

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)). However, to date there does not exist a diagnostic test that works across cancer diseases to accurately define a molecular subtype that demonstrates a deficiency in DNA damage repair, that can also predict sensitivity to any drug that directly or indirectly targets DNA damage repair across diseases.

What is therefore needed is a test that identifies DNA repair deficient tumors with sufficient accuracy to allow the stratification of patients into those who are likely to respond to chemotherapeutic agents that damage DNA, and those who should receive alternative therapies.

What is also needed is a molecular subtype classifier that is predictive of therapeutic responsiveness across different cancer types with sufficient accuracy.

SUMMARY OF THE INVENTION

In one aspect, the present invention refers to a method of predicting responsiveness of an individual to a DNA-damage therapeutic agent comprising

- a. measuring expression levels of at least five biomarkers in a test sample obtained from the individual, wherein the at least five biomarkers are selected from the biomarkers in Table 2B;
- b. deriving a combined test score that captures the expression levels;
- c. providing a threshold score comprising information correlating the combined test score and responsiveness;
- d. and comparing the combined test score to the threshold score; wherein responsiveness is predicted when the combined test score exceeds the threshold score.

In another aspect, the present invention refers to a method of diagnosing an individual for having a cancer associated with a DNA damage response deficiency (DDR) comprising

- a. measuring expression levels of at least five biomarkers in a test sample obtained from the individual, wherein the at least five biomarkers are selected from the biomarkers in Table 2B;
- b. deriving a combined test score that captures the expression levels;
- c. providing a threshold score comprising information correlating the combined test score and a diagnosis of the cancer;
- d. and comparing the combined test score to the threshold score; wherein the individual is diagnosed as having the cancer when the test score exceeds the threshold score.

In another aspect, the present invention refers to a method of detecting a cancer with DNA damage response deficiency related immune signalling in an individual comprising:

a. measuring expression levels of one or more biomarkers in a test sample obtained from the individual, wherein the one or more biomarkers comprise at least one biomarker 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, OR211P, EGFR, NAT1, LATS2, CYP2B6, PTPRC, PPP1R1A, and AL137218.1;

b. deriving a test score that captures the expression levels;

c. providing a threshold score comprising information correlating the test score and detection of the cancer;

d. and comparing the test score to the threshold score; wherein the cancer with DNA damage response deficiency related immune signalling is detected when the test score exceeds the threshold score.

In another aspect, the present invention refers to a method of selecting an appropriate therapy to treat a cancer with DNA damage response deficiency related immune signalling in an individual comprising:

a. measuring expression levels of one or more biomarkers in a test sample obtained from the individual, wherein the one or more biomarkers comprise at least one biomarker 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, OR211P, EGFR, NAT1, LATS2, CYP2B6, PTPRC, PPP1R1A, and AL137218.1;

b. deriving a test score that captures the expression levels;

c. providing a threshold score comprising information correlating the test score and selection of the appropriate therapy;

d. and comparing the test score to the threshold score; wherein a therapeutic agent appropriate for treatment of the individual is selected when the test score exceeds the threshold score.

In another aspect, the present invention refers to a method of selecting an appropriate therapy to treat a cancer with DNA damage response deficiency related immune signalling in an individual comprising:

a. measuring expression levels of one or more biomarkers in a test sample obtained from the individual, wherein the one or more biomarkers comprise at least one biomarker 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, OR211P, EGFR, NAT1, LATS2, CYP2B6, PTPRC, PPP1R1A, and AL137218.1;

b. deriving a test score that captures the expression levels;

c. providing a threshold score comprising information correlating the test score and selection of the appropriate therapy;

d. and comparing the test score to the threshold score; wherein a therapeutic agent that targets tumors with abnormal DNA damage response is selected when the test score exceeds the threshold score.

In another aspect, the present invention refers to a method of selecting an appropriate therapy to treat a cancer with DNA damage response deficiency related immune signalling in an individual comprising:

a. measuring expression levels of one or more biomarkers in a test sample obtained from the individual, wherein the one or more biomarkers comprise at least one biomarker 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, OR211P, EGFR, NAT1, LATS2, CYP2B6, PTPRC, PPP1R1A, and AL137218.1;

b. deriving a test score that captures the expression levels;

c. providing a threshold score comprising information correlating the test score and selection of the appropriate therapy;

d. and comparing the test score to the threshold score; wherein a therapeutic agent that inhibits expression of at least one of the biomarkers is selected when the test score exceeds the threshold score.

In another aspect, the present invention refers to the use of a therapeutic agent in the manufacture of a medicament for treating a cancer in an individual, wherein the cancer displays DNA damage response deficiency related immune signalling as determined according to the method of the present invention.

In another aspect, the present invention refers to the use of a therapeutic agent that targets tumors with abnormal DNA damage response in the manufacture of a medicament for treating a cancer in an individual, wherein the cancer displays DNA damage response deficiency related immune signalling as determined according to the method of the present invention.

In another aspect, the present invention refers to the use of a therapeutic agent that inhibits expression of at least one 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, OR211P, EGFR, NAT1, LATS2, CYP2B6, PTPRC,

PPP1R1A, and AL137218.1 in the manufacture of a medicament for treating a cancer in an individual, wherein the cancer displays DNA damage response deficiency related immune signalling as determined according to the method of the present invention.

In another aspect, the present invention refers to the use of a therapeutic agent in the manufacture of a medicament for treating a cancer in an individual, wherein the cancer displays DNA damage response deficiency related immune signalling as determined by the level of expression of one or more biomarkers 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, OR211P, EGFR, NAT1, LATS2, CYP2B6, PTPRC, PPP1R1A, and AL137218.1.

In another aspect, the present invention refers to the use of a therapeutic agent that targets tumors with abnormal DNA damage response in the manufacture of a medicament for treating a cancer in an individual, wherein the cancer displays DNA damage response deficiency related immune signalling as determined by the level of expression of one or more biomarkers 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, OR211P, EGFR, NAT1, LATS2, CYP2B6, PTPRC, PPP1R1A, and AL137218.1.

In another aspect, the present invention refers to the use of a therapeutic agent that inhibits expression of at least one 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, OR211P, EGFR, NAT1, LATS2, CYP2B6, PTPRC, PPP1R1A, and AL137218.1 in the manufacture of a medicament for treating a cancer in an individual, wherein the cancer displays DNA damage response deficiency related immune signalling as determined by the level of expression of one or more biomarkers 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, OR211P, EGFR, NAT1, LATS2, CYP2B6, PTPRC, PPP1R1A, and AL137218.1.

The invention disclosure is directed to methods of using a collection of gene product markers expressed in cancer such that when some or all of the transcripts are over or under-expressed, they identify a subtype of cancer that has a deficiency in DNA damage repair. Designation of this subtype can be considered 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 ~~invention~~disclosure also provides methods for indicating responsiveness or resistance to DNA-damage therapeutic agents. In different ~~aspects~~examples, 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, Q-PCR, 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 ~~example~~embodiment of the ~~invention~~, these genes or gene products are useful for evaluating both breast and ovarian cancer tumors.

The ~~invention~~disclosure 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 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). In specific ~~examples~~aspects—this ~~invention~~, it is useful for evaluating paclitaxel, fluorouracil, doxorubicin (Adriamycin), and cyclophosphamide (T/FAC) neoadjuvant treatment in breast cancer. In other ~~examples~~aspects ~~this invention~~, it is useful for evaluating platinum or platinum plus taxol treatment in ovarian cancer.

The present ~~invention~~disclosure 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 specific ~~embodiments~~examples, this ~~invention~~disclosure can be used to evaluate pathological complete response in breast cancer treated with FEC or FAC either alone or in the context of standard treatment, or RECIST and serum CA125 levels in ovarian cancer.

In another ~~aspect~~example, the present ~~invention~~disclosure relates to the identification of a DNA damage response deficiency (DDR^D) molecular subtype in breast and ovarian cancer. This molecular subtype can be detected by the use of two different gene classifiers – one being 40 genes in length and one being 44 genes in length. The DDR^D classifier was first defined by a classifier consisting of 53 probesets on the Almac Breast Disease Specific Array (DSATM). 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 DDR^D classifier using microarray data from independent datasets that were profiled on microarray platforms other than the Almac Breast DSATM. In order to facilitate defining the classifier at a gene level, the genes to which the Almac Breast DSATM 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 DDR^D classifier model, as this model supersedes that of the 40-gene DDR^D 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, standard breast and ovarian cancer therapeutic drug classes, including DNA damage causing agents and DNA repair targeted therapies.

In another ~~aspect~~example, the present ~~invention~~disclosure relates to kits for conventional diagnostic uses listed above such as qPCR, microarray, 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~~disclosure also provides methods for identifying DNA damage response-deficient (DDR) human tumors. It is likely that this description of examples~~invention~~ can be used to identify patients that are sensitive to and respond, or are resistant to and do not respond, to drugs that damage DNA directly, damage DNA indirectly or inhibit normal DNA damage signaling and/or repair processes.

The ~~invention~~disclosure also relates to guiding conventional treatment of patients. The ~~invention~~disclosure also relates to selecting patients for clinical trials where novel drugs of the classes that directly or indirectly affect DNA damage and/or DNA damage repair.

The present ~~invention~~disclosure 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 a 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