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(54) Title: A GENE EXPRESSION PROFILE OF BRCA-NESS THAT CORRELATES WITH RESPONSIVENESS TO CHEMOTHERAPY AND WITH OUTCOME IN CANCER PATIENTS

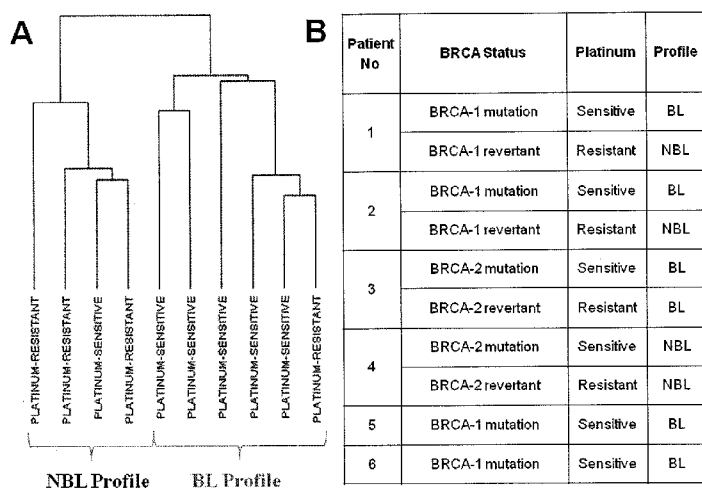


Figure 3.

(57) Abstract: Aspects of the invention provide methods and compositions for evaluating cells and tissues based on gene expression profiles to determine their sensitivity or resistance to one or more chemotherapeutic agents. A patient having a tumor or cancer can be evaluated using a gene expression profile to assist in determining the disease prognosis, selecting an appropriate therapy, and/or predicting drug resistance or sensitivity.

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This application claims the benefit under 35 U.S.C. § 119(e) of U.S. provisional application USSN 61/351,282, filed June 3, 2010, and entitled “A gene expression profile of BRCA-ness that correlates with responsiveness to chemotherapy and with outcome in cancer patients,” the entire contents of which are incorporated herein by reference.

15

In certain types of cancers, for example, certain epithelial cancers, like ovarian cancer and breast cancer, a subset of tumors exhibit a mutation in breast cancer associated gene 1 or 2 (BRCA1 or BRCA2). Both BRCA1 and BRCA2 are involved in cellular DNA repair and, without wishing to be bound by theory, it is believed that impairment of either gene's function is causally related to tumorigenesis. Importantly, impairment of DNA repair capabilities increases a tumor's sensitivity to chemotherapeutic agents, which is reflected with a generally favorable prognosis for chemotherapeutic treatment of cancers in subjects with BRCA1 or BRCA2 mutations. In contrast, sporadic cancers of the same types develop without BRCA mutations. Until now, there has been no reliable technique to predict whether such sporadic cancers will or will not respond favorably to a chemotherapeutic approach.

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In some embodiments, aspects of this invention relate to the surprising discovery that a subset of sporadic epithelial ovarian tumors sensitive to chemotherapeutic compounds can be identified based on the gene expression data obtained from these tumors resembling certain similarities to gene expression data obtained from tumors having a BRCA1 or a BRCA2 mutation (regardless of the BRCA1/2 genotype of the tumors). In some embodiments, aspects of this invention relate to methods for the classification of a tumor, for example, an epithelial ovarian tumor, as BRCA-like or non-BRCA-like based on expression profiles obtained from the tumors and in accordance with a predictive algorithm, for example, a diagonal

linear discriminant predictor. In some embodiments, aspects of this invention relate to methods of identifying genes that are informative, either alone or in combination, as marker genes for the classification of epithelial ovarian tumors as BRCA-like or non-BRCA-like. In some embodiments, aspects of this invention relate to kits and reagents useful in the classification of epithelial ovarian tumors as BRCA-like or non-BRCA-like based on expression data. In some embodiments, aspects of this invention relate to predictive classifiers useful in the classification of expression profiles as BRCA-like or non-BRCA-like.

BRIEF DESCRIPTION OF THE DRAWINGS

FIG. 1 illustrates an embodiment of the development of a BRCAness gene expression profile;

FIG. 2 shows an expression plot of 60 genes that comprise an example of a BRCAness profile;

FIG. 3 illustrates an example of a BRCAness profile distinguishing between platinum sensitive and resistant tumor biopsy specimens (n=10) from 6 patients with known BRCA germline mutation status;

FIG. 4 illustrates an example of a BRCAness profile distinguishing between RAD51 foci forming CAPAN-1 clones (n=12) and predicting PARP inhibitor sensitivity of different clones (n=4);

FIG. 5 shows an association of a BRCAness profile with DFS and OS in a combined patient cohort (n=70);

FIG. 6 shows an association of a BRCAness profile with DFS and OS in a sequenced patient cohort (n=35); and,

FIG. 7 shows an association of a BRCAness profile with DFS and OS in a non-sequenced patient cohort (n=35).

DETAILED DESCRIPTION

In some embodiments, aspects of the invention provide compositions, methods, and devices for predicting whether a tumor will be either responsive or resistant to treatment with one or more chemotherapeutic agents. In some embodiments, aspects of the invention involve evaluating the expression profiles of one or more identified genes to predict the response of cells to chemotherapeutic agents. Embodiments of the invention may be useful to assist in the diagnosis,

prognosis, and/or therapy selection for patients that have or are suspected of having cancer. As described in more detail herein, embodiments of the invention may be useful for evaluating and/or predicting the responsiveness of patients to certain chemotherapeutic agents regardless of the BRCA 1 or 2 status of the patient.

5 Accurate prediction of tumor sensitivity to chemotherapeutic agents is a cornerstone in the decision for or against the administration of chemotherapy to a subject diagnosed with a tumor. In many cancers, for example, in sporadic epithelial ovarian tumors, such a prediction cannot be made reliably to date, and the decision for or against chemotherapy is mainly based on patient health status and the expected side
10 effects of the therapeutic regimen instead of the sensitivity of the tumor itself. The lack of methods for accurately predicting a chemotherapeutic outcome results in many instances of either i) inappropriate administration of chemotherapy to subjects carrying chemotherapy-resistant (chemoresistant) tumors or ii) withholding of chemotherapy treatment from patients carrying chemotherapy-sensitive
15 (chemosensitive) tumors. For example, in the absence of information about the sensitivity or resistance to chemotherapy, a decision to withhold treatment from a patient may be made if the patient has a marginal health status for chemotherapy, particularly if the overall success rate of such a therapy is low for the patient's cancer type. Some embodiments of this invention address this unmet clinical need and
20 provide methods for accurate prediction of tumor sensitivity to chemotherapeutic compounds based on gene expression data obtained from the tumor.

 Accordingly, some methods provided herein allow for customizing a therapeutic approach not only to a specific subject, but also to a specific tumor in question, and are useful to increase the success rate of chemotherapeutic interventions
25 and/or to lower the burden of medication side effects on patients carrying chemoresistant tumors.

 One major challenge in predicting tumor response to chemotherapeutic intervention from tumor expression data is the identification of informative genes, e.g., of genes the expression level of which is correlated with tumor chemosensitivity
30 or chemoresistance. Informative genes have not been identified for many clinically relevant diagnostic distinctions, for example for the distinction between chemosensitive and chemoresistant tumors, for example, sporadic tumors (e.g., sporadic epithelial ovarian tumors). In many cases, the identification of informative genes is hampered by non-uniform expression of such genes within a subgroup of

tumors, e.g., within chemosensitive tumors, and/or because of the small differences in gene expression between tumor groups, e.g., between chemoresistant and chemosensitive tumors.

Some embodiments of this invention provide methods for the identification of informative genes for tumor classification as chemosensitive or chemoresistant based on gene expression data. Some embodiments of this invention relate to the discovery of an expression profile in sporadic tumors that correlates with tumor sensitivity to chemotherapeutic agents, for example, platinum compounds or PARP inhibitors, and/or patient outcome. Some embodiments of this invention, accordingly, provide the identities of informative genes for a classification of a tumor, for example, a sporadic epithelial tumor (e.g., ovarian tumor), as chemosensitive or chemoresistant. In some embodiments, a tumor is classified as BRCA-like or non-BRCA-like based on gene expression data, and chemosensitivity or –resistance is determined based on a tumor’s BRCAness. Examples of informative genes for the classification of epithelial ovarian tumors as BRCA-like and non-BRCA-like are shown in **Table 1**.

Some embodiments of this invention provide methods of using a predictive algorithm incorporating informative gene identities for accurate classification of tumors as BRCA-like or non-BRCA-like and/or the classification of tumors as chemosensitive or chemoresistant based on tumor expression data.

In some embodiments, tumor classification strategies are provided herein that allow the prediction of a tumor’s responsiveness to chemotherapy and/or of patient outcome. Some embodiments relate to the identification and/or definition of gene expression profiles for the classification of a tumor as BRCA-like or non-BRCA-like. In some embodiments, methods are provided to assign tumors, for example, sporadic epithelial tumors (e.g., ovarian tumors), to a class exhibiting a BRCA-like expression profile or a class exhibiting a non-BRCA-like expression profile. In some embodiments, a BRCA-like expression profile is correlated with sensitivity to chemotherapeutic agents, for example, platinum compounds or PARP inhibitors. In some embodiments, a non-BRCA-like expression profile is not correlated with chemosensitivity. In some embodiments, a non-BRCA-like expression profile is correlated with chemoresistance.

Some embodiments relate to the identification of informative genes, the differential expression of which is indicative for a given tumor being chemosensitive or chemoresistant. Some aspects relate to the building of predictive algorithms using

the identified informative genes and the use of the resulting predictors in the classification of tumors as chemosensitive or chemoresistant. In some embodiments, specific predictors and methods for their use in classifying sporadic tumors based on expression data as sensitive or resistant to chemotherapeutic agents are provided that
5 allow for a distinction of BRCA-like (BL) from non-BRCA-like (NBL) tumors. In some embodiments, classification of sporadic tumors is performed with at least 94% accuracy. In some embodiments, methods and predictors are provided that allow for a distinction between platinum sensitivity and resistance in at least 80% of patient-derived tumor specimens. In some embodiments, methods and predictors are
10 provided that allow for predictions of disease-free survival and overall survival time in subjects with sporadic tumors. In some embodiments, predictors and methods are provided for the classification of tumors based on expression profile data as part of a multivariate disease analysis, for example, with respect to one or more of patient age and health status, and tumor stage, grade, histology, and debulking status.

15 In some embodiments, a subject that does not have a mutation or defect in either the BRCA1 or BRCA2 gene may be classified as either responsive or resistant according to methods described herein. In some embodiments, a subject (e.g., a cancer patient) may first be screened to determine whether a BRCA 1 or 2 mutation or defect is present. If the answer is yes, then the subject is likely to be responsive to
20 treatment with one or more chemotherapeutic agents and may be identified as such, and/or prescribed for such treatment, and/or treated (e.g., by administration) with one or more chemotherapeutic agents. In some embodiments, such subjects may not need to be evaluated for a BL or NBL expression profile since they are already likely to be sensitive based on their BRCA status. However, in some embodiments, a subject that
25 is BRCA 1 or 2 defective still may be evaluated to determine whether a BL or NBL expression profile is present. In some embodiments, patients that have a BRCA 1 or 2 genetic defect may not be candidates for certain chemotherapeutic treatments if they have a NBL gene expression profile as described herein.

In some embodiments, a subject that is identified as not having a mutation or
30 defect in a BRCA gene may be evaluated for a BL or NBL expression profile. If the subject has a BL expression profile, the subject may be identified and/or treated as responsive to certain chemotherapeutic agents. However, if the subject has a NBL expression profile, the subject may be identified and/or treated as non-responsive to certain chemotherapeutic agents.

BRCA proteins BRCA-1 and BRCA-2 are involved in the process of homologous recombination, which mediates repair of double stranded DNA breaks¹. Cancer patients, for example, ovarian cancer patients, with germline mutations in either BRCA-1 or BRCA-2 genes exhibit an impaired ability to repair double stranded DNA breaks via homologous recombination, which may partly explain their heightened sensitivity to platinum and their more favorable survival compared to patients not carrying such mutations²⁻⁴. Furthermore, in the setting of defective homologous recombination, it has been shown that inhibition of a second DNA repair pathway such as base excision repair (BER) is a lethal event⁵⁻⁷. Based upon this observation, there has been great interest in developing inhibitors of the BER pathway for use as possible therapeutic agents in ovarian cancer patients with germline BRCA-1 or -2 mutation^{8,9}. Drugs that target BER typically inhibit poly-ADP ribose polymerase (PARP), an enzyme critical to BER, and have already shown promising activity in patients with recurrent ovarian cancer who harbor germline BRCA-1 or -2 mutation^{10,11}.

The promise of PARP inhibitors in the management of epithelial ovarian cancer (EOC) is tempered by the fact that only approximately 10% of such patients have a germline mutation in BRCA-1 or -2¹²⁻¹⁴. At first glance, this might imply that 90% of patients with this highly lethal disease would not benefit from this novel class of drugs. However, it has been speculated that a subset of sporadic EOCs may harbor abnormalities in the homologous recombination pathway that could be associated with improved response rate and survival after treatment with platinum compounds, in the absence of germline BRCA-1 or -2 mutation^{4,15}. This "BRCAness" phenotype may be due, in part, to defective homologous recombination related to several mechanisms including epigenetic hypermethylation of the BRCA-1 promoter¹⁶⁻¹⁹, somatic mutation of BRCA-1 or -2^{18,20-22}, or loss of function mutations in other homologous recombination pathway genes^{23,24}. At present, however, it has not been possible to reliably identify such patients on the basis of molecular (e.g., transcript- or protein-based) biomarkers, and the concept of BRCAness for patients with the sporadic form of the disease has remained elusive.

Some aspects of the invention are based on the discovery of a plurality of informative genes that are differentially expressed in chemosensitive and chemoresistant tumors. For example, the identity of informative genes for the classification of tumor as BRCA-like and non-BRCA-like is provided in **Table 1**.

Table 1. Identity of informative genes for the classification of tumors (e.g., epithelial ovarian tumors) as BRCA-like or non-BRCA-like

	#	Probe set ID	GenBank accession #	Gene Symbol	Unigene Cluster
	1	208668_x_at	BC003689	HMGN2	Hs.181163
5	2	204664_at	NM_001632	ALPP	Hs.284255
	3	207113_s_at	NM_000594	TNF	Hs.241570
	4	201311_s_at	AL515318	SH3BGR1	Hs.108029
	5	205075_at	NM_000934	SERPINF2	Hs.159509
	6	219606_at	NM_016018	PHF20L1	Hs.304362
10	7	202365_at	BC004815	UNC119B	Hs.127610
	8	204029_at	NM_001408	CELSR2	Hs.57652
	9	204531_s_at	NM_007295	BRCA1	Hs.194143
	10	204826_at	NM_001761	CCNF	Hs.1973
	11	214656_x_at	BE790157	MYO1C	Hs.286226
15	12	203817_at	W93728	GUCY1B3	Hs.77890
	13	204956_at	NM_002451	MTAP	Hs.193268
	14	210027_s_at	M80261	APEX1	Hs.73722
	15	206832_s_at	NM_004186	SEMA3F	Hs.32981
	16	207175_at	NM_004797	ADIPOQ	Hs.80485
20	17	209688_s_at	BC005078	CCDC93	Hs.107845
	18	201695_s_at	NM_000270	NP	Hs.75514
	19	205057_s_at	AI762782	IDUA	Hs.89560
	20	212932_at	AK022494	RAB3GAP1	Hs.306327
	21	204534_at	NM_000638	VTN	Hs.2257
25	22	201808_s_at	BE732652	ENG	Hs.76753
	23	204466_s_at	BG260394	SNCA	Hs.271771
	24	201403_s_at	NM_004528	MGST3	Hs.191734
	25	208037_s_at	NM_007164	MADCAM1	Hs.102598
	26	202701_at	NM_006129	BMP1	Hs.1274
30	27	212224_at	NM_000689	ALDH1A1	Hs.76392
	28	210809_s_at	D13665	POSTN	Hs.136348
	29	203892_at	NM_006103	WFDC2	Hs.2719
	30	204259_at	NM_002423	MMP7	Hs.2256
	31	200665_s_at	NM_003118	SPARC	Hs.111779
35	32	221004_s_at	NM_030926	ITM2C	Hs.111577
	33	205974_at	AI168371	HOXD1	Hs.83465
	34	204456_s_at	AW611727	GAS1	Hs.65029
	35	205479_s_at	NM_002658	PLAU	Hs.77274
	36	206976_s_at	NM_006644	HSPH1	Hs.36927
40	37	205849_s_at	NM_006294	UQCRB	Hs.131255
	38	207008_at	NM_001557	IL8RB	Hs.846
	39	207526_s_at	NM_003856	IL1RL1	Hs.66
	40	200650_s_at	NM_005566	LDHA	Hs.2795
	41	201858_s_at	J03223	SRGN	Hs.1908
45	42	203213_at	AL524035	CDC2	Hs.334562
	43	206605_at	NM_006025	P11	Hs.997
	44	201111_at	AF053641	CSE1L	Hs.90073
	45	204103_at	NM_002984	CCL4	Hs.75703
	46	210512_s_at	AF022375	VEGFA	Hs.73793

	47	202988_s_at	NM_002922	RGS1	Hs.75256
	48	209492_x_at	BC003679	ATP5I	Hs.85539
	49	201785_at	NM_002933	RNASE1	Hs.78224
	50	205789_at	NM_001766	CD1D	Hs.1799
5	51	202543_s_at	BC005359	GMFB	Hs.151413
	52	204927_at	NM_003475	RASSF7	Hs.72925
	53	203745_at	AI801013	HCCS	Hs.211571
	54	204086_at	NM_006115	PRAME	Hs.30743
	55	203764_at	NM_014750	DLG7	Hs.77695
10	56	201095_at	NM_004394	DAP	Hs.75189
	57	208658_at	BC000425	PDIA4	Hs.93659
	58	201693_s_at	AV733950	EGR1	Hs.326035
	59	201641_at	NM_004335	BST2	Hs.118110
	60	205365_at	AA527340	HOXB6	Hs.98428
15	61	201212_at	D55696	LGMN	Hs.18069
	62	200711_s_at	NM_003197	SKP1A	Hs.171626
	63	207442_at	NM_000759	CSF3	Hs.2233
	64	201501_s_at	NM_002092	GRSF1	Hs.309763
	65	203560_at	NM_003878	GGH	Hs.78619
20	66	208729_x_at	D83043	HLA-B	Hs.77961
	67	202812_at	NM_000152	GAA	Hs.1437
	68	201485_s_at	BC004892	RCN2	Hs.79088
	69	206589_at	NM_005263	GFI1	Hs.73172
	70	213932_x_at	AI923492	HLA-A	Hs.181244
25	71	201135_at	NM_004092	ECHS1	Hs.76394
	72	202269_x_at	BC002666	GBP1	Hs.62661
	73	205400_at	NM_000377	WAS	Hs.2157
	74	221931_s_at	AV701173	SEH1L	Hs.301048
	75	200749_at	BF112006	RAN	Hs.10842
30	76	218676_s_at	NM_021213	PCTP	Hs.285218
	77	213677_s_at	BG434893	PMS1	Hs.111749
	78	201099_at	AA824386	USP9X	Hs.77578
	79	208716_s_at	AB020980	TMCO1	Hs.93832
	80	201179_s_at	J03005	GNAI3	Hs.73799
35	81	200607_s_at	BG289967	RAD21	Hs.81848
	82	207569_at	NM_002944	ROS1	Hs.1041
	83	201209_at	NM_004964	HDAC1	Hs.88556
	84	200726_at	NM_002710	PPP1CC	Hs.79081
	85	203523_at	NM_002339	LSP1	Hs.56729
40	86	211178_s_at	AF038602	PSTPIP1	Hs.129758
	87	204168_at	NM_002413	MGST2	Hs.81874
	88	201289_at	NM_001554	CYR61	Hs.8867
	89	200046_at	NM_001344	DAD1	Hs.82890
	90	209149_s_at	BE899402	TM9SF1	Hs.91586
45	91	203455_s_at	NM_002970	SAT1	Hs.28491
	92	203964_at	NM_004688	NMI	Hs.54483
	93	201589_at	D80000	SMC1A	Hs.211602

Some embodiments are based on the discovery that tumor expression profiles including expression data for one or more informative genes, allow for accurate diagnostic classification of the tumor as chemosensitive or chemoresistant. Some embodiments relate to diagnostic methods of classifying a tumor as sensitive to a
5 chemotherapeutic compound (chemosensitive) or resistant to a chemotherapeutic compound (chemoresistant) based on gene expression data obtained from the tumor. Some embodiments relate to methods of predicting chemosensitivity or chemoresistance of a tumor based on tumor classification. Some embodiments relate to methods of administering chemotherapy to a subject carrying a tumor based on the
10 tumor's classification as chemosensitive. Some embodiments relate to methods of withholding administration from a subject carrying a tumor based on the tumor being classified as chemoresistant.

The term "tumor" as used herein, refers to a neoplastic cell growth, including benign, malignant, pre-cancerous and cancerous cell neoplasms. A tumor may be a
15 liquid tumor, for example, a leukemic tumor, or a solid tumor, for example, an ovarian epithelial tumor, a breast tumor, a colon tumor, a gastric tumor, a prostate tumor, a pancreatic tumor, a lung tumor, a liver tumor, a brain tumor, or a kidney tumor. In some embodiments, the tumor is an epithelial tumor. In some embodiments, the tumor is a tumor harboring a cell with a defect in homologous
20 recombination. In some embodiments, the tumor may be the manifestation of a cancer, for example, blood cancer, ovarian epithelial cancer, breast cancer, colon cancer, gastric cancer, prostate cancer, pancreatic cancer, lung cancer, liver cancer, brain cancer, or kidney cancer.

In some embodiments, the tumor being classified according to methods
25 provided herein is a primary tumor. In some embodiments, the tumor being classified according to methods provided herein is a secondary, metastatic, or recurrent tumor.

The term "subject", as used herein, refers to an individual that may be, but is not limited to, a human, a non-human mammal, for example, a mouse, rat, cow, sheep, cat, dog, or goat.

30 In some embodiments, a method for the diagnostic classification of a tumor as chemoresistant or chemosensitive is provided. In some embodiments, the method includes obtaining an expression profile of the tumor. An expression profile can be obtained from a cell or a tissue from a tumor, for example, from a tumor biopsy. The term "expression profile", as used herein, refers to a dataset containing gene

expression data from a cell or tissue. In some embodiments, the expression profile may consist of a single data point, for example, a quantitative or semi-quantitative value of expression of a single gene, for example, reflective of the signal obtained from a quantitative or semi-quantitative assay detecting the abundance of a gene product (e.g., a protein or a nucleic acid transcript). Suitable assays for the detection of gene expression products are well known to those of skill in the art and include, for example, western blots, ELISA, RT-PCR (e.g. end-point RT-PCR, real-time PCR, or qPCR), protein or nucleic acid microarray, and massive parallel sequencing assays. However, any suitable assay may be used based on hybridization, specific binding (e.g., antibody binding), or any other technique, as aspects of the invention are not limited in this respect. In some embodiments, an expression profile may contain a plurality of gene expression data points, for example, quantitative or semi-quantitative values of expression of two or more genes. In some embodiments, the expression profile may comprehensively cover the whole transcriptome or proteome of a given cell, tissue, or organism. Whole-transcriptome or –proteome microarrays, containing probes for the detection of substantially all transcript or protein sequences known to those of skill in the art to be transcribed or translated from a cell’s genome, e.g., for all sequences in the transcriptome of the respective organism the cell originates from, are well known to those of skill in the art and such microarrays are commercially available for various species including human. Methods for the generation of expression profiles are well known to those in the art and include, for example, western blot, northern blot, reverse northern blot, RT-PCR (e.g. endpoint, real time, or qPCR), microarray (for either protein or transcript detection) (for exemplary detection methods, see, e.g., Sambrook et al., *Molecular Cloning: A Laboratory Manual, Third Edition (3 Volume Set)*, Cold Spring Harbor Laboratory Press; 3rd edition (January 15, 2001), ISBN-10: 0879695773; Robert Grützmann (Editor), Christian Pilarsky (Editor), *Cancer Gene Profiling: Methods and Protocols (Methods in Molecular Biology)*, Humana Press; 1st edition (November 6, 2009), ISBN-10: 1934115762, both incorporated herein by reference for disclosure of gene product detection and expression profiling methods).

Further, methods to generate comprehensive transcript expression profiles from a given cell or tissue that measure the abundance of all transcripts expressed by the cell or tissue, are well known in the art and include, for example, whole-transcriptome or whole proteome microarrays and massive parallel sequencing assays

(e.g., 454 sequencing or Solexa/Illumina sequencing (see, e.g., Robert Grützmann (Editor), Christian Pilarsky (Editor), *Cancer Gene Profiling: Methods and Protocols* (*Methods in Molecular Biology*), Humana Press; 1st edition (November 6, 2009), ISBN-10: 1934115762, both incorporated herein by reference for disclosure of whole
 5 transcriptome expression profiling methods).

In some embodiments, a quantitative expression value is a value reflecting the abundance of a gene transcript in the starting sample, for example, a tumor cell or tissue sample. In some embodiments, a semi-quantitative expression value is a value reflecting the abundance of a gene transcript in the starting sample in relation to a
 10 control or reference quantity. In some embodiments, a semi-quantitative value may be a non-numeric indication of gene regulation (e.g., “up”, “down”, “+”, “+ +”, “+ + +”, “-“, “- -“, or “- - -“). In some embodiments, a semi-quantitative expression value may give a numeric dimension of gene regulation (e.g., “1.5-fold upregulated”, “2.456”, “0.32” or “-1.5”). Methods of calculating semi-quantitative expression
 15 values are well known to those in the art. Appropriate control or reference quantities for the generation of semi-quantitative expression values are well known to those in the art and include, for example, expression values of housekeeping genes (e.g., beta-actin or GAPDH), external controls (e.g., spiked in RNA or DNA controls not usually expressed in the cell to be analyzed), overall expression values (e.g., all expression
 20 values obtained from a cell added together), or historic or empiric values.

In some embodiments, an expression profile used for class prediction of a tumor includes an expression value related to an informative marker gene for the classification of the tumor as chemoresistant or chemosensitive. In some
 25 embodiments, the informative gene is a gene identified to be informative by methods provided herein. In some embodiments, the informative gene is a gene selected from the genes in any of Tables 1-4. In some embodiments, the expression profile includes expression values for a subgroup of 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, 40, 41, 42, 43, 44, 45, 46, 47, 48, 49, 50, or more genes of the genes in any of Tables 1- 4.
 30 In some embodiments, the expression profile includes expression values for a subgroup of at least 2, at least 3, at least 4, at least 5, at least 6, at least 7, at least 8, at least 9, at least 10, at least 11, at least 12, at least 13, at least 14, at least 15, at least 20, at least 25, at least 30, at least 35, at least 40, at least 45, at least 50, at least 55, or

at least 60 genes of the genes in any of Tables 1-4. In some embodiments, the expression level of all or fewer of the genes in any of Tables 1-4 are evaluated.

It should be appreciated that the expression level of one or more genes of interest may be evaluated or determined in any suitable biological sample. In some
5 embodiments, a biopsy of a tumor may be obtained and an expression profile may be obtained from a biopsy cell or tissue. In some embodiments, one or more circulating cells (e.g., one or more circulating tumor cells) may be obtained and an expression profile may be obtained from the cell or cells. In some embodiments, one or more tumor cells may be obtained from ascites fluid, peripheral blood, or from
10 cerebrospinal fluid of a subject.

In some embodiments, a method of diagnostic tumor classification includes processing a tumor expression profile in accordance to a predictive algorithm, for example, a classifier or predictor. Bioinformatic methods of classifying tumors based on gene expression data according to a predictive algorithm are well known in the art
15 (see, for example, Dudoit S, Fridlyand J, and Speed TP, *Comparison of discrimination methods for the classification of tumors using gene expression data*. Journal of the American Statistical Association 97:77-87, 2002, section 2, “discrimination methods”, pages 78-80, incorporated herein by reference for disclosure of discrimination methods based on expression profile data). In general, a
20 predictor can be built from expression data obtained from tumors known to be either chemosensitive or chemoresistant according to methods provided herein or well known to those of skill in the art. Generally, a predictor will include a list of genes identified as informative for the class distinction based on a comparison of expression profiles of tumors of both classes. In some embodiments, a predictive algorithm will
25 analyze an expression profile obtained from a tumor to be classified by filtering the profile for expression values of informative genes, and calculating a class call (e.g., BRCA-like/chemosensitive or non-BRCA-like/chemoresistant) for each informative gene based on the gene’s expression value. For example, if an expression profile obtained from a tumor indicates a downregulation of the expression of an informative
30 gene in the tumor tissue as compared to a reference or control value, and a downregulation of that gene has been determined to be correlated with a BRCA-like/chemosensitive phenotype, then the predictive algorithm would output the respective class call (e.g., BRCA-like or chemosensitive) for this specific informative gene.

Some embodiments of this invention provide a predictor for the classification of a tumor, for example, an epithelial ovarian tumor, as chemosensitive or chemoresistant, or chemosensitive or not chemosensitive, or BRCA-like or non-BRCA-like. Exemplary predictors for tumor classification as BRCA-like and non-

5 BRCA-like are given in Tables 2 and 3.

Table 2. 60-gene predictor for classification of tumors (e.g., epithelial ovarian tumors) as BRCA-like and non-BRCA-like.

10	Gene Symbol	Gene Name
	HMGN2	high-mobility group nucleosomal binding domain 2
	ALPP	alkaline phosphatase, placental (Regan isozyme)
	TNF	tumor necrosis factor (TNF superfamily, member 2)
	SH3BGRL	SH3 domain binding glutamic acid-rich protein like
15	SERPINF2	serpin peptidase inhibitor, clade F (alpha-2 antiplasmin, pigment epithelium derived factor), member 2
	SAT1	spermidine/spermine N1-acetyltransferase 1
	NMI	N-myc (and STAT) interactor
	SMC1A	structural maintenance of chromosomes 1A
20	UNC119B	unc-119 homolog B (C. elegans)
	TM9SF1	transmembrane 9 superfamily member 1
	DAD1	defender against cell death 1
	CYR61	cysteine-rich, angiogenic inducer, 61
	MGST2	microsomal glutathione S-transferase 2
25	PSTPIP1	proline-serine-threonine phosphatase interacting protein 1
	PPP1CC	protein phosphatase 1, catalytic subunit, gamma isoform
	HDAC1	histone deacetylase 1
	ROS1	v-ros UR2 sarcoma virus oncogene homolog 1 (avian)
	GUCY1B3	guanylate cyclase 1, soluble, beta 3
30	MTAP	methylthioadenosine phosphorylase
	APEX1	APEX nuclease (multifunctional DNA repair enzyme) 1
	SEMA3F	sema domain, immunoglobulin domain (Ig), short basic domain, secreted, (semaphorin) 3F
	RAD21	RAD21 homolog (S. pombe)
35	GNAI3	guanine nucleotide binding protein (G protein), alpha inhibiting activity polypeptide 3
	CCDC93	coiled-coil domain containing 93
	USP9X	ubiquitin specific peptidase 9, X-linked
	PMS1	PMS1 postmeiotic segregation increased 1 (S. cerevisiae)
40	PCTP	phosphatidylcholine transfer protein
	RAN	RAN, member RAS oncogene family
	IDUA	iduronidase, alpha-L-
	SEH1L	SEH1-like (S. cerevisiae)
	WAS	Wiskott-Aldrich syndrome (eczema-thrombocytopenia)
45	GBP1	guanylate binding protein 1, interferon-inducible, 67kDa
	VTN	vitronectin
	ECHS1	enoyl Coenzyme A hydratase, short chain, 1, mitochondrial
	HLA-A	major histocompatibility complex, class I, A

	GFI1	growth factor independent 1
	RCN2	reticulocalbin 2, EF-hand calcium binding domain
	ENG	endoglin (Osler-Rendu-Weber syndrome 1)
	SNCA	synuclein, alpha (non A4 component of amyloid precursor)
5	MGST3	microsomal glutathione S-transferase 3
	HLA-B	major histocompatibility complex, class I, B
	GGH	gamma-glutamyl hydrolase (conjugase, folylpolyglutammaglutamyl hydrolase)
	MADCAM1	mucosal vascular addressin cell adhesion molecule 1
10	SKP1A	S-phase kinase-associated protein 1A (p19A)
	LGMN	legumain
	BST2	bone marrow stromal cell antigen 2
	PDIA4	protein disulfide isomerase family A, member 4
	PRAME	preferentially expressed antigen in melanoma
15	CD1D	CD1d molecule
	RGS1	regulator of G-protein signaling 1
	VEGFA	vascular endothelial growth factor A
	CCL4	chemokine (C-C motif) ligand 4
	P11	26 serine protease
20	CDC2	cell division cycle 2, G1 to S and G2 to M
	SRGN	serglycin
	LDHA	lactate dehydrogenase A
	SPARC	secreted protein, acidic, cysteine-rich (osteonectin)
	MMP7	matrix metalloproteinase 7 (matrilysin, uterine)
25	WFDC2	WAP four-disulfide core domain 2
	POSTN	periostin, osteoblast specific factor

Table 3. 30-gene predictor for informative genes in core classifier

#	Symbol	Gene Name	Up in BL (Fold)	Up in NBL (Fold)
1	CD1D	CD1d molecule		1.59
2	GFI1	growth factor independent 1 transcription repressor		1.1
3	SNCA	synuclein, alpha (non A4 component of amyloid precursor)		1.3
4	CCDC93	coiled-coil domain containing 93		1.21
5	RGS1	regulator of G-protein signaling 1		1.57
6	CCL4	chemokine (C-C motif) ligand 4		1.3
7	P11	26 serine protease		1.09
8	LGMN	Legumain		1.16
9	WAS	Wiskott-Aldrich syndrome (eczema-thrombocytopenia)		1.06
10	CYR61	cysteine-rich, angiogenic inducer, 61		1.22
11	NMI	N-myc (and STAT) interactor	1.13	
12	TNF	tumor necrosis factor (TNF superfamily, member 2)	1.08	
13	SH3BGRL	SH3 domain binding glutamic acid-rich protein like	1.12	
14	GBP1	guanylate binding protein 1, interferon-inducible, 67kDa	1.33	
15	VEGFA	vascular endothelial growth factor A	1.3	

16	BST2	bone marrow stromal cell antigen 2	1.45	
17	SEMA3F	sema domain, immunoglobulin domain (Ig), short basic domain, secreted, (semaphorin) 3F	1.22	
18	WFDC2	WAP four-disulfide core domain 2	1.63	
19	MMP7	matrix metalloproteinase 7 (matrilysin, uterine)	2.17	
20	APEX1	APEX nuclease (multifunctional DNA repair enzyme) 1	1.24	
21	LDHA	lactate dehydrogenase A	1.28	
22	GGH	gamma-glutamyl hydrolase (conjugase, folypolygammaglutamyl hydrolase)	1.51	
23	IDUA	iduronidase, alpha-L-	1.24	
24	SMC1A	structural maintenance of chromosomes 1A	1.25	
25	TM9SF1	transmembrane 9 superfamily member 1	1.37	
26	DAD1	defender against cell death 1	1.35	
27	PPP1CC	protein phosphatase 1, catalytic subunit, gamma isoform	1.17	
28	GNAI3	guanine nucleotide binding protein (G protein), alpha inhibiting activity polypeptide 3	1.47	
29	ROS1	c-ros oncogene 1 , receptor tyrosine kinase	1.34	
30	PDIA4	protein disulfide isomerase family A, member 4	1.73	

In some embodiments, a predictor for the classification of a tumor as chemosensitive or chemoresistant, or chemosensitive or not chemosensitive, or BRCA-like or non-BRCA-like, comprises 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, 40, 41, 42, 43, 44, 45, 46, 47, 48, 49, 50, 51, 52, 53, 54, 55, 56, 57, 58, 59, 60, or more genes of the genes listed in any of Tables 1-3. In some embodiments, a predictor for the classification of a tumor, for example, an epithelial ovarian tumor, as chemosensitive or chemoresistant, or chemosensitive or not chemosensitive, or BRCA-like or non-BRCA-like, essentially consists of a subgroup of 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, 40, 41, 42, 43, 44, 45, 46, 47, 48, 49, 50, 51, 52, 53, 54, 55, 56, 57, 58, 59, 60, or more genes of the genes listed in any of Tables 1-3. In some embodiments, a predictor for the classification of a tumor, for example, an epithelial ovarian tumor, as chemosensitive or chemoresistant, or chemosensitive or not chemosensitive, or BRCA-like or non-BRCA-like, consists of a subgroup of 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, 40, 41, 42, 43, 44, 45, 46, 47, 48, 49, 50, 51, 52, 53, 54, 55, 56, 57, 58, 59, 60, or more genes of the genes listed in any of Tables 1-3.

In some embodiments, a predictor for the classification of a tumor, for example, an epithelial ovarian tumor, as chemosensitive or chemoresistant, or

chemosensitive or not chemosensitive, or BRCA-like or non-BRCA-like, comprises at least 2, at least 3, at least 4, at least 5, at least 6, at least 7, at least 8, at least 9, at least 10, at least 11, at least 12, at least 13, at least 14, at least 15, at least 20, at least 25, at least 30, at least 35, at least 40, at least 45, at least 50, at least 55, or at least 60 genes of the genes in any of Tables 1-3. In some embodiments, a predictor for the classification of a tumor, for example, an epithelial ovarian tumor, as chemosensitive or chemoresistant, or chemosensitive or not chemosensitive, or BRCA-like or non-BRCA-like, essentially consists of up to 2, up to 3, up to 4, up to 5, up to 6, up to 7, up to 8, up to 9, up to 10, up to 11, up to 12, up to 13, up to 14, up to 15, up to 20, up to 25, up to 30, up to 35, up to 40, up to 45, up to 50, up to 55, or up to 60 genes of the genes in any of Tables 1-3.

In some embodiments, a predictor for the classification of a tumor, for example, an epithelial ovarian tumor, as chemosensitive or chemoresistant, or chemosensitive or not chemosensitive, or BRCA-like or non-BRCA-like, comprises, essentially consists of, or consists of one or more genes listed in any of Tables 1-3 that are upregulated in BRCA-like tumors, and/or of 1 or more genes listed in any of Tables 1-3 that are downregulated in BRCA-like tumors, or a combination thereof.

In some embodiments, a predictive algorithm, as provided herein, classifies a tumor by calculating the sum of all class calls for all informative genes in the predictor and assigning the tumor to the class that received the most calls based on the expression data for these informative genes. In some embodiments, the calls of the informative genes included in the predictor are weighed by the predictive algorithm, for example, by assigning more weight to a call of an informative gene differential expression of which strongly correlates with tumor chemosensitivity or chemoresistance than to a call of an informative gene that shows only weak correlation with either phenotype. Statistical methods of determining correlation between differential expression and observed phenotype are well known in the art. In some embodiments employing a weighted voting scheme, a prediction may be based by the inner sum of the weights (w_i) and expression of the genes (x_i), wherein the weight is the weight assigned to each gene, for example, based on correlation of differential expression of the gene with the class distinction, and expression of the gene is expressed as a quantitative or numeric semi-quantitative value. A class call may be made, in some embodiments, based on the inner sum $\sum_i w_i x_i$ for all or a

subset of genes in a predictor exceeding or being lower than a certain threshold level. Such threshold levels can be calculated from empirical data by methods well known to those of skill in the art. In some embodiments, a single threshold level is determinative of a tumor being assigned to one or the other class in a binary class distinction. In some embodiments, two threshold levels are determined, and a tumor is assigned to one class, for example, BRCA-like, if $\sum_i w_i x_i$ exceeds the higher threshold level, and to the other class, for example, non-BRCA-like, if $\sum_i w_i x_i$ is less than the lower threshold level. In some embodiments, if $\sum_i w_i x_i$ falls between both threshold levels, the tumor may not be assigned a class. In some embodiments, using two threshold levels may result in a predictive algorithm only assigning tumors to a respective class that can be classified with high confidence.

An exemplary 30-gene weighted voting predictor is provided in Table 4. In some embodiments employing the predictor described in Table 4, a tumor, for example, an ovarian epithelial tumor, is classified as BRCA-like if, for example, the inner sum is greater than about 120 ($\sum_i w_i x_i > \sim 120$), and as non-BRCA-like, if the inner sum is less than about 120.

Table 4. Weighted voting predictor for classification of tumors, for example, epithelial ovarian tumors, as BRCA-like or non-BRCA-like

#	Gene symbol	Weight	Gene name
1	GFI1	-2.7315	growth factor independent 1 transcription repressor
2	PPP1CC	2.3313	protein phosphatase 1, catalytic subunit, gamma isoform
3	ROS1	2.285	c-ros oncogene 1, receptor tyrosine kinase
4	SMC1A	1.5789	structural maintenance of chromosomes 1A
5	PDIA4	1.5138	protein disulfide isomerase family A, member 4
6	IDUA	1.4871	iduronidase, alpha-L-
7	CD1D	-1.4667	CD1d molecule
8	P11	-1.3085	26 serine protease
9	DAD1	1.213	defender against cell death 1
10	CCDC93	-1.2001	coiled-coil domain containing 93
11	APEX1	1.1607	APEX nuclease (multifunctional DNA repair enzyme) 1
12	TM9SF1	1.1594	transmembrane 9 superfamily member 1
13	LDHA	1.0736	lactate dehydrogenase A
14	WAS	-1.0483	Wiskott-Aldrich syndrome (eczema-thrombocytopenia)
15	GNAI3	1.0425	guanine nucleotide binding protein (G protein), alpha inhibiting activity polypeptide 3

	16	SEMA3F	1.0379	sema domain, immunoglobulin domain (Ig), short basic domain, secreted, (semaphorin) 3F
	17	SNCA	-0.9457	synuclein, alpha (non A4 component of amyloid precursor)
5	18	TNF	0.836	tumor necrosis factor (TNF superfamily, member 2)
	19	SH3BGRL	0.7711	SH3 domain binding glutamic acid-rich protein like
10	20	GGH	0.7583	gamma-glutamyl hydrolase (conjugase, folylpolygamma glutamyl hydrolase)
	21	LGMN	-0.548	legumain
	22	VEGFA	0.4834	vascular endothelial growth factor A
	23	CCL4	-0.4713	chemokine (C-C motif) ligand 4
	24	BST2	0.4623	bone marrow stromal cell antigen 2
15	25	RGS1	-0.4598	regulator of G-protein signaling 1
	26	WFDC2	0.4489	WAP four-disulfide core domain 2
	27	NMI	0.4457	N-myc (and STAT) interactor
	28	GBP1	0.411	guanylate binding protein 1, interferon-inducible, 67kDa
20	29	MMP7	0.3115	matrix metalloproteinase 7 (matrilysin, uterine)
	30	CYR61	-0.2718	cysteine-rich, angiogenic inducer, 61

In some embodiments, class calls for individual genes are weighed based on the level of differential expression detected in the tumor as compared to a control of reference value, for example by giving more weight to a call based on a greater observed fold change in gene expression than to a call based on a smaller fold change (e.g., giving more weight to a call based on a 1.5-fold change than to a call based on 1.1-fold change in gene expression). In some embodiments, a predictive algorithm, as provided herein, weighs class calls for individual genes based on a combination of phenotype correlation and level of differential expression. In some embodiments, a predictive algorithm as provided herein assigns a confidence level to the overall class assignment (e.g., BRCA-like/chemosensitive or non-BRCA-like/chemoresistant). Those of skill in the art will be aware of a variety of methods and algorithms to build and use a class predictor. Types of class predictors useful for the classification of tumors in BRCA-like/chemosensitive and non-BRCA-like/chemoresistant include, for example, diagonal linear discriminant predictors, compound covariate predictors, nearest centroid predictors, or support vector machines predictors. Further, a class predictor can be combined with other statistical and/or bioinformatic procedures or algorithms to increase accuracy, for example, with hierarchical clustering of a dataset of unknown class to datasets of known class, or by using aggregation strategies, for example, bagging or boosting schemes. Methods of building and using predictive

algorithms using various approaches are well known in the art see, for example, Dudoit S, Fridlyand J, and Speed TP, *Comparison of discrimination methods for the classification of tumors using gene expression data*. Journal of the American Statistical Association 97:77-87, 2002, section 2, "discrimination methods", pages 78-80, incorporated herein by reference for disclosure of discrimination methods based on expression profile data; and Golub TR, Slonim DK, Tamayo P, Huard C, Gaasenbeek M, Mesirov JP, Coller H, Loh ML, Downing JR, Caligiuri MA, Bloomfield CD, Lander ES. *Molecular classification of cancer: class discovery and class prediction by gene expression monitoring*. Science. 1999 Oct 15;286(5439):531-7, e.g., FIG. 1 and description, and text passage from page 531, 3rd columns, last line to page 532, second full paragraph, incorporated herein by reference for disclosure of building and using a weighted voting scheme class predictor based on expression data).

In some embodiments, a predictor for the classification of tumors as chemosensitive or chemoresistant, or BRCA-like or non-BRCA-like, may comprise, essentially consist of, or consist of 1, 2, 3, 4, or 5 of the top 5 weighted genes in Table 4 (genes 1-5). In some embodiments, a predictor for the classification of tumors as chemosensitive or chemoresistant, or BRCA-like or non-BRCA-like, may comprise, essentially consist of, or consist of 1, 2, 3, 4, 5, 6, 7, 8, 9, or 10, of the top 10 weighted genes in Table 4 (genes 1-10). In some embodiments, a predictor for the classification of tumors as chemosensitive or chemoresistant, or BRCA-like or non-BRCA-like, may comprise, essentially consist of, or consist of 1, 2, 3, 4, 5, 6, 7, 8, 9, 10, 11, 12, 13, 14, or 15 of the top 15 weighted genes in Table 4 (genes 1-15). In some embodiments, a predictor for the classification of tumors as chemosensitive or chemoresistant, or BRCA-like or non-BRCA-like, may comprise, essentially consist of, or consist of 1, 2, 3, 4, 5, 6, 7, 8, 9, 10, 11, 12, 13, 14, 15, 16, 17, 18, 19, or 20 of the top 20 weighted genes in Table 4 (genes 1-20). In some embodiments, a predictor for the classification of tumors as chemosensitive or chemoresistant, or BRCA-like or non-BRCA-like, may comprise, essentially consist of, or consist of 1, 2, 3, 4, 5, 6, 7, 8, 9, 10, 11, 12, 13, 14, 15, 16, 17, 18, 19, 20, 21, 22, 23, 24, or 25 of the top 25 weighted genes in Table 4 (genes 1-25). In some embodiments, a predictor for the classification of tumors as chemosensitive or chemoresistant, or BRCA-like or non-BRCA-like, may comprise, essentially consist of, or consist of 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, or 30 of the top 30 weighted genes in Table 4 (genes 1-30).

Accordingly, in some embodiments, aspects of the invention relate to methods of interrogating the expression of one or more informative genes, or combinations of
5 genes (e.g., from any of Tables 1-4 or otherwise described herein). One or more expression levels may be evaluated in any suitable sample (or in a nucleic acid or protein preparation from a suitable biological sample). In some embodiments, the biological sample is a biopsy of a tumor or other cancerous or precancerous tissue. However, in some embodiments, biological sample may be any suitable sample that
10 contains cells that are informative of the status of a tumor or cancer. For example, a biological sample may be a blood or other fluid sample that contains sufficient tumor or cancer cells to be informative.

In some embodiments, a subject, or a biopsy or other biological sample obtained from a subject, is evaluated to determine whether a BRCA 1 or 2 genetic
15 defect (e.g., mutation) is present. In some embodiments, a gene expression analysis is performed only if no BRCA 1 or 2 genetic defect is identified. However, in other embodiments, the presence of a BRCA 1 or 2 genetic defect is not evaluated, or an expression analysis is performed even if a BRCA 1 or 2 is detected, as aspects of the invention are not limited in this respect. It should be appreciated that any of the
20 genetic and/or expression information described herein may be used alone or in combination, with or without additional patient information to assist in a prognosis, therapeutic recommendation, or other diagnostic or predictive evaluation of the health, outcome, and/or treatment for the patient.

In some embodiments, the expression level for each of a panel of genes
25 consisting (or consisting essentially) of the genes or combinations of genes described herein (e.g., in any of Tables 1-4 or otherwise described herein) is evaluated to determine the BRCAness of a cell or tissue. In some embodiments, the expression of one or a few additional genes (e.g., non-informative genes) may be evaluated to provide a control (e.g., a reference level for normal/high/low expression, an internal
30 control, or other form of control or reference). In some embodiments, the expression level for each of a panel of genes comprising the genes or combinations of genes described herein (e.g., in any of Tables 1-4 or otherwise described herein) is evaluated to determine the BRCAness of a cell or tissue. In some embodiments, the expression level of many additional genes (e.g., non-informative genes) may be determined, but

the evaluation may be based on the expression level of one or more of the informative genes or combinations thereof described herein (e.g., in any of Tables 1-4 or otherwise described herein).

It should be appreciated that the expression level of an informative gene (or set of genes) may be compared to the respective level(s) in a chemoresistant cell or tissue, a chemosensitive cell or tissue, or both, or other reference cells or tissues or combinations thereof, in order to determine or classify the cell or tissue in which the expression level(s) was measured. It should be appreciated that a comparison may include a statistical analysis and a conclusion as to the status or classification of the cell or tissue may be based on the presence of statistically significant similarities and/or differences for the expression level of each gene being evaluated relative to one or more reference expression levels.

The term “informative gene” or “informative marker gene”, as interchangeably used herein, refers to a gene which is measurably differentially expressed in cell populations exhibiting different phenotypes and differential expression of which, alone or in combination with other genes, is correlated with a specific phenotype to a greater degree than expected by chance. For example, if a gene is differentially expressed, e.g. upregulated or downregulated, in tumor cells (e.g., epithelial ovarian tumor cells) susceptible, or sensitive to a chemotherapeutic agent (e.g., a platinum compound or a PARP inhibitor), as compared to cells of the same cell type (e.g., the same tumor type) resistant to the chemotherapeutic agent, such a gene is an “informative gene”, if the differential expression is measurable, for example, by expression profiling methods known to those of skill in the art, and differential expression is correlated with chemoresistance and/or chemosensitivity to a degree greater than a degree of correlation expected by chance.

Some aspects of this invention provide the identities of informative genes for a classification of a tumor as BRCA-like or non-BRCA-like. Some aspects of this invention provide the identities of informative genes for a classification of a tumor as sensitive to a chemotherapeutic compound (chemosensitive) or resistant to a chemotherapeutic compound (chemoresistant). Examples of informative genes for the classification of epithelial ovarian tumors as BRCA-like and non-BRCA-like are shown in any of Tables 1-4.

In some embodiments, a class vote is only made by a predictive algorithm for an informative gene, if the level of upregulation or downregulation of the expression

of the informative gene is greater than a cutoff value. For example, in some embodiments, a class call will be made for a specific informative gene only, if the level of expression of the informative gene is at least a fold change shown in one of the rows of Table 3 for exemplary informative genes. Those of skill in the art will be able to determine appropriate cut off values for individual informative gene class calls, and such cutoff values may, for example be, values reflecting a minimum fraction (e.g. about 10%, about 20%, about 25%, about 30%, about 35%, about 40%, or about 50%) of the average fold regulation observed in comparing gene expression profiles from distinct classes.

In some embodiments, an informative gene is a gene differential expression of which by itself is not correlated to a specific phenotype of a cell to an extent greater than that expected by chance, but differential expression of the gene in the context of differential expression of a different gene is correlated with the phenotype to an extent greater than expected by chance.

The term “differential expression”, as used herein, refers to either up- or downregulation of gene expression to a measurable extent in cells or tissues exhibiting a different phenotype, for example, in tumor cells or tissues that are chemosensitive and tissues that are chemoresistant. Accordingly, a gene that is expressed at a measurably different level in a tumor that is sensitive to chemotherapeutic intervention as compared to the level of expression of the gene in a tumor that is resistant, or not sensitive, to such intervention, is a gene that is differentially expressed in chemosensitive and chemoresistant tumors. Methods for measuring gene expression levels of genes and comparing such measured gene expression levels are well known to those of skill in the art and include, for example, western blot, northern blot, reverse northern blot, transcript or protein microarray, RT-PCR, and massive parallel sequencing assays. However, other techniques may be used as aspects of the invention are not limited in this respect.

Some aspects of this invention relate to methods of assisting in the evaluation of a subject having a tumor for treatment with a chemotherapeutic compound. In some embodiments, a classification of the tumor is performed after the subject has been diagnosed to have the tumor, but before administration of a chemotherapeutic compound. In some embodiments, the tumor is a recurrent tumor, and classification is performed, for example, after a subject underwent initial chemotherapy for the treatment of the initial tumor, but before administration of a chemotherapeutic

compound to treat the recurrent tumor. In some embodiments, a chemotherapeutic compound, for example, a PARP inhibitor or a platinum compound, is administered to the subject based on the classification of the tumor as BRCA-like, or chemosensitive. In some embodiments, administration of chemotherapy is omitted based on

5 classification of the tumor as non-BRCA-like or chemoresistant. In some embodiments, chemotherapy is administered based on a combination of diagnostic tests, including the classification of the tumor as BRCA-like, or chemosensitive, genetic analysis (e.g. mutation analysis), in vitro tumor cell analysis (e.g., in vitro sensitivity of tumor cells to chemotherapeutic compounds), subject/disease history

10 (e.g. recurrent disease after initial chemotherapy), subject health status, and/or other diagnostic tests or assays well known to those of skill in the art.

In some embodiments, the method of diagnostic tumor classification includes determining whether the tumor expression profile is either similar to a first reference expression profile indicative of tumor sensitivity to a chemotherapeutic compound or

15 to a second reference expression profile indicative of tumor resistance to a chemotherapeutic compound. In some embodiments, methods for the identification of appropriate reference expression profiles are provided. In some embodiments, a methods of identifying one or more informative marker genes for a class distinction between “BRCA-like” and “non-BRCA-like” of tumors is provided. In some

20 embodiments, a tumor is classified as BRCA-like or non-BRCA-like based on whether the inner sum of the weighted expression levels of the genes included in the BRCA-ness predictor is above or below a specific cut-off value, for example, as described in more detail elsewhere herein.

In some embodiments, aspects of the invention relate to identifying patients

25 that are candidates for one or more chemotherapeutic treatments. In some embodiments, aspects of the invention relate to identifying patients that should not be treated with one or more chemotherapeutics agents.

The terms “therapy”, “therapeutic”, “treat” or “treatment” refer to , but are not limited to, one or more clinical intervention with an intent to prevent, ameliorate, or

30 cure a condition or symptoms of the condition in a subject, for example, a cancer or tumor, e.g., an epithelial ovarian tumor.

In some embodiments, a treatment as provided by some aspects of this invention is aimed to eliminate a tumor, to induce a decrease in the size of a tumor, to induce a decrease in the number of tumor cells, or to inhibit or halt the growth of a

tumor in a subject. Apparent to those skilled in the relevant medical arts, this can be accomplished by various approaches including, but not limited to, chemotherapeutic interventions. Suitable chemotherapeutic methods and administration schedules of chemotherapeutic compounds, alone or in combination with other therapeutics, will
5 be apparent to those of skill in the relevant medical art.

Some methods for killing or inhibiting the proliferation of tumor cells, according to some embodiments of this invention, feature contacting such cells with a chemotherapeutic agent, for example, a cytotoxic or cytostatic agent. In some
10 embodiments, the cells are contacted with a chemotherapeutic agent, for example, a cytotoxic or cytostatic agent, that selectively targets tumor cells. By “selectively targeting” is meant that the agent or combination of agents selectively recognizes, binds, or acts upon tumor cells. In some embodiments, the agent or combination of agents can effectively kill tumor cells by one or more of several mechanisms, such as by induction of apoptosis, or by attracting other cells such as cytotoxic T lymphocytes
15 or macrophages that can kill or inhibit proliferation of the targeted cells. By “cytotoxic or cytostatic agent” is meant an agent (for example a molecule) that kills or reduces proliferation of cells. Some examples of cytotoxic agents include, but are not limited to, cytotoxic radionuclides, chemical toxins, and protein toxins.

In some embodiments, the chemotherapeutic agent is a cytotoxic radionuclide
20 or radiotherapeutic isotope, for example, an alpha-emitting isotope such as ²²⁵Ac, ²¹¹At, ²¹²Bi, ²¹³Bi, ²¹²Pb, ²²⁴Ra or ²²³Ra. Alternatively, the cytotoxic radionuclide may be a beta-emitting isotope such as ¹⁸⁶Rh, ¹⁸⁸Rh, ¹⁷⁷Lu, ⁹⁰Y, ¹³¹I, ⁶⁷Cu, ⁶⁴Cu, ¹⁵³Sm or ¹⁶⁶Ho. Further, the cytotoxic radionuclide may emit Auger and low energy electrons and may be one of the isotopes ¹²⁵I, ¹²³I or ⁷⁷Br.

25 Chemotherapeutic compounds are well known in the art and non-limiting examples of suitable chemotherapeutic agents include, but are not limited to alkylating agents, for example platinum compounds (e.g., carboplatin, cisplatin and oxaliplatin), mechlorethamine, cyclophosphamide, chlorambucil, and ifosfamide. PARP inhibitors are well known in the art and non-limiting examples of PARP
30 inhibitors include BSI201, AZD2281, ABT888, AG014699, MK4827, INO-1001, NU1025.

Other chemotherapeutic compounds are also well known to those of skill in the art and non-limiting examples of such compounds include members of the enediyne family of molecules, such as calicheamicin and esperamicin. Chemical

toxins can also be taken from the group consisting of methotrexate, doxorubicin, melphalan, chlorambucil, ARA-C, vindesine, mitomycin C, cis-platinum, etoposide, bleomycin and 5-fluorouracil. Examples of antineoplastic agents include, but are not limited to, dolastatins (U.S. Patent Nos. 6,034,065 and 6,239,104) and derivatives thereof, for example, dolastatin 10 (dolavalline-valine-dolaisoleuine-dolaproine-dolaphenine) and the derivatives auristatin PHE (dolavalline-valine-dolaisoleuine-dolaproine-phenylalanine-methyl ester) (Pettit, G.R. et al., *Anticancer Drug Des.* 13(4):243-277, 1998; Woyke, T. et al., *Antimicrob. Agents Chemother.* 45(12):3580-3584, 2001), and auristatin E and the like. Other chemotherapeutic agents are known to those skilled in the art.

However, it should be appreciated that other chemotherapeutic compounds, and/or combinations of compounds (e.g., two or more compounds described herein alone or with other compounds) may be used as aspects of the invention are not limited in this respect.

Therapeutic compositions of the present invention may be administered in pharmaceutically acceptable preparations. Such preparations may contain pharmaceutically acceptable concentrations of salt, buffering agents, preservatives, compatible carriers, supplementary immune potentiating agents such as adjuvants and cytokines, and optionally other therapeutic agents.

As used herein, the term "pharmaceutically acceptable" means a non-toxic material that does not interfere with the effectiveness of the biological activity of the active ingredients. The term "physiologically acceptable" refers to a non-toxic material that is compatible with a biological system such as a cell, cell culture, tissue, or organism. The characteristics of the carrier will depend on the route of administration. Examples of physiologically and pharmaceutically acceptable carriers include, without being limited to, diluents, fillers, salts, buffers, stabilizers, solubilizers, and other materials which are well known in the art. The term "carrier" denotes an organic or inorganic ingredient, natural or synthetic, with which the active ingredient is combined to facilitate the application. The components of the pharmaceutical compositions also are capable of being co-mingled with the molecules of the present invention, and with each other, in a manner such that there is no interaction which would substantially impair the desired pharmaceutical efficacy.

Therapeutics according to some embodiments of the invention can be administered by any conventional route, for example injection or gradual infusion

over time. The administration may, for example, be oral, intravenous, intratumoral, intraperitoneal, intramuscular, intracavity, subcutaneous, or transdermal, or by pulmonary aerosol.

The compositions of some embodiments of the invention are administered in effective amounts. An “effective amount” is that amount of a composition that alone, or together with further doses, produces the desired clinical response. In some cases of treating a particular disease or condition, for example, a cancer manifested in a tumor, the desired response is inhibiting the progression of the disease, for example, the growth of the tumor or the spread of a primary tumor to secondary sites via metastasis. This may involve slowing the progression of the disease temporarily, although more preferably, it involves halting the progression of the disease permanently. In some cases, the desired response to treatment is a permanent eradication of tumor cells. In some cases, the desired response to treatment can be delaying or preventing the manifestation of clinical symptoms, for example, of recurrent tumors.

The effect of treatment can be monitored by routine methods or can be monitored according to diagnostic methods of the invention discussed herein.

The effective amount of a chemotherapeutic compound or a combination of such compounds will depend, of course, on the particular tumor being treated, the severity of the condition, the individual patient parameters including age, physical condition, size and weight, the duration of the treatment, the nature of concurrent therapy (if any), the specific route of administration and like factors within the knowledge and expertise of the health practitioner. These factors are well known to those of ordinary skill in the art and can be addressed with no more than routine experimentation. It is generally preferred that a maximum dose of the individual components or combinations thereof be used, that is, the highest safe dose according to sound medical judgment. It will be understood by those of ordinary skill in the art, however, that a patient may insist upon a lower dose or tolerable dose for medical reasons, psychological reasons or for virtually any other reasons.

In some embodiments, a kit is provided, comprising reagents useful for determining an expression level of an informative gene, for example, an informative gene listed in any of Tables 1-4. A reagent useful for determining expression of an informative gene may, in some embodiments, be a detectable agent that binds to an expression product of an informative gene. Detectable agents, their generation and/or

purification and their use are well known to those of skill in the art and non-limiting, exemplary detection agents include detectable binding agents, for example antibodies, antibody fragments, nucleic acids complementary to a sequence comprised in a transcript of the informative gene, aptamers, and adnectins. In some embodiments, a kit may comprise a plurality of different nucleic acid molecules that correspond to different informative gene transcripts. In some embodiments, the plurality of nucleic acid molecules is attached to a solid support. In some embodiments, a kit is provided that includes a focused microarray for the detection of expression levels of all or some of the informative genes described herein, for example, the informative genes listed in any of Tables 1-4. In some embodiments, a plurality of primer pairs is provided for determining an expression level of a plurality of informative genes, for example, of all or some of the genes listed in any of Tables 1-4.

These and other aspects of the invention are illustrated by the following non-limiting examples.

EXAMPLES

Given the heterogeneous mechanism(s) by which an ovarian cancer cell might develop defective HR, it was reasoned that a broad-based approach that makes few assumptions about mechanism might have the highest chance of identifying patients with a BRCAness phenotype. Microarray gene expression profiling lends itself to this goal, because it is not mechanism based and has already been proposed in ovarian cancer as both a prognostic as well as predictive tool²⁵⁻²⁷. In this study it is shown that it is possible to define a gene expression profile of BRCAness, associated with responsiveness to platinum and PARP inhibitors, and that this profile is correlated with important outcome measures in patients with the sporadic form of the disease.

Example 1: Development of a gene expression profile of BRCAness

For the purpose of profile development, a publicly available microarray dataset was used that included tumor expression data from 61 patients with pathologically confirmed epithelial ovarian cancer (EOC), including 34 with BRCA-1 or -2 germline mutation (18 BRCA-1 and 16 BRCA-2 mutations), and 27 without either mutation (i.e. sporadic cancers)²⁸. Genome wide hierarchical clustering was used to define BRCA-like and non-BRCA-like tumors as described in detail elsewhere herein and in **FIG. 1**.

Example 2: Patient samples

Two patient cohorts were used in this study. The first included 6 EOC patients with BRCA-1 or -2 germline mutations and have been previously described
5 29,30. Four patients had paired samples, pre and post the development of platinum resistance, and 2 had samples obtained only at the time of platinum sensitive disease.

The second patient cohort included 70 patients treated at Beth Israel Deaconess Medical Center, Memorial Sloan Kettering Medical Center, and Cedars-Sinai Medical Center, who underwent exploratory laparotomy for diagnosis, staging,
10 and debulking, followed by first-line platinum-based chemotherapy. Standard post-chemotherapy surveillance included serial physical examination, serum CA-125 level, and computed tomography scanning as clinically indicated.

The study protocol for collection of tissue and clinical information for all patients was approved by the institutional review boards at all three institutions, and
15 patients provided written informed consent authorizing the collection and use of the tissue for study purposes. Additional details are provided elsewhere herein.

Example 3: Cell lines

Twelve cisplatin-resistant clones of the BRCA-2-mutated pancreatic cancer
20 cell line Capan-1 have been previously described ²⁹.

Example 4: RNA Isolation and Affymetrix GeneChip Hybridization

Total RNA isolation, microarray hybridization (U133 Plus 2.0 Array GeneChip, Affymetrix, Santa Clara, CA), and data processing were performed as
25 previously described ^{26,27,31}.

Example 5: Statistical Analysis

The statistical significance of the association between the BRCAness profile and various clinicopathologic factors was assessed by the Fisher's exact test. The p
30 values of all statistical tests were two-sided. Overall and disease free survival curves were generated by the Kaplan-Meier method, and differences between survival curves were assessed for statistical significance with the log-rank test. Multivariate analyses to adjust for known prognostic factors were performed using a Cox proportional hazards regression model that included grade (1-2 versus 3), age (< 65 years versus

≥65 years), stage (2 versus 3 or 4), histology (clear cell, papillary serous, endometrioid), debulking status (optimal, less than or equal to 1 cm; or suboptimal, greater than 1 cm residual disease) and BRCAness profile (BRCA-like versus non-BRCA-like).

5

Example 6: Development of a gene expression profile of BRCAness

For the purpose of profile development, a publicly available microarray dataset was used that included tumor expression data from 61 patients with pathologically confirmed epithelial ovarian cancer (EOC), including 34 with BRCA-1 or -2 germline mutation (18 BRCA-1 and 16 BRCA-2 mutations), and 27 without either mutation (i.e. sporadic cancers)²⁸. BRCA-like and non-BRCA-like tumors were defined using genome wide hierarchical clustering as illustrated in **FIG. 1**. FIG. 1 describes the development of the BRCAness gene expression profile. A publicly available microarray dataset was used that included tumor expression data from 61 patients with pathologically confirmed epithelial ovarian cancer (EOC), including 34 with BRCA-1 or -2 germline mutation (18 BRCA-1 and 16 BRCA-2 mutations), and 27 without either mutation (i.e. sporadic cancers)²⁸. Although previous investigators have described gene expression differences between BRCA-mutated and sporadic cancers, these studies grouped all sporadic tumors together without taking into consideration that some of these sporadic tumors might indeed have a BRCAness phenotype^{40,53}. Thus, the group of sporadic patients in such analyses is “contaminated” by patients who may exhibit a gene expression profile more consistent with a BRCA-1 or -2 germline mutation carrier, and vice versa. In order to address this issue, genome-wide hierarchical clustering of all 61 tumors was performed first. It was found that patients clustered into three groups, representing BRCA-1, BRCA-2, and sporadic clusters, respectively (**FIG. 2A**). The BRCA-1 cluster contained 22 patients, of which 9 actually had sporadic (non-mutated) disease. The BRCA-2 cluster contained 14 patients, of which 4 had sporadic disease. The sporadic cluster contained 25 patients, of which 6 had BRCA-1 and 5 had BRCA-2 germline mutation. The clustering reproducibility index (R) was 0.934 and the 3 clusters did not change even if clear cell or mucinous samples were excluded from the analysis. For the purpose of defining the profile, these outliers (e.g., a BRCA-1 patient contaminating the sporadic cluster, or a sporadic patient contaminating the BRCA cluster) were removed from the analysis. A 60-gene diagonal linear

30

discriminant predictor was developed next that distinguished the BRCA clusters (BRCA-like tumors) from the sporadic cluster (non-BRCA-like tumors) (**FIG. 2B**).

The predictor that distinguished BRCA-like from non-BRCA-like tumors was developed using the diagonal linear discriminant algorithm⁴⁸. The classifier was trained by selecting genes with the highest fold-change difference between the two classes (BRCA-like and non-BRCA-like tumors). Classifier accuracy and statistical significance were assessed using leave-one-out cross-validation and a 1000 random permutation test to control for over-fitting^{49,50}. In order to ascertain that classifier accuracy was not an artifact of the optimal 60 gene predictor, the performance of predictors from a range of 40 to 90 genes was assessed and it was found that they demonstrated very good performance with accuracy of 89-92%. The BRCAness profile was mapped across different platforms using Affymetrix annotation files before being applied to patient and cell line samples (www.affymetrix.com/analysis/index.affx). Non-biologic experimental variation ("batch effect") across datasets was adjusted using empirical Bayes methods as previously described³⁶.

Example 7: Patient samples

Two patient cohorts were used in this study. The first was comprised of 6 EOC patients with BRCA-1 or BRCA-2 germline mutations treated at Cedars-Sinai Medical Center and have been previously described^{29,30}. Four patients from this group had paired samples, pre and post the development of platinum resistance (two patients with germline BRCA-1 mutation and two with germline BRCA-2 mutation). In each case, the development of platinum resistance was associated with reversion to functional BRCA-1 and -2 protein^{23,30}. Tumor from the two other patients was obtained at the time of platinum sensitive disease, without a follow-up specimen at the time of platinum resistance. In both of these cases the tumor specimen contained a germline mutation in BRCA-1.

The second patient cohort consisted of 70 EOC patients, in which tumor was obtained at the time of diagnostic exploratory laparotomy. Twenty eight of these patients were diagnosed between November 1994 and June 2005 and treated at Cedars-Sinai Medical Center, and had sporadic EOC as determined by negative BRCA-1 or -2 sequencing. The remaining 42 patients were diagnosed between January 1995 and October 2000 and treated at Beth Israel Deaconess Medical Center

and Memorial Sloan-Kettering Cancer Center, and represent a subset of those previously reported²⁷. Seven of the 42 patients were sequenced and found to be negative for a BRCA-1 or -2 mutation. The remaining 35 of these 42 patients were selected on the basis of criteria that are expected to enrich for sporadic disease.

- 5 Specifically, these 35 patients had no family history of ovarian cancer, no family history of breast cancer at age<50, no family history of more than 1 breast cancer at any age, and were not of Ashkenazi Jewish ethnicity. Ovarian cancer samples from this patient cohort were collected at the time of primary debulking surgery and frozen at -80°C. Tumor samples were pulverized in liquid nitrogen and homogenized in
- 10 Trizol solution (Invitrogen Corp, Carlsbad, CA), followed by RNA isolation.

Example 8: Cell lines

- Twelve cisplatin-resistant clones of the originally cisplatin-sensitive BRCA-2-mutated pancreatic cancer cell line Capan-1 have been previously described²⁹. The
- 15 parent Capan-1 line harbors a 6174delT mutation, associated with loss of heterozygosity⁵¹. As a result of platinum-induced selection pressure, 6 of these 12 clones had acquired secondary genetic events that restored nearly full-length, functional BRCA-2 protein and RAD51 foci formation in response to ionizing radiation (IR)²⁹. The remaining 6 clones showed persistent evidence of mutated
- 20 BRCA-2 (6174delT), lacked BRCA-2 protein expression and exhibited impaired IR-induced RAD51 foci formation (except one which had proficient RAD51 foci formation). Two of the clones with restored functional BRCA-2 protein were tested for PARP inhibitor sensitivity and found to be resistant to PARP inhibition, while two of the clones with restored functional BRCA-2 protein were tested for PARP inhibitor
- 25 sensitivity and found to be resistant to PARP inhibition²⁹.

Example 9: RNA Isolation and Affymetrix GeneChip Hybridization

- Total RNA was isolated from patient tumor samples using Trizol reagent (Invitrogen, Carlsbad, CA) and from Capan-1 cell lines using the RNeasy Mini Kit
- 30 (Qiagen Valencia, CA, USA) according to manufacturer's instructions. cDNA synthesis and hybridization on oligonucleotide microarrays (U133 Plus 2.0 Array GeneChip, Affymetrix, Inc., Santa Clara, CA) containing approximately 54,700 transcripts were carried out using standard protocols. Microarray experiments were performed at the Dana Farber Cancer Institute Microarray Core Facility

(chip.dfci.harvard.edu/). Raw data were processed using Robust Multi-Array (RMA) analysis. All raw microarray data are provided in GEO (Gene Expression Omnibus).

Example 10: Statistical Analysis

5 The statistical significance of the association between the BRCAness profile and various clinicopathologic factors was assessed by the Fisher's exact test. Unsupervised hierarchical clustering was performed using the average linkage method and the one minus centered correlation as a distance metric in all cases⁵². The p values of all statistical tests were two-sided. The SPSS version 16.0 and STATA
10 version 10.1 packages were used for statistical tests. All bioinformatic analyses were performed using the BRB-ArrayTools Version 3.8 [developed by Dr Richard Simon (Biometrics Research Branch, National Cancer Institute, Bethesda, MD)].

Example 11: Characteristics of the BRCAness profile

15 The strategy for developing the BRCAness profile is described in detail in **FIG. 1**. The optimal classifier was a 60-gene diagonal linear discriminant predictor that distinguished BRCA-like from non-BRCA-like tumors with 94% accuracy, as assessed by leave-one-out cross-validation and 1000 random permutations test (**FIG. 2**)($p < 0.001$). **FIG. 2** displays an expression plot of the 60 genes that comprise the
20 BRCAness profile. Columns: Training set samples. Rows: Gene expression levels (normalized). Complete information regarding gene identity is provided in **Table 1**. Dark shading: Overexpressed genes. Light shading: Underexpressed genes. The gene expression signature that correlates with BRCA-like tumors is defined as the "BL" profile, and the signature that correlates with non-BRCA-like tumors is defined as the
25 "NBL" profile.

Other predictive algorithms performed similarly, such as compound covariate predictor (92%), nearest centroid (92%) and support vector machines (92%)³²⁻³⁵. For the analyses described below, the gene expression signature that correlates with BRCA-like tumors is defined as the "BL" profile, and the signature that correlates
30 with non-BRCA-like tumors is defined as the "NBL" profile. The identities of all BRCAness profile genes are provided in **Table 1**.

Example 12: BRCAness profile distinguishes between platinum-sensitive and -resistant tumor biopsy samples

It was first investigated whether the BRCAness profile could correlate with platinum responsiveness in patients with known BRCA germline mutation. For this purpose, 10 tumor biopsy specimens from 6 patients with either BRCA-1 or -2 germline mutation were used, four of whom were initially platinum sensitive but eventually developed platinum resistance (with pre and post biopsy pairs). These patients formed the basis of a previous report in which reversion of the BRCA genotype occurred (with re-establishment of BRCA function) upon the development of platinum resistance²⁹. Thus, these samples provided an opportunity to determine how the BRCAness profile correlated with both platinum responsiveness and BRCA functional status (e.g., mutant versus revertant BRCA gene).

The BRCAness profile was applied to these 10 platinum sensitive and resistant tumors specimens, with the results shown in **FIG. 3**. **FIG. 3A** shows that hierarchical clustering based on the expression pattern of the 60 genes of the BRCAness profile distinguished between platinum resistant and platinum sensitive tumor biopsy samples. Abbreviations: NBL, non BRCA-like; BL, BRCA-like. Five out of 6 tumor samples with the BL signature were platinum-sensitive, whereas 3 out of 4 tumor samples with the NBL signature were platinum-resistant. **FIG. 3B** shows the correlation of the BRCAness profile with platinum sensitivity and BRCA germline mutation status in the 10 tumor biopsy specimens from 6 patients. The BRCAness profile accurately distinguished between platinum sensitivity and platinum resistance in 8 out of 10 tumor specimens, which in turn correlated with presence of mutated versus functional BRCA gene status, respectively. In the first two patients, the BRCAness profile dynamically tracked the development of platinum resistance over the course of therapy (e.g., the profile changed from BL to NBL following the development of platinum resistance, associated with reversion to functional BRCA-1 or 2).

The BRCAness profile could accurately distinguish between platinum sensitive and platinum resistance in 8 out of 10 tumor specimens, which in turn correlated with presence of mutated versus functional BRCA gene status, respectively. Specifically, 5 out of 6 tumors with the BL signature were platinum sensitive (and were BRCA-1 or -2 mutated), whereas 3 out of 4 tumors with the NBL signature were platinum resistant (and had reverted to functional BRCA-1 or -2)^{29,30}. Furthermore, patients were observed in which the BRCAness profile dynamically tracked the development of platinum resistance over the course of therapy (e.g., the

profile changed from BL to NBL following the development of platinum resistance, associated with reversion to functional BRCA-1 or -2, **FIG. 3B**).

Example 13: BRCAness profile correlates with PARP inhibitor responsiveness and RAD51 foci formation

The above data suggest that the BRCAness profile might correlate with platinum responsiveness in patient-derived tumor samples. As another surrogate of BRCAness, it was next investigated whether the profile could also correlate with the ability to form RAD51 foci after ionizing radiation, which is a surrogate of intact homologous recombination, as well as with responsiveness to PARP inhibitors. For this purpose, 12 clones of the BRCA-2-mutated pancreatic cancer cell line Capan-1 was used, a cell line previously characterized²⁹. These clones were generated by exposing the parent Capan-1 cell line to platinum-selection pressure, eventually isolating 12 platinum resistant clones. Seven of these clones formed intact RAD51 foci after IR (6 of these regained functional BRCA-2 due to secondary BRCA-2 mutations which cancel the effect of the inherited BRCA-2 mutation), and the remaining 5 exhibited deficient RAD51 foci formation (all of which were BRCA-2 deficient). PARP inhibitor sensitivity had been determined for 4 of these clones, 2 being PARP inhibitor sensitive, and 2 being PARP inhibitor resistant (Sakai W, Swisher EM, Karlan BY, et al: *Secondary mutations as a mechanism of cisplatin resistance in BRCA2-mutated cancers*. Nature 451:1116-20, 2008). When applied to these cell lines, the BRCAness profile correlated with RAD51 foci formation in 9 out of 12 Capan-1 clones, and between presence of mutated versus functional BRCA-2 gene status in 10 out of 12 Capan-1 clones (**FIG. 4**). Importantly, the BRCAness profile accurately distinguished between 2 PARP inhibitor resistant clones (NBL signature) and 2 PARP inhibitor sensitive clones (BL signature) (**FIG. 4**).

Example 14: The relationship between BRCAness profile and clinical outcome in patients with sporadic EOC

The above data suggest that the BRCAness profile may correlate with platinum and PARP-inhibitor responsiveness in the context of a known BRCA germline mutation, but they do not address whether the profile correlates with outcome in patients with sporadic disease. In order to test this, the profile was applied to tumor samples from 35 patients with invasive EOC who had been sequenced and

known to be wildtype for BRCA-1 and -2, and 35 patients enriched for sporadic disease on the basis of the following characteristics: no family history of ovarian cancer, no family history of breast cancer under the age of 50 years, no family history of more than 1 breast cancer at any age, and not of Ashkenazi Jewish ethnicity^{37,38}.

- 5 The clinical and pathologic characteristics of all 70 patients are shown in **Table 5**.

Table 5. Clinical and Pathological Characteristics

Characteristic		Sequenced Cohort ^a (n=35)		Non-Sequenced Cohort ^b (n=35)		Combined Cohort ^c (n=70)	
		No.	%	No.	%	No.	%
Age	Median (range)	62.3 (44-89)		58.7 (39-80)		60.5 (39-89)	
Grade	1-2	4	11.4	6	17.1	10	14.3
	3	31	88.6	29	82.9	60	85.7
Histology	Serous	34	97.1	31	88.6	65	92.9
	Clear Cell	0	0	2	5.7	2	2.9
	Endometrioid	1	2.9	2	5.7	3	4.3
Stage	2	2	5.7	1	2.9	3	4.3
	3	28	80	29	82.9	57	81.4
	4	5	14.3	5	14.3	10	14.3
Debulking Status^{d, e}	Optimal	27	79.4	22	62.9	49	71
	Suboptimal	7	20.6	13	37.1	20	29

^a All 35 patients underwent sequencing and were wildtype for BRCA-1 and -2.

- 10 ^b Not sequenced but enriched for sporadic disease on the basis of the following: no family history of ovarian cancer, no family history of breast cancer under the age of

50 years, no family history of more than 1 breast cancer at any age, and not of Ashkenazi Jewish ethnicity.

^c All patients received first line platinum-based chemotherapy

^d Debulking status was unknown for 1 patient. Optimal, less than or equal to 1 cm.;

5 suboptimal, greater than 1 cm.

^e Difference in debulking status between sequenced and non-sequenced cohorts was not statistically significant ($p=0.19$).

Overall, 20 of the 70 patient cohort (29%) demonstrated the BL profile (8 out
10 of 35 in the sequenced group, and 12 out of 35 in the non-sequenced group, $p=0.43$). Compared to the non-sequenced cohort, the sequenced cohort was enriched with patients with optimally debulked disease although this did not reach statistical significance (two-sided Fisher's exact $p = 0.19$). As shown in **Table 6**, there were no differences in age, stage, grade, histology, or debulking status between the BL and the
15 NBL signature groups. The ability to achieve a clinical remission for the BL and NBL groups was 90% compared to 74%, although this did not reach statistical significance (two-sided Fisher's exact $p = 0.2$).

Table 6. Association of BRCAness profile with clinical characteristics and remission status after first line therapy

Characteristic		NBL Profile ^a (n=50)		BL Profile ^a (n=20)		p value
		No.	%	No.	%	
Age	Median (range)	61 (39-89)		59.25 (44-80)		0.55
Grade	1-2	9	18	1	5	0.477
	3	41	82	19	95	
Histology	Serous	46	92	19	95	0.412
	Clear Cell	1	2	1	5	
	Endometrioid	3	6	0	0	
Stage	2	3	6	0	0	0.479
	3	41	82	16	80	
	4	6	12	4	20	
Debulking Status ^b	Optimal	33	67.3	16	80	0.386
	Suboptimal	16	32.7	4	20	
Achievement of CR ^c after first- line therapy		74%		90%		0.2

^a NBL, non-BRCA-like; BL, BRCA-like.

5 ^b Debulking status was unknown for 1 patient. Optimal, less than or equal to 1 cm.; suboptimal, greater than 1 cm.

^c CR, complete response.

10 For the entire 70 patient cohort, the BRCAness profile was capable of discriminating between long and short median disease free survival (DFS), with the BL and NBL patients having a median DFS of 34 months and 15 months, respectively (log-rank $P = 0.013$) (**FIG. 5A**). In addition, the percent of patients disease-free at 18

months for the BL and NBL groups was 65% and 29%, respectively (two-sided $p = 0.007$). Similarly, the percent disease free at 24 months for the BL and NBL groups was 50% and 22%, respectively (two-sided $p=0.042$). Finally, the BRCAness profile distinguished between long and short median overall survival (OS), with the BL and NBL patients having a median OS of 72 and 41 months, respectively (log-rank $P = 0.006$) (**FIG. 5B**). Similar findings were observed when applying the profile separately to the group of 35 sequenced patients (**Table 5**) who had undergone formal mutation testing and were found to have wildtype BRCA-1 and -2 genes, or to the group of the 35 non-sequenced patients. **FIG. 6A** shows DFS in the sequenced patient cohort. The median DFS for patients with the BL and NBL profile was not yet reached at a median follow up of 53.5 months and 20 months, respectively (log-rank $P = 0.03$). **FIG. 6B** shows OS in the sequenced patient cohort. The median OS for patients with the BL and NBL profile was 72 months and 48 months, respectively (log-rank $P = 0.1$). **FIG. 7** shows DFS in the non-sequenced patient cohort. The median DFS for patients with the BL and NBL profile was 22 and 7 months, respectively (log-rank $P = 0.013$). **FIG. 7B** shows OS in the non-sequenced patient cohort. The median OS for patients with the BL and NBL profile was not yet reached at a median follow up of 39 months and 30 months, respectively (log-rank $P = 0.009$).

In univariate analysis, the hazard ratio for recurrence (NBL versus BL group) was 2.47 ($P = 0.018$, 95% CI, 1.17 to 5.2) and the hazard ratio for death (NBL versus BL group) was 3.29 ($P = 0.009$, 95% CI, 1.34 to 8.09) (**Table 7**). Multivariate analysis including the BRCAness profile, age, stage, grade, histology, and debulking status, demonstrated that the profile maintained an independent association with DFS and OS. The hazard ratio for recurrence (NBL versus BL group) was 2.65 ($P = 0.016$, 95% CI, 1.2 to 5.86) and the hazard ratio for death (NBL versus BL group) was 3.39 ($P = 0.009$, 95% CI, 1.35 to 8.5) (**Table 7**). The fact that characteristics such as stage, grade, and histology did not correlate with outcome in either univariate or multivariate analysis is likely related to the fact that the vast majority of patients were stage III (81%), grade III (89%), and serous histology (94%).

Table 7. Predictive value of BRCAness profile adjusted for Grade, Age, Stage and Debulking Status

Factor	Univariate P Value		Multivariate P Value	
	DFS	OS	DFS	OS
Age	0.44	0.35	0.96	0.8
Grade	0.9	0.34	0.87	0.32
Histology	0.97	0.61	0.9	0.96
Stage	0.43	0.54	0.4	0.61
Debulking Status	0.39	0.05 (HR=1.84) ^a	0.53	0.67
NBL/BL profile	0.018 (HR=2.47)	0.009 (HR=3.29)	0.016 (HR=2.65)	0.009 (HR=3.39)

^a Debulking status was unknown for 1 patient. Values in bold are statistically significant at p less than or equal to 0.05. homologous recombination (hazard ratio for death) represented in parentheses (comparing NBL versus BL groups), for statistically significant associations.

PARP inhibitors have been evaluated in patients with germline BRCA-1 and -2 mutations, with impressive results as single agents^{10,11}. In addition to patients with germline BRCA-1 or -2 mutations, however, it has been suggested that PARP inhibition might be a useful therapeutic strategy for the treatment of patients with sporadic cancers that have a BRCAness phenotype, characterized by defective homologous recombination¹⁵. In this regard, a number of mechanisms have been identified in sporadic ovarian cancer that might implicate the homologous

recombination pathway in pathogenesis and in drug responsiveness. Such mechanisms include mutations or epigenetic silencing of genes involved in the Fanconi Anemia protein complex, intrinsic homologous recombination genes, or other DNA damage response genes^{5,15,17,19,23}. Amplification of genes that encode for proteins that inactivate BRCA-2 function, such as EMSY, has also been described²⁴. BRCA-1 promoter methylation, FANCF promoter methylation, and EMSY amplification have been identified in 5-31%, 21%, and 17% of sporadic EOCs respectively^{15,17,19,23,24}, supporting the notion that at least some patients with sporadic disease might harbor defects in HR, independent of the presence of a germline BRCA-1 or -2 mutation.

Although it is possible to identify individual molecular mechanisms by which the homologous recombination pathway might be disrupted in sporadic ovarian cancer, only a handful of studies have explored the relationship between homologous recombination and response to platinum or PARP-inhibitors. D'Andrea et al showed that inhibition of the FANCF gene in ovarian cancer cell lines through promoter methylation is associated with enhanced sensitivity to DNA damaging agents such as platinum, while demethylation of the FANCF promoter results in platinum resistance²³. McCabe et al showed that cells deficient in the expression of genes involved in homologous recombination (e.g., RAD51, ATR, ATM, CHK2) are sensitive to PARP inhibitors⁵. Teodoridis et al used methylation-specific PCR and showed that BRCA-1 promoter hypermethylation is associated with improved response to platinum-based chemotherapy¹⁶. In addition, Quinn et al used siRNA knock-down to decrease the expression of the BRCA-1 gene in two separate ovarian cancer cell lines, showing that lower levels of BRCA-1 mRNA correlated with enhanced *in vitro* sensitivity to cisplatin³⁹.

In the experiments described herein, the concept of BRCAness has been broadened by identifying a gene expression profile that is associated with platinum and PARP-inhibitor responsiveness, as well as RAD51 foci formation. In addition, when applied to a population of patients enriched for sporadic disease, the profile correlated with clinical outcome, independent of standard prognostic factors such as age, grade, histology, stage, and debulking status.

The present data does not determine with certainty whether the correlation between the BL signature and improved survival is indicative of enhanced platinum responsiveness, or conversely might identify patients with a more indolent natural

history. In this regard, it is intriguing that the proportion of patients rendered into a complete clinical remission at the end of first line chemotherapy was higher in patients with a BL signature (90%), compared to those with the NBL signature (74%), although this was not statistically significant ($p = 0.2$). Accordingly, without wishing
5 to be bound by theory, in some embodiments, improved survival and/or complete remission in patients with a tumor classified as BRCA-like is, at least partially, due to increased responsiveness to chemotherapeutic compounds.

Although previous microarray studies have reported gene expression differences between BRCA-mutated and sporadic breast and ovarian cancers, none of
10 them was specifically designed to identify a BRCAness profile with predictive or prognostic potential in patients with sporadic disease^{28,40}. In order to define the profile described herein, hierarchical clustering was first used to identify those sporadic patients who migrated with the BRCA-mutated group, these were then re-categorized them appropriately, prior to generating the profile (**FIG. 1**). From the
15 standpoint of profile development, this provided two comparison groups, based not simply on the presence or absence of BRCA germline mutation, but rather based upon how patients clustered with one another, partly independent of their mutation status. Not only was the resulting profile capable of tracking with platinum response, PARP-inhibitor response, and clinical outcome, but it contained genes such as APEX1,
20 MGST3 and PMS1, that have been previously associated with platinum resistance or DNA repair (**FIG. 2**)⁴¹⁻⁴⁶. It is noteworthy that neither BRCA-1 or -2 was part of the gene expression profile, perhaps indirectly supporting the notion that at least for some patients, genes other than BRCA-1 or 2 may sometimes be responsible for BRCAness in sporadic disease.

25 Although the BRCAness profile was developed in ovarian tumors, it was also capable of predicting PARP inhibitor sensitivity and RAD51 foci formation in the pancreatic cancer cell line Capan-1, suggesting that the profile may be detecting a pattern of gene expression that more globally reflects the status of HR, independent of cell lineage. In this regard, the predictive value of this profile in triple negative breast
30 cancer is currently being investigated, which is thought to be enriched for BRCAness and a high response to platinum-containing chemotherapy⁴⁷. Ultimately, it should be possible to apply this profile in the context of a clinical trial involving sporadic ovarian cancer patients treated with a PARP inhibitor, in order to gain further insight into the predictive value of this approach. In this regard, studies are currently being

performed to explore the potential value of PARP inhibitors in patients with ovarian cancer, independent of BRCA mutation status. These include a placebo-controlled, randomized phase II trial of maintenance AZD2281 in patients with relapsed ovarian cancer who are responding to platinum-containing chemotherapy (NCT00753545), as well as a phase II trial of ABT888 in combination with topotecan in patients with relapsed disease (NCT01012817). Accordingly, gene expression profiling may be useful as an eligibility criterion in such studies, to enrich for sporadic patients that may benefit the most from this novel class of agents. Although further study may provide additional information, in some embodiments the identification of a gene expression profile that correlates with BRCAness may be useful to identify cancer patients (e.g., patients with epithelial ovarian cancer) to be treated with certain chemotherapeutic agents (including, but not limited to, PARP inhibitors), regardless of the BRCA-1 or -2 mutation status of the patients. This may allow certain chemotherapeutic agents to be used more effectively in a broader range of patients.

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In addition, it should be appreciated that the reference to a computer program which, when executed, performs the above-discussed functions, is not limited to an
15 application program running on a host computer. Rather, the term computer program is used herein in a generic sense to reference any type of computer code (e.g., software or microcode) that can be employed to program a processor to implement the above-discussed aspects of the present invention.

It should be appreciated that in accordance with several embodiments of the
20 present invention wherein processes are implemented in a computer readable medium, the computer implemented processes may, during the course of their execution, receive input manually (e.g., from a user).

The terms "program" or "software" are used herein in a generic sense to refer to any type of computer code or set of computer-executable instructions that can be
25 employed to program a computer or other processor to implement various aspects of the present invention as discussed above. Additionally, it should be appreciated that according to one aspect of this embodiment, one or more computer programs that when executed perform methods of the present invention need not reside on a single computer or processor, but may be distributed in a modular fashion amongst a number
30 of different computers or processors to implement various aspects of the present invention.

Computer-executable instructions may be in many forms, such as program modules, executed by one or more computers or other devices. Generally, program modules include routines, programs, objects, components, data structures, etc. that

performs particular tasks or implement particular abstract data types. Typically the functionality of the program modules may be combined or distributed as desired in various embodiments.

Also, data structures may be stored in computer-readable media in any suitable
 5 form. For simplicity of illustration, data structures may be shown to have fields that are related through location in the data structure. Such relationships may likewise be achieved by assigning storage for the fields with locations in a computer-readable medium that conveys relationship between the fields. However, any suitable
 mechanism may be used to establish a relationship between information in fields of a
 10 data structure, including through the use of pointers, tags or other mechanisms that establish relationship between data elements.

Transcripts named herein are well known to those of skill in the art, and the skilled artisan will be able to identify nucleotide sequences associated with the transcripts provided herein, for example, by retrieving transcript-associated database
 15 entries from a sequence database (e.g. NCBI or Ensembl at <http://www.ncbi.nlm.nih.gov/> and <http://uswest.ensembl.org/index.html>, respectively). Non-limiting examples of representative sequences related to some transcripts disclosed herein are given below:

>gil3lemblA00003.1| B.taurus DNA sequence 2 from patent application EP0238993
 20 (SEQ ID NO: 1)
 >gil178742|gb|M80261.1|HUMAPE Human apurinic endonuclease (APE) mRNA, complete cds (SEQ ID NO: 2)
 >gil183183|gb|J03005.1|HUMGIAB Human alternative guanine nucleotide-binding regulatory protein (G) alpha-inhibitory-subunit mRNA, complete cds (SEQ ID NO: 3)
 25 >gil190419|gb|J03223.1|HUMPROLEU Human secretory granule proteoglycan peptide core mRNA, complete cds (SEQ ID NO: 4)
 >gil393318|dbj|D13665.1|HUMOSF2P1 Homo sapiens osf-2 mRNA for osteoblast specific factor 2 (OSF-2p1), complete cds (SEQ ID NO: 5)
 >gil1871135|dbj|D83043.1| Homo sapiens HLA-B mRNA, allele A*2711, complete
 30 cds (SEQ ID NO: 6)
 >gil1890049|dbj|D55696.1| Homo sapiens mRNA for cysteine protease, complete cds (SEQ ID NO: 7)
 >gil2921548|gb|AF038602.1| Homo sapiens CD2 binding protein 1 short form mRNA, complete cds (SEQ ID NO: 8)

- >gil3560556|gb|AF053641.1| Homo sapiens brain cellular apoptosis susceptibility protein (CSE1) mRNA, complete cds (SEQ ID NO: 9)
- >gil3719220|gb|AF022375.1| Homo sapiens vascular endothelial growth factor mRNA, complete cds (SEQ ID NO: 10)
- 5 >gil6005805|ref|NM_007164.1| Homo sapiens mucosal vascular addressin cell adhesion molecule 1 (MADCAM1), mRNA (SEQ ID NO: 11)
- >gil6006030|ref|NM_003197.2| Homo sapiens transcription elongation factor B (SIII), polypeptide 1-like (TCEB1L), mRNA (SEQ ID NO: 12)
- >gil6467174|dbj|AB020980.1| Homo sapiens mRNA for putative membrane protein, complete cds (SEQ ID NO: 13)
- 10 >gil7262372|ref|NM_004335.2| Homo sapiens bone marrow stromal cell antigen 2 (BST2), mRNA (SEQ ID NO: 14)
- >gil10433912|dbj|AK022494.1| Homo sapiens cDNA FLJ12432 fis, clone NT2RM1000018, highly similar to Human mRNA for KIAA0066 gene (SEQ ID NO: 15)
- 15 >gil13128859|ref|NM_004964.2| Homo sapiens histone deacetylase 1 (HDAC1), mRNA (SEQ ID NO: 16)
- >gil13277543|gb|BC003679.1| Homo sapiens ATP synthase, H⁺ transporting, mitochondrial F₀ complex, subunit E, mRNA (cDNA clone MGC:12532 IMAGE:4052832), complete cds (SEQ ID NO: 17)
- 20 >gil13277559|gb|BC003689.1| Homo sapiens high-mobility group nucleosomal binding domain 2, mRNA (cDNA clone IMAGE:3455121) (SEQ ID NO: 18)
- >gil13435956|gb|BC004815.1| Homo sapiens unc-119 homolog B (C. elegans), mRNA (cDNA clone IMAGE:3448346), complete cds (SEQ ID NO: 19)
- 25 >gil13477224|gb|BC005078.1| Homo sapiens coiled-coil domain containing 93, mRNA (cDNA clone IMAGE:3609481) (SEQ ID NO: 20)
- >gil13529184|gb|BC005359.1| Homo sapiens glia maturation factor, beta, mRNA (cDNA clone MGC:12462 IMAGE:3681628), complete cds (SEQ ID NO: 21)
- >gil19924164|ref|NM_002944.2| Homo sapiens c-ros oncogene 1 , receptor tyrosine kinase (ROS1), mRNA (SEQ ID NO: 22)
- 30 >gil20521835|dbj|D80000.2| Homo sapiens KIAA0178 mRNA, complete cds (SEQ ID NO: 23)
- >gil22035639|ref|NM_002413.3| Homo sapiens microsomal glutathione S-transferase 2 (MGST2), mRNA (SEQ ID NO: 24)

- >gil22035640|reflNM_004528.2| Homo sapiens microsomal glutathione S-transferase 3 (MGST3), mRNA (SEQ ID NO: 25)
- >gil25777722|reflNM_000689.3| Homo sapiens aldehyde dehydrogenase 1 family, member A1 (ALDH1A1), mRNA (SEQ ID NO: 26)
- 5 >gil25952110|reflNM_000594.2| Homo sapiens tumor necrosis factor (TNF), mRNA (SEQ ID NO: 27)
- >gil27894327|reflNM_003856.2| Homo sapiens interleukin 1 receptor-like 1 (IL1RL1), transcript variant 2, mRNA (SEQ ID NO: 28)
- >gil33873105|gb|BC004892.2| Homo sapiens reticulocalbin 2, EF-hand calcium
- 10 binding domain, mRNA (cDNA clone MGC:1650 IMAGE:3505241), complete cds (SEQ ID NO: 29)
- >gil33876989|gb|BC002666.2| Homo sapiens guanylate binding protein 1, interferon-inducible, 67kDa, mRNA (cDNA clone MGC:3949 IMAGE:3606865), complete cds (SEQ ID NO: 30)
- 15 >gil38197148|gb|BC000425.2| Homo sapiens protein disulfide isomerase family A, member 4, mRNA (cDNA clone MGC:8346 IMAGE:2819726), complete cds (SEQ ID NO: 31)
- >gil42544158|reflNM_006644.2| Homo sapiens heat shock 105kDa/110kDa protein 1 (HSPH1), mRNA (SEQ ID NO: 32)
- 20 >gil46249365|reflNM_006115.3| Homo sapiens preferentially expressed antigen in melanoma (PRAME), transcript variant 1, mRNA (SEQ ID NO: 33)
- >gil47132621|reflNM_002451.3| Homo sapiens methylthioadenosine phosphorylase (MTAP), mRNA (SEQ ID NO: 34)
- >gil48675809|reflNM_003118.2| Homo sapiens secreted protein, acidic, cysteine-rich
- 25 (osteonectin) (SPARC), mRNA (SEQ ID NO: 35)
- >gil56682943|reflNM_002922.3| Homo sapiens regulator of G-protein signaling 1 (RGS1), mRNA (SEQ ID NO: 36)
- >gil56699494|reflNM_006103.3| Homo sapiens WAP four-disulfide core domain 2 (WFDC2), mRNA (SEQ ID NO: 37)
- 30 >gil60302915|reflNM_030926.4| Homo sapiens integral membrane protein 2C (ITM2C), transcript variant 1, mRNA (SEQ ID NO: 38)
- >gil61742787|reflNM_002339.2| Homo sapiens lymphocyte-specific protein 1 (LSP1), transcript variant 1, mRNA (SEQ ID NO: 39)

- >gil63252872|reflNM_007295.2| Homo sapiens breast cancer 1, early onset (BRCA1), transcript variant BRCA1b, mRNA (SEQ ID NO: 40)
- >gil75709180|reflNM_002423.3| Homo sapiens matrix metalloproteinase 7 (matrilysin, uterine) (MMP7), mRNA (SEQ ID NO: 41)
- 5 >gil88853068|reflNM_000638.3| Homo sapiens vitronectin (VTN), mRNA (SEQ ID NO: 42)
- >gil90704850|reflNM_002984.2| Homo sapiens chemokine (C-C motif) ligand 4 (CCL4), transcript variant 1, mRNA (SEQ ID NO: 43)
- >gil94721245|reflNM_001632.3| Homo sapiens alkaline phosphatase, placental
- 10 (Regan isozyme) (ALPP), mRNA (SEQ ID NO: 44)
- >gil110618228|reflNM_001766.3| Homo sapiens CD1d molecule (CD1D), mRNA (SEQ ID NO: 45)
- >gil111120330|reflNM_016018.4| Homo sapiens PHD finger protein 20-like 1 (PHF20L1), transcript variant 1, mRNA (SEQ ID NO: 46)
- 15 >gil118572587|reflNM_001761.2| Homo sapiens cyclin F (CCNF), mRNA (SEQ ID NO: 47)
- >gil119393890|reflNM_000152.3| Homo sapiens glucosidase, alpha; acid (GAA), transcript variant 1, mRNA (SEQ ID NO: 48)
- >gil124053441|reflNM_000934.3| Homo sapiens serpin peptidase inhibitor, clade F
- 20 (alpha-2 antiplasmin, pigment epithelium derived factor), member 2 (SERPINF2), transcript variant 1, mRNA (SEQ ID NO: 49)
- >gil149193320|reflNM_002092.3| Homo sapiens G-rich RNA sequence binding factor 1 (GRSF1), transcript variant 1, mRNA (SEQ ID NO: 50)
- >gil149999367|reflNM_004394.2| Homo sapiens death-associated protein (DAP),
- 25 mRNA (SEQ ID NO: 51)
- >gil156151414|reflNM_021213.2| Homo sapiens phosphatidylcholine transfer protein (PCTP), transcript variant 1, mRNA (SEQ ID NO: 52)
- >gil156938332|reflNM_002933.4| Homo sapiens ribonuclease, RNase A family, 1 (pancreatic) (RNASE1), transcript variant 4, mRNA (SEQ ID NO: 53)
- 30 >gil157389013|reflNM_001408.2| Homo sapiens cadherin, EGF LAG seven-pass G-type receptor 2 (flamingo homolog, Drosophila) (CELSR2), mRNA (SEQ ID NO: 54)
- >gil168693661|reflNM_001344.2| Homo sapiens defender against cell death 1 (DAD1), mRNA (SEQ ID NO: 55)

- >gil169658386|reflNM_006294.3| Homo sapiens ubiquinol-cytochrome c reductase binding protein (UQCRB), nuclear gene encoding mitochondrial protein, mRNA (SEQ ID NO: 56)
- 5 >gil187608471|reflNM_000377.2| Homo sapiens Wiskott-Aldrich syndrome (eczema-thrombocytopenia) (WAS), mRNA (SEQ ID NO: 57)
- >gil187761347|reflNM_005263.3| Homo sapiens growth factor independent 1 transcription repressor (GFI1), transcript variant 1, mRNA (SEQ ID NO: 58)
- >gil188219598|reflNM_004688.2| Homo sapiens N-myc (and STAT) interactor (NMI), mRNA (SEQ ID NO: 59)
- 10 >gil188497702|reflNM_004186.3| Homo sapiens sema domain, immunoglobulin domain (Ig), short basic domain, secreted, (semaphorin) 3F (SEMA3F), mRNA (SEQ ID NO: 60)
- >gil194097322|reflNM_004092.3| Homo sapiens enoyl CoA hydratase, short chain, 1, mitochondrial (ECHS1), nuclear gene encoding mitochondrial protein, mRNA (SEQ ID NO: 61)
- 15 >gil194097416|reflNM_002710.2| Homo sapiens protein phosphatase 1, catalytic subunit, gamma isozyme (PPP1CC), mRNA (SEQ ID NO: 62)
- >gil197313774|reflNM_001554.4| Homo sapiens cysteine-rich, angiogenic inducer, 61 (CYR61), mRNA (SEQ ID NO: 63)
- 20 >gil207028465|reflNM_005566.3| Homo sapiens lactate dehydrogenase A (LDHA), transcript variant 1, mRNA (SEQ ID NO: 64)
- >gil215422332|reflNM_003878.2| Homo sapiens gamma-glutamyl hydrolase (conjugase, folylpolyglutamyl hydrolase) (GGH), mRNA (SEQ ID NO: 65)
- >gil221218992|reflNM_003475.3| Homo sapiens Ras association (RalGDS/AF-6) domain family (N-terminal) member 7 (RASSF7), transcript variant 1, mRNA (SEQ ID NO: 66)
- 25 >gil222537757|reflNM_002658.3| Homo sapiens plasminogen activator, urokinase (PLAU), transcript variant 1, mRNA (SEQ ID NO: 67)
- >gil226371666|reflNM_014750.4| Homo sapiens discs, large (Drosophila) homolog-associated protein 5 (DLGAP5), transcript variant 1, mRNA (SEQ ID NO: 68)
- 30 >gil239835753|reflNM_002970.2| Homo sapiens spermidine/spermine N1-acetyltransferase 1 (SAT1), transcript variant 1, mRNA (SEQ ID NO: 69)
- >gil269973857|reflNM_001557.3| Homo sapiens chemokine (C-X-C motif) receptor 2 (CXCR2), transcript variant 1, mRNA (SEQ ID NO: 70)

- >gil270288734|reflNM_000270.3| Homo sapiens purine nucleoside phosphorylase (PNP), mRNA (SEQ ID NO: 71)
- >gil289063431|reflNM_006025.3| Homo sapiens endonuclease, polyU-specific (ENDOU), transcript variant 2, mRNA (SEQ ID NO: 72)
- 5 >gil291463261|reflNM_006129.4| Homo sapiens bone morphogenetic protein 1 (BMP1), transcript variant 3, mRNA (SEQ ID NO: 73)
- >gil295317373|reflNM_004797.3| Homo sapiens adiponectin, C1Q and collagen domain containing (ADIPOQ), transcript variant 2, mRNA (SEQ ID NO: 74)
- >gil296011050|reflNM_000759.3| Homo sapiens colony stimulating factor 3 (granulocyte) (CSF3), transcript variant 1, mRNA (SEQ ID NO: 75)
- 10 >gil1422918|gb|W93728.1|W93728 zd96a11.s1 Soares_fetal_heart_NbHH19W Homo sapiens cDNA clone IMAGE:357308 3', mRNA sequence (SEQ ID NO: 76)
- >gil2269409|gb|AA527340.1|AA527340 ng36d08.s1 NCI_CGAP_Co3 Homo sapiens cDNA clone IMAGE:936879 3', mRNA sequence (SEQ ID NO: 77)
- 15 >gil2896270|gb|AA824386.1|AA824386 aj29c05.s1 Soares_testis_NHT Homo sapiens cDNA clone 1391720 3', mRNA sequence (SEQ ID NO: 78)
- >gil3701541|gb|AI168371.1|AI168371 qa24h02.s1 Soares_NhHMPu_S1 Homo sapiens cDNA clone IMAGE:1687731 3', mRNA sequence (SEQ ID NO: 79)
- >gil5178449|gb|AI762782.1|AI762782 wi04c05.x1 NCI_CGAP_CLL1 Homo sapiens cDNA clone IMAGE:2389256 3' similar to gb:M74715 ALPHA-L-IDURONIDASE PRECURSOR (HUMAN);, mRNA sequence (SEQ ID NO: 80)
- 20 >gil5366485|gb|AI801013.1|AI801013 wg15d09.x1 Soares_NSF_F8_9W_OT_PA_P_S1 Homo sapiens cDNA clone IMAGE:2365169 3', mRNA sequence (SEQ ID NO: 81)
- 25 >gil5659456|gb|AI923492.1|AI923492 wn86a02.x1 NCI_CGAP_Ut1 Homo sapiens cDNA clone IMAGE:2452682 3' similar to gb:X13111 HLA CLASS I HISTOCOMPATIBILITY ANTIGEN, A-11 A*1101/A*1102 ALPHA (HUMAN);contains element L1 repetitive element ;, mRNA sequence (SEQ ID NO: 82)
- 30 >gil7316913|gb|AW611727.1|AW611727 hg86h08.x1 NCI_CGAP_Kid11 Homo sapiens cDNA clone IMAGE:2952543 3' similar to SW:GAS1_HUMAN P54826 GROWTH-ARREST-SPECIFIC PROTEIN 1 ;, mRNA sequence (SEQ ID NO: 83)
- >gil10146644|gb|BE732652.1|BE732652 601571266F1 NIH_MGC_21 Homo sapiens cDNA clone IMAGE:3925601 5', mRNA sequence (SEQ ID NO: 84)

- >gil10211355|gb|BE790157.1|BE790157 601482981F1 NIH_MGC_68 Homo sapiens cDNA clone IMAGE:3885236 5', mRNA sequence (SEQ ID NO: 85)
- >gil10367069|gb|BE899402.1|BE899402 601681419F1 NIH_MGC_9 Homo sapiens cDNA clone IMAGE:3951724 5', mRNA sequence (SEQ ID NO: 86)
- 5 >gil10717503|gb|AV701173.1|AV701173 AV701173 ADA Homo sapiens cDNA clone ADAAGH04 5', mRNA sequence (SEQ ID NO: 87)
- >gil10851495|gb|AV733950.1|AV733950 AV733950 cdA Homo sapiens cDNA clone cdAADG12 5', mRNA sequence (SEQ ID NO: 88)
- >gil10941619|gb|BF112006.1|BF112006 7137e05.x1
- 10 Soares_NSF_F8_9W_OT_PA_P_S1 Homo sapiens cDNA clone IMAGE:3523665 3', mRNA sequence (SEQ ID NO: 89)
- >gil12770210|gb|BG260394.1|BG260394 602371523F1 NIH_MGC_93 Homo sapiens cDNA clone IMAGE:4479556 5', mRNA sequence (SEQ ID NO: 90)
- >gil13046289|gb|BG289967.1|BG289967 602381386F1 NIH_MGC_93 Homo sapiens cDNA clone IMAGE:4499085 5', mRNA sequence (SEQ ID NO: 91)
- 15 >gil13341399|gb|BG434893.1|BG434893 602507842F1 NIH_MGC_79 Homo sapiens cDNA clone IMAGE:4604891 5', mRNA sequence (SEQ ID NO: 92)
- >gil45652030|gb|AL515318.3|AL515318 AL515318 Homo sapiens NEUROBLASTOMA Homo sapiens cDNA clone CL0BB030ZH05 5-PRIME, mRNA sequence (SEQ ID NO: 93)
- 20 >gil45699297|gb|AL524035.3|AL524035 AL524035 Homo sapiens NEUROBLASTOMA COT 25-NORMALIZED Homo sapiens cDNA clone CS0DC003YN06 3-PRIME, mRNA sequence (SEQ ID NO: 94)
- >gil6005805|ref|NM_007164.1| Homo sapiens mucosal vascular addressin cell adhesion molecule 1 (MADCAM1), mRNA (SEQ ID NO: 95)
- 25 Below is an additional sequence representative of MADCAM1:
- >gil109633021|ref|NM_130760.2| Homo sapiens mucosal vascular addressin cell adhesion molecule 1 (MADCAM1), transcript variant 1, mRNA (SEQ ID NO: 96)
- >gil6006030|ref|NM_003197.2| Homo sapiens transcription elongation factor B (SIII), polypeptide 1-like (TCEB1L), mRNA (SEQ ID NO: 97)
- 30 >gil63252872|ref|NM_007295.2| Homo sapiens breast cancer 1, early onset (BRCA1), transcript variant BRCA1b, mRNA (SEQ ID NO: 98)
- Other representative BRCA1-gene sequences known in the art include, for example:

- >gil237757283|ref|NM_007294.3| Homo sapiens breast cancer 1, early onset (BRCA1), transcript variant 1, mRNA (SEQ ID NO: 99)
- >gil237681126|ref|NR_027676.1| Homo sapiens breast cancer 1, early onset (BRCA1), transcript variant 6, non-coding RNA (SEQ ID NO: 100)
- 5 >gil237681124|ref|NM_007299.3| Homo sapiens breast cancer 1, early onset (BRCA1), transcript variant 5, mRNA (SEQ ID NO: 101)
- >gil237681122|ref|NM_007298.3| Homo sapiens breast cancer 1, early onset (BRCA1), transcript variant 4, mRNA (SEQ ID NO: 102)
- >gil237681120|ref|NM_007297.3| Homo sapiens breast cancer 1, early onset
- 10 (BRCA1), transcript variant 3, mRNA (SEQ ID NO: 103)
- >gil237681118|ref|NM_007300.3| Homo sapiens breast cancer 1, early onset (BRCA1), transcript variant 2, mRNA (SEQ ID NO: 104)

Various aspects of the present invention may be used alone, in combination, or

15 in a variety of arrangements not specifically discussed in the embodiments described in the foregoing and is therefore not limited in its application to the details and arrangement of components set forth in the foregoing description or illustrated in the drawings. For example, aspects described in one embodiment may be combined in any manner with aspects described in other embodiments.

20 Also, the invention may be embodied as a method, of which an example has been provided. The acts performed as part of the method may be ordered in any suitable way. Accordingly, embodiments may be constructed in which acts are performed in an order different than illustrated, which may include performing some acts simultaneously, even though shown as sequential acts in illustrative

25 embodiments.

Use of ordinal terms such as "first," "second," "third," etc., in the claims to modify a claim element does not by itself connote any priority, precedence, or order of one claim element over another or the temporal order in which acts of a method are performed, but are used merely as labels to distinguish one claim element having a

30 certain name from another element having a same name (but for use of the ordinal term) to distinguish the claim elements.

Also, the phraseology and terminology used herein is for the purpose of description and should not be regarded as limiting. The use of "including," "comprising," or "having," "containing," "involving," and variations thereof herein, is

meant to encompass the items listed thereafter and equivalents thereof as well as additional items.

Having described several embodiments of the invention in detail, various modifications and improvements will readily occur to those skilled in the art. Such
5 modifications and improvements are intended to be within the spirit and scope of the invention. Accordingly, the foregoing description is by way of example only, and is not intended as limiting. The invention is limited only as defined by the following claims and the equivalents thereto.

10 What is claimed is:

CLAIMS

1. A method of assisting in the evaluation of a subject having a tumor for treatment with a chemotherapeutic compound, the method comprising
 - 5 (a) obtaining an expression profile of the tumor, the profile comprising at least the expression level of one gene selected from the group of genes consisting of all or of some of the genes listed in any of Tables 1-4; and
 - (b) determining whether the tumor expression profile is either similar to a first reference expression profile indicative of tumor sensitivity to the chemotherapeutic
 - 10 compound or to a second reference expression profile indicative of tumor resistance to the chemotherapeutic compound.
2. The method of claim 1, wherein the tumor is an epithelial ovarian tumor.
- 15 3. The method of claim 1 or 2, further comprising
 - (c) administering the chemotherapeutic compound to the subject based on the tumor expression profile being similar to the first reference expression profile, or
 - (d) not administering the chemotherapeutic compound to the subject based on the tumor expression profile being similar to the second reference expression profile.
- 20 4. The method of any of claims 1-3, wherein the chemotherapeutic compound is a platinum compound.
5. The method of any of claims 1-4, wherein the chemotherapeutic compound is a
- 25 PARP inhibitor.
6. The method of any of claims 1-5, wherein the subject does not carry a germline mutation of the BRCA1 or the BRCA2 gene.
- 30 7. A method of classifying a tumor as "BRCA-like" or "non-BRCA-like", the method comprising,
 - (a) obtaining an expression profile of the tumor, the profile comprising at least one gene selected from the group of genes consisting of the genes listed in any of Tables 1-4;

(b) assigning the tumor to the class “BRCA-like” or the class “non-BRCA-like” based on the expression profile in accordance with a classifier comprising the at least one gene.

5 8. The method of claim 7, wherein the classifier is a diagonal linear discriminant predictor.

9. The method of claim 7 or 8, wherein the classifier is a compound covariate predictor.

10

10. The method of any of claims 7-9, wherein the classifier is a nearest centroid predictor.

11. The method of any of claims 7-10, wherein the classifier is a support vector
15 machines predictor.

12. The method of any of claims 7-11, further comprising

(c) assigning a confidence value to the classification of the tumor as “BRCA-like” or “non-BRCA-like”.

20

13. The method of claim 12, wherein

if the tumor is classified as “BRCA-like”, then the tumor is indicated to be sensitive to a chemotherapeutic compound, or

if the tumor is classified as “non-BRCA-like”, then the tumor is indicated to
25 be resistant to the chemotherapeutic compound.

14. The method of claim 12, wherein

if the tumor is classified as “BRCA-like” with a confidence of at least about 25%, at least about 30%, at least about 40%, at least about 50%, at least about 60%, at
30 least about 70%, at least about 80%, at least about 90%, at least about 95%, or at least about 99%, then the tumor is indicated to be sensitive to a chemotherapeutic compound, or

if the tumor is classified as “non-BRCA-like” with a confidence at least about 25%, at least about 30%, at least about 40%, at least about 50%, at least about 60%, at least

about 70%, at least about 80%, at least about 90%, at least about 95%, or at least about 99%, then the tumor is indicated to be resistant to the chemotherapeutic compound.

5 15. The method of claim 13, wherein the chemotherapeutic compound is a platinum compound.

16. The method of claim 13, wherein the chemotherapeutic compound is a PARP inhibitor.

10

17. The method of any of claims 7-16, wherein the tumor is an epithelial ovarian tumor.

18. A method of identifying one or more informative marker genes for a class distinction between "BRCA-like" and "non-BRCA-like" of tumors, the method comprising

15 (a) obtaining expression profiles from a plurality of tumors known to harbor a BRCA1 or BRCA2 mutation or harbor neither BRCA1 or BRCA2 mutation (sporadic tumors) wherein the expression profiles comprise a plurality of candidate marker
20 genes

(b) performing hierarchical clustering analysis to define

i) which sporadic tumors co-cluster with BRCA1 or BRCA2 tumors

ii) which BRCA1 or BRCA2 tumors co-cluster with sporadic tumors

(c) excluding tumors identified in i)+ii) above

25 (d) defining as "BRCA-like" tumors, the remaining BRCA1 or BRCA2 after exclusion of ii)

(e) defining as "non-BRCA-like" tumors, the remaining sporadic tumors after exclusion of i)

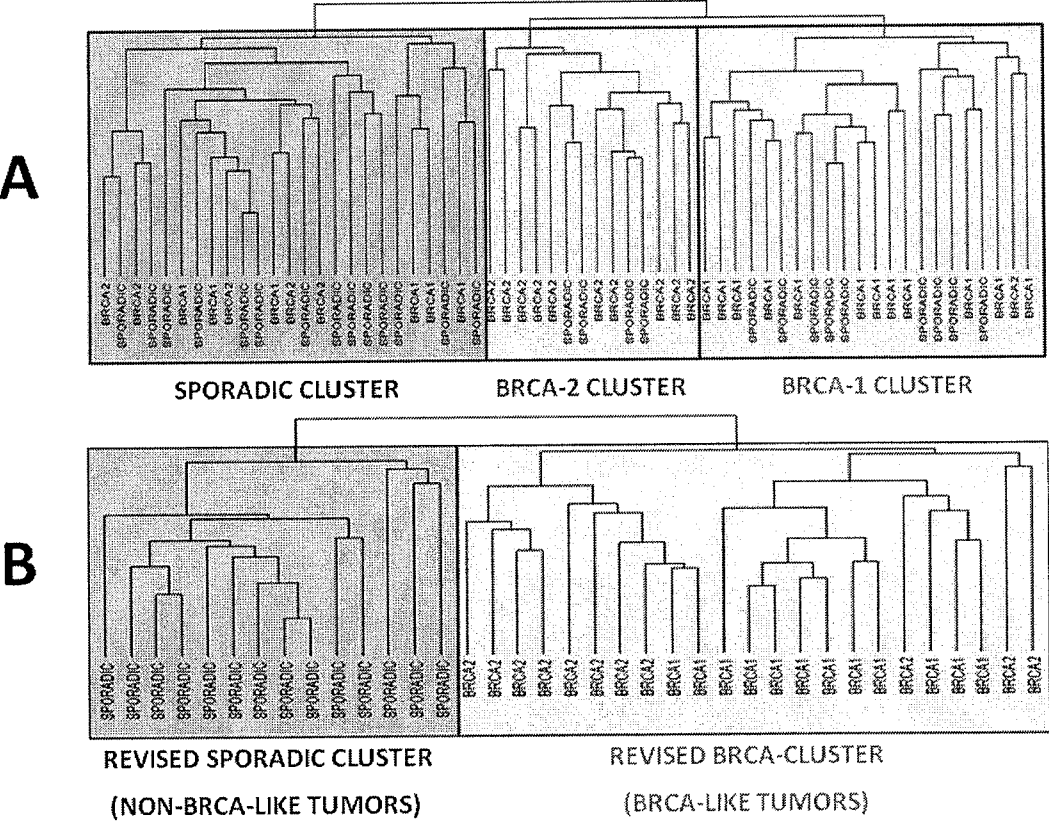
(f) sorting the candidate marker genes using a neighborhood analysis algorithm, wherein the genes are sorted by degree to which their expression in the
30 profiled tumors correlates with the class distinction; and

(g) determining whether said correlation is stronger than expected by chance, wherein marker genes for which the correlation with the class distinction is stronger than expected by chance are informative marker genes.

19. The method of claim 18, further comprising
(d) building a classifier comprising at least one of the informative marker genes.
- 5
20. The method of claim 19, wherein the classifier is a diagonal linear discriminant predictor.
21. The method of claim 19, wherein the classifier is a compound covariate predictor.
- 10
22. The method of claim 19, wherein the classifier is a nearest centroid predictor.
23. The method of claim 19, wherein the classifier is a support vector machines predictor.
- 15
24. The method of claim 19, further comprising
(e) obtaining an expression profile from a tumor of unknown class, wherein the expression profile comprises the at least one informative marker gene, and
(f) classifying the tumor as either "BRCA-like" or "non-BRCA-like" based on
20 the expression profile in accordance to the classifier.
25. A kit for the generation of a tumor expression profile for the classification of a tumor as "BRCA-like" or "non-BRCA-like", the kit comprising
a plurality of nucleic acids corresponding to a plurality of informative marker
25 genes for the classification of a tumor as "BRCA-like" or "non-BRCA-like", wherein the plurality of informative marker genes is chosen from the group consisting of the genes listed in any of Tables 1-4.
26. A kit for the generation of a tumor expression profile for the classification of a
30 tumor as "BRCA-like" or "non-BRCA-like", the kit comprising
a detection agent for the determining the expression level of at least one of the genes of any of Tables 1-4, or
a binding agent that binds to a gene product expressed from an informative gene, wherein the informative gene is a gene listed in any of Tables 1-4.

27. The kit of claim 25 or 26, wherein the detection agent is an antibody or a fragment thereof that selectively binds to a protein expressed from the at least one gene.
- 5
28. The kit of claim 25 or 26, wherein the detection agent is a nucleic acid molecule that selectively binds to a nucleic acid expressed from the at least one gene.
29. The kit of any of claims 25-28, wherein the kit comprises
- 10 a plurality of nucleic acids corresponding to a plurality of informative marker genes for the classification of a tumor as “BRCA-like” or “non-BRCA-like”, wherein the plurality of informative marker genes is chosen from the group consisting of the genes listed in any of Tables 1-4.
- 15 30. The kit of any of claims 25, 28 or 29, wherein the nucleic acids are immobilized on a solid support.
31. The kit of any of claims 25-30, further comprising
- reagents for the generation of the tumor expression profile, the reagents comprising
- 20 RNA extraction reagents, a reverse transcriptase, a DNA polymerase, a buffering reagent, nucleotides, a fluorescent label, and/or a hybridization reagent.

Figure 1.



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Figure 2.

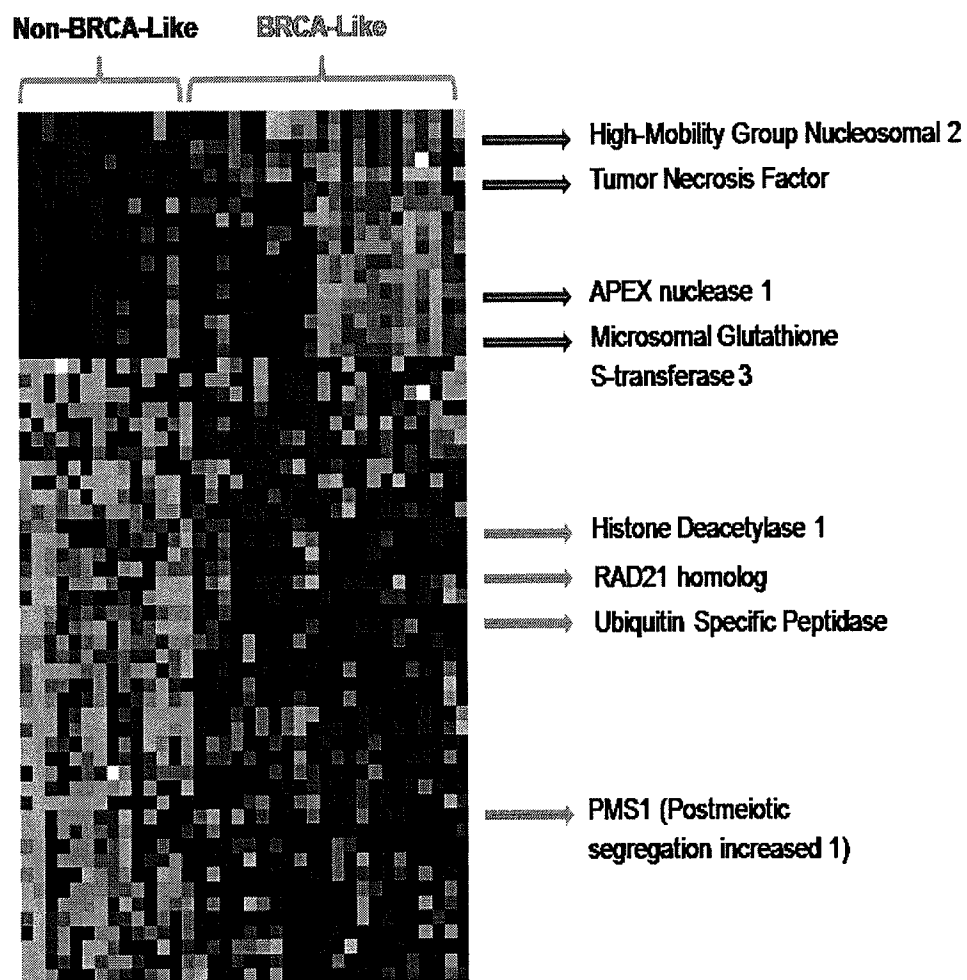


Figure 3.

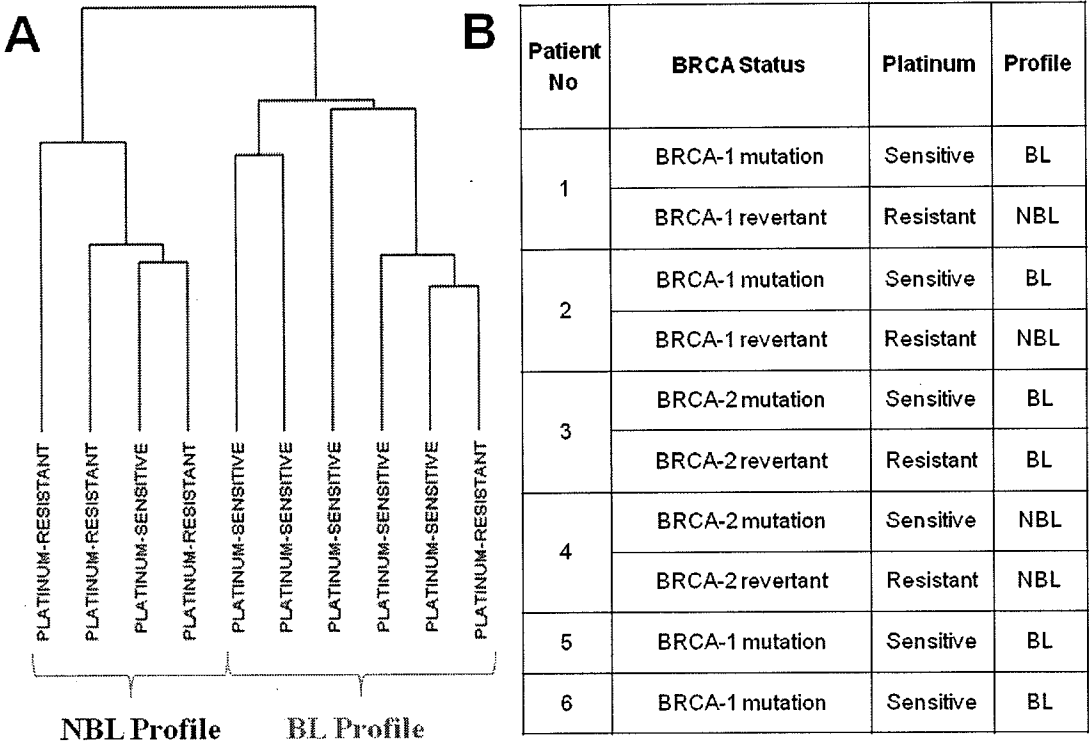


Figure 4.

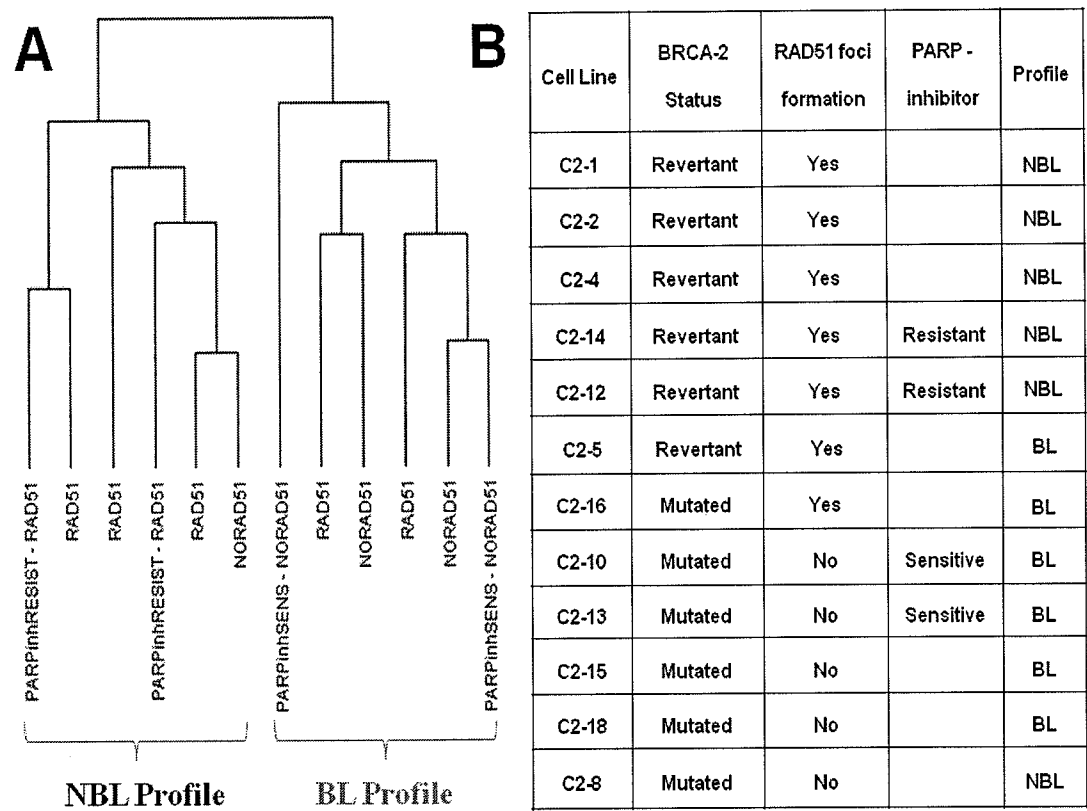


Figure 5.

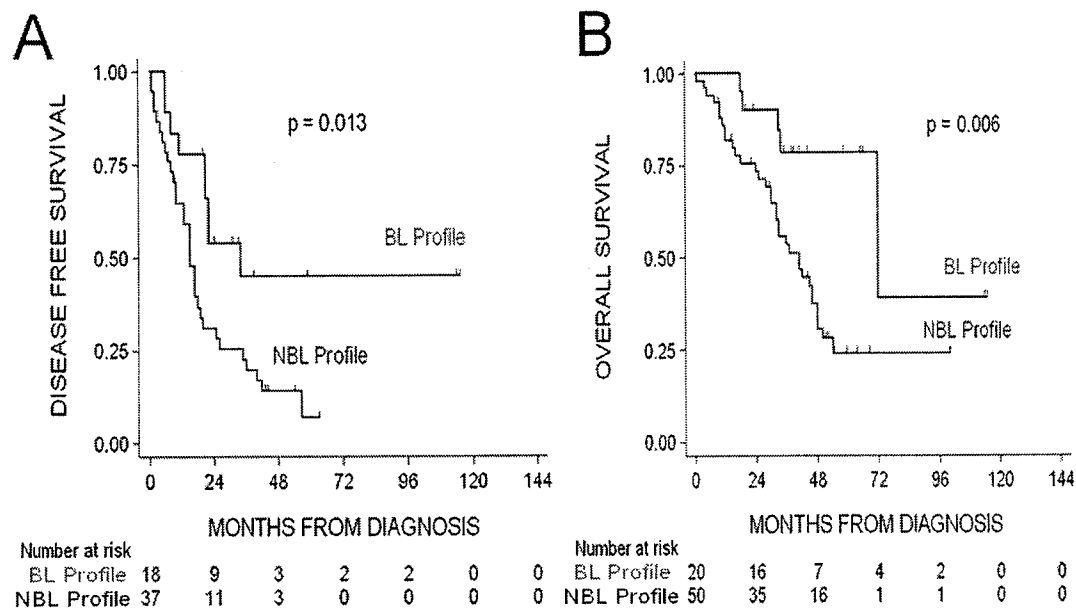
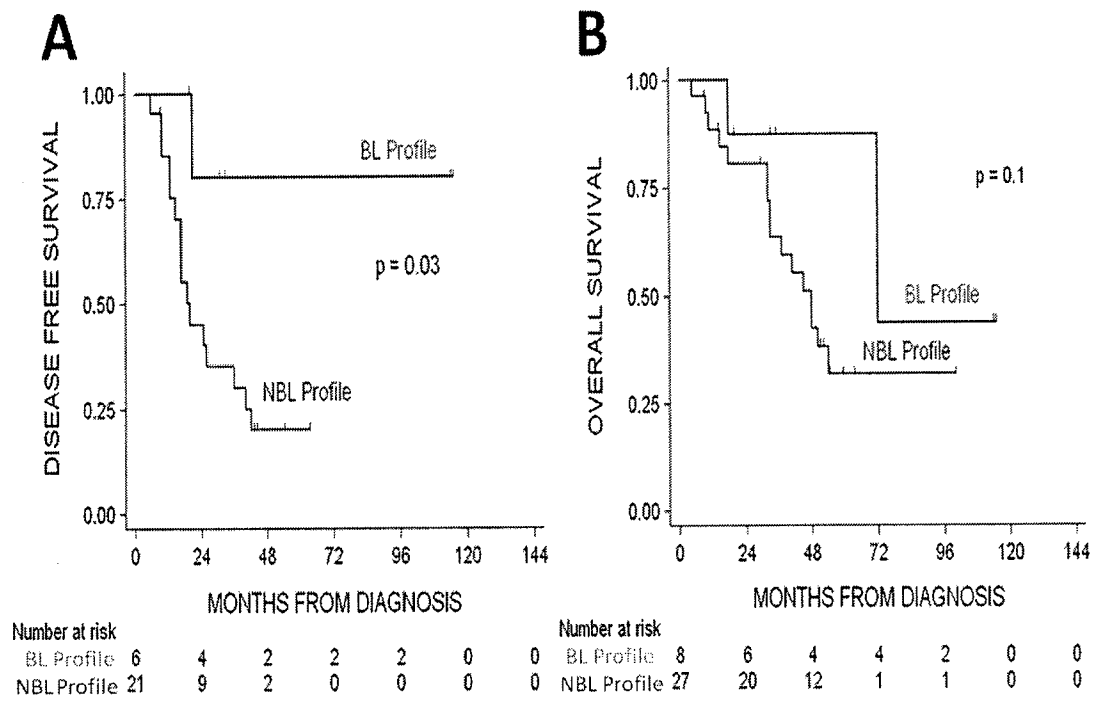


Figure 6.



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Figure 7.

