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(54) **Title**: DNA REPAIR OR BRCA1-LIKE GENE SIGNATURE

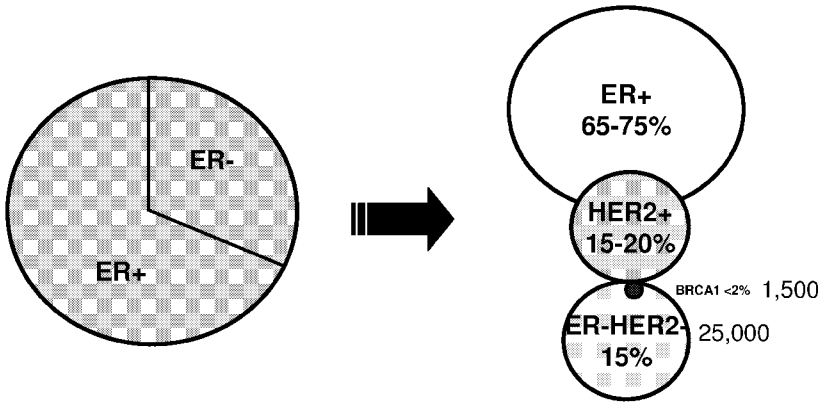


FIG. 1

(57) **Abstract**: The present invention concerns the identification of individuals that have triple negative breast cancer and/or identification of an appropriate treatment therefor. In certain cases, the identification includes determining the expression levels of a multitude of genes.

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DNA REPAIR OR BRCA1-LIKE GENE SIGNATURE

STATEMENT REGARDING FEDERALLY SPONSORED RESEARCH OR DEVELOPMENT

[0001] This invention was made with government support under Grant Nos. 5RO1CA112305, 5RO1CA138197, and SPORE P50 CA50183, awarded by National Institutes of Health/National Cancer Institute, and US Army Medical Research and Materiel Command DAMD17-01-0132 and W81XWH-04-1-0468. The government has certain rights in the invention.

[0002] This application claims priority to U.S. Provisional Application Serial No. 61/182,349, filed May 29, 2009, and U.S. Provisional Application Serial No. 61/267,977, filed December 9, 2009, both of which applications are incorporated by reference herein in their entirety.

TECHNICAL FIELD

[0003] The present invention concerns at least the fields of molecular genetics, cell biology, molecular biology, and medicine.

BACKGROUND OF THE INVENTION

[0004] Approximately 10% to 15% of breast carcinomas are considered "triple–receptor-negative" for lacking expression of estrogen receptor (ER) and progesterone receptor (PR) and lacking overexpression and/or gene amplification of HER2/neu). Triple-negative breast cancers include about 85% of all basal-type tumors. It is characterized by its unique molecular profile, aggressive behavior, particular patterns of metastasis, and scarcity of targeted therapies. In certain cases, the majority of triple-negative breast cancers carry the "basal-like" molecular profile on gene expression arrays. Mutations in the BRCA1 gene can result in breast cancer. The majority of these BRCA1-associated breast cancers are triple-negative and basal-like. Epidemiologic studies illustrate a high prevalence of triple-negative breast cancers among younger women and those of African descent. Increasing evidence suggests that the risk factor profile differs between this subtype and the more common luminal subtypes and within this subtype. Although sensitive to chemotherapy (including anthracycline- and taxane-based treatments), it is common for individuals to have an early relapse and an inclination for visceral

metastasis, including brain metastasis, is observed. Some patients do not respond to standard therapy and have a poor prognosis.

[0005] Most BRCA1-associated breast cancers are triple-negative, and dysfunctional BRCA1 renders cancer cells deficient in double-stranded DNA break repair mechanisms and sensitive to DNA damaging agents (for example, platinum salts and topoisomerase I inhibitors). In particular, BRCA1 function is a sensor for DNA damage and is involved in double-strand DNA break repair. It is involved in cell cycle checkpoint control, apoptosis in response to DNA damage, and it is a transcription factor involved in hormone receptor regulated gene expression (Brody, 2005).

[0006] The histological characteristics of tumors from individuals carrying BRCA1 mutation are shared with tumors from some individuals not carrying the BRCA1 mutation, particularly the high grade and high proliferation. Classic BRCA1 phenotype involves the following: negative hormonal receptor status; negative HER-2/neu status; histological grade 3; high proliferation rate; pushing margins; lymphocytic infiltrate*; CK5/6+ and/or EGFR+, p53+ (Marcus *et al.*, 1996) Germline BRCA1 mutations account for 20% of breast cancers that appear to be inherited, which is only <2% of all breast cancers. Also, tumors from BRCA1 carriers have somatic inactivation of their second wild-type allele. The present invention addresses a need in the art at least to provide guidance for therapy for individuals with breast cancer, including triple negative breast cancer.

BRIEF SUMMARY OF THE INVENTION

[0007] In a certain embodiment, the present invention concerns personalizing treatment for individuals with triple negative breast cancer. The present invention, in specific embodiments, concerns identification of sporadic triple negative breast cancers with BRCA1 deficiency or DNA repair deficiences. In further specific embodiments, the present invention concern identification of individuals with BRCA1 deficiency or DNA repair deficiences or concerns stratification of patients with BRCA1 deficiency or DNA repair deficiences in therapeutic trials. In some embodiments of the invention, the present invention concerns determination of effective therapy for an individual with breast cancer, such as triple negative breast cancer.

[0008] Using a public database of triple negative breast cancers and BRCA1 mutation carriers, the inventors have identified a gene signature that can differentiate two groups of sporadic triple negative breast cancer: 1) highly sensitive to anthracycline-based chemotherapy due to BRCA1 deficiency or DNA repair deficiences; and 2) anthracycline-resistant group that exhibits sensitivity to dasatinib.

[0009] In certain embodiments of the invention, there are methods and compositions for determining which patients will benefit from DNA-damaging agents (for example, cisplatin, cyclophosphamide, irinotecan hydrochloride, gemcitabine hydrochloride, Temozolomide) or PARP inhibitors (for example, AZD2281 or AG14361, NU1025, ABT-888, KU-0059436 (AZD2281), MK4827, AG014699, BSI-201, E7016) versus those who will benefit more from taxane-based therapy (for example, paclitaxel, docetaxel, BMS-275183).

[0010] In certain embodiments, the present invention concerns identification of the expression of 1 or more; 2 or more, 3 or more, 4 or more, 5 or more, 6 or more, 7 or more, 8 or more, 9 or more, 10 or more, 11 or more, 12 or more, 13 or more, 14 or more, 15 or more, 16 or more, 17 or more, 18 or more, 19 or more, 20 or more, 21 or more, 22 or more, 23 or more, 24 or more, 25 or more, 26 or more, 27 or more, 28 or more, 29 or more, 30 or more, 31 or more, 32 or more, 33 or more, 34 or more, 35 or more, 36 or more, 37 or more, 38 or more, 39 or more, 40 or more, 41 or more, 42 or more, 43 or more, 44 or more, 45 or more, 46 or more, 47 or more, 48 or more, 49 or more, 50 or more, 51 or more, 52 or more, 53 or more, 54 or more, 55 or more, 56 or more, 57 or more, 58 or more, 59 or more, 60 or more, 61 or more, 62 or more, 63 or more, 64 or more, 65 or more, 66 or more, 67 or more, or 68 or more of the 69 genes listed in Table 1 to identify triple negative breast cancer. In certain embodiments, the present invention concerns identification of the expression of at least 96%, at least 95%, at least 90%, at least 85%, at least 80%, at least 75%, at least 70%, at least 65%, at least 60%, at least 55%, at least 50%, at least 45%, at least 40%, at least 35%, at least 30%, at least 25%, at least 20%, at least 15%, at least 10%, or at least 5% of the genes listed in Table 1 to identify triple negative breast cancer.

[0011] In specific embodiments, the methods and compositions of the present invention are utilized in lieu of or in addition to other methods and compositions for identification of triple negative breast cancer, for example immunohistochemistry.

[0012] In other embodiments, the present invention provides a quantitative test for prognosis determination in cancer patients. The test concerns measurements of the tumor levels

of certain messenger RNAs (mRNAs). These mRNA levels are inserted into an algorithm that yields a numerical recurrence score, which indicates identification of triple negative breast cancer and/or a particular optimal course of therapy.

[0013] In one embodiment of the invention, there is a method of identifying triple negative breast cancer from a sample from an individual that has triple negative breast cancer, is suspected of having triple negative breast cancer, or is receiving or has received treatment for breast cancer, including triple negative breast cancer, comprising the step of assaying the expression of two or more sequences from breast cells of the individual, said sequences selected from the group consisting of genes listed in Table 1, or the complement of said sequences.

[0014] In another embodiment of the invention, there is a method of determining a therapy for an individual with triple negative breast cancer, who is suspected of having triple negative breast cancer, or who is receiving or has received treatment for triple negative breast cancer, comprising the step of assaying the expression of two or more sequences from breast cells of the individual, said sequences selected from the group consisting of genes listed in Table 1, or the complement of said sequences.

[0015] In an additional embodiment of the invention, there is a plurality of primers for polymerizing at least two or more sequences selected from the group consisting of genes listed in Table 1, or the complement of said sequence or of a sequence capable of hybridizing to the sequence under stringent conditions.

[0016] In one embodiment of the invention, there is a collection of oligonucleotides that correspond to two or more, three or more, four or more, five or more, six or more, seven or more, eight or more, nine or more, ten or more, eleven or more, twelve or more, thirteen or more, fourteen or more, fifteen or more, sixteen or more, seventeen or more, eighteen or more, nineteen or more, twenty or more, twenty-one or more, twenty-two or more, twenty-three or more, twenty-four or more, twenty-five or more, twenty-six or more, twenty-seven or more, twenty-eight or more, twenty-nine or more, thirty or more, thirty-one or more, thirty-two or more, thirty-three or more, thirty-four or more, thirty-five or more, thirty-six or more, thirty-seven or more, thirty-eight or more, thirty-nine or more, forty or more, forty-one or more, forty-two or more, forty-three or more, forty-four or more, forty-five or more, forty-six or more, forty-seven or more, forty-eight or more, forty-nine or more, fifty or more, fifty-one or more, fifty-two or more, fifty-three or more, fifty-four or more, fifty-five or more, fifty-six or more, fifty-seven or more,

fifty-eight or more, fifty-nine or more, sixty or more, sixty-one or more, sixty-two or more, sixty-three or more, sixty-four or more, sixty-five or more, sixty-six or more, sixty-seven or more, or sixty-eight or more of the genes listed in Table 1, said oligonucleotides housed on a substrate. The term "oligonucleotide" in certain aspects refers to a molecule of between about 3 and about 100 nucleobases in length, for example. The oligonucleotides may be considered to correspond to a gene by encompassing a fragment of the gene or the complement thereof. Thus, the oligonucleotide in specific embodiments may hybridize to an mRNA expressed from the gene. In particular embodiments, the oligonucleotide is at least 10, 12, 15, 20, 25, 30, 35, 40, 45, 50, 55, 60, 65, 70, 75, 80, 85, 90, or 95 nucleotides in length. In certain aspects, the oligonucleotide encompasses a range of lengths, for example, from 8-15, 10-15, 12-15, 10-20, 15-20, 18-20, 20-25, 22-25, 20-30, 25-30, or 27-30 nucleotides in length, and so on.

[0017] In an additional embodiment of the invention, there is a collection of two or more, three or more, four or more, five or more, six or more, seven or more, eight or more, nine or more, ten or more, twelve or more, fifteen or more, twenty or more, twenty-two or more, twenty-five or more, thirty or more, thirty-five or more, forty or more, forty-five or more, fifty or more, fifty-five or more, sixty or more, sixty-five or more, sixty-seven or more, or all of the genes listed in Table 1, said collection housed on a substrate.

[0018] In another embodiment, there is as a composition of matter, a breast cancer RNA expression profile comprising two or more, three or more, four or more, five or more, six or more, seven or more, eight or more, nine or more, ten or more, twelve or more, fifteen or more, twenty or more, twenty-two or more, twenty-five or more, thirty or more, thirty-five or more, forty or more, forty-five or more, fifty or more, fifty-five or more, sixty or more, sixty-five or more, sixty-seven or more, or all of the genes listed in Table 1.

[0019] In an additional embodiment, there is as a composition of matter, isolated expressed polynucleotides the levels of which are indicative of the presence of triple negative breast cancer or indicative of a therapy for triple negative breast cancer, wherein two or more, three or more, four or more, five or more, six or more, seven or more, eight or more, nine or more, ten or more, twelve or more, fifteen or more, twenty or more, twenty-two or more, twenty-five or more, thirty or more, thirty-five or more, forty or more, forty-five or more, fifty or more, fifty-five or more, sixty or more, sixty-five or more, sixty-seven or more, or all of the expressed polynucleotides are listed in Table 1, for example.

[0020] In one embodiment of the invention, there is a method for determining the likelihood of breast cancer response to DNA-damaging therapy such as anthracycline or platinum based or to therapies affecting DNA repair such as PARP(poly-ADP ribose polymerase)inhibitors in a mammalian subject comprising: (a) measuring the expression levels of the RNA transcripts of APOBEC3B, NAP1L3, CXCL10, HMGA1, IRF1, ISG20, USP13, IL32, HSP14A, TP53BP2, FBLN1, CDH5, LAMA4, PCOLCE, COL15A1, SERPINF1, PDGFRA, EFEMP2, LHFP, HTRA1, ITGB5, CTSK, FBN1, PDGFRB, and IGFBP4, or their expression products in a biological sample containing tumor cells obtained from said subject; (b) creating the following gene subsets comprising: (i) underexpressed genes subset: CDH5, LAMA4, PCOLCE, COL15A1, SERPINF1, PDGFRA, EFEMP2, LHFP, HTRA1, ITGB5, CTSK, FBN1, PDGFRB, and IGFBP4, and (ii) overexpressed genes subset: APOBEC3B, NAP1L3, CXCL10, HMGA1, IRF1, ISG20, USP13, IL32, HSP14A, TP53BP2, (c) calculating a likelihood score for said subject by weighting the measured expression levels of each of the gene subsets by contribution to response to DNA targeted therapy; (d) using said score to determine the likelihood of response to therapy; and (e) creating a report summarizing the result of said determination.

[0021] For the purposes of certain embodiments of this invention, triple negative breast cancer may be categorized into BRCA1-like (at least having DNA repair deficiency and being sensitive to DNA damaging agents) and non-BRCA1-like (at least having normal DNA repair and being resistant to DNA damaging agents) cancers, which may be tumors. In particular embodiments of the invention, certain genes are overexpressed in BRCA1-like (DNA repair-deficient tumors) triple negative tumors: APOBEC3B, USP13, HSP14A, HMGA1, SLC5A6, CXCL10, ISG20, TP53BP2, NAP1L3, and HDGF, and any combinations thereof. All of the other genes listed in Table 2 herein are overexpressed in nonBRCA1-like (normal DNA repair tumors).

BRIEF DESCRIPTION OF THE DRAWINGS

[0022] FIG. 1 illustrates that breast cancer is not one disease.

[0023] FIG. 2 demonstrates a BRCA-associated expression pattern.

[0024] FIG. 3 shows that BRCA1-associated tumors are more sensitive to anthracyclines than sporadic triple negatives.

[0025] FIG. 4 illustrates redirecting therapies in triple negative breast cancer.

[0026] FIG. 5 concerns the BRCA1 gene expression signature in a Nature 2002 paper (van't Veer *et al.*, 2002).

[0027] FIG. 6 shows convergence of 182 genes that overlap from a previously published BRCA1 gene expression signature that was applied to 3 datasets of triple negative breast cancer.A-BCM (Baylor College of Medicine), B-Wang (publically available), C-Netherlands (publically available) From each dataset, a new list of genes had been selected by obtaining the most differentially expressed genes between those tumors that exhibited the pattern most like "sporadic" tumors versus those tumors that exhibited the pattern of BRCA1 mutation carrier tumors.

[0028] FIGS. 7A-7C show that the gene signature was applied to 3 different datasets that contain preoperative anthracycline response data. Blue 1 = no cancer after treatment with anthracycline.

[0029] FIG. 8 shows that BRCA1-like embodiments correlate with lymphocytic infiltrate.

[0030] FIG. 9 demonstrates that lymphocytic infiltrate correlates with good prognosis.

[0031] FIG. 10 shows that in order to validate the gene list obtained, it was applied to dataset of archived tumor biopsy samples at Baylor College of Medicine, and the figure shows that 30 samples were analyzed by RT-QPCR and by low density microarray analysis.

[0032] FIGS. 11 and 12 demonstrate RT-QPCR of 7 exemplary genes in the list.

[0033] FIG. 13 illustrates a custom low-density microarray card that was created to analyze the 80 most differentially expressed genes out of the 180 gene list.

[0034] FIG. 14 shows further refinement of the list by selecting the 25 most differentially expressed genes between these two groups, with the samples being in order of a BRCAness score (left is most consistent with BRCA1 pattern, right least consistent with BRCA1 pattern).

[0035] FIG. 15 demonstrates PARP1 microarray expression data of four triple negative breast cancer datasets. High PARP1 expression level correlated with BRCA1-like signature pattern.

[0036] FIG. 16 shows clustering of 68 sporadic triple negative tumors using Ingenuity BRCA1 pathway genes.

[0037] FIG. 17 shows that non-BRCA1-like tumors exhibit Dasatinib sensitivity, in certain embodiments of the invention.

[0038] FIG. 18 illustrates particular cases and clinical treatment for triple negative breast cancer, in certain embodiments.

[0039] FIG. 19 shows confirmation of particular microarray gene expression with low density array.

[0040] FIGS. 20A-20B show identification of samples with BRCA1-like signature. FIG. 20A is a heat map of 68 triple negative tumors from BCM ranked according to previously published BRCA1 gene signature. The samples are ranked according to an algorithm which places the tumors with a gene expression pattern most similar to that of sporadic tumors to the left, labeled with a green S, and the tumors with a BRCA1-like gene expression pattern to the right, labeled with a red B. FIG. 20 B shows that three gene lists form each datasets (BCM1, Wang, NKI) were obtained. They were composed of the most differentially expressed genes between sporadic triple negative tumors with BRCA1-like gene expression pattern versus a sporadic (also referred to as non-BRCA1-like in the context of gene expression) pattern. The signature of 334 genes is derived from overlap of these three gene lists.

[0041] FIGS. 21A and 21B show increased expression of known DNA repair genes in BRCA1-like tumors *vs.* other non-BRCA1-like TN cancers. In FIG. 21A (by microarray) known DNA repair pathway genes (PARP1, RAD51, FANCA, CHK1) have increased gene expression in tumors identified as having defective DNA repair signature. BCM1, Wang, NKI2 Datasets combined. In FIG. 21B, (QRT-PCRNA) DNA repair-related genes (PARP1, CHEK1, and RAD51) had higher RNA expression in tumors identified as having defective DNA repair signature. High: tumors with BRCA1-like signature: Low: tumors with non-BRCA1-like signature. BCM1 Dataset

[0042] FIGS. 22A-22B show ROC curves for FEC and TET using gene expression microarrays. FIG. 22A shows for FEC chemotherapy – six cycles of anthracycline-based therapy. FIG. 22B shows for TET chemotherapy - primarily "taxane-based" chemotherapy.

[0043] FIG. 23 shows Receiver Operating Characteristic (ROC) curves for AC chemotherapy using the 69-gene LDA.

DETAILED DESCRIPTION OF THE INVENTION

I. Definitions

[0044] As used herein the specification, "a" or "an" may mean one or more. As used herein in the claim(s), when used in conjunction with the word "comprising", the words "a" or "an" may mean one or more than one. As used herein "another" may mean at least a second or more. Some embodiments of the invention may consist of or consist essentially of one or more elements, method steps, and/or methods of the invention. It is contemplated that any method or composition described herein can be implemented with respect to any other method or composition described herein.

[0045] The term "expressed RNAs" as used herein refers to RNAs that are transcribed from a polynucleotide. In specific embodiments, the polynucleotide is a gene, such as a gene on a chromosome or mitochondrial DNA. In further embodiments, the expressed RNAs may be isolated from one or more cancer cells, such as one or more cancer cells suspected of being resistant to a hormonal therapy or that are known to be resistant to a hormone therapy. In specific embodiments, the level of the expressed RNA may be determined by determining the level of the RNA molecule or by determining the level of a polypeptide translated from the expressed RNA, such as determining the level by immunoblot, for example.

[0046] The term "microarray" as used herein refers to a collection of expressed RNAs, in particular comprised on a substrate, such as a microchip.

[0047] The terms "overexpress," "overexpressed," or overexpressing" as used herein refers to the level of expression of an RNA being greater than one fold higher compared to a control sample or compared to the expression of a housekeeping gene, for example. For example, expression may be compared to one or more genes normalized to ribosomal RNA, such as 18S ribosomal RNA.

[0048] The term "sample" as used herein refers to any biological fluid or tissue that contains breast cancer cells. Such samples may further be diluted with saline, buffer or a physiologically acceptable diluent. In some cases, such samples are concentrated by conventional means.

[0049] The terms "underexpress," "underexpressed," or underexpressing" as used herein refers to the level of expression of an RNA being less than one fold higher compared to a control sample, or compared to the expression of a housekeeping gene, for example. For example, expression may be compared to one or more genes normalized to ribosomal RNA, such as 18S ribosomal RNA.

II. Certain Embodiments of the Invention

[0050] In certain embodiments, the present invention concerns a DNA repair signature that is associated with anthracycline response in triple negative breast cancer patients.

[0051] In particular embodiments of the invention, a subset of sporadic triple negative (TN) breast cancer patients whose tumors have defective DNA repair similar to BRCA1-associated tumors are more likely to exhibit up-regulation of DNA repair-related genes, anthracycline-sensitivity, and taxane-resistance. The inventors derived a defective DNA repair gene expression signature of 334 genes by applying a previously published BRCA1-associated expression pattern to three datasets of sporadic TN breast cancers. A subset of 69 of the most differentially expressed genes was confirmed by quantitative RT-PCR using a low density custom array (LDA). Next, the association of this DNA repair microarray signature expression was tested with pathologic response in neoadjuvant anthracycline trials of FEC (n=50) and AC (n=16), or taxane-based TET chemotherapy (n=39). Paraffin-fixed, formalin-embedded biopsies were collected from TN patients who had received neoadjuvant AC (n=28), and the utility of the LDA to discriminate response was tested. Correlation between RNA expression measured by the microarrays and 69-gene LDA was ascertained. This defective DNA repair microarray gene expression pattern was significantly associated with anthracycline response and taxane resistance, with the area under the ordinary receiver operating characteristic curve (AUC) of 0.61 (95% CI=0.45-0.77), and 0.65 (95% CI=0.46-0.85), respectively. From the FFPE samples, the 69-gene LDA could discriminate AC responders, with AUC of 0.79 (95% CI=0.59-0.98). Thus, the present invention provides one or more defective DNA repair gene expression signatures that differentiate TN breast cancers that are sensitive to anthracyclines and resistant to taxane-based

chemotherapy, and in specific embodiments is useful with other DNA-damaging agents and PARP-1 inhibitors. Table 1 identifies the expression levels of the 69 genes in 20 BRCA-like and 7 sporadic samples.

TABLE 1

Gene name	ITGB5	EFEMP2	LAMA4	HTRA1	FBN1	PDGFRB	CTSK	PRSS23	HMGA1	IGFBP4
Avg. of 20 BRCA1-like samples	15.33332887	25.80981396	24.74656955	18.18032844	11.46728326	20.41688366	13.3408103	11.44707566	8.685526851	8.623998159
Avg. of 7 sporadic samples	8.994541204	10.78992957	12.25675862	8.934396332	7.726206959	9.153028942	8.05800314	7.982128789	13.58588787	7.393481743
score (Avg. of 7 sporadic samples minus average of 20 BRCA1-like samples)	-6.338787663	-15.01988439	-12.48981093	-9.245932106	-3.741076299	-11.26385472	-5.282807164	-3.464946875	4.900361017	-1.230516416
RANK SUM	0.0001	0.0001	0.0001	0.0001	0.0001	0.0001	0.0001	0.0001	0.0001	0.001

Gene name	SERPINF1	COL5A2	CPE	RUNX1T1	TIMP3	COL15A1	LHFP	CDH5	HDGF	FLRT2
Avg. of 20 BRCA1-like samples	10.338448	17.58189	18.05518	23.74299	9.884444	16.58189	20.82126	28.33054	7.791855	67.04563
Avg. of 7 sporadic samples	7.8263239	10.50429	8.972251	8.821878	7.772314	9.518099	10.42123	13.61555	21.05764	17.81521
score (Avg. of 7 sporadic samples minus average of 20 BRCA1-like samples)	-2.5121238	-7.07759	-9.08293	-14.9211	-2.11213	-7.06379	-10.4	-14.715	13.74767	-49.2304
RANK SUM	0.001	0.001	0.001	0.001	0.002	0.002	0.003	0.004	-7.30997	0.005

Gene name	PDGFRL	PDGFRA	PCOLCE	LAMB1	COPZ2	NID2	FBLN1	LRP1	KANK2	OLFML3
Avg. of 20 BRCA1-like samples	25.92	31.36674	9.979115	12.09548	18.72602	19.18522	13.2893	9.252037	21.09038	15.04997
Avg. of 7 sporadic samples	10.12443	13.21078	7.852814	9.020169	10.71132	11.65764	8.614172	7.959885	12.86233	10.03272
score (Avg. of 7 sporadic samples minus average of 20 BRCA1-like samples)	-15.7956	-18.156	-2.1263	-3.07531	-8.0147	-7.52758	-4.67513	-1.29215	-8.22805	-5.01724
RANK SUM	0.005	0.007	0.01	0.011	0.013	0.013	0.015	0.017	0.017	0.017

Gene name	USP13	APOBEC3B	LRRC32	SRPX2	VCAN	STXBP1	HSPA14	SEMA5A	SLC5A6	IL1R1
Avg. of 20 BRCA1-like samples	11.99148	17.58828	44.09873	48.36437	39.31509	28.39208	13.39728	59.02135	12.46246	15.82219
Avg. of 7 sporadic samples	18.77994	35.36648	18.31021	21.25738	21.04994	17.05655	18.90991	27.63959	16.43655	11.19585
score (Avg. of 7 sporadic samples minus average of 20 BRCA1-like samples)	6.788451	17.7782	-25.7885	-27.107	-18.2652	-11.3355	5.512631	-31.3818	3.974091	-4.62634

TABLE 1

RANK SUM	0.017	0.017	0.02	0.02	0.023	0.023	0.023	0.026	0.026	0.034

Gene name	TP53BP2	CXCL10	ISG20	SRPX	NAP1L3	ITGBL1	NUAK1	EDNRA	CPA3	CCRL1
Avg. of 20 BRCA1-like samples	11.69559	9.20705	9.219157	20.64139	30.95764	21.05764	32.23838	18.57787	40.65215	56.95725
Avg. of 7 sporadic samples	13.93706	12.65916	11.5258	11.53622	33.11801	13.74767	20.26564	12.82347	17.66131	32.53385
score (Avg. of 7 sporadic samples minus average of 20 BRCA1-like samples)	2.241471	3.452107	2.306642	-9.10516	2.16037	-7.30997	-11.9727	-5.7544	-22.9908	-24.4234
RANK SUM	0.038	0.039	0.039	0.041	0.05	x	x	x	x	x

Gene name	ATXN1	GRP	FHL1	BDKRB2	WARS	NOX4	EXO1	IL32	PELI2	FKBP1B
Avg. of 20 BRCA1-like samples	22.70634	47.42161	35.36476	18.89529	8.669793	26.95693	23.66381	8.07231	42.7533	37.57288
Avg. of 7 sporadic samples	16.09255	25.3446	21.78182	32.57946	9.910818	20.90468	37.30459	8.730685	27.68262	23.292
score (Avg. of 7 sporadic samples minus average of 20 BRCA1-like samples)	-6.6138	-22.077	-13.5829	13.68417	1.241025	-6.05225	13.64078	0.658374	-15.0707	-14.2809
RANK SUM	x	x	x	x	x	x	x	x	x	x

Gene name	NOVA1	USP18	C1orf112	GEM	SLCO2A1	PRKD1	LRRC17	LAG3	ERCC6L
Avg. of 20 BRCA1-like samples	18.79982	29.62192	32.70785	17.92934	30.17402	51.51748	56.41239	30.03219	35.22792
Avg. of 7 sporadic samples	41.63735	19.37419	26.66255	14.72665	24.21147	44.45921	39.3484	39.42365	30.85361
score (Avg. of 7 sporadic samples minus average of 20 BRCA1-like samples)	22.83753	-10.2477	-6.0453	-3.20269	-5.96255	-7.05828	-17.064	9.391466	-4.37431
RANK SUM	x	x	x	x	x	x	x	x	x

Genes with positive score are overexpressed in BRCA1-like tumors

Genes with RANK SUM p value <0.05 are the 45 relevant genes that are differentially expressed and statistically significant

III. Nucleic Acid Detection

[0052] In certain embodiments of the invention, nucleic acids are detected, for example using methods to identify particular mRNAs, such as with the use of oligonucleotides that hybridize to the mRNA.

A. Hybridization

[0053] The use of a probe or primer (which may be referred to as an oligonucleotide) of between 13 and 100 nucleotides, preferably between 17 and 100 nucleotides in length, or in some aspects of the invention up to 1-2 kilobases or more in length, allows the formation of a duplex molecule that is both stable and selective. Molecules having complementary sequences over contiguous stretches greater than about 20 bases in length may be employed, to increase stability and/or selectivity of the hybrid molecules obtained. One will generally prefer to design nucleic acid molecules for hybridization having one or more complementary sequences of 20 to 30 nucleotides, or even longer where desired. Such fragments may be readily prepared, for example, by directly synthesizing the fragment by chemical means or by introducing selected sequences into recombinant vectors for recombinant production.

[0054] Accordingly, the nucleotide sequences of the invention may be used for their ability to selectively form duplex molecules with complementary stretches of DNAs and/or RNAs or to provide primers for amplification of DNA or RNA from samples. Depending on the application envisioned, one would desire to employ varying conditions of hybridization to achieve varying degrees of selectivity of the probe or primers for the target sequence.

[0055] For applications requiring high selectivity, one will typically desire to employ relatively high stringency conditions to form the hybrids. For example, relatively low salt and/or high temperature conditions, such as provided by about 0.02 M to about 0.10 M NaCl at temperatures of about 50°C to about 70°C. Such high stringency conditions tolerate little, if any, mismatch between the probe or primers and the template or target strand and would be particularly suitable for isolating specific genes or for detecting specific mRNA transcripts. It is generally appreciated that conditions can be rendered more stringent by the addition of increasing amounts of formamide.

[0056] For certain applications, for example, it is appreciated that lower stringency conditions are preferred. Under these conditions, hybridization may occur even though the

sequences of the hybridizing strands are not perfectly complementary, but are mismatched at one or more positions. Conditions may be rendered less stringent by increasing salt concentration and/or decreasing temperature. For example, a medium stringency condition could be provided by about 0.1 to 0.25 M NaCl at temperatures of about 37°C to about 55°C, while a low stringency condition could be provided by about 0.15 M to about 0.9 M salt, at temperatures ranging from about 20°C to about 55°C. Hybridization conditions can be readily manipulated depending on the desired results.

[0057] In other embodiments, hybridization may be achieved under conditions of, for example, 50 mM Tris-HCl (pH 8.3), 75 mM KCl, 3 mM $MgCl_2$, 1.0 mM dithiothreitol, at temperatures between approximately 20°C to about 37°C. Other hybridization conditions utilized could include approximately 10 mM Tris-HCl (pH 8.3), 50 mM KCl, 1.5 mM $MgCl_2$, at temperatures ranging from approximately 40°C to about 72°C.

[0058] In certain embodiments, it will be advantageous to employ nucleic acids of defined sequences of the present invention in combination with an appropriate means, such as a label, for determining hybridization. A wide variety of appropriate indicator means are known in the art, including fluorescent, radioactive, enzymatic or other ligands, such as avidin/biotin, which are capable of being detected. In preferred embodiments, one may desire to employ a fluorescent label or an enzyme tag such as urease, alkaline phosphatase or peroxidase, instead of radioactive or other environmentally undesirable reagents. In the case of enzyme tags, colorimetric indicator substrates are known that can be employed to provide a detection means that is visibly or spectrophotometrically detectable, to identify specific hybridization with complementary nucleic acid containing samples.

[0059] In general, it is envisioned that the probes or primers described herein will be useful as reagents in solution hybridization, as in PCR™, for detection of expression of corresponding genes, as well as in embodiments employing a solid phase. In embodiments involving a solid phase, the test DNA (or RNA) is adsorbed or otherwise affixed to a selected matrix or surface. This fixed, single-stranded nucleic acid is then subjected to hybridization with selected probes under desired conditions. The conditions selected will depend on the particular circumstances (depending, for example, on the G+C content, type of target nucleic acid, source of nucleic acid, size of hybridization probe, *etc.*). Optimization of hybridization conditions for the particular application of interest is well known to those of skill in the art. After washing of

the hybridized molecules to remove non-specifically bound probe molecules, hybridization is detected, and/or quantified, by determining the amount of bound label. Representative solid phase hybridization methods are disclosed in U.S. Patent Nos. 5,843,663, 5,900,481 and 5,919,626. Other methods of hybridization that may be used in the practice of the present invention are disclosed in U.S. Patent Nos. 5,849,481, 5,849,486 and 5,851,772. The relevant portions of these and other references identified in this section of the Specification are incorporated herein by reference.

B. Amplification of Nucleic Acids

[0060] Nucleic acids used as a template for amplification may be isolated from cells, tissues or other samples according to standard methodologies (Sambrook *et al.*, 1989). In certain embodiments, analysis is performed on whole cell or tissue homogenates or biological fluid samples without substantial purification of the template nucleic acid. The nucleic acid may be genomic DNA or fractionated or whole cell RNA. Where RNA is used, it may be desired to first convert the RNA to a complementary DNA.

[0061] The term "primer," as used herein, is meant to encompass any nucleic acid that is capable of priming the synthesis of a nascent nucleic acid in a template-dependent process. Typically, primers are oligonucleotides from ten to twenty and/or thirty base pairs in length, but longer sequences can be employed. Primers may be provided in double-stranded and/or single-stranded form, although the single-stranded form is preferred.

[0062] Pairs of primers designed to selectively hybridize to nucleic acids corresponding to the genes in FIG. 14 are contacted with the template nucleic acid under conditions that permit selective hybridization. Depending upon the desired application, high stringency hybridization conditions may be selected that will only allow hybridization to sequences that are completely complementary to the primers. In other embodiments, hybridization may occur under reduced stringency to allow for amplification of nucleic acids contain one or more mismatches with the primer sequences. Once hybridized, the template-primer complex is contacted with one or more enzymes that facilitate template-dependent nucleic acid synthesis. Multiple rounds of amplification, also referred to as "cycles," are conducted until a sufficient amount of amplification product is produced.

[0063] The amplification product may be detected or quantified. In certain applications, the detection may be performed by visual means. Alternatively, the detection may involve indirect identification of the product via chemiluminescence, radioactive scintigraphy of incorporated radiolabel or fluorescent label or even via a system using electrical and/or thermal impulse signals (Affymax technology; Bellus, 1994).

[0064] A number of template dependent processes are available to amplify the oligonucleotide sequences present in a given template sample. One of the best known amplification methods is the polymerase chain reaction (referred to as PCR™) which is described in detail in U.S. Patent Nos. 4,683,195, 4,683,202 and 4,800,159, and in Innis *et al.*, 1988, each of which is incorporated herein by reference in their entirety.

[0065] A reverse transcriptase PCR™ amplification procedure may be performed to quantify the amount of mRNA amplified. Methods of reverse transcribing RNA into cDNA are well known (see Sambrook *et al.*, 1989). Alternative methods for reverse transcription utilize thermostable DNA polymerases. These methods are described in WO 90/07641. Polymerase chain reaction methodologies are well known in the art. Representative methods of RT-PCR are described in U.S. Patent No. 5,882,864.

[0066] Another method for amplification is ligase chain reaction ("LCR"), disclosed in European Application No. 320 308, incorporated herein by reference in its entirety. U.S. Patent 4,883,750 describes a method similar to LCR for binding probe pairs to a target sequence. A method based on PCR™ and oligonucleotide ligase assy (OLA), disclosed in U.S. Patent 5,912,148, may also be used.

[0067] Alternative methods for amplification of target nucleic acid sequences that may be used in the practice of the present invention are disclosed in U.S. Patent Nos. 5,843,650, 5,846,709, 5,846,783, 5,849,546, 5,849,497, 5,849,547, 5,858,652, 5,866,366, 5,916,776, 5,922,574, 5,928,905, 5,928,906, 5,932,451, 5,935,825, 5,939,291 and 5,942,391, GB Application No. 2 202 328, and in PCT Application No. PCT/US89/01025, each of which is incorporated herein by reference in its entirety.

[0068] Qbeta Replicase, described in PCT Application No. PCT/US87/00880, may also be used as an amplification method in the present invention. In this method, a replicative sequence of RNA that has a region complementary to that of a target is added to a sample in the

presence of an RNA polymerase. The polymerase will copy the replicative sequence which may then be detected.

[0069] An isothermal amplification method, in which restriction endonucleases and ligases are used to achieve the amplification of target molecules that contain nucleotide 5'-[alpha-thio]-triphosphates in one strand of a restriction site may also be useful in the amplification of nucleic acids in the present invention (Walker *et al.*, 1992). Strand Displacement Amplification (SDA), disclosed in U.S. Patent No. 5,916,779, is another method of carrying out isothermal amplification of nucleic acids which involves multiple rounds of strand displacement and synthesis, i.e., nick translation.

[0070] Other nucleic acid amplification procedures include transcription-based amplification systems (TAS), including nucleic acid sequence based amplification (NASBA) and 3SR (Kwoh *et al.*, 1989; Gingeras *et al.*, PCT Application WO 88/10315, incorporated herein by reference in their entirety). European Application No. 329 822 disclose a nucleic acid amplification process involving cyclically synthesizing single-stranded RNA ("ssRNA"), ssDNA, and double-stranded DNA (dsDNA), which may be used in accordance with the present invention.

[0071] PCT Application WO 89/06700 (incorporated herein by reference in its entirety) disclose a nucleic acid sequence amplification scheme based on the hybridization of a promoter region/primer sequence to a target single-stranded DNA ("ssDNA") followed by transcription of many RNA copies of the sequence. This scheme is not cyclic, i.e., new templates are not produced from the resultant RNA transcripts. Other amplification methods include "race" and "one-sided PCR" (Frohman, 1990; Ohara *et al.*, 1989).

C. Detection of Nucleic Acids

[0072] Following any amplification, it may be desirable to separate the amplification product from the template and/or the excess primer. In one embodiment, amplification products are separated by agarose, agarose-acrylamide or polyacrylamide gel electrophoresis using standard methods (Sambrook *et al.*, 1989). Separated amplification products may be cut out and eluted from the gel for further manipulation. Using low melting point agarose gels, the separated band may be removed by heating the gel, followed by extraction of the nucleic acid.

[0073] Separation of nucleic acids may also be effected by chromatographic techniques known in art. There are many kinds of chromatography which may be used in the practice of the present invention, including adsorption, partition, ion-exchange, hydroxylapatite, molecular sieve, reverse-phase, column, paper, thin-layer, and gas chromatography as well as HPLC.

[0074] In certain embodiments, the amplification products are visualized. A typical visualization method involves staining of a gel with ethidium bromide and visualization of bands under UV light. Alternatively, if the amplification products are integrally labeled with radio- or fluorometrically-labeled nucleotides, the separated amplification products can be exposed to x-ray film or visualized under the appropriate excitatory spectra.

[0075] In one embodiment, following separation of amplification products, a labeled nucleic acid probe is brought into contact with the amplified marker sequence. The probe preferably is conjugated to a chromophore but may be radiolabeled. In another embodiment, the probe is conjugated to a binding partner, such as an antibody or biotin, or another binding partner carrying a detectable moiety.

[0076] In particular embodiments, detection is by Southern blotting and hybridization with a labeled probe. The techniques involved in Southern blotting are well known to those of skill in the art (see Sambrook *et al.*, 1989). One example of the foregoing is described in U.S. Patent No. 5,279,721, incorporated by reference herein, which discloses an apparatus and method for the automated electrophoresis and transfer of nucleic acids. The apparatus permits electrophoresis and blotting without external manipulation of the gel and is ideally suited to carrying out methods according to the present invention.

[0077] Other methods of nucleic acid detection that may be used in the practice of the instant invention are disclosed in U.S. Patent Nos. 5,840,873, 5,843,640, 5,843,651, 5,846,708, 5,846,717, 5,846,726, 5,846,729, 5,849,487, 5,853,990, 5,853,992, 5,853,993, 5,856,092, 5,861,244, 5,863,732, 5,863,753, 5,866,331, 5,905,024, 5,910,407, 5,912,124, 5,912,145, 5,919,630, 5,925,517, 5,928,862, 5,928,869, 5,929,227, 5,932,413 and 5,935,791, each of which is incorporated herein by reference.

IV. Kits of the Invention

[0083] All the essential materials and/or reagents required for detecting the genes in FIG. 14 or Table 1 in a sample may be assembled together in a kit. This generally will comprise a probe or primers (such as oligonucleotides) designed to hybridize specifically to individual nucleic acids of interest in the practice of the present invention, including one or more of the genes listed in FIG. 14 or Table 1. Also included may be enzymes suitable for amplifying nucleic acids, including various polymerases (reverse transcriptase, Taq, *etc.*), deoxynucleotides and buffers to provide the necessary reaction mixture for amplification. Such kits may also include enzymes and other reagents suitable for detection of specific nucleic acids or amplification products. Such kits generally will comprise, in suitable means, distinct containers for each individual reagent or enzyme as well as for each probe or primer pair.

V. Collection of Samples

[0084] In aspects of the invention, samples are obtained from an individual for subjecting to the methods, such as from an individual suspected of having triple negative breast cancer or needing an appropriate therapy therefor. Any suitable methods for obtaining the samples are within the scope of the invention, and exemplary methods include by fine needle aspirates obtained *via* a biopsy procedure, for example.

[0085] One or more cells of the samples may be isolated and used to prepare the RNA from said cell(s). In specific embodiments of the invention, the isolation of one or more cells may be performed by microdissection, such as, but not limited to, laser capture microdissection (LCM) or laser microdissection (LMD). The levels and/or activities of the RNA(s) may be assayed directly or indirectly, or may be amplified in whole or in part prior to detection.

VI. Examples

[0086] The following examples are included to demonstrate preferred embodiments of the invention. It should be appreciated by those of skill in the art that the techniques disclosed in the examples which follow represent techniques discovered by the inventor to function well in the practice of the invention, and thus can be considered to constitute preferred modes for its practice. However, those of skill in the art should, in light of the present disclosure, appreciate that many changes can be made in the specific embodiments which are disclosed and still obtain a like or similar result without departing from the spirit and scope of the invention.

[0087] In the following exemplary examples, the inventors first set out to identify a gene expression signature that distinguishes triple negative (TN) breast cancers into those that exhibit DNA repair defects similar to tumors with BRCA1 mutations (BRCA1-like) from TN breast cancers that may not carry a deficiency in homologous-recombination DNA repair (non-BRCA1-like). Secondly, they confirmed these expression results by two different RNA platforms (gene expression microarray *vs.* an exemplary 69-gene low density custom array, LDA). Thirdly, they tested this defective DNA repair microarray gene expression signature and its association with treatment response in TN breast cancers with the consideration that patients with this signature demonstrate sensitivity to agents that affect DNA repair like anthracyclines, but not to non-DNA damaging agents, like taxanes. Finally, the 69-gene LDA was tested on formalin-fixed, paraffin-embedded (FFPE) core biopsies obtained from women that received neoadjuvant anthracycline chemotherapy (n=28).

EXAMPLE 1

[0088] FIG. 1 shows that breast cancer is not one disease. Currently, breast cancer is stratified in the clinic as ER+HER2-, ER+HER2+, ER-HER2+, and ER-HER2-. Less than 2% of all breast cancers are from BRCA1 mutation carriers. These tumors are generally ER-HER2- and because of BRCA1's function in DNA repair, these tumors are more sensitive to DNA damaging drugs like anthracyclines and are also dependent on an enzyme called PARP1 to repair its DNA. Targeting PARP1 by inhibiting its action has been a novel approach to treat the minority of breast cancer.

[0089] In a specific embodiment of the invention, there is identification of a subset of ER-HER2- tumors from non-BRCA1 mutation carriers that are biologically similar to the tumors from BRCA1 carriers and hence would have the same properties described above. If successful, selection of patients with ER-HER2- with BRCA1 deficient properties (or BRCA1-like) may enhance efficacy results of DNA-damaging agents and PARP1 inhibitors.

[0090] FIG. 2 demonstrates that expression pattern similarities are also shared with tumors from BRCA1 mutation carriers and non-carriers, both in general cluster as basal-like tumors (Sorlie *et al.*, 2003).

[0091] FIG. 3 indicates that BRCA1-associated tumors are more sensitive to anthracyclines than sporadic triple negatives (Delaloge *et al.*, 2008). Tumors from BRCA1

mutation carriers are more sensitive to DNA-damaging drugs than tumors from non-carriers (controls) After treatment with preoperative anthracycline containing regimen, 47% of the BRCA1 mutation carriers had no residual cancer at surgery whereas only 22% of the patients with ER-HER2- had no cancer (PCR = pathological complete response).

[0092] Hereditary BRCA1 breast tumors and basal-like sporadic breast tumors have a similar phenotype and gene expression signature, suggesting involvement of BRCA1 in the pathogenesis of sporadic basal-like breast cancer. In certain embodiments of the invention, sporadic triple negative tumors have BRCA-like qualities. BRCA1 familial cancers show: 1) phenotypic similarities to sporadic triple negative breast cancers; 2) gene expression similarities to sporadic triple negative breast cancers (Foulkes *et al.*, 2003; Sorlie *et al.*, 2003; Lakhani *et al.*, 2005). These two observations suggest there may be an underlying defect in BRCA1-related pathways in a subset of sporadic triple negative breast cancers.

[0093] FIG. 4 concerns redirecting therapies in triple negative breast cancer. Selection of $ER^{-}HER2^{-}$ patients who are most likely to respond to PARP inhibitor will not only expand its current use in breast cancer to not just BRCA1 mutation carriers, but also maintain enhanced efficacy in the ER-HER2 negative patients by not selecting those patients who are unlikely to respond.

[0094] FIG. 5 concerns a Nature 2002 publication of a 430 gene expression signature was identified that could potentially identify ER- tumors from BRCA1 mutation carriers (van't Veer *et al.*, 2002). In the present invention, the inventors considered that if this gene expression profile was used on all triple negative tumors, one could identify ER-HER2- tumors with acquired BRCA1 dysfunction or DNA repair deficiencies and hence sensitivity to anthracycline and PARP inhibitors.

[0095] In a certain embodiment of the invention, there is identification of a molecular signature that differentiates between two subsets of sporadic triple negative breast cancer: one that may benefit from chemotherapy, particularly DNA damaging agents, such as anthracyclines, platinums, or PARP inhibitors; and another that may exhibit chemoresistance and poor prognosis and would therefore be ideal candidates for testing novel targeted agents in TN breast cancer, *i.e.* dasatinib.

[0096] In FIG. 6, the previously published BRCA1-associated gene expression signature was applied to 3 datasets of triple negative breast cancer. A-BCM (Baylor College of Medicine), B-Wang (publically available), C-Netherlands (publically available). From each dataset, a new list of genes was selected by obtaining the most differentially expressed genes between those tumors that exhibited the pattern most like "sporadic" tumors versus those tumors that exhibited the pattern of BRCA1-associated mutation carrier tumors. Then, the gene list was refined to 182 by selecting only those genes that overlapped in all 3 lists.

[0097] In order to further investigate certain aspects of the invention, the gene signature was applied to 3 different datasets that contain preoperative anthracycline response data. Blue 1 = no cancer after treatment with anthracycline. In all 3 datasets those tumors that exhibited the BRCA1 expression pattern were more likely to have no cancer at surgery after preoperative anthracycline (see FIG. 7). In FIG. 7A, there is higher pCR rate (1) *vs.* non-pCR (0) in patients with BRCA1-like tumors (B) signature, receiving neoadjuvant FAC-containing chemotherapy.9 Patients (6/9) with BRCA1-like signature (B) achieved pCR, *vs.* only 2/7 in the 'sporadic" (S) group, p=0.15. In FIG. 7B, there is higher pCR rate (1) *vs.* non-pCR (0) in patients receiving neoadjuvant AC (BCM dataset 2). 0/3 patients with sporadic (S) pattern vs. 6/9 patients with BRCA1-like (B) pattern achieved pCR, $p<0.05$. In FIG. 7C, there is higher pCR (1) *vs.* non-pCR (0) was observed in patients with BRCA1-like (B) signature, in patients receiving neoadjuvant FEC chemotherapy.27 13/25 patients with BRCA1-like signature (B) achieved pCR, *vs.* 8/28 in the 'sporadic" (S) group, p=0.035.

[0098] Tumors with a BRCA1-like pattern were most likely to have lymphocytic infiltrate, a characteristic of BRCA1-associated tumors. FIG. 8 shows that BRCA1-like correlates with lymphocytic infiltrate.

[0099] FIG. 9 shows that lymphocytic infiltrate was associated with an improved prognosis in these chemotherapy treated patients. Metastasis-free survival of 71 patients with triple-negative breast carcinomas comparing amount of lymphocytic infiltrate (Kreike *et al.*, 2007).

[0100] In order to validate the gene list obtained, the list was applied to dataset of archived tumor biopsy samples at Baylor College of Medicine. FIG. 10 shows that 30 samples were analyzed by RTQ PCR and by low density microarray analysis.

[0101] In exemplary RT-QPCR, FIGS. 11 and 12 show that 7 genes selected for their availability in the lab were measured by RT-QPCR. B = Tumor has BRCA1-like pattern and S = tumor has sporadic pattern. All 7 genes were differentially expressed and statistically significant. One sporadic sample was an outlier in all 7 genes analyzed.

[0102] In FIG. 13, a custom low-density microarray card was created to analyze the 80 most differentially expressed genes out of the 180 gene list. The genes were able to differentiate the two groups.

[0103] In FIG. 14, the gene list was refined further by selecting the 25 most differentially expressed genes between these two groups. Here the samples are in order of a BRCA1-like score, left is most consistent with BRCA1-associated pattern, right least consistent with BRCA1-associated pattern.

[0104] In specific embodiments, there may be two or more expressed genes identified in FIG. 14 that are associated with triple negative breast cancer and/or therapy therefor and therefore is useful for an individual suspected of having breast cancer, suspected of having triple negative breast cancer, or in need of therapy for triple negative breast cancer. In additional embodiments, there may be combinations of expressed genes identified in FIG. 14 as being indicative of identifying triple negative breast cancer and/or therapy therefor and therefore is useful for an individual suspected of having breast cancer, suspected of having triple negative breast cancer, or in need of therapy for triple negative breast cancer. There may be combinations of two expressed genes, three expressed genes, four expressed genes, five expressed genes, six expressed genes, seven expressed genes, eight expressed genes, nine expressed genes, ten expressed genes, twelve expressed genes, fifteen expressed genes, twenty expressed genes, twenty-five expressed genes, thirty expressed genes, thirty-five expressed genes, forty expressed genes, forty-five expressed genes, fifty expressed genes, fifty-five expressed genes, sixty expressed genes, sixty-five expressed genes, or more expressed genes, for example.

[0105] FIG. 15 shows PARP1 microarray expression data of 4 triple negative breast cancer datasets. High PARP1 expression level correlated with BRCA1-like signature pattern. PARP1 appears to be overexpressed in the BRCA1-like group when compared to the Sporadic group. However, PARP1 measurement alone by microarray data was unable to differentiate an anthracycline sensitive group.

[0106] FIG. 16 shows clustering of 68 sporadic triple negative tumors using Ingenuity BRCA1 pathway genes. Upregulation of BRCA1 was observed in the tumors exhibiting the BRCA1-like gene expression pattern.

[0107] FIG. 17 shows that non-BRCA1-like tumors exhibit dasatinib sensitivity, in certain embodiments (Dizdar *et al.*, 2008; Finn *et al.*, 2007)

[0108] In certain embodiments the inventors have identified and validated a set of genes by microarray analysis and RT-QPCR that can identify two groups of sporadic triple negative breast cancer: 1) anthracycline sensitive, likely due to acquired BRCA1 deficiency or DNA repair deficiency; and 2) Anthracycline-resistant which has the potential of being sensitive to dasatinib. In particular embodiments, the set of genes is conveniently measured on formalin-fixed paraffin embedded tissue. In certain aspects, this set of genes is used, for example in the clinic, to predict which individuals will respond to anthracyclines, PARP inhibitors, or other DNA-damaging drugs, for example. FIG. 18 illustrates particular embodiments for therapy.

[0109] FIG. 19 shows confirmation of microarray gene expression with low density array. Provided is a heat map showing mRNA relative expression by LDA (Ct values normalized to ACTB,IPO8, and POLR2A), demonstrating 45 of 69 genes (Table 2) that correlate with microarray data, red = high Ct value or low mRNA expression.

[0110] Table 2: Triple Negative Breast Cancer-Related Polynucleotides

Gene	GenBank® Accession No.	SEQ ID NO	p-value	prior 25
ITGB5	NM_002213	1	0.0001	1
EFEMP2	AF109121	2	0.0001	1
LAMA4	NM_001105206	3	0.0001	1
HTRA1	NG_011554	4	0.0001	1
FBN1	NM_000138	5	0.0001	1
PDGFRB	NM_002609	6	0.0001	1
CTSK	NM_000396	7	0.0001	1
PRSS23	NM_007173	8	0.0001	
HMGA1	NM_145901	9	0.0001	1
IGFBP4	NM_001552	10	0.001	1
SERPINF1	NM_002615	11	0.001	1
COL5A2	NM_000393	12	0.001	
CPE	NM_001873	13	0.001	
RUNX1T1	NM_004349	14	0.001	
TIMP3	NM_000362	15	0.002	
COL15A1	NM_001855	16	0.002	1
LHFP	AF098807	17	0.003	1

CDH5	NM_001795	18	0.004	1
HDGF	NM_004494	19	0.004	
FLRT2	NM_013231	20	0.005	
PDGFRL	NM_006207	21	0.005	
PDGFRA	NM_006206	22	0.007	1
PCOLCE	NM_002593	23	0.01	1
LAMB1	NM_002291	24	0.011	
COPZ2	NM_016429	25	0.013	
NID2	NM_007361	26	0.013	
FBLN1	NM_006486	27	0.015	1
LRP1	NM_002332	28	0.017	
KANK2	NM_001136191	29	0.017	
OLFML3	NM_020190	30	0.017	
USP13	NM_003940	31	0.017	1
APOBEC3B	NM_004900	32	0.017	1
LRRC32	NM_005512	33	0.02	
SRPX2	NM_014467	34	0.02	
VCAN	NM_004385	35	0.023	
STXBP1	NM_003165	36	0.023	
HSPA14	NM_016299	37	0.023	1
SEMA5A	NM_003966	38	0.026	
SLC5A6	NM_021095	39	0.026	
IL1R1	NM_000877	40	0.034	
TP53BP2	NM_001031685	41	0.038	1
CXCL10	NM_001565	42	0.039	1
ISG20	NM_002201	43	0.039	1
SRPX	NM_006307	44	0.041	
NAP1L3	NM_004538	45	0.05	1

[0111] The GenBank® Accession numbers of genes referred to in Table 1 that are not identified in Table 2 are as follows: ITGBL1 (BC036788); NUAK1 (NM_014840); EDNRA (NM_001957); CPA3 (NM_001870); CCRL1 (NM_178445); ATXN1 (NM_000332); GRP (NM_000332); FHL1 (NM_001159704); BDKRB2 (NM_000623); WARS (NM_201263); NOX4 (NM_001143837); EXO1 (NM_130398); IL32 (NM_001012631); PEL12 (NM_021255); FKBP1B (NM_054033); NOVA1 (NM_002515); USP18 (NM_017414); C1orf112 (NM_018186); GEM (NM_005261); SLCO2A1 (NM_005630); PRKD1 (NM_002742); LRRC17 (NM_005824); LAG3 (NM_002286); ERCC6L (NM_017669)

EXAMPLE 2

DNA REPAIR SIGNATURE IS ASSOCIATED WITH ANTHRACYCLINE RESPONSE IN TRIPLE NEGATIVE BREAST CANCER PATIENTS

Exemplary Methods

[0112] The inventors used six gene expression datasets obtained by microarray analysis of tumor specimens from a total of 307 patients with primary triple-negative breast cancer.

[0113] The training sets used to obtain the candidate genes were the Baylor College of Medicine (BCM) dataset 1 (BCM1), the Nederlands Kanker Instituut (NKI2) (van de Vijver et al., 2002), and the Wang dataset (GSE2034) (Mohsin et al., 2005). The two anthracycline-treated validation sets used were from Baylor College of Medicine dataset 2 (BCM2), and EORTC (GSE6861) (Farmer et al., 2009; Bonnefoi et al., 2007). The BCM1 and BCM2 datasets consist of information obtained from a total of 84 patients with primary invasive triple-negative breast cancer, whose frozen tumor specimens were archived at BCM. The other 4 datasets are publically available. Microarray and clinical data for the Wang and EORTC patients are available at the Gene Expression Omnibus database on the world wide web), using the associated GSE accession codes, GSE2034 and GSE6861, respectively. The NKI2 dataset was downloaded from the Rosetta Web site. The BCM1 and BCM2 dataset contained 68 and 16 triple negative breast cancer samples, as defined by immunohistochemistry (IHC). The Wang, NKI2, and EORTC datasets contained data from 57, 49, and 89 primary breast tumor samples, respectively, and were ER-negative and PR-negative by IHC. As HER2 status was unavailable in the Wang and NKI2 dataset, HER2-negative patients were identified by microarray data, excluding those samples with ERBB2 and GRB7 overexpression. As such, from the 69 ER-negative and PR-negative samples in the NKI2 dataset, 20 samples were excluded due to overexpression of ERBB2 and GRB7 and 19 out of 76 samples were excluded from the Wang dataset.

[0114] The validation neoadjuvant gene expression microarray studies were conducted on two datasets: BCM2 and EORTC contained data from 16 and 89 triple-negative breast tumor samples, respectively. The treatment received by patients in the BCM2 dataset was 4 cycles of doxorubicin and cyclophosphamide, 60 mg/m2 and 600 mg/m2 respectively, every 3 weeks (AC). The patients in the EORTC dataset were randomized to receive anthracycline chemotherapy of FEC (6 cycles of 500mg/m2 fluorouracil, 100mg/m2 epirubicin, and 500mg/m2 cyclophosphamide every 3 weeks), or primarily taxane-based chemotherapy of TET (3 cycles of 100 mg/m2 docetaxel, followed by 3 cycles of 90 mg/m2 epirubicin plus 70 mg/m2 docetaxel). Pathologic response (pCR) was defined as the complete disappearance of all tumor in the breast in all data sets except BCM2 which also included minute foci of residual disease (<0.1 cm).

[0115] Gene Expression Analysis

[0116] For BCM1 and BCM2 datasets, microarray analysis was performed with Affymetrix U133A GeneChips (Affymetrix, Santa Clara, CA), as previously published (Chang et al., 2003; Chang et al., 2005). These datasets contained samples from BCM, Houston, TX, and Mt Vernon Hospital, United Kingdom. RNA samples from U.K. were shipped on dry ice for processing. The quality of the RNA obtained from each tumor sample was assessed via the RNA profile generated by the Agilent bioanalyzer. Samples with a total area under the 28S and 18S bands of less than 15% of the total RNA band area, as well as a 28S/18S ratio of less than 1.1, were considered to be degraded and were not analyzed further (approximately 20% of the samples). Only tumor samples with good quality RNA were considered for further analysis. RNA amplification, hybridization, and scanning were done according to standard Affymetrix protocols. Image analysis and probe quantification was done with Affymetrix software that produced raw probe intensity data in Affymetrix CEL files. Normalization was done with the program dChip, which processes a group of CEL files simultaneously. The default options of RMA (with background correction, quantile normalization, and log transformation) were used. The CEL files were normalized separately in two groups, according to the dataset, BCM1 and BCM2. The publicly available datasets consisted of both Affymetrix (Wang and EORTC) and Agilent arrays (NKI2), with several different chip designs. To simplify analysis, the inventors used only the gene probes that were common in all datasets.

[0117] Identification of samples with a high likelihood of having defective DNA repair

[0118] BRCA1-associated triple-negative tumors are more likely to have a deficiency in homologous recombination and DNA repair deficiency than sporadic triple-negative tumors. Van't Veer et al. published a set of 430 genes found by microarray data to be differentially expressed between BRCA1-associated ER-negative tumors and sporadic ER-negative tumors, and an optimal set of 100 genes was found to discriminate between BRCA1-associated and sporadic cases. Although these results have not been externally validated or disproven, the inventors considered that using the set of 430 genes, one could identify a subset of triple-negative tumors likely to have defective DNA repair, similar to BRCA1-associated tumors and hence are more likely to exhibit anthracycline-sensitivity, taxane-resistance, and up-regulation of DNA repair-related genes (Martin et al., 2007). These candidate genes were used

and applied it to the BCM1, NKI2, and Wang training datasets which included 68, 49, and 57 samples of triple negative tumors.

[0119] An algorithm was then introduced to rank the samples in each heat map (BCM1, Wang, and NKI2). The genes for each sample were computed as the standardized gene-wise z-scores (underexpressed gene were multiplied by -1), and a total score was determined as the sum. The samples were then ranked according to the total score. The samples with the highest overall score have the gene expression pattern most similar to BRCA1-associated tumors, and those with the lowest score similar to "sporadic" tumors (FIG. 20, BCM1 Dataset). This ranking system was used in order to classify the samples in an objective manner. This algorithm was chosen, rather than metagene analysis, as a straightforward ranking system of differentially expressed genes equally, instead of metagene analysis where complex combinations of many genes and pathways are factored into the analysis. The ranked samples were then divided into high and low expression of genes with DNA repair signature based on the heat-map generated.

[0120] This same algorithm was then applied to the Wang and NKI datasets (N=57, and N=49 samples, respectively). For each of the datasets the samples were ranked from low score to high score. A sample with a high score had a gene expression profile most similar to BRCA1-associated tumors, and thus was considered to have a high likelihood of having defective DNA repair signature. Three gene lists from each dataset were obtained. They were composed of the most differentially expressed genes between sporadic triple negative tumors with BRCA1-like gene expression pattern versus non-BRCA1-like pattern using a false discovery rate of <5%, $p<0.01$, 1.5-fold change. The signature of 334 genes is derived from overlap of these three gene lists, with 136 genes overexpressed in and 198 underexpressed genes (FIG. 20B).

[0121] Receiver operating characteristic (ROC) curves were used to assess the accuracy of predictions. The association between expression and pathological complete response was examined by Fisher's exact test. All statistical tests were two-sided. Sensitivity and specificity were calculated based on the optimal cut-off value as the shortest Euclidean distance obtained from the ROC curves. The Youden index (sensitivity + specificity–1) was used to select a threshold for estimation of sensitivity and specificity.

[0122] Confirmation of expression measurements by single gene Q-RTPCR and by low density QPCR array (LDA)

[0123] To confirm measurement of RNA levels, expression values derived from normalized Affymetrix data were correlated with values from semi-quantitative RT-PCR for six genes normalized to 18S. Next, measurements of these microarray RNA levels were confirmed by low density arrays (LDA), based on real time quantitative RT-PCR (QRT-PCR) of 69 most differentially expressed genes.

[0124] Confirmation study in neoadjuvant AC patients with 69-gene LDA

[0125] The validation neoadjuvant AC study was conducted with the 69-gene LDA was conducted by identifying triple negative patients (n=28) from the database of 145 patients from the University of Louisville, Kentucky, USA, who had received 6 cycles of standard AC chemotherapy. Pathologic response was assessed by a breast pathologist (SS) without prior knowledge of patient outcome, and pCR was defined as the complete disappearance of all invasive cancer in the breast. The LDA was then applied to RNA extracted form the pretreatment FFPE core biopsies. The AUC, sensitivity, and specificity were then calculated, as above.

Results

[0126] The inventors have derived a gene expression profile that is associated with DNA repair deficiency in sporadic TN breast cancers. Van't Veer et al. published a gene expression signature that can potentially distinguish breast tumors from germline BRCA1 mutation carriers from sporadic tumors. Using this gene signature and the genetic profiles of sporadic TN from three datasets, the overlap yielded a signature of 334 with 136 genes overexpressed in and 198 underexpressed genes (FIG. 20).

[0127] Increased expression of known DNA repair genes in "BRCA1-like" tumors

[0128] The inventors selected four known and commonly cited DNA repair genes (PARP-1, RAD51, FANCA, and CHK1) and measured the expression levels of these genes in triple-negative breast cancers to demonstrate an increased expression of these genes in BRCA1-like tumors. By microarray, all four genes had increased expression in BRCA1-like tumors (FIG. 21A). Additionally, they confirmed the expression of PARP-1, RAD51, and CHK1 by single gene QRT-PCR, of which PARP1 and CHEK1 were significantly increased ($p<0.05$), while RAD51 showed a trend towards increased expression in BRCA1-like tumors ($p=0.056$) (FIG.

21B). These data are consistent with up-regulation of known DNA repair genes in these sporadic TN cancers that bear the BRCA1-like signature (Martin et al., 2007).

[0129] Confirmation of expression measurements by single gene Q-RTPCR and by low density QPCR array

[0130] To confirm measurement of RNA levels, expression values derived from normalized Affymetrix data were correlated with values from semi-quantitative RT-PCR for six genes normalized to 18S. Spearman rank correlations were positive for all 6 genes (SERPINF1, PDGRA, HSP14, EFEMP2, COL15A1, and CDH5), and significantly positive for 5 of 6 genes ($p<0.05$).

[0131] Next, they confirmed measurements of these microarray RNA levels by the correlation of normalized Affymetrix data *vs.* a 69-gene low density array (LDAs). Low density arrays (LDAs), based on real time quantitative RT-PCR (QRT-PCR), enable a more focused and sensitive approach to the study of gene expression than gene chips, while offering higher throughput than single gene RT-PCR. To compare expression profiles between specimens, normalization based on three reference genes was used. An average of three references genes was used for normalization in a manner previously described (Cronin et al., 2004; Vandesompele et al., 2002). Relative mRNA was expressed as $2^{\Delta CT}+7.1$, where $\Delta C_T = C_T$(test gene) - C_T (mean of three reference genes). The average expression of the mean of the three reference genes is 10, corresponding to a C_T of 29.6. They confirmed the expression of 69 most differentially expressed genes normalized to ACTB, IPO8, and POLR2A at $p<0.05$. The correlation coefficients between the two methods were significantly positive for 45 of 69, 65.2% of the genes ($p<0.05$). In specific embodiments of the invention, this grouping of 45 is useful as a gene expression signature for identifying triple negative breast cancer from a sample from an individual that has breast cancer, is suspected of having breast cancer, or is receiving or has received treatment for breast cancer,

[0132] Defective DNA repair microarray gene expression signature is associated with anthracycline response and suggests taxane resistance

[0133] The inventors considered that those tumors exhibiting the presumptive defective DNA repair pattern would be most sensitive to DNA-damaging drugs, particularly doxorubicin, and would show relative resistance to taxanes. They then confirmed the value of

this signature in association with response to neoadjuvant chemotherapy in independent clinical trials.

[0134] Consistent with these tumors having defective DNA repair, a higher pathologic response rate (pCR) to anthracycline chemotherapy was observed in those tumors that exhibited the defective DNA repair pattern (FIG. 22). In the first data set, 80 patients were enrolled in a prospective trial at BCM (BCM2 dataset) who were treated with neoadjuvant AC. Evaluating patients (N=16) with TN breast cancer, a higher pCR or near pCR rate (*vs.* non-pCR) was observed in patients in patients with high likelihood of defective DNA repair (7/8 vs. 2/8), p=0.04.

[0135] In the second validation data set involving 50 TN patients receiving neoadjuvant FEC chemotherapy and again, a higher pCR to FEC was observed in patients with high likelihood of defective DNA repair. The area under the ordinary receiver operating characteristic (ROC) curve is 0.61, 95% CI=0.45-0.77 (FIG. 22A), with a sensitivity and specificity of 0.62and 0.62, respectively.

[0136] Interestingly, this second validation neoadjuvant trial randomized patients to FEC vs. a primarily taxane-based regimen, TET. The TET regimen was administered to 39 women with TN breast cancer. Here, patients received six full cycles of docetaxel, while epirubicin was given for only three cycles at a low dose of 90 mg/m^2, which is less than half the usually prescribed adjuvant dose. The defective DNA repair signature was associated, conversely, with relative taxane resistance. The area under the ordinary receiver operating characteristic (ROC) curve is 0.65, 95% CI=0.46-0.85 (FIG. 22B), and the sensitivity and specificity of 0.61 and 0.76 respectively, indicating that this expression pattern was not representative of general chemosensitivity.

[0137] The utility of the 69-gene LDA in predicting anthracycline response

[0138] Of the 28 TN patients, 25% (7/28) achieved pathologic complete response. From FFPE core biopsies, sufficient RNA was isolated from 21 samples, which were then used to interrogate the 69-gene low density array (LDA). This 69-gene LDA could predict anthracycline response, with an AUC of 0.79 (95% CI=0.59-0.98), with a sensitivity of 0.86, and a specificity of 0.64 (FIG. 23).

EXAMPLE 3

SIGNIFICANCE OF CERTAIN EMBODIMENTS OF THE PRESENT INVENTION

[0139] There are no currently approved targeted therapies in TN breast cancer patients, who traditionally have a poor prognosis. Patients with chemotherapy-refractory disease after neoadjuvant treatment have a high chance of distant relapse and death (Liedtke et al., 2008). In this invention there is a gene expression pattern that identifies patients whose tumors may have defective DNA repair similar to BRCA1-associated breast cancer. This expression pattern was confirmed with two other RNA platforms, QRT-PCR and a 69-gene low density array (LDA). This signature was associated with sensitivity to DNA-damaging chemotherapy (anthracyclines) and relative taxane resistance, consistent with published preclinical data in BRCA1-deficient tumors (Delaloge et al., 2008; Wysocki et al., 2008; Tassone et al., 2005; Gilmore et al., 2004).

[0140] In neoadjuvant chemotherapy studies, pathologic complete response (pCR) is associated with improved patient outcome. Despite TN cancers as a whole having poor prognosis, paradoxically, TN breast cancer patients generally achieve a higher rate of pCR. Additionally, BRCA1 mutation carriers with breast cancer achieve a higher rate of pCR. A plausible explanation is that TN breast cancer is a heterogeneous disease (Teschendorff et al., 2007; Kreike et al., 2007; Schneider et al,. 2007) with some tumors characterized by defective DNA repair similar to BRCA1-associated tumors, a defect that can be therapeutically exploited as these have an enhanced response to DNA-damaging agents. The inventors recognized this expression pattern in sporadic TN breast cancers that have a deficiency in DNA repair, and hence, show a differential improved response to agents like anthracyclines, and, in certain cases, other DNA-damaging agents.

[0141] In a hereditary mouse model of breast cancer where mice spontaneously develop mammary tumors in which BRCA1 protein has been lost, differential responses to chemotherapy (doxorubicin, docetaxel, and cisplatin) have been observed (Tassone et al,. 2009; Murray et al,. 2007; Kennedy et al., 2004; Tassone et al., 2003; Tassone et al., 2005; Gilmore et al., 2004; Sgagias et al., 2004). These mice demonstrated resistance to docetaxel, yet were highly sensitive to DNA-damaging drugs like cisplatin and doxorubicin. Additionally, sensitivity to PARP-1 inhibitors has also been shown (Ashworth, 2008; Farmer et al., 2005). PARP-1 is a group of proteins that contribute to the survival of both proliferating and non-proliferating cells

following DNA damage. It is involved in the first immediate cellular response to DNA damage, and its activation leads to DNA repair through the base excision repair (BER) pathway. Based on these observations, PARP-1 inhibitors have been reported to have high single agent activity in germline BRCA mutation carriers (Fong et al., 2009). These findings have recently been extrapolated to sporadic TN breast cancer patients in combination with chemotherapy in metastatic triple negative patients (O'Shaughnessy et al., 2009).

[0142] Low density arrays (LDAs) have recently been introduced as a novel approach to confirm gene expression profiling results (Abruzzo et al., 2005). Based on QRT-PCR, these LDAs can be used on routinely processed, formalin-fixed, paraffin-embedded (FFPE) tissue and represent a valuable approach for sensitive and quantitative gene expression profiling of multiple genes. In embodiments of this invention, the inventors confirmed with the gene expression pattern with small amounts of FFPE tissue. Successful application of these LDAs in breast cancer may assist in the selection of patients who might, or more importantly, might not benefit from anthracycline chemotherapy and other DNA damaging agents like PARP-1 inhibitors, and who might be better treated with taxane-based chemotherapy.

[0143] Limitations in this study would include the relatively small patient numbers in these analyses, as triple negative tumors account for only 15% of all breast cancers, thus increasing the difficulty in acquiring large datasets. Nonetheless, the inventors have demonstrated a defective DNA repair signature that is associated with anthracycline response and taxane resistance in TN breast cancer patients.

EXAMPLE 4

EXEMPLARY CLINICAL USE OF THE INVENTION

[0144] In an example of use of the invention in a clinical setting, an individual suspected of having breast cancer, known to have breast cancer, or having an increased risk for having breast cancer is subjected to a biopsy. In some cases, when cancer has been confirmed, histochemistry or gene expression analysis may be performed to determine what kind of breast cancer the individual has, and if it is triple negative breast cancer, a sample from the individual is subjected to a method of the invention. Whether or not the triple negative cancer is BRCA1-like determines the course of therapy. When the triple negative cancer is BRCA1-like, there is a deficiency in DNA repair, and the cancer is sensitive to DNA damaging agents. When the triple

negative breast cancer is non-BRCA1-like, the DNA repair is normal, and the cancer is resistant to DNA damaging agents. In the non-BRCA1-like cancers, therapy other than DNA damaging agents is employed, such as surgery, radiation, chemotherapy, hormone therapy, and so forth.

REFERENCES

[0145] Abruzzo LV, Lee KY, Fuller A, et al: Validation of oligonucleotide microarray data using microfluidic low-density arrays: a new statistical method to normalize real-time RT-PCR data. Biotechniques 38:785-92, 2005

[0146] Ashworth A: A synthetic lethal therapeutic approach: poly(ADP) ribose polymerase inhibitors for the treatment of cancers deficient in DNA double-strand break repair. J Clin Oncol 26:3785-90, 2008

[0147] Bernstein C, Bernstein H, Payne CM, et al: DNA repair/pro-apoptotic dual-role proteins in five major DNA repair pathways: fail-safe protection against carcinogenesis. Mutat Res 511:145-78, 2002

[0148] Bonnefoi H, Potti A, Delorenzi M, et al: Validation of gene signatures that predict the response of breast cancer to neoadjuvant chemotherapy: a substudy of the EORTC 10994/BIG 00-01 clinical trial. Lancet Oncol 8:1071-8, 2007

[0149] Brody LC: Treating cancer by targeting a weakness. N Engl J Med 353:949-50, 2005

[0150] Byrski T, Huzarski T, Dent R, et al: Response to neoadjuvant therapy with cisplatin in BRCA1-positive breast cancer patients. Breast Cancer Res Treat, 2008

[0151] Byrski T, Gronwald J, Huzarski T, et al: Response to neo-adjuvant chemotherapy in women with BRCA1-positive breast cancers. Breast Cancer Res Treat 108:289-96, 2008

[0152] Carey LA, Dees EC, Sawyer L, et al: The triple negative paradox: primary tumor chemosensitivity of breast cancer subtypes. Clin Cancer Res 13:2329-34, 2007

[0153] Chang JC, Wooten EC, Tsimelzon A, et al: Gene expression profiling for the prediction of therapeutic response to docetaxel in patients with breast cancer. Lancet 362:362-9, 2003

[0154] Chang JC, Wooten EC, Tsimelzon A, et al: Patterns of resistance and incomplete response to docetaxel by gene expression profiling in breast cancer patients. J Clin Oncol 23:1169-77, 2005

[0155] Chappuis PO, Goffin J, Wong N, et al: A significant response to neoadjuvant chemotherapy in BRCA1/2 related breast cancer. J Med Genet 39:608-10, 2002

[0156] Cronin M, Pho M, Dutta D, et al: Measurement of gene expression in archival paraffin-embedded tissues: development and performance of a 92-gene reverse transcriptase-polymerase chain reaction assay. Am J Pathol 164:35-42, 2004

[0157] Delaloge S et al., ASCO 2008 Abstract 574

[0158] Dizdar O et al, Dasatinib may also inhibit c-Kit in triple negative breast cancer cell lines., Breast Cancer Res Treat. 2008 Jan;107(2):303

[0159] Farmer H, McCabe N, Lord CJ, et al: Targeting the DNA repair defect in BRCA mutant cells as a therapeutic strategy. Nature 434:917-21, 2005

[0160] Farmer P, Bonnefoi H, Anderle P, et al: A stroma-related gene signature predicts resistance to neoadjuvant chemotherapy in breast cancer. Nat Med 15:68-74, 2009

[0161] Finn et al., Dasatinib, an orally active small molecule inhibitor of both the src and abl kinases, selectively inhibits growth of basal-type/"triple-negative" breast cancer cell lines growing in vitro. Breast Cancer Res Treat. 2007 Nov;105(3):319-26

[0162] Fong PC, Boss DS, Yap TA, et al: Inhibition of poly(ADP-ribose) polymerase in tumors from BRCA mutation carriers. N Engl J Med 361:123-34, 2009

[0163] Foulkes et al., 2003

[0164] Gilmore PM, McCabe N, Quinn JE, et al: BRCA1 interacts with and is required for paclitaxel-induced activation of mitogen-activated protein kinase kinase kinase 3. Cancer Res 64:4148-54, 2004

[0165] Hess KR, Anderson K, Symmans WF, et al: Pharmacogenomic predictor of sensitivity to preoperative chemotherapy with paclitaxel and fluorouracil, doxorubicin, and cyclophosphamide in breast cancer. J Clin Oncol 24:4236-44, 2006

[0166] Hubert A, Mali B, Hamburger T, et al: Response to neo-adjuvant chemotherapy in BRCA1 and BRCA2 related stage III breast cancer. Fam Cancer, 2008

[0167] J. O'Shaughnessy CO, J. Pippen, M. Yoffe, D. Patt, G. Monaghan, C. Rocha, V. Ossovskaya, B. Sherman, C. Bradley: Efficacy of BSI-201, a poly (ADP-ribose) polymerase-1 (PARP1) inhibitor, in combination with gemcitabine/carboplatin (G/C) in patients with metastatic triple-negative breast cancer (TNBC): Results of a randomized phase II trial. Journal of Clinical Oncology Abstract 3 27, 2009

[0168] James CR, Quinn JE, Mullan PB, et al: BRCA1, a potential predictive biomarker in the treatment of breast cancer. Oncologist 12:142-50, 2007

[0169] Kennedy RD, Quinn JE, Mullan PB, et al: The role of BRCA1 in the cellular response to chemotherapy. J Natl Cancer Inst 96:1659-68, 2004

[0170] Kreike B, van Kouwenhove M, Horlings H, et al: Gene expression profiling and histopathological characterization of triple-negative/basal-like breast carcinomas. Breast Cancer Res 9:R65, 2007

[0171] Lakhani et al., 2005

[0172] Liedtke C, Mazouni C, Hess KR, et al: Response to neoadjuvant therapy and long-term survival in patients with triple-negative breast cancer. J Clin Oncol 26:1275-81, 2008

[0173] LJ van't Veer et al, Gene Expression Profiling Predicts Clinical Outcome of Breast Cancer, Nature 2002: 415, 530-536

[0174] Marcus JN et al, Cancer. 1996 Feb 15;77(4):697-709

[0175] Martin RW, Orelli BJ, Yamazoe M, et al: RAD51 up-regulation bypasses BRCA1 function and is a common feature of BRCA1-deficient breast tumors. Cancer Res 67:9658-65, 2007

[0176] Mohsin SK, Weiss HL, Gutierrez MC, et al: Neoadjuvant trastuzumab induces apoptosis in primary breast cancers. J Clin Oncol 23:2460-8, 2005

[0177] Mueller CR, Roskelley CD: Regulation of BRCA1 expression and its relationship to sporadic breast cancer. Breast Cancer Res 5:45-52, 2003

[0178] Murray MM, Mullan PB, Harkin DP: Role played by BRCA1 in transcriptional regulation in response to therapy. Biochem Soc Trans 35:1342-6, 2007

[0179] S. Delaloge FB, Y. El Masmoudi, B. Bressac de Paillerets, O. Caron, C. Bourgier, J. Garbay, M. Spielmann, and F. André: BRCA1 germ-line mutation: Predictive of sensitivity to anthracyclin alkylating agents regimens but not to taxanes? Journal of Clinical Oncology Abstract 574 26, 2008

[0180] Schneider BP, Winer EP, Foulkes WD, et al: Triple-negative breast cancer: risk factors to potential targets. Clin Cancer Res 14:8010-8, 2008

[0181] Sgagias MK, Wagner KU, Hamik B, et al: Brca1-deficient murine mammary epithelial cells have increased sensitivity to CDDP and MMS. Cell Cycle 3:1451-6, 2004

[0182] Sorlie T, Tibshirani R, Parker J, et al: Repeated observation of breast tumor subtypes in independent gene expression data sets. Proc Natl Acad Sci U S A 100:8418-23, 2003

[0183] Tassone P, Tagliaferri P, Perricelli A, et al: BRCA1 expression modulates chemosensitivity of BRCA1-defective HCC1937 human breast cancer cells. Br J Cancer 88:1285-91, 2003

[0184] Tassone P, Blotta S, Palmieri C, et al: Differential sensitivity of BRCA1-mutated HCC1937 human breast cancer cells to microtubule-interfering agents. Int J Oncol 26:1257-63, 2005

[0185] Tassone P, Di Martino MT, Ventura M, et al: Loss of BRCA1 function increases the antitumor activity of cisplatin against human breast cancer xenografts in vivo. Cancer Biol Ther 8:648-53, 2009

[0186] Teschendorff AE, Miremadi A, Pinder SE, et al: An immune response gene expression module identifies a good prognosis subtype in estrogen receptor negative breast cancer. Genome Biol 8:R157, 2007

[0187] Turner N, Tutt A, Ashworth A: Hallmarks of 'BRCAness' in sporadic cancers. Nat Rev Cancer 4:814-9, 2004

[0188] Turner NC, Reis-Filho JS: Basal-like breast cancer and the BRCA1 phenotype. Oncogene 25:5846-53, 2006

[0189] Turner NC, Reis-Filho JS, Russell AM, et al: BRCA1 dysfunction in sporadic basal-like breast cancer. Oncogene 26:2126-32, 2007

[0190] van de Vijver MJ, He YD, van't Veer LJ, et al: A gene-expression signature as a predictor of survival in breast cancer. N Engl J Med 347:1999-2009, 2002

[0191] Vandesompele J, De Preter K, Pattyn F, et al: Accurate normalization of real-time quantitative RT-PCR data by geometric averaging of multiple internal control genes. Genome Biol 3:RESEARCH0034, 2002

[0192] von Minckwitz G KM, Kümmel S, Fasching P, Eiermann W, Blohmer J-U, Costa SD, Sibylle L, Dietmar V, Untch M: Integrated meta-analysis on 6402 patients with early breast cancer receiving neoadjuvant anthracycline-taxane +/- trastuzumab containing chemotherapy. SABCS Abstract 79, 2008

[0193] Wysocki PJ, Korski K, Lamperska K, et al: Primary resistance to docetaxel-based chemotherapy in metastatic breast cancer patients correlates with a high frequency of BRCA1 mutations. Med Sci Monit 14:SC7-10, 2008

CLAIMS

What is claimed is:

1. A method of identifying triple negative breast cancer from a sample from an individual that has breast cancer, is suspected of having breast cancer, or is receiving or has received treatment for breast cancer, comprising the step of assaying the expression of two or more sequences from breast cells of the individual, said sequences selected from the group consisting of genes listed in Table 1, or the complement of said sequences.

2. A method of determining a therapy for an individual with triple negative breast cancer, who is suspected of having triple negative breast cancer, or who is receiving or has received treatment for triple negative breast cancer, comprising the step of assaying the expression of two or more sequences from breast cells of the individual, said sequences selected from the group consisting of genes listed in Table 1, or the complement of said sequences.

3. A plurality of primers for polymerizing at least two or more sequences selected from the group consisting of genes listed in Table 1, or the complement of said sequence or of a sequence capable of hybridizing to the sequence under stringent conditions.

4. A collection of oligonucleotides that correspond to two or more of the genes listed in Table 1, said oligonucleotides housed on a substrate.

5. The collection of claim 4, further defined as comprising oligonucleotides that correspond to three or more of the genes listed in Table 1.

6. The collection of claim 4, further defined as comprising oligonucleotides that correspond to four or more of the genes listed in Table 1.

7. The collection of claim 4, further defined as comprising oligonucleotides that correspond to five or more of the genes listed in Table 1.

8. The collection of claim 4, further defined as comprising oligonucleotides that correspond to six or more of the genes listed in Table 1.

9. The collection of claim 4, further defined as comprising oligonucleotides that correspond to seven or more of the genes listed in Table 1.

10. The collection of claim 4, further defined as comprising oligonucleotides that correspond to eight or more of the genes listed in Table 1.

11. The collection of claim 4, further defined as comprising oligonucleotides that correspond to nine or more of the genes listed in Table 1.

12. The collection of claim 4, further defined as comprising oligonucleotides that correspond to ten or more of the genes listed in Table 1.

13. The collection of claim 4, further defined as comprising oligonucleotides that correspond to fifteen or more of the genes listed in Table 1.

14. The collection of claim 4, further defined as comprising oligonucleotides that correspond to twenty or more of the genes listed in Table 1.

15. The collection of claim 4, further defined as comprising oligonucleotides that correspond to twenty-two or more of the genes listed in Table 1.

16. The collection of claim 4, further defined as comprising oligonucleotides that correspond to twenty-five of the genes listed in Table 1.

17. The collection of claim 4, further defined as comprising thirty or more of the genes listed in Table 1.

18. The collection of claim 4, further defined as comprising thirty-five or more of the genes listed in Table 1.

19. The collection of claim 4, further defined as comprising forty or more of the genes listed in Table 1.

20. The collection of claim 4, further defined as comprising all forty-five of the genes listed in Table 1.

21. A collection of two or more of the genes listed in Table 1, said collection housed on a substrate.

22. The collection of claim 21, further defined as comprising three or more of the genes listed in Table 1.

23. The collection of claim 21, further defined as comprising four or more of the genes listed in Table 1.

24. The collection of claim 21, further defined as comprising five or more of the genes listed in Table 1.

25. The collection of claim 21, further defined as comprising six or more of the genes listed in Table 1.

26. The collection of claim 21, further defined as comprising seven or more of the genes listed in Table 1.

27. The collection of claim 21, further defined as comprising eight or more of the genes listed in Table 1.

28. The collection of claim 21, further defined as comprising nine or more of the genes listed in Table 1.

29. The collection of claim 21, further defined as comprising ten or more of the genes listed in Table 1.

30. The collection of claim 21, further defined as comprising fifteen or more of the genes listed in Table 1.

31. The collection of claim 21, further defined as comprising twenty or more of the genes listed in Table 1.

32. The collection of claim 21, further defined as comprising twenty-five or more of the genes listed in Table 1.

33. The collection of claim 21, further defined as comprising thirty or more of the genes listed in Table 1.

34. The collection of claim 21, further defined as comprising thirty-five or more of the genes listed in Table 1.

35. The collection of claim 21, further defined as comprising forty or more of the genes listed in Table 1.

36. The collection of claim 1, further defined as comprising all forty-five of the genes listed in Table 1.

37. As a composition of matter, a breast cancer RNA expression profile comprising two or more of the genes listed in Table 1.

38. As a composition of matter, isolated expressed polynucleotides the levels of which are indicative of the presence of triple negative breast cancer or indicative of a therapy for triple negative breast cancer, wherein two or more of the expressed polynucleotides are listed in Table 1.

39. A kit, housed in a suitable container, comprising one or both of the following:

(1) an array comprising polynucleotides corresponding to the genes listed in Table 1, or the complement of said sequences; and

(2) a collection of oligonucleotides that correspond to two or more of the genes listed in Table 1.

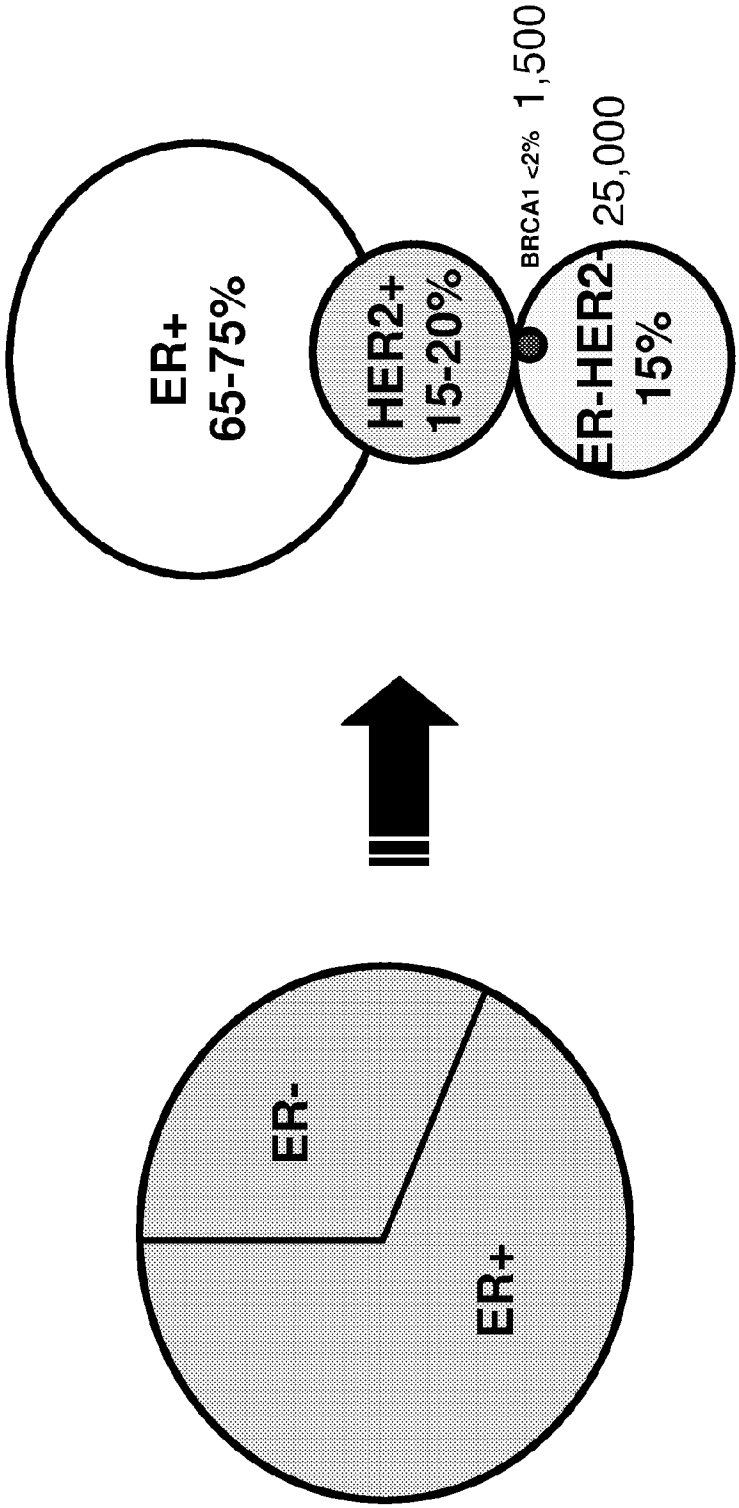
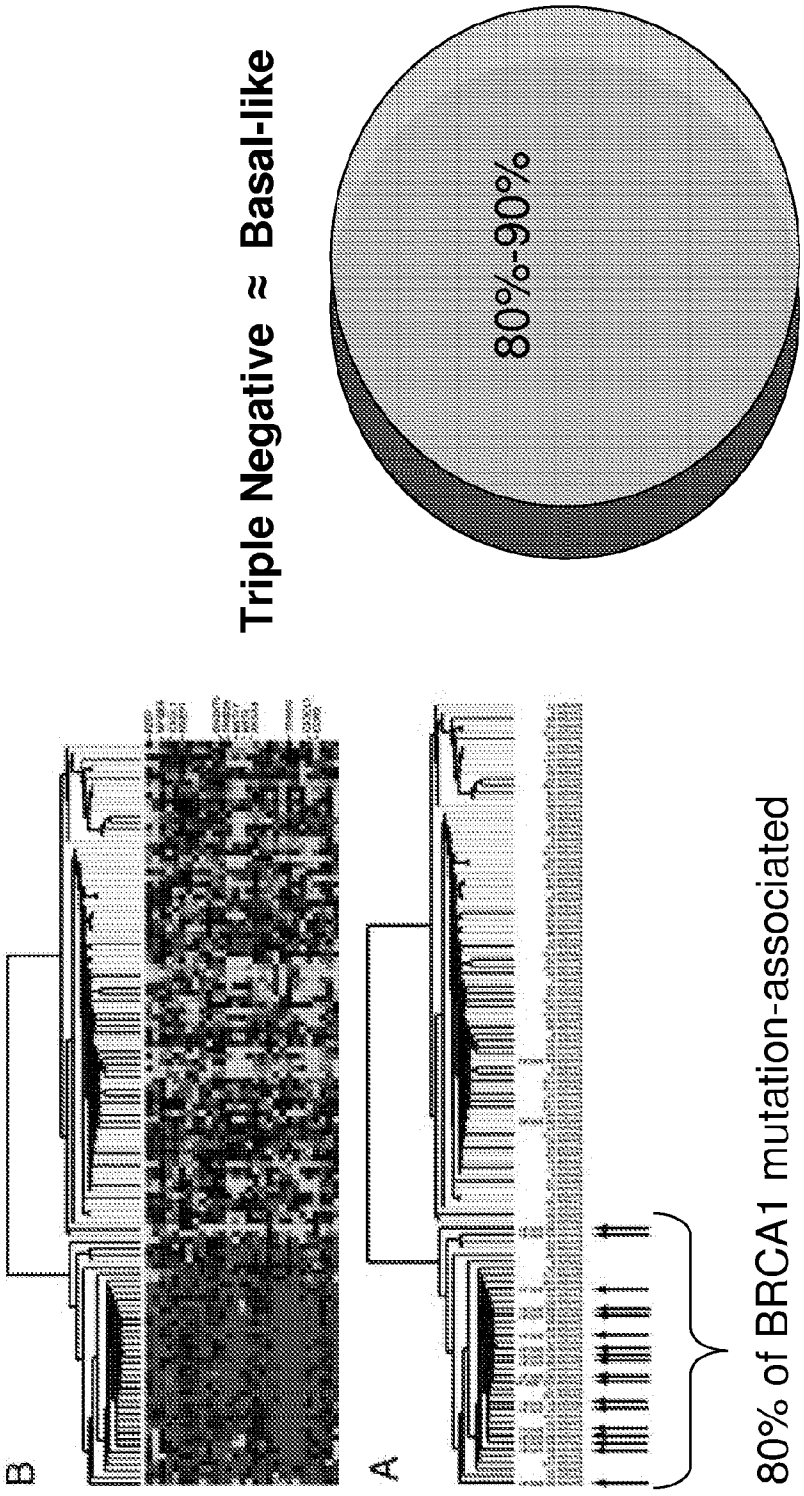
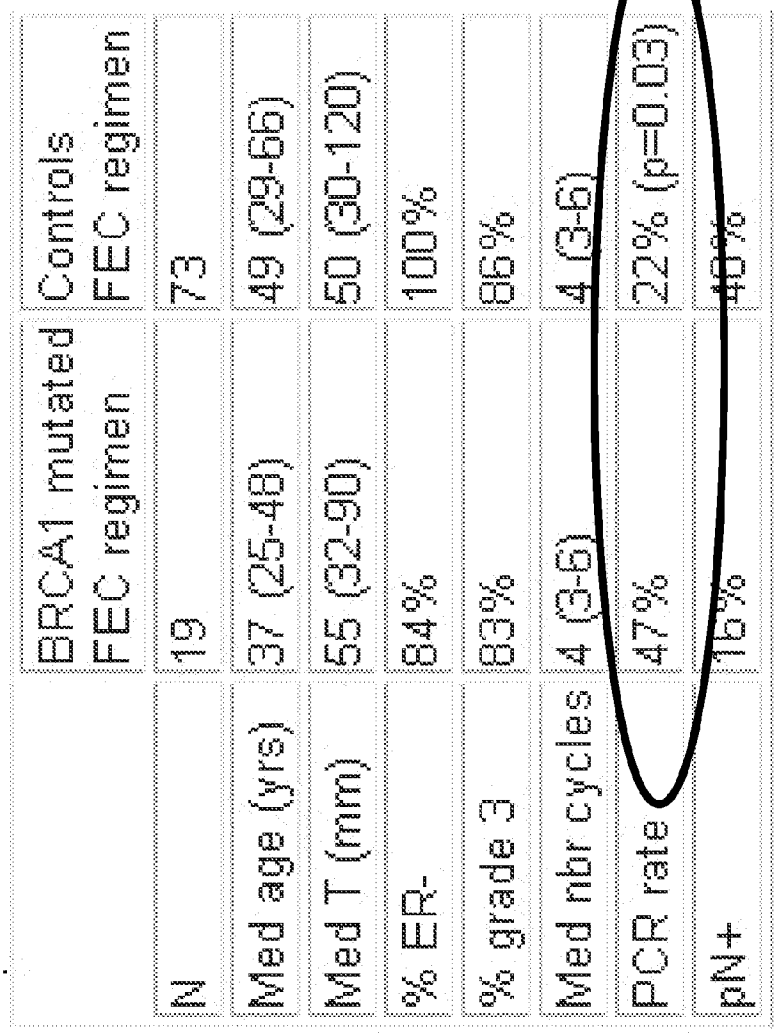


FIG. 1



Sorlie et al, Proc Natl Acad Sci U S A. 2003 July 8; 100(14): 8418–8423

FIG. 2



	BRCA1 mutated FEC regimen	Controls FEC regimen
N	19	73
Med age (yrs)	37 (25-48)	49 (29-66)
Med T (mm)	55 (32-90)	50 (30-120)
% ER-	84%	100%
% grade 3	83%	86%
Med nbr cycles	4 (3-6)	4 (3-6)
PCR rate	47%	22% (p=0.03)
pN+	16%	40%

Delaloge S et al., ASCO 2008 Abstract 574

FIG. 3

Redirecting Therapies in Triple Negative Breast Cancer

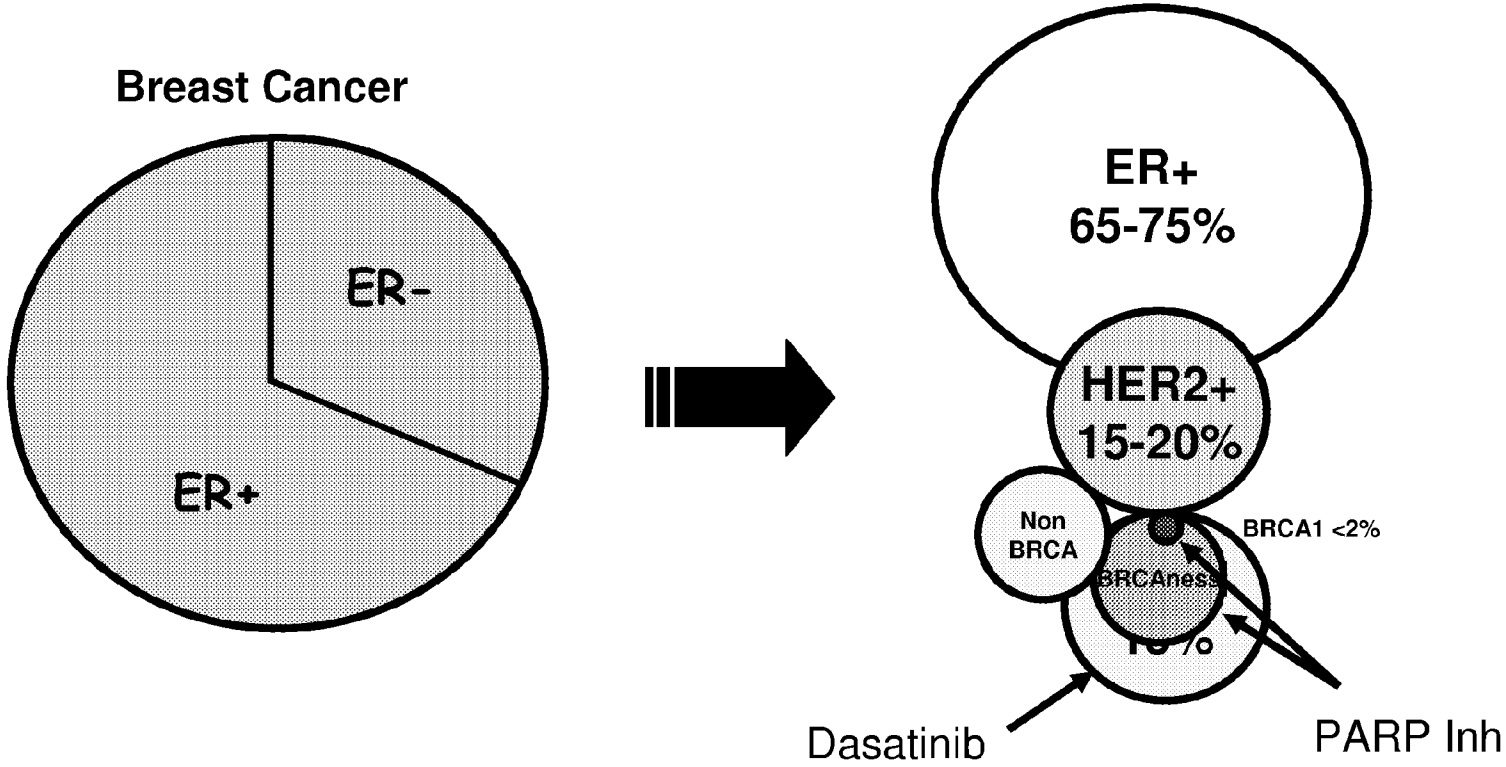
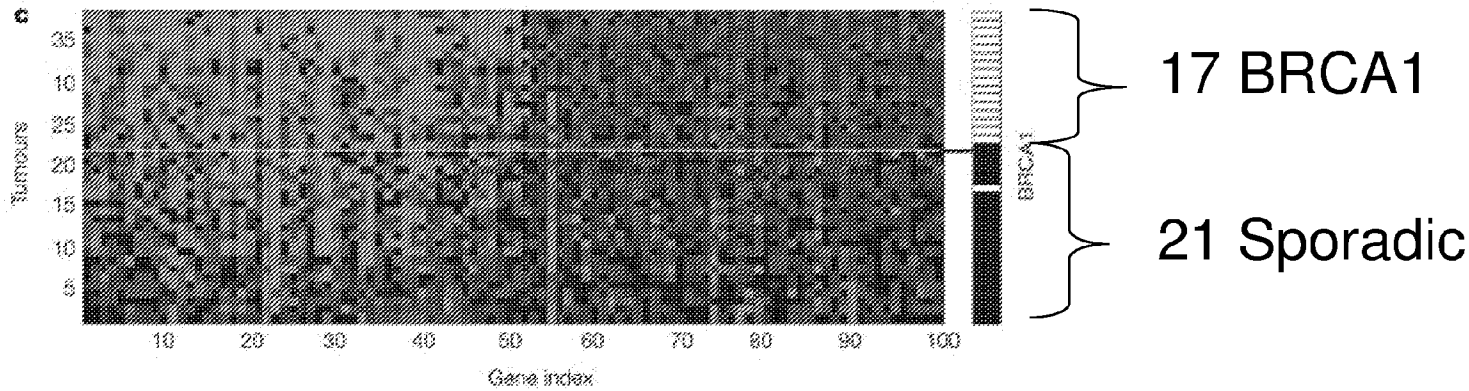


FIG. 4

38 ER- Tumors



- Largest number of samples with BRCA1 mutation
- Classification into sporadic and BRCA1 tumors was caused mainly by the differences in levels of gene expression(amplitude)

LJ van't Veer et al, Gene Expression Profiling Predicts Clinical Outcome of Breast Cancer, Nature 2002: 415, 530-536

FIG. 5

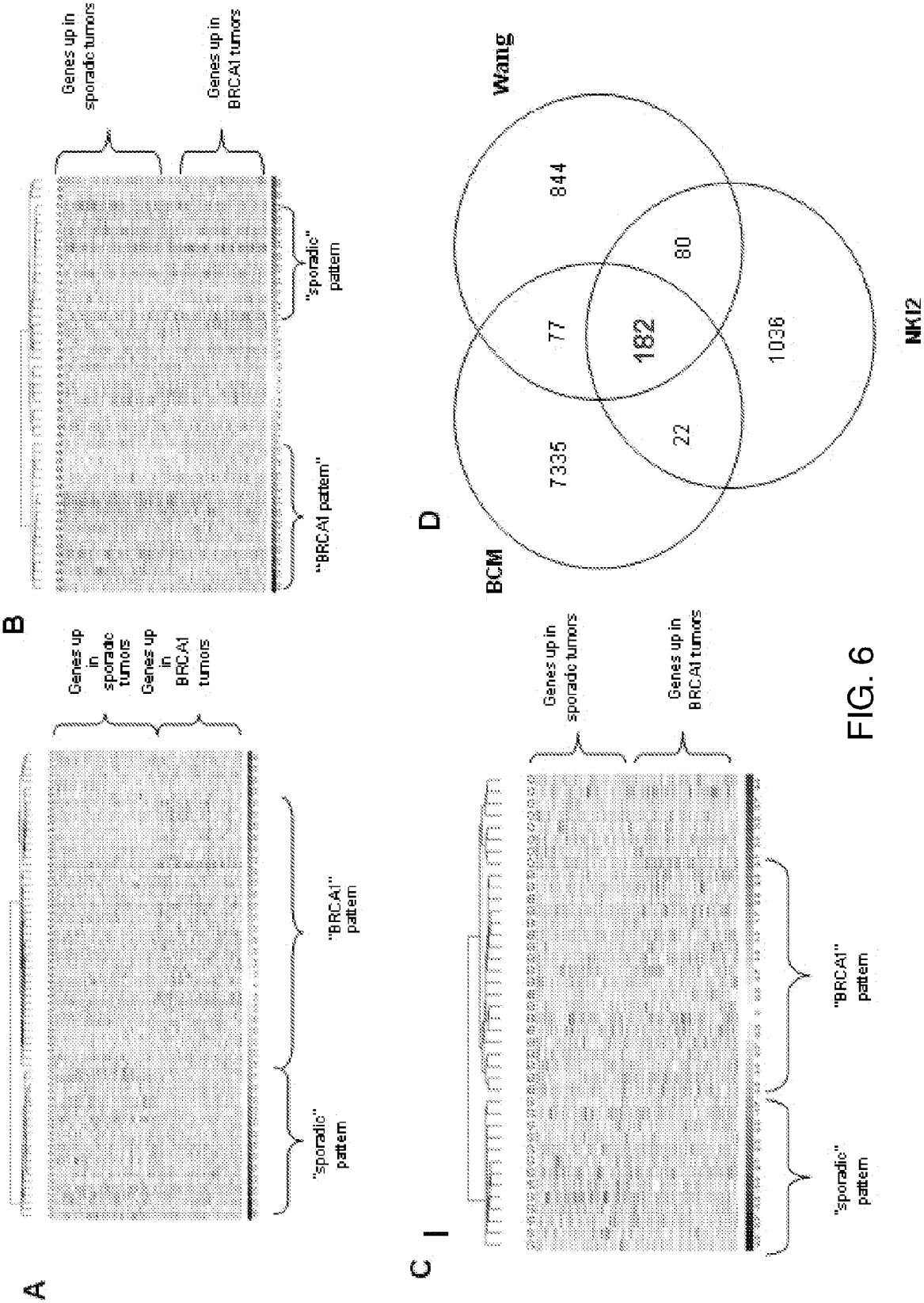

A
Genes up in sporadic tumors
Genes up in BRCA1 tumors
"sporadic" pattern
"BRCA1" pattern
B
Genes up in sporadic tumors
Genes up in BRCA1 tumors
"sporadic" pattern
"BRCA1 pattern"
C
Genes up in sporadic tumors
Genes up in BRCA1 tumors
"sporadic" pattern
"BRCA1" pattern
D
Wang
BCM
NKI2
844
77
182
80
7335
22
1036

FIG. 6

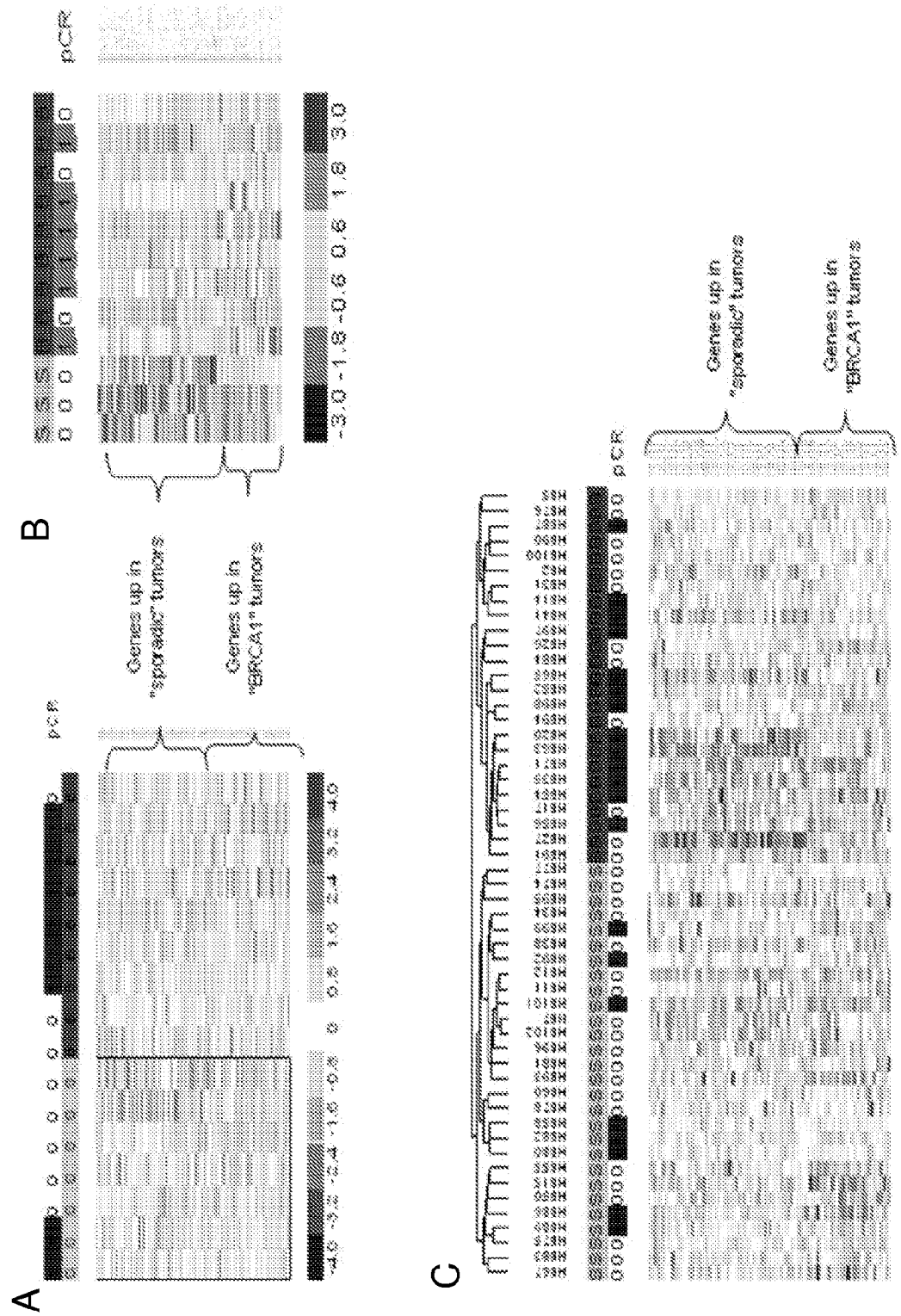
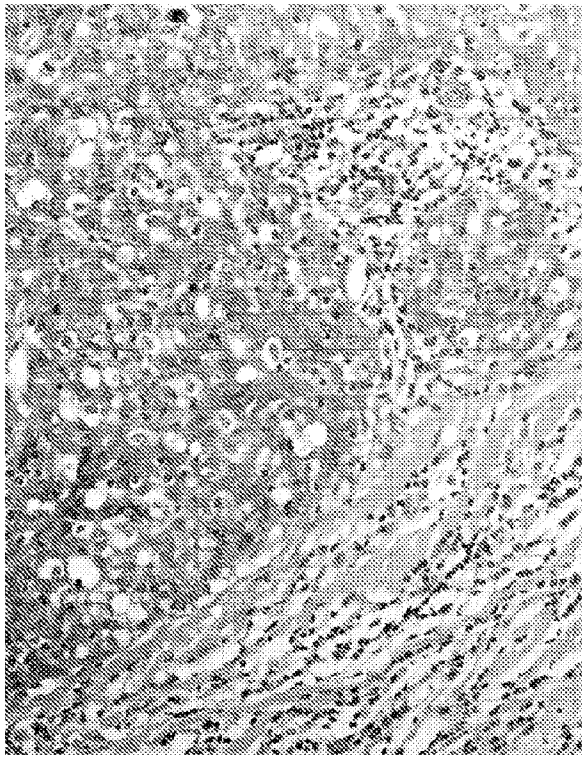

A
pCR
Genes up in "sporadic" tumors
Genes up in "BRCA1" tumors
-4.8 -3.2 -2.4 -1.6 -0.8 0 0.8 1.6 2.4 3.2 4.8
B
pCR
-3.0 -1.8 -0.6 0.6 1.8 3.0
C
pCR
Genes up in "sporadic" tumors
Genes up in "BRCA1" tumors

FIG. 7

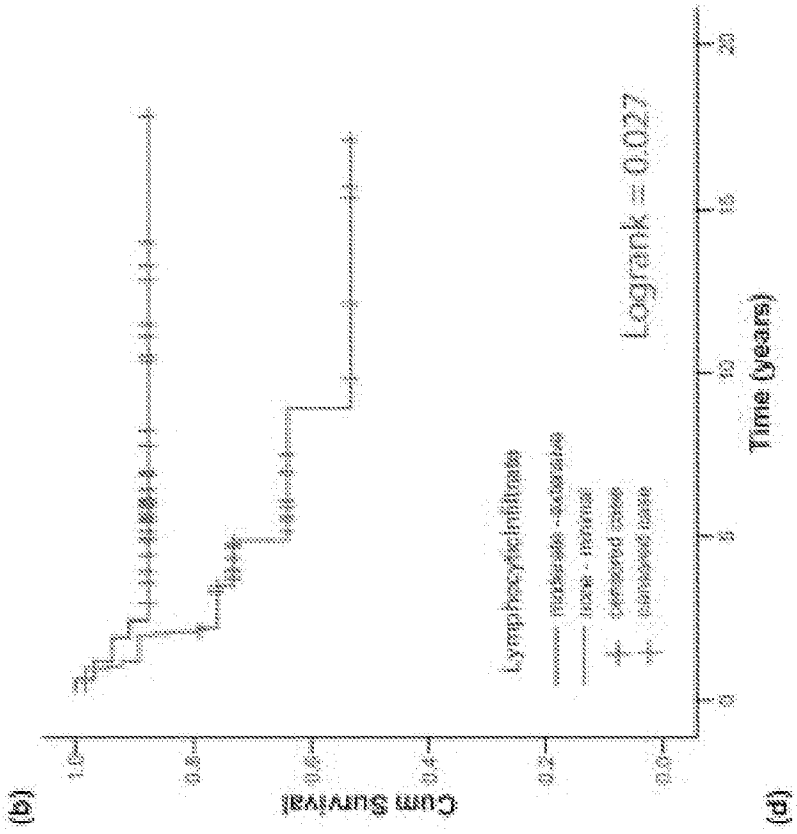


Sporadic Only Samples

	BRCA1-like	Non-BRCA1
Lymphocytic Infiltrate	6	4
No Lymph Infiltrate	1	7
Total	7	11

$P<0.05$

FIG. 8



Metastasis-free survival of 71 patients with triple-negative breast carcinomas comparing amount of lymphocytic infiltrate,

B Kreike et al, *Breast Cancer Res.* 2007; 9(5)

FIG. 9

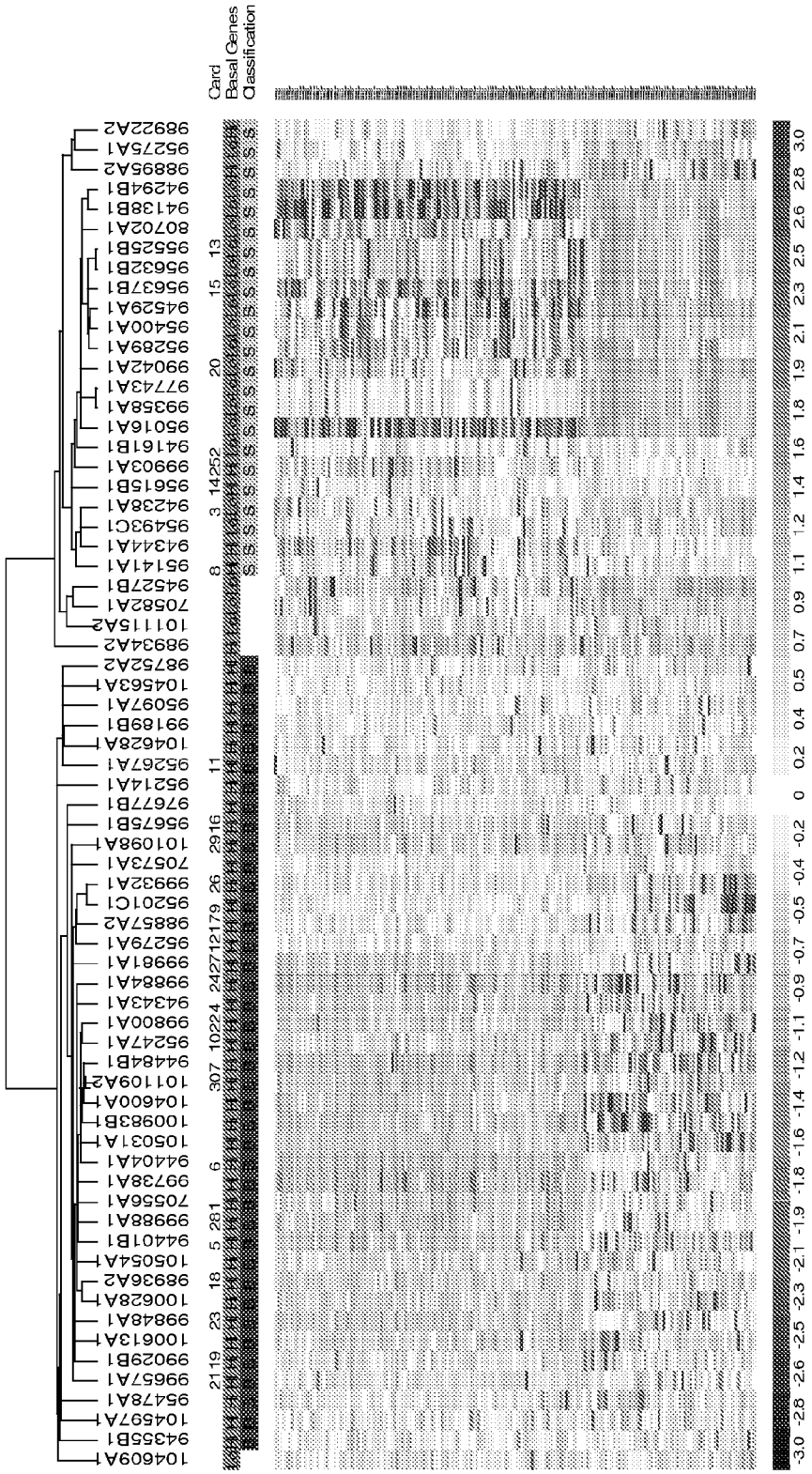

Card
Basal Genes
Classification
-3.0 -2.8 -2.6 -2.5 -2.3 -2.1 -1.9 -1.8 -1.6 -1.4 -1.2 -1.1 -0.9 -0.7 -0.5 -0.4 -0.2 0 0.2 0.4 0.5 0.7 0.9 1.1 1.2 1.4 1.6 1.8 1.9 2.1 2.3 2.5 2.6 2.8 3.0

FIG. 10

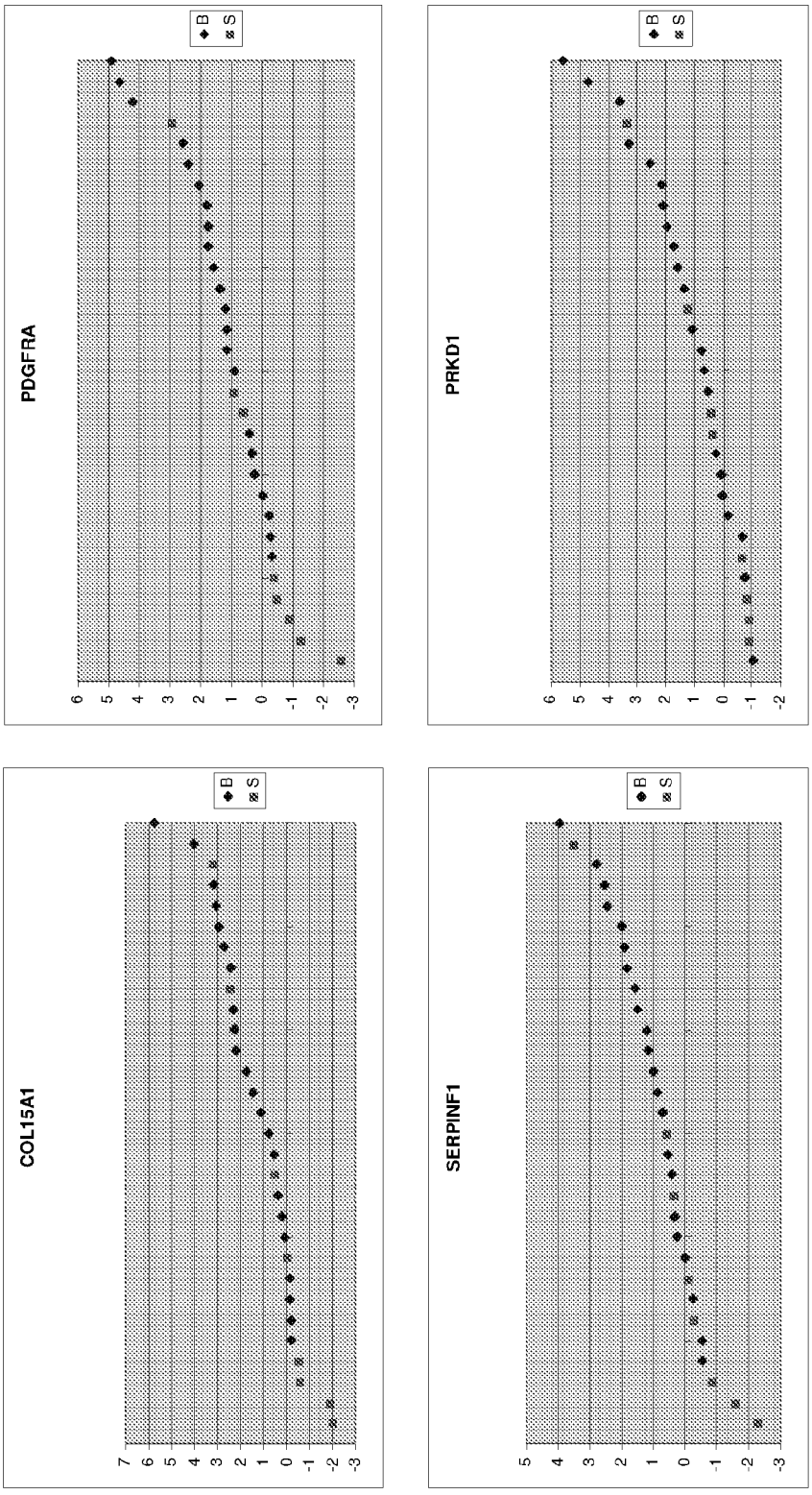
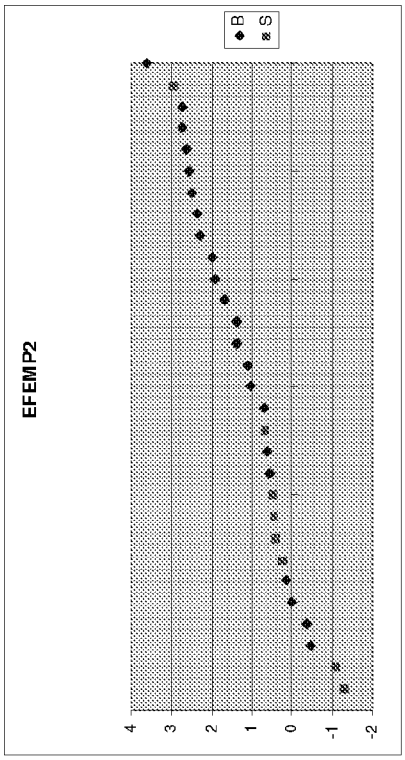
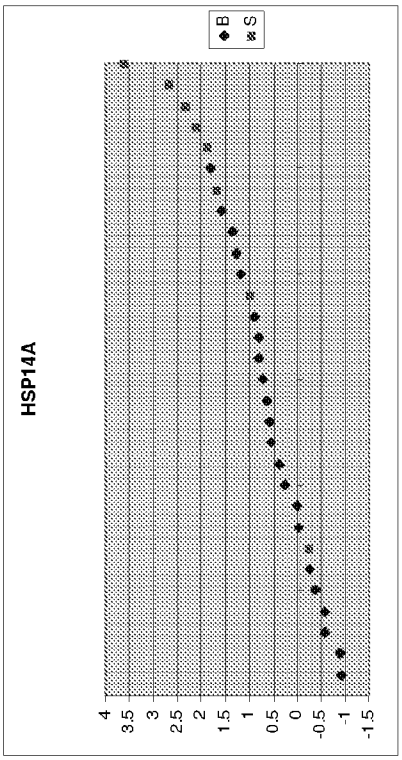


FIG. 11



probe set	Sporadic mean	BRCAn mean	F stat	P
PDGFRA	-0.16	1.53	7.0	0.01
HSP14A	1.87	0.41	15.4	0.001
SERPINF1	-0.1	1.17	5.3	0.03
PRKD1	0.23	1.42	2.9	0.1
COL15A1	0.12	1.65	5.0	0.03
EFEMP2	0.33	1.5	5.9	0.02
CDH5	1.22	2.16	0.6	.4



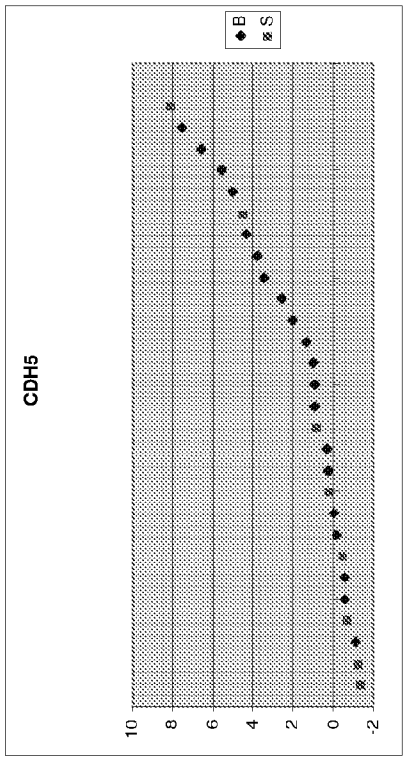


FIG. 12

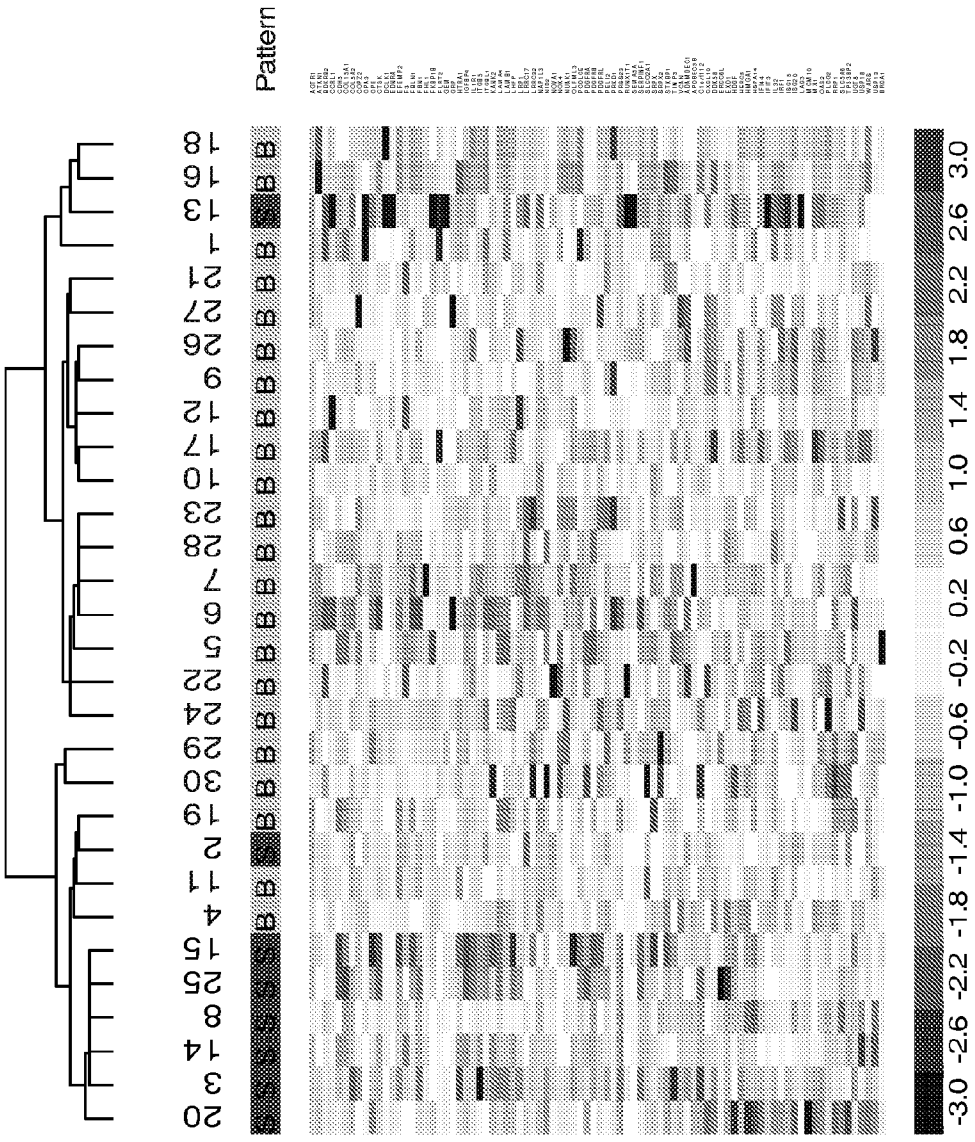
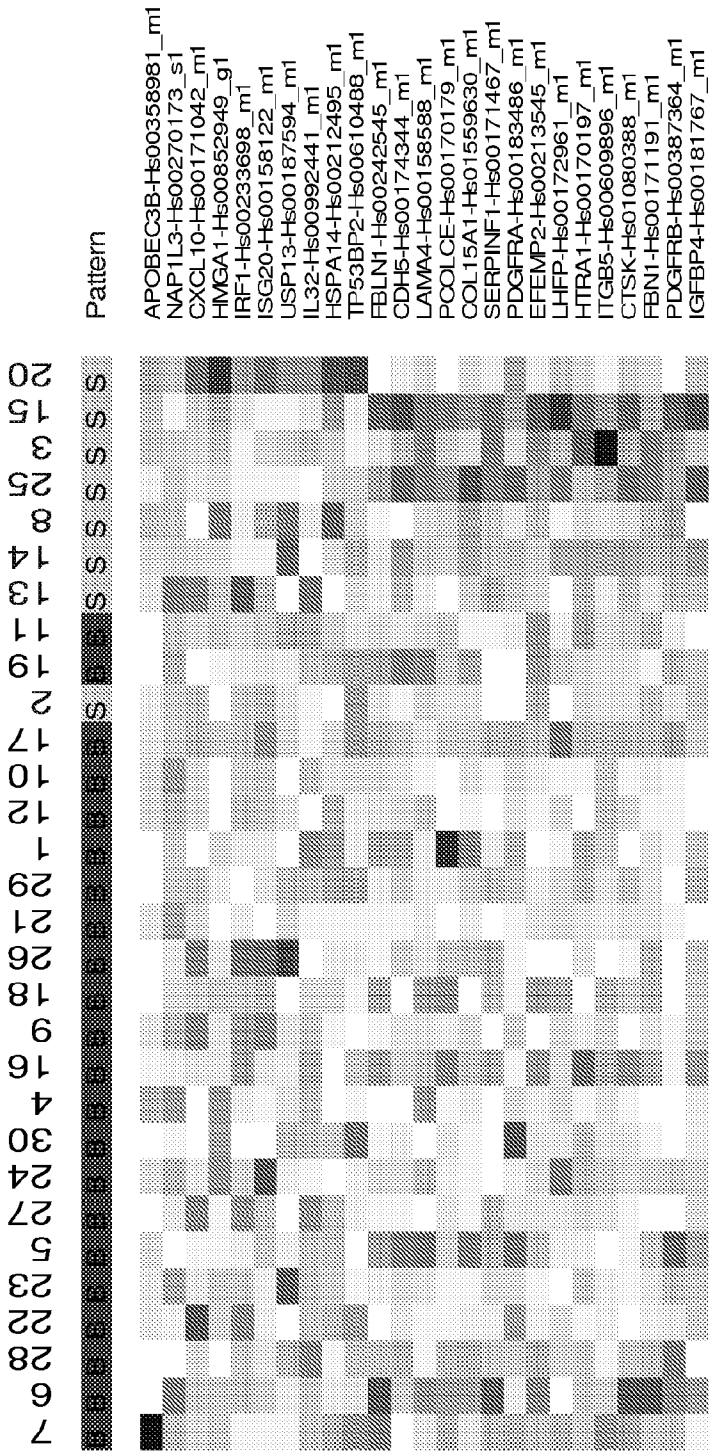

Pattern
18 16 13 1 21 27 26 9 12 17 10 23 28 7 6 5 22 24 29 30 19 2 11 4 15 25 8 14 3 20
-3.0 -2.6 -2.2 -1.8 -1.4 -1.0 -0.6 -0.2 0.2 0.6 1.0 1.4 1.8 2.2 2.6 3.0

FIG. 13



25 genes, Ct diff >1, p<0.05

FIG. 14

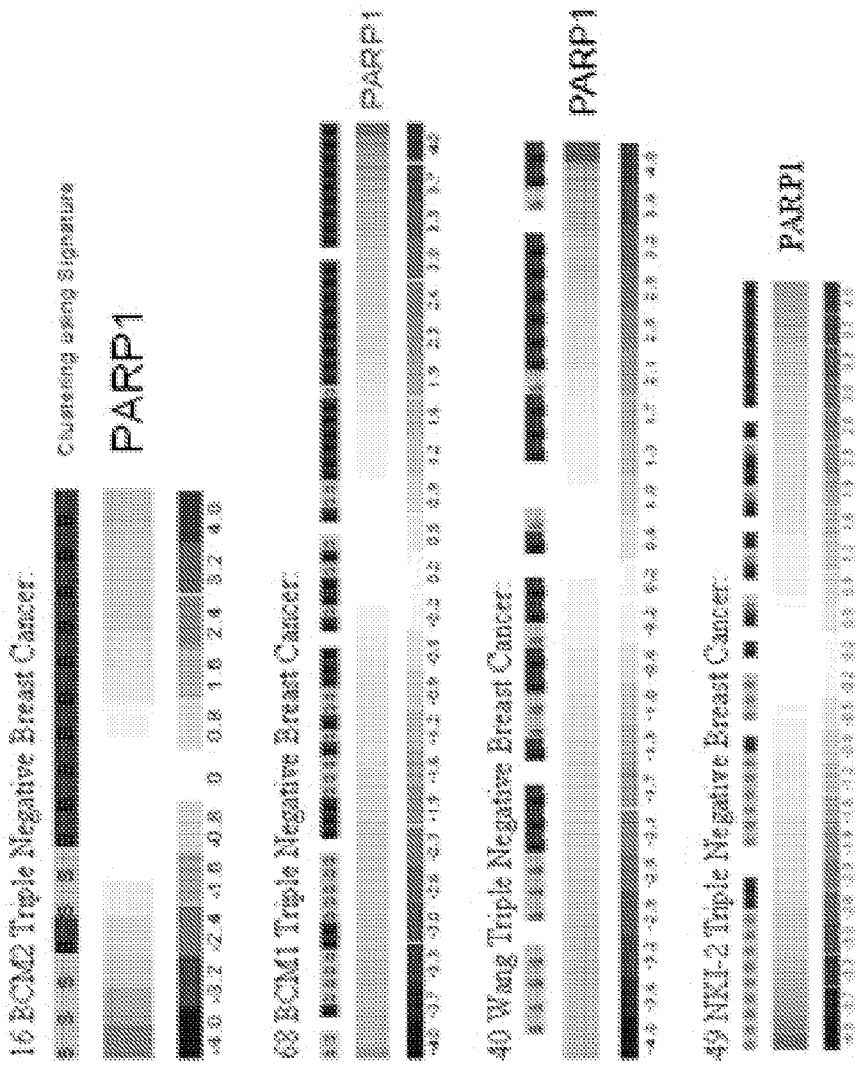

16 BCM2 Triple Negative Breast Cancer
Clustering using Signature
PARP1
68 BCM1 Triple Negative Breast Cancer
PARP1
40 Wang Triple Negative Breast Cancer
PARP1
49 NKI-2 Triple Negative Breast Cancer
PARP1

FIG. 15

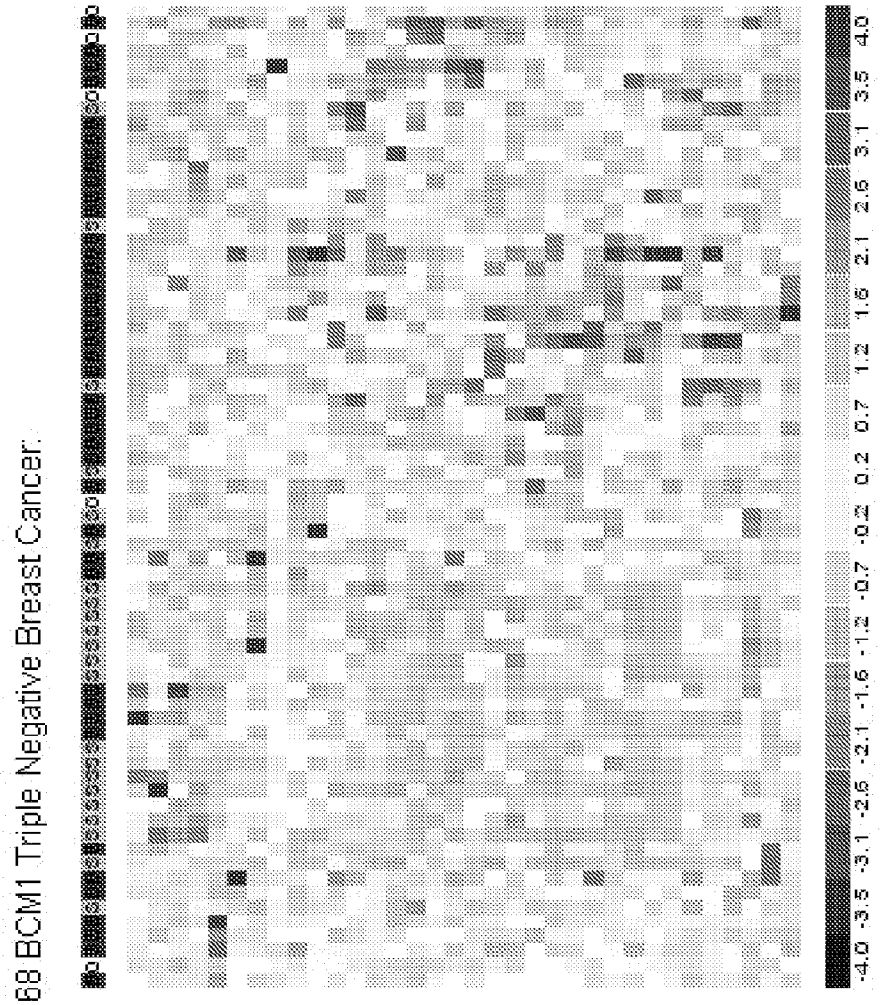
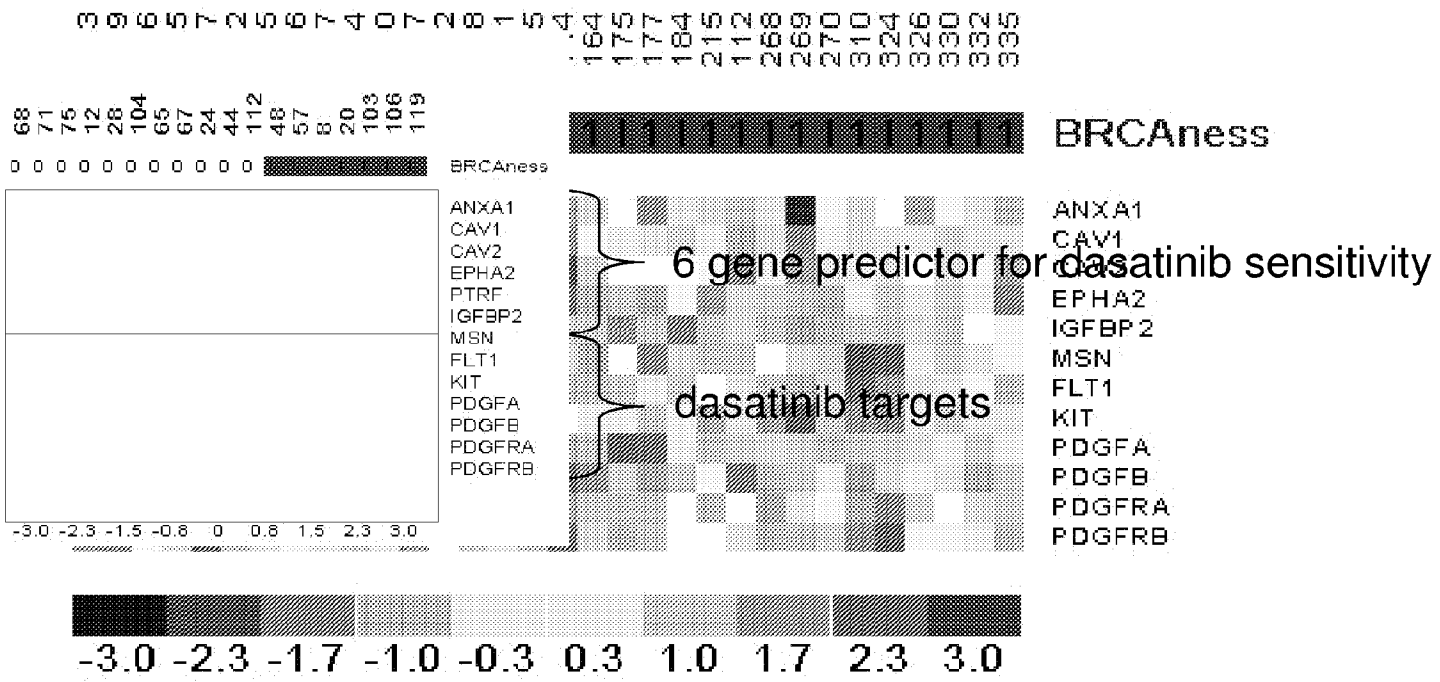

68 BCM1 Triple Negative Breast Cancer.
-4.0 -3.5 -3.1 -2.6 -2.1 -1.6 -1.2 -0.7 -0.2 0.2 0.7 1.2 1.6 2.1 2.6 3.1 3.5 4.0

FIG. 16



Dizdar O et al, Dasatinib may also inhibit c-Kit in triple negative breast cancer cell lines., Breast Cancer Res Treat. 2008 Jan;107(2):303

Finn et al., Dasatinib, an orally active small molecule inhibitor of both the src and abl kinases, selectively inhibits growth of basal-type/"triple-negative" breast cancer cell lines growing in vitro. Breast Cancer Res Treat. 2007 Nov;105(3):319-26

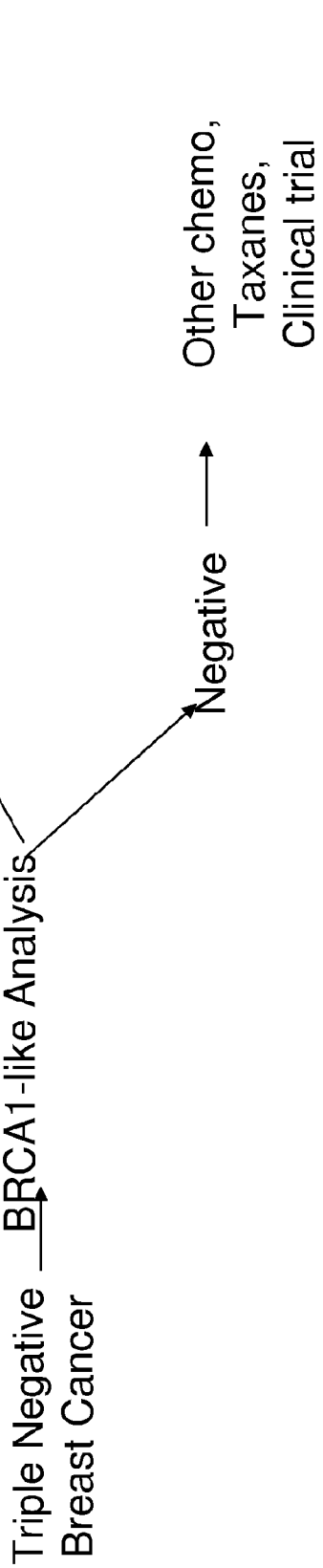

Triple Negative
Breast Cancer
BRCA1-like Analysis
High
Score
DNA-damaging chemo
+ PARP Inh
Negative
Other chemo,
Taxanes,
Clinical trial

FIG. 18

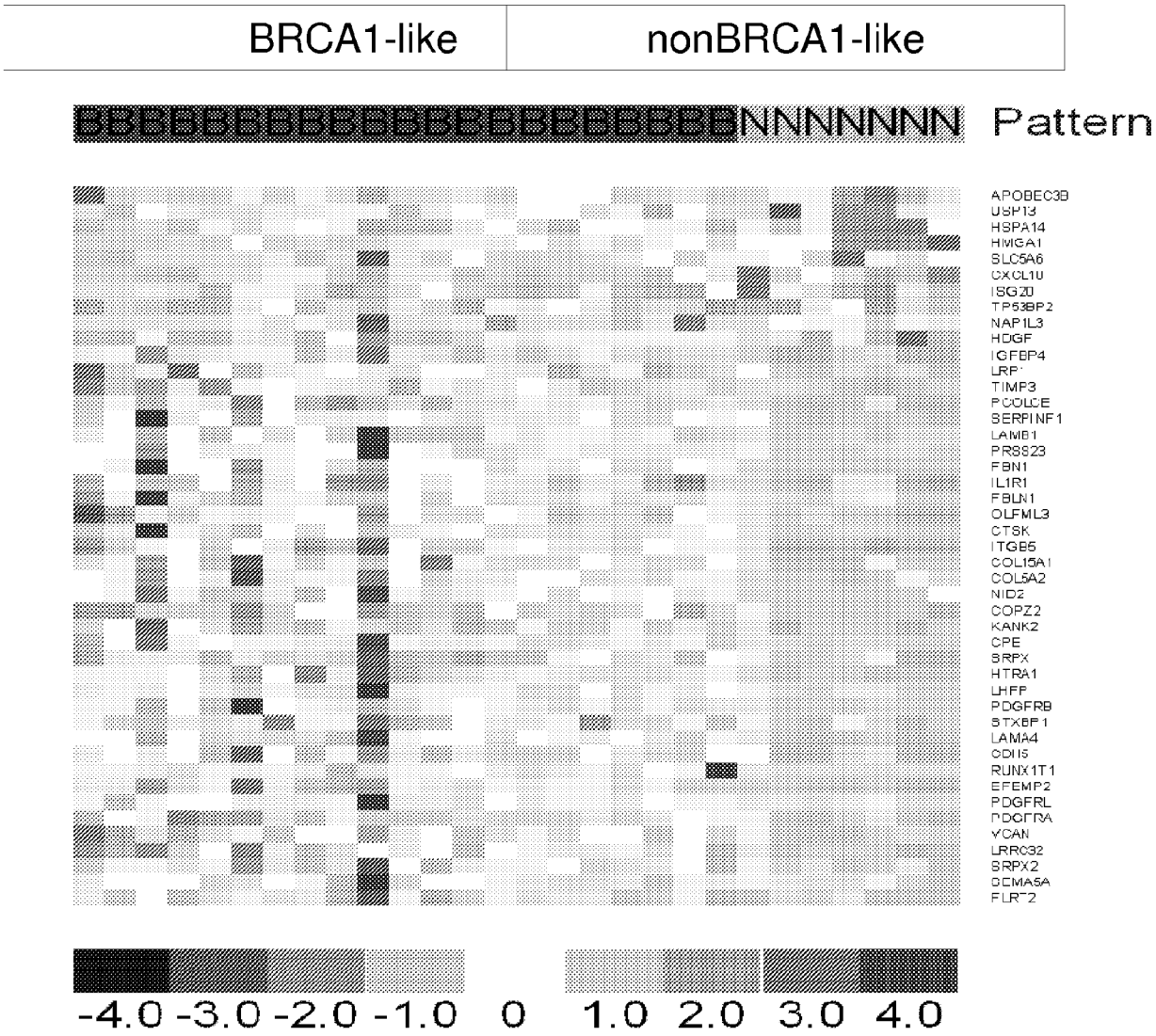
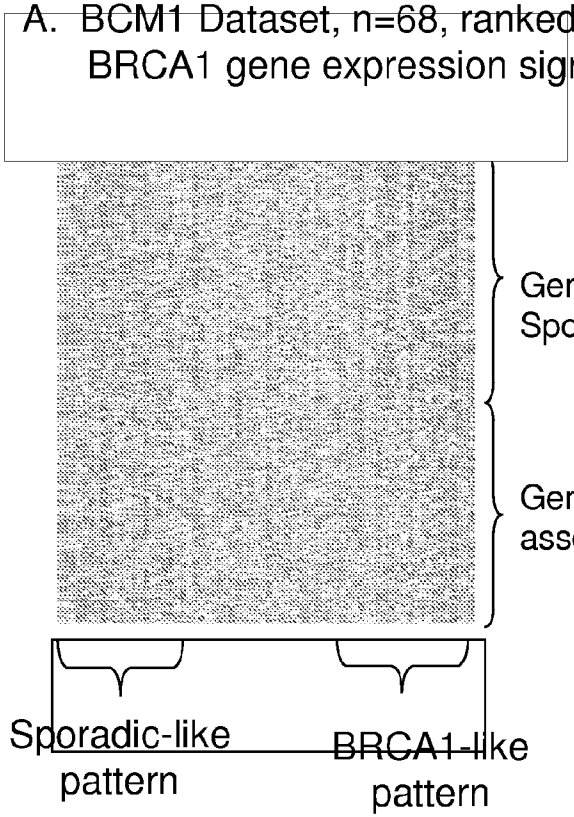

BRCA1-like
nonBRCA1-like
BBBBBBBBBBBBBBBBBBBBBBBBNNNNNNNN
Pattern
APOBEC3B
USP13
HSPA14
HMGA1
SLC5A6
CXCL10
ISG20
TP53BP2
NAP1L3
HDGF
IGFBP4
LRP1
TIMP3
PCOLCE
SERPINF1
LAMB1
PRSS23
FBN1
IL1R1
FBLN1
OLFML3
CTSK
ITGB5
COL15A1
COL5A2
NID2
COPZ2
KANK2
CPE
SRPX
HTRA1
LHFP
PDGFRB
STXBP1
LAMA4
CDH5
RUNX1T1
EFEMP2
PDGFRL
PDGFRA
VCAN
LRRC32
SRPX2
SEMA5A
FLRT2
-4.0 -3.0 -2.0 -1.0 0 1.0 2.0 3.0 4.0

FIG. 19


A. BCM1 Dataset, n=68, ranked with BRCA1 gene expression signature
Genes overexpressed in Sporadic ER-negative tumors
Genes overexpressed in BRCA1-associated ER-negative tumors
Sporadic-like pattern
BRCA1-like pattern

B

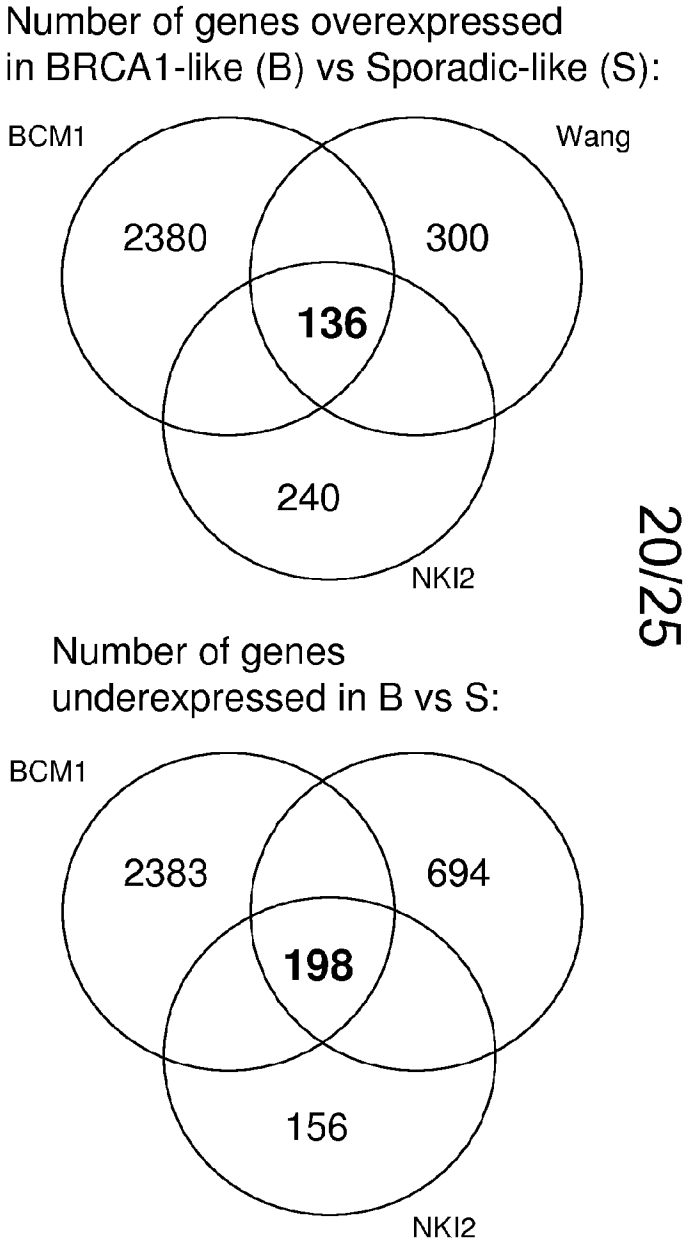

Number of genes overexpressed in BRCA1-like (B) vs Sporadic-like (S):
BCM1
Wang
NKI2
2380
300
136
240
Number of genes underexpressed in B vs S:
BCM1
NKI2
2383
694
198
156

FIG. 20

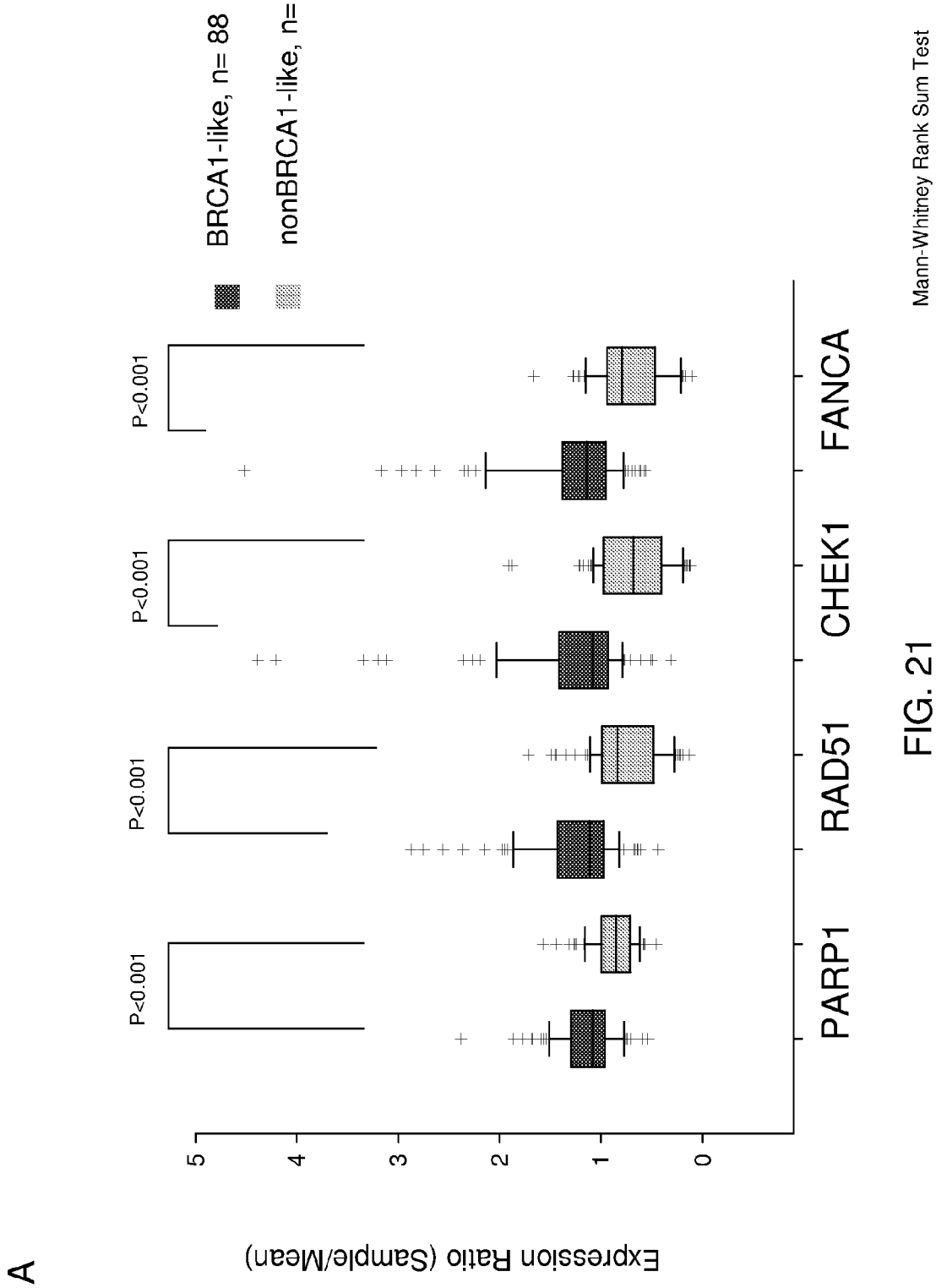

A
Expression Ratio (Sample/Mean)
5
4
3
2
1
0
BRCA1-like, n= 88
nonBRCA1-like, n= 86
P<0.001
P<0.001
P<0.001
PARP1
RAD51
CHEK1
FANCA
Mann-Whitney Rank Sum Test

FIG. 21

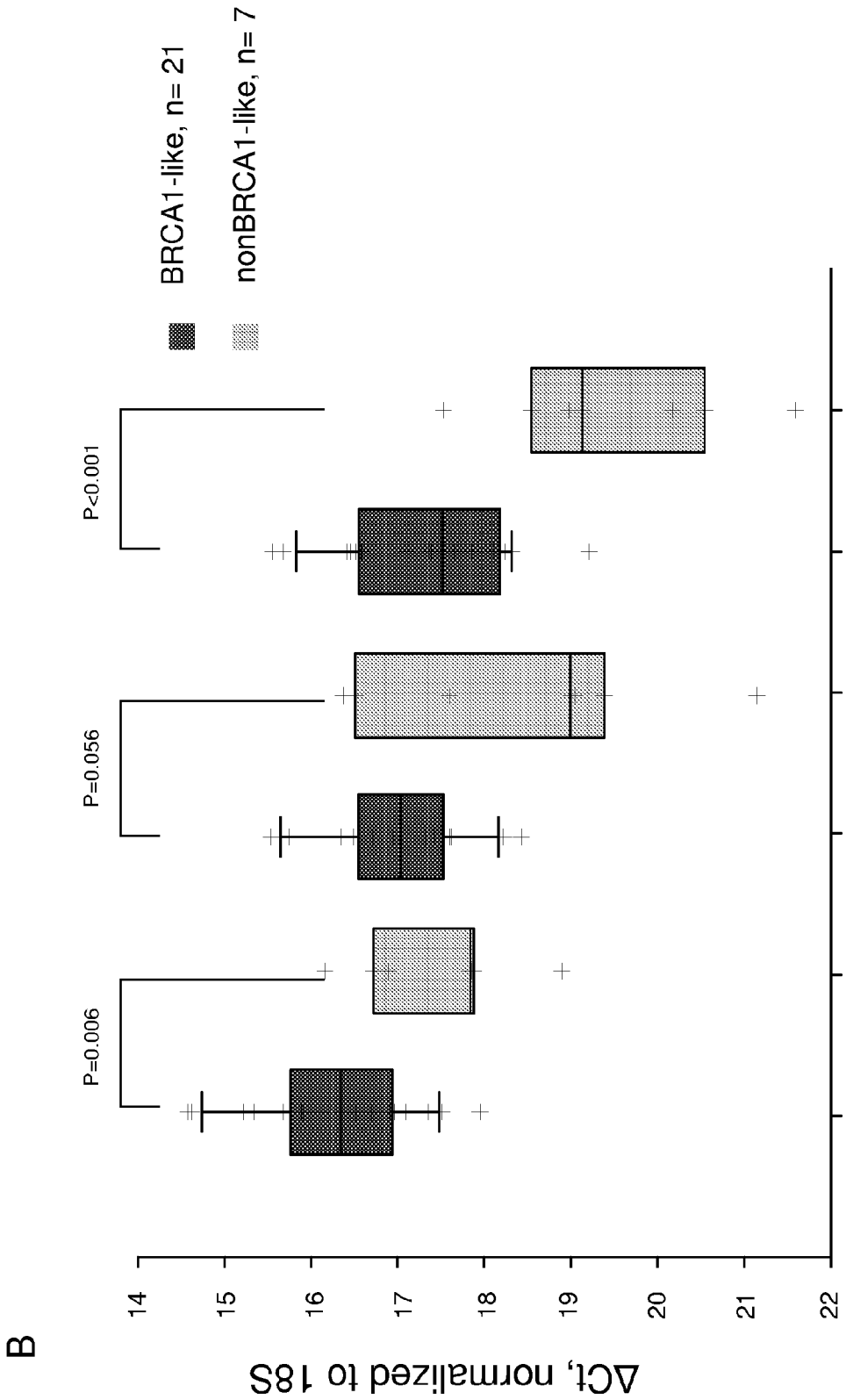
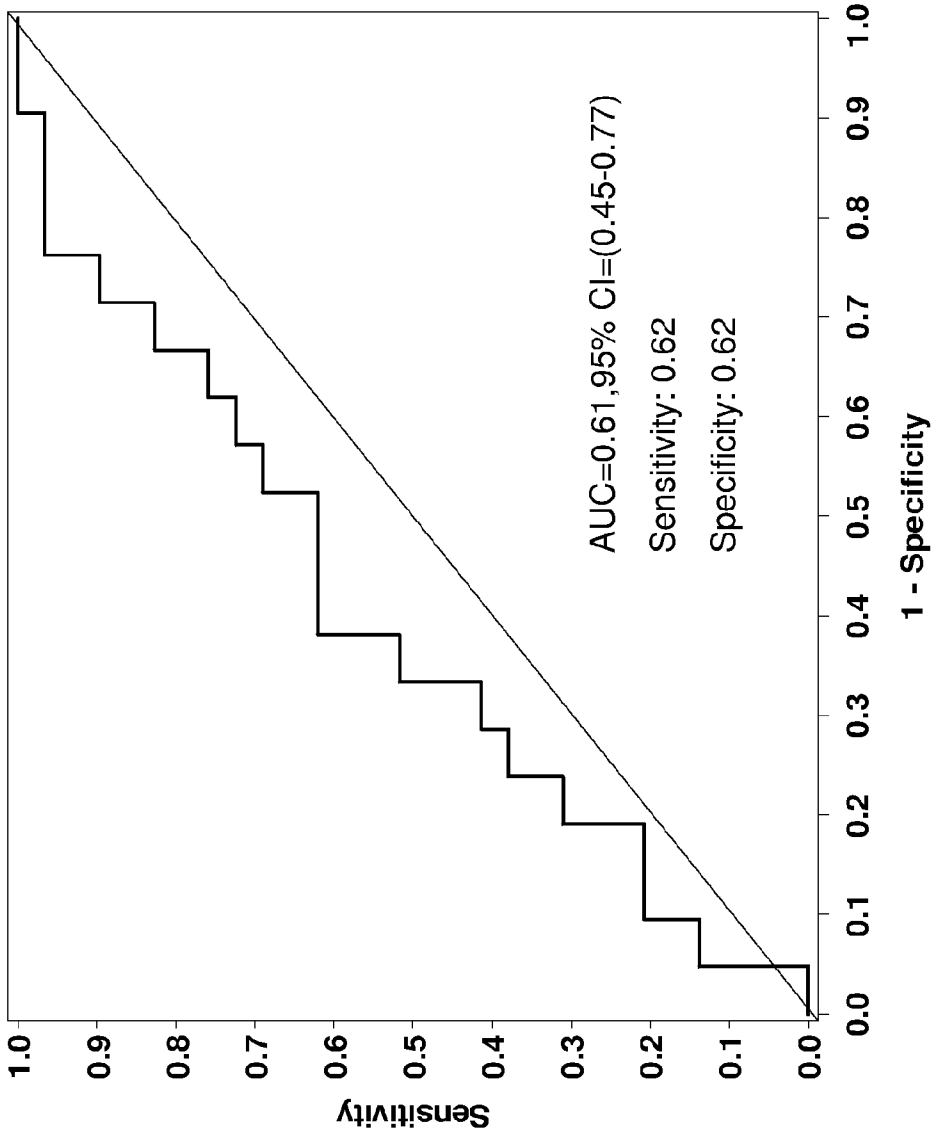

B
ΔCt, normalized to 18S
14
15
16
17
18
19
20
21
22
BRCA1-like, n= 21
nonBRCA1-like, n= 7
P=0.006
P=0.056
P<0.001
PARP1
RAD51
CHEK1

FIG. 21

Figure 22 A. ROC curve (FEC) chemotherapy



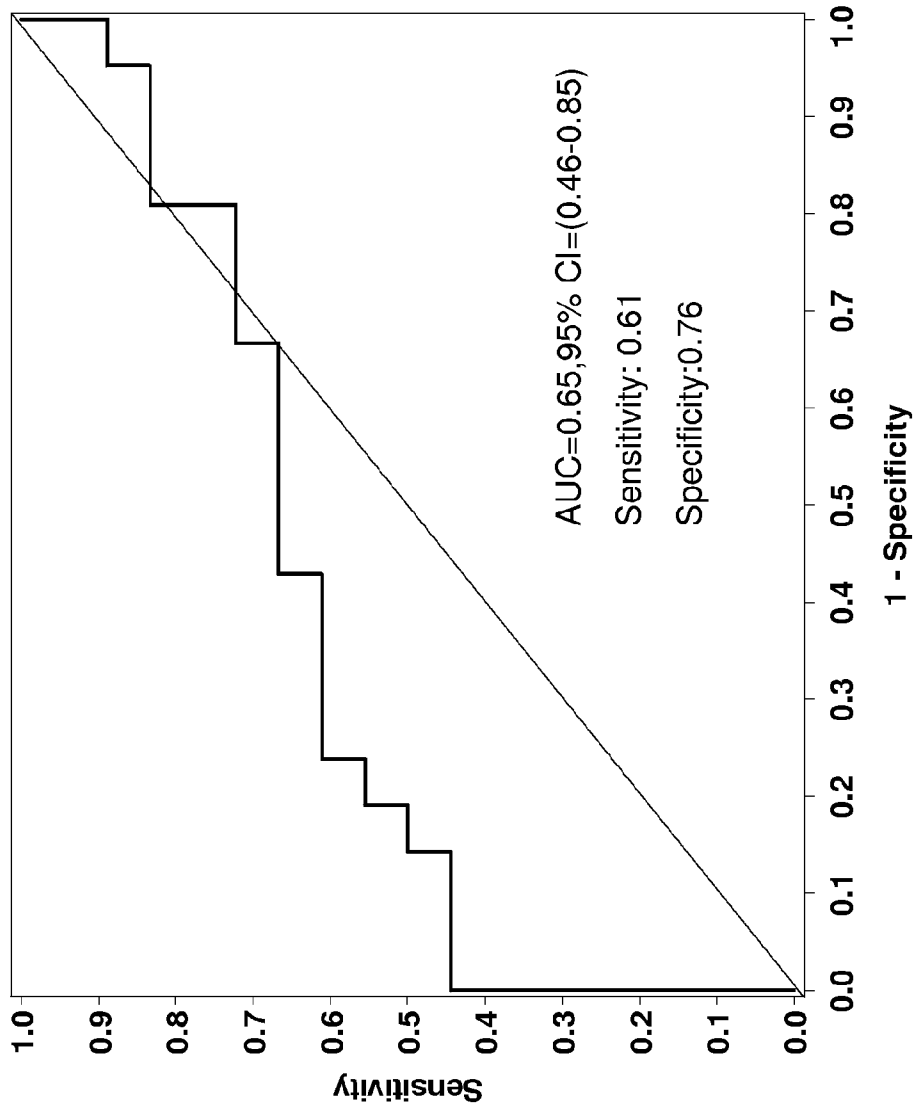


Figure 22 B. ROC curve TET chemotherapy

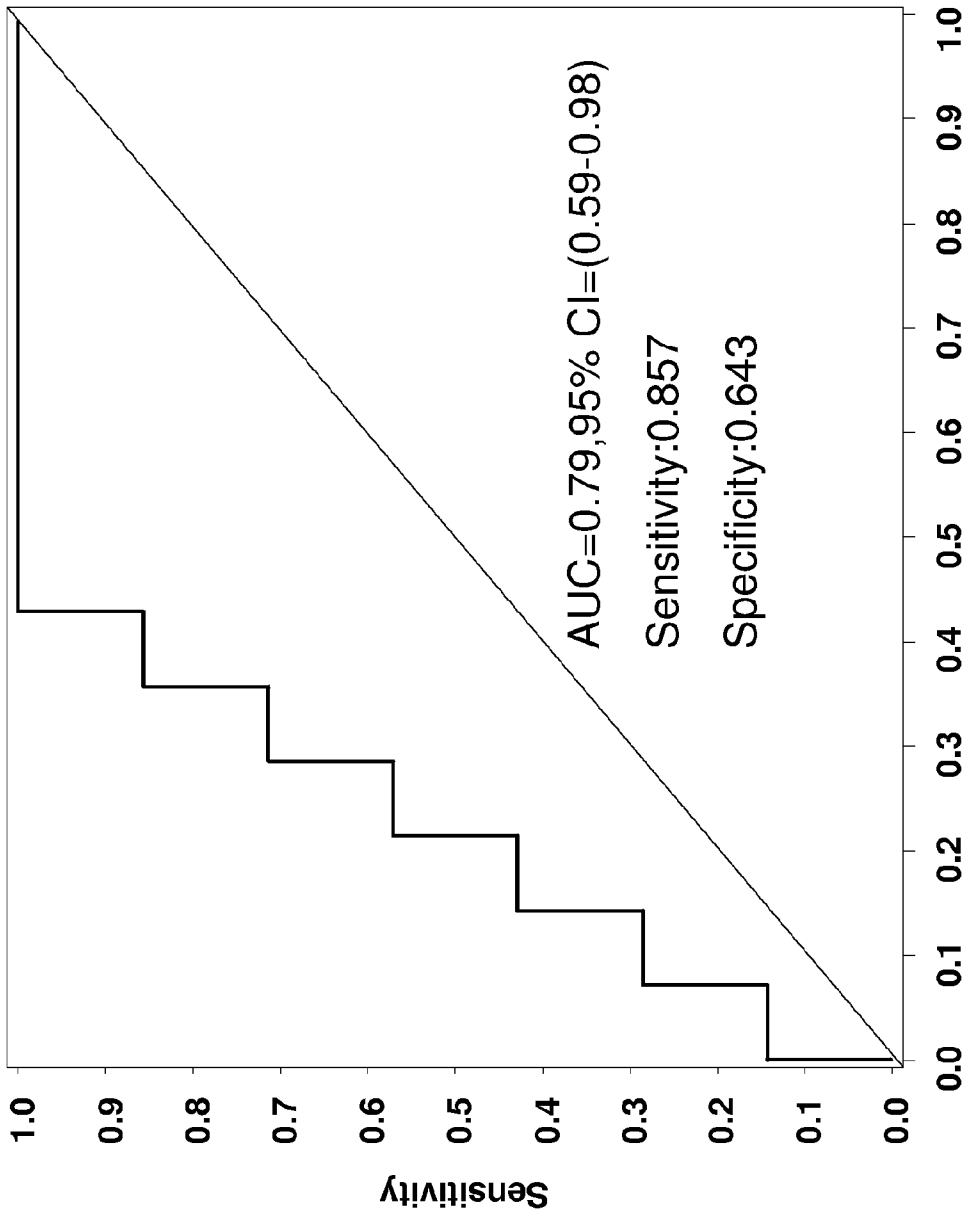

AUC=0.79,95% CI=(0.59-0.98)
Sensitivity:0.857
Specificity:0.643
Sensitivity
1 - Specificity
0.0
0.1
0.2
0.3
0.4
0.5
0.6
0.7
0.8
0.9
1.0

FIG. 23