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

(19) World Intellectual Property Organization
International Bureau

(43) International Publication Date
28 April 2011 (28.04.2011)

PCT

(10) International Publication Number
WO 2011/048495 A1

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

(21) International Application Number:
PCT/IB2010/002870

(22) International Filing Date:
19 October 2010 (19.10.2010)

(25) Filing Language: English

(26) Publication Language: English

(30) Priority Data:
61/279,564 19 October 2009 (19.10.2009) US

(71) Applicant *(for all designated States except US)*: **STICHTING HET NEDERLANDS KANKER INSTITUUT** [NL/NL]; Plesmanlaan 121, NL-1066 CX Amsterdam (NL).

(72) Inventors; and
(75) Inventors/Applicants *(for US only)*: **LINN, Sabine Charlotte** [NL/NL]; Plesmanlaan 121, NL-1066 CX Amsterdam (NL). **NEDERLOF, Petra Marleen** [NL/NL]; Plesmanlaan 121, NL-1066 CX Amsterdam (NL). **VOLLEBERGH, Marieke Anne** [NL/NL]; Plesmanlaan 121, NL-1066 CX Amsterdam (NL).

(74) Agents: **NEDERLANDSCH OCTROOIBUREAU** et al.; P.O. Box 29720, NL-2502 LS The Hague (NL).

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

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

Declarations under Rule 4.17:

— *as to the identity of the inventor (Rule 4.17(i))*

[Continued on next page]

(54) **Title**: PREDICTING BENEFIT OF ANTI-CANCER THERAPY VIA ARRAY COMPARATIVE GENOMIC HYBRIDIZATION

FIGURE 1A

Chromosome	Begin BAC	END BAC	chrom band	chrom band	region start	Region end	size Mb	characteristic
2	RP11-560C7	RP5-960D23	2p24.1	2p16.3	22998568	47897750	24.9	
2	RP11-86O17	RP11-263G22	2q36.3	2q37.1	226629534	234701766	8.1	loss in SPOR
3	RP11-510B7	RP11-12A13	3p12.3	3q11.2	80611850	96250469	15.6	
4	RP11-227F19	RP11-416A5	4p13	4p12	41340065	45778656	4.4	loss in SPOR
6	GS-62-L11	RP11-199A24	6p25.3	6p11.1	135996	58788604	58.7	gain in BRCA2
6	RP11-349P19	RP11-256L9	6q12	6q13	65158448	73217550	8.1	gain in BRCA2
7	RP4-756H11	RP4-635O5	7q11.21	7q11.22	65647490	71274716	5.6	
7	RP4-811H12	GS-3-K23	7q35	7q36.3	146787501	158864424	12.1	
10	RP11-118K6	RP13-355A21	10p15.2	10p12.1	3035538	28141734	25.1	
10	RP11-90J7	RP11-436O19	10q22.3	10q26.13	79687950	124268654	44.6	loss in BRCA2
11	RP11-113A6	RP11-327O2	11p15.5	11p15.4	2261568	10454001	8.2	
11	RP11-569N5	RP11-137O10	11q13.2	11q14.2	68072319	87028461	19.0	
11	RP11-108O10	GS-26-N8	11q23.1	11q25	111095144	134437384	23.3	
13	RP11-125I23	RP11-384G23	13q12.2	13q21.1	26843991	52955532	26.1	loss in BRCA2
13	RP11-95C14	RP11-255P5	13q31.3	13q33.1	91284428	102377477	11.1	gain in BRCA2
14	RP11-468E2	RP11-168D12	14q12	14q21.2	23572376	41500293	17.9	
14	RP11-544I20	RP11-73M18	14q23.2	14q32.33	63342178	103382895	40.0	loss in BRCA2
16	RP11-429K17	RP11-2C24	16p12.3	16p11.2	20151302	30747839	10.6	gain in SPOR
16	RP11-283C7	RP11-370P15	16q12.1	16q21	45627546	62396983	16.8	loss in SPOR
17	RP1-27J12	RP11-121A13	17p12	17p11.2	14285924	20230382	5.9	
17	RP11-260A9	RP11-445F12	17q11.1	17q12	22287134	32371528	10.1	loss in SPOR
17	RP5-1110E20	RP5-971F3	17q21.2	17q21.31	36260511	41939592	5.7	loss in SPOR
22	RP11-80O7	RP1-172B20	22q11.23	22q13.1	22510132	38559115	16.0	
23	GS-839-D20	RP11-576G22	23p22.33	23p11.3	110000	45268722	45.2	
23	RP5-965E19	RP5-1087L19	23q26.2	23q28	131271371	153547872	22.3	

(57) **Abstract**: Array comparative genomic hybridization classifiers, arrays comprising the classifiers, and related methods of using the same for predicting the therapeutic efficacy of anti-cancer therapy by detecting phenotypic genetic traits using comparative genomic hybridization are disclosed.

WO 2011/048495 A1

— *as to applicant's entitlement to apply for and be granted a patent (Rule 4.17(ii))*

— *as to the applicant's entitlement to claim the priority of the earlier application (Rule 4.17(iii))*

Published:

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

— *before the expiration of the time limit for amending the claims and to be republished in the event of receipt of amendments (Rule 48.2(h))*

PREDICTING BENEFIT OF ANTI-CANCER THERAPY VIA ARRAY COMPARATIVE GENOMIC HYBRIDIZATION

Cross-Reference to Related Applications

5 [001] This Patent Cooperation Treaty patent application claims priority to U.S. Provisional Patent Application No. 61/279,564 filed October 19, 2009, which is incorporated by reference herein for all purposes in its entirety.

Field

[002] Array comparative genomic hybridization classifiers, arrays comprising the 10 classifiers, and related methods provided by the present disclosure may be used to predict a patient's response to anti-cancer therapy by detecting phenotypic genetic traits using comparative genomic hybridization.

Background

[003] Breast cancer is the most frequently occurring cancer among women in the 15 western world. It is a heterogeneous cancer disease, consisting of several subtypes. Molecular biology has greatly enhanced our understanding of the heterogeneity of breast cancer, but few molecular tumor features are actually used in the clinic to guide the choice of a systemic treatment strategy.

[004] (Neo)adjuvant systemic therapy has become a widely used treatment strategy 20 for patients with early, or locally advanced, breast cancer. Despite its early and late toxicities, this treatment strategy reduces the risk of breast cancer relapse and mortality by approximately half.

[005] In spite of this advantage, a disadvantage to the use of (neo)adjuvant systemic therapy is the lack of predictive tests to individualize the choice of certain combinations of 25 drugs for an individual breast cancer patient to ensure maximal benefit with minimal toxicity. For example, for highly toxic adjuvant treatment regimens, such as high dose alkylating chemotherapy with hematopoietic stem-cell rescue, the survival benefit when compared with standard chemotherapy is approximately 10% for patients with 10 or more positive axillary lymph nodes. It would thus be advantageous to be able to target those 10% of patients who 30 would benefit from high dose alkylating chemotherapy. However, no such predictive test presently exists. Because of the relatively high toxicity and the low level of efficacy in unselected breast cancer patients, alkylating agents are not commonly used in the treatment of breast cancer, with the exception of cyclophosphamide.

[006] Alkylating chemotherapy and platinating agents work by causing interstrand 35 DNA crosslinking, which cause DNA double strand breaks. In normal cells, these double

strand breaks are repaired by a process called homologous recombination. If this process is unavailable or impaired, a situation referred to as "homologous recombination deficiency" exists and alternative, error-prone DNA repair mechanisms take over, leading to genomic instability. The breast cancer genes BRCA1 and BRCA2 are involved in normal homologous recombination and tumors of patients carrying germ-line inactivating mutations in one or both of these genes show homologous recombination deficiency. BRCA1 and BRCA2 can also be inactivated in sporadic cancers as well, a phenomenon sometimes referred to as BRCA-likeness (or BRCAness). Emerging preclinical evidence shows that breast cancers with a defective DNA repair system, such as a mutation in the BRCA1or BRCA2 genes, may be extremely sensitive to DNA damaging agents, such as platinum compounds and bifunctional alkylating agents. It therefore appears that patients with breast cancers harboring a defective DNA repair system may specifically benefit from high dose alkylating chemotherapy, a DNA double strand break (DSB)-inducing regimen.

[007] Tumors with homologous recombination deficiency have been shown to be particularly sensitive to DNA double strand break (DSB)-inducing agents, such as alkylators and platinum drugs or platinating agents. Both classes of drugs are employed in metastatic breast cancer. The novel poly(ADP-ribose) polymerase inhibitors (PARP inhibitors) are specifically effective in homologous recombination deficient tumors as well, and have shown impressive activity in clinical studies recently. Unfortunately, no clinical tests exist which can reliably determine homologous recombination deficiency in tumor biopsies.

Summary

[008] Therefore, methods of predicting the therapeutic efficacy of anti-cancer therapies by identifying patients who would benefit from one or more anti-cancer therapies, including, without limitation, DNA double strand break-inducing regimens such as high dose alkylating chemotherapy, by reliably determining homologous recombination deficiency in tumor biopsies, and by identifying patients with breast cancers harboring a defective DNA repair system, are useful.

[009] In various aspects, the present disclosure is based on the discovery that certain chromosomal copy number aberrations in tumor cells allow tumors to be classified as BRCA2- like tumors or non-BRCA2-like (originally called 'sporadic') tumors. The classification of a tumor in this manner allows for the prospective prediction of responsiveness of the patient from which the tumor was removed to anti-cancer therapy.

[010] In a first aspect, methods for using a BRCA2 aCGH classifier to detect genomic copy number variations in a test sample, as compared to a reference sample, in the

genomic loci selected from 2p24.1-16.3, 2q36.3-37.1, 3p12.3-3q11.2, 4p13-12, 6p25.3-11.1, 6q12-13, 7q11.21-11.22, 7q35-36.3, 10p15.2-12.1, 10q22.3-26.13, 11p15.5-15.4, 11q13.2-14.2, 11q23.1-25, 13q12.2-21.1, 13q31.3-33.1, 14q12-21.2, 14q23.2-32.33, 16p12.3-11.2, 16q12.1-21, 17p12-11.2, 17q11.1-12, 17q21.2-21.31, 22q11.23-13.1, 23p22.33-11.3 and 23q26.2-28 are disclosed. The methods comprise detecting genomic copy number variations in a test sample, wherein the copy number variations are detected in at least one, or in some embodiments a plurality, of the genomic loci selected from 2p24.1-16.3, 2q36.3-37.1, 3p12.3-3q11.2, 4p13-12, 6p25.3-11.1, 6q12-13, 7q11.21-11.22, 7q35-36.3, 10p15.2-12.1, 10q22.3-26.13, 11p15.5-15.4, 11q13.2-14.2, 11q23.1-25, 13q12.2-21.1, 13q31.3-33.1, 14q12-21.2, 14q23.2-32.33, 16p12.3-11.2, 16q12.1-21, 17p12-11.2, 17q11.1-12, 17q21.2-21.31, 22q11.23-13.1, 23p22.33-11.3 and 23q26.2-28, wherein a variation in copy number at any one or more of the genomic loci, as compared to the number of copies of DNA from a reference sample, classifies the cell sample as from either a BRCA2-like tumor or a non-BRCA2-like tumor, and wherein such classification can be used to predict an individual subject's response to anti-cancer therapy. In some embodiments, the genomic copy number variations are detected at all 25 genomic loci. In some embodiments, the genomic copy number variations are detected at a number of genomic loci selected from greater than 1, greater than 2, greater than 3, greater than 4, greater than 5, greater than 6, greater than 7, greater than 8, greater than 9, greater than 10, greater than 11, greater than 12, greater than 13, greater than 14, greater than 15, greater than 16, greater than 17, greater than 18, greater than 19, greater than 20, greater than 21, greater than 22, greater than 23, and greater than 24. In some embodiments, the genomic copy number variations are detected at a number of genomic loci selected from less than 25, less than 24, less than 23, less than 22, less than 21, less than 20, less than 19, less than 18, less than 17, less than 16, less than 15, less than 14, less than 13, less than 12, less than 11, less than 10, less than 9, less than 8, less than 7, less than 6, less than 5, less than 4, less than 3, and less than 2.

[011] In a second aspect, methods for using a BRCA2 aCGH classifier to detect genomic copy number variations in a test sample, as compared to a reference sample, in the genomic loci selected from 4p13-12, 13q12.2-21.1, 13q31.3-33.1, 14q23.2-32.33, 16q12.1-21, 17q11.1-12 and 17q21.2-21.31 are disclosed. The methods comprise detecting genomic copy number variations in a test sample, wherein the copy number variations are detected in one, or in some embodiments a plurality, of the genomic loci selected from 4p13-12, 13q12.2-21.1, 13q31.3-33.1, 14q23.2-32.33, 16q12.1-21, 17q11.1-12 and 17q21.2-21.31, and wherein a variation in copy number at any one or more of the genomic loci, as compared to

the number of copies of DNA from a reference sample, classifies the cell sample as from either a BRCA2-like tumor or a non-BRCA2-like tumor, and wherein such classification can be used to predict whether an individual will benefit from anti-cancer therapy. In some embodiments, the genomic copy number variations are detected at all 7 genomic loci. In some embodiments, the genomic copy number variations are detected at a number of genomic loci selected from greater than 1, greater than 2, greater than 3, greater than 4, greater than 5, and greater than 6. In some embodiments, the genomic copy number variations are detected at a number of genomic loci selected from less than 7, less than 6, less than 5, less than 4, less than 3, and less than 2.

[012] In a third aspect, methods for using a BRCA2 aCGH classifier to detect genomic copy number variations in a test sample, as compared to a reference sample, are disclosed, wherein the classifier comprises at least one of the BAC clones set forth in **Fig. 2**. The methods comprise detecting genomic copy number variations in a test sample, wherein the copy number variations are detected using at least one, or in some embodiments a plurality, of the BAC clones of **Fig. 2**, wherein a variation in copy number at any one or more of the BAC clones, as compared to the number of copies of DNA from a reference sample, classifies the cell sample as from either a BRCA2-like tumor or a non-BRCA2-like tumor, and wherein such classification can be used to predict whether an individual will benefit from anti-cancer therapy. In some embodiments, the genomic copy number variations are detected using all 704 of the BAC clones set forth in **Fig. 2**. In some embodiments, the genomic copy number variations are detected using a number of the BAC clones set forth in **Fig. 2** selected from greater than 1, greater than 10, greater than 20, greater than 25, greater than 50, greater than 75, greater than 100, greater than 125, greater than 150, greater than 175, greater than 200, greater than 225, greater than 250, greater than 275, greater than 300, greater than 325, greater than 350, greater than 375, greater than 400, greater than 425, greater than 450, greater than 475, greater than 500, greater than 525, greater than 550, greater than 575, greater than 600, greater than 625, greater than 650, greater than 675, and greater than 700. In some embodiments, the genomic copy number variations are detected using a number of the BAC clones set forth in **Fig. 2** selected from less than 704, less than 700, less than 675, less than 650, less than 625, less than 600, less than 575, less than 550, less than 525, less than 500, less than 475, less than 450, less than 425, less than 400, less than 375, less than 350, less than 325, less than 300, less than 275, less than 250, less than 225, less than 200, less than 175, less than 150, less than 125, less than 100, less than 75, les than 50, less than 25, less than 20, and less than 10.

[013] In a fourth aspect, methods for using a BRCA2 aCGH classifier to detect genomic copy number variations in a test sample, as compared to a reference sample, in the genomic loci selected from 6p25.3-11.1, 6q12-13 and 13q31.3-33.1 are disclosed. The methods comprise detecting genomic copy number variations in a test sample, wherein the copy number variations are detected in at least one, or in some embodiments a plurality, of the genomic loci selected from 6p25.3-11.1, 6q12-13 and 13q31.3-33.1, wherein an increase in copy number at any one or more of the genomic loci, as compared to the number of copies of DNA from a reference sample, classifies the cell sample as from a BRCA2-like tumor, and wherein such classification can be used to predict whether an individual will benefit from anti-cancer therapy. In some embodiments, the genomic copy number variations are detected at all 3 genomic loci. In some embodiments, the genomic copy number variations are detected at a number of genomic loci selected from greater than 1 and greater than 2. In some embodiments, the genomic copy number variations are detected at a number of genomic loci selected from less than 3, and less than 2.

[014] In a fifth aspect, methods for using a BRCA2 aCGH classifier to detect genomic copy number variations in a test sample, as compared to a reference sample, in the genomic loci selected from 10q22.3-26.13, 13q12.2-21.1 and 14q23.2-32.33 are disclosed. The methods comprise detecting genomic copy number variations in a test sample, wherein the copy number variations are detected in at least one, or in some embodiments a plurality, of the genomic loci selected from 10q22.3-26.13, 13q12.2-21.1 and 14q23.2-32.33, wherein a decrease in copy number at any one or more of the genomic loci, as compared to the number of copies of DNA from a reference sample, classifies the cell sample as from a BRCA2-like tumor, and wherein such classification can be used to predict whether an individual will benefit from anti-cancer therapy. In some embodiments, the genomic copy number variations are detected at all 3 genomic loci. In some embodiments, the genomic copy number variations are detected at a number of genomic loci selected from greater than 1 and greater than 2. In some embodiments, the genomic copy number variations are detected at a number of genomic loci selected from less than 3, and less than 2.

[015] In a sixth aspect, methods for using a BRCA2 aCGH classifier to detect genomic copy number variations in a test sample, as compared to a reference sample, in the genomic locus 16p12.3-11.2 are disclosed. The methods comprise detecting genomic copy number variations in a test sample, wherein the copy number variations are detected at the genomic locus 16p12.3-11.2, wherein an increase in copy number at 16p12.3-11.2, as compared to the number of copies of DNA from a reference sample, classifies the cell sample

as from a non-BRCA2-like tumor, and wherein such classification can be used to predict whether an individual will benefit from anti-cancer therapy.

[016] In a seventh aspect, methods for using a BRCA2 aCGH classifier to detect genomic copy number variations in a test sample, as compared to a reference sample, in one, or a plurality, of the genomic loci selected from 2q36.3-37.1, 4p13-12, 16q12.1-21, 17q11.1-12 and 17q21.2-21.31 are disclosed. The methods comprise detecting genomic copy number variations in a test sample, wherein the copy number variations are detected in at least one, or in some embodiments a plurality, of the genomic loci selected from 2q36.3-37.1, 4p13-12, 16q12.1-21, 17q11.1-12 and 17q21.2-21.31, wherein a decrease in copy number at any one or more of the genomic loci, as compared to the number of copies of DNA from a reference sample, classifies the cell sample as from a non-BRCA2-like tumor, and wherein such classification can be used to predict whether an individual will benefit from anti-cancer therapy. In some embodiments, the genomic copy number variations are detected at all 5 genomic loci. In some embodiments, the genomic copy number variations are detected at a number of genomic loci selected from greater than 1, greater than 2, greater than 3, and greater than 4. In some embodiments, the genomic copy number variations are detected at a number of genomic loci selected from less than 5, less than 4, less than 3, and less than 2.

Brief Description of the Drawings

[017] Those skilled in the art will understand that the drawings, described herein, are for illustration purposes only. The drawings are not intended to limit the scope of the present disclosure.

[018] **Fig. 1A** depicts the BRCA2-like genomic loci used to identify breast cancers with a BRCA2-deficient DNA repair system. 'SPOR' means non-BRCA2-like ('sporadic').

[019] **Fig. 1B** depicts a subset of the BRCA2-like genomic loci of **Fig. 1A**. 'SPOR' means non-BRCA2-like ('sporadic').

[020] **Fig. 2** depicts exemplary BAC clones that may be used to detect, or to generate probes to detect, copy number aberrations in the genomic loci of **Figs. 1A** and **1B**.

[021] **Fig. 3** depicts the Kaplan-Meier curves for recurrence-free and overall survival of BRCA2-like^{CGH} and non-BRCA2-like^{CGH} breast cancer patients randomized between conventional adjuvant chemotherapy and high-dose, platinum-based adjuvant chemotherapy in the validation series of Example 2.

[022] **Fig. 4** depicts patient characteristics distributed by treatment arm and BRCA2-classification of the stage-III series for Example 3.

Detailed Description

Definitions

[023] "Anti-cancer therapy" means any one, or a plurality, of therapies and/or drugs used to treat cancer, or any combinations thereof, including a) homologous recombination deficiency-targeted drugs and/or treatments; and b) drugs or treatments that directly or indirectly cause double strand DNA breaks. This definition includes, without limitation, high dose platinum-based alkylating chemotherapy, platinum compounds, thiotepa, cyclophosphamide, iphosphamide, nitrosureas, nitrogen mustard derivatives, mitomycins, epipodophyllotoxins, camptothecins, anthracyclines, poly(ADP-ribose) polymerase (PARP) inhibitors, ionizing radiation, ABT-888, olaparib (AZT-2281), gemcitabine, CEP-9722, AG014699, AG014699 with Temozolomide, and BSI-201.

[024] "Array" refers to an arrangement, on a substrate surface, of multiple nucleic acid probes (as defined herein) of predetermined identity. In various embodiments, the sequences of each of the multiple nucleic acid probes are known. In general, an array comprises a plurality of target elements, each target element comprising one or more nucleic acid probes immobilized on one or more solid surfaces, to which sample nucleic acids can be hybridized. In various embodiments, each individual probe is immobilized to a designated, discrete location (*i.e.*, a defined location or assigned position) on the substrate surface. In various embodiments, each nucleic acid probe is immobilized to a discrete location on an array and each has a sequence that is either specific to, or characteristic of, a particular genomic locus. A nucleic acid probe is specific to, or characteristic of, a genomic locus when it contains a nucleic acid sequence that is unique to that genomic locus. Such a probe preferentially hybridizes to a nucleic acid made from that genomic locus, relative to nucleic acids made from other genomic loci.

[025] The nucleic acid probes can contain sequence(s) from specific genes or clones. In various embodiments, at least some of the nucleic acid probes contain sequences from any one or more of the specific genomic regions recited in **Fig. 1A**. In various embodiments, at least some of the nucleic acid probes contain sequences from any one or more of the specific genomic regions recited in **Fig. 1B**. In various embodiments, at least some of the nucleic acid probes contain sequences of known, reference genes or clones. In various embodiments, the nucleic acid probes in a single array contain both sequences from any one or more of the specific genomic regions recited in **Fig. 1A** and sequences of known, reference genes or clones. In various embodiments, the nucleic acid probes in a single array contain both

sequences from any one or more of the specific genomic regions recited in **Fig. 1B** and sequences of known, reference genes or clones.

[026] The probes may be arranged on the substrate in a single density, or in varying densities. The density of each of the probes can be varied to accommodate certain factors such as, for example, the nature of the test sample, the nature of a label used during hybridization, the type of substrate used, and the like. Each probe may comprise a mixture of nucleic acids of varying lengths and, thus, varying sequences. For example, a single probe may contain more than one copy of a cloned nucleic acid, and each copy may be broken into fragments of different lengths. Each length will thus have a different sequence.

[027] The length, sequence and complexity of the nucleic acid probes may be varied. In various embodiments, the length, sequence and complexity are varied to provide optimum hybridization and signal production for a given hybridization procedure, and to provide the required resolution among different genes or genomic locations.

[028] "BRCA2-like tumor" means a tumor having cells containing a mutation of the BRCA2 locus or a deficiency in the homologous recombination-dependent double strand break DNA repair pathway that alters BRCA2 activity or function, either directly or indirectly.

[029] "CGH" or "Comparative Genomic Hybridization" refers generally to molecular-cytogenetic techniques for the analysis of copy number changes, gains and/or losses, in the DNA content of a given subject's DNA. CGH can be used to identify chromosomal alterations, such as unbalanced chromosomal changes, in any number of cells including, for example, cancer cells. In various embodiments, CGH is utilized to detect one or more chromosomal amplifications and/or deletions of regions between a test sample and a reference sample.

[030] "Chromosomal locus" refers to a specific, defined portion of a chromosome.

[031] "Genome" refers to all nucleic acid sequences, coding and non-coding, present in each cell type of a subject. The term also includes all naturally occurring or induced variation of these sequences that may be present in a mutant or disease variant of any cell type, including, for example, tumor cells. Genomic DNA and genomic nucleic acids are thus nucleic acids isolated from a nucleus of one or more cells, and include nucleic acids derived from, isolated from, amplified from, or cloned from genomic DNA, as well as synthetic versions of all or any part of a genome.

[032] For example, the human genome consists of approximately 3.0×10^9 base pairs of DNA organized into 46 distinct chromosomes. The genome of a normal human

diploid somatic cell consists of 22 pairs of autosomes (chromosomes 1 to 22) and either chromosomes X and Y (male) or a pair of X chromosomes (female) for a total of 46 chromosomes. A genome of a cancer cell may contain variable numbers of each chromosome in addition to deletions, rearrangements and amplification of any sub-chromosomal region or DNA sequence.

[033] "Genomic locus" refers to a specific, defined portion of a genome.

[034] "HBOC tumors" refers to tumors present in a group of patients with a high risk for BRCA2-like breast cancer (patients from Hereditary Breast and Ovarian Cancer families), who display a negative screen result for BRCA1 and/or BRCA2 mutation. Such patients have a family history that include at least two diagnoses for breast cancer and one diagnosis for ovarian cancer.

[035] "Hybridization" refers to the binding of two single stranded nucleic acids via complementary base pairing. Extensive guides to the hybridization of nucleic acids can be found in: Tijssen, Laboratory Techniques in Biochemistry and Molecular Biology-Hybridization with Nucleic Acid Probes Part I, Ch. 2, "Overview of principles of hybridization and the strategy of nucleic acid probe assays" (1993), Elsevier, N.Y.; and Sambrook *et al.*, Molecular Cloning: A Laboratory Manual (3rd ed.) Vol. 1-3 (2001), Cold Spring Harbor Laboratory, Cold Spring Harbor Press, N.Y. The phrases "hybridizing specifically to", "specific hybridization", and "selectively hybridize to", refer to the preferential binding, duplexing, or hybridizing of a nucleic acid molecule to a particular probe under stringent conditions. The term "stringent conditions" refers to hybridization conditions under which a probe will hybridize preferentially to its target subsequence, and to a lesser extent, or not at all, to other sequences in a mixed population (*e.g.*, a DNA preparation from a tissue biopsy). "Stringent hybridization" and "stringent hybridization wash conditions" are sequence-dependent and are different under different environmental parameters.

[036] Generally, highly stringent hybridization and wash conditions are selected to be about 5° C lower than the thermal melting point (Tm) for a specific sequence at a defined ionic strength and pH. The Tm is the temperature at which 50% of the target sequence hybridizes to a perfectly matched probe. Very stringent conditions are selected to be equal to the Tm for a particular probe. Often, a high stringency wash is preceded by a low stringency wash to remove background probe signal. An example of stringent hybridization conditions for hybridization of complementary nucleic acids which have more than 100 complementary residues on an array is 42° C using standard hybridization solutions, with the hybridization

being carried out overnight. An example of highly stringent wash conditions is a 0.15 M NaCl wash at 72° C for 15 minutes. An example of stringent wash conditions is a wash in 0.2X Standard Saline Citrate (SSC) buffer at 65° C for 15 minutes. An example of a medium stringency wash for a duplex of, for example, more than 100 nucleotides, is 1X SSC at 45° C for 15 minutes. An example of a low stringency wash for a duplex of, for example, more than 100 nucleotides, is 4X to 6X SSC at 40° C for 15 minutes.

[037] "Micro- array" refers to an array that is miniaturized so as to require microscopic examination for visual evaluation. In various embodiments, the arrays used in the methods of the present disclosure are micro-arrays.

[038] "Nucleic acid" refers to a deoxyribonucleotide or ribonucleotide in either single- or double-stranded form and includes all nucleic acids comprising naturally occurring nucleotide bases as well as nucleic acids containing any and/or all analogues of natural nucleotides. This term also includes nucleic acid analogues that are metabolized in a manner similar to naturally occurring nucleotides, but at rates that are improved for the purposes desired. This term also encompasses nucleic-acid-like structures with synthetic backbone analogues including, without limitation, phosphodiester, phosphorothioate, phosphorodithioate, methylphosphonate, phosphoramidate, alkyl phosphotriester, sulfamate, 3'-thioacetal, methylene(methylimino), 3'-N-carbamate, morpholino carbamate, and peptide nucleic acids (PNAs) (*see, e.g.*: "Oligonucleotides and Analogues, a Practical Approach," edited by F. Eckstein, IRL Press at Oxford University Press (1991); "Antisense Strategies," Annals of the New York Academy of Sciences, Volume 600, Eds. Baserga and Denhardt (NYAS 1992); Milligan (1993) J. Med. Chem. 36:1923-1937; and "Antisense Research and Applications" (1993, CRC Press)). PNAs contain non-ionic backbones, such as N-(2-aminoethyl) glycine units. Phosphorothioate linkages are described in: WO 97/03211; WO 96/39154; and Mata (1997) Toxicol. Appl. Pharmacol. 144:189-197. Other synthetic backbones encompassed by this term include methyl-phosphonate linkages or alternating methyl-phosphonate and phosphodiester linkages (Strauss-Soukup (1997) Biochemistry 36: 8692-8698), and benzyl-phosphonate linkages (Samstag (1996) Antisense Nucleic Acid Drug Dev 6: 153-156).

[039] "Probe" or "nucleic acid probe" refer to one or more nucleic acid fragments whose specific hybridization to a sample can be detected. In various embodiments, probes are arranged on a substrate surface in an array. The probe may be unlabelled, or it may contain one or more labels so that its binding to a nucleic acid can be detected. In various embodiments, a probe can be produced from any source of nucleic acids from one or more

particular, pre-selected portions of a chromosome including, without limitation, one or more clones, an isolated whole chromosome, an isolated chromosome fragment, or a collection of polymerase chain reaction (PCR) amplification products.

[040] In some embodiments, the probe may be a member of an array of nucleic acids as described in WO 96/17958. Techniques capable of producing high density arrays can also be used for this purpose (*see, e.g.*, Fodor (1991) Science 767-773; Johnston (1998) Curr. Biol. 8: Rl 71 -Rl 74; Schummer (1997) Biotechniques 23: 1087-1092; Kern (1997) Biotechniques 23: 120-124; and U.S. Patent No. 5,143,854).

[041] The sequence of the probes can be varied. In various embodiments, the probe sequence can be varied to produce probes that are substantially identical to the probes disclosed herein, but that retain the ability to hybridize specifically to the same targets or samples as the probe from which they were derived.

[042] "Reference sample" refers to nucleic acids comprising sequences whose quantity or degree of representation, copy number, and/or sequence identity are known. Such nucleic acids serve as a reference to which one or more test samples are compared.

[043] "Sample" refers to a material, or mixture of materials, containing one or more components of interest. Samples include, but are not limited to, material obtained from an organism and may be directly obtained from a source, such as from a biopsy or from a tumor, or indirectly obtained such as after culturing and/or processing.

[044] "Test sample" refers to nucleic acids comprising sequences whose quantity or degree of representation, copy number, and/or sequence identity are unknown. In various embodiments, the present disclosure is directed to the detection of the quantity or degree of representation, copy number, and/or sequence identity of one or more test samples.

[045] Reference is now made in detail to certain embodiments of arrays and methods. The disclosed embodiments are not intended to be limiting of the claims. To the contrary, the claims are intended to cover all alternatives, modifications, and equivalents.

Arrays, Micro-Arrays and Probes

[046] In various aspects, the present disclosure relates to the determination of copy number changes in the DNA content of a given test sample, as compared to one or more reference samples. In some embodiments, the copy number changes comprise gains or increases in the DNA content of a test sample. In some embodiments, the copy number changes comprise losses or decreases in the DNA content of a test sample. In some embodiments, the copy number changes comprise both gains or increases and losses or decreases in the DNA content of a test sample.

[047] Determination of copy number changes can be determined by hybridizations that are performed on a solid support. For example, probes that selectively hybridize to specific chromosomal regions can be spotted onto a surface. In various aspects, the spots of probes are placed in an ordered pattern, or array, and the pattern is recorded to facilitate correlation of results. Once an array is generated, one or more test samples can be hybridized to the array. In various aspects, arrays comprise a plurality of nucleic acid probes immobilized to discrete spots (*i.e.*, defined locations or assigned positions) on a substrate surface.

[048] Thus, in several aspects, copy number changes of genomic loci are analyzed in an array-based approach. In some embodiments, copy number changes of genomic loci are analyzed using comparative genomic hybridization. In some embodiments, copy number changes of genomic loci are analyzed using array-based comparative genomic hybridization.

[049] Any of a variety of arrays may be used. A number of arrays are commercially available for use from Vysis Corporation (Downers Grove, Ill), Spectral Genomics Inc. (Houston, TX), and Affymetrix Inc. (Santa Clara, CA). Arrays can also be custom made for one or more hybridizations.

[050] Methods of making and using arrays are well known in the art (*see, e.g.*, Kern *et al.*, Biotechniques (1997), 23:120-124; Schummer *et al.*, Biotechniques (1997), 23:1087-1092; Solinas-Toldo *et al.*, Genes, Chromosomes & Cancer (1997), 20: 399-407; Johnston, Curr. Biol. (1998), 8: R171-R174; Bowtell, Nature Gen. (1999), Supp. 21:25-32; Watson *et al.*, Biol. Psychiatry (1999), 45: 533-543; Freeman *et al.*, Biotechniques (2000), 29: 1042-1046 and 1048-1055; Lockhart *et al.*, Nature (2000), 405: 827-836; Cuzin, Transfus. Clin. Biol. (2001), 8:291-296; Zarrinkar *et al.*, Genome Res. (2001), 11: 1256-1261; Gabig *et al.*, Acta Biochim. Pol. (2001), 48: 615-622; and Cheung *et al.*, Nature (2001), 40: 953-958; *see also, e.g.*, U.S. Patent Nos. 5,143,854; 5,434,049; 5,556,752; 5,632,957; 5,700,637; 5,744,305; 5,770,456; 5,800,992; 5,807,522; 5,830,645; 5,856,174; 5,959,098; 5,965,452; 6,013,440; 6,022,963; 6,045,996; 6,048,695; 6,054,270; 6,258,606; 6,261,776; 6,277,489; 6,277,628; 6,365,349; 6,387,626; 6,458,584; 6,503,711; 6,516,276; 6,521,465; 6,558,907; 6,562,565; 6,576,424; 6,587,579; 6,589,726; 6,594,432; 6,599,693; 6,600,031; and 6,613,893).

[051] Substrate surfaces suitable for use in the generation of an array can be made of any rigid, semi-rigid or flexible material that allows for direct or indirect attachment (*i.e.*, immobilization) of nucleic acid probes to the substrate surface. Suitable materials include, without limitation, cellulose (*see, e.g.*, U.S. Patent No. 5,068,269), cellulose acetate (*see, e.g.*,

U.S. Patent No. 6,048,457), nitrocellulose, glass (*see, e.g.*, U.S. Patent No. 5,843,767), quartz and/or other crystalline substrates such as gallium arsenide, silicones (*see, e.g.*, U.S. Patent No. 6,096,817), plastics and plastic copolymers (*see, e.g.*, U.S. Patent Nos. 4,355,153; 4,652,613; and 6,024,872), membranes and gels (*see, e.g.*, U.S. Patent No. 5,795,557), and
5 paramagnetic or supramagnetic microparticles (*see, e.g.*, U.S. Patent No. 5,939,261). When fluorescence is to be detected, arrays comprising cyclo-olefin polymers may be used (*see, e.g.*, U.S. Patent No. 6,063,338). The presence of reactive functional chemical groups (such as, for example, hydroxyl, carboxyl, and amino groups) present on the surface of the substrate material can be used to directly or indirectly attach nucleic acid probes to the substrate
10 surface.

[052] More than one copy of each nucleic acid probe may be spotted onto an array. For example, each nucleic acid probe may be spotted onto an array once, in duplicate, in triplicate, or more, depending on the desired application. Multiple spots of the same probe allows for assessment of the reproducibility of the results obtained.

15 [053] Related nucleic acid probes may also be grouped together, in probe elements, on an array. For example, a single probe element may include a plurality of spots of related nucleic acid probes, which are of different lengths but that comprise substantially the same sequence or that are derived from the sequence of a specific genomic locus. Alternatively, a single probe element may include a plurality of spots of related nucleic acid probes that are
20 fragments of different lengths resulting from digestion of more than one copy of a cloned nucleic acid. An array may contain a plurality of probe elements and probe elements may be arranged on an array at different densities.

[054] Array-immobilized nucleic acid probes may be nucleic acids that contain sequences from genes (*e.g.*, from a genomic library) including, for example, sequences that
25 collectively cover a substantially complete genome, or any one or more subsets of a genome. In various embodiments, the sequences of the nucleic acid probes on an array comprise those for which comparative copy number information is desired. In some embodiments, to obtain DNA sequence copy number information across an entire genome, an array comprising nucleic acid probes covering a whole genome or a substantially complete genome is used. In
30 some embodiments, at least one relevant genomic locus has been determined and is used in an array, such that there is no need for genome-wide hybridization. In some embodiments, a plurality of relevant genomic loci have been determined and are used in an array, such that there is no need for genome-wide hybridization. In some embodiments, the array comprises a plurality of specific nucleic acid probes that originate from a discrete set of genes or genomic

loci and whose copy number, in association with the type of condition or tumor is to be tested, is known. Additionally, the array may comprise nucleic acid probes that will serve as positive or negative controls. In some embodiments, the array comprises a plurality of nucleic acid sequences derived from karyotypically normal genomes.

5 [055] The probes may be generated by any number of known techniques (*see, e.g.*, Tijssen, Laboratory Techniques in Biochemistry and Molecular Biology-Hybridization with Nucleic Acid Probes Part I, Ch. 2, "Overview of principles of hybridization and the strategy of nucleic acid probe assays" (1993), Elsevier, N.Y.; Sambrook *et al.*, Molecular Cloning: A Laboratory Manual (3rd ed.) Vol. 1-3 (2001), Cold Spring Harbor Laboratory, Cold Spring
10 Harbor Press, N.Y.; Innis (Ed.) "PCR Strategies" (1995), Academic Press: New York, N.Y.; and Ausubel (Ed.), "Short Protocols in Molecular Biology" 5th Ed. (2002), John Wiley & Sons). Nucleic acid probes may be obtained and manipulated by cloning into various vehicles. They may be screened and re-cloned or amplified from any source of genomic DNA.

15 [056] Nucleic acid probes may also be obtained and manipulated by cloning into vehicles including, for example, recombinant viruses, cosmids, or plasmids. Nucleic acid probes may also be synthesized *in vitro* by chemical techniques (*see, e.g.*, Nucleic Acids Res. (1997), 25: 3440-3444; Blommers *et al.*, Biochemistry (1994), 33: 7886-7896; and Frenkel *et al.*, Free Radic. Biol. Med. (1995), 19: 373-380). Probes may vary in size from synthetic
20 oligonucleotide probes and/or PCR-type amplification primers of a few base pairs in length to artificial chromosomes of more than 1 megabases in length. In various embodiments, probes comprise at least 10, at least 12, at least 15, at least 18, at least 20, at least 22, at least 30, at least 50 or at least 100 contiguous nucleotides of a sequence present in a BAC clone set forth in **Fig. 2**. In various embodiments, probes also comprise at least 10, at least 12, at least 15, at
25 least 18, at least 20, at least 22, at least 30, at least 50 or at least 100 contiguous nucleotides of a sequence present in one or more reference samples. In some embodiments, probes comprise a sequence that is unique in a genome. In some embodiments, probes comprise a sequence that is unique in the human genome.

[057] Probes may be obtained from any number of commercial sources. For
30 instance, several P1 clones are available from the DuPont P1 library (*see, e.g.*, Shepard *et al.*, Proc. Natl. Acad. Sci. USA (1994), 92: 2629), and available commercially from Incyte Corporation (Wilmington, DE). Various libraries spanning entire chromosomes are available commercially from Clontech Laboratories, Inc. (Mountain View, CA), or from the Los Alamos National Laboratory (Los Alamos, CA). In various aspects, the present disclosure

relates to the use of the human 3600 BAC/PAC genomic clone set, covering the full human genome at 1 Mb spacing, obtained from the Wellcome Trust Sanger Institute (Hinxton, Cambridge, UK).

[058] In some embodiments, the nucleic acid probes are derived from mammalian artificial chromosomes (MACs) and/or human artificial chromosomes (HACs), which can contain inserts from about 5 to 400 kilobases (kb) (*see, e.g.*, Roush, Science (1997), 276: 38-39; Rosenfeld, Nat. Genet. (1997), 15: 333-335; Ascenzioni *et al.*, Cancer Lett. (1997), 118: 135-142; Kuroiwa *et al.*, Nat. Biotechnol. (2000), 18: 1086-1090; Meija *et al.*, Am. J. Hum. Genet. (2001), 69: 315-326; and Auriche *et al.*, EMBO Rep. (2001), 2: 102-107).

[059] In some embodiments, the nucleic acid probes are derived from satellite artificial chromosomes or satellite DNA-based artificial chromosomes (SATACs). SATACs can be produced by inducing *de novo* chromosome formation in cells of varying mammalian species (*see, e.g.*, Warburton *et al.*, Nature (1997), 386: 553-555; Csonka *et al.*, J. Cell. Sci. (2000), 113: 3207-3216; and Hadlaczky, Curr. Opin. Mol. Ther. (2001), 3: 125-132).

[060] In some embodiments, the nucleic acid probes are derived from yeast artificial chromosomes (YACs), 0.2-1 megabses in size. YACs have been used for many years for the stable propagation of genomic fragments of up to one million base pairs in size (*see, e.g.*, Feingold *et al.*, Proc. Natl. Acad. Sci. USA (1990), 87:8637-8641; Adam *et al.*, Plant J. (1997), 11: 1349-1358; Tucker *et al.*, Gene (1997), 199: 25-30; and Zeschnigk *et al.*, Nucleic Acids Res. (1999), 27: E30).

[061] In some embodiments, the nucleic acid probes are derived from bacterial artificial chromosomes (BACs) up to 300 kb in size. BACs are based on the E. coli F factor plasmid system and are typically easy to manipulate and purify in microgram quantities (*see, e.g.*, Asakawa *et al.*, Gene (1997), 191: 69-79; and Cao *et al.*, Genome Res. (1999), 9: 763-774).

[062] In some embodiments, the nucleic acid probes are derived from P1 artificial chromosomes (PACs), about 70-100 kb in size. PACs are bacteriophage P1 -derived vectors (*see, e.g.*, Ioannou *et al.*, Nature Genet. (1994), 6: 84-89; Boren *et al.*, Genome Res. (1996), 6: 1123-1130; Nothwang *et al.*, Genomics (1997), 41: 370-378; Reid *et al.*, Genomics (1997), 43: 366-375; and Woon *et al.*, Genomics (1998), 50: 306-316).

[063] In some embodiments, the array comprises a series of separate wells or chambers on the substrate surface, into which probes may be immobilized as described herein. The probes can be immobilized in the separate wells or chambers and hybridization can take place within the wells or chambers. In various embodiments, the arrays can be

selected from chips, microfluidic chips, microtiter plates, Petri dishes, and centrifuge tubes. Robotic equipment has been developed for these types of arrays that permit automated delivery of reagents into the separate wells or chambers which allow the amount of the reagents used per hybridization to be sharply reduced. Examples of chip and microfluidic chip techniques can be found, for example, in U.S. Patent No. 5,800,690; Orchid, "Running on Parallel Lines" New Scientist (1997); McCormick *et al.*, Anal. Chem. (1997), 69:2626-30; and Turgeon, "The Lab of the Future on CD-ROM?" Medical Laboratory Management Report. December 1997, p. 1.

[064] In some embodiments, arrays may be generated by isolating DNA from one or more artificial chromosomes, such as for example BACs, according to standard procedures. For example, in some embodiments, DNA can be isolated from one or more BACs using a Qiawell plasmid kit (Qiagen, Chatsworth, CA). Total DNA can be amplified from the insert sites of the BACs via degenerate oligonucleotide primed PCR using a set of degenerate primers with a C6-NH_2 modification at their 5' end for covalent attachment to a substrate surface. The substrates may be any type suitable for such use including, for example, CODELINK™ glass slides (Corning, Cambridge, UK). Covalent attachment to the substrate can occur via the manufacturer's suggested protocols, or via other detailed protocols (such as those described in Pinkel *et al.*, Nature Genetics (1998), 20:207-211) with some modifications (such as those described in Alers et al. 1999). The DNA obtained after PCR amplification can then be spotted onto the substrate surface for covalent attachment thereto. The DNA may be spotted as a single site, in duplicate or in triplicate on the substrate surface.

BRCA2 Arrays

[065] In various aspects, the present disclosure relates to the use of a BRCA2 array to identify breast cancers with a deficient homologous recombination-dependent double strand break DNA repair system due to BRCA2 dysfunction and to thus identify patients, from whom the cancers have been excised, who will be highly sensitive to certain anti-cancer therapy. Therefore, in various aspects, the present disclosure relates to the use of a BRCA2 array comprising the unique BRCA2 aCGH profile disclosed herein to prospectively optimize the therapeutic efficacy of anti-cancer therapy in an individual subject by detecting phenotypic genetic traits associated with deficiencies in the BRCA2 gene. In further aspects, the present disclosure relates to the use of a BRCA2 array comprising the unique BRCA2 aCGH profile disclosed herein to prospectively optimize the therapeutic efficacy of anti-cancer therapy in an individual subject by detecting phenotypic genetic traits associated with deficiencies in non-BRCA2 genes, wherein the deficiencies negatively affect the homologous

recombination-dependent double strand break DNA repair pathway of which BRCA2 is a component.

[066] In various embodiments, a BRCA2 array comprising a BRCA2 aCGH profile for identifying individual subjects who will experience a therapeutic benefit from anti-cancer therapy, is provided. In various aspects, arrays provided by the present disclosure, which in some embodiments are BRCA2 arrays, can comprise at least one, or in some embodiments a plurality, of the BAC clones of **Fig. 2** immobilized on a substrate surface. In various aspects, arrays provided by the present disclosure, which in some embodiments are BRCA2 arrays, can comprise at least one, or in some embodiments a plurality, of the BAC clones of **Fig. 2** immobilized to discrete spots on a substrate surface. In some embodiments, an array comprises all 704 of the BAC clones set forth in **Fig. 2** immobilized on a substrate surface. In some embodiments, an array comprises all 704 of the BAC clones set forth in **Fig. 2**, immobilized to a plurality of discrete spots on a substrate surface. In some embodiments, arrays provided by the present disclosure comprise a number of the BAC clones set forth in **Fig. 2** selected from greater than 1, greater than 10, greater than 20, greater than 25, greater than 50, greater than 75, greater than 100, greater than 125, greater than 150, greater than 175, greater than 200, greater than 225, greater than 250, greater than 275, greater than 300, greater than 325, greater than 350, greater than 375, greater than 400, greater than 425, greater than 450, greater than 475, greater than 500, greater than 525, greater than 550, greater than 575, greater than 600, greater than 625, greater than 650, greater than 675 and greater than 700. In some embodiments, the BAC clones comprising the arrays of the preceding sentence are immobilized to a plurality of discrete spots on a substrate surface. In some embodiments, arrays provided by the present disclosure comprise a number of the BAC clones set forth in **Fig. 2** selected from less than 704, less than 700, less than 675, less than 650, less than 625, less than 600, less than 575, less than 550, less than 525, less than 500, less than 475, less than 450, less than 425, less than 400, less than 375, less than 350, less than 325, less than 300, less than 275, less than 250, less than 225, less than 200, less than 175, less than 150, less than 125, less than 100, less than 75, les than 50, less than 25, less than 20, and less than 10. In some embodiments, the BAC clones comprising the arrays of the preceding sentence are immobilized to a plurality of discrete spots on a substrate surface. In various aspects, arrays provided by the present disclosure can also comprise at least one, or in some embodiments a plurality, of nucleic acid probes from a reference sample immobilized on a substrate surface. In various aspects, arrays provided by the present disclosure can also comprise at least one, or in some embodiments a plurality, of nucleic acid

probes from a reference sample immobilized to discrete spots on a substrate surface. In some embodiments, a BRCA2 array is used to detect BRCA2-like genomic copy number variations in a test sample, as compared to a reference sample, at one, or a plurality, of the genomic loci selected from 2p24.1-16.3, 2q36.3-37.1, 3p12.3-3q11.2, 4p13-12, 6p25.3-11.1, 6q12-13, 7q11.21-11.22, 7q35-36.3, 10p15.2-12.1, 10q22.3-26.13, 11p15.5-15.4, 11q13.2-14.2, 11q23.1-25, 13q12.2-21.1, 13q31.3-33.1, 14q12-21.2, 14q23.2-32.33, 16p12.3-11.2, 16q12.1-21, 17p12-11.2, 17q11.1-12, 17q21.2-21.31, 22q11.23-13.1, 23p22.33-11.3 and 23q26.2-28. In some embodiments, a BRCA2 array is used to detect BRCA2-like genomic copy number variations in a test sample, as compared to a reference sample, at one, or a plurality, of the genomic loci selected from 4p13-12, 13q12.2-21.1, 13q31.3-33.1, 14q23.2-32.33, 16q12.1-21, 17q11.1-12 and 17q21.2-21.31. In each of the aforementioned embodiments, detection of genomic copy number variations in the test sample, as compared to the reference sample, classifies the subject from whom the test sample was excised as an individual who will experience a therapeutic benefit from anti-cancer therapy.

[067] In some embodiments, a BRCA2 array is used to detect an increase in genomic copy numbers in a test sample, as compared to a reference sample, at one, or a plurality, of the genomic loci selected from 6p25.3-11.1, 6q12-13 and 13q31.3-33.1. In some embodiments, a BRCA2 array is used to detect a decrease in genomic copy numbers in a test sample, as compared to a reference sample, at one, or a plurality, of the genomic loci selected from 10q22.3-26.13, 13q12.2-21.1 and 14q23.2-32.33. In each of the aforementioned embodiments, detection of genomic copy number variations in the test sample, as compared to the reference sample, the subject from whom the test sample was excised as an individual who will experience a therapeutic benefit from anti-cancer therapy.

[068] In some embodiments, a BRCA2 array is used to detect an increase in genomic copy numbers in a test sample, as compared to a reference sample, at the genomic locus 16p12.3-11.2. In some embodiments, a BRCA2 array is used to detect a decrease in genomic copy numbers in a test sample, as compared to a reference sample, at one, or a plurality, of the genomic loci selected from 2q36.3-37.1, 4p13-12, 16q12.1-21, 17q11.1-12 and 17q21.2-21.31. In each of the aforementioned embodiments, detection of genomic copy number variations in the test sample, as compared to the reference sample, classifies the subject from whom the test sample was excised as an individual who will experience a therapeutic benefit from anti-cancer therapy.

[069] The genomic loci may be detected individually, or in any combination of two or more loci. In some embodiments, a BRCA2 array is used that is capable of detecting

BRCA2-like genomic copy number variations in all 25 of the above-listed chromosomal loci. In some embodiments, a BRCA2 array is used that is capable of detecting BRCA2-like genomic copy number variations at a number of the above-listed genomic loci selected from greater than 1, greater than 2, greater than 3, greater than 4, greater than 5, greater than 6, greater than 7, greater than 8, greater than 9, greater than 10, greater than 11, greater than 12, greater than 13, greater than 14, greater than 15, greater than 16, greater than 17, greater than 18, greater than 19, greater than 20, greater than 21, greater than 22, greater than 23, and greater than 24. In some embodiments, a BRCA2 array is used that is capable of detecting BRCA2-like genomic copy number variations at a number of the above-listed genomic loci selected from less than 25, less than 24, less than 23, less than 22, less than 21, less than 20, less than 19, less than 18, less than 17, less than 16, less than 15, less than 14, less than 13, less than 12, less than 11, less than 10, less than 9, less than 8, less than 7, less than 6, less than 5, less than 4, less than 3, and less than 2. In some embodiments, a BRCA2 array is used that is capable of detecting BRCA2-like genomic copy number variations in all 25 of the BRCA2-like genomic loci set forth in **Fig. 1A**. In some embodiments, a BRCA2 array is used that is capable of detecting BRCA2-like genomic copy number variations in all 7 of the BRCA2-like genomic loci set forth in **Fig. 1B**. In some embodiments, a BRCA2 array is used that is capable of detecting BRCA2-like genomic copy number variations in at least one, or a plurality, of the genomic loci selected from 2p24.1-16.3, 2q36.3-37.1, 3p12.3-3q11.2, 4p13-12, 6p25.3-11.1, 6q12-13, 7q11.21-11.22, 7q35-36.3, 10p15.2-12.1, 10q22.3-26.13, 11p15.5-15.4, 11q13.2-14.2, 11q23.1-25, 13q12.2-21.1, 13q31.3-33.1, 14q12-21.2, 14q23.2-32.33, 16p12.3-11.2, 16q12.1-21, 17p12-11.2, 17q11.1-12, 17q21.2-21.31, 22q11.23-13.1, 23p22.33-11.3 and 23q26.2-28. In some embodiments, a BRCA2 array is used that is capable of detecting BRCA2-like genomic copy number variations in at least one, or a plurality, of the genomic loci selected from 4p13-12, 13q12.2-21.1, 13q31.3-33.1, 14q23.2-32.33, 16q12.1-21, 17q11.1-12 and 17q21.2-21.31. In some embodiments, a BRCA2 array is used that is capable of detecting BRCA2-like genomic copy number variations in at least one, or a plurality, of the genomic loci selected from 6p25.3-11.1, 6q12-13 and 13q31.3-33.1. In some embodiments, a BRCA2 array is used that is capable of detecting BRCA2-like genomic copy number variations in at least one, or a plurality, of the genomic loci selected from 10q22.3-26.13, 13q12.2-21.1 and 14q23.2-32.33. In some embodiments, a BRCA2 array is used that is capable of detecting BRCA2-like genomic copy number variations in at the genomic locus 16p12.3-11.2. In some embodiments, a BRCA2 array is used that is capable of detecting BRCA2-like genomic copy number variations in at least one, or a plurality, of

the genomic loci selected from 2q36.3-37.1, 4p13-12, 16q12.1-21, 17q11.1-12 and 17q21.2-21.31. In each of the aforementioned embodiments, detection of BRCA2-like genomic copy number variations classifies the test sample as from either a BRCA2-like tumor or from a sporadic tumor and classifies the subject from whom the test sample was excised as an individual who will or will not experience a therapeutic benefit from anti-cancer therapy.

[070] The BRCA2 arrays comprise at least one probe. In various embodiments, the BRCA2 arrays comprise a plurality of probes. In some embodiments, the BRCA2 arrays comprise a plurality of probes, wherein the probes comprise nucleic acid sequences derived from BAC clones. The BRCA2-like genomic loci set forth in **Fig. 1A** are bounded by the BAC probes set forth in **Fig 2**. The BRCA2-like genomic loci set forth in **Fig. 1B** are bounded by a sub-set of the BAC probes set forth in **Fig 2**. In some embodiments, arrays capable of detecting BRCA2-like genomic copy number variations comprise at least one, or a plurality, probes derived from the BAC clones of **Fig. 2**. The BAC clones set forth in **Fig. 2** are not intended to be limiting in any way, and other probes within the BRCA2-like genomic loci of **Figs. 1A** and **1B** can also be used in the BRCA2 arrays. In some embodiments, arrays capable of detecting BRCA2-like genomic copy number variations comprise all 704 of the BAC clones set forth in **Fig. 2**. In some embodiments, arrays capable of detecting BRCA2-like genomic copy number variations comprise a number of the BAC clones set forth in **Fig. 2** selected from greater than 1, greater than 10, greater than 20, greater than 25, greater than 50, greater than 75, greater than 100, greater than 125, greater than 150, greater than 175, greater than 200, greater than 225, greater than 250, greater than 275, greater than 300, greater than 325, greater than 350, greater than 375, greater than 400, greater than 425, greater than 450, greater than 475, greater than 500, greater than 525, greater than 550, greater than 575, greater than 600, greater than 625, greater than 650, greater than 675, and greater than 700. In some embodiments, arrays capable of detecting BRCA2-like genomic copy number variations comprise a number of the BAC clones set forth in **Fig. 2** selected from less than 704, less than 700, less than 675, less than 650, less than 625, less than 600, less than 575, less than 550, less than 525, less than 500, less than 475, less than 450, less than 425, less than 400, less than 375, less than 350, less than 325, less than 300, less than 275, less than 250, less than 225, less than 200, less than 175, less than 150, less than 125, less than 100, less than 75, les than 50, less than 25, less than 20, and less than 10.

[071] In some embodiments, a BRCA2 array capable of detecting BRCA2-like genomic copy number variations comprises at least one, or a plurality, of probes that independently hybridize to a genomic locus selected from 2p24.1-16.3, 2q36.3-37.1, 3p12.3-

3q11.2, 4p13-12, 6p25.3-11.1, 6q12-13, 7q11.21-11.22, 7q35-36.3, 10p15.2-12.1, 10q22.3-26.13, 11p15.5-15.4, 11q13.2-14.2, 11q23.1-25, 13q12.2-21.1, 13q31.3-33.1, 14q12-21.2, 14q23.2-32.33, 16p12.3-11.2, 16q12.1-21, 17p12-11.2, 17q11.1-12, 17q21.2-21.31, 22q11.23-13.1, 23p22.33-11.3 and 23q26.2-28. In some embodiments, a BRCA2 array capable of detecting BRCA2-like genomic copy number variations comprises at least one, or a plurality, of probes that independently hybridize to a genomic locus selected from 4p13-12, 13q12.2-21.1, 13q31.3-33.1, 14q23.2-32.33, 16q12.1-21, 17q11.1-12 and 17q21.2-21.31. In some embodiments, a BRCA2 array capable of detecting BRCA2-like genomic copy number variations comprises at least one, or a plurality, of probes that independently hybridize to a genomic locus selected from 6p25.3-11.1, 6q12-13 and 13q31.3-33.1. In some embodiments, a BRCA2 array capable of detecting BRCA2-like genomic copy number variations comprises at least one, or a plurality, of probes that independently hybridize to a genomic locus selected from 10q22.3-26.13, 13q12.2-21.1 and 14q23.2-32.33. In some embodiments, a BRCA2 array capable of detecting BRCA2-like genomic copy number variations comprises at least one, or a plurality, of probes that independently hybridize to the genomic locus 16p12.3-11.2. In some embodiments, a BRCA2 array capable of detecting BRCA2-like genomic copy number variations comprises at least one, or a plurality, of probes that independently hybridize to a genomic locus selected from 2q36.3-37.1, 4p13-12, 16q12.1-21, 17q11.1-12 and 17q21.2-21.31. In these embodiments, the number of probes used can be determined as described above, the probes are as defined above and/or the probes may be obtained in methods as described above.

[072] In some embodiments, BRCA2 arrays capable of detecting BRCA2-like genomic copy number variations comprise at least one, or a plurality, of probes, wherein the probes comprise at least one, or a plurality of the distinct BAC clones of **Fig. 2**. In some embodiments, BRCA2 arrays capable of detecting BRCA2-like genomic copy number variations comprise at least one, or a plurality of probes, wherein the probes comprise at least one, or a plurality, of the BAC clones of **Fig. 2**, and wherein the probes specifically hybridize to at least 1, at least 2, at least 3, at least 4, at least 5, at least 6, at least 7, at least 8, at least 9, at least 10, at least 11, at least 12, at least 13, at least 14, at least 15, at least 16, at least 17, at least 18, at least 19, at least 20, at least 21, at least 22, at least 23, at least 24 or at least 25 of the genomic loci set forth in **Fig. 1A**. In some embodiments, BRCA2 arrays capable of detecting BRCA2-like genomic copy number variations comprise a plurality of probes, wherein the nucleic acid sequences of the probes are unique to the genomic loci set forth in **Fig. 1A**. In some embodiments, BRCA2 arrays capable of detecting BRCA2-like genomic

copy number variations comprise a plurality of probes, wherein the probes comprise a plurality of BAC clones specific to all of the genomic loci set forth in **Fig. 1A**. In some embodiments, BRCA2 arrays capable of detecting BRCA2-like genomic copy number variations comprise at least one, or a plurality of probes, wherein the probes comprise at least one, or a plurality, of the BAC clones of **Fig. 2**, and wherein the probes specifically hybridize to at least 1, at least 2, at least 3, at least 4, at least 5, at least 6 or at least 7 of the genomic loci set forth in **Fig. 1B**. In some embodiments, BRCA2 arrays capable of detecting BRCA2-like genomic copy number variations comprise a plurality of probes, wherein the nucleic acid sequences of the probes are unique to the genomic loci set forth in **Fig. 1B**. In some embodiments, BRCA2 arrays capable of detecting BRCA2-like genomic copy number variations comprise a plurality of probes, wherein the probes comprise a plurality of BAC clones specific to all of the genomic loci set forth in **Fig. 1B**. In some embodiments, BRCA2 arrays capable of detecting BRCA2-like genomic copy number variations comprise at least one, or a plurality, of probes, wherein the probes comprise greater than 1, greater than 10, greater than 20, greater than 25, greater than 50, greater than 75, greater than 100, greater than 125, greater than 150, greater than 175, greater than 200, greater than 225, greater than 250, greater than 275, greater than 300, greater than 325, greater than 350, greater than 375, greater than 400, greater than 425, greater than 450, greater than 475, greater than 500, greater than 525, greater than 550, greater than 575, greater than 600, greater than 625, greater than 650, greater than 675, or greater than 700 of the distinct BAC clones of **Fig. 2**. In some embodiments, BRCA2 arrays capable of detecting BRCA2-like genomic copy number variations comprise at least three probes, wherein the probes comprise greater than 1, greater than 10, greater than 20, greater than 25, greater than 50, greater than 75, greater than 100, greater than 125, greater than 150, greater than 175, greater than 200, greater than 225, greater than 250, greater than 275, greater than 300, greater than 325, greater than 350, greater than 375, greater than 400, greater than 425, greater than 450, greater than 475, greater than 500, greater than 525, greater than 550, greater than 575, greater than 600, greater than 625, greater than 650, greater than 675, or greater than 700 distinct BAC clones of **Fig. 2** that specifically hybridize to at least 1, at least 2, at least 3, at least 4, at least 5, at least 6, at least 7, at least 8, at least 9, at least 10, at least 11, at least 12, at least 13, at least 14, at least 15, at least 16, at least 17, at least 18, at least 19, at least 20, at least 21, at least 22, at least 23, at least 24 or at least 25 of the genomic loci set forth in **Fig. 1A**. In some embodiments, BRCA2 arrays capable of detecting BRCA2-like genomic copy number variations comprise at least one, or a plurality, of probes, wherein the probes comprise greater

than 1, greater than 10, greater than 20, greater than 25, greater than 50, greater than 75, greater than 100, greater than 125, greater than 150, greater than 175, greater than 200, greater than 225, greater than 250, greater than 275, greater than 300, greater than 325, greater than 350, greater than 375, greater than 400, greater than 425, greater than 450, greater than 475, greater than 500, greater than 525, greater than 550, greater than 575, greater than 600, greater than 625, greater than 650, greater than 675, or greater than 700 distinct BAC clones of **Fig. 2** that specifically hybridize to at least 1, at least 2, at least 3, at least 4, at least 5, at least 6 or at least 7 of the genomic loci set forth in **Fig. 1B**.

[073] In various embodiments, BRCA2 arrays capable of detecting BRCA2-like genomic copy number variations that comprise at least one, or a plurality, of probes, and/or that comprise at least one, or a plurality, of distinct BAC clones, allow for the individual analysis of at least one, or a plurality, of distinct genomic loci. Therefore, in some embodiments, the probes, and/or the distinct BAC clones, capable of detecting BRCA2-like genomic copy number variations are arranged on the BRCA2 arrays in a positionally-addressable manner.

[074] In various embodiments, BRCA2 arrays capable of detecting BRCA2-like genomic copy number variations comprise at least one, or a plurality, of distinct BAC clones, wherein the distinct BAC clones represent at least 1, at least 2, at least 3, at least 4, at least 5, at least 6, at least 7, at least 8, at least 9, at least 10, at least 11, at least 12, at least 13, at least 14, at least 15, at least 16, at least 17, at least 18, at least 19, at least 20, at least 21, at least 22, at least 23, at least 24 or at least 25 of the genomic loci set forth in **Fig. 1A**. In various embodiments, BRCA2 arrays capable of detecting BRCA2-like genomic copy number variations comprise at least one, or a plurality, of distinct BAC clones, wherein the distinct BAC clones represent at least 1, at least 2, at least 3, at least 4, at least 5, at least 6 or at least 7 of the genomic loci set forth in **Fig. 1B**. In various embodiments, BRCA2 arrays capable of detecting BRCA2-like genomic copy number variations comprise at least one, or a plurality, of distinct BAC clones, wherein the distinct BAC clones represent all 25 of the genomic loci set forth in **Fig. 1A**. In various embodiments, BRCA2 arrays capable of detecting BRCA2-like genomic copy number variations comprise at least one, or a plurality, of distinct BAC clones, wherein the distinct BAC clones represent all 7 of the genomic loci set forth in **Fig. 1B**.

Array Comparative Genomic Hybridization

[075] In various aspects, the present disclosure relates to the analysis of tumor cell samples by array-based comparative genomic hybridization. Array comparative genomic

hybridization (aCGH) is a technique that is used to detect genomic copy number variations at a higher level of resolution than chromosome-based comparative genomic hybridization. In aCGH, nucleic acids from a test sample and nucleic acids from a reference sample are labelled differentially. The test sample and the reference sample are then hybridized to an array comprising a plurality of probes. The ratio of the signal intensity of the test sample to that of the reference sample is then calculated, to measure the copy number changes for a particular location in the genome. The difference in the signal ratio determines whether the total copy numbers of the nucleic acids in the test sample are increased or decreased as compared to the reference sample. The test sample and the reference sample may be hybridized to the array separately or they may be mixed together and hybridized simultaneously. Exemplary methods of performing aCGH can be found, for example, in U.S. Patent Nos. 5,635,351; 5,665,549; 5,721,098; 5,830,645; 5,856,097; 5,965,362; 5,976,790; 6,159,685; 6,197,501; and 6,335,167; European Patent Nos. EP 1 134 293 and EP 1 026 260; van Beers *et al.*, Brit. J. Cancer (2006), 20; Joosse *et al.*, BMC Cancer (2007), 7:43; Pinkel *et al.*, Nat. Genet. (1998), 20: 207-211; Pollack *et al.*, Nat. Genet. (1999), 23: 41-46; and Cooper, Breast Cancer Res. (2001), 3: 158-175.

[076] Samples that are labelled differentially are labelled such that one of the two samples is labelled with a first detectable agent and the other of the two samples is labelled with a second detectable agent, wherein the first detectable agent and the second detectable agent produce distinguishable signals. Detectable agents that produce distinguishable signals can include, for example, matched pairs of fluorescent dyes.

[077] In some embodiments, the methods of the present disclosure comprise analyzing at least one test sample of tumor DNA from a subject by array-based comparative genomic hybridization to obtain information relating to the copy number aberrations present in the sample(s), if any; and based on the information obtained, classifying the tumor as a BRCA2-like tumor, a BRCAlikeness tumor or a non-BRCA2-like tumor.

[078] Information relating to the copy number aberrations present in a sample can include, for example, a gain of genetic material at one or more genomic loci, a loss of genetic material at one or more genomic loci, chromosomal abnormalities at one or more genomic loci, and genome copy number changes at one or more genomic loci. This information is obtained by analyzing the difference in signal intensity between the test sample and a reference sample at one or more genomic loci. The analysis can be performed using any of a variety of methods, means and variations thereof for carrying out array-based comparative genomic hybridization.

[079] In various embodiments, the reference sample is a nucleic acid sample that is representative of a normal, non-diseased state, for example a non-tumor/non-cancer cell, and contains a normal amount of copy numbers of the complement of the genomic loci being tested. The reference sample may be derived from a genomic nucleic acid sample from a normal and/or healthy individual or from a pool of such individuals. In various embodiments, the reference sample does not comprise any tumor or cancerous nucleic acids. In some embodiments, the reference sample is derived from a pool of female subjects. In some embodiments, the reference sample comprises pooled genomic DNA isolated from tissue samples (*e.g.* lymphocytes) from a plurality (*e.g.* at least 4-10) of healthy female subjects. In some embodiments, the reference sample comprises an artificially-generated population of nucleic acids designed to approximate the copy number level from each tested genomic region, or fragments of each tested genomic region. In some embodiments, the reference sample is derived from normal, non-cancerous cell lines or from cell line samples.

[080] Test samples may be obtained from a biological source comprising tumor cells, and reference samples may be obtained from a biological source comprising normal reference cells, by any suitable method of nucleic acid isolation and/or extraction. In various aspects, the test sample and the reference sample are DNA. Methods of DNA extraction are well known in the art. A classical DNA isolation protocol is based on extraction using organic solvents, such as a mixture of phenol and chloroform, followed by precipitation with ethanol (*see, e.g.*, Sambrook *et al.*, *supra*). Other methods include salting out DNA extraction, trimethylammonium bromide salt extraction, and guanidinium thiocyanate extraction. Additionally, there are numerous DNA extraction kits that are commercially available from, for example, BD Biosciences Clontech (Palo Alto, CA), Epicentre Technologies (Madison, WI), Gentra Systems, Inc. (Minneapolis, MN), MicroProbe Corp. (Bothell, WA), Organon Teknika (Durham, NC), and Qiagen Inc. (Valencia, CA).

[081] The test samples and the reference samples may be differentially labelled with any detectable agents or moieties. In various embodiments, the detectable agents or moieties are selected such that they generate signals that can be readily measured and such that the intensity of the signals is proportional to the amount of labelled nucleic acids present in the sample. In various embodiments, the detectable agents or moieties are selected such that they generate localized signals, thereby allowing resolution of the signals from each spot on an array.

[082] Methods for labeling nucleic acids are well-known in the art. For exemplary reviews of labeling protocols, label detection techniques and recent developments in the field,

see: Kricka, Ann. Clin. Biochem. (2002), 39: 114-129; van Gijlswijk *et al.*, Expert Rev. Mol. Diagn. (2001), 1: 81-91; and Joos *et al.*, J. Biotechnol. (1994), 35: 135-153. Standard nucleic acid labeling methods include: incorporation of radioactive agents, direct attachment of fluorescent dyes or of enzymes, chemical modification of nucleic acids to make them
5 detectable immunochemically or by other affinity reactions, and enzyme-mediated labeling methods including, without limitation, random priming, nick translation, PCR and tailing with terminal transferase. Other suitable labeling methods include psoralen-biotin, photoreactive azido derivatives, and DNA alkylating agents. In various embodiments, test sample and reference sample nucleic acids are labelled by Universal Linkage System, which
10 is based on the reaction of monoreactive cisplatin derivatives with the N7 position of guanine moieties in DNA (*see, e.g.*, Heetebrij *et al.*, Cytogenet. Cell. Genet. (1999), 87: 47-52).

[083] Any of a wide variety of detectable agents or moieties can be used to label test and/or reference samples. Suitable detectable agents or moieties include, but are not limited to: various ligands; radionuclides such as, for example, ^{32}P, ^{35}S, ^{3}H, ^{14}C, ^{125}I, ^{131}I, and others;
15 fluorescent dyes; chemiluminescent agents such as, for example, acridinium esters, stabilized dioxetanes, and others; microparticles such as, for example, quantum dots, nanocrystals, phosphors and others; enzymes such as, for example, those used in an ELISA, horseradish peroxidase, beta-galactosidase, luciferase, alkaline phosphatase and others; colorimetric labels such as, for example, dyes, colloidal gold and others; magnetic labels such as, for
20 example, Dynabeads™; and biotin, dioxigenin or other haptens and proteins for which antisera or monoclonal antibodies are available.

[084] In some embodiments, the test samples and the reference samples are labelled with fluorescent dyes. Suitable fluorescent dyes include, without limitation, Cy-3, Cy-5, Texas red, FITC, Spectrum Red, Spectrum Green, phycoerythrin, rhodamine, and fluorescein,
25 as well as equivalents, analogues and/or derivatives thereof. In some embodiments, the fluorescent dyes selected display a high molar absorption coefficient, high fluorescence quantum yield, and photostability. In some embodiments, the fluorescent dyes exhibit absorption and emission wavelengths in the visible spectrum (i.e., between 400nm and 750nm) rather than in the ultraviolet range of the spectrum (i.e., lower than 400nm). In some
30 embodiments, the fluorescent dyes are Cy-3 (3-N,N'-diethyltetramethylindo-dicarbocyanine) and Cy-5 (5-N,N'-diethyltetramethylindo-dicarbocyanine). Cy-3 and Cy-5 form a matched pair of fluorescent labels that are compatible with most fluorescence detection systems for array-based instruments. In some embodiments, the fluorescent dyes are Spectrum Red and Spectrum Green.

[085] A key component of aCGH is the hybridization of a test sample and a reference sample to an array. Exemplary hybridization and wash protocols are described, for example, in Sambrook *et al.* (2001), *supra*; Tijssen (1993), *supra*; and Anderson (Ed.), "Nucleic Acid Hybridization" (1999), Springer Verlag: New York, N.Y. In some embodiments, the hybridization protocols used for aCGH are those of Pinkel *et al.*, Nature Genetics (1998), 20:207-211. In some embodiments, the hybridization protocols used for aCGH are those of Kallioniemi, Proc. Natl. Acad. Sci. USA (1992), 89:5321-5325.

[086] Methods of optimizing hybridization conditions are well known in the art (*see, e.g.*, Tijssen, (1993), *supra*). To create competitive hybridization conditions, the array may be contacted simultaneously with differentially labelled nucleic acid fragments of the test sample and the reference sample. This may be done by, for example, mixing the labelled test sample and the labelled reference sample together to form a hybridization mixture, and contacting the array with the mixture.

[087] The specificity of hybridization may be enhanced by inhibiting repetitive sequences. In some embodiments, repetitive sequences (*e.g.*, Alu sequences, L1 sequences, satellite sequences, MRE sequences, simple homo-nucleotide tracts, and/or simple oligonucleotide tracts) present in the nucleic acids of the test sample, reference sample and/or probes are either removed, or their hybridization capacity is disabled. Removing repetitive sequences or disabling their hybridization capacity can be accomplished using any of a variety of well-known methods. These methods include, but are not limited to, removing repetitive sequences by hybridization to specific nucleic acid sequences immobilized to a solid support (*see, e.g.*, Brison *et al.*, Mol. Cell. Biol. (1982), 2: 578- 587); suppressing the production of repetitive sequences by PCR amplification using adequately designed PCR primers; inhibiting the hybridization capacity of highly repeated sequences by self-reassociation (*see, e.g.*, Britten *et al.*, Methods of Enzymology (1974), 29: 363-418); or removing repetitive sequences using hydroxyapatite which is commercially available from a number of sources including, for example, Bio-Rad Laboratories, Richmond, VA. In some embodiments, the hybridization capacity of highly repeated sequences in a test sample and/or in a reference sample is competitively inhibited by including, in the hybridization mixture, unlabelled blocking nucleic acids. The unlabelled blocking nucleic acids are therefore mixed with the hybridization mixture, and thus with a test sample and a reference sample, before the mixture is contacted with an array. The unlabelled blocking nucleic acids act as a competitor for the highly repeated sequences and bind to them before the hybridization mixture is contacted with an array. Therefore, the unlabelled blocking nucleic acids prevent labelled

repetitive sequences from binding to any highly repetitive sequences of the nucleic acid probes, thus decreasing the amount of background signal present in a given hybridization. In some embodiments, the unlabelled blocking nucleic acids are Human Cot-1 DNA. Human Cot-1 DNA is commercially available from a number of sources including, for example, Gibco/BRL Life Technologies (Gaithersburg, MD).

[088] Once hybridization is complete, the ratio of the signal intensity of the test sample as compared to the signal intensity of the reference sample is calculated. This calculation quantifies the amount of copy number aberrations present in the genomic DNA of the test sample, if any. In some embodiments, this calculation is carried out quantitatively or semi-quantitatively. In several aspects, it is not necessary to determine the exact copy number aberrations present in the genomic loci tested, as detection of an aberration, *i.e.* a gain or loss of genetic material, from the copy number in normal, non-cancerous genomic DNA is indicative of the presence of a disease state and is thus sufficient. Therefore, in several embodiments the quantification of the amount of copy number aberrations present in the genomic DNA of a test sample comprises an estimation of the copy number aberrations, as a semi-quantitative or relative measure usually suffices to predict the presence of a disease state and thus prospectively direct the determination of therapy for a subject.

[089] Quantitative techniques may be used to determine the copy number aberrations per cell present in a test sample. Several quantitative and semi-quantitative techniques to determine copy number aberrations exist including, for example, semi-quantitative PCR analysis or quantitative real-time PCR. The Polymerase Chain Reaction (PCR) *per se* is not a quantitative technique, however PCR-based methods have been developed that are quantitative or semi-quantitative in that they give a reasonable estimate of original copy numbers, within certain limits. Examples of such PCR techniques include, for example, quantitative PCR and quantitative real-time PCR (also known as RT-PCR, RQ-PCR, QRT-PCR or RTQ-PCR). In addition, many techniques exist that give estimates of relative copy numbers, as calculated relative to a reference. Such techniques include many array-based techniques. Absolute copy number estimates may be obtained by *in situ* hybridization techniques such as, for example, fluorescence *in situ* hybridization or chromogenic *in situ* hybridization.

[090] Fluorescence *in situ* hybridization permits the analysis of copy numbers of individual genomic locations and can be used to study copy numbers of individual genetic loci or particular regions on a chromosome (*see, e.g.*, Pinkel et al., Proc. Natl. Acad. Sci. U.S.A. (1988), 85, 9138-42). Comparative genomic hybridization can also be used to probe

for copy number changes of chromosomal regions (*see, e.g.*, Kallioniemi *et al.*, Science (1992), 258: 818-21; and Houldsworth *et al.*, Am. J. Pathol. (1994), 145: 1253-60).

[091] Copy numbers of genomic locations may also be determined using quantitative PCR techniques such as real-time PCR (*see, e.g.*, Suzuki *et al.*, Cancer Res. (2000), 60:5405-9). For example, quantitative microsatellite analysis can be performed for rapid measurement of relative DNA sequence copy numbers. In quantitative microsatellite analysis, the copy numbers of a test sample relative to a reference sample is assessed using quantitative, real-time PCR amplification of loci carrying simple sequence repeats. Simple sequence repeats are used because of the large numbers that have been precisely mapped in numerous organisms. Exemplary protocols for quantitative PCR are provided in Innis *et al.*, PCR Protocols, A Guide to Methods and Applications (1990), Academic Press, Inc. N.Y. Semi -quantitative techniques that may be used to determine specific DNA copy numbers include, for example, multiplex ligation-dependent probe amplification (*see, e.g.*, Schouten *et al.* Nucleic Acids Res. (2002), 30(12):e57; and Sellner *et al.*, Human Mutation (2004), 23(5):413-419) and multiplex amplification and probe hybridization (*see, e.g.*, Sellner *et al.* (2004), *supra*).

BRCA2 Array Comparative Genomic Hybridization

[092] In various aspects, the present disclosure relates to the use of a BRCA2 aCGH classifier capable of identifying BRCA2-like tumors in predicting whether an individual will benefit from anti-cancer therapy. In various aspects, a BRCA2 aCGH classifier capable of identifying BRCA2-like tumors is set forth on a BRCA2 array, as described herein.

[093] Using the methods described above, in various aspects, a BRCA2 aCGH classifier, which in some embodiments is present in an array as described herein, is capable of detecting genomic copy number variations in a test sample, as compared to a reference sample, wherein the copy number variations are detected in at least one, or a plurality, of the genomic loci selected from 2p24.1-16.3, 2q36.3-37.1, 3p12.3-3q11.2, 4p13-12, 6p25.3-11.1, 6q12-13, 7q11.21-11.22, 7q35-36.3, 10p15.2-12.1, 10q22.3-26.13, 11p15.5-15.4, 11q13.2-14.2, 11q23.1-25, 13q12.2-21.1, 13q31.3-33.1, 14q12-21.2, 14q23.2-32.33, 16p12.3-11.2, 16q12.1-21, 17p12-11.2, 17q11.1-12, 17q21.2-21.31, 22q11.23-13.1, 23p22.33-11.3 and 23q26.2-28. Using the methods described above, in various aspects, a BRCA2 aCGH classifier, which in some embodiments is present in an array as described herein, is capable of detecting genomic copy number variations in a test sample, as compared to a reference sample, wherein the copy number variations are detected in at least one, or a plurality, of the genomic loci selected from 4p13-12, 13q12.2-21.1, 13q31.3-33.1, 14q23.2-32.33, 16q12.1-

21, 17q11.1-12 and 17q21.2-21.31. Using the methods described above, in various aspects, a BRCA2 aCGH classifier, which in some embodiments is present in an array as described herein, is capable of detecting genomic copy number variations in a test sample, as compared to a reference sample, wherein the copy number variations are detected in at least one, or a 5 plurality, of the genomic loci selected from 6p25.3-11.1, 6q12-13 and 13q31.3-33.1. Using the methods described above, in various aspects, a BRCA2 aCGH classifier, which in some embodiments is present in an array as described herein, is capable of detecting genomic copy number variations in a test sample, as compared to a reference sample, wherein the copy number variations are detected in at least one, or a plurality, of the genomic loci selected 10 from 10q22.3-26.13, 13q12.2-21.1 and 14q23.2-32.33. Using the methods described above, in various aspects, a BRCA2 aCGH classifier, which in some embodiments is present in an array as described herein, is capable of detecting genomic copy number variations in a test sample, as compared to a reference sample, wherein the copy number variations are detected in the genomic locus 16p12.3-11.2. Using the methods described above, in various aspects, a 15 BRCA2 aCGH classifier, which in some embodiments is present in an array as described herein, is capable of detecting genomic copy number variations in a test sample, as compared to a reference sample, wherein the copy number variations are detected in at least one, or a plurality, of the genomic loci selected from 2q36.3-37.1, 4p13-12, 16q12.1-21, 17q11.1-12 and 17q21.2-21.31. Using the methods described above, in various aspects, a BRCA2 aCGH 20 classifier, which in some embodiments is present in an array as described herein, is capable of detecting genomic copy number variations in a test sample, as compared to a reference sample, wherein the copy number variations are detected in at least one, or a plurality, of the genomic loci set forth in **Fig. 1A**. Using the methods described above, in various aspects, a BRCA2 aCGH classifier, which in some embodiments is present in an array as described 25 herein, is capable of detecting genomic copy number variations in a test sample, as compared to a reference sample, wherein the copy number variations are detected in at least one, or a plurality, of the genomic loci set forth in **Fig. 1B**. In some embodiments, a BRCA2 aCGH classifier, which in some embodiments is present in an array as described herein, is capable of detecting genomic copy number variations at a number of the above-listed genomic loci 30 selected from greater than 1, greater than 2, greater than 3, greater than 4, greater than 5, greater than 6, greater than 7, greater than 8, greater than 9, greater than 10, greater than 11, greater than 12, greater than 13, greater than 14, greater than 15, greater than 16, greater than 17, greater than 18, greater than 19, greater than 20, greater than 21, greater than 22, greater than 23, and greater than 24. In some embodiments, a BRCA2 aCGH classifier, which in

some embodiments is present in an array as described herein, is capable of detecting genomic copy number variations at a number of the above-listed genomic loci selected from less than 25, less than 24, less than 23, less than 22, less than 21, less than 20, less than 19, less than 18, less than 17, less than 16, less than 15, less than 14, less than 13, less than 12, less than 11, less than 10, less than 9, less than 8, less than 7, less than 6, less than 5, less than 4, less than 3, and less than 2.

[094] Using the methods described above, in various aspects, a BRCA2 aCGH classifier, which in some embodiments is present in an array as described herein, is capable of detecting genomic copy number variations in a test sample using at least one, or a plurality, of probes that independently hybridize to at least one, or a plurality, of the genomic loci set forth in **Fig. 1A**. Using the methods described above, in various aspects, a BRCA2 aCGH classifier, which in some embodiments is present in an array as described herein, is capable of detecting genomic copy number variations in a test sample using at least one, or a plurality, of probes that independently hybridize to at least one, or a plurality, of the genomic loci set forth in **Fig. 1B**. Using the methods described above, in various aspects, a BRCA2 aCGH classifier, which in some embodiments is present in an array as described herein, is capable of detecting genomic copy number variations in a test sample, as compared to a reference sample, using at least one, or a plurality, of the distinct BAC clones set forth in **Fig. 2**. In some embodiments, a BRCA2 aCGH classifier, which in some embodiments is present in an array as described herein, capable of detecting genomic copy number variations in a test sample comprises all 704 of the BAC clones set forth in **Fig. 2**. In some embodiments, a BRCA2 aCGH classifier, which in some embodiments is present in an array as described herein, capable of detecting genomic copy number variations in a test sample comprises a number of the BAC clones set forth in **Fig. 2** selected from greater than 1, greater than 10, greater than 20, greater than 25, greater than 50, greater than 75, greater than 100, greater than 125, greater than 150, greater than 175, greater than 200, greater than 225, greater than 250, greater than 275, greater than 300, greater than 325, greater than 350, greater than 375, greater than 400, greater than 425, greater than 450, greater than 475, greater than 500, greater than 525, greater than 550, greater than 575, greater than 600, greater than 625, greater than 650, greater than 675, and greater than 700. In some embodiments, a BRCA2 aCGH classifier, which in some embodiments is present in an array as described herein, capable of detecting genomic copy number variations in a test sample comprises a number of the BAC clones set forth in **Fig. 2** selected from less than 704, less than 700, less than 675, less than 650, less than 625, less than 600, less than 575, less than 550, less than 525, less

than 500, less than 475, less than 450, less than 425, less than 400, less than 375, less than 350, less than 325, less than 300, less than 275, less than 250, less than 225, less than 200, less than 175, less than 150, less than 125, less than 100, less than 75, les than 50, less than 25, less than 20, and less than 10.

5

Therapeutic Uses

[095] In various aspects, the BRCA2 classifiers, which in some embodiments are present in one or more arrays as described herein, can be used to predict whether an individual will benefit from anti-cancer therapy.

[096] Using the methods described above, in various aspects, the BRCA2 classifiers
10 are capable of determining whether an individual metastatic breast cancer patient, in continuous complete remission after one or more anti-cancer therapies, has a BRCA2-like tumor. Using the methods described above, in various aspects, the BRCA2 classifiers are capable of determining whether a metastatic breast cancer patient with a BRCA2-like tumor has a significantly higher complete remission rate. The BRCA2 classifiers are therefore
15 capable of predicting whether an individual patient will benefit from anti-cancer therapy. Using the methods described above, in various aspects, the BRCA2 classifiers are capable of predicting improved outcome after anti-cancer therapy by identifying breast cancer patients specifically benefiting from one or more anti-cancer therapies.

[097] The BRCA2 classifiers can be used as pre-selection tools, to prospectively
20 detect subjects with a high risk of carrying a BRCA2-mutation and/or a BRCA2-like tumor. Additionally, the BRCA2 classifiers can be used as predictive tests to identify breast cancer patients likely to benefit from anti-cancer therapy.

[098] In further aspects, the present disclosure relates to kits for use in the diagnostic applications described above. The kits can comprise any or all of the reagents to perform the
25 methods described herein. The kits can comprise one or more of the BRCA2 classifiers, which in some embodiments are present in one or more arrays as described herein. In the diagnostic applications such kits may include any or all of the following: assay reagents, buffers, nucleic acids such as hybridization probes and/or primers that specifically bind to at least one of the genomic locations described herein, as well as arrays comprising such nucleic
30 acids. In addition, the kits may include instructional materials containing directions (i.e., protocols) for the practice of the methods of this disclosure. While the instructional materials typically comprise written or printed materials they are not limited to such. Any medium capable of storing such instructions and communicating them to an end user is contemplated by this disclosure. Such media include, but are not limited to electronic storage media (e.g.,

magnetic discs, tapes, cartridges, chips), optical media (e.g., CD ROM), and the like. Such media may include addresses to internet sites that provide such instructional materials.

Examples

[099] The following examples describe in detail certain embodiments of the BRCA2 arrays and the BRCA2 aCGH classifiers. It will be apparent to those skilled in the art that many modifications, both to materials and methods, may be practiced without departing from the scope of the disclosure.

Example 1

Homologous recombination deficiency in breast cancer and association with response to neo-adjuvant chemotherapy

[0100] Tumors with homologous recombination deficiency (HRD), such as BRCA2 associated breast cancers, are not able to reliably repair DNA double strand breaks (DSBs), and are highly sensitive to alkylating agents and PARP inhibitors. In this Example, markers that may indicate the presence of HRD in patients with HER2-negative breast cancer, scheduled to receive neoadjuvant chemotherapy, were studied. Forty-three triple negative (TN) and 91 estrogen receptor positive (ER+) pre-treatment biopsies from sporadic breast cancer patients were examined. In ER+ tumors, an aCGH "BRCA2-like" pattern and the amplification of the BRCA2 inhibiting gene EMSY were frequently observed (37% and 15% respectively). A "BRCA2-like" aCGH pattern was associated with a significantly higher response rate to neoadjuvant chemotherapy with doxorubicin and cyclophosphamide. In addition, EMSY amplification and a "BRCA2-like" pattern rarely occurred together, raising doubts about the assumption that EMSY amplification inactivates BRCA2 and causes HRD. In conclusion, in ER+/HER2- tumors, a 'BRCA2-like' aCGH profile may be predictive of chemotherapy response.

[0101] **Introduction**

[0102] The breast cancer genes BRCA1 and BRCA2 are involved in homologous recombination and tumors of patients carrying germ-line mutations in these genes, show HRD. BRCA1 and BRCA2 can be inactivated in sporadic cancers as well (Joosse,S.A., van Beers,E.H., Tielen,I.H., et al Prediction of BRCA1-association in hereditary non-BRCA1/2 breast carcinomas with array-CGH, Breast Cancer Res Treat, 2008; and Turner,N., Tutt,A. and Ashworth,A. Hallmarks of 'BRCAness' in sporadic cancers, Nat Rev Cancer, 4: 814-819, 2004), a phenomenon sometimes referred to as "BRCA-likeness" (or "BRCAness"). Many other genes are involved in homologous recombination, including the Fanconi anemia genes and the BRCA2 inactivating gene EMSY (Hughes-Davies,L., Huntsman,D., Ruas,M., et al

EMSY links the BRCA2 pathway to sporadic breast and ovarian cancer, Cell, 115: 523-535, 2003).

[0103] It has been previously shown that breast cancers of BRCA1 mutation carriers have a characteristic pattern of DNA gains and losses in an array comparative genomic hybridization (aCGH) assay (Wessels,L.F., van Welsem,T., Hart,A.A., Van't Veer,L.J., Reinders,M.J. and Nederlof,P.M. Molecular classification of breast carcinomas by comparative genomic hybridization: a specific somatic genetic profile for BRCA1 tumors, Cancer Res, 62: 7110-7117, 2002). This pattern is also found in a subgroup of HER-2 negative sporadic breast cancers that do not contain a BRCA1 mutation. The BRCA1-like pattern accurately identified tumors benefitting from intensive alkylating chemotherapy in two recent retrospective studies (Vollebergh,M.A., Lips E.H., Nederlof,P.M., et al. An aCGH classifier derived from BRCA1-mutated breast cancer and benefit of high-dose platinum-based chemotherapy in HER2-negative breast cancer patients, Ann Oncol, 2010, in press). An aCGH classifier that recognizes breast cancers of BRCA2 mutation carriers has been found as well (Joosse,S.A., Brandwijk,K.I.M., Devilee,P., et al Prediction of BRCA2-association in hereditary breast carcinomas with array-CGH, Breast Cancer Res Treat. 2010 Jul 8. PubMed PMID: 20614180). Each of the above mentioned tests could be useful to detect HRD in clinical samples.

[0104] In this Example, the frequency in which these possibly HRD-associated features occur in untreated patients with breast cancer was prospectively determined. The findings were correlated with beneficial response to chemotherapy that causes DNA DSBs. If HRD is confirmed to be the "Achilles heel" of certain sporadic tumors, such tests could eventually serve to individualize drug treatment.

[0105] **Patients and methods**

[0106] **Patients**

[0107] Pre-treatment biopsies of primary breast tumors from 134 women with HER2 negative breast cancer were collected. All patients had received neoadjuvant treatment at the Netherlands Cancer Institute between 2000 and 2007 as part of two ongoing clinical trials, or were treated off protocol according to the standard arm of one of these studies. Both studies had been approved by the ethical committee and written informed consent was obtained. For eligibility, breast carcinoma with either a primary tumor size of at least 3 cm was required, or the presence of fine needle aspiration (FNA) -proven axillary lymph node metastases. Biopsies were taken using a 14G core needle under ultrasound guidance. After collection, specimens were snap-frozen in liquid nitrogen and stored at -70°C. Each patient had two or

three biopsies taken to assure that enough tumor material was available for both diagnosis and further study.

[0108] Depending on the particular study, a treatment regimen was assigned to each patient, which consisted of one of the following: 1.) Six courses of dose-dense Doxorubicin/Cyclophosphamide (ddAC) or 2.) Six courses of Capecitabine/Docetaxel (CD) or 3.) Three courses of ddAC followed by three courses CD (or vice versa) if the therapy response was considered unfavorable by MRI evaluation after three courses. For the response analysis, only patients who started with ddAC (group 1 and group 3) were considered.

[0109] **Response evaluation**

[0110] The response of the primary tumor to chemotherapy was evaluated by contrast-enhanced MRI (Loo,C.E., Teertstra,H.J., Rodenhuis,S., et al Dynamic contrast-enhanced MRI for prediction of breast cancer response to neoadjuvant chemotherapy: initial results, AJR Am J Roentgenol, 191: 1331-1338, 2008) after 3 courses of chemotherapy, and after surgery by pathologic evaluation of the resection specimen. The primary end point of both studies was a pCR, defined as the complete absence of residual invasive tumor cells seen at microscopy. If only non-invasive tumor (carcinoma in situ) was detected, this was considered a pCR as well. When a small number of scattered tumor cells were seen, the samples were classified as 'near pCR' (npCR). Because the aim of this study was to determine if HRD was correlated with a higher sensitivity to chemotherapy, tumors with a npCR were included in the group of complete remission for analytical purposes. Patients with larger amounts of residual tumor left were classified as non-responders (NR).

[0111] **Array-CGH**

[0112] Tumor DNA and reference DNA were co-hybridized using two different CyDyes to a microarray containing 3.5k BAC/PAC derived DNA segments covering the whole genome with an average spacing of 1MB and processed as described before (Joosse,S.A., van Beers,E.H. and Nederlof,P.M. Automated array-CGH optimized for archival formalin-fixed, paraffin-embedded tumor material, BMC Cancer, 7: 43, 2007). Classification of subtypes was performed using the aCGH BRCA2 classifiers disclosed herein (**Fig. 2**) and developed by Joosse et al. (Joosse,S.A., van Beers,E.H., Tielen,I.H., et al Prediction of BRCA1-association in hereditary non-BRCA1/2 breast carcinomas with array-CGH, Breast Cancer Res Treat, 2008; and Joosse,S.A., Brandwijk,K.I.M., Devilee,P., et al Prediction of BRCA2-association in hereditary breast carcinomas using array-CGH, Breast Cancer Res Treat. 2010 Jul 8. PubMed PMID: 20614180). When the BRCA2 score was 0.50 or higher the tumour was qualified as BRCA2-like (Joosse,S.A., Brandwijk,K.I.M.,

Devilee,P., et al Prediction of BRCA2-association in hereditary breast carcinomas using array-CGH, Breast Cancer Res Treat. 2010 Jul 8. PubMed PMID: 20614180). Under this cut-off a tumour was called non-BRCA2-like.

[0113] **MLPA**

5 [0114] Amplification of EMSY (C11orf30) was determined using a custom MLPA set, containing seven different EMSY probes and nine reference probes (MRC Holland, The Netherlands; X025). This EMSY MLPA set was first validated by an EMSY FISH assay (Dako, Glostrup, Denmark). From the comparison of the EMSY FISH assay and the MLPA, it was concluded that an average of the seven probes above 1.5 corresponded to EMSY
10 amplification, as detected by at least 6 copies of the probe at the FISH assay. DNA fragments were analyzed on a 3730 DNA Analyzer (AB, USA). For normalization and analysis the Coffalizer program was used (MRC-Holland, The Netherlands).

[0115] **Statistical tests**

[0116] The Fisher's exact test was used to assess association between the
15 dichotomized HRD characteristics and treatment response. The Mann-Whitney U test was used to analyze means of variables and relate it to treatment response. All data analyses were performed using SPSS version 15.

[0117] **Results**

[0118] **Overview of samples**

20 [0119] In the series of patients described in this Example, the frequency of features associated with HRD in pre-treatment biopsies was studies, and possible relationships with beneficial response to chemotherapy known to cause DNA DSBs were explored. HER2+ tumors were not investigated in this study, because they were treated with regimens based on trastuzumab and taxanes, agents that do not cause DNA DSBs. The choice for EMSY
25 amplification was pragmatic, since this test can be performed reliably on small pretreatment biopsies. aCGH was used to assess "BRCA-ness". If the pattern of genomic alterations resembled those in BRCA2 associated tumors, the sample was called BRCA2-like. If no pattern was recognized the tumor was called non-BRCA2-like. A total of 134 tumors were studied, of which 91 were ER+ and 43 were Triple Negative tumors. See table 1 for an
30 overview of the different patients.

Table 1 Patient and tumor characteristics

	TN	ER+
Number of patients	43	91
Median age (sd)	45 (11.18)	50.5 (9.14)

Progesterone receptor	Positive	0	0 %	58	64%
	Negative	100	100 %	33	36%
T-stage	T1	2	5%	12	13%
	T2	29	67%	51	56%
	T3	11	26%	25	28%
	T4	1	2%	3	3%
N-stage	Node negative	28	65%	22	24%
	Node positive	15	35%	69	76%
Initial chemotherapy	AC	38	88%	81	89%
	DC	2	5%	7	8%
	other	3	7%	3	3%
Response	pCR	15	34%	6	7%
	npCR	7	16%	12	13%
	NR	19	44%	67	74%
	unknown	2	5%	6	7%

AC=doxorubicin, cyclophosphamide; DC=docetaxel, capecitabine; (n)pCR=(near) pathological complete remission; NR= non response.

[0120] Array comparative genomic hybridization was performed in 37 TN and 75 ER+ tumors. A BRCA2-like profile was observed in both TN and ER+ tumors (32% and 37% respectively) (Table 2). The BRCA2 inhibiting gene EMSY was only amplified in ER+ tumors, in this tumor group the frequency was 15%. This initial analysis shows that EMSY amplification is specific for ER+ tumors and a BRCA2-like profile occurs in both TN and ER+ tumors. This is in concordance with the fact that tumors in BRCA2 carriers are often ER+ (Chappuis,P.O., Nethercot,V. and Foulkes,W.D. Clinico-pathological characteristics of B, Semin Surg Oncol, 18: 287-295, 2000).

Table 2 Summary of HRD characteristics

	TN (n=43)	ER+ (n=91)	p-value
aCGH BRCA2-like			
B2-like	12 (32%)	28 (37%)	
non-B2-like	25 (66%)	47 (63%)	0.832
EMSY Amplification			
Amplification	0 (0%)	9 (15%)	
No amplification	23 (100%)	51 (85%)	**0.057**

[0121] **ER+ tumors and BRCA2-like profile and EMSY amplification**

[0122] Table 3 gives an overview of HRD characteristics in ER+ tumors. As mentioned earlier, many ER+ tumors show a BRCA2-like pattern or an amplification of the BRCA2 inactivating protein EMSY. Interestingly, a BRCA2-like pattern and EMSY amplification occur together in only one tumor sample (Table 3).

Table 3. Overview of HRD characteristics in ER+ tumors*

Sample Number	BRCA2 like	EMSY amplification
2055	-	-
2105	-	-
2099	+	+
2013	+	
2016	+	
2017	+	
2032	+	
2044	+	
2114	+	
2138	+	
2147	+	
100	+	-
158	+	-
2062	+	-
2065	+	-
2071	+	-
2073	+	-
2075	+	-
2077	+	-
2085	+	-
2098	+	-
2117	+	-
2122	+	-
2128	+	-
2143	+	-
2144	+	-
2151	+	-
2153	+	-
2081	+	-
2100	+	-
2086	-	+
2087	-	+
110	-	+
112	-	+
2038	-	+
2058	-	+
2084	-	+
2120	-	+
2023	-	.

*Only samples with at least one characteristic are shown

5 [0123] Table 4 gives an overview of HRD characteristics related to clinical pathological factors. Investigation into whether BRCA2 and EMSY were related to PR positivity, T-stage, N-stage and response to neoadjuvant treatment was performed. For a BRCA2 pattern no association was observed for PR positivity, T-stage and N-stage. There was a significant association between BRCA2-like pattern and a higher response rate to
10 alkylating neoadjuvant chemotherapy (35% vs. 12%, p=0.033). No differences in response to therapy between tumors with an EMSY amplification or without was observed.

Table 4 Association between BRCA2 pattern and EMSY amplification and clinical pathological variables in ER+ tumor samples.

	BRCA2 like pattern			EMSY		
	BRCA2-like	Sporadic-like	p-value	Amplification	No amplification	p-value
PRpos	15/27 (56%)	36/47 (77%)	0.072	7/9 (78)	34/51 (68)	0.71
T-stage						
1	2/28 (7%)	8/48 (17%)		0 (0%)	8/51 (16%)	
2	18/28 (64%)	26/48 (54%)		5/9 (56%)	28/51(55%)	
3	7/28 (25%)	13/48 (27%)		4/9 (44%)	14/51 (28%)	
4	1/28 (4%)	1/48 (2%)		0	1/51 (2%)	
N-stage						
Pos	19/28 (68%)	41/48 (83%)	0.086	7/9 (78%)	41/51 (80%)	1
Response on A/C*						
pCR+npCR	9/26 (35%)	5/42 (12%)	**0.033**	2/7 (29%)	8/46 (18%)	0.604

* Response was measured only on samples from patients treated with A/C

5 [0124] **Discussion**

[0125] Classical chemotherapeutic agents that cause DNA double-strand breaks (DSBs) are thought to be particularly effective in tumors with HRD (Kennedy,R.D., Quinn,J.E., Mullan,P.B., Johnston,P.G. and Harkin,D.P. The role of BRCA1 in the cellular response to chemotherapy, J Natl Cancer Inst, 96: 1659-1668, 2004; Fedier,A., Steiner,R.A.,
10 Schwarz,V.A., Lenherr,L., Haller,U. and Fink,D. The effect of loss of Brca1 on the sensitivity to anticancer agents in p53-deficient cells, Int J Oncol, 22: 1169-1173, 2003; Helleday,T., Petermann,E., Lundin,C., Hodgson,B. and Sharma,R.A. DNA repair pathways as targets for cancer therapy, Nat Rev Cancer, 8: 193-204, 2008; Moynahan,M.E., Cui,T.Y. and Jasin,M. Homology-directed dna repair, mitomycin-c resistance, and chromosome
15 stability is restored with correction of a Brca1 mutation, Cancer Res, 61: 4842-4850, 2001; and Powell,S.N. and Kachnic,L.A. Therapeutic exploitation of tumor cell defects in homologous recombination, Anticancer Agents Med Chem, 8: 448-460, 2008) and the novel class of PARP inhibiting drugs has been shown to have marked antitumor activity with very little toxicity (Bryant,H.E., Schultz,N., Thomas,H.D., et al Specific killing of BRCA2-
20 deficient tumours with inhibitors of poly(ADP-ribose) polymerase, Nature, 434: 913-917, 2005; and Farmer,H., McCabe,N., Lord,C.J., et al Targeting the DNA repair defect in BRCA mutant cells as a therapeutic strategy, Nature, 434: 917-921, 2005). Unfortunately, a demonstration of HRD in clinical tumor samples is problematic. One reported assay measures DSB repair pathways, but requires short-term cultures of primary breast cancer cells
25 (Keimling,M., Kaur,J., Bagadi,S.A., Kreienberg,R., Wiesmuller,L. and Ralhan,R. A sensitive test for the detection of specific DSB repair defects in primary cells from breast cancer specimens, Int J Cancer, 123: 730-736, 2008). Immunohistochemical methods have been proposed as well, aiming to detect CHK1 and RAD51 localization in the cytoplasm and/or

the nucleus (Honrado,E., Osorio,A., Palacios,J., et al Immunohistochemical expression of DNA repair proteins in familial breast cancer differentiate BRCA2-like tumors, J Clin Oncol, 23: 7503-7511, 2005), but reliable immunohistochemical staining results can be difficult to obtain. Others have used methylation assays for BRCA1 (Esteller,M., Silva,J.M.,
5 Dominguez,G., et al Promoter hypermethylation and BRCA1 inactivation in sporadic breast and ovarian tumors, J Natl Cancer Inst, 92: 564-569, 2000; and Catteau,A., Harris,W.H., Xu,C.F. and Solomon,E. Methylation of the BRCA1 promoter region in sporadic breast and ovarian cancer: correlation with disease characteristics, Oncogene, 18: 1957-1965, 1999), FancC and FancD and have studied EMSY amplification (Rodriguez,C., Hughes-Davies,L.,
10 Valles,H., et al Amplification of the BRCA2 pathway gene EMSY in sporadic breast cancer is related to negative outcome, Clin Cancer Res, 10: 5785-5791, 2004), for example by an in situ hybridization assay (Turner,N., Tutt,A. and Ashworth,A. Hallmarks of 'BRCAness' in sporadic cancers, Nat Rev Cancer, 4: 814-819, 2004). The sensitivity and specificity of these approaches is unknown and a possible association of these features with neoadjuvant
15 treatment response has not been reported.

[0126] High-dose alkylating chemotherapy has previously been employed in the treatment of patients with breast cancer, with either a high risk of relapse (Rodenhuis,S., Bontenbal,M., Beex,L.V., et al High-dose chemotherapy with hematopoietic stem-cell rescue for high-risk breast cancer, N Engl J Med, 349: 7-16, 2003) or with distant metastases
20 (Schrama,J.G., Baars,J.W., Holtkamp,M.J., Schornagel,J.H., Beijnen,J.H. and Rodenhuis,S. Phase II study of a multi-course high-dose chemotherapy regimen incorporating cyclophosphamide, thiotepa, and carboplatin in stage IV breast cancer, Bone Marrow Transplant, 28: 173-180, 2001). In both studies, a modest survival advantage for patients who had received this intensive treatment was observed, a result which has also been documented
25 in meta-analyses of the randomized studies (Berry,D.A., Ueno,N.T., Johnson,M.M., et al High-dose chemotherapy with autologous stem-cell support versus standard-dose chemotherapy: meta-analysis of individual patient data from 6 randomized metastatic breast cancer trials, Proc.San Antonio Breast Cancer Symp, Abstract 6113:2008). These observations are consistent with the existence of a putative subgroup of breast cancers that is
30 highly responsive to alkylating drugs, as has been previously speculated based on clinical observations (Rodenhuis,S. The status of high-dose chemotherapy in breast cancer, Oncologist, 5: 369-375, 2000; and Rodenhuis,S. High-dose chemotherapy in breast cancer--interpretation of the randomized trials, Anticancer Drugs, 12: 85-88, 2001). This subgroup could overlap or even be identical with the subgroup of tumors that show HRD. To study this

hypothesis, Vollebergh et al. have recently applied the aCGH test to search for the 'BRCA1 like' pattern (Joosse,S.A., van Beers,E.H., Tielen,I.H., et al Prediction of BRCA1-association in hereditary non-BRCA1/2 breast carcinomas with array-CGH, Breast Cancer Res Treat, 2008; Wessels,L.F., van Welsem,T., Hart,A.A., Van't Veer,L.J., Reinders,M.J. and
5 Nederlof,P.M. Molecular classification of breast carcinomas by comparative genomic hybridization: a specific somatic genetic profile for BRCA1 tumors, Cancer Res, 62: 7110-7117, 2002; and van Beers,E.H., van Welsem,T., Wessels,L.F., et al Comparative genomic hybridization profiles in human BRCA1 and BRCA2 breast tumors highlight differential sets of genomic aberrations, Cancer Res, 65: 822-827, 2005) in metastatic tumors and related it to
10 the treatment results of intensive alkylating chemotherapy (Vollebergh,M.A., Lips E.H., Nederlof,P.M., et al. An aCGH classifier derived from BRCA1-mutated breast cancer and benefit of high-dose platinum-based chemotherapy in HER2-negative breast cancer patients, Ann Oncol, 2010, in press). It was observed that all long-term survivors of stage IV breast cancer had tumors with the BRCA1-like signature. It was shown in a second retrospective
15 study, that triple-negative tumors with the BRCA1-signature benefitted markedly from high-dose therapy in the adjuvant setting, while the triple-negative tumors with a non-BRCA1-like like profile did not (Vollebergh,M.A., Lips E.H., Nederlof,P.M., et al. An aCGH classifier derived from BRCA1-mutated breast cancer and benefit of high-dose platinum-based chemotherapy in HER2-negative breast cancer patients, Ann Oncol, 2010, in press). The
20 recent aCGH test to detect a pattern of DNA gains and losses associated with breast cancers in BRCA2 carriers (Joosse,S.A., Brandwijk,K.I.M., Devilee,P., et al Prediction of BRCA1- and BRCA2-association in hereditary breast carcinomas with array-CGH, Submitted for publication, 2009) has not yet been studied in relation to chemotherapy response.

[0127] In the series of patients described herein, the frequency of certain features
25 associated with HRD in untreated breast cancers was studied and possible relationships with neoadjuvant treatment response were explored. HER2+ tumors were not investigated in this study, because they are treated with different agents, such as trastuzumab and taxanes, which do not cause DSBs. BRCA2 inactivation, shown by a BRCA2 like aCGH profile and EMSY amplification, was specifically observed in ER+ tumors. A significantly higher response rate
30 of ER+ tumors with the BRCA2 profile to DSBs-causing chemotherapy was observed.

[0128] **Features of BRCA2 inactivation**

[0129] Of the ER+ and TN tumors combined, roughly one-third had a BRCA2-like profile, while EMSY amplification was exclusively found in the ER+ tumors. In a series of 183 breast tumors from BRCA2 mutation carriers and from sporadic breast tumors, BRCA2

methylation has been assessed, but methylation has not been found in any of the samples (Joosse,S.A., Brandwijk,K.I.M., Devilee,P., et al Prediction of BRCA2-association in hereditary breast carcinomas with array-CGH, Breast Cancer Res Treat. 2010 Jul 8. PubMed PMID: 20614180). In the literature, BRCA2 promotor methylation has been sporadically observed in ovarian cancer (Hilton,J.L., Geisler,J.P., Rathe,J.A., Hattermann-Zogg,M.A., DeYoung,B. and Buller,R.E. Inactivation of BRCA1 and BRCA2 in ovarian cancer, J Natl Cancer Inst, 94: 1396-1406, 2002), but not in breast cancer. An alternative mechanism for BRCA2 inactivation involves amplification of the EMSY gene. Interestingly, the present study did not identify any overlap between tumors showing a BRCA2-like profile and EMSY amplification, except in one case (Table 3). This observation points at two different routes or levels of BRCA2 inactivation. In tumors with EMSY amplification, usually a lower degree of chromosomal gains and losses is observed than in the BRCA2-like tumors. This does not support the hypothesis that EMSY is a HRD characteristic and would consequently show a high level of genomic instability. Moreover, in a different series of 52 sporadic tumors from which aCGH data are available, 7 ER+ tumors have been detected with a gain at the EMSY locus, and none of these showed a BRCA2 like profile. This supports the finding that EMSY and the BRCA2 like profile only rarely occur together and that EMSY amplification is not associated with the same degree of chromosomal instability as BRCA2 mutation. In vitro assays have shown that the EMSY protein can bind BRCA2 protein and inactivate its function (Raouf,A., Brown,L., Vrcelj,N., et al Genomic instability of human mammary epithelial cells overexpressing a truncated form of EMSY, J Natl Cancer Inst, 97: 1302-1306, 2005). An increase in chromosomal instability was observed after EMSY overexpression.

[0130] ER+ tumors with a BRCA2-like profile show higher response rates to neoadjuvant chemotherapy with cyclophophamide and doxorubicin than ER+ tumors with a non-BRCA2-like profile. This is remarkable as ER+ tumors usually show a low pCR rate (5-10%) after neoadjuvant therapy. In the present study, ER+ tumors with a BRCA2-like profile had a (near)pCR rate of 35% versus 12% for ER+ tumors with a non-BRCA2-like profile. EMSY amplified tumors did not show a difference in response rates, which is in line with the finding that these tumors have a lower degree of chromosomal instability and thus are not HRD. If confirmed, these findings could have important implications for neoadjuvant chemotherapy selection in ER+ tumors.

[0131] **Conclusion**

[0132] A BRCA2 aCGH pattern appears to be a strong predictor of response in ER+ HER2- tumors. EMSY amplification is not correlated with a BRCA2 like profile, indicating that it may not signify HRD.

Example 2

5 [0133] The predictive value of one or more of the BRCA2-classifiers disclosed herein (see, for example, **Fig. 2**) was evaluated for selective benefit of high-dose (HD) alkylating chemotherapy, a DNA double strand break-inducing regimen, with autologous stem cell rescue in the subgroup of hormone receptor positive, HER2-negative patients who have participated in the RODENHUIS trial (Rodenhuis,S., Bontenbal,M., Beex,L.V., et al High-
10 dose chemotherapy with hematopoietic stem-cell rescue for high-risk breast cancer, N Engl J Med, 349: 7-16, 2003).

[0134] **Methods**

[0135] To determine whether the BRCA2-classifiers disclosed herein predict benefit from HD-chemotherapy, a study comprising a random sample (N=249) taken from the HER2
15 negative subpopulation (N=621) who participated in a randomized controlled trial of standard adjuvant chemotherapy (5 courses of 5-fluorouracil, epirubicin, cyclophosphamide (FEC)) versus 4 courses of FEC followed by high dose cyclophosphamide, thiotepa and carboplatin (CTC) with autologous stem cell support was performed. BRCA2-probability scores were obtained for every sampled patient according to the methods disclosed herein. The cut-off of
20 the BRCA2-probability score used in this study was as had been reported before (Joosse,S.A., Brandwijk,K.I.M., Devilee,P., et al Prediction of BRCA2-association in hereditary breast carcinomas using array-CGH, Breast Cancer Res Treat. 2010 Jul 8. PubMed PMID: 20614180; Lips et al. Ann Oncol 2010 in press). To assess whether this cut-off of the reported BRCA2-classifier could also serve as a predictive marker for benefit of DNA DSB-
25 inducing anticancer therapies, the interaction between the BRCA2-classifier and benefit of HD-chemotherapy (CTC) with autologous stem cell support was evaluated.

[0136] The trial described in this Example has previously received the approval of the Institutional Review board of the Netherlands Cancer Institute. This study has been designed following the REMARK guidelines.

30 [0137] **Random patient sample (stage-III series)**

[0138] Patients were randomly sampled from the HER2-negative subpopulation that had participated in a large, randomized, controlled, multicentre trial performed in the Netherlands between 1993 and 1999. Inclusion criteria have been published previously (Rodenhuis,S., Bontenbal,M., Beex,L.V., et al High-dose chemotherapy with hematopoietic

stem-cell rescue for high-risk breast cancer, N Engl J Med, 349: 7-16, 2003). Eligible patients were randomized between either conventional chemotherapy (five courses FEC), or HD-chemotherapy, which is identical except that instead of the fifth course of FEC, a course of CTC was given. Based on prior experience, it is known that HER2-positive breast cancer patients did not derive any benefit of HD-PB-chemotherapy (Rodenhuis,S., Bontenbal,M., Beex,L.V., et al High-dose chemotherapy with hematopoietic stem-cell rescue for high-risk breast cancer, N Engl J Med, 349: 7-16, 2003). Therefore, patients with HER2-positive breast cancer have been omitted from this selection. In addition, these patients now typically receive highly effective trastuzumab-based adjuvant systemic therapy in the clinic.

[0139] Cases were only included when their FFPE primary tumor tissue was available and contained more than 60% of tumor cells.

[0140] **Comparative Genomic Hybridization and mutation analyses**

[0141] aCGH patterns of the BRCA1-like^{CGH} pattern of 230 patients that had been previously generated were used in this study (Vollebergh,M.A., Lips E.H., Nederlof,P.M., et al. An aCGH classifier derived from BRCA1-mutated breast cancer and benefit of high-dose platinum-based chemotherapy in HER2-negative breast cancer patients, Ann Oncol, 2010, in press). Tumours of 19 patients could additionally be analyzed in this series. In short, genomic DNA was extracted from FFPE primary tumours (van Beers EH, Joosse SA, Ligtenberg MJ et al. A multiplex PCR predictor for aCGH success of FFPE samples. Br J Cancer 2006; 94(2):333-337). Of seven of these 19 patients, only lymph-node tissue containing primary tumour tissue, removed at first diagnosis, was available. Three of these 19 samples contained DNA concentrations that were too low for direct aCGH-analysis and these samples were amplified with the BioScore™ Screening and Amplification Kit (42440, Enzo Life Sciences). Tumor and reference DNA was labelled according to the manufacturers' instructions (Kreatech Biotechnology, Amsterdam) and used for aCGH as previously described (Joosse SA, van Beers EH, Nederlof PM. Automated Array-CGH Optimized for Archival Formalin-Fixed, Paraffin-Embedded Tumor Material. BMC Cancer 2007; 7: 43). Slides were scanned with an Agilent DNA Microarray Scanner BA on the same day. The quality of each aCGH pattern was determined using a profile-quality and hybridization quality score, as previously published (Vollebergh,M.A., Lips E.H., Nederlof,P.M., et al. An aCGH classifier derived from BRCA1-mutated breast cancer and benefit of high-dose platinum-based chemotherapy in HER2-negative breast cancer patients, Ann Oncol, 2010, in press). The data of 230 of the 249 patients have been deposited in NCBI's Gene Expression Omnibus, with the data of the remaining 19 patients to be added.

[0142] **Histopathology**

[0143] Two pathologists (JW and MvdV) had previously reviewed all tumors and scored whole Haematoxylin & Eosin (H&E)-slides for tumor percentages. Oestrogen-receptor (ER), progesterone-receptor (PR), P53, and HER2 status were determined by immunohistochemistry (IHC) as described previously (Rodenhuis S, Bontenbal M, Beex LV et al. High-Dose Chemotherapy With Hematopoietic Stem-Cell Rescue for High-Risk Breast Cancer. N Engl J Med 2003; 349 (1): 7-16; van de Vijver MJ, Peterse JL, Mooi WJ et al. Neu-Protein Overexpression in Breast Cancer. Association With Comedo-Type Ductal Carcinoma in Situ and Limited Prognostic Value in Stage II Breast Cancer. N Engl J Med 1988; 319 (19): 1239-1245).

[0144] **Statistical analysis**

[0145] Groups of interest were tested for differences using Fisher's exact tests. Recurrence-free survival (RFS) was defined as the time between randomization and appearance of local or regional recurrence, metastases or death from any cause (Rodenhuis S, Bontenbal M, Beex LV et al. High-Dose Chemotherapy With Hematopoietic Stem-Cell Rescue for High-Risk Breast Cancer. N Engl J Med 2003; 349 (1): 7-16); all other events were censored. Overall survival (OS) was calculated from randomization to death from any cause, or end of follow-up. Patients alive at last follow-up were censored at that time. Median RFS and OS were 7.7 and 8.3 years, respectively, for all 249 patients. Survival analyses were performed using the Kaplan-Meier method for the making of curves and compared using log-rank tests; Cox regression methods were used to calculate hazard ratios (HR).

[0146] To ensure a direct correlation between aCGH pattern and treatment received, only patients who completed their assigned treatment were analyzed (per-protocol analysis). Whether the treatment effect on survival of HD-PB-chemotherapy compared to conventional chemotherapy, expressed as the HR, was significantly different between BRCA2-likeCGH and non-BRCA2-like^{CGH} patients was assessed. This was evaluated with multivariate Cox regression analyses with an interaction term, adjusting for potential confounders.

[0147] To address non-proportionality of hazards, the Cox model was stratified for the number of lymph nodes (4-9 vs. >=10) and double negative ER/PR status (ER<10% and PR<10% vs. other). Regression coefficients for stratification variables were not explicitly estimated. Instead, separate baseline hazards were non-parametrically estimated for each combination of values of the stratification variables (4 strata). There was no evidence of non-proportional hazards in the stratified model, based on an evaluation using Schoenfeld residuals as well as interaction terms with follow-up time. In all models fitted during the

evaluation of non-proportional hazards (different stratifications, time-interactions), the estimated effects for treatment, the BRCA2 classifier and their interaction were very stable (Therneau TM, Grambsch PM: Modeling survival data: extending the Cox model. Springer, New York 2000).

5 [0148] All calculations were performed using the statistical package SPSS 15.0 and SAS 9.1 (for Windows, respectively SAS Institute Inc., Cary, NC, USA).

[0149] **Results**

[0150] **Stage-III Series**

[0151] In total, for 249 patients an aCGH profile could be obtained. Characteristics 10 and treatments of these 249 patients did not differ from those HER2-negative patients of the randomized controlled trial not in the current analysis (Table 5). Based on the aCGH tumor profiles, tumors of 51 patients (51/249, 20%) were scored as BRCA2-like^{CGH}. Within the patients with BRCA2- or non-BRCA2-like^{CGH} tumors patient characteristics did not differ by treatment arm (**Fig. 4**). Patients with BRCA2-like^{CGH} tumors were generally younger and 15 their tumors were more often poorly differentiated (**Fig. 4**) compared to non-BRCA2-like^{CGH} patients.

Table 5. Distribution of clinicopathological variables between randomly sampled HER2-negative patients and patients not in the current analysis from the stage-III series

Variable	Total		In analysis with aCGH classifier		Not in current analysis		p values*
	n	(%)	n	(%)	n	(%)	
Total	**592**	**100.0**	**249**	**42.1**	**343**	**57.9**	
Treatment							
Conventional chemotherapy	298	50.3	122	49.0	176	51.3	0.618
High Dose chemotherapy	294	49.7	127	51.0	167	48.7	
Age in categories							
≤ 40 years	153	25.8	69	27.7	84	24.5	0.393
> 40 years	439	74.2	180	72.3	259	75.5	
Type of surgery							
Breast conserving therapy	135	22.8	51	20.5	84	24.5	0.276
Mastectomy	457	77.2	198	79.5	259	75.5	
Tumor classification							
T1	136	23.0	47	18.9	89	25.9	
T2	357	60.3	163	65.5	194	56.6	0.222#
T3	90	15.2	37	14.9	53	15.5	
Unknown	9	1.5	2	0.8	7	2.0	
No. of positive lymph nodes							
4-7	289	48.8	127	51.0	162	47.2	
8-10	145	24.5	58	23.3	87	25.4	0.458#
≥ 11	158	26.7	64	25.7	94	27.4	
Histologic grade							

I	137	23.1	55	22.1	82	23.9	
II	221	37.3	93	37.3	128	37.3	0.918
III	217	36.7	92	36.9	125	36.4	
Not determined	17	2.9	9	3.6	8	2.3	
Estrogen receptor status							
Negative (<10%)	140	23.6	65	26.1	75	21.9	0.242
Positive (≥10%)	451	76.2	184	73.9	267	77.8	
Unknown	1	0.2	0	0.0	1	0.3	
Progesterone receptor status							
Negative (<10%)	213	36.0	101	40.6	112	32.7	0.081
Positive (≥10%)	368	62.2	146	58.6	222	64.7	
Unknown	11	1.9	2	0.8	9	2.6	
P53 status							
Negative (<10%)	331	55.9	142	57.0	189	55.1	0.861
Positive (≥10%)	225	38.0	94	37.8	131	38.2	
Unknown	36	6.1	13	5.2	23	6.7	

* p values: patients with unknown values were omitted and p values were calculated using the Fisher exact, except for #Chi square test for trend.; test.

[0152] **Survival According to Treatment in Stage-III Series by BRCA2-like^{CGH} pattern**

[0153] In the multivariate analyses, tumor size according to TNM classification, number of positive lymph nodes, Bloom Richardson grade (BR-grade), triple-negative status and treatment were included, since these variables were significantly associated with RFS in univariate analysis (Table 6).

Table 6. Univariate Cox proportional-hazard regression analysis of the risk of Recurrence (RFS) after randomization in the stage-III series

Variable	No. Events / No. patients	Hazard Ratio	95% CI	p values
Age				
Continuously	112 / 249	0.99	0.97 – 1.02	0.695
Type of surgery				
Breast conserving therapy	23 / 51	1.00		
Mastectomy	89 / 198	0.98	0.62 – 1.55	0.934
Pathological tumor classification				
T1	18 / 47	1.00		
T2	70 / 163	1.10	0.66 – 1.85	0.708
T3	24 / 37	2.36	1.28 – 4.34	0.006
No. of positive lymph nodes				
4-7	52 / 127	1.00		
8-10	23 / 58	0.98	0.60 – 1.60	0.937
≥ 11	37 / 64	1.80	1.18 – 2.74	0.006
Histologic grade				

I	20 / 55	1.00		
II	43 / 93	1.40	0.83 – 2.38	0.212
III	47 / 92	1.83	1.09 – 3.09	0.024
Estrogen receptor status				
Negative (<10%)	34 / 65	1.00		
Positive (≥10%)	78 / 184	0.57	0.38 – 0.86	0.008
Progesterone receptor status				
Negative (<10%)	49 / 101	1.00		
Positive (≥10%)	62 / 146	0.71	0.49 – 1.04	0.076
P53 status				
Negative (<10%)	66 / 142	1.00		
Positive (≥10%)	40 / 94	0.91	0.62 – 1.35	0.648
Treatment				
Conventional Chemotherapy	68 / 122	1.00		
High Dose Chemotherapy	44 / 127	0.50	0.34 – 0.73	<0.001
aCGH BRCA2-pattern				
Non-BRCA2-like^{CGH} tumor	86 / 198	1.00		
BRCA2-like^{CGH} tumor	26 / 51	1.28	0.83 – 1.99	0.266

Number of events is not equal for all variables, since some patients have missing data; maximum missing variables (i.e. events) is 6 /112.*Abbreviations:* CI, confidence interval.

[0154] A significantly greater benefit of HD-PB-chemotherapy compared to conventional chemotherapy was observed with regard to RFS in patients with BRCA2-like^{CGH} tumors (**Fig. 3** panel B, adjusted HR 0.22, 95%CI: 0.09-0.55; Table 7). In patients with non-BRCA2-like^{CGH} tumors this benefit was maintained although non-significantly (**Fig. 3** panel A, adjusted HR 0.67, 95%CI: 0.44-1.04; Table 7). The difference observed between treatment arms was significantly different between patients with BRCA2-like^{CGH} tumor and non-BRCA2-like^{CGH} tumors (Table 7; p-interaction: 0.032). Similar results were obtained for overall survival (**Fig. 3**, panels C and D, p-interaction: 0.037; Table 8).

Table 7. Multivariate Cox proportional-hazard analysis of the risk of recurrence (RFS) in the stage-III series and the BRCA2-classifier

Variable	No. Events / No. patients	Hazard Ratio	95% CI	p values
p T-stage				
pT1	16 / 43	1.00		
pT2	69 / 157	1.01	0.58 – 1.76	0.967
pT3	24 / 37	1.93	1.00 – 3.73	0.052
Histologic grade				
I	20 / 55	1.00		
II	43 / 92	1.15	0.66 – 2.01	0.622
III	46 / 90	1.65	0.91 – 2.99	0.101
aCGH pattern				
Non-BRCA2-like^{CGH} tumour	84 / 189	1.00		
BRCA2-like^{CGH} tumour	25 / 48	1.90	1.06 – 3.42	0.032
BRCA2-like^{CGH} tumour				
Conventional chemotherapy	18 / 25	1.00		
High-dose chemotherapy	7 / 23	0.22*	0.09 – 0.53	0.001
Non-BRCA2-like^{CGH} tumour				
Conventional chemotherapy	48 / 94	1.00		
High-dose chemotherapy	36 / 95	0.65*	0.42 – 1.01	0.056

Hazard ratios for high-dose vs. conventional chemotherapy differ significantly by BRCA2-like^{CGH} status (interaction p=0.032). Cox model stratified for number of lymph nodes (4-9 vs. >=10) and double negative ER/PR status (ER<10% and PR<10% vs. other) and based on 237 patients (12 patients contributing 3 events were excluded due to missing values for at least one of the variables shown).

Abbreviations: No, number; CI, confidence interval.

Table 8. Multivariate Cox proportional-hazard analysis of the risk of death (OS) in the stage-III series and the BRCA2-classifier

Variable	No. Events / No. patients	Hazard Ratio	95% CI	p values
p T-stage				
pT1	14 / 43	1.00		
pT2	51 / 157	0.98	0.54 – 1.79	0.940
pT3	22 /37	2.11	1.06 – 4.22	0.035
Histologic grade				
I	15 /55	1.00		
II	33 /92	1.12	0.59 – 2.12	0.739
III	39 / 90	1.55	0.79 – 3.04	0.200
aCGH pattern				
Non-BRCA2-like^{CGH} tumour	67 / 189	1.00		
BRCA2-like^{CGH} tumour	20 / 48	1.75	0.94 – 3.26	0.078
BRCA2-like^{CGH}				
Conventional chemotherapy	16 / 25	1.00		
High-dose chemotherapy	4 /23	0.18*	0.06 – 0.55	0.003
Non-BRCA2-like^{CGH} tumour				
Conventional chemotherapy	39 / 94	1.00		
High-dose chemotherapy	28 / 95	0.67*	0.41 – 1.11	0.118

Hazard ratios for high-dose vs. conventional chemotherapy differ significantly by BRCA2-like^{CGH} status (interaction p=0.037). Cox model stratified for number of lymph nodes (4-9 vs. >=10) and double negative ER/PR status (ER<10% and PR<10% vs. other) and based on 237 patients (12 patients contributing 3 events were excluded due to missing values for at least one of the variables shown).

Abbreviations: No, number; CI, confidence interval.

[0155] **Discussion**

5 [0156] In this Example, a BRCA2-like^{CGH} pattern, a genomic pattern originated from *BRCA2*-mutated tumors, was investigated. We observed that patients with a BRCA2-like^{CGH} tumor had a significant better recurrence-free and overall survival after HD-PB-

chemotherapy compared to anthracycline-based conventional chemotherapy, while this was not observed for patients with non-BRCA2-like^{CGH} tumors (significant p-interactions, RFS and OS). These data suggest that the BRCA2-like^{CGH} pattern is a predictive marker for HD-PB-chemotherapy benefit.

5 [0157] Tumors with a BRCA2-like^{CGH} pattern displayed a similar distribution of hormone-receptor negativity (16/51, 31%) as *BRCA2*-mutated breast cancers and as the general breast cancer population (Lakhani SR, van de Vijver MJ, Jacquemier J et al. The Pathology of Familial Breast Cancer: Predictive Value of Immunohistochemical Markers Estrogen Receptor, Progesterone Receptor, HER-2, and P53 in Patients With Mutations in 10 BRCA1 and BRCA2. J Clin Oncol 2002; 20 (9): 2310-2318; Palacios J, Honrado E, Osorio A et al. Phenotypic Characterization of BRCA1 and BRCA2 Tumors Based in a Tissue Microarray Study With 37 Immunohistochemical Markers. Breast Cancer Res Treat 2005; 90 (1): 5-14). In a previous report regarding the BRCA1-like^{CGH} pattern, it was observed that 25% of the BRCA1-like^{CGH} tumors harbored a *BRCA1*-mutation 15 (Vollebergh,M.A., Lips E.H., Nederlof,P.M., et al. An aCGH classifier derived from BRCA1-mutated breast cancer and benefit of high-dose platinum-based chemotherapy in HER2-negative breast cancer patients, Ann Oncol, 2010, in press). An additional one-third of these BRCA1-like^{CGH} tumors showed hypermethylation of the *BRCA1*-promoter, a plausible cause for a disturbed BRCA1-pathway other than mutations. A similar strategy was 20 used in a recent publication in which a profile for BRCAness was developed using *BRCA1/2*-mutated ovarian cancer, however that study used gene expression instead of aCGH (Konstantinopoulos PA, Spentzos D, Karlan BY et al. Gene Expression Profile of BRCAness That Correlates With Responsiveness to Chemotherapy and With Outcome in Patients With Epithelial Ovarian Cancer. J Clin Oncol 2010). Sporadic ovarian cancer patients who scored 25 as BRCAness (29%) with this profile showed a significantly longer disease-free survival after platinum agents.

[0158] In this Example a BRCA2-like^{CGH} classifier disclosed herein (**Fig. 2**) was used to identify HER2-negative patients with a selective improved outcome after HD-PB-chemotherapy, a DSB-inducing regimen. A variety of other methods have been applied to 30 select patients benefiting from DSB-inducing agents, such as RAD51 staining (Asakawa H, Koizumi H, Koike A et al. Prediction of Breast Cancer Sensitivity to Neoadjuvant Chemotherapy Based on Status of DNA Damage Repair Proteins. Breast Cancer Res 2010; 12 (2): R17), gene expression profiling (Konstantinopoulos PA, Spentzos D, Karlan BY et al. Gene Expression Profile of BRCAness That Correlates With Responsiveness to

Chemotherapy and With Outcome in Patients With Epithelial Ovarian Cancer. J Clin Oncol 2010), methylation and *BRCA1* gene expression measurement (Silver DP, Richardson AL, Eklund AC et al. Efficacy of Neoadjuvant Cisplatin in Triple-Negative Breast Cancer. J Clin Oncol 2010). These methods are based on an indirect link with either *BRCA1* or *BRCA2* as in the BRCAness phenotype concept described by Turner (Turner N, Tutt A, Ashworth A. Hallmarks of 'BRCAness' in Sporadic Cancers. Nat Rev Cancer 2004; 4 (10): 814-819), as are the methods disclosed herein. However, most of the other studies performed to date were in triple negative (TN) breast cancer, as TN tumors share both histological and molecular features with *BRCA1*-mutated tumors (Lakhani SR, van de Vijver MJ, Jacquemier J et al. The Pathology of Familial Breast Cancer: Predictive Value of Immunohistochemical Markers Estrogen Receptor, Progesterone Receptor, HER-2, and P53 in Patients With Mutations in BRCA1 and BRCA2. J Clin Oncol 2002; 20 (9): 2310-2318; Pathology of Familial Breast Cancer: Differences Between Breast Cancers in Carriers of BRCA1 or BRCA2 Mutations and Sporadic Cases. Breast Cancer Linkage Consortium. Lancet 1997; 349 (9064): 1505-1510). It is believes that BRCAness is not restricted to TN tumors, and thus this Example studied the BRCA2-like^{CGH} pattern which is also able to identify ER-positive tumors. This in contrast to the BRCA1-like^{CGH} pattern of which most tumors are TN (34/39, 87%) (Vollebergh,M.A., Lips E.H., Nederlof,P.M., et al. An aCGH classifier derived from BRCA1-mutated breast cancer and benefit of high-dose platinum-based chemotherapy in HER2-negative breast cancer patients, Ann Oncol, 2010, in press). By performing a randomized controlled trial, the selective benefit of platinum-based chemotherapy and general chemotherapy benefit could be distinguished by studying the association between aCGH patterns and survival.

[0159] Breast cancers are typically characterized by many large regions of genomic gains and losses that can readily be detected by aCGH arrays such as those disclosed herein. Another advantage of the methods disclosed herein is that they require only minimal amounts of DNA derived from FFPE tissue, which is a prerequisite for a clinical application in many jurisdictions.

[0160] In conclusion, in a series of 249 patients it was shown that a BRCA2-like^{CGH} classifier **(Fig. 2)** was able to both select ER-positive and TN breast cancer patients for selective benefit of intensified DSB-inducing chemotherapy. Patients with this BRCA2-like^{CGH} tumour phenotype had a five times lower risk of recurrence and death after the high-dose platinum-based chemotherapy than patients without this tumour phenotype. Therefore this BRCA2-like^{CGH} test can be used as a clinical chemotherapy prediction test.

[0161] Finally, it should be noted that there are alternative ways of implementing the embodiments disclosed herein. Accordingly, the present embodiments are to be considered as illustrative and not restrictive. Furthermore, the claims are not to be limited to the details given herein, and are entitled their full scope and equivalents thereof.

Claims

What is claimed is:

1. A method for predicting whether a patient will benefit from anti-cancer therapy, comprising:

obtaining a test sample from a patient;

detecting the copy numbers of DNA in the test sample in at least one genomic locus selected from 2p24.1-16.3, 2q36.3-37.1, 3p12.3-3q11.2, 4p13-12, 6p25.3-11.1, 6q12-13, 7q11.21-11.22, 7q35-36.3, 10p15.2-12.1, 10q22.3-26.13, 11p15.5-15.4, 11q13.2-14.2, 11q23.1-25, 13q12.2-21.1, 13q31.3-33.1, 14q12-21.2, 14q23.2-32.33, 16p12.3-11.2, 16q12.1-21, 17p12-11.2, 17q11.1-12, 17q21.2-21.31, 22q11.23-13.1, 23p22.33-11.3 and 23q26.2-28; and

comparing the copy numbers in the test sample to corresponding copy numbers in a reference sample;

wherein a variation in the copy numbers in the test sample indicates that the patient will benefit from anti-cancer therapy.

2. The method of claim 1, wherein a variation in the copy numbers in the test sample is detected in at least one genomic locus selected from 4p13-12, 13q12.2-21.1, 13q31.3-33.1, 14q23.2-32.33, 16q12.1-21, 17q11.1-12 and 17q21.2-21.31.

3. The method of claim 1, wherein an increase in the copy numbers in the test sample is detected in at least one genomic locus selected from 6p25.3-11.1, 6q12-13 and 13q31.3-33.1.

4. The method of claim 1, wherein a decrease in the copy numbers in the test sample is detected in at least one genomic locus selected from 10q22.3-26.13, 13q12.2-21.1 and 14q23.2-32.33.

5. The method of claim 1, wherein a variation in the copy numbers in the test sample is detected in at least one genomic locus selected from 13q12.2-21.1, 13q31.3-33.1 and 14q23.2-32.33.

6. The method of claim 1, wherein an increase in the copy numbers in the test sample is detected in the genomic locus 13q31.3-33.1.

7. The method of claim 1, wherein a decrease in the copy numbers in the test sample is detected in at least one genomic locus selected from 13q12.2-21.1 and 14q23.2-32.33.

8. The method of claim 1, wherein:

an increase in the copy numbers in the test sample is detected in at least one genomic locus selected from 6p25.3-11.1, 6q12-13 and 13q31.3-33.1;

a decrease in the copy numbers in the test sample is detected in at least one
5 genomic locus selected from 10q22.3-26.13, 13q12.2-21.1 and 14q23.2-32.33;

an increase in the copy numbers in the test sample is detected in the genomic locus 13q31.3-33.1; and

a decrease in the copy numbers in the test sample is detected in at least one genomic locus selected from 13q12.2-21.1 and 14q23.2-32.33.

9. The method of any one of claims 1-8, wherein the anti-cancer therapy is selected from homologous recombination deficiency-targeted drugs, drugs that directly cause double strand DNA breaks, and drugs that indirectly cause double strand DNA breaks.

10. The method of any one of claims 1-9, wherein the detecting is performed by array comparative genomic hybridization using an array.

11. The method of claim 10, wherein the array comprises a plurality of probes immobilized on a substrate, wherein the probes hybridize to DNA from at least one genomic locus selected from 2p24.1-16.3, 2q36.3-37.1, 3p12.3-3q11.2, 4p13-12, 6p25.3-11.1, 6q12-13, 7q11.21-11.22, 7q35-36.3, 10p15.2-12.1, 10q22.3-26.13, 11p15.5-15.4, 11q13.2-14.2,
5 11q23.1-25, 13q12.2-21.1, 13q31.3-33.1, 14q12-21.2, 14q23.2-32.33, 16p12.3-11.2, 16q12.1-21, 17p12-11.2, 17q11.1-12, 17q21.2-21.31, 22q11.23-13.1, 23p22.33-11.3 and 23q26.2-28.

12. The method of claim 11, wherein the probes hybridize to DNA from the genomic loci 6p25.3-11.1, 6q12-13 and 13q31.3-33.1.

13. The method of claim 12, wherein the probes detect an increase in copy number of the DNA from the genomic loci.

14. The method of claim 11, wherein the probes hybridize to DNA from the genomic loci 10q22.3-26.13, 13q12.2-21.1 and 14q23.2-32.33.

15. The method of claim 14, wherein the probes detect a decrease in copy number of the DNA from the genomic loci.

16. The method of claim 11, wherein the probes hybridize to DNA from the genomic locus 13q31.3-33.1.

17. The method of claim 16, wherein the probes detect an increase in copy number of the DNA from the genomic locus.

18. The method of claim 11, wherein the probes hybridize to DNA from the genomic loci 13q12.2-21.1 and 14q23.2-32.33.

19. The method of claim 18, wherein the probes detect a decrease in copy number of the DNA from the genomic loci.

20. The method of claim 11, wherein the probes hybridize to DNA from the genomic loci 6p25.3-11.1, 6q12-13, 13q31.3-33.1, 10q22.3-26.13, 13q12.2-21.1, 14q23.2-32.33, 13q31.3-33.1, 13q12.2-21.1 and 14q23.2-32.33.

21. The method of claim 20, wherein the probes:

detect an increase in copy number of the DNA from the genomic loci 6p25.3-11.1, 6q12-13 and 13q31.3-33.1;

detect a decrease in copy number of the DNA from the genomic loci 10q22.3-
5 26.13, 13q12.2-21.1 and 14q23.2-32.33;

detect an increase in copy number of the DNA from the genomic locus 13q31.3-33.1; and

detect a decrease in copy number of the DNA from the genomic loci 13q12.2-21.1 and 14q23.2-32.33.

22. The method of any one of claims 11-21, wherein the array comprises a plurality of probes derived from at least one of the BAC clones of **Fig. 2**.

23. The method of any one of claims 11-22, wherein the probes are derived from at least 50 of the BAC clones of **Fig. 2**.

24. The method of any one of claims 11-23, wherein the probes are derived from all 704 of the BAC clones of **Fig. 2**.

25. The method of any one of claims 1-24, wherein the detecting is performed prior to administration of the anti-cancer therapy.

26. A BRCA2 classifier, comprising:

a plurality of probes, wherein the probes hybridize to DNA from at least one genomic locus selected from 2p24.1-16.3, 2q36.3-37.1, 3p12.3-3q11.2, 4p13-12, 6p25.3-11.1, 6q12-13, 7q11.21-11.22, 7q35-36.3, 10p15.2-12.1, 10q22.3-26.13, 11p15.5-15.4, 11q13.2-14.2,
5 11q23.1-25, 13q12.2-21.1, 13q31.3-33.1, 14q12-21.2, 14q23.2-32.33, 16p12.3-11.2, 16q12.1-21, 17p12-11.2, 17q11.1-12, 17q21.2-21.31, 22q11.23-13.1, 23p22.33-11.3 and 23q26.2-28; and

wherein the probes detect a variation in copy number of the DNA from the at least one genomic locus.

27. The classifier of claim 26, wherein the probes hybridize to DNA from the genomic loci 6p25.3-11.1, 6q12-13 and 13q31.3-33.1.

28. The classifier of claim 26, wherein the probes hybridize to DNA from the genomic loci 10q22.3-26.13, 13q12.2-21.1 and 14q23.2-32.33.

29. The classifier of claim 26, wherein the probes hybridize to DNA from the genomic locus 13q31.3-33.1.

30. The classifier of claim 26, wherein the probes hybridize to DNA from the genomic loci 13q12.2-21.1 and 14q23.2-32.33.

31. The classifier of claim 30, wherein the probes hybridize to DNA from the genomic loci 6p25.3-11.1, 6q12-13, 13q31.3-33.1, 10q22.3-26.13, 13q12.2-21.1, 14q23.2-32.33, 13q31.3-33.1, 13q12.2-21.1 and 14q23.2-32.33.

32. The classifier of claim 31, wherein the probes:

detect an increase in copy number of the DNA from the genomic loci 6p25.3-11.1, 6q12-13 and 13q31.3-33.1;

detect a decrease in copy number of the DNA from the genomic loci 10q22.3-
5 26.13, 13q12.2-21.1 and 14q23.2-32.33;

detect an increase in copy number of the DNA from the genomic locus 13q31.3-33.1; and

detect a decrease in copy number of the DNA from the genomic loci 13q12.2-21.1 and 14q23.2-32.33.

33. The classifier of any one of claims 26-32, wherein the probes are derived from at least 50 of the BAC clones of **Fig. 2**.

34. The classifier of any one of claims 26-33, wherein the probes are derived from all 704 of the BAC clones of **Fig. 2**.

FIGURE 1A

Chromosome	Begin BAC	END BAC	chrom band	chrom band	region start	Region end	size Mb	characteristic
2	RP11-560C7	RP5-960D23	2p24.1	2p16.3	22998568	47897750	24.9	
2	RP11-86O17	RP11-263G22	2q36.3	2q37.1	226629534	234701766	8.1	loss in SPOR
3	RP11-510B7	RP11-12A13	3p12.3	3q11.2	80611850	96250469	15.6	
4	RP11-227F19	RP11-416A5	4p13	4p12	41340065	45778656	4.4	loss in SPOR
6	GS-62-L11	RP11-199A24	6p25.3	6p11.1	135996	58788604	58.7	gain in BRCA2
6	RP11-349P19	RP11-256L9	6q12	6q13	65158448	73217550	8.1	gain in BRCA2
7	RP4-756H11	RP4-635O5	7q11.21	7q11.22	65647490	71274716	5.6	
7	RP4-811H12	GS-3-K23	7q35	7q36.3	146787501	158864424	12.1	
10	RP11-118K6	RP13-355A21	10p15.2	10p12.1	3035538	28141734	25.1	
10	RP11-90J7	RP11-436O19	10q22.3	10q26.13	79687950	124268654	44.6	loss in BRCA2
11	RP11-113A6	RP11-327O2	11p15.5	11p15.4	2261568	10454001	8.2	
11	RP11-569N5	RP11-137O10	11q13.2	11q14.2	68072319	87028461	19.0	
11	RP11-108O10	GS-26-N8	11q23.1	11q25	111095144	134437384	23.3	
13	RP11-125I23	RP11-384G23	13q12.2	13q21.1	26843991	52955532	26.1	loss in BRCA2
13	RP11-95C14	RP11-255P5	13q31.3	13q33.1	91284428	102377477	11.1	gain in BRCA2
14	RP11-468E2	RP11-168D12	14q12	14q21.2	23572376	41500293	17.9	
14	RP11-544I20	RP11-73M18	14q23.2	14q32.33	63342178	103382895	40.0	loss in BRCA2
16	RP11-429K17	RP11-2C24	16p12.3	16p11.2	20151302	30747839	10.6	gain in SPOR
16	RP11-283C7	RP11-370P15	16q12.1	16q21	45627546	62396983	16.8	loss in SPOR
17	RP1-27J12	RP11-121A13	17p12	17p11.2	14285924	20230382	5.9	
17	RP11-260A9	RP11-445F12	17q11.1	17q12	22287134	32371528	10.1	loss in SPOR
17	RP5-1110E20	RP5-971F3	17q21.2	17q21.31	36260511	41939592	5.7	loss in SPOR
22	RP11-80O7	RP1-172B20	22q11.23	22q13.1	22510132	38559115	16.0	
23	GS-839-D20	RP11-576G22	23p22.33	23p11.3	110000	45268722	45.2	
23	RP5-965E19	RP5-1087L19	23q26.2	23q28	131271371	153547872	22.3	

FIGURE 1B

Chromosome	Begin BAC	END BAC	chrom band	chrom band	region start	Region end	size Mb	characteristic
4	RP11-227F19	RP11-416A5	4p13	4p12	41340065	45778656	4.4	loss in SPOR
13	RP11-125I23	RP11-384G23	13q12.2	13q21.1	26843991	52955532	26.1	loss in BRCA2
13	RP11-95C14	RP11-255P5	13q31.3	13q33.1	91284428	102377477	11.1	gain in BRCA2
14	RP11-544I20	RP11-73M18	14q23.2	14q32.33	63342178	103382895	40.0	loss in BRCA2
16	RP11-283C7	RP11-370P15	16q12.1	16q21	45627546	62396983	16.8	loss in SPOR
17	RP11-260A9	RP11-445F12	17q11.1	17q12	22287134	32371528	10.1	loss in SPOR
17	RP5-1110E20	RP5-971F3	17q21.2	17q21.31	36260511	41939592	5.7	loss in SPOR

FIGURE 2

Clone	Centroids 704 BRCA2	GRCh37 Ensembl56	start	end
RP4-575L21	-0.021125	1	10154043	10246367
RP4-669K10	-0.152518	1	28962129	28962785
RP11-114B7	-0.029022	1	33102443	33273241
RP5-1043G4	0.030203	1	55270329	55270865
RP1-97P20	0.202631	1	169821804	169863408
RP11-100E13	-0.035699	1	224697138	224858861
RP11-528D17	-0.039751	1	221148264	221328294
RP11-399B15	-0.004144	1	245156672	245363768
RP11-438H8	-0.160698	1	247948203	248113393
RP11-560C7	-0.048528	2	23146100	23328443
RP11-557N21	-0.212993	2	23817707	23926090
RP11-557N21	-0.212993	2	23817707	23926090
RP11-169L20	-0.14628	2	24850139	25036031
RP11-404P12	-0.14628	2	25844534	26016317
RP11-106G13	-0.008506	2	26968701	27135673
RP11-373D23	-0.008506	2	28560131	28717580
RP11-328L16	-0.008506	2	29408613	29599239
RP11-559D11	-0.115671	2	32631123	32795421
RP11-299C5	-0.044483	2	42481333	42664230
RP11-421J10	-0.470412	2	45401278	45595215
RP11-27C22	-0.064912	2	46041314	46230526
RP11-110G2	-0.173062	2	46252606	46442514
RP11-436K12	-0.049791	2	47840483	47841282
RP5-960D23	-0.117312	2	47921241	48043172
RP11-30C22	-0.033203	2	54896322	55064464
RP11-304A15	-0.107136	2	69007759	69169681
RP11-304A15	-0.107136	2	69007759	69169681
RP11-542D13	-0.126564	2	98108186	98108876
RP11-547F10	-0.126564	2	98646525	98831782
RP11-549H5	-0.124728	2	100589799	100760360
RP11-30G7	-0.124728	2	100751733	100924841
RP11-298E18	-0.198838	2	124953149	125053149
RP11-48K7	-0.03105	2	127373737	127536405
RP11-535B12	-0.000561	2	180238113	180408717
RP11-507C18	0.001235	2	205007999	205205934
RP11-15J24	0.001235	2	205467646	205638019
RP11-325M10	0.001235	2	206347149	206533169
RP11-86O17	0.294376	2	226921290	227031126
RP11-86O17	0.294376	2	226921290	227031126
RP11-211K17	0.112976	2	227332561	227495985
RP11-149O3	0.017067	2	228659456	228832792
RP11-70L16	0.017067	2	229609402	229766511
RP11-419H23	0.040678	2	232079718	232080385

FIGURE 2

RP11-91N19	0.218862	2	233280034	233434643
RP11-534J17	0.012665	2	234273852	234435410
RP11-534J17	0.012665	2	234273852	234435410
RP11-263G22	0.012665	2	234888946	235035976
RP11-263G22	0.058667	2	234888946	235035976
RP11-97C16	0.035866	3	1373065	1541440
RP11-95E11	0.035866	3	3160172	3328965
RP11-10H6	0.035866	3	3182042	3350580
RP11-183N22	0.035866	3	4517889	4518650
RP11-238A9	0.035866	3	4516514	4676956
RP11-25C10	0.024778	3	12446266	12614814
RP11-163D23	0.024778	3	12616855	12759917
RP11-165B2	0.024778	3	13823746	13988636
RP11-27J5	0.015562	3	20662018	20852567
RP11-170K19	0.002219	3	59727302	59899910
RP11-20B7	0.099057	3	73773215	73942284
RP11-510B7	0.076236	3	80530160	80712228
RP11-442C9	0.023143	3	81727298	81728106
RP11-425D6	0.023143	3	81566843	81567635
RP11-425D6	0.023143	3	81566843	81567635
RP11-206J21	0.023143	3	82574172	82715711
RP11-382L10	0.073911	3	83685332	83685896
RP11-474M18	0.456238	3	84541156	84541982
RP11-447J13	0.37679	3	86006748	86184860
RP11-81P15	0.37679	3	86962850	87140031
RP11-81p15	0.37679	3	86962850	87140031
RP11-312H1	0.228484	3	87488189	87646565
RP11-88I7	0.016342	3	94037937	94222721
RP11-12A13	0.016342	3	94611500	94766779
RP11-58D2	0.057191	3	114797967	114946260
RP11-249J17	0.057191	3	115458843	115626868
RP11-165B13	0.037539	3	117760127	117760888
RP11-572O17	0.347197	4	1626433	1627536
RP11-565I3	-0.12499	4	6945137	6962926
RP11-24H13	0.145506	4	34786915	34955520
RP11-227F19	0.110111	4	41646350	41831334
RP11-109E24	0.255604	4	42636077	42813830
RP11-229G4	0.074035	4	43207054	43307054
RP11-55C6	0.063409	4	44835262	44979919
RP11-416A5	0.004672	4	45914433	46084878
RP11-400D2	0.077091	4	135245617	135420334
RP11-127A9	0.077091	4	136568108	136733118
RP11-63M2	0.013785	4	137796004	137955859
RP11-84H6	-0.046445	4	179170140	179349717
RP11-45F23	-0.056703	4	190284043	190430413
1129-C9	-0.127659	5	50	100050
RP11-57I6	-0.106146	5	23644140	23795207
RP11-57I6	-0.106146	5	23644140	23795207
CTD-2219P12	-0.0508	5	27325194	27427057
RP11-37M16	-0.081588	5	28716023	28881237
CTD-2052F19	-0.160737	5	34894786	35006515

FIGURE 2

CTD-2007F2	-0.035823	5	38165822	38269649
RP11-15A14	-0.035823	5	39191269	39385812
CTD-2276O24	0.199417	5	50026715	50131413
RP4-592P18	0.108382	5	51049080	51195687
RP11-506H20	0.038879	5	54527948	54727328
RP11-210O14	0.071366	5	60167981	60168520
RP11-34J15	-0.074893	5	61624859	61796141
RP11-494C5	0.066886	5	152838083	153003938
RP11-292M11	-0.377792	5	169915012	170086458
84-C11	-0.460991	5	180714187	180814187
GS-62-L11	0.216322	6	135996	235996
GS-196-I5	0.216322	6	250000	350000
RP11-13J16	0.216322	6	1297036	1357295
RP11-15N12	0.216322	6	3348078	3525148
RP11-174B19	0.118521	6	5084698	5271233
RP11-232H4	0.208053	6	6175859	6334512
RP11-163I22	0.208053	6	6273515	6436301
RP11-320C15	0.146965	6	6703139	6859877
RP3-336K20	0.024784	6	7483281	7629676
RP11-196B15	0.208588	6	7954163	8132797
RP3-365E2	0.197931	6	14429170	14564812
RP1-232K4	0.197931	6	15325618	15521703
RP1-13D10	0.375748	6	16058670	16209663
RP11-68J15	0.304161	6	17628621	17771850
RP11-408C8	0.304161	6	19023271	19023868
RP4-625H18	0.218811	6	19726252	19909568
RP3-444C7	0.085716	6	20523819	20650057
RP11-204E9	0.011555	6	21405056	21589578
RP11-33I5	0.202226	6	22473443	22627331
RP1-278E11	0.077917	6	39870200	39969544
RP11-162O6	0.077917	6	40753403	40929365
RP11-533O20	0.180169	6	41890906	42063209
RP11-501I18	0.180169	6	42780788	42846928
RP11-227E22	0.026242	6	43858965	44040979
RP11-554O14	0.030765	6	44880799	45055750
RP3-341E18	0.151441	6	52889011	52979483
RP11-362K18	0.279115	6	53985202	54145854
RP11-524H19	0.279115	6	54613786	54801265
RP11-472M19	0.302324	6	56640449	56817727
RP3-422B11	0.109123	6	57185560	57342705
RP11-199A24	0.016344	6	58503276	58679639
RP11-349P19	0.069561	6	65035120	65097390
RP1-40C9	0.008236	6	65783270	65784054
RP3-324B8	0.225932	6	66821093	66963834
RP11-409K15	0.225932	6	68157449	68297367
RP1-46B1	0.079193	6	69383014	69539129
RP11-462G2	0.169344	6	71009571	71198194
RP11-111D8	0.169344	6	72101590	72284545
RP11-256L9	0.178213	6	73038504	73218079
RP3-443C4	-0.221279	6	152053838	152145741
RP11-450E24	-0.250487	6	152084343	152290880

FIGURE 2

RP1-130E4	-0.250487	6	152478376	152478910
RP4-546K19	-0.110507	6	153064308	153174576
GS-57-H24	0.198508	6	170569992	170669992
RP11-41A3	-0.154226	7	35169796	35253695
RP5-1091E12	-0.207312	7	55042213	55042908
RP4-756H11	0.154232	7	66011128	66144029
RP11-458F8	0.27486	7	66298311	66453955
RP4-736H5	0.383203	7	67030260	67176862
RP11-358M3	0.383203	7	67591342	67591923
RP11-156A14	0.383203	7	67909149	68064977
RP11-3P22	0.383203	7	68614075	68779535
RP11-409J21	0.273299	7	71050338	71203941
RP4-635O5	0.273299	7	71507839	71635768
RP11-101N13	0.027188	7	94478109	94641349
RP5-1145A22	0.0244	7	97264652	97386239
RP11-10E6	0.184172	7	109251622	109404921
RP11-10E6	0.262054	7	109251622	109404921
RP11-391H5	-0.06984	7	127601775	127799120
RP4-811H12	-0.045176	7	147318092	147318704
RP11-302C22	-0.064168	7	147454336	147627247
RP11-24N19	-0.277116	7	148595274	148756871
RP4-800G7	-0.277116	7	148832677	148994476
RP11-511P7	-0.11727	7	150020921	150206768
RP4-548K24	-0.11727	7	150539825	150662642
RP4-548D19	-0.11727	7	150861149	150998990
RP11-312C1	-0.215169	7	152459778	152639200
RP11-312C1	-0.362118	7	152459778	152639200
RP11-317C13	-0.154646	7	153589527	153641465
RP11-269M19	-0.003701	7	154634176	154782516
RP5-1015O24	-0.003701	7	155295097	155490615
RP11-69O3	-0.060486	7	155501974	155654553
RP4-764O12	-0.062889	7	157229624	157342427
RP11-452C13	-0.062889	7	157614278	157824565
GS-3-K23	-0.066477	7	158764424	158864424
GS-580-L5	0.025745	8	200000	300000
GS-77-L23	0.025745	8	300000	400000
RP11-338B22	0.025745	8	488653	667324
RP4-593A12	0.025745	8	1542145	1691889
RP11-297N6	0.326732	8	11578760	11702671
RP11-363L24	0.085846	8	31006261	31006261
RP11-301H15	0.085846	8	32411506	32594737
RP11-11N9	0.008963	8	32908355	32909082
RP11-197P20	-0.30227	8	37086323	37251083
RP11-197P20	-0.30227	8	37086323	37251083
RP11-350N15	-0.653063	8	38170901	38368835
RP11-44K6	-0.143054	8	39718456	39865432
RP11-414L17	-0.00916	8	61514660	61687626
RP11-227F6	-0.130359	8	62249646	62409175
RP11-51M18	0.037368	8	84216776	84386516
RP11-419L20	0.134382	8	110368642	110542334
RP11-28I2	-0.665709	8	127495448	127694132

FIGURE 2

RP11-343P9	0.048876	8	136402189	136583198
RP11-172M18	0.048876	8	139239227	139383045
RP11-526P7	0.057715	8	140217248	140373496
RP11-370K2	-0.067661	8	142544371	142720368
GS-261-I1	-0.118929	8	146054826	146154826
GS-489-D14	-0.118929	8	146054876	146154876
RP11-48M17	0.00548	9	2147364	2305360
RP11-509J21	0.097571	9	3544199	3705631
RP11-125K10	0.220999	9	4830733	5000796
RP11-509D8	0.152012	9	4922574	5130406
RP11-23D5	0.004467	9	11181428	11350967
RP11-123J20	0.282633	9	17850221	18022839
RP11-15P13	0.096009	9	20183465	20360121
RP11-149I2	0.141668	9	21862433	22055818
RP11-264J11	0.328498	9	28850269	28851013
RP11-326F20	-0.029936	9	33086353	33247752
RP11-195F19	-0.029936	9	34634468	34820900
RP11-112J3	-0.029936	9	35670117	35865495
RP11-8N6	-0.029936	9	36632473	36774305
RP11-397D12	-0.029936	9	37263640	37455975
RP11-274B18	0.022824	9	71129855	71297835
RP11-71A24	0.440962	9	75619763	75786792
RP11-439A18	-0.158439	9	86102092	86295020
RP11-59M22	-0.050124	9	86991741	87146621
RP11-172F7	-0.078098	9	88058064	88058760
RP11-440G5	-0.178714	9	94131899	94300747
RP11-23J9	0.11168	9	100042032	100205448
RP11-23B15	0.228212	9	100545008	100703779
RP11-205K6	-0.090263	9	129217477	129380045
GS-135-I17	0.086991	9	140158252	140258252
RP11-118K6	-0.209449	10	3035538	3133935
RP11-154P11	-0.060717	10	4333070	4506152
RP11-336A10	-0.174232	10	5638110	5803775
RP11-298K24	-0.174232	10	6238668	6435633
RP4-542G16	-0.047156	10	7157764	7295862
GS1-756B1	-0.146199	10	8180572	8305520
RP1-249K20	-0.167377	10	9060753	9157064
RP11-189M8	-0.171631	10	9995827	10161417
RP11-566K1	-0.19338	10	10791781	10979028
RP1-251M9	-0.19338	10	10915523	11053430
RP11-401F24	-0.161555	10	11766246	11970786
RP11-730A19	-0.274991	10	13019504	13213671
RP11-275E20	-0.185994	10	14282735	14473313
RP11-2K17	-0.183957	10	14913569	15099778
RP11-37P5	-0.251641	10	15999930	16155788
RP11-337F21	-0.251641	10	16967978	17153153
RP11-16O1	-0.251641	10	17719035	17880741
RP11-383B4	-0.330187	10	18803312	18997209
RP11-188P8	-0.330187	10	19986417	20148469
RP11-337N19	-0.330187	10	20522279	20700003
RP11-165O3	-0.330187	10	21281276	21281882

FIGURE 2

RP11-108B14	-0.330187	10	22360413	22534888
RP11-379L21	-0.40624	10	23084792	23278752
RP11-162E8	-0.376429	10	24120160	24300421
RP11-129O7	-0.323034	10	25101996	25265814
RP11-307B23	-0.100013	10	25930725	26091525
RP11-128B16	-0.003461	10	26758309	26900962
RP13-355A21	-0.096552	10	27896302	28100727
RP11-38B21	-0.008561	10	44545781	44703284
RP11-79I23	-0.083928	10	60426379	60599657
RP11-210G22	0.246152	10	67730039	67909272
RP11-90J7	-0.267551	10	80018953	80173990
RP11-574P20	-0.304665	10	80803824	80971399
RP11-40F6	-0.200148	10	82028900	82029577
RP11-20E23	-0.329272	10	82490215	82657699
RP4-684F13	-0.172354	10	83646484	83647175
RP11-95M17	-0.172354	10	84435533	84586780
RP11-219F10	-0.247379	10	85578672	85756585
RP11-470J18	-0.18939	10	86237532	86399151
RP11-113E21	-0.246808	10	87201090	87382234
RP11-396M20	-0.246808	10	88065908	88231673
RP11-165M8	-0.020621	10	89704489	89704995
RP11-380G5	-0.020621	10	89608026	89807449
RP11-162K11	-0.129274	10	95785188	95933132
RP11-248J23	-0.05225	10	97623987	97789364
RP11-196N24	-0.05225	10	98432174	98534683
RP11-19C6	-0.086945	10	99631313	99790502
RP11-704L16	0.284322	10	100952271	101170323
RP11-287G8	-0.071512	10	101712730	101881251
RP11-324L3	-0.071512	10	102906124	103083779
RP11-165P9	-0.030806	10	106759204	106921788
RP11-596L14	-0.030806	10	107694481	107873707
RP11-699H2	-0.030806	10	108411165	108583820
RP11-478K18	-0.030806	10	109301761	109497716
RP11-163F15	-0.030806	10	110246657	110410183
RP11-271I13	-0.030806	10	111508658	111672127
RP11-364E8	-0.182928	10	112361447	112508693
RP11-381K7	-0.182928	10	112974154	113145927
RP11-426E5	-0.182928	10	113871121	114065761
RP11-357H24	-0.106844	10	114885414	115067205
RP11-411P18	-0.170779	10	115844971	116003352
RP11-427L15	-0.188532	10	120370659	120567875
RP11-359E7	-0.233664	10	121501561	121679100
RP11-323P17	-0.267136	10	122477477	122477980
RP11-62L18	-0.204763	10	123225110	123397490
RP11-45D20	-0.174421	10	123344229	123515458
RP11-436O19	-0.174421	10	124094555	124277632
RP11-113A6	-0.160888	11	2306063	2473158
RP11-19N21	-0.132046	11	2790044	2962612
RP11-19N21	-0.132046	11	2790044	2962612
RP11-120E20	-0.245995	11	3618360	3800415
RP11-438N5	-0.316319	11	3898611	4048193

FIGURE 2

RP11-309J20	-0.449527	11	4790316	4990480
RP11-364G22	-0.37097	11	5798145	5969718
RP11-89D4	-0.353816	11	7444669	7445456
RP11-379P15	-0.248686	11	8189128	8410723
RP11-152H18	-0.248686	11	8679389	8838111
RP11-243M7	-0.209085	11	9709739	9710446
RP11-327O2	-0.209085	11	10333863	10496425
RP4-607I7	-0.064034	11	35030956	35182961
RP11-472K20	-0.019876	11	35698571	35767187
RP11-115P8	-0.019876	11	35701895	35853383
RP11-324K6	-0.036946	11	37718145	37881309
RP11-307P14	-0.003385	11	40229424	40421930
RP11-100E23	-0.12214	11	51294869	51450781
RP11-163K24	-0.153594	11	62514295	62515049
RP11-231P15	-0.153594	11	62474381	62646249
RP11-138N3	-0.010573	11	67395614	67616066
RP11-569N5	-0.209221	11	68316743	68521009
RP11-804L21	-0.331709	11	69589482	69628306
RP11-21D20	-0.071687	11	70559975	70712757
RP11-598K3	-0.238339	11	70629079	70629611
RP11-512I24	-0.240256	11	70919961	71111150
RP11-31L22	-0.240256	11	72316067	72504103
RP11-93M11	-0.314981	11	72701572	72895150
RP11-28L18	-0.314981	11	74000318	74169974
RP11-28L18	-0.548878	11	74000318	74169974
RP11-167F22	-0.383148	11	74029417	74188898
RP11-535A19	-0.431652	11	75431286	75594844
RP11-25F7	-0.138308	11	76288376	76451702
RP11-25F7	-0.51236	11	76288376	76451702
RP11-30J7	-0.367048	11	76397807	76553748
RP11-321B9	-0.465223	11	77235154	77335154
RP11-321B9	-0.465223	11	77235204	77335204
RP11-7H7	-0.669823	11	78357714	78528143
RP11-153F6	-0.669823	11	78965063	79113170
RP11-187P2	-0.118564	11	80741408	80892446
RP11-90K17	-0.136405	11	85460902	85663450
RP11-320L11	-0.215729	11	86042427	86042949
RP11-137O10	-0.323576	11	87243850	87390553
RP11-49M9	0.481285	11	100108958	100251249
RP11-45C5	0.058039	11	100641187	100793328
RP11-25I9	-0.003696	11	108722050	108887096
RP11-108O10	-0.127936	11	111590961	111769918
RP11-107P10	-0.264486	11	112293289	112467692
RP11-87N22	-0.264486	11	113066688	113257885
RP11-212D19	-0.20639	11	114236228	114372375
RP11-136I14	-0.1532	11	115319527	115487638
RP11-114K7	-0.1532	11	116089651	116251062
RP11-4N9	-0.021098	11	116700206	116862391
RP11-35P15	-0.021098	11	117518653	117688362
RP11-215D10	-0.041798	11	119858549	119859218
RP11-10N17	-0.084891	11	124983596	125140038

FIGURE 2

RP11-100P11	-0.171307	11	125081210	125255316
RP11-432I22	-0.171307	11	126323274	126500239
RP11-106O22	-0.171307	11	126457851	126653816
RP11-168K9	-0.196147	11	126916438	127061799
RP11-264E20	-0.196147	11	128426391	128584603
RP11-567M21	-0.340183	11	129822295	130008331
RP11-567M21	-0.340183	11	129822295	130008331
RP11-340L13	-0.233068	11	131468786	131642985
RP11-419F8	-0.233068	11	132711687	132885208
RP11-545G16	-0.233068	11	132947448	133120135
RP11-149G17	-0.233068	11	134010524	134199934
RP11-469N6	-0.233068	11	134479818	134650277
GS-26-N8	-0.233068	11	134337384	134437384
GS-8-M16	0.161087	12	50000	150000
GS-124-K20	-0.043453	12	50050	150050
RP11-283I3	-0.043453	12	282273	282962
RP11-283I3	-0.043453	12	282273	282962
GS-496-A11	-0.043453	12	665787	765787
RP11-359b12	-0.167774	12	989387	1213323
RP11-359B12	-0.014577	12	989387	1213323
RP11-21K20	-0.014577	12	1850721	1983928
RP5-1096D14	-0.014577	12	1985931	2204029
RP3-461F17	0.090641	12	7139412	7139702
RP11-239A17	0.028678	12	16852561	17016324
RP11-437F6	0.073297	12	23719467	23892262
RP11-707G18	0.073297	12	25379658	25555543
RP11-318G8	0.073297	12	25503820	25664999
RP11-522D14	0.073297	12	25662693	25871054
RP11-350G2	0.006343	12	26604593	26784417
RP11-290I21	0.038341	12	64567977	64568730
RP11-2K12	-0.080029	12	92681767	92842830
RP1-46F2	-0.046075	12	111316270	111463931
RP1-261P5	-0.023583	12	112617924	112753566
RP11-25E2	-0.061054	12	115454531	115641805
RP11-8A1	-0.061054	12	116211328	116380488
RP11-125I23	-0.327073	13	27946897	28110985
RP11-153M24	-0.153731	13	28516250	28516756
RP11-95G6	-0.052618	13	28927701	29071943
RP11-218E6	-0.4846	13	29940843	30109462
RP11-550P23	-0.267297	13	31017797	31149599
RP11-95N14	-0.371997	13	32170305	32341545
RP11-37E23	-0.41993	13	32806968	32971598
RP11-141M1	-0.41993	13	33893732	34048408
RP11-87G1	-0.395778	13	33945471	34126319
RP11-266E6	-0.396421	13	35098112	35260357
RP11-98D3	-0.351275	13	36012940	36181870
RP11-10M8	-0.268906	13	38319921	38320551
RP11-131P10	-0.266521	13	38900297	39052253
RP11-131F1	-0.266521	13	39553249	39709557
RP11-407E23	-0.31579	13	40599391	40600139
RP11-2P5	-0.359128	13	40367434	40566000

FIGURE 2

RP11-125A7	-0.359128	13	42364233	42364987
RP11-117I13	-0.293188	13	43258420	43402191
RP11-168P13	-0.293188	13	44251302	44406457
RP11-442J21	-0.223623	13	45224309	45402217
RP11-351K3	-0.126528	13	46042385	46225345
RP11-408L13	-0.126528	13	48083033	48083775
RP11-305D15	-0.032156	13	48999120	48999915
RP11-185C18	-0.17702	13	50006928	50169848
RP11-40A8	-0.315746	13	51431527	51572831
RP11-327P2	-0.306071	13	52345110	52345932
RP11-431O22	-0.049524	13	53286623	53457898
RP11-384G23	-0.120568	13	53907332	54074848
RP11-319L6	0.017969	13	91545055	91725016
RP11-95C14	0.133857	13	92487444	92653120
RP11-632L2	0.214622	13	93702796	93882311
RP11-62D23	0.253884	13	94432522	94604151
RP11-74A12	0.253884	13	95708902	95709479
RP11-318K19	0.21319	13	96536179	96699791
RP11-235O20	0.21319	13	97346660	97498461
RP11-383H17	0.261407	13	98471162	98668143
RP11-442I9	0.305449	13	100004819	100196340
RP11-279D17	0.305449	13	100320129	100320731
RP11-118F16	0.175092	13	101516431	101681729
RP11-564N10	0.231489	13	102521693	102708370
RP11-255P5	0.231489	13	103461685	103625759
RP11-562E17	0.097007	13	105475125	105643083
RP11-468E2	0.120775	14	24442949	24678228
RP11-89K22	0.186468	14	25479462	25644546
RP11-330O19	0.186468	14	26469450	26686358
RP11-144C18	0.186468	14	27492538	27674153
RP11-419C10	0.168081	14	30187580	30332351
RP11-159L20	0.168081	14	31089528	31254500
RP11-187E13	0.168081	14	32374441	32531022
RP11-501E21	0.168081	14	33310161	33470003
RP11-114L8	0.252033	14	33766983	33928745
RP11-561B11	0.252033	14	35770456	35951901
RP11-259K15	0.197884	14	36544890	36707936
RP11-356O9	0.022831	14	37941646	38143220
RP11-138H18	0.204578	14	38268488	38417016
RP11-506K19	0.204578	14	39279990	39458242
RP11-34O18	0.088668	14	40300279	40467221
RP11-332O9	0.14966	14	41294945	41492454
RP11-168D12	0.14966	14	42272635	42429509
RP11-333K19	0.08548	14	42928208	43100812
RP11-134J10	0.08548	14	44219870	44382366
RP11-99L13	0.08548	14	44838106	44972901
RP11-27P2	-0.435706	14	48816525	48975173
RP11-58E21	-0.001522	14	50538326	50538992
RP11-544I20	-0.309625	14	64273459	64429832
RP11-430G13	-0.309625	14	65129659	65130424
RP11-66E7	-0.262014	14	65958994	66105007

FIGURE 2

RP11-50H7	-0.337473	14	66586729	66754780
RP11-125H8	-0.404435	14	67628639	67799662
RP11-204K16	-0.463173	14	68753875	68924811
RP11-226F19	-0.463173	14	69341229	69505464
RP11-486O13	-0.419185	14	70525284	70729505
RP11-164G17	-0.222319	14	71488026	71488577
RP4-816G1	-0.222319	14	72108120	72237250
RP11-233L14	-0.372673	14	72224012	72270400
RP3-514A23	-0.562377	14	72974679	73155647
RP3-449M8	-0.640902	14	74862252	75000616
RP11-173A8	-0.607955	14	75228320	75408161
RP11-316E14	-0.607955	14	75518904	75519627
RP11-368K8	-0.607955	14	76141892	76325728
RP11-368K8	-0.607955	14	76141892	76325728
RP11-361H10	-0.392555	14	76584595	76762495
RP11-361H10	-0.328973	14	76584595	76762495
RP11-463C8	-0.328973	14	77583617	77584324
RP11-61F4	-0.328973	14	78259832	78442470
RP11-285P21	-0.392422	14	78389382	78593925
RP11-526N18	-0.258069	14	80271909	80450002
RP11-114N19	-0.161968	14	81429028	81587318
RP11-226P1	-0.161968	14	82122485	82305627
RP11-203D9	-0.007058	14	84784824	84930394
RP11-504I1	-0.007058	14	85099070	85099879
RP11-96H16	-0.031253	14	87007447	87008243
RP11-2E15	-0.209689	14	87765087	87915830
RP11-300J18	-0.209689	14	88420711	88596020
RP11-300J18	-0.322333	14	88420711	88596020
RP11-507K2	-0.100872	14	88933009	89112709
RP11-79J20	-0.306695	14	89750111	89916280
RP11-257P13	-0.200003	14	90840977	91024003
RP11-257P13	-0.321917	14	90840977	91024003
RP11-353N19	-0.321917	14	92321034	92490892
RP11-371E8	-0.371428	14	93495784	93496428
RP11-262P9	-0.506966	14	94877177	94877796
RP11-725G5	-0.6878	14	94732236	94885168
RP11-298I23	-0.632062	14	95833001	95996781
RP11-164H13	-0.56186	14	96130887	96294959
RP11-185P18	-0.401862	14	95364095	95527190
RP11-433J8	-0.401862	14	97248817	97249589
RP11-76E12	-0.322019	14	97996975	98163374
RP11-68I8	-0.338241	14	98766862	98940846
RP11-543C4	-0.482754	14	100012907	100176983
RP11-123M6	-0.370752	14	101227262	101390233
RP11-168L7	-0.412084	14	101787803	101975142
RP11-365N19	-0.412084	14	103251182	103408708
RP11-73M18	-0.129459	14	104148594	104312132
RP11-13O24	-0.043714	15	8632	331718
RP11-152F13	0.060857	15	83070992	83226151
RP11-90B9	0.014425	15	83506249	83551620
RP11-365F16	0.014425	15	84215221	84394713

FIGURE 2

RP11-565O12	0.014425	15	85106013	85106544
RP11-356B18	0.027459	15	88671166	88838784
RP3-443N8	0.027959	15	88547448	88647448
RP1-138O23	0.027959	15	88823992	88923992
RP11-405A15	0.011219	15	91506979	91507589
RP11-90E5	0.00282	15	100571562	100756032
GS-124-5	0.021806	15	99988915	100088915
RP11-167B4	-0.049414	16	6226968	6398846
RP11-490O6	-0.032808	16	11888024	11888819
RP11-489O1	-0.091205	16	15505454	15664993
CTD-2504F3	-0.091205	16	16077437	16283248
RP11-378B23	-0.085028	16	16808566	16908566
RP11-429K17	-0.183049	16	20151302	20315827
RP11-489A11	-0.152316	16	21397775	21564932
RP11-101E7	-0.115789	16	22029146	22029952
RP11-105C19	-0.17161	16	22597131	22771241
RP11-105C19	-0.17161	16	22597131	22771241
RP11-548B6	-0.147802	16	23672609	23847849
CTD-2515A14	-0.147802	16	24731063	24947950
RP11-142A12	-0.170707	16	26688287	26819858
RP11-142A12	-0.009298	16	26688287	26819858
F21283	-0.137249	16	28604078	28704078
RP11-74E23	-0.107586	16	29644284	29822033
RP11-2C24	-0.107586	16	30633463	30839321
RP11-388M20	-0.018326	16	31048088	31282150
RP11-5L1	0.091194	16	46510743	46672574
RP11-283C7	0.117577	16	46946360	47129544
RP11-283C7	0.117577	16	46946360	47129544
RP11-523L20	0.127342	16	47889993	48046856
RP11-452G23	0.619446	16	48682426	48839286
RP11-452G23	0.558504	16	48682426	48839286
RP11-147B17	0.363561	16	50525390	50694156
RP11-424K7	0.610472	16	51061729	51235705
RP11-122K22	0.610472	16	51873878	51874527
RP11-467J12	0.610472	16	52909551	53111596
RP11-357N13	0.148594	16	53871620	54067953
RP11-357N13	0.258625	16	53871620	54067953
RP11-7O2	0.339914	16	55267280	55417386
RP11-165M2	0.076808	16	55869021	56001451
RP11-325K4	0.248781	16	56813097	57000794
RP11-405F3	0.103068	16	57622904	57772978
RP11-481J2	0.024436	16	58395492	58581463
RP11-481J2	0.024436	16	58395492	58581463
RP11-457D20	0.171614	16	60220086	60434930
RP11-157H19	0.07654	16	61801773	61802422
RP11-467O15	0.152921	16	62541767	62728484
RP11-148F12	0.155872	16	63387399	63522174
RP11-370P15	0.151156	16	63838775	63839447
RP11-298C15	0.028886	16	65378281	65583547
RP11-298C15	0.028886	16	65378281	65583547
RP11-311C24	-0.108689	16	69607426	69608066

FIGURE 2

RP11-296I10	-0.414787	16	70133673	70312186
RP11-296I10	-0.305336	16	70133673	70312186
RP11-556H2	-0.009703	16	79043072	79223295
RP1-27J12	0.017801	17	14423818	14424490
RP11-64B12	0.018149	17	14593233	14657803
RP11-385D13	0.087973	17	15381603	15568444
RP11-219A15	0.196775	17	16587021	16777061
RP11-524F11	0.198667	17	17398429	17579737
RP11-189D22	0.151592	17	18000668	18172954
CTB-1187M2	0.024885	17	19272101	19316085
RP11-78O7	0.246235	17	19674485	19807021
RP5-836L9	0.042467	17	20073751	20192547
RP11-121A13	0.068523	17	20172437	20288709
RP11-64J19	-0.001939	17	21055083	21191541
RP11-260A9	0.304594	17	25440972	25441520
RP11-229K15	0.368536	17	29271460	29427884
RP11-142O6	0.285307	17	29550205	29550969
RP11-474K4	0.146122	17	30393209	30570951
RP11-215E13	0.133727	17	32489785	32642786
RP5-837J1	0.034409	17	33317034	33416269
RP11-47L3	0.490027	17	33500468	33660812
RP11-445F12	0.4177	17	35116399	35296332
RP11-94L15	-0.137472	17	37812853	37972567
RP5-1110E20	0.528741	17	38984203	39119156
RP11-156E6	0.572504	17	39940099	40116322
RP11-506G7	0.572504	17	40884763	41073084
RP11-376M2	0.572504	17	40968824	41171464
RP11-948G15	0.572504	17	41171833	41172398
RP11-392O1	0.572504	17	41572478	41735005
RP5-905N1	0.156688	17	41826877	41827638
RP5-1169K15	0.107933	17	43339849	43430323
RP5-843B9	0.107933	17	44084882	44094836
RP5-971F3	0.210633	17	41818827	41939592
RP11-506D12	-0.045637	17	48817562	49024474
RP11-217N19	-0.019738	17	52399470	52566958
RP11-283E7	-0.012275	17	66771269	66941683
RP11-166M16	0.024014	17	69658425	69814539
RP11-478P5	0.034421	17	72268412	72462136
RP11-141D15	-0.009074	17	76125635	76301246
GS-52-M11	-0.251919	18	0	100000
GS-74-G18	-0.251919	18	162949	262949
RP11-324G2	-0.251919	18	179428	349445
RP11-267C19	-0.039286	18	967589	968333
RP11-308J14	-0.094755	18	8806684	8982445
RP11-99M10	-0.043828	18	10447604	10596116
RP11-164M8	0.114787	18	39052514	39227568
RP11-748M14	0.037744	18	45336521	45490381
CTD-3149D2	-0.06166	19	17701434	17925502
RP11-943H6	-0.229612	19	17702637	17802637
RP11-165F1	0.111524	19	22342884	22496031
CTD-2579N5	0.201129	19	22984141	23157068

FIGURE 2

RP11-359H18	0.203608	19	23764233	23939462
RP11-521I20	-0.009738	19	49834840	50025458
CTD-2545M3	0.27721	19	50897959	51060255
GS-82-O2	0.195473	20	130000	230000
RP5-852M4	-0.036076	20	328014	475783
RP11-314N13	-0.036076	20	1181792	1371966
RP5-1140M3	-0.285629	20	8209614	8379068
RP4-811H13	-0.285629	20	9250717	9391955
RP11-204H22	-0.071623	20	10451757	10633167
RP4-742J24	-0.071623	20	11865380	12030405
RP11-234K24	-0.062688	20	34706346	34882247
RP3-470L14	-0.140544	20	47636060	47809221
RP4-715N11	0.010711	20	51284969	51394314
RP11-304D2	0.154417	21	19303137	19461093
RP11-509A1	0.197586	21	22203093	22354855
RP1-50A12	0.197586	21	22316309	22488461
RP11-258A5	0.039774	21	23231053	23410836
RP11-15H23	0.039774	21	25587202	25587712
RP1-255P7	0.039732	21	36361439	36609975
RP11-155F20	-0.148898	22	19310306	19458514
RP11-80O7	-0.201505	22	24181132	24458754
LL22NC03-95F10	-0.179077	22	25057726	25058244
CTA-221G9	-0.179077	22	23879817	23982359
CTA-125H2	-0.179077	22	24560701	24734213
CTA-445C9	-0.179077	22	25228499	25359896
RP3-353E16	-0.218972	22	28168322	28391737
RP11-329J7	-0.15257	22	28774294	28986685
CTA-57G9	-0.15257	22	27842535	27956406
RP4-539M6	-0.191813	22	30788544	30978289
RP3-515N1	-0.359607	22	31568783	31701831
RP1-127L4	-0.233942	22	30835421	30883232
XXbac-677f7	-0.233942	22	33169418	33296100
CTA-415G2	-0.142924	22	31695938	31825894
LL22NC01-132D12	-0.004841	22	35478908	35505203
CTA-390B3	-0.058909	22	36120349	36212755
CTA-228A9	-0.150749	22	38482376	38575714
CTA-150C2	-0.150749	22	39281250	39480329
LL22NC03-10C3	-0.288987	22	37938484	37976499
RP1-172B20	-0.161693	22	40016508	40228143
RP3-388M5	-0.019282	22	42473525	42651092
GS-839-D20	0.428842	23	110000	210000
GS-98-C4	0.643112	23	440000	540000
RP11-418N20	0.112672	23	2930526	3111894
RP11-23N11	0.062576	23	3707732	3754886
RP11-483M24	0.210165	23	6948788	7125832
RP11-323F16	0.076683	23	7446566	7622822
RP1-108M6	0.015246	23	9601995	9723171
RP6-1O2	0.015246	23	10308319	10476613
RP11-1J4	0.273793	23	12540561	12632714
RP11-1J4	0.171176	23	12540561	12632714
RP1-164K3	0.13605	23	13043422	13147981

FIGURE 2

RP1-122K4	0.13605	23	13906016	14104117
RP1-93D11	0.13605	23	14852690	15010369
RP11-431J24	0.042102	23	16119734	16331718
RP5-1129A6	0.09684	23	18172945	18317643
RP11-558P14	0.09684	23	18365515	18542396
RP11-421K1	0.09684	23	19245771	19246392
RP11-406A18	0.154731	23	21539344	21690173
RP11-261M11	0.192769	23	24268031	24430811
RP11-272N24	0.192769	23	25337706	25338461
RP11-26L4	0.216937	23	26894928	27060256
RP11-489K4	0.051206	23	28120950	28276180
RP11-37E19	0.128159	23	29018586	29019209
RP6-27C10	0.128159	23	29102825	29232829
RP4-662N3	0.201728	23	29593002	29593694
RP11-122N14	0.201728	23	31518157	31679330
RP4-639D23	0.041451	23	32233440	32366887
RP5-1147O16	0.041451	23	32438265	32568505
RP11-12J5	0.085112	23	37669959	37830488
RP11-12J5	0.085112	23	37669959	37830488
RP1-169I5	0.130957	23	41107931	41241498
RP5-879N19	0.086982	23	43468696	43563277
RP11-576G22	0.102115	23	45256642	45434291
RP11-253L3	-0.020798	23	73471329	73638506
RP3-380C13	0.03489	23	94692551	94829928
RP11-274M8	0.054965	23	96464293	96621678
RP5-965E19	0.129764	23	131357146	131514495
RP6-198C21	0.129764	23	132395417	132536303
RP11-481F23	0.440201	23	134603533	134803789
RP1-196E23	0.287047	23	135361435	135484339
RP5-833B2	0.245947	23	137995743	138080362
RP13-34G21	0.245947	23	138760670	138948618
RP4-595A18	0.245947	23	139603351	139745257
RP11-518F7	0.27191	23	140385198	140548576
RP4-552K20	0.450723	23	140891246	140891811
RP3-324C6	0.182749	23	141533809	141648115
GS1-91O18	0.120938	23	142294977	142411207
RP1-29A6	0.282421	23	143614272	143617166
RP11-390O24	0.33793	23	144177088	144353695
RP11-387H19	0.297334	23	145042542	145222156
RP1-73A14	0.229363	23	145778695	145884842
RP3-433G13	0.320371	23	146664917	146721650
RP6-224C24	0.320371	23	146697323	146873742
RP5-892C22	0.043261	23	150436146	150546295
RP11-54I20	0.145657	23	152707082	152884336
RP11-54I20	0.259044	23	152707082	152884336
RP5-1087L19	0.151835	23	153752486	153893678
RP11-296N8	0.072446	23	154131702	154308447
RP11-218L14	0.009194	23	154682277	154828136

Figure 3

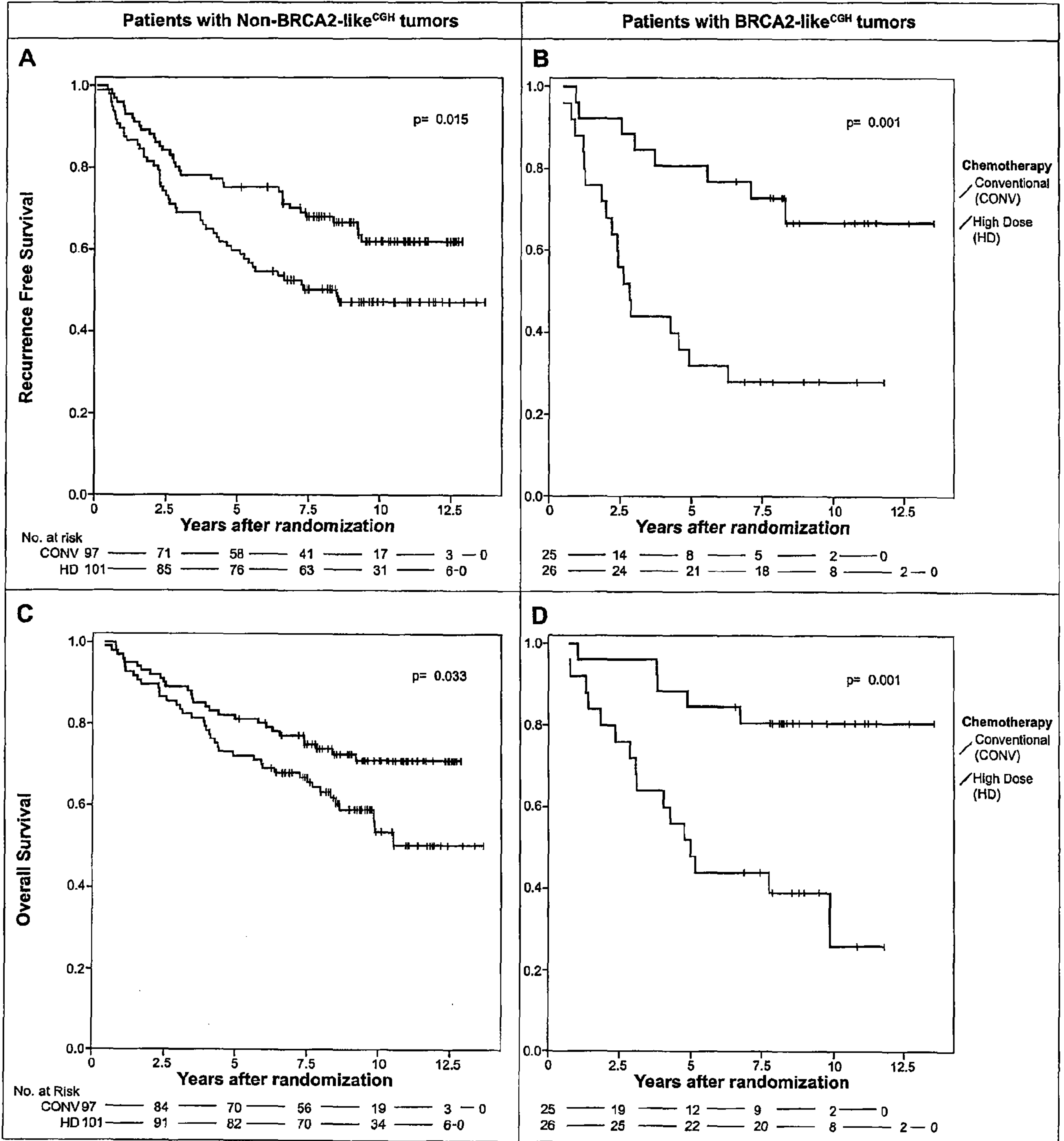

Figure 4. Patient characteristics distributed by treatment arm and BRCA2-classification of the stage-III series

Variable	Patients with Non-BRCA2-like^{CGH} tumours							Patients with BRCA2-like^{CGH} tumours							p values†
	Conventional Chemotherapy		High-Dose Chemotherapy		Total		p values*	Conventional Chemotherapy		High-Dose Chemotherapy		Total		p values*	
	n	%	n	%	n	%		n	%	n	%	n	%		
Total	97	49.0	101	51.0	198	100.0		25	49.0	26	51.0	51	100.0		1.000
Age in categories															
≤ 40 years	20	20.6	29	28.7	49	24.7	0.193	12	48.0	8	30.8	20	39.2	0.258	0.053
> 40 years	77	79.4	72	71.3	149	75.3		13	52.0	18	69.2	31	60.8		
Type of surgery															
Breast conserving therapy	20	20.6	23	22.8	43	21.7	0.733	5	20.0	3	11.5	8	15.7	0.465	0.437
Mastectomy	77	79.4	78	77.2	155	78.3		20	80.0	23	88.5	43	84.3		
Tumour classification															
T1	17	17.5	22	21.8	39	19.7		5	20.0	3	11.5	8	15.7		
T2	66	68.0	65	64.4	131	66.2	0.537#	14	56.0	18	69.2	32	62.7	1.000#	0.281#
T3	14	14.4	13	12.9	27	13.6		6	24.0	4	15.4	10	19.6		
Unknown	0	0.0	1	1.0	1	0.5		0	0.0	1	3.8	1	2.0		
No. of positive lymph nodes															
4-7	45	46.4	57	56.4	102	51.5		13	52.0	12	46.2	25	49.0		
8-10	27	27.8	15	14.9	42	21.2	0.563#	8	32.0	8	30.8	16	31.4	0.595#	0.710#
≥ 11	25	25.8	29	28.7	54	27.3		4	16.0	6	23.1	10	19.6		
Histologic grade															
I	21	21.6	30	29.7	51	25.8		2	8.0	2	7.7	4	7.8		
II	39	40.2	34	33.7	73	36.9	0.363	9	36.0	11	42.3	20	39.2	0.826	0.009
III	35	36.1	32	31.7	67	33.8		14	56.0	11	42.3	25	49.0		
Not determined	2	2.1	5	5.0	7	3.5		0	0.0	2	7.7	2	3.9		
Estrogen receptor status															
Negative (<10%)	24	24.7	25	24.8	49	24.7	1.000	9	36.0	7	26.9	16	31.4	0.555	0.372
Positive (≥10%)	73	75.3	76	75.2	149	75.3		16	64.0	19	73.1	35	68.6		
Progesterone receptor status															
Negative (<10%)	38	39.2	38	37.6	76	38.4	0.884	14	56.0	11	42.3	25	49.0	0.406	0.203
Positive (≥10%)	58	59.8	62	61.4	120	60.6		11	44.0	15	57.7	26	51.0		
Unknown	1	1.0	1	1.0	2	1.0		0	0.0	0	0.0	0	0.0		
P53 status															
Negative (<10%)	47	48.5	62	61.4	109	55.1	0.078	13	52.0	20	76.9	33	64.7	0.103	0.093
Positive (≥10%)	46	47.4	35	34.7	81	40.9		9	36.0	4	15.4	13	25.5		
Unknown	4	4.1	4	4.0	8	4.0		3	12.0	2	7.7	5	9.8		

p values: patients with unknown values were omitted and *P* values were calculated using the Fisher's exact test, except for #Chi square test for trend.; * Association within subgroup; † Association between subgroups.

INTERNATIONAL SEARCH REPORT

International application No
PCT/IB2010/002870

A. CLASSIFICATION OF SUBJECT MATTER
INV. C12Q1/68
ADD.

According to International Patent Classification (IPC) or to both national classification and IPC

B. FIELDS SEARCHED

Minimum documentation searched (classification system followed by classification symbols)
C12Q

Documentation searched other than minimum documentation to the extent that such documents are included in the fields searched

Electronic data base consulted during the international search (name of data base and, where practical, search terms used)

EPO-Internal, BIOSIS, Sequence Search, EMBASE, WPI Data

C. DOCUMENTS CONSIDERED TO BE RELEVANT

Category*	Citation of document, with indication, where appropriate, of the relevant passages	Relevant to claim No.
X	WO 2008/082643 A2 (ABBOTT LAB [US]; MURRAY WILLIAM E [US]) 10 July 2008 (2008-07-10) the whole document	1-25
X	YASUI K ET AL: "Alteration in copy numbers of genes as a mechanism for acquired drug resistance", CANCER RESEARCH, AMERICAN ASSOCIATION FOR CANCER REREARCH, US, vol. 64, 15 February 2004 (2004-02-15), pages 1403-1410, XP002980904, ISSN: 0008-5472, DOI: DOI:10.1158/0008-5472.CAN-3263-2 the whole document	1

-/--

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

[X] See patent family annex.

* Special categories of cited documents :

"A" document defining the general state of the art which is not considered to be of particular relevance

"E" earlier document but published on or after the international filing date

"L" document which may throw doubts on priority claim(s) or which is cited to establish the publication date of another citation or other special reason (as specified)

"O" document referring to an oral disclosure, use, exhibition or other means

"P" document published prior to the international filing date but later than the priority date claimed

"T" later document published after the international filing date or priority date and not in conflict with the application but cited to understand the principle or theory underlying the invention

"X" document of particular relevance; the claimed invention cannot be considered novel or cannot be considered to involve an inventive step when the document is taken alone

"Y" document of particular relevance; the claimed invention cannot be considered to involve an inventive step when the document is combined with one or more other such documents, such combination being obvious to a person skilled in the art.

"&" document member of the same patent family

Date of the actual completion of the international search	Date of mailing of the international search report
28 February 2011	11/03/2011

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

1

C(Continuation). DOCUMENTS CONSIDERED TO BE RELEVANT

Category*	Citation of document, with indication, where appropriate, of the relevant passages	Relevant to claim No.
A	JAIN AJAY N ET AL: "Quantitative analysis of chromosomal CGH in human breast tumors associates copy number abnormalities with p53 status and patient survival", PROCEEDINGS OF THE NATIONAL ACADEMY OF SCIENCES OF THE UNITED STATES OF AMERICA,, vol. 98, no. 14, 3 July 2001 (2001-07-03), pages 7952-7957, XP002624741, the whole document -----	1-25
A	SIMON A JOOSSE ET AL: "Prediction of BRCA1-association in hereditary non-BRCA1/2 breast carcinomas with array-CGH", BREAST CANCER RESEARCH AND TREATMENT, KLUWER ACADEMIC PUBLISHERS, BO, vol. 116, no. 3, 14 August 2008 (2008-08-14), pages 479-489, XP019727887, ISSN: 1573-7217 the whole document -----	1-25
A	OLDENBURG ROGIER A ET AL: "Genome-wide linkage scan in dutch hereditary non-BRCA 1/2 breast cancer families identifies 9q21-22 as a putative breast cancer susceptibility locus", GENES CHROMOSOMES & CANCER, vol. 47, no. 11, November 2008 (2008-11), pages 947-956, XP002625152, ISSN: 1045-2257 the whole document -----	1-25
A	STAFF SYNNOVE ET AL: "Multiple copies of mutant BRCA1 and BRCA2 alleles in breast tumors from germ-line mutation carriers", GENES CHROMOSOMES AND CANCER, vol. 28, no. 4, August 2000 (2000-08), pages 432-442, XP002625153, ISSN: 1045-2257 see whole doc. esp. Tables 2 and 4 -----	1-25

1

INTERNATIONAL SEARCH REPORT

International application No.
PCT/IB2010/002870

Box No. II Observations where certain claims were found unsearchable (Continuation of item 2 of first sheet)

This international search report has not been established in respect of certain claims under Article 17(2)(a) for the following reasons:

1. ☐ Claims Nos.:
because they relate to subject matter not required to be searched by this Authority, namely:

2. ☒ Claims Nos.: 26-34
because they relate to parts of the international application that do not comply with the prescribed requirements to such an extent that no meaningful international search can be carried out, specifically:
see FURTHER INFORMATION sheet PCT/ISA/210

3. ☐ Claims Nos.:
because they are dependent claims and are not drafted in accordance with the second and third sentences of Rule 6.4(a).

Box No. III Observations where unity of invention is lacking (Continuation of item 3 of first sheet)

This International Searching Authority found multiple inventions in this international application, as follows:

1. ☐ As all required additional search fees were timely paid by the applicant, this international search report covers all searchable claims.

2. ☐ As all searchable claims could be searched without effort justifying an additional fees, this Authority did not invite payment of additional fees.

3. ☐ As only some of the required additional search fees were timely paid by the applicant, this international search report covers only those claims for which fees were paid, specifically claims Nos.:

4. ☐ No required additional search fees were timely paid by the applicant. Consequently, this international search report is restricted to the invention first mentioned in the claims; it is covered by claims Nos.:

Remark on Protest

☐ The additional search fees were accompanied by the applicant's protest and, where applicable, the payment of a protest fee.

☐ The additional search fees were accompanied by the applicant's protest but the applicable protest fee was not paid within the time limit specified in the invitation.

☐ No protest accompanied the payment of additional search fees.

FURTHER INFORMATION CONTINUED FROM PCT/ISA/ 210

Continuation of Box II.2

Claims Nos.: 26-34

Present claim 26 relates to a product defined (inter alia) by reference to the following unusual parameter: namely to probes which hybridize to DNA from at least one genomic locus selected from 2p24.1... The use of this unusual parameter in the present context is considered to lead to a lack of clarity because the claim does not clearly identify the products encompassed by it as the parameter cannot be clearly and reliably determined by indications in the description or by objective procedures which are usual in the art. This makes it impossible to compare the claim/s to the prior art. As a result, the application does not comply with the requirement of clarity under Article 6 PCT. The lack of clarity is to such an extent, that no search was performed taking into consideration the non-compliance in determining the extent of the search of claim 26 The same applies to thereon dependent claims 27-34. The reference made to Fig.2 in claims 33 and 34 also lacks clarity because the therein identified BAC clone are not defined by structural feature nor does the description provides support for the structural features of the BAC clone designations (Article 5 PCT).

The applicant's attention is drawn to the fact that claims relating to inventions in respect of which no international search report has been established need not be the subject of an international preliminary examination (Rule 66.1(e) PCT). The applicant is advised that the EPO policy when acting as an International Preliminary Examining Authority is normally not to carry out a preliminary examination on matter which has not been searched. This is the case irrespective of whether or not the claims are amended following receipt of the search report or during any Chapter II procedure. If the application proceeds into the regional phase before the EPO, the applicant is reminded that a search may be carried out during examination before the EPO (see EPO Guideline C-VI, 8.2), should the problems which led to the Article 17(2) declaration be overcome.

INTERNATIONAL SEARCH REPORT

Information on patent family members

International application No

PCT/IB2010/002870

Patent document cited in search report	Publication date	Patent family member(s)	Publication date
WO 2008082643 A2	10-07-2008	WO 2008082673 A2	10-07-2008

Form PCT/ISA/210 (patent family annex) (April 2005)