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Molecular Cancer Therapeutics

High *XIST* and Low 53BP1 Expression Predict Poor Outcome after High-Dose Alkylating Chemotherapy in Patients with a *BRCA1*-like Breast Cancer

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Abstract

In previous studies, high expression of *XIST* and low expression of *53BP1* were respectively associated with poor systemic therapy outcome in patients and therapy resistance in *BRCA1*-deficient mouse tumor models, but have not been evaluated in *BRCA1*-deficient patients. Previously, we demonstrated that classifying breast cancer copy number profiles as *BRCA1*-like or non–*BRCA1*-like identified patients enriched for defects in *BRCA1* that benefit from high-dose (HD) alkylating chemotherapy compared with a conventional standard regimen. We investigated whether *XIST* and 53BP1 expression predicted poor outcome of HD chemotherapy within 28 *BRCA1*-like patients from a trial randomizing between HD [4 cycles 5-fluorouracil, epirubicin, cyclophosphamide (FEC) followed by 1 cycle HD carboplatin, thiotepa, cyclophosphamide] or conventional chemotherapy (5 cycles FEC), for which both *XIST* and 53BP1 statuses were available. High RNA expression of *XIST* ($n = 5$) and low protein expression of 53BP1 ($n = 3$) expression did not coincide. Patients with either one had poor outcome after treatment with HD chemotherapy, whereas patients with low expression of *XIST* and high expression of 53BP1 derived substantial benefit of this regimen on recurrence-free survival, disease-free survival, and overall survival, corroborating preclinical findings. *XIST* and 53BP1 may be predictive biomarkers in BRCA1-like breast cancer. *Mol Cancer Ther; 15(1); 190–8. ©2015 AACR.*

Introduction

Germline mutations in the *BRCA1* gene confer a substantially increased breast cancer risk (1, 2). The genomic instability associated with such cancers is driven by defects in DNA repair, because BRCA1 is critical in the homologous recombination (HR) pathway. This pathway is the only known error-free repair mechanism for DNA double-strand breaks (DSB). In the absence of HR, repair of these lesions is error-prone and mainly performed by nonhomologous end-joining (NHEJ; refs. 3–6). In preclinical models, it has been extensively demonstrated that BRCA1-deficient tumor cells are sensitive to specific therapies. These therapies either damage the DNA by introducing DNA DSBs, such as alkylating agents, or through a synthetic lethal approach by PARP1 inhibition (4, 7–10).

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Despite the sensitivity of *BRCA1*-deficient tumors to DNA DSB-inducing chemotherapy and PARP inhibitors, both intrinsic and acquired resistances can occur and thwart effective treatment (11). Recently, two biomarkers associated with resistance were identified in *BRCA1*-deficient model systems (12, 13). The first biomarker, X inactive–specific transcript (*XIST*), was found to be differentially expressed in *BRCA1*-deficient mouse tumors that had early and late relapses after treatment with cisplatin. Specifically, low gene expression of *XIST* was associated with late relapse, suggesting that this may be a predictive biomarker (12). Subsequently, *XIST* expression was investigated in HER2-negative patients from a randomized trial comparing high-dose (HD) alkylating chemotherapy and conventional (CONV) chemotherapy. We will use the trial's abbreviation of the arms (HD and CONV), but the observed effects may be due to the alkylating agents, the HD, or a combination. Low expression of *XIST* correlated with longer recurrence-free survival (RFS) after HD alkylating chemotherapy compared with conventional chemotherapy. Thus, the patients in the clinical study did not represent the BRCA1 deficiency of the mouse model, because many patients were included without features of BRCA1 deficiency (12, 14, 15). Furthermore, because associations between dysfunction of BRCA1 and *XIST* have been frequently described, it may be that BRCA1 deficiency and low *XIST* expression identify a similar group of patients (12, 16–21). For these two reasons, it is important to study *XIST* expression in the presence of BRCA1 deficiency, to recapitulate the findings in a *brca1*-deficient mouse model as closely as possible in the human situation (12).

The second biomarker associated with resistance of *BRCA1*-deficient cells was 53BP1. The choice of repair pathway of DNA

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DSBs is tightly regulated to ensure the proper activation of NHEJ and HR. 53BP1 loss has been shown to partially restore HR in *BRCA1*-deficient cells. Loss of 53BP1 allowed end resection of breaks ends, which is an important step in preparing the DNA strand for HR rather than NHEJ (13, 22, 23) This partial restoration rendered tumor cells more resistant to cisplatin, mitomycin C, and olaparib (a PARP inhibitor; refs. 13, 24). Subsequently, 53BP1 expression was investigated in two cohorts of patients. In these cohorts, associations with clinical characteristics and survival analysis were found (13). The clinical analyses for both biomarkers did not investigate whether these markers could be used as predictive biomarkers for therapy responses within *BRCA1*-deficient patients, leaving critical questions on the applicability of the findings.

We previously investigated a *BRCA1*-like copy number classifier as predictive biomarker for benefit of HD alkylating chemotherapy (25, 26). This test classifies tumors as *BRCA1*-like or non–*BRCA1*-like based on the presence of characteristic DNA gains and losses associated with *BRCA1*-mutated and promoter methylated breast cancers (14, 27). Patients with a *BRCA1*-like tumor had a 6-fold lower risk of recurrence and death when treated with HD chemotherapy compared with conventional chemotherapy. This benefit was not observed in patients with a non–*BRCA1*-like tumor (14). We found similar estimates in an independent cohort, in which *BRCA1*-like patients treated with HD chemotherapy had a 6-fold lower risk of recurrence and death (28).

We assessed whether the expression of *XIST* and 53BP1 can be used to identify a population of patients with a BRCA1-like breast cancer that do not benefit from HD chemotherapy. Our hypothesis was that matching the targetable defect of the model system to patients with that defect would result in good concordance of the preclinical and clinical findings and thus create opportunities for translation toward patients.

Materials and Methods

Patients

The patients in these studies have been reported before (14, 29) and were treated in a randomized controlled trial comparing HD alkylating to conventional chemotherapy. The conventional regimen consisted of five courses of 500 mg/m^2 5-fluorouracil, 90 mg/m^2 epirubicin, and 500 mg/m^2 cyclophosphamide (FEC). Patients in the HD treatment cohort were administered four courses of FEC, followed by stem cell harvesting and one course of 1,600 mg/m^2 carboplatin, 480 mg/m^2 thiotepa, and 6,000 mg/m^2 cyclophosphamide. All patients have given written informed consent to be included in the study that was approved by the Institutional Review Committee (29). According to Dutch law, this implied consent for the analysis of residual tissue specimens obtained for diagnostic purposes and anonymized publication of the results (http://www.federa.org/code-goed-gebruik-van-lichaamsmateriaal-2011).

BRCA1-like copy number classification

Patients were classified based on their copy number profile to belong to the *BRCA1*-like class or the non–*BRCA1*-like class as described before (14, 26). These classifiers assign a probability of being *BRCA1*-mutated (similar to being *BRCA1*-mutated, thus "*BRCA1*-like") or non–*BRCA1*-mutated class (not similar to BRCA1-mutated, thus non–*BRCA1*-like). The *BRCA1*-like class is enriched for *BRCA1* mutation carriers as well as patients with tumors that have *BRCA1* promoter methylation. Patients with a *BRCA1*-like copy number profile benefited from receiving HD chemotherapy (14, 27). Because the preclinical models do not provide evidence for prediction in a *BRCA1*-proficient system, we did not investigate non–*BRCA1*-like patients.

Genomic loss of *XIST* and 53BP1

We segmented 3.2K bacterial artificial chromosome (BAC) genome-wide aCGH profiles with the cghseg package (30). We then used the segmented log ratio of the BAC clone in which 53BP1 is located and the average of the two probes surrounding the *XIST* location for further calculations. We used a cutoff of <-0.1 segmented log ratio for identifying regions that may have lost the locus. To calculate the fraction of the chromosome lost, we counted the number of probes with segmented log ratio <-0.1 and divided that by the total number of probes on the chromosome.

Immunohistochemistry for 53BP1

Immunohistochemistry for 53BP1 was performed using a standardized protocol on the Ventana Benchmark Ultra system. Antigen retrieval was performed with citrate buffer for 44 minutes followed by antibody incubation for 32 minutes (Bethyl A300-272A dilution 1:2,000). The stainings were scored on a percentage of positive tumor cells by M. Opdam and J. Wesseling, discordant cases were resolved by consensus. A cutoff for loss of expression was based on the histogram of 53BP1 expression of tumors of 523 patients present in the tissue microarray (TMA). The distribution of the marker was bimodal, and we put a cutoff for low expression between the two groups, independent of survival analyses. The histogram of the data and representative pictures of a TMA core without 53BP1 expression and one with 53BP1 expression are shown in Fig. 1.

RT-MLPA *XIST*

The gene expression analysis of *XIST* was performed with an Reverse Transcriptase–Multiplex Ligation-dependent Probe Amplification (RT-MLPA) kit (MRC Holland) as described before (12). We used the same cutoff for aberrant expression as this study.

Statistical analysis

We investigated the influence of *XIST* and 53BP1 to predict outcome after HD chemotherapy compared with conventional chemotherapy by dividing them into two groups: (i) "*XIST*-low-and-53BP1-high," predicted to be sensitive, and (ii) "*XIST*-high-or-53BP1-low," predicted to be resistant. We also report on 53BP1 status as independent predictive biomarker in the whole cohort as this analysis has not been described elsewhere. Furthermore, we investigated independent value of 53BP1 in the triple-negative (TN) subgroup because aberrant expression has been reported to be enriched in this subgroup of patients (13, 31–33).

We used the Kaplan–Meier method to visualize the survival [disease-free survival (DFS), RFS, and overall survival (OS), as defined in ref. 29] curves comparing HD and conventional chemotherapy of all *BRCA1*-like patients by *XIST* and 53BP1 status with exact log rank tests. We performed unadjusted Cox proportional hazards regression to estimate the effect of HD chemotherapy in *XIST*-low–53BP1-high and *XIST*-high-or-53BP1-low groups. To adjust for confounding, we performed a logistic regression of chemotherapy group on estrogen receptor (ER), grade, tumor size,

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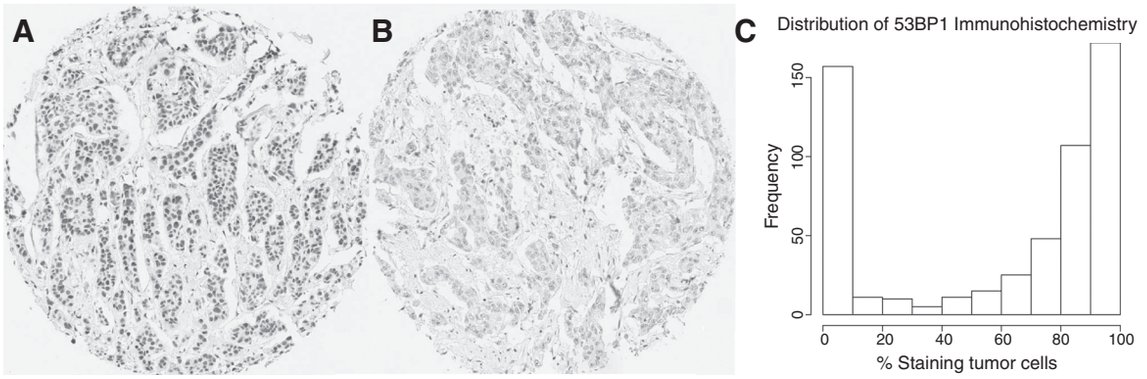

Figure 1.
A, microscopy image of a TMA core with positive 53BP1 staining. B, microscopy image a TMA core with negative 53BP1 staining. C, histogram of 53BP1 percentage of staining tumor cells in 413 patients with interpretable staining. A bimodal distribution was observed, and a cutoff was set at $\leq$30%.

and lymph node status, and used the fitted propensity score as adjustment factor in the Cox proportional hazards model. This way we could adjust for the characteristics of the four biomarker-treatment groups with only one extra covariate given the sample size. We used a treatment by biomarker interaction to attribute prognostic and/or predictive value of the biomarkers. In the analysis of 53BP1 as independent biomarker, we adjusted for ER, PR, HER2 status (except in the TN analysis), tumor size, lymph node status, and grade. Supplementary Table S1 shows a description of this study according to the reporting recommendations for tumor marker prognostic studies (REMARK) criteria.

Sensitivity analysis

We chose to present the patients for which all biomarkers were available. However, we also performed the same analyses on either of the biomarkers separately. Furthermore, we checked whether changing the cutoff for 53BP1 from 30% (main analysis) to 20% or 40% would influence the analysis. We did not change the *XIST* cutoff from the already published article (12).

All calculations were performed with R version 3.0.2 (34).

Results

Array CGH-based *BRCA1*-like classification was available for 230 patients of the randomized trial; *XIST* expression was available for 60 patients that had either a *BRCA1*-like or TN cancer as described before (12, 14). We stained TMAs of the series with 53BP1 antibodies. We focused on the *BRCA1*-like patients with *XIST* and 53BP1 status to investigate the predictions of the preclinical model. Forty-one of the 230 patients had *BRCA1*-like cancers. We obtained immunohistochemistry of 53BP1 for 37 patients, dropout being caused by missing TMA cores or TMA cores that did not contain tumor cells for reliable assessment. We drew histograms of the data of 413 (after dropout of missing or non–tumor-containing cores) patients, found a bimodal distribution of the immunohistochemical scores, and determined the cutoff to be 30% (Fig. 1).

XIST RNA expression was available in 32 of 41 patients, the dropout being due to low RNA quality. Twenty-eight patients had available *BRCA1*-like status, *XIST*, and 53BP1 expression status. Table 1 describes the clinical characteristics of these 28 patients.

As previously described, the cohort of *BRCA1*-like patients has a good outcome after HD chemotherapy, although some patients have an event (Fig. 2A and D). Figure 2B and C and Fig. 2E and F split the overall *BRCA1*-like group into a group that according to the preclinical models should be sensitive (*XIST*-low-and-53BP1-high) and one that is resistant, either *XIST*-high-or-53BP1-low. This selects a subset of *BRCA1*-like patients that seem to have equally poor outcome after HD as after conventional therapy.

We further investigated this by calculating the event rates and comparing these for the subset of patients with exact log rank tests (Table 2). *XIST*-low, 53BP1-high patients have lower rates of events compared with *XIST*-high-or-53BP1-low patients, irrespective of chemotherapy. Furthermore, among *XIST*-low, 53BP1-high patients, those treated with HD chemotherapy had significantly lower event rates than those treated with conventional chemotherapy on DFS, RFS, and OS.

We then estimated HRs of HD versus conventional chemotherapy in the *BRCA1*-like sensitive and resistant group and the interaction HR, adjusting for ER status, tumor size, lymph node status, and grade by means of the propensity score (Table 3). *BRCA1*-like, *XIST*-low, 53BP1-high patients have significantly better outcomes after HD than after conventional chemotherapy in event rate analysis as well as Cox models.

Cox regression of RFS and OS showed the same direction of effects, but with unreliable estimates of the effect sizes due to 0 event in the HD-treated *BRCA1*-like, *XIST*-low, 53BP1-high patients. We performed sensitivity analysis with similar results when using cutoffs of 20% or 40% for 53BP1 and when analyzing both genes separately (data not shown). In non–*BRCA1*-like patients, the chemotherapy effect did not differ by *XIST*-53BP1 status on DFS, RFS, and OS (Supplementary Fig. S1 and Supplementary Table S2).

Although our interest in performing these analyses was driven by preclinical observations, knowing that this would result in a small subgroup analysis, we have data on 53BP1 protein expression for many more patients. Therefore, we calculated whether 53BP1 expression as an independent marker has any prognostic or predictive value in this cohort (Supplementary Table S3). 53BP1 did not have significant prognostic or predictive value (for HD chemotherapy) in the whole cohort (Supplementary Table S4). Patients with a 53BP1-expressing tumor in the TN subgroup (Supplementary Table S5) had better RFS, DFS, and OS on HD

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Table 1. Clinical characteristics of the *BRCA1*-like cohort

	***BRCA1*-like, *XIST*-low, 53BP1-high, Conv**	**%**	***BRCA1*-like, *XIST*-low, 53BP1-high, HD**	**%**	***BRCA1*-like, *XIST*-high-or-53BP1-low, Conv**	**%**	***BRCA1*-like, *XIST*-high-or-53BP1-low, HD**	**%**
Tumor size								
T1	2	25	4	33	1	25	1	25
T2	4	50	8	67	2	50	2	50
T3	2	25	0	0	1	25	1	25
Missing	0	0	0	0	0	0	0	0
Lymph node stage								
N1	5	63	8	67	2	50	1	25
N2	3	38	4	33	2	50	3	75
Grade								
2	2	25	1	8	0	0	0	0
3	6	75	9	75	4	100	3	75
Missing	0	0	2	17	0	0	1	25
ER status								
Negative	7	88	11	92	3	75	4	100
Positive	1	13	1	8	1	25	0	0
PR status								
Negative	6	75	9	75	4	100	3	75
Positive	0	0	1	8	0	0	0	0
HER2 status								
Negative	8	100	12	100	4	100	4	100
XIST								
Low	8	100	12	100	1	25	2	50
High	0	0	0	0	3	75	2	50
53BP1								
High	8	100	12	100	3	75	2	50
Lost	0	0	0	0	1	25	2	50
RFS								
No recurrence or death	2	25	12	100	0	0	0	0
Recurrence or death	6	75	0	0	4	100	4	100
Missing	0	0	0	0	0	0	0	0
DFS								
No recurrence, second primary, or death	2	25	10	83	0	0	0	0
Recurrence, second primary, or death	6	75	2	17	4	100	4	100
Missing	0	0	0	0	0	0	0	0
OS								
No death	2	25	12	100	0	0	0	0
Death	6	75	0	0	4	100	4	100
Missing	0	0	0	0	0	0	0	0

NOTE: Clinical characteristics of 28 *BRCA1*-like patients with *XIST* expression and 53BP1 expression available. The patients are split in the four groups (number and percentage shown) that were used in the analysis, respectively, the samples predicted sensitive, treated with conventional and HD chemotherapy, and the samples predicted to be resistant, treated with conventional and HD chemotherapy.

chemotherapy than on conventional chemotherapy [RFS hazard rate 0.34; 95% confidence interval (CI), 0.15–0.78; $P = 0.01$; Supplementary Table S6, similar for DFS and OS). The *P* values for interaction were not significant (0.65, 0.33, 0.18). We also calculated whether 53BP1 may be predictive for the conventionally dosed regimen. We did not find significant prognostic or predictive value in the whole cohort (Supplementary Table S7). In the TN subgroup, conventionally treated patients have poorer responses than HD-treated patients, but 53BP1 does not seem to be a prognostic or predictive modifier of this effect (Supplementary Table S8).

We investigated whether we could find evidence for genomic loss of *XIST* and *53BP1* in the aCGH profiles, which could result in, respectively, low RNA and protein expression of these genes. The mechanism of low expression of *XIST* was previously investigated by overlapping RNA FISH, aCGH, and RT-MLPA in a subset of patients (12). These findings suggested that low *XIST* expression was related to loss of the inactive X chromosome. In some cases, the active X chromosome was duplicated (12). We plotted the aCGH log ratios and the fraction of the chromosome lost by the *XIST* RNA and 53BP1 protein expression (Fig. 3). Patients that lost *XIST* expression had evidence for losing substantial parts of the X chromosome. However, this loss was not found for all patients, which could be obscured by duplication of the X chromosome. Of the 3 patients with 53BP1 loss, 2 patients showed indications that the *53BP1* locus might be lost, but these deletions seemed restricted to only a part of the chromosome (Fig. 4). The analyses on all patients with aCGH and, respectively, *XIST* and 53BP1 expression yielded similar conclusions (Supplementary Figs. S2 and S3).

Discussion

In this study, we investigated whether *XIST* and 53BP1 expression, two potential markers of resistance to HD alkylating chemotherapy in *BRCA1*-deficient tumors identified in preclinical

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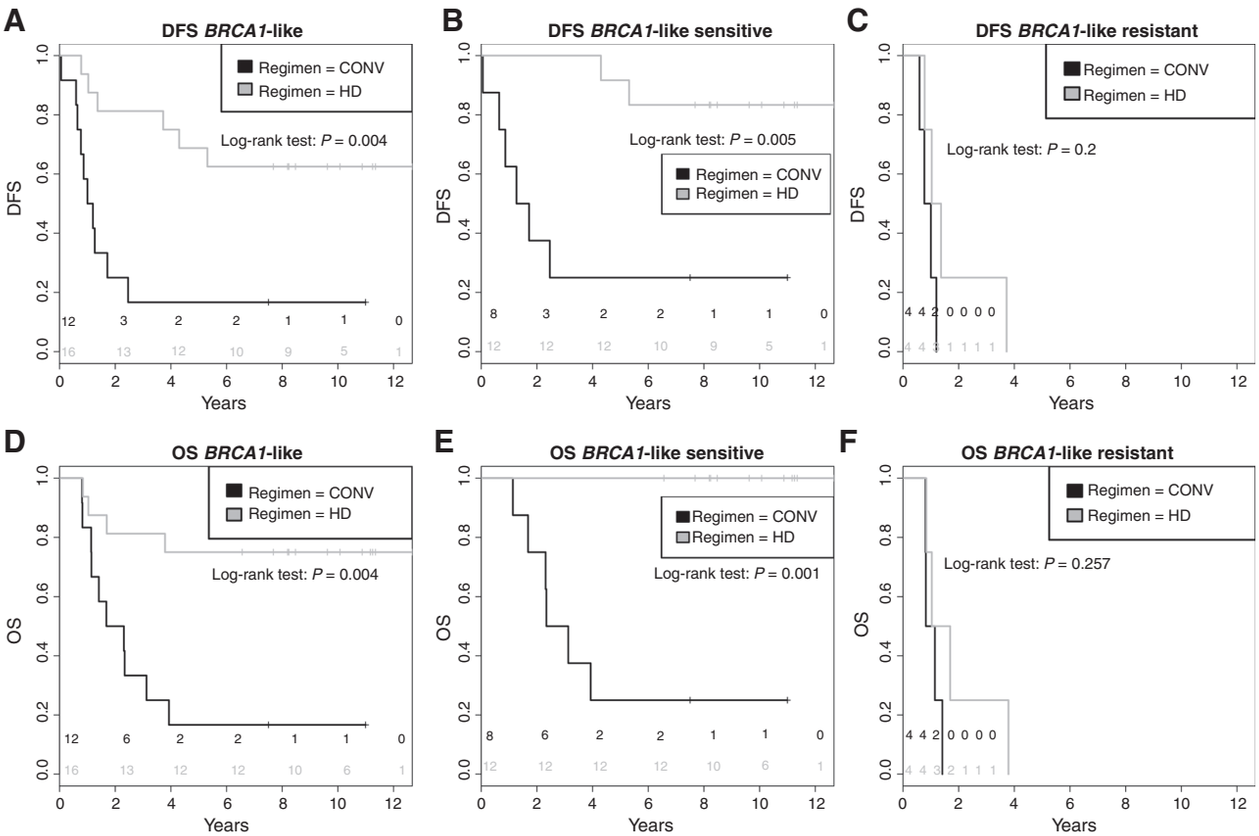

Figure 2.
Kaplan–Meier curves of the *BRCA1*-like cohort analyzed with several biomarker combinations. Patients were treated with HD alkylating or CONV chemotherapy. An exact log rank test was performed to test whether the treatment arms differed significantly. A, Kaplan–Meier curve of *BRCA1*-like patients for DFS. B, Kaplan–Meier curve for DFS of *BRCA1*-like, *XIST*-low, 53BP1-high (predicted sensitive) patients. C, Kaplan–Meier curve for DFS of *BRCA1*-like, *XIST*-high-or-53BP1-low patients (predicted resistant). D, Kaplan–Meier curve of *BRCA1*-like patients for OS. E, Kaplan–Meier curve for OS of *BRCA1*-like, *XIST*-low-or-53BP1-high (predicted sensitive) patients. F, Kaplan–Meier curve for OS of *BRCA1*-like, *XIST*-high-or-53BP1-low patients (predicted resistant).

models (12, 13), could be used as predictive markers in breast cancer patients. We present early and preliminary evidence that high *XIST* expression or low 53BP1 expression could be used to identify *BRCA1*-like tumors that have early events and poorer outcome than *BRCA1*-like tumors that have low *XIST* and high 53BP1 expression.

The mechanism of loss of *XIST* expression seems to be through loss of the inactive X chromosome (12). We observed that the copy number profiles of some patients hinted at losing an X chromosome. However, we are unable to tell whether this is the inactive X chromosome and whether those without apparent loss may have copy number neutral DNA loss. These

Table 2. Survival analysis of the *BRCA1*-like cohort by *XIST*/53BP1 status and chemotherapy

	Conv				HD				
	Event	*n*	PY	Rate	Event	*n*	PY	Rate	*P* exact lr HD vs. CONV
DFS									
XIST-low-and-53BP1-high	6	8	25.52	0.235	2	12	108.05	0.019	0.005
XIST-high-or-53BP1-low	4	4	3.56	1.124	4	4	6.90	0.580	0.150
RFS									
XIST-low-and-53BP1-high	6	8	25.52	0.235	0	12	116.16	0.000	0.001
XIST-high-or-53BP1-low	4	4	3.56	1.124	4	4	6.90	0.580	0.150
OS									
XIST-low-and-53BP1-high	6	8	33.03	0.182	0	12	116.16	0.000	0.001
XIST-high-or-53BP1-low	4	4	4.19	0.955	4	4	7.36	0.543	0.220

NOTE: Survival analysis of sensitive and resistant patients in the cohort. The number of events (event), total number of patients (*n*), person years (PY), and the event rate (rate = event/py) are shown for the conventionally treated and HD-treated patients. An exact log rank test was performed comparing HD with the conventionally treated patients. DFS, RFS, and OS are shown. For example, for patients with *XIST*-low-and-53BP1-high, 6 recurrences occurred among the 8 patients treated conventionally for an event rate of 0.235/year, whereas no recurrence occurred among the 12 patients who received HD chemotherapy for an event rate of 0/year, a significant difference in event rates ($P = 0.001$).
Abbreviation: lr, log rank.

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Table 3. Unadjusted and adjusted Cox proportional hazards model for DFS of the *BRCA1*-like cohort by *XIST*/53BP1 status and chemotherapy

		Events/*n*	HR (95% CI)	*P*	Interaction *P*
Unadjusted Cox proportional hazards					
XIST-high-or-53BP1-low			4.84 (1.09–21.42)	0.038	
Chemotherapy among *XIST*-high-or-53BP1-low	CONV	4/4	1.00		
	HD	4/4	0.38 (0.08–1.69)	0.201	
Chemotherapy among *XIST*-low-and-53BP1-high	CONV	6/8	1.00		
	HD	2/12	0.10 (0.02–0.49)	0.005	0.22
Adjusted Cox proportional hazards					
Propensity score			0.13 (0.00–11.92)	0.380	
XIST-high-or-53BP1-low			5.55 (1.16–26.55)	0.032	
Chemotherapy among *XIST*-high-or-53BP1-low	CONV	4/4	1.00		
	HD	4/4	0.53 (0.11–2.56)	0.430	
Chemotherapy among *XIST*-low-and-53BP1-high	CONV	6/8	1.00		
	HD	2/12	0.14 (0.03–0.71)	0.018	0.24

NOTE: A Cox proportional hazards model was fit with and without a propensity score (which adjusts for confounders). The hazard rates, 95% CIs, and *P* values for HD vs. conventional treatment in patients with *XIST*-high-or-53BP1-low (resistant) tumors and in patients with *XIST*-low and 53BP1-high (sensitive) tumors are shown. A *P* value for interaction was calculated to determine whether HD vs. conventional treatment outcomes differed significantly between patients with sensitive tumors and patients with resistant tumors. The propensity score row provides the hazard rate and CI of the adjustment variable. Example in the unadjusted analysis, resistant patients have poorer outcome than sensitive patients (4.84 times). Among sensitive patients, HD chemotherapy significantly reduced the hazard by 90%, whereas there is no significant difference between HD and conventional chemotherapy among the resistant patients.

findings fit with the literature in which *XIST* expression, deregulation, and loss of the inactive X chromosome have been described in basal-like breast cancer over the last few years (16–21). Unfortunately, this does not shed light on how *XIST* deregulation or the loss of an inactive X chromosome confers a resistance phenotype. A link between BRCA1 and *XIST* has been investigated intensively (35–39). Furthermore, the deletion of *Xist* in mice leads to hematologic malignancies (40) and genomic instability of the X chromosome (41). One hypothesis would be that true BRCA1-loss–driven cancers require (a sequence of) events or multiple functions of BRCA1 that results in losing the inactive X chromosome. A tumor that does not lose the inactive X chromosome would not be true BRCA1-loss–driven, but may retain characteristics associated with the dysfunction of a single gene.

The mechanism of action of 53BP1 has been described better, with it being an important factor skewing DNA repair to NHEJ rather than homology-directed DNA repair in the absence of BRCA1. Losing both genes restores this balance and restores some level of HR (42). Genomic loss of the *53BP1* locus may contribute to the low expression of 53BP1, although the size of the aberrations in our series was to be certain that 53BP1 was driving the loss.

Several biomarkers to identify *BRCA1* deficiency have been proposed (mutation status, gene expression (classifiers), methylation, copy number classifiers, etc.; ref. 43). All of these should enrich a patient population for *BRCA1* defects. Clinical data from retrospective studies and early trials suggest that *BRCA1*-mutated tumors are sensitive to DNA DSB-inducing therapy, demonstrated by high pathologic complete remission rates, DFS, or OS benefits (44, 45). However, for none of these markers there is conclusive evidence as many studies have (combinations of) caveats, such as nonrandomized designs, lack of control groups to properly assess whether *BRCA1* status is a prognostic marker or a predictive marker, the use of mixed chemotherapy regimens or lack of replication in independent studies. In this study, we used the genomic signature of *BRCA1*-mutated breast cancer, as it seems to have several advantages over other markers. First, it identifies both *BRCA1*-mutated cancers and tumors with *BRCA1* promoter methylation (14, 27). This combination enlarges the group of patients with a potential defect in *BRCA1*. This is similar to our current finding that *XIST* and 53BP1 both seem to contribute to part of the poor outcome cases of HD chemotherapy. Secondly, *BRCA1*-like status is a replicated predictor of benefit of HD chemotherapy in a randomized controlled trial (14, 28). This enables us to investigate extra markers on

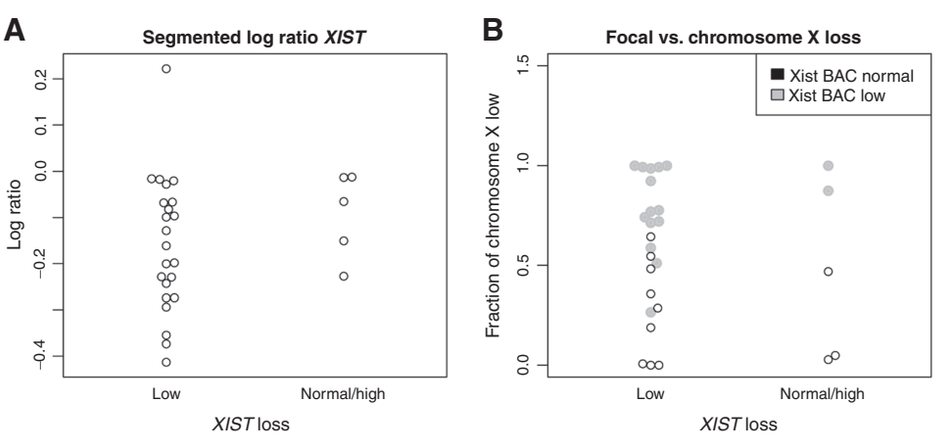

Figure 3.
A, segmented copy number log ratios split by the *XIST* RNA expression groups. A copy number higher than −0.1 was considered no indication for loss of the locus. B, fraction of chromosome X lost split by *XIST* RNA expression groups. 0 indicates 0% of the probes being < −0.1; 1 indicates 100% of the probes being < −0.1. The samples are color-coded based on the call of the *XIST* locus: black-outlined circles for samples in which the *XIST* locus was not lost, grey circles for the samples in which the *XIST* locus was lost.

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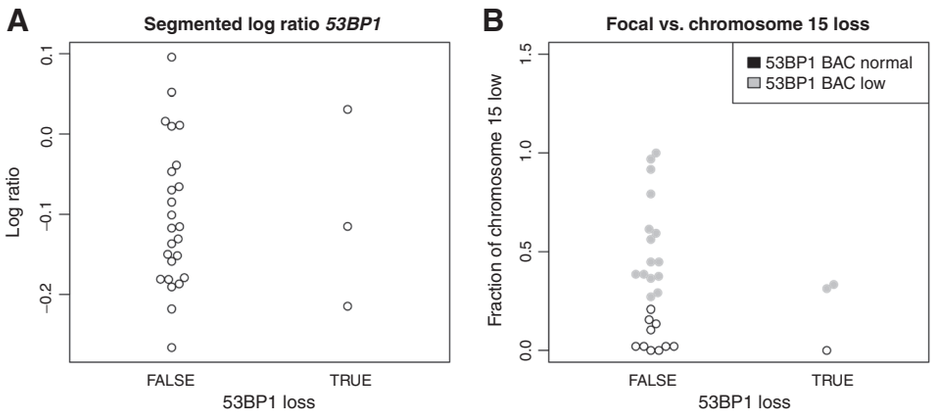

Figure 4.
A, segmented copy number log ratios split by the 53BP1 protein expression groups. A copy number higher than −0.1 was considered no indication for loss of the locus. B, fraction of chromosome 15 lost split by 53BP1 protein expression groups. 0 indicates 0% of the probes being < −0.1; 1 indicates 100% of the probes being < −0.1. The samples are color-coded based on the call of the *53BP1* locus: black-outlined circles for samples in which the *53BP1* locus was not lost, grey circles for the samples in which the *53BP1* locus was lost.

top of *BRCA1* deficiency identified through *BRCA1*-like classification of copy number profiles. Because separate biomarkers usually differ (hopefully slightly if aiming to identify the same population), it is prudent to separately investigate overlaying *XIST* and 53BP1 with other genomic scars (46) that could identify cancers with deficient HR repair until a formal comparison has been made. It is uncertain whether the dose, the specific agents, or the combination of drug and dose confer the benefit of *BRCA1*-like patients for the HD regimen (14, 28, 15, 47). Furthermore, it could be that drugs with a less heavy treatment burden than HD chemotherapy, such as carboplatin or PARP1 inhibitors, may be predicted with similar clinical outcome (48, 49).

Previously, *XIST* and 53BP1 were not assessed in a preselected background enriching for *BRCA1*-deficient tumors based on *BRCA1*-like status. The aim of the study that identified *XIST* was the identification of biomarkers of response to cisplatin (12). However, this finding was subsequently tested in a more general population (HER2-negative breast cancer) than the initial preclinical setting (*BRCA1*-deficient mouse tumors). We would argue that the good outcome observed by Rottenberg and colleagues, who assessed *XIST* as independent marker, can be explained by a strong correlation with *BRCA1*-like status. In the 53BP1 study, only prognostic value was investigated, despite data that suggest a role as predictive rather than prognostic biomarker (13). Unfortunately, investigating putative biomarkers in relation to chemotherapy outcome within a specific genetic defect automatically limits the number of patients, but may be very valuable in evaluating more personalized treatment options. In this case, for example, the heavy burden of HD chemotherapy could be prevented if the poor outcome could be expected, as predicted by the preclinical data and as our clinical data suggest. A robust preclinical modeling system that is indicative of human cancers could thus be very valuable. On the other hand, one could argue that only high prevalence markers with solid statistical proof in large cohorts will ultimately be clinically important (50). Unfortunately, in these large cohorts, the context of the preclinical modeling system is often forgotten. It could be that many biomarkers derived from such modeling systems get lost in translation when the cohorts are not sufficiently matched to the characteristics of the preclinical system. We investigated 53BP1 in the whole cohort (a reasonable powered situation) and found no association and a minor effect in the TN subgroup, in which those patients with 53BP1-expressing tumors benefit from HD regimen. Although potentially interesting, we are not aware of any other evidence explaining the inverse relationship between therapy responses. This association follows the analysis in the *BRCA1*-like patients: TN or *BRCA1*-like patients with a 53BP1-expressing tumor benefit from the HD regimen compared with the conventional regimen. We found similar association of 53BP1 loss in hormone receptor–negative tumors as others did for TN status (although only a trend in our dataset; refs. 13, 33).

As has been discussed for *BRCA1* mutation status, it is extremely rare to have a randomized controlled trial and high numbers of patients to investigate the response of *BRCA1*-associated breast cancer to a specific therapy (51). Even combining all trials that randomized between a HD and conventional therapy regimen would not yield a large number of patients that have the specific combination of biomarkers. Therefore, it seems more feasible to follow these findings up further in prospective studies that investigate therapy that targets a *BRCA1* defect.

For concluding, we show that *XIST* and 53BP1 can be used to identify *BRCA1*-like breast cancer patients that have higher event rates and poor outcome after HD chemotherapy. These observations have to be confirmed in trials with similar questions that have been conducted in the past, or as exploratory analysis in future randomized trials.

Disclosure of Potential Conflicts of Interest

S.C. Linn has ownership interest as co-inventor on a patent application for a BRCAness gene expression classifier. No potential conflicts of interest were disclosed by the other authors.

Authors' Contributions

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Administrative, technical, or material support (i.e., reporting or organizing data, constructing databases): M. Jonkers
Study supervision: J. Wesseling, S.C. Linn

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