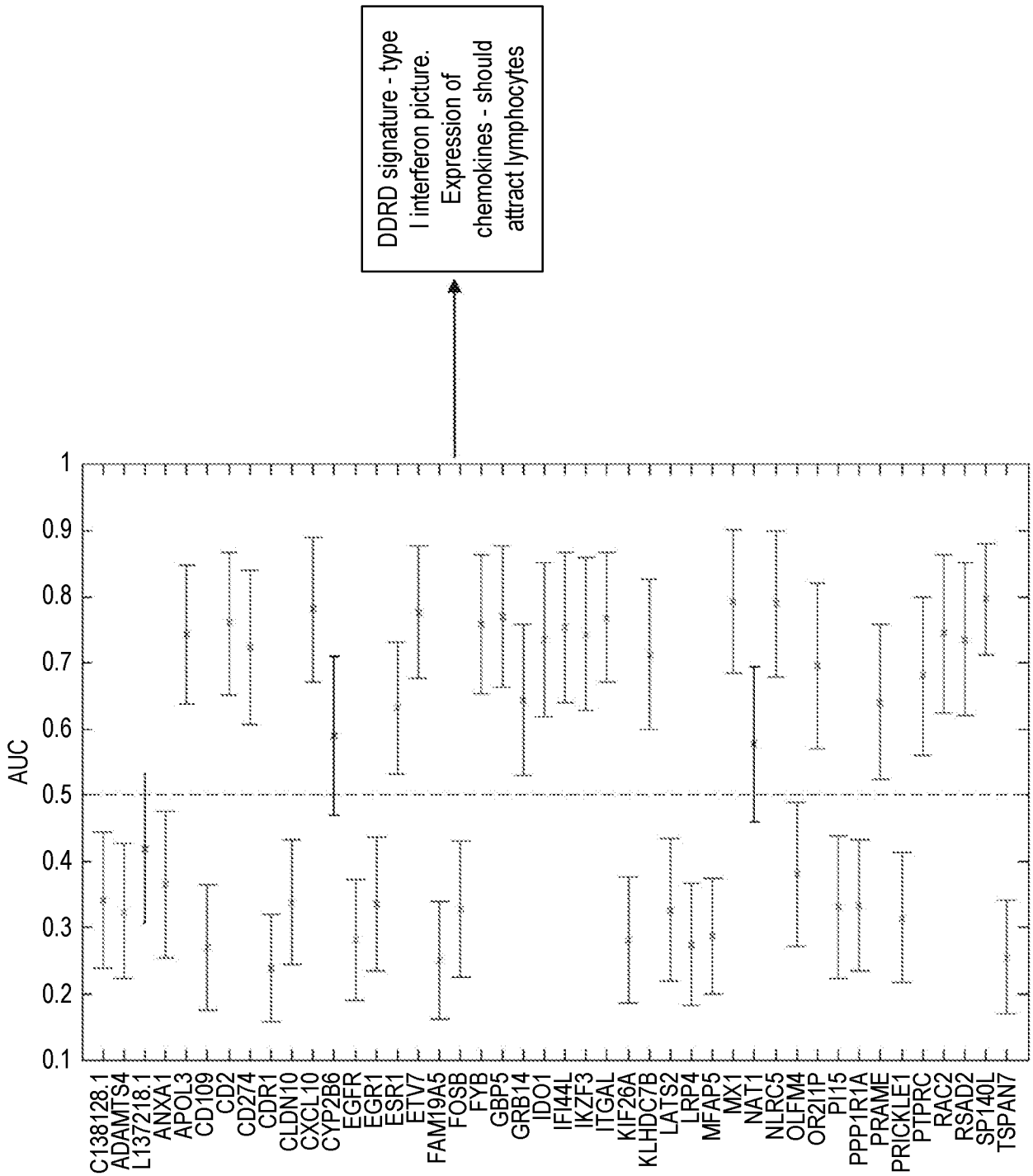

DDRD signature - type I interferon picture. Expression of chemokines - should attract lymphocytes
AUC
1
0.9
0.8
0.7
0.6
0.5
0.4
0.3
0.2
0.1
C138128.1
ADAMTS4
L137218.1
ANXA1
APOL3
CD109
CD2
CD274
CDR1
CLDN10
CXCL10
CYP2B6
EGFR
EGR1
ESR1
ETV7
FAM19A5
FOSB
FYB
GBP5
GRB14
IDO1
IFI44L
IKZF3
ITGAL
KIF26A
KLHDC7B
LATS2
LRP4
MFAP5
MX1
NAT1
NLRC5
OLFM4
OR2I1P
PI15
PPP1R1A
PRAME
PRICKLE1
PTPRC
RAC2
RSAD2
SP140L
TSPAN7

FIG. 10A

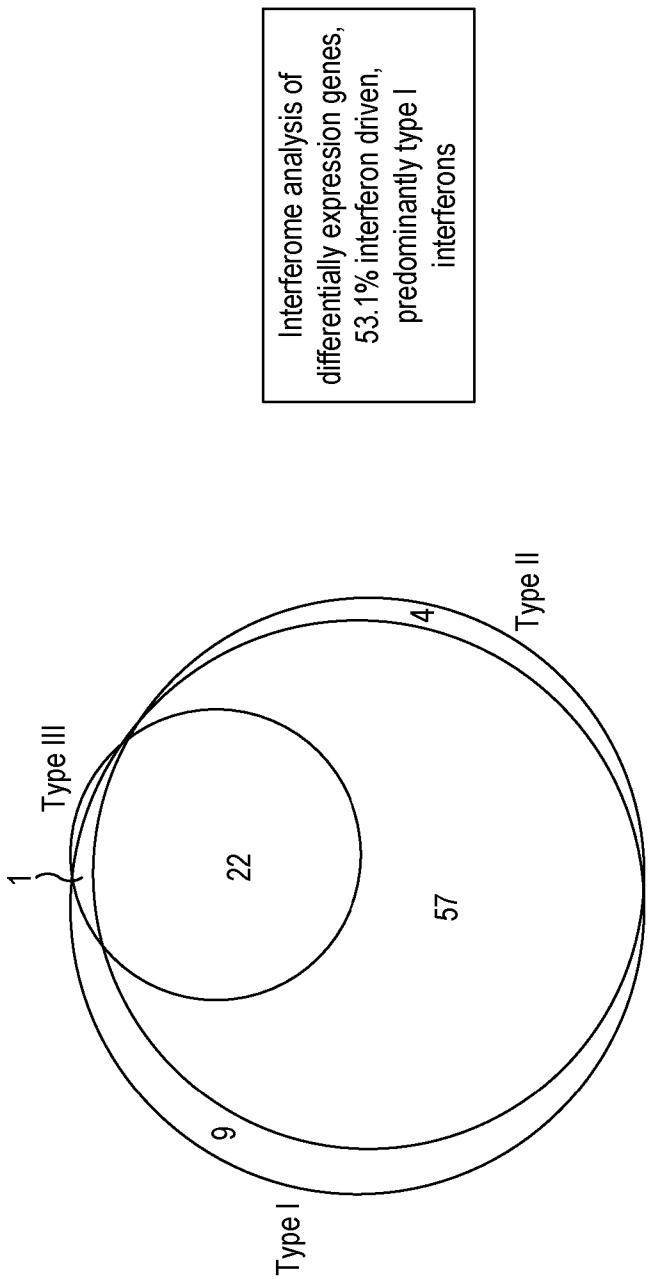

Interferome analysis of differentially expression genes, 53.1% interferon driven, predominantly type I interferons
Type III
Type II
Type I
1
22
4
57
9

FIG. 10B

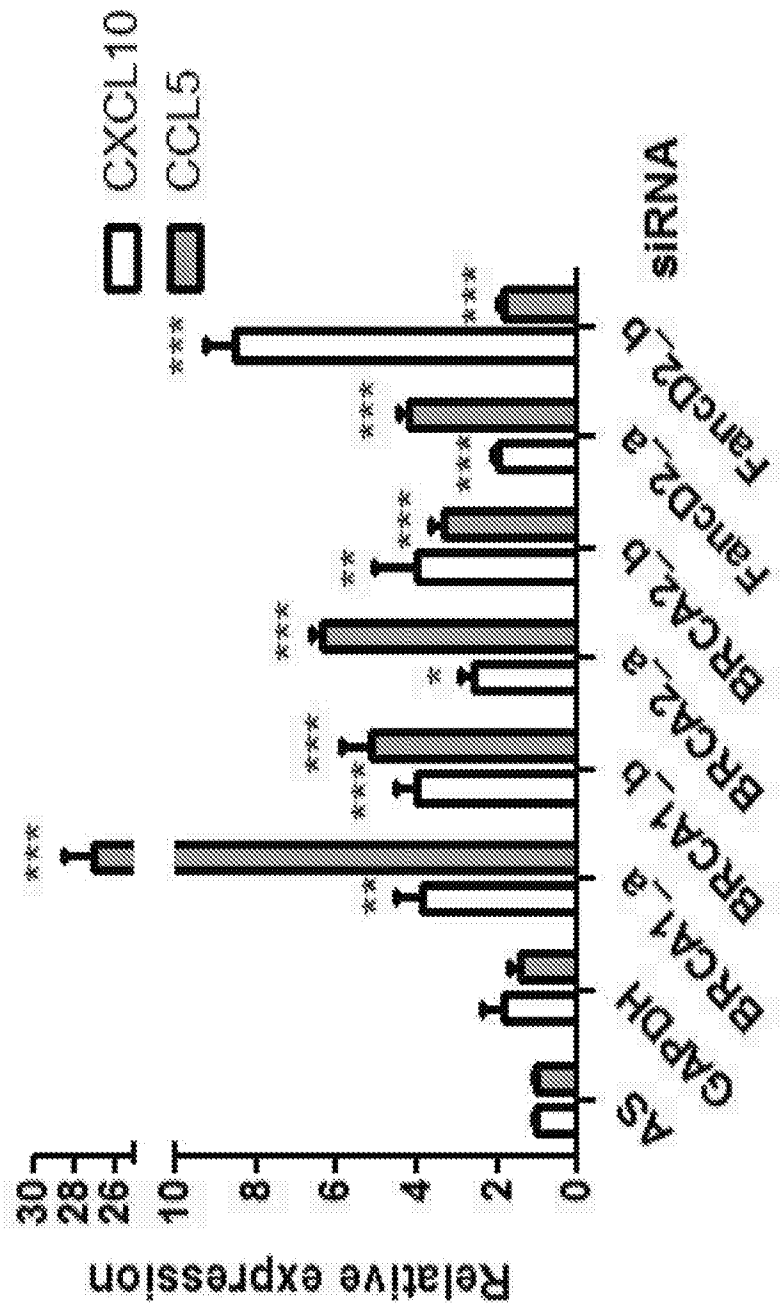

CXCL10
CCL5
Relative expression
30
28
26
10
8
6
4
2
0
AS
GAPDH
BRCA1_a
BRCA1_b
BRCA2_a
BRCA2_b
FancD2_a
FancD2_b
siRNA

FIG. 11

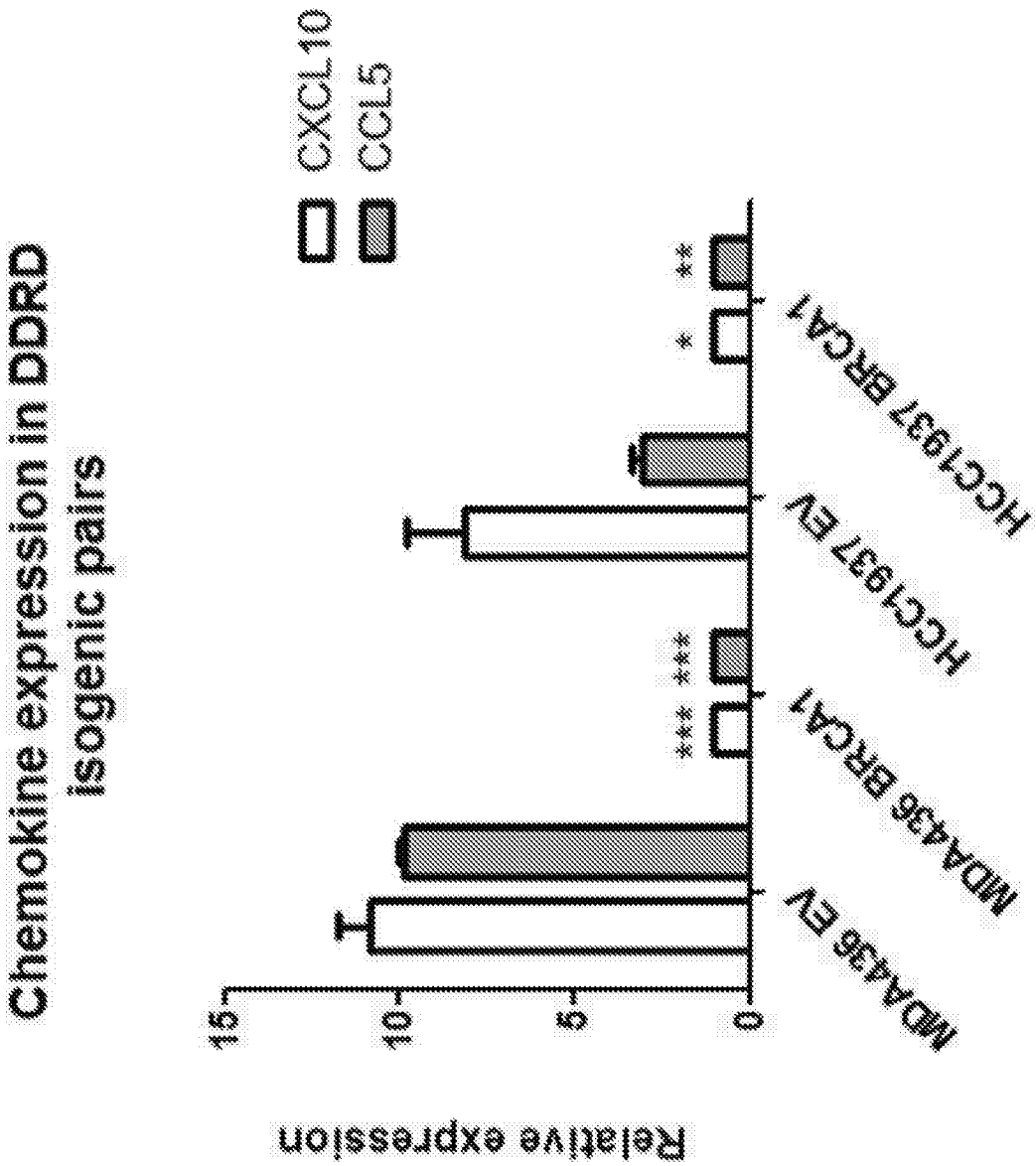

Chemokine expression in DDRD
isogenic pairs
CXCL10
CCL5
Relative expression
15
10
5
0
MDA436 EV
MDA436 BRCA1
HCC1937 EV
HCC1937 BRCA1
***
***
*
**

FIG. 12A

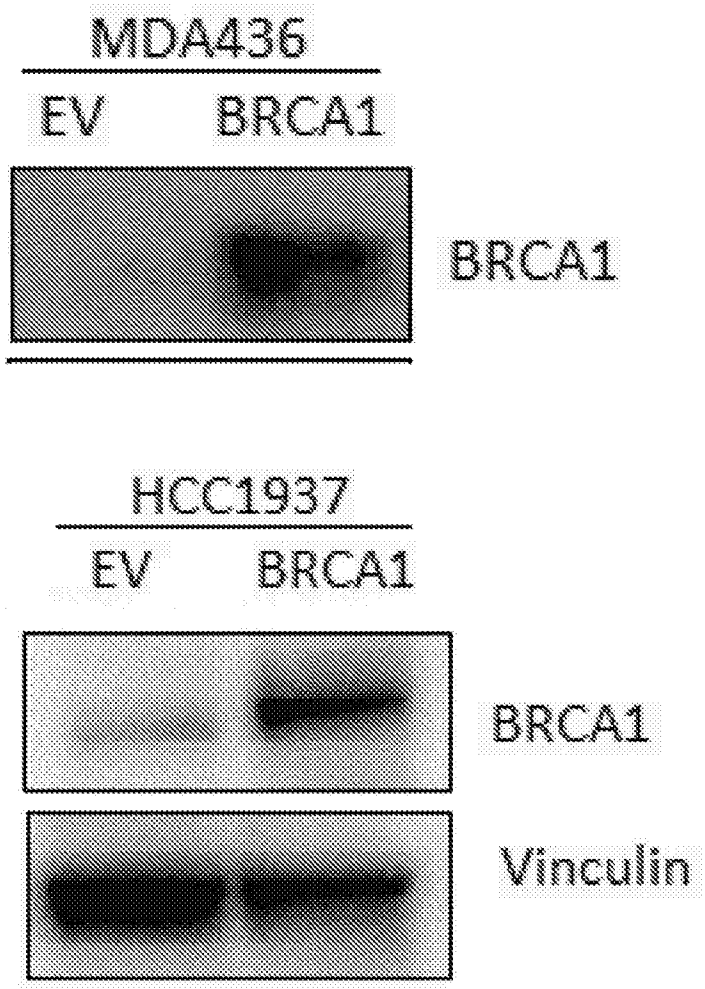


FIG. 12B

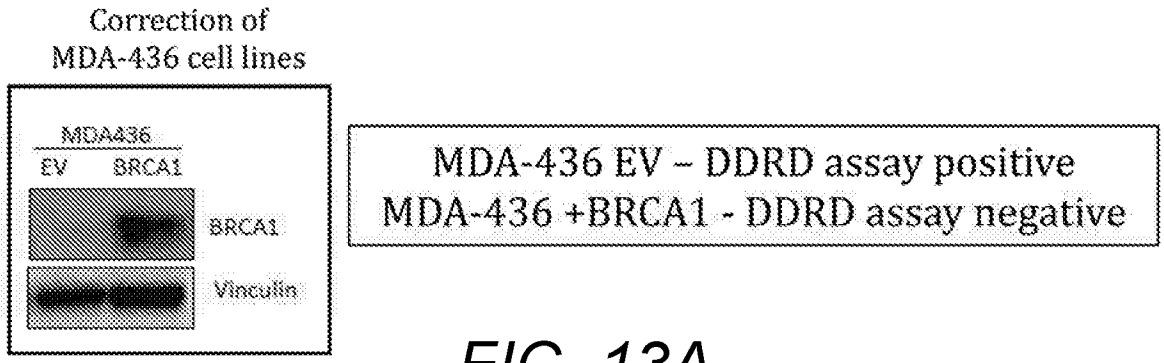


FIG. 13A

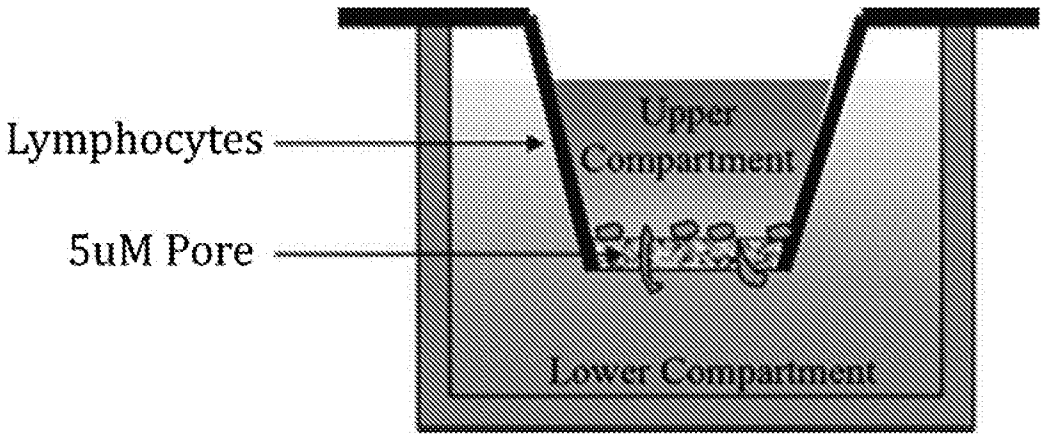


FIG. 13B

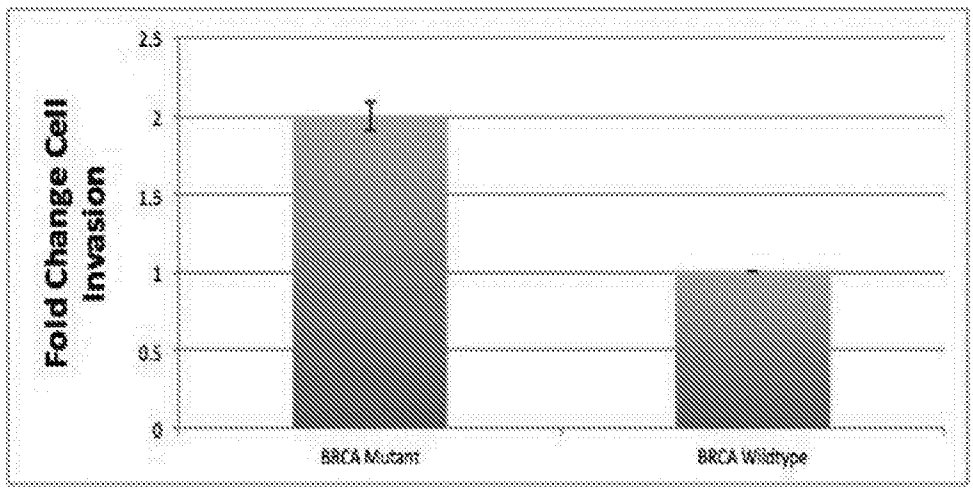


FIG. 13C

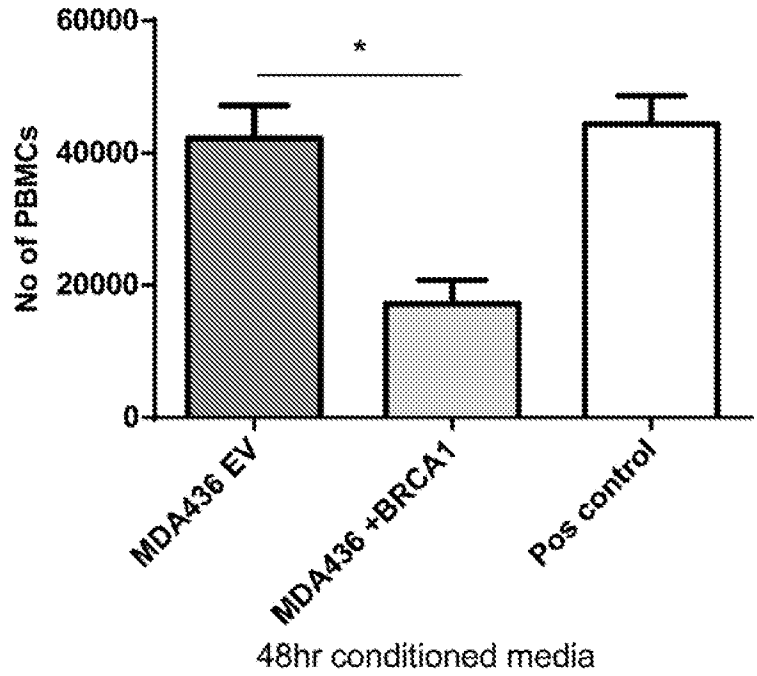


FIG. 13D

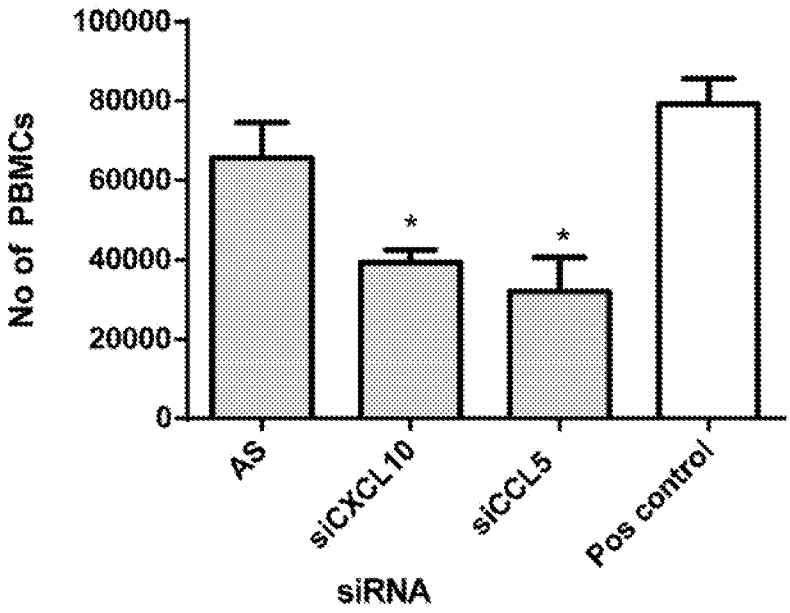


FIG. 13E

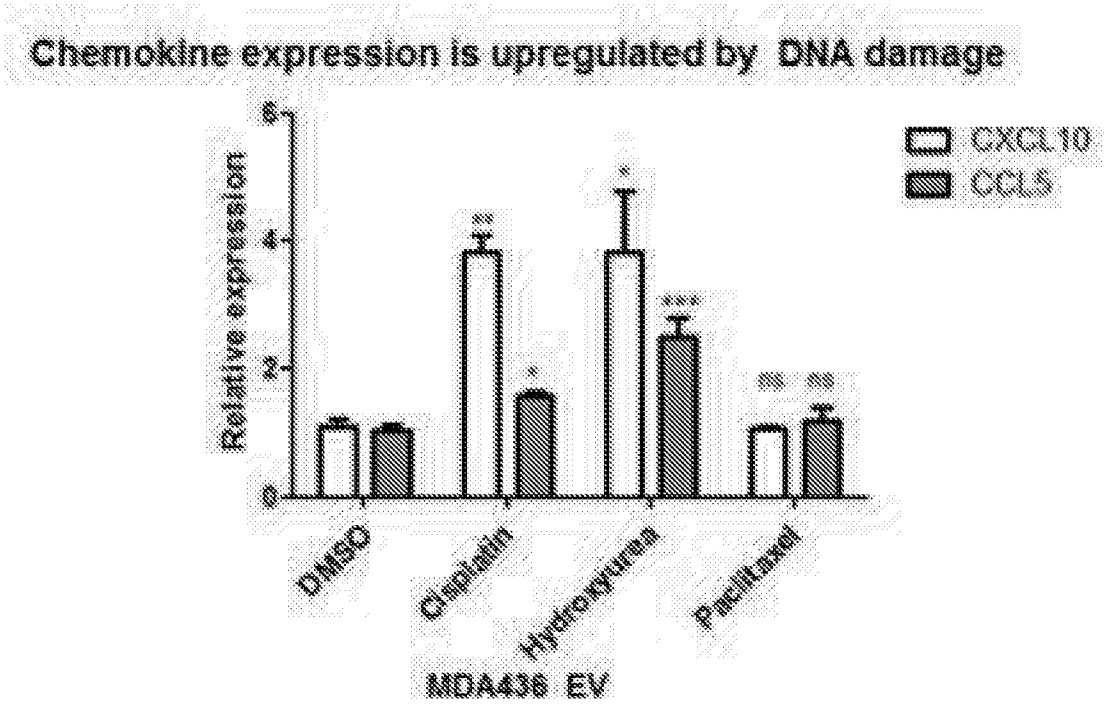


FIG. 14A

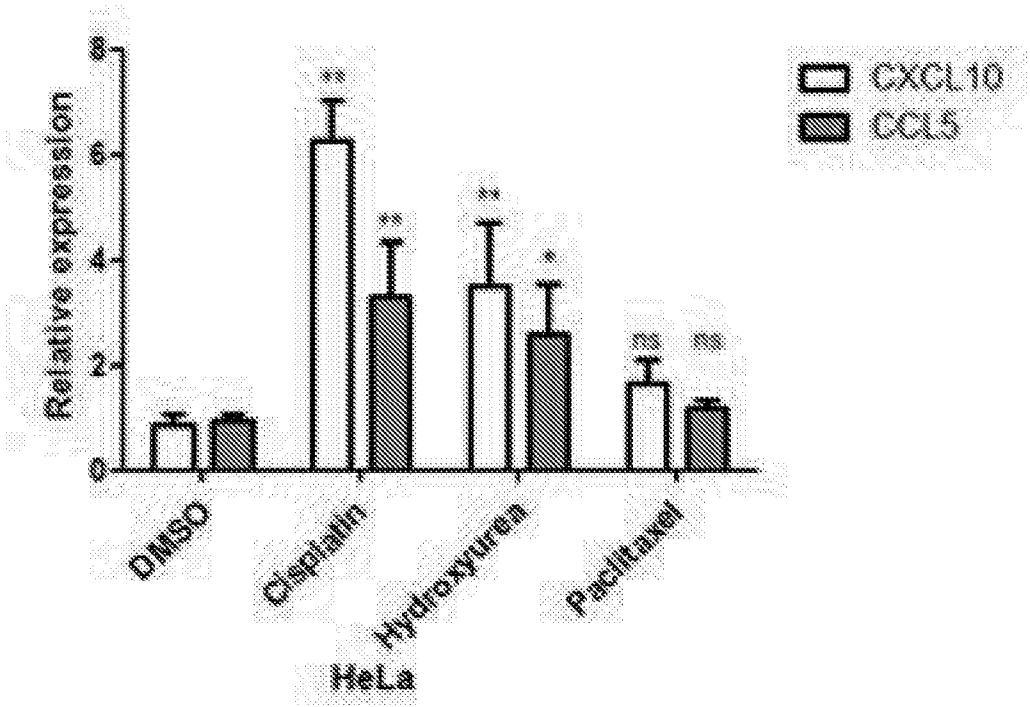


FIG. 14B

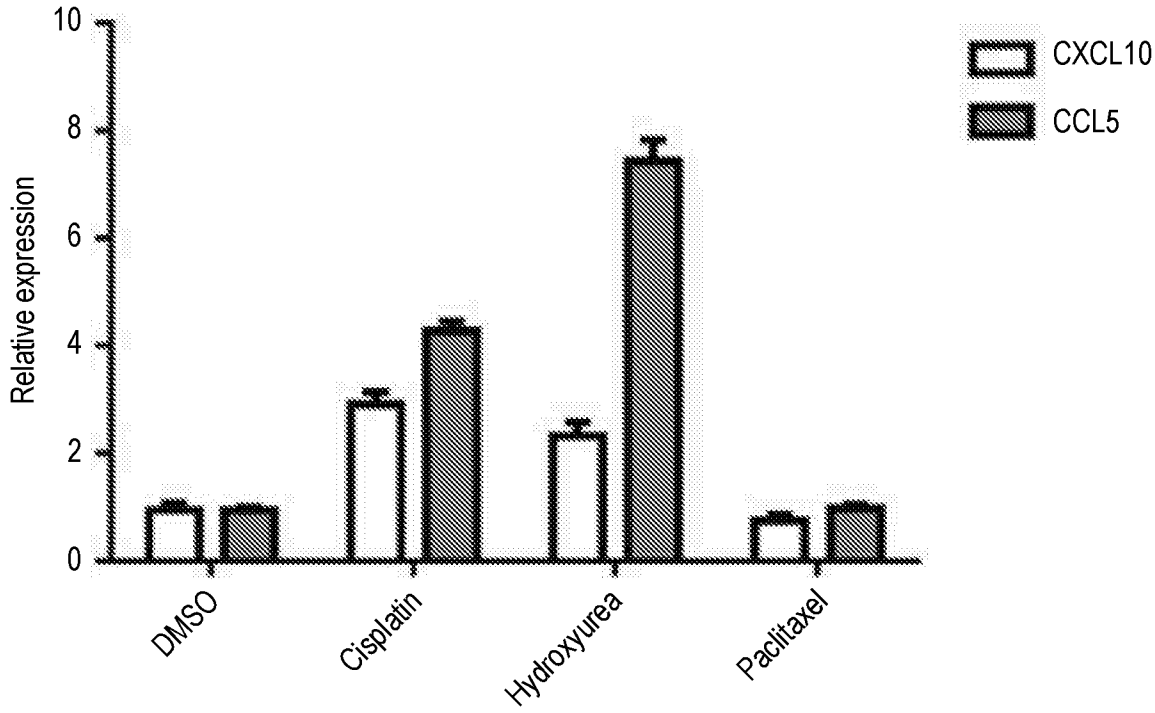


FIG. 14C

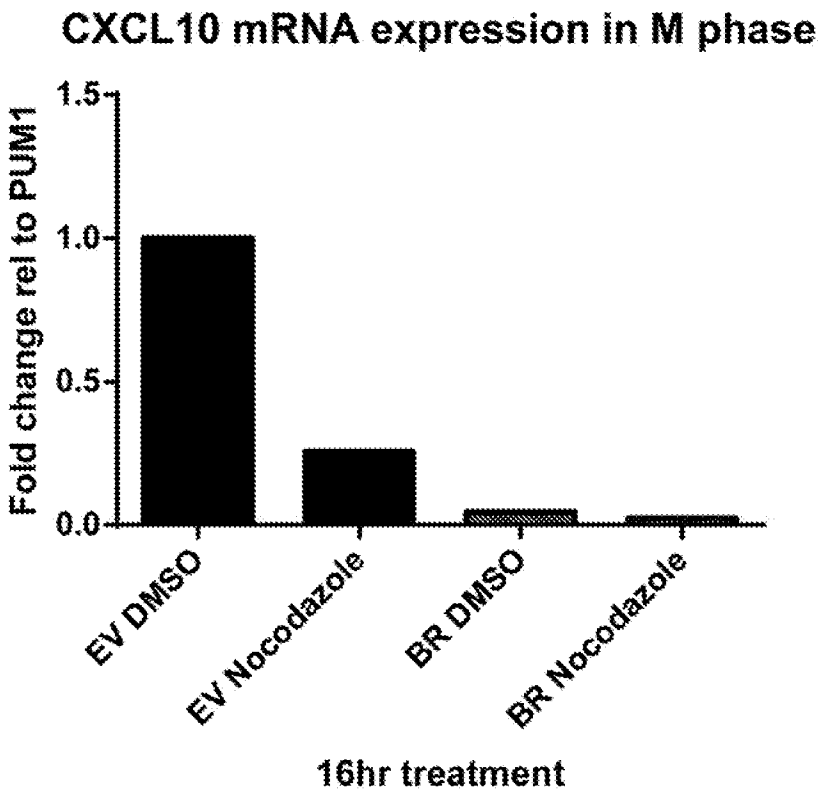


FIG. 15A

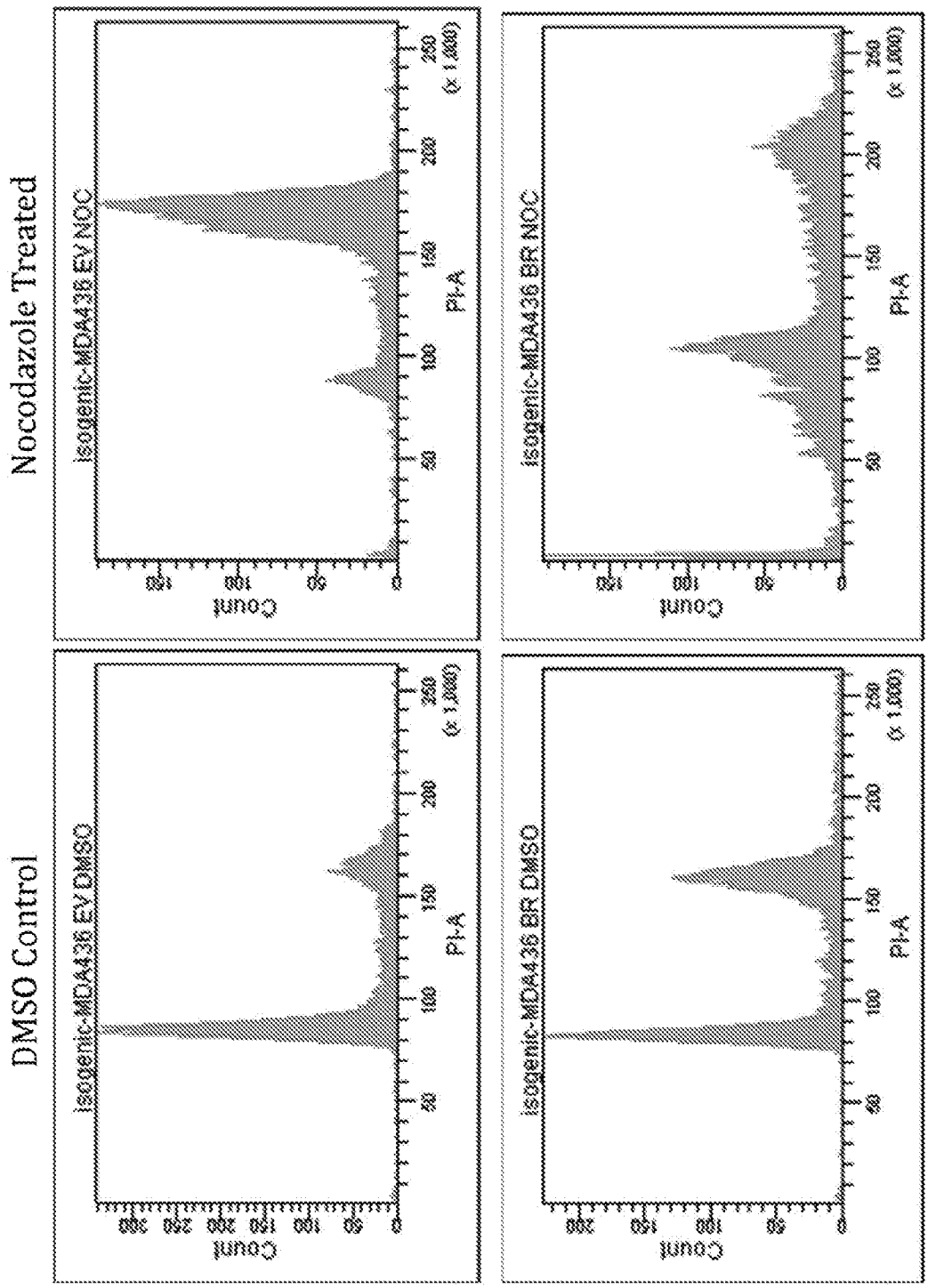

Nocodazole Treated
DMSO Control
isogenic-MDA436 EV NOC
isogenic-MDA436 BR NOC
isogenic-MDA436 EV DMSO
isogenic-MDA436 BR DMSO
Count
PI-A
(x 1,000)
Confirmation of M Phase Block

FIG. 15B

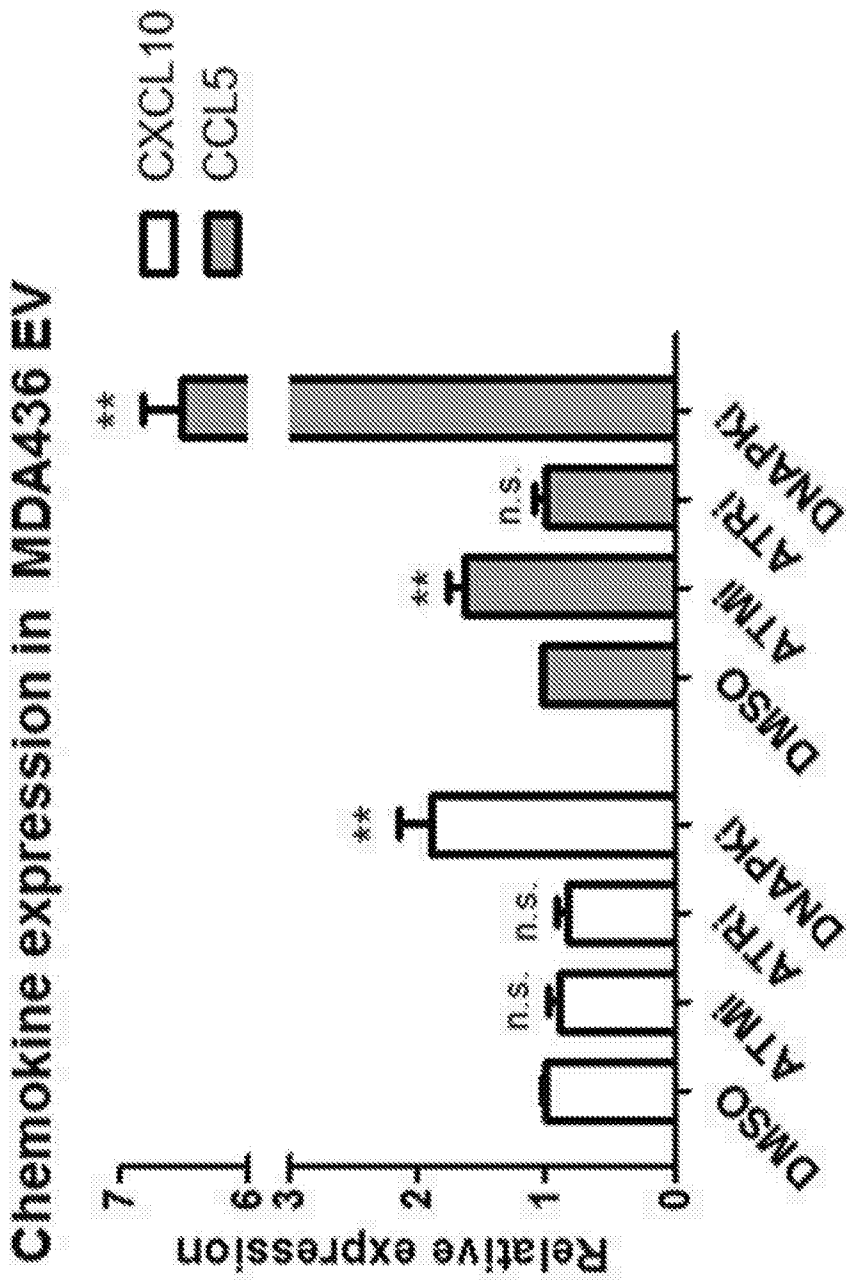

Chemokine expression in MDA436 EV
CXCL10
CCL5
Relative expression
0
1
2
3
6
7
**
n.s.
n.s.
**
**
n.s.
DMSO
ATMi
ATRi
DNAPKi
DMSO
ATMi
ATRi
DNAPKi

FIG. 16

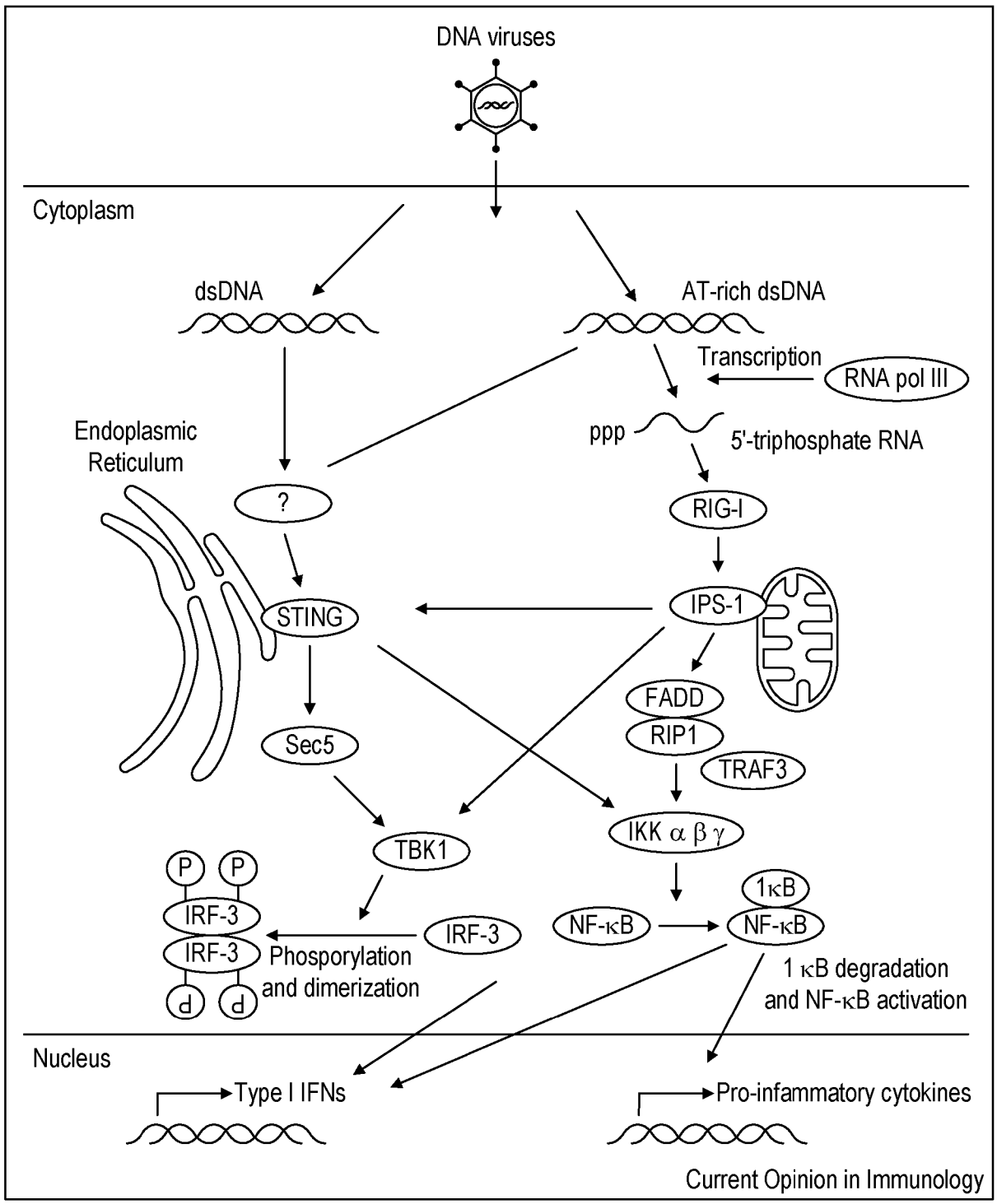

DNA viruses
Cytoplasm
dsDNA
AT-rich dsDNA
Transcription
RNA pol III
Endoplasmic Reticulum
ppp
5'-triphosphate RNA
?
RIG-I
STING
IPS-1
FADD
RIP1
TRAF3
Sec5
TBK1
IKK α β γ
P
P
IRF-3
IRF-3
IRF-3
Phosporylation and dimerization
1κB
NF-κB
NF-κB
1 κB degradation and NF-κB activation
Nucleus
Type I IFNs
Pro-infammatory cytokines
Current Opinion in Immunology

FIG. 17A

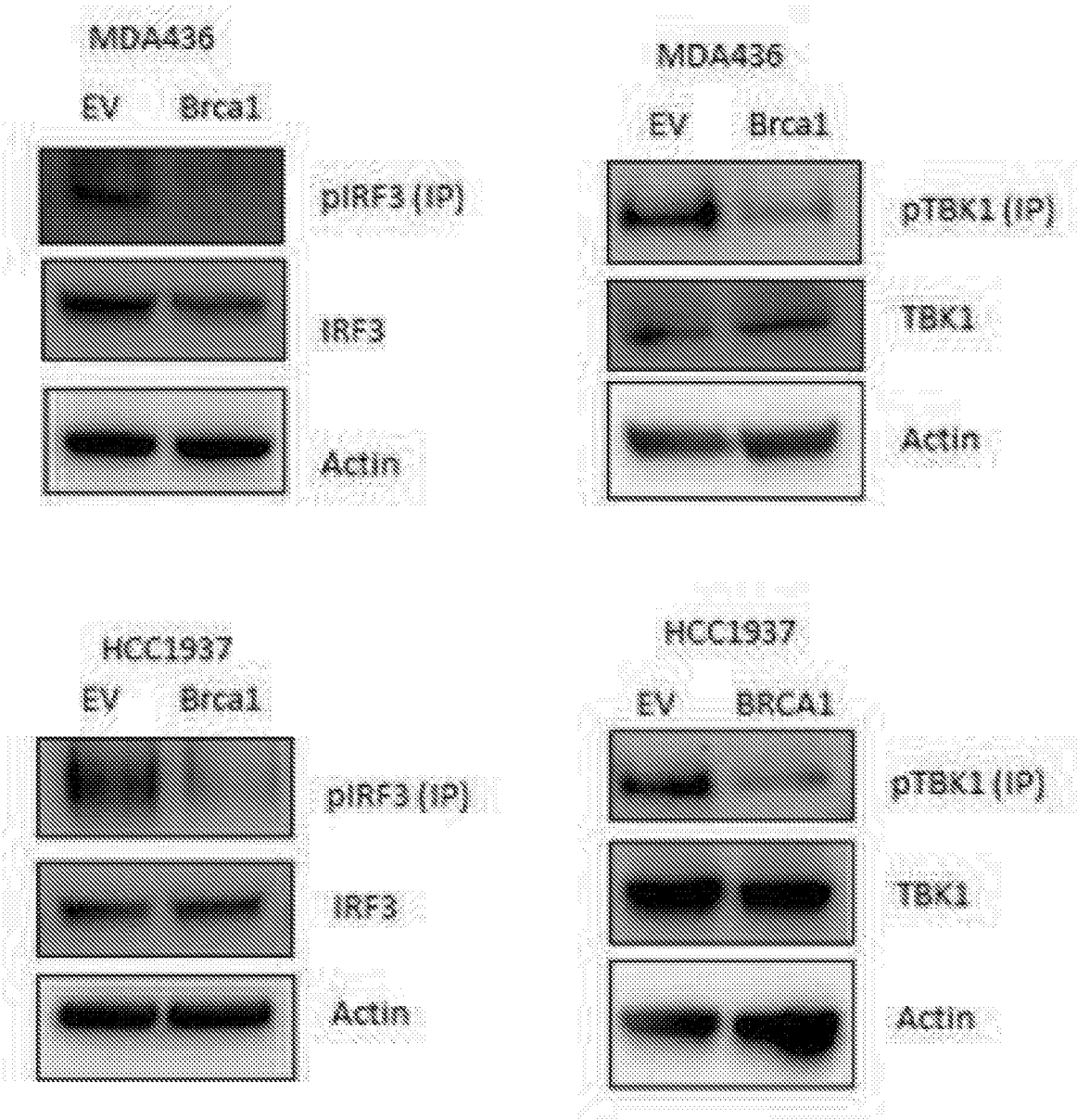
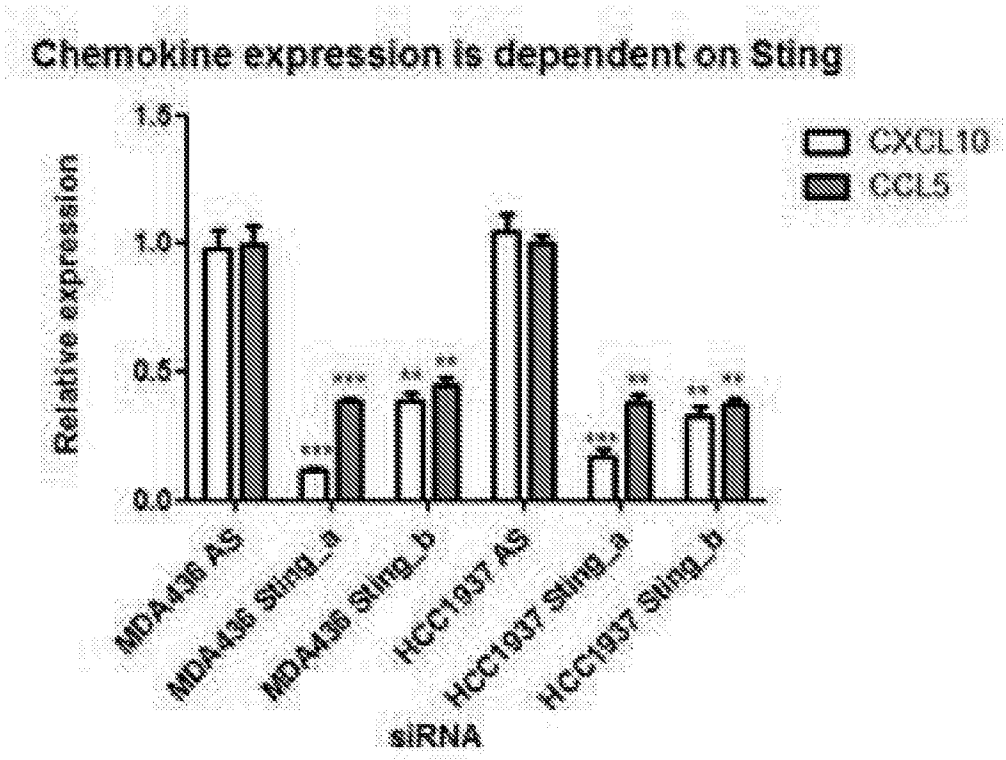

MDA436
EV
Brca1
pIRF3 (IP)
IRF3
Actin
MDA436
EV
Brca1
pTBK1 (IP)
TBK1
Actin
HCC1937
EV
Brca1
pIRF3 (IP)
IRF3
Actin
HCC1937
EV
BRCA1
pTBK1 (IP)
TBK1
Actin

FIG. 17B


Chemokine expression is dependent on Sting
CXCL10
CCL5
Relative expression
1.5
1.0
0.5
0.0
MDA436 AS
MDA436 Sting_a
MDA436 Sting_b
HCC1937 AS
HCC1937 Sting_a
HCC1937 Sting_b
siRNA

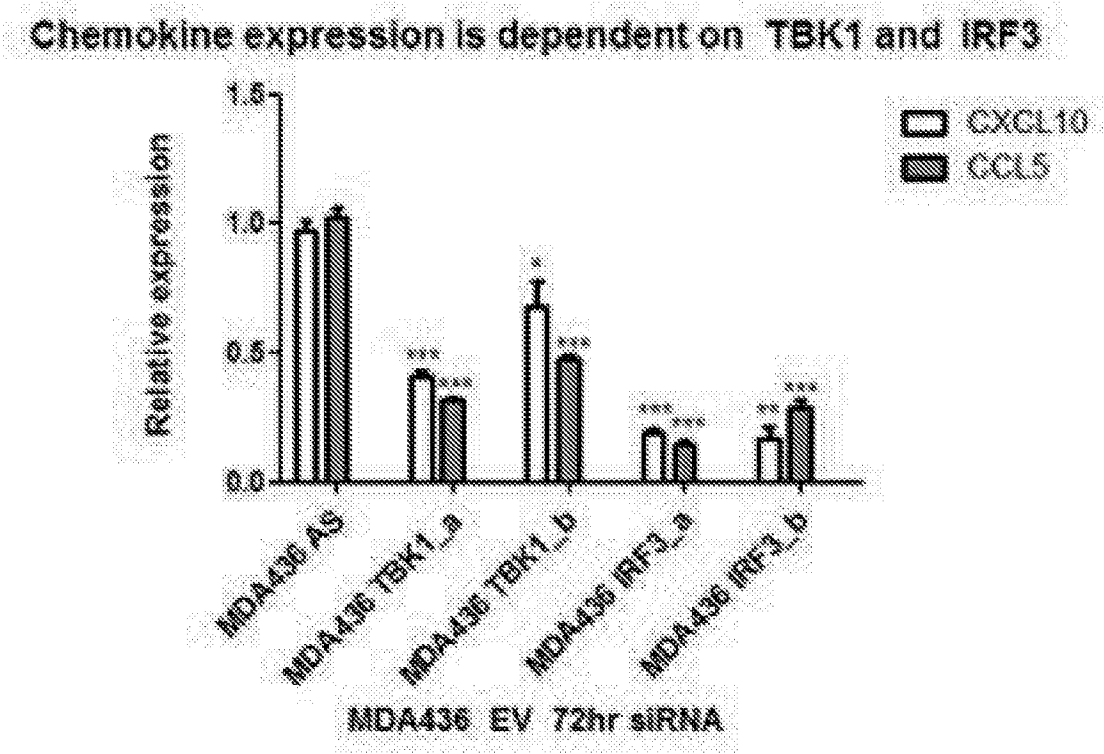

Chemokine expression is dependent on TBK1 and IRF3
CXCL10
CCL5
Relative expression
1.5
1.0
0.5
0.0
MDA436 AS
MDA436 TBK1_a
MDA436 TBK1_b
MDA436 IRF3_a
MDA436 IRF3_b
MDA436 EV 72hr siRNA

FIG. 17C

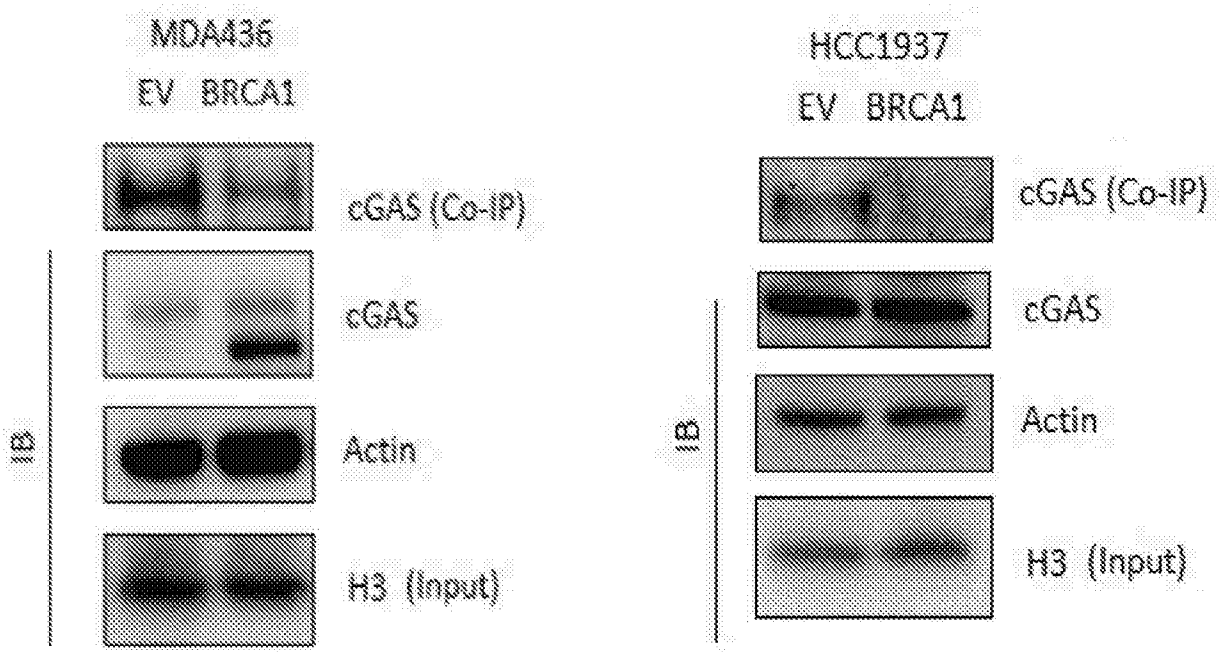

MDA436
EV BRCA1
cGAS (Co-IP)
cGAS
Actin
H3 (Input)
IB
HCC1937
EV BRCA1
cGAS (Co-IP)
cGAS
Actin
H3 (Input)
IB

FIG. 18A

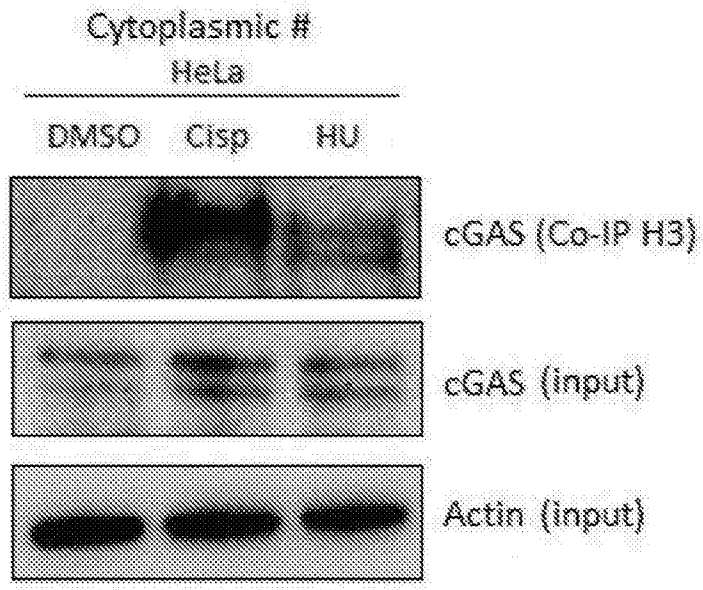


FIG. 18B

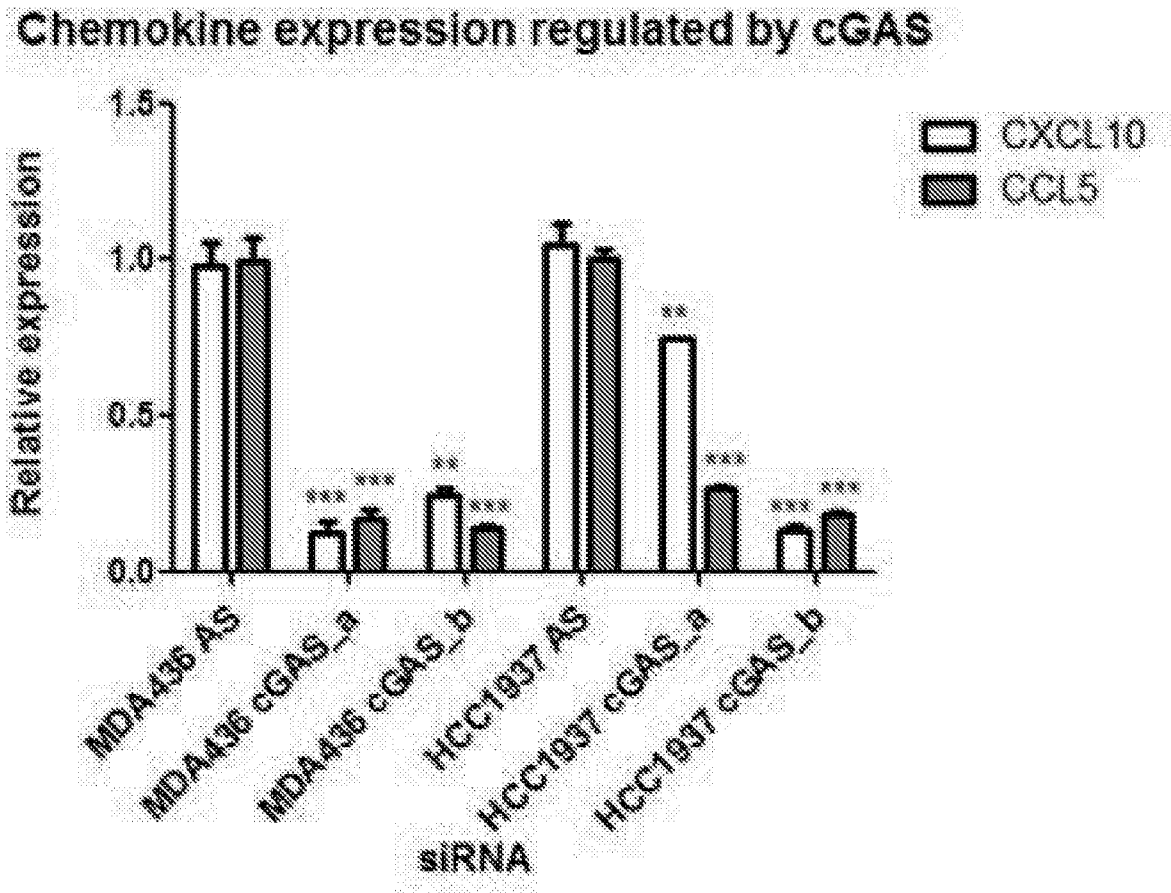


FIG. 18C

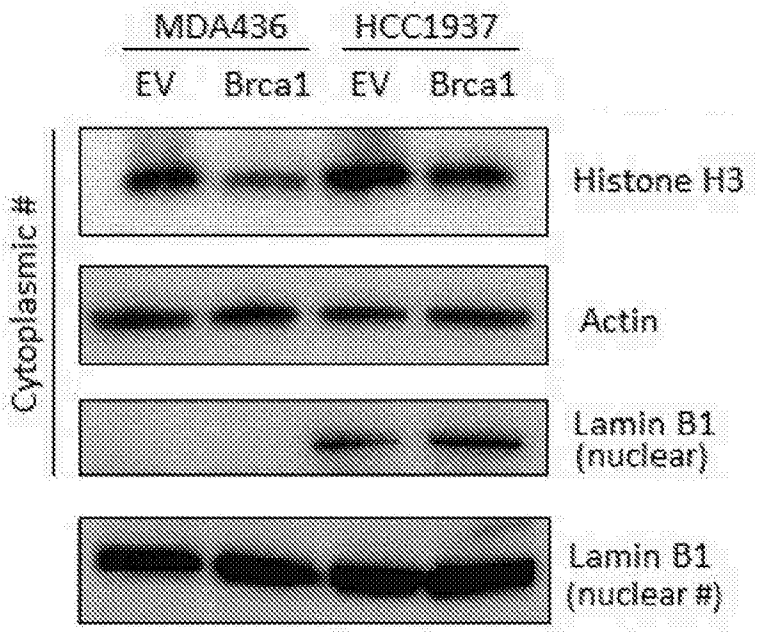


FIG. 19A

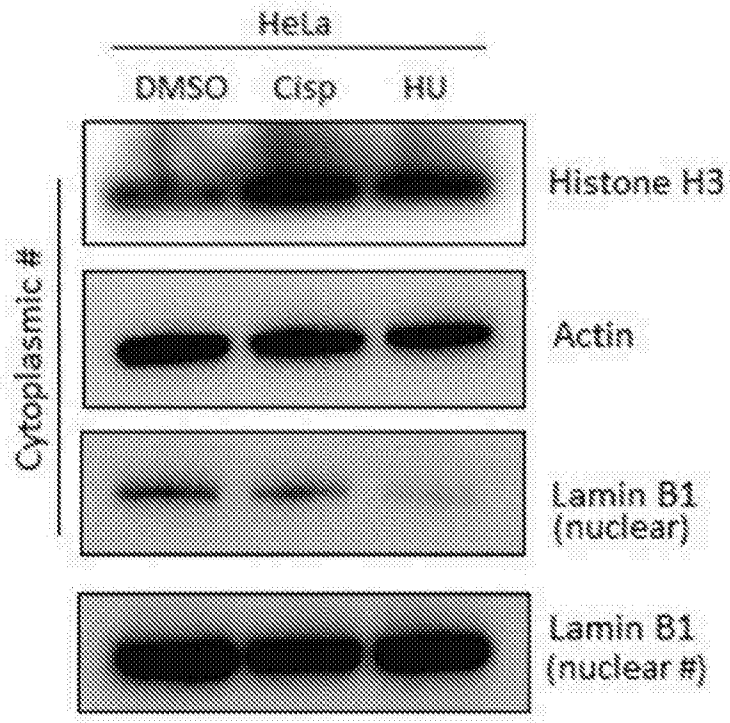


FIG. 19B

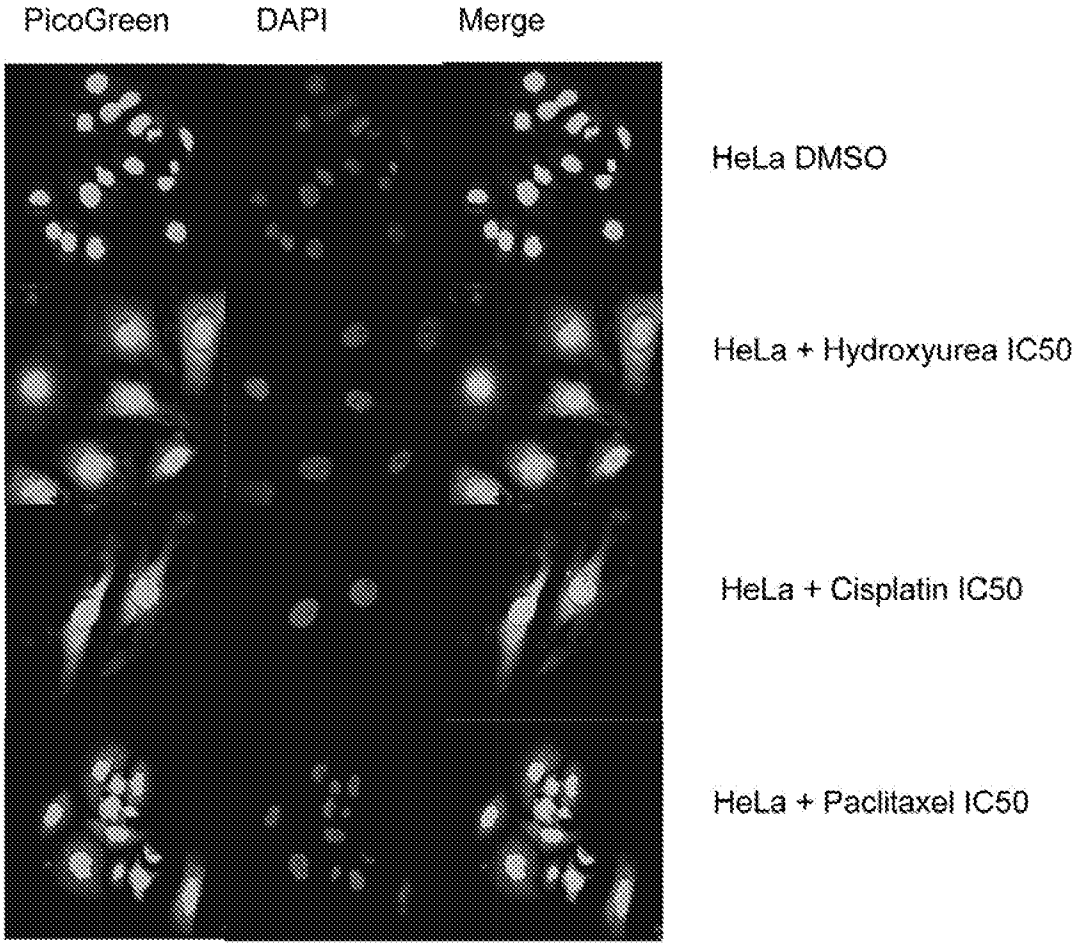

PicoGreen
DAPI
Merge
HeLa DMSO
HeLa + Hydroxyurea IC50
HeLa + Cisplatin IC50
HeLa + Paclitaxel IC50

FIG. 19C


>1% cut off
TUMOUR DDRD pos DDRD neg
PDL1 pos 46.2% (30) 4.4% (5)
PDL1 neg 53.8% (35) 95.6% (108)
p<0.0001
LYMPHOCYTES DDRD pos DDRD neg
PDL1 pos 75.4% (49) 8.85% (10)
PDL1 neg 24.6% (16) 91.2% (103)
p<0.0001
>5% cut off
TUMOUR DDRD pos DDRD neg
PDL1 pos 21.5% (14) 3.6% (4)
PDL1 neg 78.5% (51) 96.5% (109)
P=0.0004
LYMPHOCYTES DDRD pos DDRD neg
PDL1 pos 40.0% (26) 2.7% (3)
PDL1 neg 60.0% (39) 97.3% (110)
p<0.0001

FIG. 20A

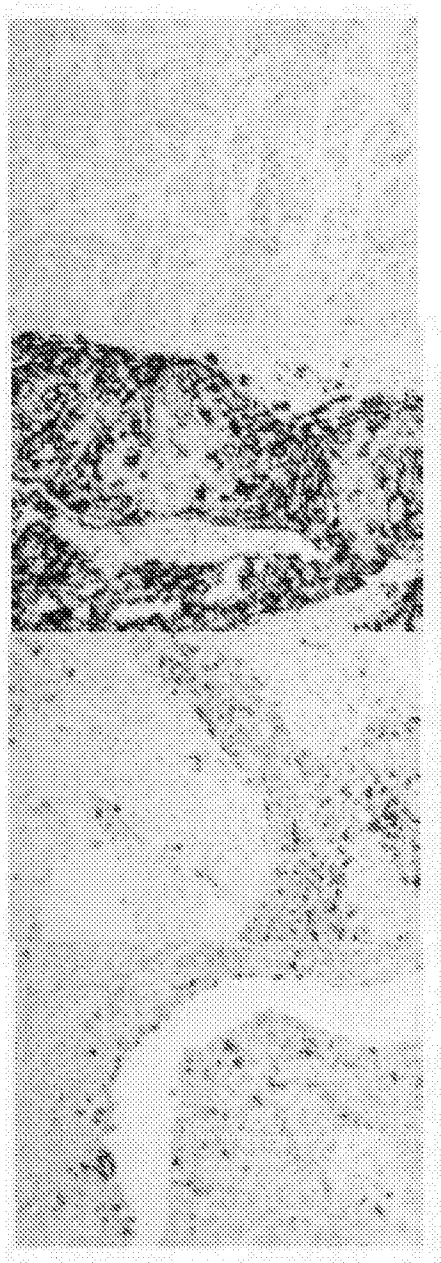
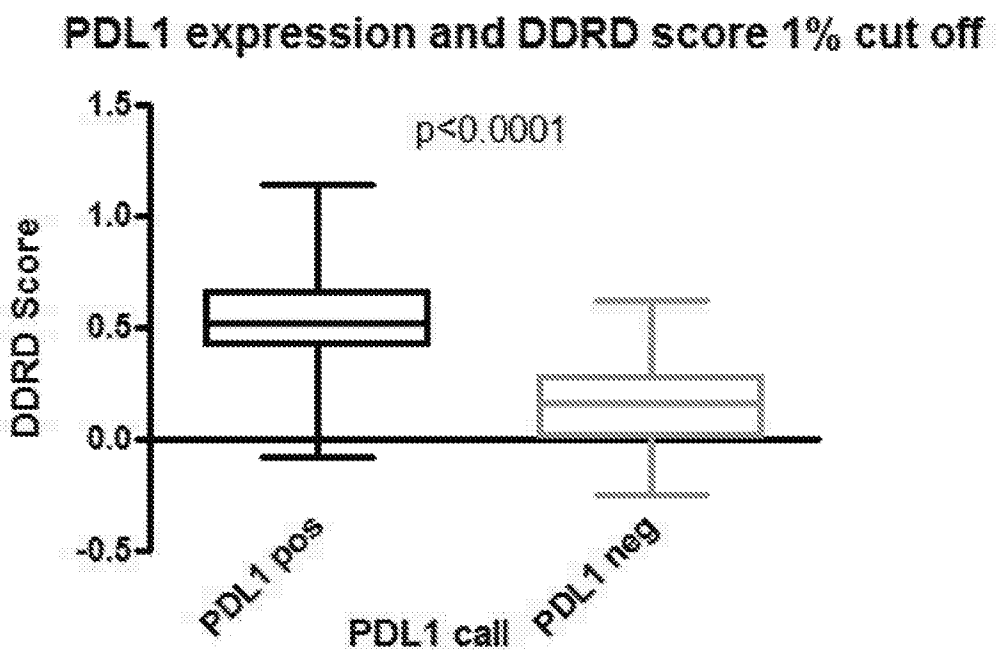

Negative PDL1 expression
Strong tumour PDL1 expression
Lymphocytic PDL1 expression
Tumour and Lymphocytic PDL1 expression

FIG. 20B


PDL1 expression and DDRD score 1% cut off
p<0.0001
DDRD Score
1.5
1.0
0.5
0.0
-0.5
PDL1 pos
PDL1 neg
PDL1 call

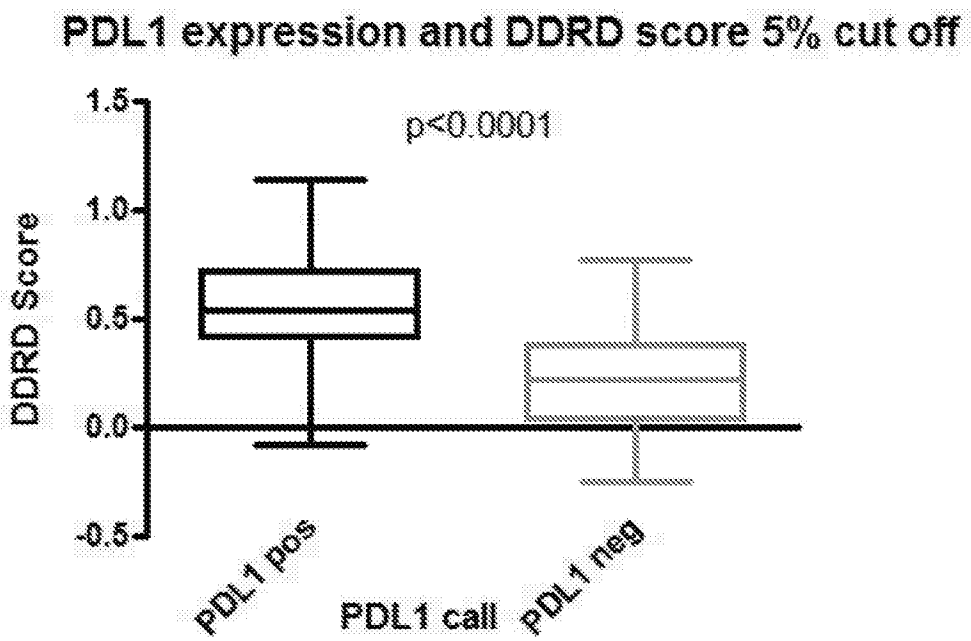

PDL1 expression and DDRD score 5% cut off
p<0.0001
DDRD Score
1.5
1.0
0.5
0.0
-0.5
PDL1 pos
PDL1 neg
PDL1 call

FIG. 21

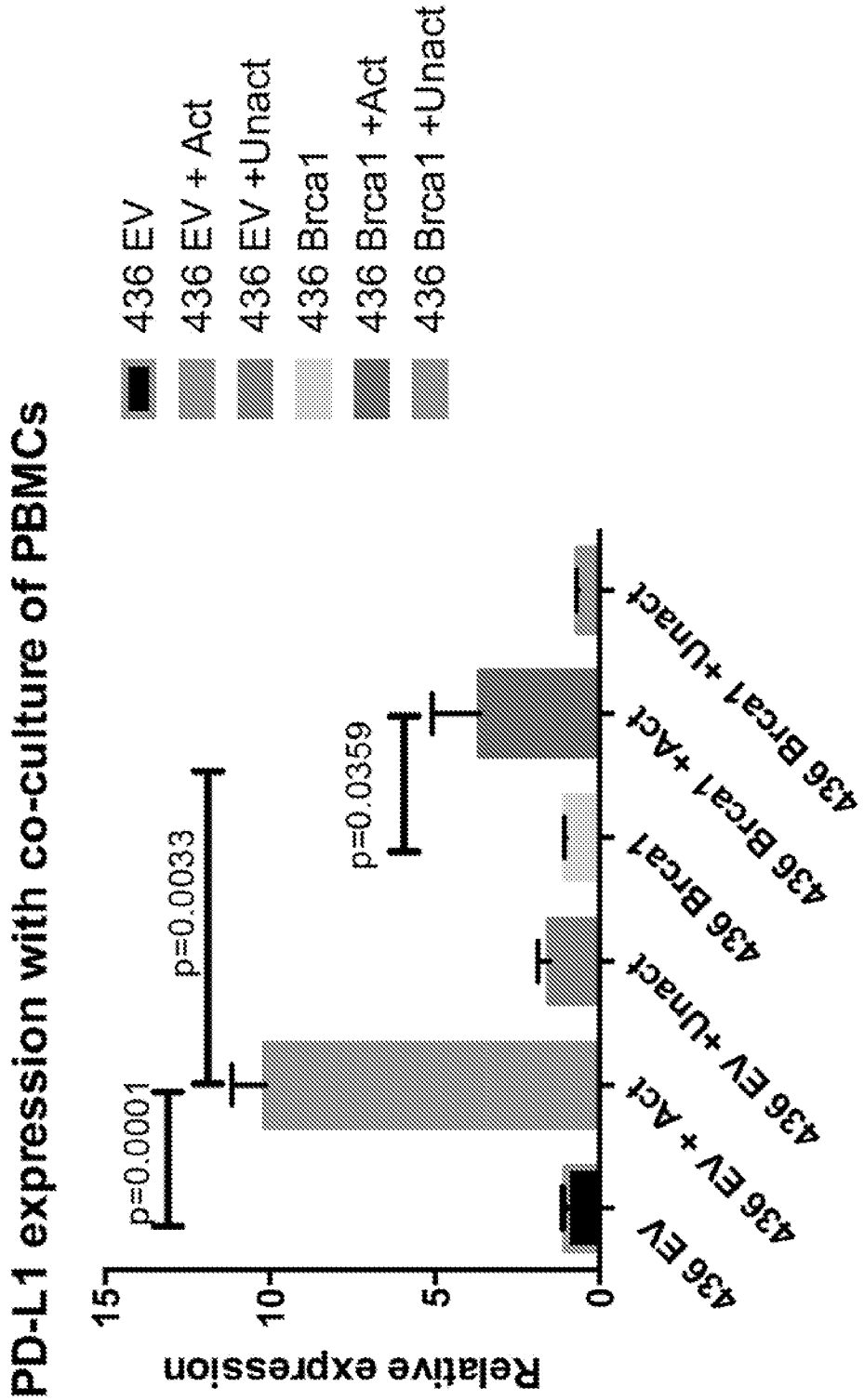

PD-L1 expression with co-culture of PBMCs
436 EV
436 EV + Act
436 EV +Unact
436 Brca1
436 Brca1 +Act
436 Brca1 +Unact
p=0.0001
p=0.0033
p=0.0359
Relative expression
15
10
5
0
436 EV
436 EV + Act
436 EV +Unact
436 Brca1
436 Brca1 +Act
436 Brca1 +Unact
24 hour co-culture

FIG. 22

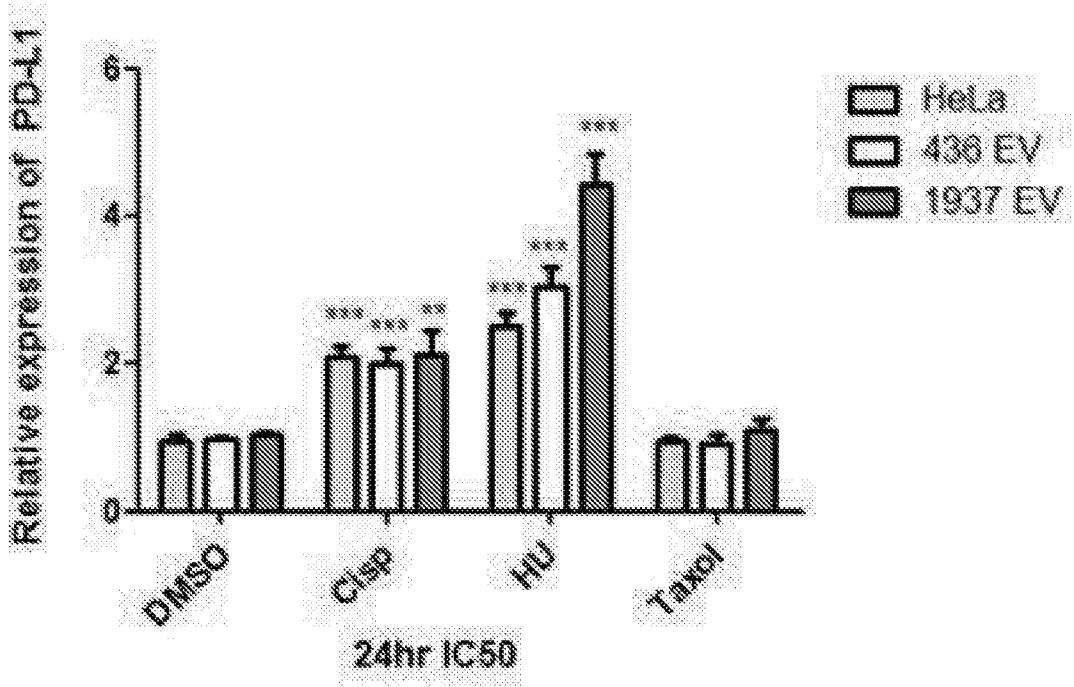


FIG. 23A

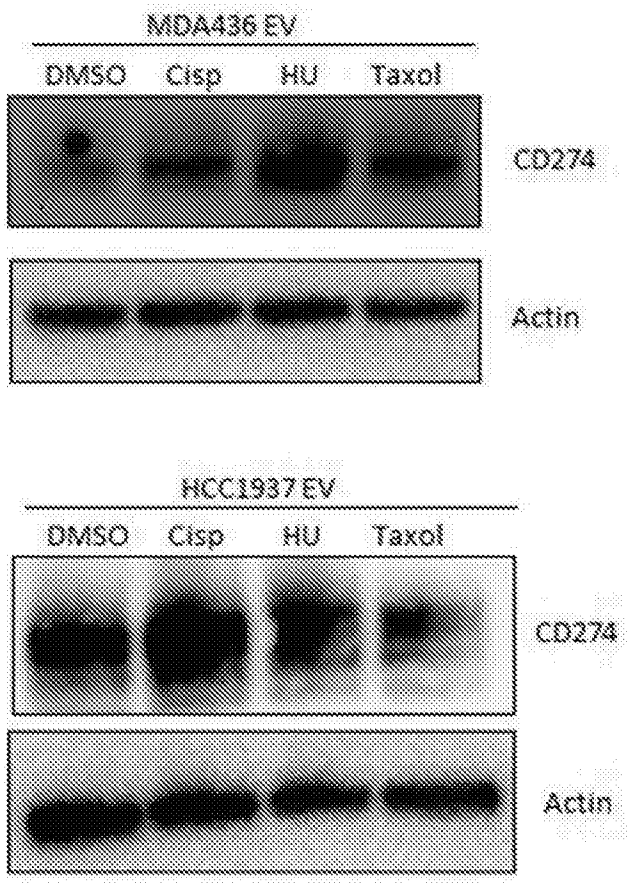


FIG. 23B

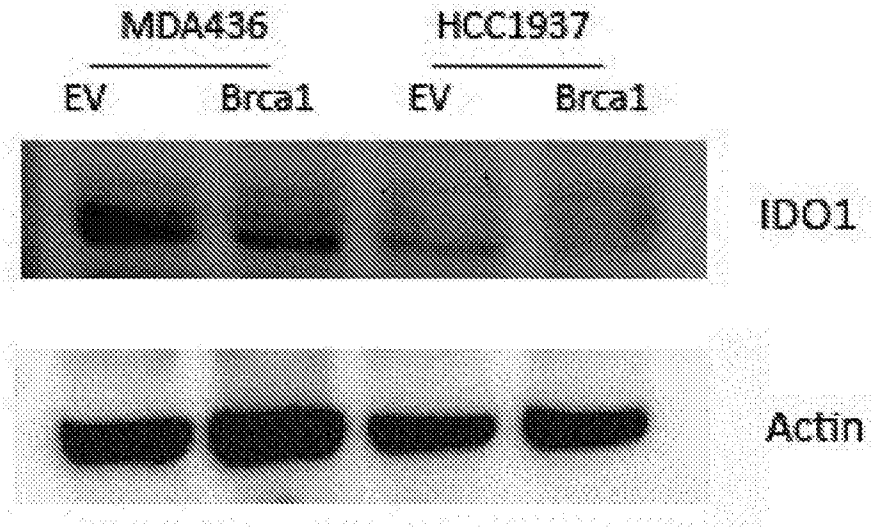


FIG. 24

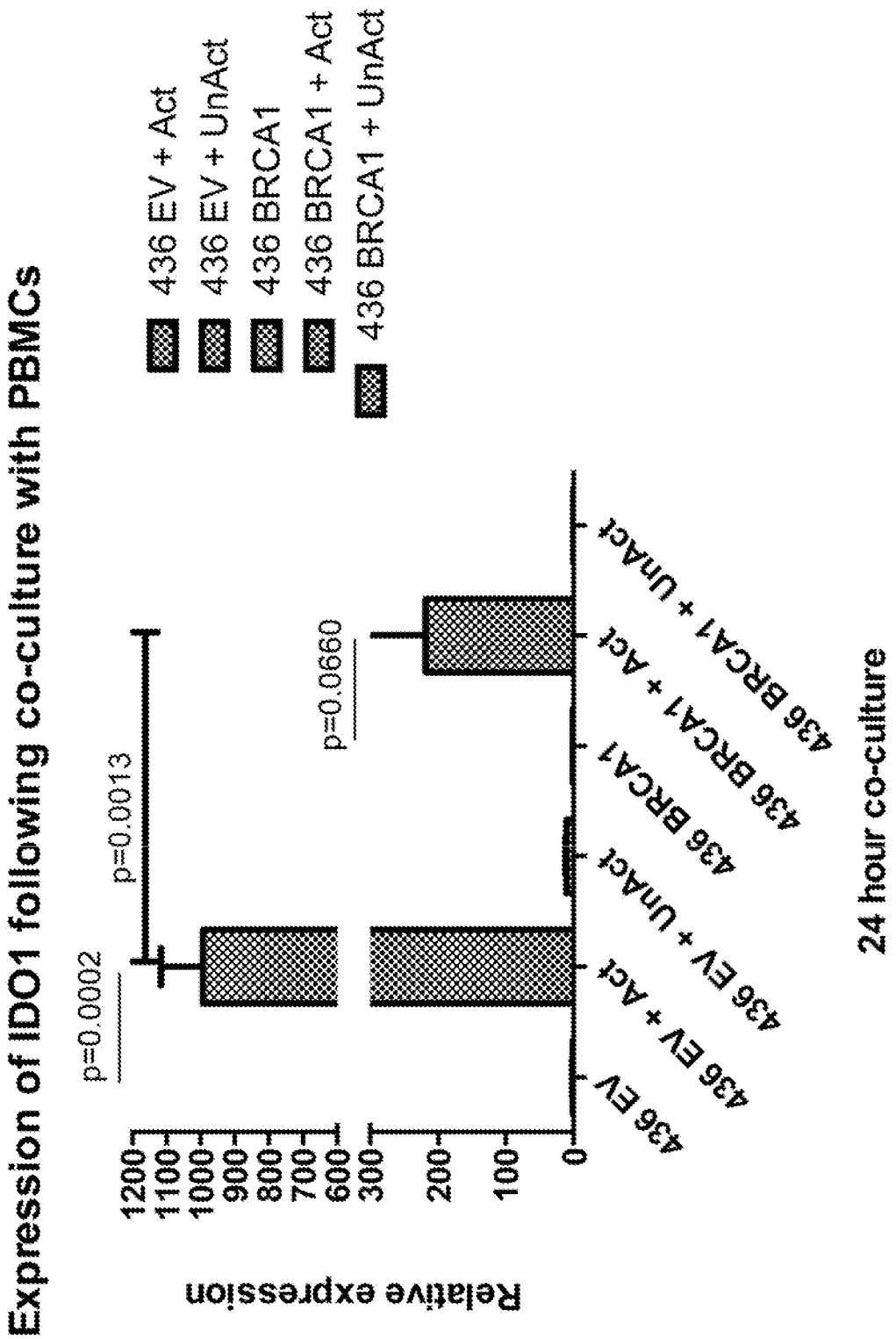

Expression of IDO1 following co-culture with PBMCs
436 EV + Act
436 EV + UnAct
436 BRCA1
436 BRCA1 + Act
436 BRCA1 + UnAct
p=0.0002
p=0.0013
p=0.0660
Relative expression
1200
1100
1000
900
800
700
600
300
200
100
0
436 EV
436 EV + Act
436 EV + UnAct
436 BRCA1
436 BRCA1 + Act
436 BRCA1 + UnAct
24 hour co-culture

FIG. 25

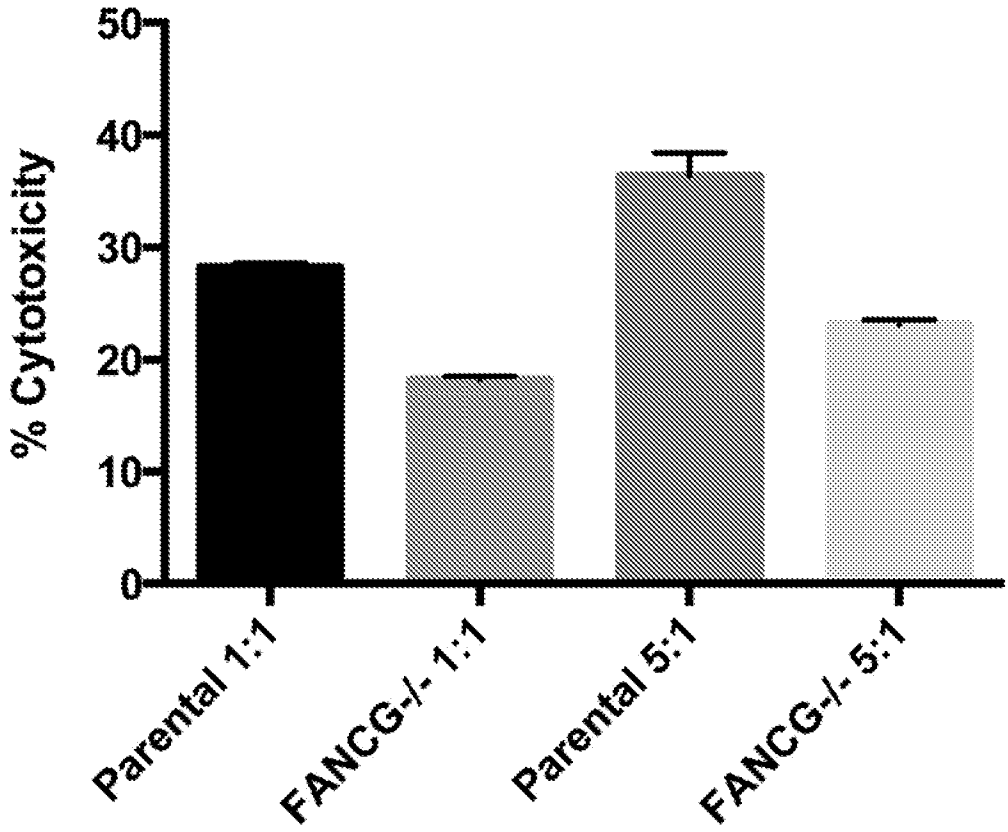

% Cytotoxicity
50
40
30
20
10
0
Parental 1:1
FANCG-/- 1:1
Parental 5:1
FANCG-/- 5:1

FIG. 26

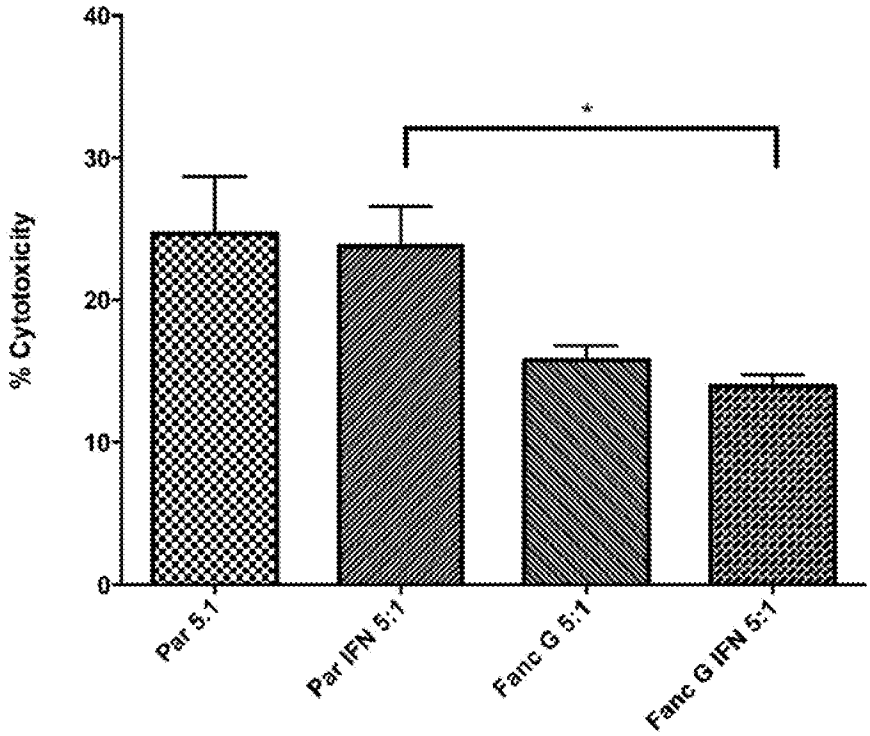


FIG. 27A

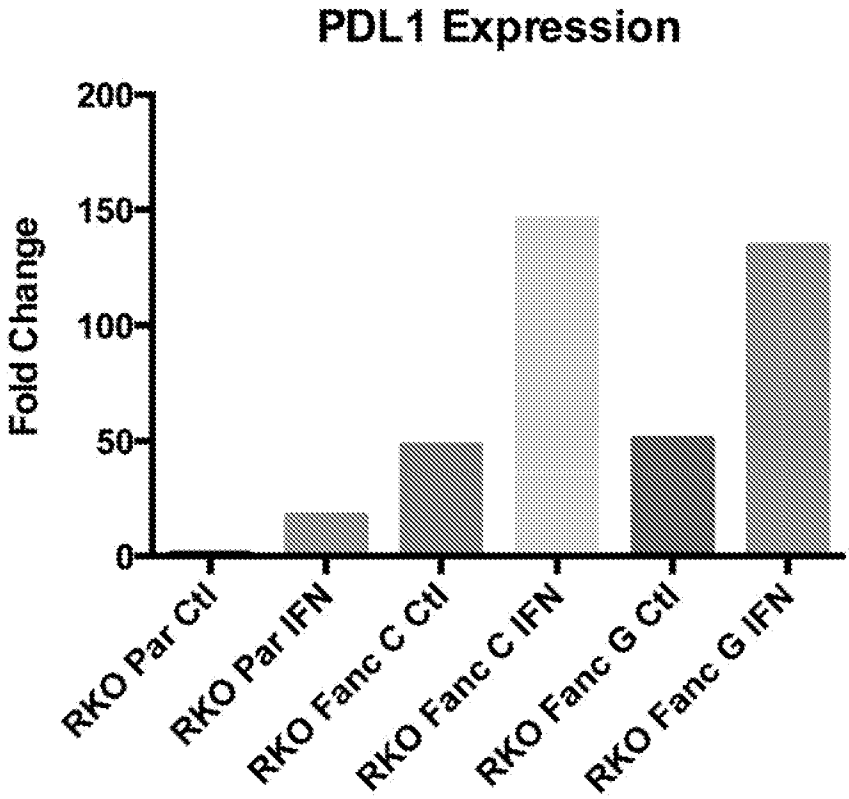


FIG. 27B

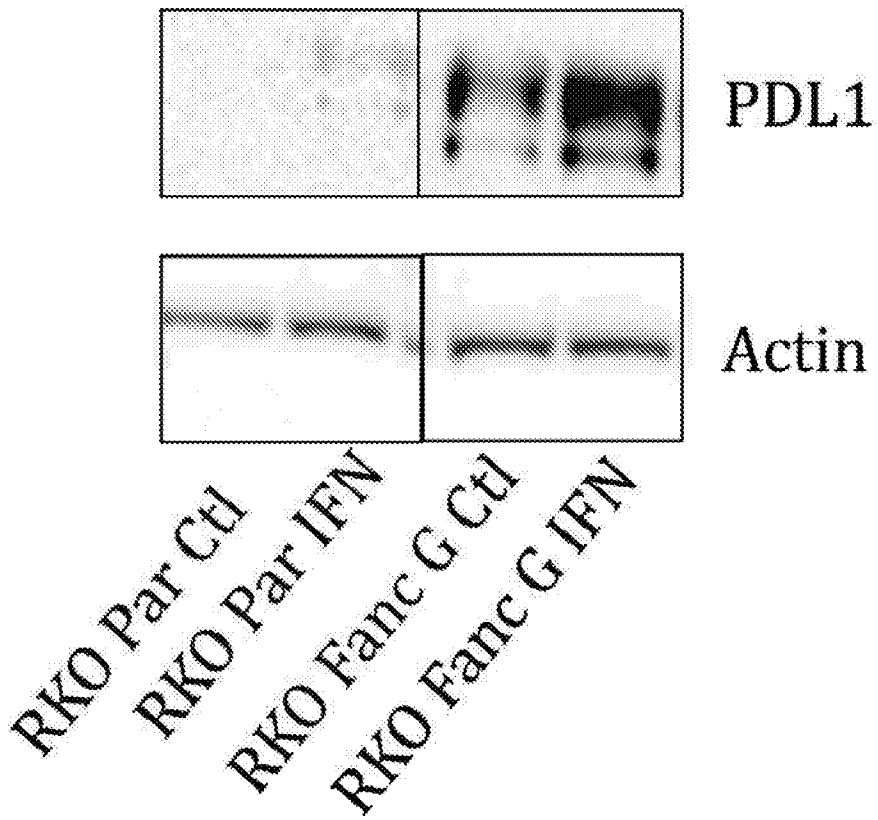


FIG. 27C

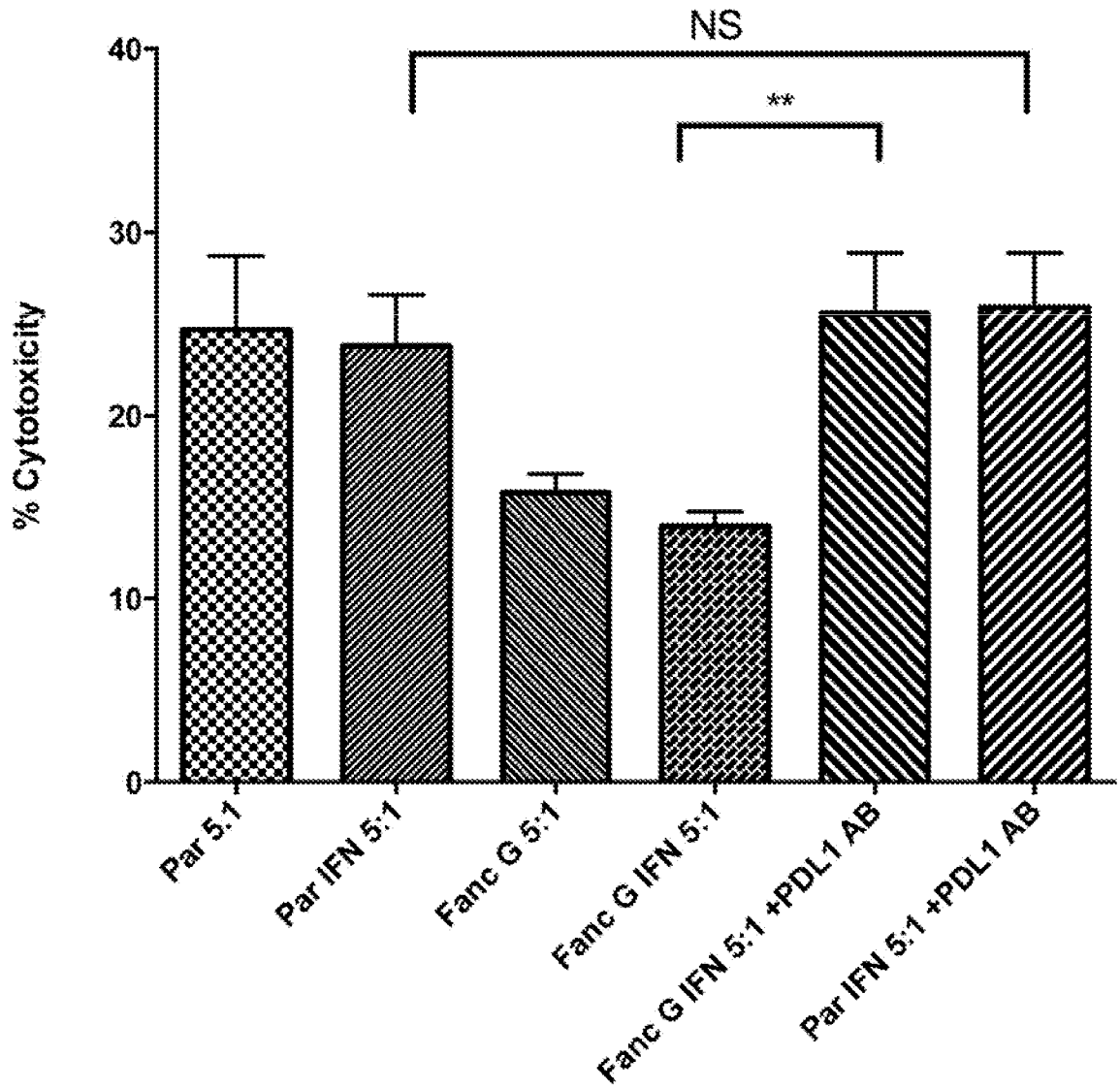
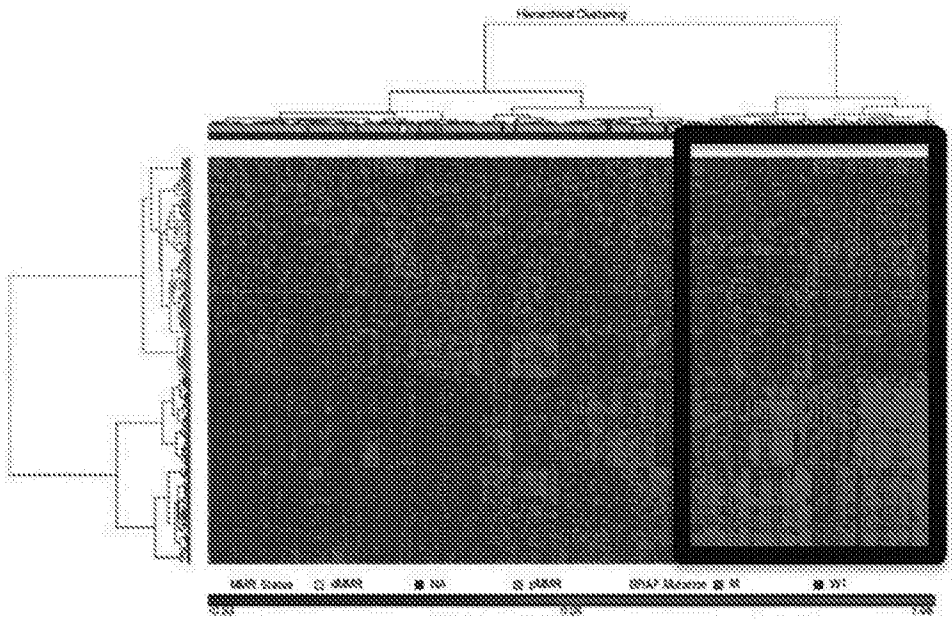
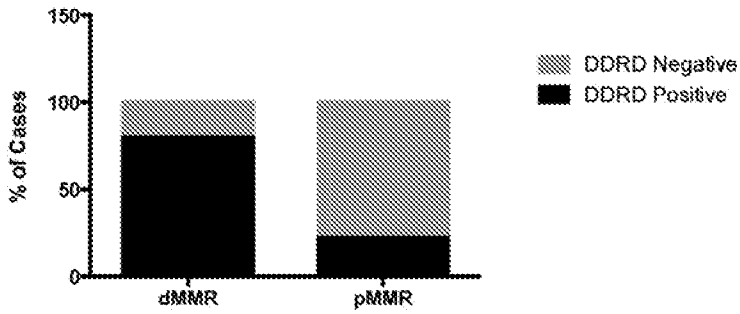

NS
**
40
30
20
10
0
% Cytotoxicity
Par 5:1
Par IFN 5:1
Fanc G 5:1
Fanc G IFN 5:1
Fanc G IFN 5:1 +PDL1 AB
Par IFN 5:1 +PDL1 AB

FIG. 28



DDRD molecular subgroup highly enriched for MSI colorectal cancers

FIG. 29A



MSI MSS

80% of MSI tumours are DDRD positive

	dMMR	pMMR
DDRD Positive	60	101
DDRD Negative	15	343

FIG. 29B

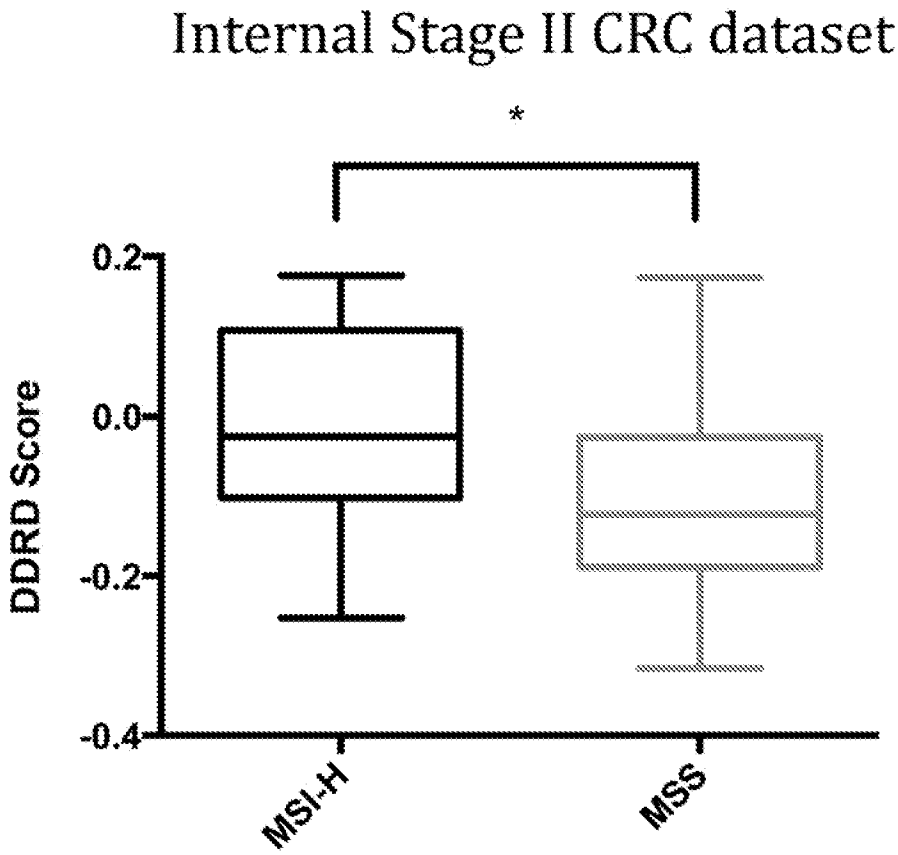


FIG. 29C

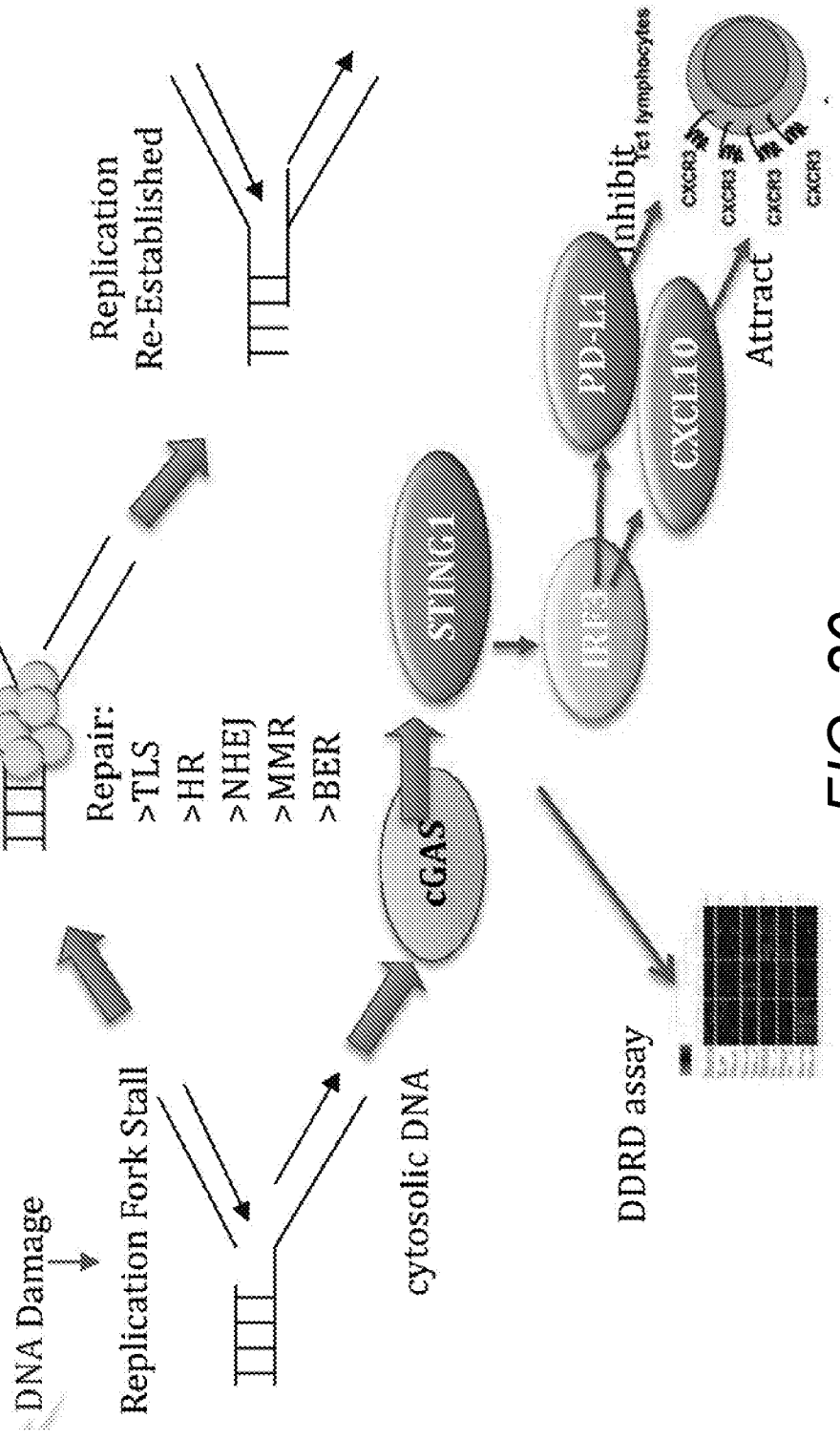

DNA Damage
Replication Fork Stall
cytosolic DNA
cGAS
STING1
DDRD assay
Repair:
>TLS
>HR
>NHEJ
>MMR
>BER
Replication
Re-Established
PD-L1
CXCL10
Attract
cxcr3

FIG. 30

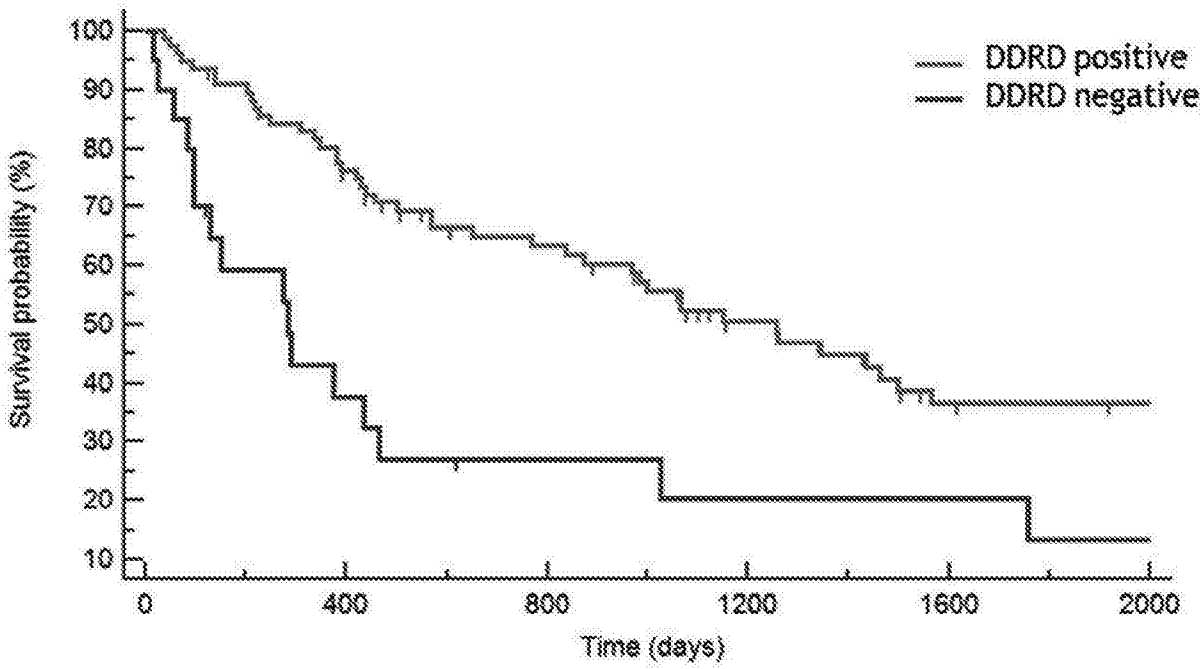

DDRD positive
DDRD negative
Survival probability (%)
100
90
80
70
60
50
40
30
20
10
0
400
800
1200
1600
2000
Time (days)

FIG. 31

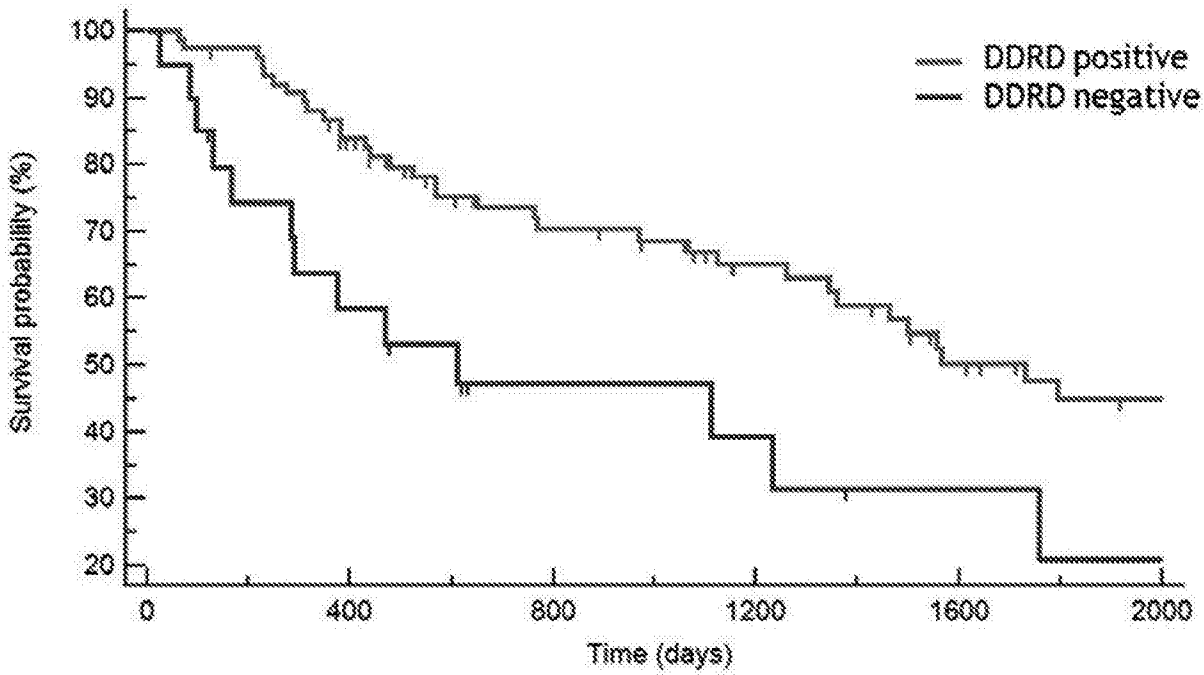

DDRD positive
DDRD negative
Survival probability (%)
100
90
80
70
60
50
40
30
20
0
400
800
1200
1600
2000
Time (days)

FIG. 32

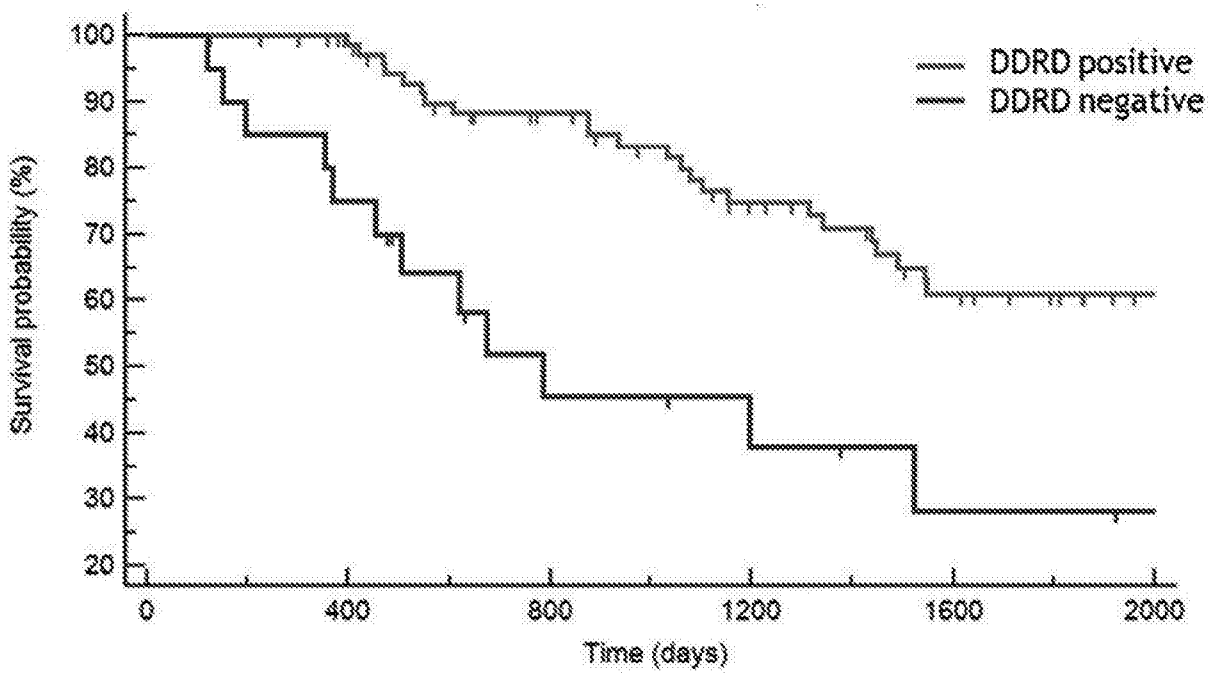

DDRD positive
DDRD negative
Survival probability (%)
100
90
80
70
60
50
40
30
20
0
400
800
1200
1600
2000
Time (days)

FIG. 33

INTERNATIONAL SEARCH REPORT

International application No
PCT/GB2016/052213

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, BIOSIS, EMBASE, WPI Data

C. DOCUMENTS CONSIDERED TO BE RELEVANT

Category*	Citation of document, with indication, where appropriate, of the relevant passages	Relevant to claim No.
X	SHARMA P: "Identification and Validation of an Anthracycline/Cyclophosphamide-Based Chemotherapy Response Assay in Breast Cancer Mulligan JM, Hill LA, Deharo S, et al (Queen's Univ Belfast, UK; et al)J Natl Cancer Inst106:1-10, 2014", BREAST DISEASES: A YEAR BOOK QUARTERLY, MOSBY, ST. LOUIS, MO, US, vol. 25, no. 3, 19 September 2014 (2014-09-19), pages 235-237, XP029061770, ISSN: 1043-321X, DOI: 10.1016/J.BREASTDIS.2014.07.045 the whole document -/--	1-60

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

[X] See patent family annex.

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

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"&" document member of the same patent family

Date of the actual completion of the international search	Date of mailing of the international search report
6 October 2016	13/10/2016

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	Botz, Jürgen

1

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C(Continuation). DOCUMENTS CONSIDERED TO BE RELEVANT

Category*	Citation of document, with indication, where appropriate, of the relevant passages	Relevant to claim No.
X	& Jude M. Mulligan: "Identification and Validation of an Anthracycline/Cyclophosphamide-Based Chemotherapy Response Assay in Breast Cancer Supplementary Information SUPPLEMENTARY EXPERIMENTAL PROCEDURES", jnci, 19 September 2014 (2014-09-19), pages 1-235, XP055308129, Oxford Retrieved from the Internet: URL:http://jnci.oxfordjournals.org/content/106/1/djt335/suppl/DC1 [retrieved on 2016-10-06]	1-60
Y	PETER BOUWMAN ET AL: "The effects of deregulated DNA damage signalling on cancer chemotherapy response and resistance", NATURE REVIEWS. CANCER, vol. 12, no. 9, 24 August 2012 (2012-08-24), pages 587-598, XP055307814, GB ISSN: 1474-175X, DOI: 10.1038/nrc3342 the whole document	1-60
X	WO 2012/037378 A2 (ALMAC DIAGNOSTICS LTD [GB]; O'DONNELL JUDE [GB]; BYLESJO MAX [GB]; MCD) 22 March 2012 (2012-03-22) the whole document	1-31, 34-41, 43-60
Y	Anonymous: "78398-102", , 1 January 2011 (2011-01-01), XP055307805, Retrieved from the Internet: URL:http://meetinglibrary.asco.org/print/574090 [retrieved on 2016-10-05] the whole document	1-60
A	WO 2012/153187 A2 (XENTECH [FR]; JUDDE JEAN-GABRIEL [FR]; LEGRIER MARIE-EMMANUELLE [FR];) 15 November 2012 (2012-11-15) the whole document	1-60
A	WO 2008/005281 A2 (ROSETTA INPHARMATICS LLC [US]; MERCK & CO INC [US]; DAI HONGYUE [US];) 10 January 2008 (2008-01-10) the whole document	1-60

1

INTERNATIONAL SEARCH REPORT

Information on patent family members

International application No
PCT/GB2016/052213

Patent document cited in search report	Publication date	Patent family member(s)	Publication date
WO 2012037378 A2	22-03-2012	AU 2011302004 A1	04-04-2013
		AU 2016200494 A1	07-04-2016
		CA 2811015 A1	22-03-2012
		CN 103299188 A	11-09-2013
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		US 2013042333 A1	14-02-2013
		WO 2012153187 A2	15-11-2012
WO 2008005281 A2	10-01-2008	US 2010284915 A1	11-11-2010
		WO 2008005281 A2	10-01-2008