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PRECLINICAL STUDY

# Cross-platform pathway-based analysis identifies markers of response to the PARP inhibitor olaparib

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**Abstract** Poly(ADP-ribose) polymerase (PARP) is an enzyme involved in DNA repair. PARP inhibitors can act as chemosensitizers, or operate on the principle of synthetic lethality when used as single agent. Clinical trials have shown drugs in this class to be promising for BRCA mutation carriers. We postulated that inability to demonstrate response in non-BRCA carriers in which BRCA is inactivated by other mechanisms or with deficiency in homologous recombination for DNA repair is due to lack of molecular markers that define a responding subpopulation. We identified candidate markers for this purpose for olaparib (AstraZeneca) by measuring inhibitory effects of nine concentrations of olaparib in 22 breast cancer cell lines and identifying features in transcriptional and genome copy number profiles that were significantly correlated with response. We emphasized in this discovery process genes involved in DNA repair. We found that the cell lines that were sensitive to olaparib had a significant lower copy number of BRCA1 compared to the resistant cell lines ($p$ value 0.012). In addition, we discovered seven genes from DNA repair pathways whose transcriptional levels were associated with response. These included five genes (*BRCA1*, *MRE11A*, *NBS1*, *TDG*, and *XPA*) whose transcript levels were associated with resistance and two genes (*CHEK2* and *MK2*) whose transcript levels were associated with sensitivity. We developed an algorithm to predict response using the seven-gene transcription levels and applied it to 1,846 invasive breast cancer samples from 8 U133A/plus 2 (Affymetrix) data sets and found that 8–21 % of patients would be predicted to be responsive to olaparib. A similar response frequency was predicted in 536 samples analyzed on an Agilent platform. Importantly, tumors predicted to respond were enriched in basal subtype tumors. Our studies support clinical evaluation of the utility of our seven-gene signature as a predictor of response to olaparib.

**Keywords** PARP · Olaparib · Breast cancer · Biomarker · Bioinformatics

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## Introduction

Several mechanisms have been identified in mammalian cells that function to maintain genome integrity [1, 2]. Mechanisms for the repair of single strand breaks (SSB) include base excision repair (BER), mismatch repair

(MMR), and nucleotide excision repair (NER). DNA damage due to double strand breaks (DSB) is repaired via the homologous recombination (HR) pathway or with non-homologous end joining (NHEJ). The Fanconi anemia (FA)/BRCA pathway is involved in repair of DSBs or DNA interstrand cross links, and the DNA damage response (DDR) pathway is a network of DDRs for the regulation of many steps in the DNA repair process [3].

These repair pathways are frequently deregulated in cancer cells, motivating efforts to develop drugs that are preferentially effective in cells with defective repair. One emerging class of therapeutic agents for this purpose is based on the principle of synthetic lethality. Synthetic lethality is defined as cell death that results from complete inactivation of two genes in cells where inactivation of either alone does not result in death [4, 5]. The first therapy to reach the clinic was based on the concept that synthetic lethality would result from coordinate inactivation of HR repair (due to a genomic aberration) and poly(ADP-ribose) polymerase (PARP). This approach was based on studies showing that cells with HR repair deficiency caused by *BRCA1/2* mutation [6, 7], *PTEN* mutation [8, 9], *RAD51D* loss of function [10], and *PALB2* loss of function [11] exhibited synthetic lethality with an inhibitor of one or more PARP family proteins, differentiating cancerous from non-cancerous cells by only targeting cells with complete loss of the former genes. The PARP family consists of 18 PARP domain enzymes [12], with the most well-studied members being *PARP1*, *PARP2*, *VPARP* (*PARP4*), tankyrase 1 (*TNKS*, *PARP5a*), and *TNKS2* (*PARP5b*). *PARP1* is the most ubiquitous member. This protein rapidly binds to sites of damaged DNA to modulate a variety of proteins involved in DNA repair and other cellular processes, making it a key protein in the BER pathway for SSB repair.

PARP is involved in SSB DNA repair and PARP inhibitors cause some of them to be converted into DSBs at replication forks [13, 14]. In HR competent cells, DSBs are repaired so that the cells can survive. However, in HR-deficient cells, DSBs are repaired via the less accurate NHEJ pathway or the single strand annealing subpathway of HR, resulting in chromatid aberrations that usually lead to cell death. These conditions therefore make cells with BRCA mutations or other HR defects [11, 15, 16] preferentially sensitive to (i.e., to show synthetic lethality with) PARP inhibitors. PARP inhibitors also have been proposed as possibly useful for treatment of triple negative breast cancers that exhibit "BRCAness" [7, 17]. BRCAness is defined as the spectrum of phenotypes that some sporadic tumors share with *BRCA* mutated cancers, reflecting the underlying distinctive DNA repair defects arising from loss of HR; for example, by epigenomic down regulation of *BRCA1* and *FANCF* [17].

PARP inhibitors in clinical studies for BRCA-associated, triple negative and/or basal-like breast cancer include olaparib (AstraZeneca, London), ABT-888 (also known as Veliparib; Abbott Laboratories, IL), and PF-01367338 (AG014699; Pfizer Inc., NY) [14, 18, 19]. These agents are licensed for monotherapy in DNA repair deficient patients or as chemo-potentiating agents after SSBs are created by common anticancer treatments such as radiotherapy and DNA damaging agents [19]. Results obtained from clinical trials so far, however, appear to vary depending on the specific breast cancer patient population, the specificity of the PARP inhibitor, and the nature of the therapeutic agent used in combination with the PARP inhibitor [20, 21]. A multicenter phase 2 trial showed that olaparib as monotherapy led to objective response rates in 41 % of BRCA1/2 mutation carriers who had previously received several courses of chemotherapy [22]. Results for triple negative breast cancer patients without known *BRCA1/2* mutations have been inconsistent. Preclinical studies and phase 1 trials suggested that PARP inhibitors can increase cell death in these patients when combined with paclitaxel [23], whilst triple negative breast cancer patients largely did not respond to olaparib monotherapy in a phase 2 trial [24]. Thus, our aim in this study was to identify candidate biomarkers that can be tested for their ability to better identify subsets of sporadic cancers with defects in HR-directed repair that will respond to PARP inhibitors.

We focused in this study on olaparib, a small-molecule, reversible, oral inhibitor of both *PARP1* and *PARP2* [25]. We identified candidate biomarkers associated with response to olaparib by correlating responses to nine concentrations of olaparib in a panel of well-characterized breast cancer cell lines with the transcription levels of genes involved in aspects of DNA repair. Genes tested for correlation with olaparib response included those reported in the literature to be directly relevant to PARP inhibitor response or involved more generally in some aspect of DNA repair (Fig. 1). We applied this signature to primary tumor data to identify the frequency and characteristics of tumors that might be expected to respond to olaparib. These studies set the stage for a clinical test of the sensitivity and specificity of this predictor and indicate known subtypes of breast cancers that might be preferentially sensitive to olaparib.

## Materials and methods

### Breast cancer cell lines, assay, and molecular data

The sensitivity of a panel of 22 breast cancer cell lines to KU0058948 (olaparib; KuDOS Pharmaceuticals/AstraZeneca) was measured with a growth inhibition assay as

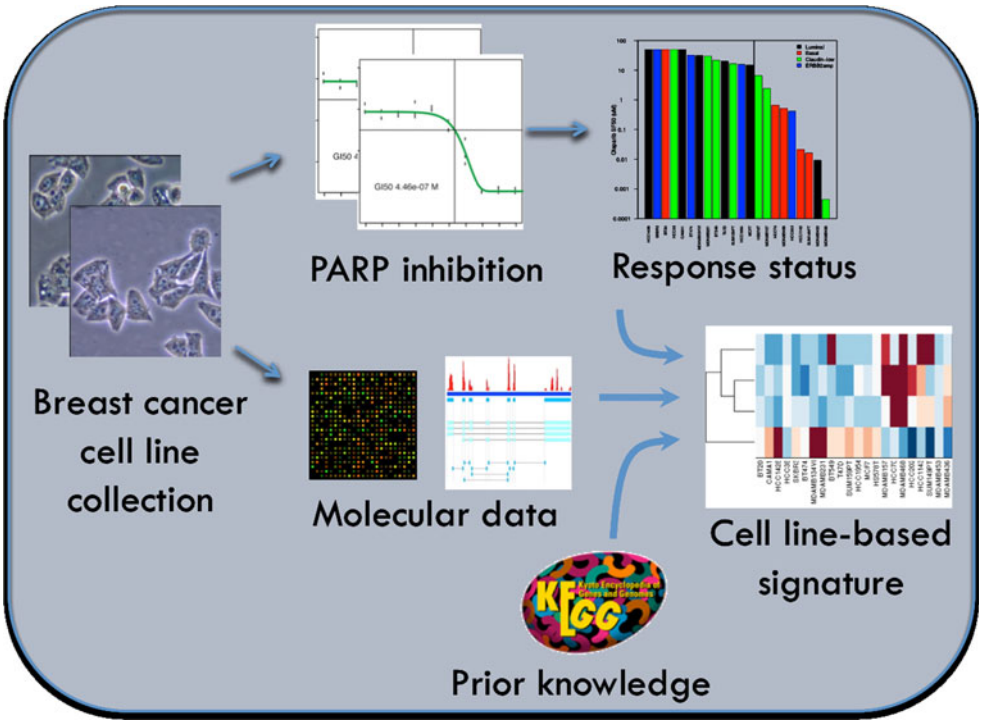



**Fig. 1** Approach for the development of a predictor of olaparib response in a breast cancer cell line panel with inclusion of prior knowledge of DNA repair pathways. For 22 breast cancer cell lines, growth inhibition assays were used to measure their sensitivity to olaparib, expressed as the surviving fraction at 50 % (SF50). For these cell lines, expression data were obtained with three different platforms (Affymetrix U133A, Affymetrix Exon 1.0 ST, and whole transcriptome shotgun sequencing). The bottom-up approach was used for biomarker selection, incorporating prior knowledge of the principal DNA repair pathways BER, NER, MMR, HR/FA, NHEJ, and DDR. Biomarkers from [31] were systematically expanded with genes assigned to any of these pathways in the KEGG database, resulting in 118 genes. For each DNA repair pathway and expression data set the most important markers were obtained with LR in combination with forward feature selection, followed by reduction to those selected with consistent pattern of sensitivity for all three platforms

described in Supplementary Material and [7, 26]. The following molecular data were collected for the panel: copy number (Affymetrix SNP6), gene expression (Affymetrix U133A, Affymetrix Exon 1.0 ST), transcriptome sequencing (Illumina GAII), methylation (Illumina Methylation27), protein abundance (reverse protein lysate array), and mutation status (COSMIC, [27]). A detailed description of the availability and preprocessing of all molecular data sets is provided in Supplementary Material and [28].

### Statistical analyses

The Wilcoxon rank sum test was used to test the association of drug response with individual biomarkers. Drug response was associated with subtype, triple negativity, and mutation status with use of the Fisher's exact test. Due to the small sample size, a $p < 0.05$ was deemed significant, whilst a $p < 0.1$ was considered a trend. Logistic regression (LR) with forward feature selection (fivefold CV) was used to identify candidate biomarkers and was applied to each considered DNA repair pathway separately. The resulting biomarkers were combined into a predictor using a weighted voting algorithm [29]. The Matlab code used for signature development and validation is provided in Supplementary Material. A Chi-square test was used to test for associations of breast cancer subtype with response to olaparib. We refer to Supplementary Material for a detailed description of the statistical methods.

## Results

### Olaparib response in a panel of 22 breast cancer cell lines

Twenty-two breast cancer cell lines previously profiled for RNA transcript levels were tested for response to nine concentrations of olaparib (see Table 1). These cells mirror many of the transcriptional and genomic characteristics of primary breast tumors and have been used to model responses to a large number of experimental and approved therapeutic compounds [28, 30]. The concentration of olaparib needed to reduce survival to 50 % (SF50) was

**Table 1** Overview of the breast cancer cell line panel with response to olaparib expressed as SF50 (μM); *ER*, *PR*, and *ERBB2* expression with + indicating up-regulation relative to the other cell lines, − down-regulation, and NC no change in expression; and availability of the different molecular data sets indicated with N for unavailability and Y for availability

| Cell line | Olaparib SF50 (μM) | Doubling time (h) | *ER*[a] | *PR*[a] | *ERBB2*[a] | COSMIC | SNP6 | RPPA | Methylation | RNA-seq | Exon array | U133A |
|---|---|---|---|---|---|---|---|---|---|---|---|---|
| HCC1428 | 50 | 88.5 | + | + | − | N | Y | Y | Y | Y | Y | Y |
| SKBR3 | 50 | 56.2 | − | + | + | Y | Y | Y | Y | Y | Y | Y |
| BT20 | 50 | 66.1 | − | NC | − | Y | Y | Y | Y | Y | Y | Y |
| HCC38 | 50 | 51.0 | − | − | − | Y | Y | Y | Y | Y | Y | Y |
| CAMA1 | 50 | 72.9 | + | NC | NC | Y | Y | Y | Y | Y | Y | Y |
| BT474 | 31.99 | 92.5 | − | − | − | Y | Y | Y | Y | Y | Y | Y |
| MDAMB134VI | 30.90 | 82.7 | + | + | − | Y | N | N | Y | Y | Y | Y |
| MDAMB231 | 29.96 | 25.0 | − | − | − | Y | Y | Y | Y | Y | Y | Y |
| BT549 | 21.43 | 25.5 | − | − | + | Y | Y | Y | Y | Y | Y | Y |
| T47D | 19.95 | 55.8 | + | + | NC | Y | Y | Y | Y | Y | Y | Y |
| SUM159PT | 16.29 | 21.7 | − | + | − | Y | Y | Y | Y | Y | Y | Y |
| HCC1954 | 15.49 | 43.8 | − | − | − | Y | Y | Y | Y | Y | Y | Y |
| MCF7 | 14.69 | 56.5 | − | − | − | Y | Y | Y | Y | Y | Y | Y |
| HS578T | 6.55 | 32.3 | − | − | − | Y | Y | Y | Y | Y | Y | Y |
| MDAMB157 | 2.41 | 67.0 | − | + | + | Y | Y | Y | Y | Y | Y | Y |
| HCC70 | 0.655 | 67.8 | − | − | NC | Y | Y | Y | Y | Y | Y | Y |
| MDAMB468 | 0.514 | 79.8 | − | − | − | Y | Y | Y | Y | N | Y | Y |
| HCC202 | 0.413 | 212.5 | − | NC | NC | N | Y | Y | Y | Y | Y | Y |
| HCC1143 | 0.0211 | 54.6 | − | − | − | Y | Y | Y | Y | Y | Y | Y |
| SUM149PT | 0.0161 | 33.9 | + | + | − | Y | Y | Y | Y | Y | Y | Y |
| MDAMB453 | 0.00915 | 62.5 | − | + | + | Y | Y | Y | Y | Y | Y | Y |
| MDAMB436 | 0.00044 | 89.3 | − | NC | − | Y | Y | Y | Y | N | Y | Y |
| # cell lines | | | | | | 20 | 21 | 21 | 22 | 20 | 22 | 22 |

Doubling times were estimated for each cell line from measurements of the number of doublings of untreated cells that occurred in 72 h during the course of assessing responses to 123 therapeutic compounds (Heiser et al. [28], PNAS 2012)

[a] For ER, probe 205225_at on the Affymetrix U133A array was investigated; for PR, probe 208305_at; and for ERBB2 probes 210930_s_at and 216836_s_at

used as a quantitative measure of sensitivity and ranged from 0.44 nM to 32 μM. The SF50 was not reached for five cell lines at the maximum treatment concentration of 50 μM olaparib. Olaparib response obtained with the growth inhibition assay was not influenced by growth rate assessed as doubling time (Spearman correlation coefficient −0.036, *p* value 0.874). Figure 2 shows the waterfall plot of SF50 with cell lines ordered from most resistant at the left to most sensitive at the right. Cell lines were divided into a group of 15 resistant and 7 sensitive cell lines, based on an SF50 threshold of 1 μM. Drug response was not significantly associated with breast cancer subtype (*p* value luminal vs. basal 0.136; Fig. 3), and did not differ between ERBB2-amplified and non-ERBB2-amplified cell lines (*p* value 1), with transcriptional subtypes assigned to cell lines as previously reported [28]. Four of the seven sensitive cell lines (57 %) were triple negative, compared to 5 of 15 (33 %) resistant cell lines (*p* value 0.376). Table 1 summarizes characteristics for the 22 cell lines, with SF50, doubling time, transcriptional ER, PR, and ERBB2 status, and the molecular data available for each of them.

## Molecular features involved in DNA repair associate with olaparib response

We selected candidate molecular features that might be developed as biomarkers for prediction of response to olaparib as those features involved in DNA repair activities that were associated with quantitative response to olaparib in the cell line panel. Molecular features included pre-treatment RNA transcript levels, mutation status, copy number variation, and promoter methylation status. Specific genes tested involved aspects of DNA repair listed by Wang and Weaver [31]; *ER*, *PR* and *ERBB2* due to the

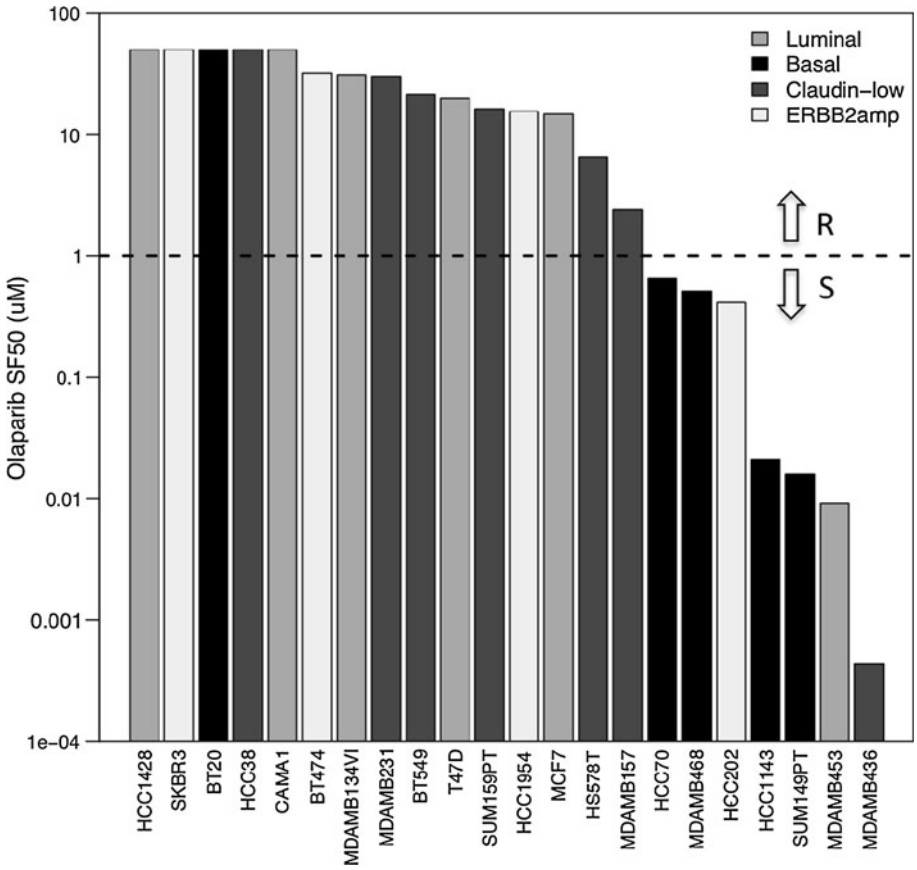



**Fig. 2** Waterfall plot of the response to olaparib (expressed as SF50 in μM) for 22 breast cancer cell lines, ordered from most resistant at the left to most sensitive at the right, with *bars colored* according to subtype (luminal in *light grey*, basal in *black*, claudin-low in *dark grey*, and ERBB2 amplified in *white*). The threshold of 1 μM used to divide the cell lines into a group of 15 resistant cell lines (indicated with *R*) and a group of 7 sensitive cell lines (indicated with *S*) is represented with a *horizontal dashed line*

importance of PARP inhibition for triple negative breast cancer [17, 19]; and PARP family members *PARP1*, *PARP2*, *VPARP*, *TNKS*, and *TNKS2*. This approach is based on observations that in vitro models showing high sensitivity to PARP inhibitors often have *BRCA* and *PTEN* deficiencies [7, 8], copy number variations involving *BRCA1* and *PARP1* [32], and/or hypermethylation of the promoter regions of genes *BRCA1* and *FANCF* [20]. Molecular features showing statistically significant associations with SF50 values are summarized in Supplementary Table 1 and illustrated in Fig. 4.

The transcription levels of *MRE11A*, *NBS1*, *TNKS*, *TNKS2*, *XPA*, and *XRCC5* were significantly lower ($p < 0.05$; fold-change >2) in the sensitive compared to the resistant cell lines for at least one expression platform (U133A, exon array and RNA-seq), whilst transcription levels for *BRCA1*, *ERCC4*, *FANCD2*, and *PR* tended to be lower in sensitive lines ($p < 0.1$). We refer to Supplementary Table 1a for the list of significant associations per platform. PR protein levels measured using reverse phase protein lysate arrays [33] were also significantly reduced in the sensitive cell lines ($p < 0.05$). Transcript levels for *CHEK2* and *MK2* were significantly higher in the sensitive compared to the resistant lines ($p < 0.05$), with a similar

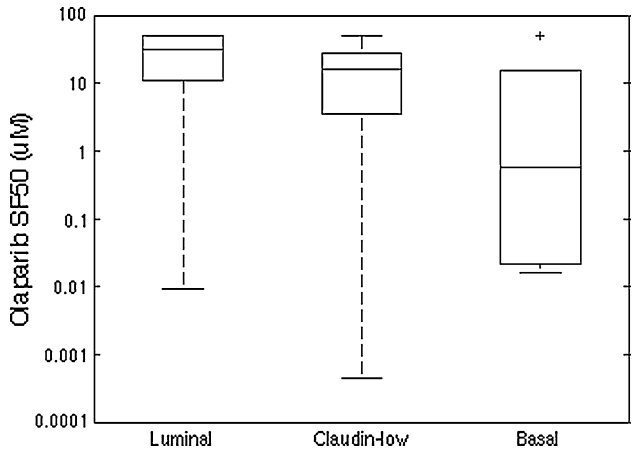
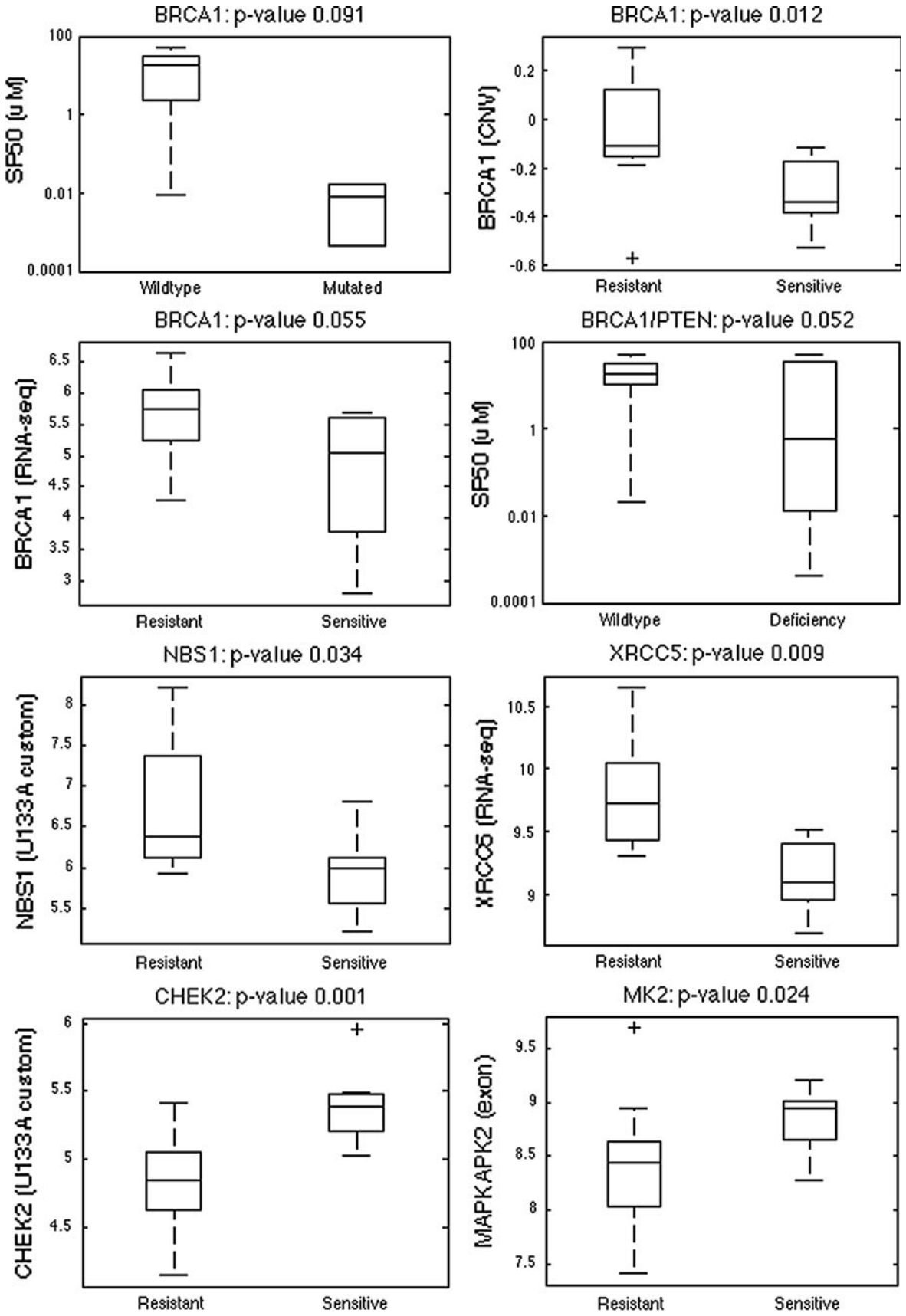



**Fig. 3** *Boxplot* of SF50 for the cell lines divided according to breast cancer subtype (9 luminal, 7 claudin-low, and 6 basal lines). No association was found between breast cancer subtype and response to olaparib in the cell line panel (Fisher's exact test for basal vs. luminal, *p* value 0.136)

trend for *PARP2* and *XRCC3* ($p < 0.1$). Although *PARP1* has been shown to be overexpressed in 58 % of invasive breast cancer samples [34] and upregulated at protein level in 82 % of *BRCA1*-associated breast cancer samples [35],


BRCA1: p-value 0.091
SP50 (u M)
100
1
0.01
0.0001
Wildtype
Mutated
BRCA1: p-value 0.012
BRCA1 (CNV)
0.2
0
-0.2
-0.4
-0.6
Resistant
Sensitive
BRCA1: p-value 0.055
BRCA1 (RNA-seq)
6.5
6
5.5
5
4.5
4
3.5
3
Resistant
Sensitive
BRCA1/PTEN: p-value 0.052
SP50 (u M)
100
1
0.01
0.0001
Wildtype
Deficiency
NBS1: p-value 0.034
NBS1 (U133A custom)
8
7.5
7
6.5
6
5.5
Resistant
Sensitive
XRCC5: p-value 0.009
XRCC5 (RNA-seq)
10.5
10
9.5
9
Resistant
Sensitive
CHEK2: p-value 0.001
CHEK2 (U133A custom)
6
5.5
5
4.5
Resistant
Sensitive
MK2: p-value 0.024
MAPKAPK2 (exon)
9.5
9
8.5
8
7.5
Resistant
Sensitive

◀ **Fig. 4** Overview of individual DNA repair-associated markers that are significantly associated with or do trend towards an association with response to olaparib in the 22 breast cancer cell lines, based on mutation, copy number, and expression data (see Supplementary Table 1 for the complete list of markers). The four *boxplots* at the *top* show the association results for *BRCA1*. The *BRCA1*-mutated cell lines MDAMB436 and SUM149PT tend to be more sensitive to olaparib compared to the wild-type cell lines ($p$ value 0.091). The sensitive cell lines are also characterized by a significant lower copy number of *BRCA1* ($p$ value 0.012) and by *BRCA1* down-regulation (RNA-seq, $p$ value 0.055). Cell lines with a deficiency in *BRCA1* and/or *PTEN* tend to be more sensitive to olaparib than cell lines with functional *BRCA1* and *PTEN* ($p$ value 0.052). The *boxplots* at the *bottom* show the association for genes *NBS1* and *XRCC5* that are significantly down-regulated and for genes *CHEK2* and *MK2* that are significantly up-regulated in the sensitive compared to the resistant cell lines

there is no consensus on its importance as a biomarker of response to PARP inhibitors [36, 37]. In our cell line panel, expression of *PARP1* mRNA levels were not significantly higher in the sensitive lines compared to the resistant lines (median $p$ value 0.277) (Supplementary Table 1a).

The *BRCA1*-mutated cell lines MDAMB436 and SUM149PT had a trend to be more sensitive to olaparib compared to the wild-type cell lines ($p$ value 0.091) (Supplementary Table 1b). Likewise, cells with reduced BRCA1 copy number were significantly more sensitive to olaparib than cells with normal copy number at this locus ($p$ value 0.012) (Supplementary Table 1c). *PTEN* loss of function, which was defined as mutation and/or lack of expression, was not significantly associated with olaparib SF50 response ($p$ value 0.145), even though previous studies from our group suggested that PTEN deficiency can cause olaparib sensitivity [8, 9]. Lack of association in the cell line panel could be ascribed to the small sample size and/or to the possibility that the univariate associations do not take into account important multigene effects. Since BRCA1 mutations have been associated with reduced PTEN expression [38], we tested for association of either BRCA1 mutation or PTEN deficiency with olaparib sensitivity. We found that cell lines with a deficiency in either gene tended to be more sensitive to olaparib than cell lines with functional *BRCA1* and *PTEN* ($p$ value 0.052) (Supplementary Table 1b). No association was found between *TP53* mutation status and drug response ($p$ value 0.376).

## Cell line-based seven-transcript signature predicts response to olaparib

We used a breast cancer cell line panel comprised luminal, basal, and claudin-low cell lines to develop a multi-transcript predictor of sensitivity to olaparib according to the REMARK recommendations [39]. We limited the predictor to transcript levels to facilitate clinical application. We considered all breast cancer subtypes for the development of the predictor based on a study of RAD51 focus formation in cells responding to a PARP inhibitor. That study showed that 30–40 % of triple negative breast cancers appeared not to have defective HR and therefore might not benefit from a PARP inhibitor whilst ~20 % of non-triple negative breast cancers appeared to have defective HR and therefore might respond to a PARP inhibitor [40]. Thus, we reasoned that a predictor developed using the complete cell line panel might be applicable to the full spectrum of breast cancer covered by the cell line panel. As shown in Fig. 1, the molecular features tested as candidate biomarkers were limited to genes involved in DNA repair pathways BER, NER, MMR, HR/FA, NHEJ, and DDR as defined by Wang and Weaver [31] and in the Kyoto Encyclopedia of Genes and Genomes (KEGG) database release 55.1 [41]. This led to the selection of 118 genes (see Supplementary Table 2) that were tested for association between transcript levels and response to olaparib. These transcript levels were measured using three different mRNA analysis platforms (Affymetrix U133A arrays, Affymetrix exon arrays and Illumina RNA-seq).

We identified the most important transcripts by applying LR with forward feature selection (fivefold CV) 100 times. Markers significantly associated with olaparib response in over half of the iterations are shown in Table 2. These were further reduced to seven gene transcripts that were significantly associated with olaparib response in at least 2 out of 3 mRNA analysis platforms. Five transcript levels (candidate resistance markers *BRCA1*, *MRE11A*, *NBS1*, *TDG*, and *XPA*) were inversely associated with predicted probability of response and two transcript levels (candidate sensitivity markers *CHEK2* and *MK2*) were positively associated with predicted probability of response (see Table 3). *BRCA1* is involved in DSB repair via *RAD51*-mediated HR [42, 43]. *CHEK2* is a kinase with signal transduction function in cell-cycle regulation and checkpoint responses [2], and is involved in the major parallel DDR pathway ATM-CHEK2 [31]. *CHEK2* has also been reported as an intermediate-level breast cancer risk gene, regardless of family history [44, 45]. Besides the standard DDR pathways, the cell-cycle checkpoint pathway p38MAPK/MK2 is additionally activated in TP53 mutant cells [46]. MK2 activity is critical for prolonged checkpoint maintenance through a process of posttranscriptional regulation of gene expression [47]. *MRE11A* and *NBS1* are part of the MRN complex, a multifaceted molecular machine for DSB recognition [48]. Finally, *TDG* is part of the BER pathway, whilst *XPA* encodes a zinc finger protein that is part of the NER complex.

We combined information on the seven-transcript levels to form a predictive signature using a weighted voting algorithm (Supplementary Material and [28]). This algorithm assigns a weight and decision boundary to each of the seven genes, based on their expression distribution for the class of sensitive versus resistant cell lines (see Table 3). For

**Table 2** Overview per expression platform of genes from six principal DNA repair pathways that are selected with the LR approach in over half of the iterations

| Biomarker source | Platform | # Genes | Genes selected in >250/500 iterations[a] | Avg. AUC (std)[b] |
|---|---|---|---|---|
| DNA repair biomarkers (Wang and Weaver [31]) | U133A (standard) | 11/29 | **BRCA1**, BRCA2, **CHEK2**, DSS1, **MRE11A**, **NBS1**, PALB2, PARP2, PTEN, TP53, **XPA** | 0.793 (0.083) |
| | U133A (custom) | 7/29 | **BRCA1**, BRCA2, **CHEK2**, DSS1, **NBS1**, RAD51, **XPA** | 0.945 (0.059) |
| | Exon array | 12/29 | BRCA2, **CHEK2**, DSS1, ERCC1, ERCC4, FANCD2, **MK2**, **MRE11A**, **NBS1**, USP11, **XPA**, XRCC5 | 0.717 (0.084) |
| | RNA-seq | 14/29 | ATM, **BRCA1**, DSS1, FANCD2, JTB, **MK2**, **MRE11A**, **NBS1**, PALB2, PARP1, PARP2, **XPA**, XRCC5, XRCC6 | 0.715 (0.132) |
| KEGG | U133A (standard) | 5/103 | DNTT, MUTYH, POLM, RPA2, TOP3B | 0.745 (0.075) |
| | U133A (custom) | 9/103 | DNTT, FEN1, MUTYH, NBS1, POLD1, POLM, RAD51, RAD51C, XRCC5 | 0.725 (0.092) |
| | Exon array | 4/103 | DNTT**, MRE11A**, **TDG**, UNG | 0.753 (0.083) |
| | RNA-seq | 5/103 | DCLRE1C, FEN1, RPA4, **TDG**, XRCC5 | 0.839 (0.054) |

[a] Genes with consistent pattern of sensitivity for all three platforms (U133A, exon array, RNA-seq) and for both measures of class comparison (mean, median) are shown in bold

[b] Average fivefold CV area under the receiver operating characteristics curve (AUC) (standard deviation) across 100 randomizations for a LR model with optimized coefficients and inclusion of the platform-specific genes selected in >1/2 of the iterations

**Table 3** Overview of the seven genes selected for prediction of response to treatment with olaparib based on breast cancer cell line expression data

| Gene symbol | Gene name | Pathway | Entrez gene ID | Marker | Probe | Weight ($w_g$) | Decision boundary ($b_g$) |
|---|---|---|---|---|---|---|---|
| *BRCA1* | Breast cancer 1, early onset | HR | 672 | Resistance | 204531_s_at | −0.5320 | −0.0153 |
| *CHEK2* | CHK2 checkpoint homolog | DDR | 11200 | Sensitivity | 210416_s_at | 0.5806 | −0.0060 |
| *MK2* | Mitogen-activated protein kinase-activated protein kinase 2 | DDR | 9261 | Sensitivity | 201461_s_at | 0.0713 | 0.0031 |
| *MRE11A* | MRE11 meiotic recombination 11 homolog A | DDR/HR | 4361 | Resistance | 205395_s_at | −0.1396 | −0.0044 |
| *NBS1* | Nibrin | DDR | 4683 | Resistance | 202906_s_at | −0.1976 | 0.0014 |
| *TDG* | Thymine-DNA glycosylase | BER | 6996 | Resistance | 203743_s_at | −0.3937 | −0.0165 |
| *XPA* | Xeroderma pigmen-tosum, complemen-tation group A | NER | 7507 | Resistance | 205672_at | −0.2335 | −0.0126 |

The weights and decision boundaries were determined with data from the U133A expression array platform measured for the 22 cell lines used to assess response to olaparib. For each of the five resistance and two sensitivity markers, gene symbol is shown together with gene name, the DNA repair pathway the gene belongs to, entrez gene identifier, corresponding probe set from the Affymetrix U133A array, and weight and decision boundary obtained with the weighted voting algorithm

this signature to work on external samples, the transcript levels were normalized to the geometric mean of seven control genes, followed by median normalization across the cell lines (see Supplementary Material). The larger the weight for a gene transcript level, the more influence this gene has on predicted probability of response. Positive weights were assigned for sensitivity markers and negative weights were assigned for resistance markers.

## Prevalence of 8–21 % of predicted responding patients, with trend towards the basal subtype

We analyzed expression profiles measured for breast cancer patients not treated with PARP inhibitors to understand which patients would have a likelihood of response to olaparib according to our seven-transcript predictor. We used seven U133A and one U133 plus two data sets on 1,846 primary breast tumors with or without metastasis, heterogeneous in treatment and ER/PR/LN status. Our seven-transcript response algorithm predicted that 8–21 % of patients in the eight data sets would be responsive to olaparib (Table 4), using threshold 0.0372 obtained from the cell lines to distinguish sensitive from resistant (see Supplementary Material). The fraction predicted to respond was inversely related to the fraction of ER-positive patients in each data set (Pearson correlation coefficient −0.614, $p$ value 0.1). We also tested the seven-transcript predictor in Agilent mRNA transcript profiles measured for 536

**Table 4** Prevalence of the seven-gene signature in tumor samples from 9 different studies on primary breast tumors with or without metastasis, heterogeneous in treatment and ER/PR/LN status

| Data set | Platform | # Samples | Characteristics | Treatment | Event rate (%) | # Predicted responders (%)[a] |
|---|---|---|---|---|---|---|
| GSE2034 | U133A | 286 | 73.1 % ER+<br>58 % PR+<br>18.2 % ERBB2+<br>0 % LN+ | Untreated | 37.4 Distant metastasis | 55 (19.2) |
| GSE20271 | U133A | 177 | 55.7 % ER+<br>46.9 % PR+<br>14.2 % ERBB2+ | 49.2 % FAC;<br>50.8 % T/FAC | 14.1 pCR | 26 (14.7) |
| GSE23988 | U133A | 61 | 52.5 % ER+<br>0 % ERBB2+<br>65.6 % LN+<br>Median tumor size 6 cm (2–17.5) | FEC/wTx | 32.8 pCR | 9 (14.8) |
| GSE4922 | U133A + B | 289 | 86.1 % ER+<br>33.7 % LN+<br>Median tumor size 2 cm (0.2–13) | 37.7 % Systematic adjuvant therapy | 35.7 Local/distant recurrence or death | 24 (8.3) |
| GSE25066 | U133A | 508 | 58.9 % ER+<br>69.1 % LN+<br>31.5 % LumA<br>15.3 % LumB<br>37.2 % Basal-like<br>7.3 % HER2-enr<br>8.7 % Normal-like | Neoadj. taxane & anthra-cycline-based regimen | 19.5 pCR | 94 (18.5) |
| GSE7390 | U133A | 198 | 67.7 % ER+<br>14.1 % ERBB2+<br>0 % LN+<br>Median tumor size 2 cm (0.6–5) | Untreated | 31.3 Distant metastasis | 33 (16.7) |
| GSE11121 | U133A | 200 | 78 % ER+<br>65 % PR+<br>12.3 % ERBB2+<br>0 % LN+<br>Median tumor size 2 cm (0.1–6.0) | Untreated | 23 Distant metastasis | 20 (10.0) |
| GSE5460 | U133 plus 2 | 127 | 58.3 % ER+<br>23.6 % ERBB2+<br>49.6 % LN+<br>Median tumor size 2.2 cm (0.8–8.5) | Untreated | – | 27 (21.3) |

**Table 4** continued

| Data set | Platform | # Samples | Characteristics | Treatment | Event rate (%) | # Predicted responders (%)[a] |
|---|---|---|---|---|---|---|
| TCGA | Agilent G4502A | 536 | 44.0 % LumA<br>25.2 % LumB<br>18.5 % Basal-like<br>10.8 % HER2-enr<br>1.5 % Normal-like | Heterogeneous | – | 67 (12.5) |

*FAC* neoadjuvant chemotherapy regimen with 5-fluorouracil, docorubicin, and cyclophosphamide; *T/FAC* neoadjuvant chemotherapy regimen with paclitaxel and 5-fluorouracil, docorubicin, and cyclophosphamide; *FEC/wTx* neoadjuvant chemotherapy regimen with four courses of 5-fluorouracil, docorubicin, and cyclophosphamide, followed by four additional courses of weekly docetaxel and capecitabine

[a] Number and percentage of patients predicted to respond to treatment with a PARP inhibitor according to the seven-gene predictor with use of threshold 0.0372 for response assignment for Affymetrix data, and threshold 0.174 for Agilent data

breast invasive carcinoma samples collected by The Cancer Genome Atlas (TCGA) [49]. This required that an Agilent-specific threshold distinguishing sensitive from resistant be established. We accomplished this using a set of Affymetrix and Agilent mRNA transcript profiles measured for 80 I-SPY 1 samples [50, 51]. The Agilent threshold was set so that the fraction of I-SPY 1 samples in the Agilent data set predicted to be sensitive was the same as that predicted to be sensitive using the Affymetrix data (see Supplementary Materials). The fraction of samples predicted to be sensitive in the TCGA data set was 12 % (Table 4). We assessed the transcriptional subtypes of the patient populations predicted to respond to olaparib in 464 samples from GSE25066 and in 528 TCGA tumor samples after exclusion of the normal-like samples. The tumors predicted to respond were enriched in samples classified as basal-like compared to samples classified as luminal A, luminal B or HER2 ($p$ value 0.002 and $2.6 \times 10^{-28}$ for GSE25066 and TCGA, respectively; Table 5).

## Discussion

In this hypothesis generating study, our overall aim was to use quantitative measurements of response to olaparib in 22 breast cancer cell lines to identify molecular features associated with response as a first step towards development of a molecular signature to predict clinical responses. We limited our search for features associated with olaparib response to copy number, DNA sequence abnormalities or transcription levels for 42 genes suggested in [31] for their association with DNA repair. Molecular features associated with 15 of these 42 genes were found to be significantly associated or to show a trend of association with olaparib response. Specifically, cell lines that were sensitive to olaparib were enriched in *BRCA1* mutations or deletions, *PARP1* amplification, reduced expression of *BRCA1*, *ERCC4*, *FANCD2*, *MRE11A*, *NBS1*, *PR*, *TNKS*, *TNKS2*, *XPA*, and *XRCC5* and increased expression of *CHEK2*, *MK2*, *PARP2*, and *XRCC3*.

Since multiple mechanisms may contribute to olaparib sensitivity, we developed a weighted voting signature to combine influences from multiple markers. We included only transcript levels in our algorithm since most molecular features associated with response were apparent at the transcript level. We limited the search space to molecular features of 118 genes from six principal DNA repair pathways in order to increase statistical power. Associations of transcript levels for 118 genes and responses to olaparib for 22 breast cancer cell lines resulted in a seven-gene predictive signature that included five resistance markers (*BRCA1*, *MRE11A*, *NBS1*, *TDG*, and *XPA*) and two response markers (*CHEK2* and *MK2*).

**Table 5** Association of breast cancer subtype with predicted response to olaparib in 464 GSE25066 and 528 TCGA tumor samples, after exclusion of the normal-like samples

| GSE25066 | Non-responders *N* (%) | Responders *N* (%) | TCGA | Non-responders *N* (%) | Responders *N* (%) |
|---|---|---|---|---|---|
| Luminal A | 120 (75.0) | 40 (25.0) | Luminal A | 233 (98.7) | 3 (1.3) |
| Luminal B | 72 (92.3) | 6 (7.7) | Luminal B | 126 (93.3) | 9 (6.7) |
| Basal-like | 155 (82.0) | 34 (18.0) | Basal-like | 54 (54.5) | 45 (45.5) |
| HER2-enriched | 35 (94.6) | 2 (5.4) | HER2-enriched | 50 (86.2) | 8 (13.8) |
| *p* value | 0.002 | | *p* value | $2.6 \times 10^{-28}$ | |
| Chi-square test | | | Chi-square test | | |

The transcript levels of the seven genes in the predictor were consistent with expectations from the literature. Mutations in *BRCA1*, loss of heterozygosity at the *BRCA1* locus and deregulated expression have been described in the literature as potential markers for prediction of response to PARP inhibitors [17]. These studies are consistent with our finding that reduced *BRCA1* transcript levels are associated with olaparib sensitivity. PARP1 is required for rapid accumulation of MRE11A at DSB sites. Due to the direct interaction between PARP1 and MRE11A, deficiency in *MRE11A* has been suggested as a mechanism of sensitizing cells to PARP1 inhibition based on the concept of synthetic lethality [52]. Moreover, a dominant negative mutation in *MRE11A* in MMR deficient cancers has been shown to sensitize cells to agents causing replication fork stress [53]. These reports are consistent with our finding that reduced *MRE11A* transcription is associated with olaparib sensitivity. Experimental disruption of the HR pathway protein *NBS1* by RNAi has been reported to increase sensitivity to PARP inhibitors [15]. This is consistent with our finding that reduced transcription of *NBS1* is associated with olaparib sensitivity. Cells with defective NER have been shown to be hypersensitive to platinum agents, with low XPA protein levels in testis tumor cell lines explaining the low capacity to repair cisplatin-induced DNA damage [54]. PARP inhibitors also enhance lethality in XPA-deficient cells after UV irradiation [55]. Tumor cells with deficiency of the DDR pathway have been suggested to be hypersensitive to PARP inhibitors, with the DNA repair biomarker *CHEK1* shown to be overexpressed in BRCA1-like versus non-BRCA1-like triple negative breast cancer [56]. This is consistent with our finding that increased *CHEK2* transcription is associated with olaparib sensitivity.

Our seven-gene transcript algorithm suggests that 8–21 % of patients with primary breast cancers may respond to olaparib and that the responsive tumors are enriched in basal-like breast cancers. This represents a hypothesis that can now be tested in clinical trials. Since the signature has not yet been tested clinically, it is inappropriate to use it to select patients for treatment with olaparib or other PARP inhibitors or to use it in any way to manage breast cancer treatment. However, it does present a signature that can be tested in planned translational analyses of ongoing clinical trials of PARP inhibitors and that can be used to determine whether clinical trials are properly sized to detect a response of the magnitude predicted by this signature.

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**Conflict of interest** AA and CJL may benefit financially from the development of PARP inhibitors through patents held jointly with AstraZeneca through the Institute of Cancer Research "rewards to inventors" scheme. All other authors declare that they have no conflict of interest.

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