Breast cancer 1 (BRCA1) protein expression as a prognostic marker in sporadic epithelial ovarian carcinoma: an NCIC CTG OV.16 correlative study

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Background: Breast cancer 1 (BRCA1) protein inactivation in sporadic ovarian carcinoma (OC) is common and low BRCA1 expression is linked with platinum sensitivity. The clinical validation of BRCA1 as a prognostic marker in OC remains unresolved.

Patients and methods: In 251 patient samples from the NCIC CTG clinical trial, OV.16, BRCA1 protein expression was determined by immunohistochemistry.

Results: For all patients, when BRCA1 score was analyzed as a continuous variable, there was no significant correlation between BRCA1 protein expression and progression-free survival (PFS) [adjusted hazard ratio (HR) = 1.15 (0.96–1.37), P = 0.12] or response rate [HR = 0.89 (0.70–1.12), P = 0.32]. In the 116 patients with minimal residual disease (RD), higher BRCA1 expression correlated significantly with worse PFS [HR = 1.40 (1.04–1.89), P = 0.03]. Subgroup analysis divided patients with minimal RD into low (BRCA1 ≤2.5) and high (BRCA1 >2.5) expression groups. Patients with low BRCA1 expression had a more favorable outcome [median PFS was 24.7 and 16.6 months in patients with low and high BRCA1, respectively; HR = 0.56 (0.35–0.89), P = 0.01].

Conclusions: This study suggests that BRCA1 protein is a prognostic marker in sporadic OC patients with minimal RD. Further research is needed to evaluate BRCA1 as a predictive biomarker and to target BRCA1 expression to enhance chemotherapeutic sensitivity.

Key words: BRCA1, immunohistochemistry, ovarian carcinoma, prognostic marker, sporadic

introduction

Epithelial ovarian carcinoma (OC) is the fifth leading cause of cancer death in North American women [1]. The poor prognosis is mainly due to the lack of an effective screening test, leading many patients to present with advanced stage disease. The mainstay of treatment consists of surgical debulking and chemotherapy with a combined platinum and taxane regimen. Despite a favorable initial response rate to treatment, early recurrences and platinum resistance are frequent problems in patient care, necessitating the discovery of novel biomarkers and molecular targets to help define subsets of patients that may benefit most from treatment. In spite of well-accepted clinicopathologic prognostic factors in OC [2], there is currently no clinically validated tumor biomarker before initiating platinum-based chemotherapy, which correlates with prognosis or chemosensitivity.

Women with mutations in the breast cancer 1 (BRCA1) tumor suppressor gene are at significant risk of developing breast cancer and OC [3, 4]. The specific mechanism of BRCA1-related tumorigenesis is unclear, but BRCA1 binds to numerous proteins and is implicated in several cellular processes including the recognition and response to DNA damage and the activation of specific DNA repair pathways [5]. Women with OC and an inherited mutation of the BRCA1 or BRCA2 genes are believed to have a better prognosis compared with women with sporadic OC as a result of an increased tumor sensitivity to DNA damaging chemotherapeutic drugs, such as cisplatin, due to defective repair of DNA double-strand breaks through the conservative mechanism of homologous recombination [6].

More than 90% of OCs are sporadic events, without an identified germline mutation. The concept of ‘BRCAness’ has evolved to reflect the traits that some sporadic cancers share with those occurring in BRCA1- and BRCA2-mutation carriers.
The silencing or dysfunction of genes in BRCA-related DNA repair pathways is believed to account for the similar phenotype between hereditary and sporadic OCs [7]. The inactivation of BRCA1 in sporadic OC occurs through a number of epigenetic mechanisms such as loss of heterozygosity [8] and aberrant methylation of the BRCA1 promoter [9], resulting in the ability to characterize patient tumors according to their relative BRCA1 deficiency, irrespective of mutation status. BRCA1 has emerged as having broader clinical relevance as a potential prognostic and/or predictive marker in a number of malignancies, including OC [10–12], breast cancer [13, 14] and non-small-cell lung cancer [15].

Clinical validation of BRCA1 as a prognostic and predictive biomarker in sporadic OC patients remains unresolved. BRCA1 protein expression by immunohistochemistry (IHC) may be a clinically useful tool to provide important information on prognosis and to direct patients toward specific targeted therapies, such as poly ADP(ribose) polymerase (PARP) inhibitors, which are dependent on BRCA1 deficiency. The REPorting recommendations for tumor MARKer prognostic studies (REMARK) guidelines were created with the intent to develop rigorouss and consistency of data and ultimately to improve the quality of tumor marker reporting and conduct [16]. The aim of this study was to apply REMARK guidelines to evaluate the BRCA1 protein as a prognostic marker in sporadic OC patients.

**patients and methods**

**patient tumors**

Tissue samples were obtained from patients who participated in OV.16, a phase III trial of cisplatin plus topotecan followed by paclitaxel (Taxol, Bristol-Myers Squibb Canada, Montreal, Canada) plus carboplatin versus standard carboplatin plus paclitaxel as first-line chemotherapy in women with newly diagnosed advanced epithelial ovarian cancer, a Gynecologic Cancer Intergroup Study of the NCIC CTG, European Organization for Research and Treatment of Cancer, the Gynecologic Cancer Group and the Spanish Ovarian Cancer Research Group. Criteria for entry into the study included newly diagnosed OC, fallopian tube or primary peritoneal cancer, International Federation of Gynecology and Obstetrics (FIGO) stage IIb–IV, completed all prechemotherapy surgeries and Eastern Cooperative Oncology Group (ECOG) performance status 0 or 1. The primary end point of the study was progression-free survival (PFS). Eight hundred and nineteen patients were randomized in the study and 650 patients had a progression event. The results of the trial showed that the topotecan arm did not improve efficacy compared with standard therapy and was more toxic [17]. There were 251 tumor samples from patients in Canada available for analysis. Of these samples, there were 116 patients with no macroscopic residual disease (RD) or with minimum RD (macroscopic RD <1 cm).

**tissue microarrays and immunohistochemistry**

Formalin-fixed paraffin-embedded patient tumor samples were collected from various centers participating in the study. Four cores (0.6 mm) of tumor were punched per block by a manual tissue arrayer (MTA-II, Beecher Instruments, Sun Prairie, WI). The completed array included 251 patients with advanced epithelial OC, normal human tissue controls (breast, kidney, spleen, lung, pancreas and myometrium), an unrelated cancer control (mesothelioma) and a BRCA1-wildtype cell line control (MCF7, American Type Culture Collection (ATCC), Manassas, VA).

Sections (5 μm) of the completed tissue microarray were analyzed by standard immunohistochemistry protocols using the intelliPATH FLX automated slide stainer (Biocare Medical, Concord, CA). Staining was carried out with a mouse monoclonal BRCA1 antibody [18] (MS110, Calbiochem, Darmstadt, Germany) at 1:100 dilution in DaVinci universal diluent (Biocare Medical) for 1 h at room temperature. A breast cancer tissue microarray was used as a positive control and the primary antibody was omitted for a negative control. Two independent pathologists were blinded to each other’s scores and to the clinical outcome data. Immunoreactivity to the BRCA1 protein was carried out on duplicate slides and scoring was based on both distribution and staining intensity. The composite IHC scores of the four most representative cores were averaged for each patient tumor and then correlated with outcome parameters.

**statistical analysis**

BRCA1 expression was first analyzed as a continuous variable. To reduce the skewness of the data, a square root transformation was applied to the original BRCA1 expression scores. Exploratory analysis with a minimum P value approach was used then to identify a cut-off, which would divide patients into ‘high’ and ‘low’ BRCA1-expressing group. A Cox model with a single treatment covariate was used to study the correlation between BRCA1 and PFS and response in univariate analyses. Since primary analysis in OV.16 showed debulking with no macroscopic RD or with macroscopic RD (<1 cm), FIGO stage II and performance status were independent prognostic factors for better PFS and multivariate analysis was carried out by adding these three variables in the Cox model. Since a prior study with messenger RNA (mRNA) suggested that BRCA1 expression may be prognostic only for those with less RD [12], and analysis of all patients with BRCA1 expression showed debulking with no macroscopic RD or with macroscopic RD (<1 cm) as an independent factor for longer PFS, subgroup analyses were also carried out in this group of patients with BRCA1 expression data.

**results**

**patient tumors**

Results from 251 tumor samples were received in December 2009 and used for analyses. The clinical data for this analysis were based on the final OV.16 dataset locked in March 2008 for final analysis. The median follow-up of all 819 patients at the time of database lock was 43 months and the numbers of progression events and responses observed were 650 and 282, respectively. For 251 patients with BRCA1 expression data, the median follow-up was 45 months and the numbers of progression events and responses observed were 215 and 118, respectively. The characteristics of all patients and those with BRCA1 expression data are presented in Table 1. There was no apparent difference between the two groups except more patients with BRCA1 expression data had advanced stage disease (IIIc and IV) and more than 70% of the tumors used in this analysis were of papillary serous histology.

**BRCA1 IHC and scoring**

Two hundred and fifty-one patient tumors were tested for BRCA1 immunoreactivity with representative staining, illustrated in Figure 1A. The distribution of IHC score for all tumors is shown in Figure 1B. The mean (standard deviation) and median (range) of the BRCA1 expression scores were 2.3 (2.0) and 1.75 (0–9), respectively. Forty-one (16%) tumors...
had no staining and 122 (49%) tumors had mild staining resulting in 63% of the tumors with an IHC score between 0 and 2.75. In comparison, 35% of tumors showed moderate or strong immunoreactivity to BRCA1. Sixty-one (24%) tumors had moderate staining with an IHC score between 3 and 4.75, while only 27 (11%) tumors had strong staining with an IHC score ≥5.0.

### Analysis with BRCA1 expression as a continuous variable

In the multivariate analysis, BRCA1 expression as a continuous variable was not significantly related to treatment arm \((P = 1.0)\), age \((565 \text{ versus } 665, \ P = 0.12)\), prerandomization surgery \([\text{no debulking versus debulking}\) with no macroscopic RD and debulking with macroscopic RD \((<1 \text{ cm}), \ P = 0.82\) and no debulking versus debulking with macroscopic RD \((>1 \text{ cm}), \ P = 0.83\), stage \((\text{II versus III or IV}, \ P = 0.82)\), grade \((\text{well or moderate versus poor/undifferentiated or unknown}, \ P = 0.90)\), histology \((\text{serous adenocarcinoma versus others}, \ P = 0.07)\) or performance status \((0 \text{ versus } 1, \ P = 0.74)\).

In the univariate analysis, BRCA1 expression had a trend to significant correlation with PFS \([HR = 1.19 \text{ with } 95\% \text{ confidence interval (CI)} \text{ from } 1.00 \text{ to } 1.42, \ P = 0.053]\), but no significant correlation was found with response rate \((HR = 0.91 \text{ with } 95\% \text{ CI from } 0.72 \text{ to } 1.15, \ P = 0.43)\). In the 251 tumors analyzed in the multivariate analysis after adjusting for RD, stage and performance status, correlation was not significant between BRCA1 expression and PFS (adjusted HR = 1.15 with 95% CI from 0.96 to 1.37, \(P = 0.12\)) or response rate (adjusted HR = 0.89 with 95% CI from 0.70 to 1.12, \(P = 0.32\)) (Table 4). Because of issues with multiple comparisons, this cut-off value was 0.08, which was reached when the cut-off point for BRCA1 was 2.5 (i.e. patients were considered as having low BRCA1 expression if their scores were ≤2.5). This implied no significant correlation between BRCA1 expression level and PFS was found with this approach.

When patients were restricted to those with no macroscopic RD or with macroscopic RD \((<1 \text{ cm})\) after debulking (Table 3). In this subgroup of patients, higher BRCA1 expression correlated significantly with worse PFS in both univariate \((HR = 1.39 \text{ with } 95\% \text{ CI from } 1.04 \text{ to } 1.85, \ P = 0.03)\) and multivariate analysis adjusting for stage and performance status \((\text{adjusted HR} = 1.40 \text{ with } 95\% \text{ CI from } 1.04 \text{ to } 1.89, \ P = 0.03)\). Again, no significant correlation was found with response rate \((HR = 0.98 \text{ with } 95\% \text{ CI from } 0.59 \text{ to } 1.63, \ P = 0.94)\) in univariate analysis and in multivariate analysis \((\text{adjusted HR} = 0.91 \text{ with } 95\% \text{ CI from } 0.55 \text{ to } 1.52, \ P = 0.73)\) in this group of patients.

### Exploratory analyses with minimum \(P\) value approach

When all patients were included in the multivariate analysis, the smallest \(P\) value was 0.08, which was reached when the cut-off point for BRCA1 was 2.5 (i.e. patients were considered as having low BRCA1 expression if their scores were ≤2.5). This implied no significant correlation between BRCA1 expression level and PFS was found with this approach.

When patients were restricted to those with no macroscopic RD or with macroscopic RD \((<1 \text{ cm})\) after debulking, the cut-off with minimum \(P\) value was found again to be 2.5. The corresponding \(P\) value was 0.02 in univariate analysis and 0.01 in multivariate analysis. The adjusted HR between patients with low and high BRCA1 expressions defined with this cut-off was 0.56 with 95% CI from 0.35 to 0.89 from multivariate analysis adjusting for FIGO stage and ECOG performance status (Table 4). Because of issues with multiple comparisons, this cut-off should be further validated in other studies. The Kaplan–Meier curves of PFS by BRCA1 expression status determined by this cut-off are presented in Figure 2. The median PFS was 24.7 and 16.6 months for patients in the low BRCA1-expressing groups relative to the high BRCA1-expressing group.

### BRCA1 as a predictive marker for topotecan sensitivity

The results of OV.16 showed that after a median follow-up of 43 months, topotecan did not improve the efficacy of standard treatment with carboplatin and taxol [median PFS 14.6 versus 16.2 months; \(HR = 1.10 \text{ (0.94–1.28), } P = 0.25\)] [17]. In the
present study, among all patients with BRCA1 ≤2.5, those who received topotecan/carboplatinum/taxol had a significantly worse PFS compared with carboplatinum/taxol alone [median 15.0 versus 19.6 months; HR = 1.52 (1.07–2.16), \( P = 0.02 \)]. In contrast, among the patients with BRCA1 >2.5, there was no significant difference in PFS between patients treated with topotecan/carboplatinum/taxol and carboplatinum/taxol [median 14.6 versus 14.7 months; HR = 1.08 (0.70–1.67), \( P = 0.73 \)]. Importantly, in the subset of patients who received topotecan/carboplatinum/taxol, who had RD <1 cm, those with BRCA1 ≤2.5 had a significantly better PFS than those with BRCA1 >2.5 [median 24.3 versus 14.8 months; HR = 0.39 (0.21–0.72), \( P = 0.002 \)].

**discussion**

This NCIC CTG-led OV.16 correlative study is the largest correlative study to date of BRCA1 protein expression by immunohistochemistry with corresponding clinical outcome data in OC. This study supports that the BRCA1 protein is a valid biomarker of prognosis only in optimally debulked OC patients, irrespective of BRCA1 mutation status. In the 116 patients with RD <1 cm, lower BRCA1 expression significantly correlated with a better PFS (HR = 0.56). There are a number
of reports which substantiate the findings of this study [10, 20]. A correlative study of Gynecologic Oncology Group 172, in which all patients had no residual mass >1 cm, examined 152 sporadic OCs and showed that minimal BRCA1 protein expression was protective for PFS and overall survival (OS) [10]. A more recent study in 115 primary sporadic OC also provided evidence that BRCA1 protein loss in primary neoplasms was associated with better survival [20]. This group also demonstrated that in paired specimens, BRCA1 protein expression increased in 62% of recurrent carcinomas with low or intermediate protein expression in the paired primary, suggesting a mechanism for mediating platinum resistance during the course of disease.

However, there are also conflicting reports [21, 22], revealing that there are several obstacles in the comparison of studies on prognostic markers. First, there may be inconsistencies in the antibody used between reports. In the study of 87 OC patients, there was no association between BRCA1 expression and survival using a different BRCA1 antibody from the current study, Ab No. 345P [21]. A comparative study between various BRCA1 antibodies obtained superior results with the anti-BRCA1, MS 110 (Ab-1) monoclonal antibody in the correlation between immunohistochemistry and RT-PCR data [23]. In addition, the method of BRCA1 immunohistochemistry scoring may vary between reports. For instance, the previously reported method adopted by Wilson et al. [24] does not incorporate staining intensity [10]. Finally, there is considerable subjectivity between studies in the classification of relatively low, intermediate and high BRCA1-expressing tumors, with varying cut-off points in the ‘high’ group from >30% to >70% [20] used in the present study. Application of the principles outlined in the REMARK guidelines will undoubtedly improve in overcoming such inconsistencies in tumor marker studies [16].

### Table 2. Multivariate analysis based on BRCA1 expression as a continuous variable for all patients with BRCA1 expression data

<table>
<thead>
<tr>
<th>Factor</th>
<th>n</th>
<th>Adjusted HR for PFS (95% CI), P</th>
<th>Adjusted HR for response (95% CI), P</th>
</tr>
</thead>
<tbody>
<tr>
<td>Square root of BRCA1 expression</td>
<td>251</td>
<td>1.15 (0.96–1.37), 0.12</td>
<td>0.89 (0.70–1.12), 0.32</td>
</tr>
<tr>
<td>Prerandomization surgery</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Debulking with no macroscopic RD</td>
<td>116</td>
<td>0.59 (0.39–0.90), 0.01</td>
<td>0.34 (0.19–0.62), 0.0004</td>
</tr>
<tr>
<td>and debulking with macroscopic RD (&lt;1 cm)</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Debulking with macroscopic RD (≥1 cm)</td>
<td>99</td>
<td>1.12 (0.74–1.70), 0.60</td>
<td>1.07 (0.65–1.75), 0.80</td>
</tr>
<tr>
<td>No debulking</td>
<td>32</td>
<td>1.00</td>
<td>1.00</td>
</tr>
<tr>
<td>FIGO stage</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>II</td>
<td>24</td>
<td>0.38 (0.21–0.71), 0.003</td>
<td>0.27 (0.06–1.12), 0.07</td>
</tr>
<tr>
<td>III or IV</td>
<td>227</td>
<td>1.00</td>
<td>1.00</td>
</tr>
<tr>
<td>ECOG performance status</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>0</td>
<td>92</td>
<td>0.98 (0.70–1.37), 0.90</td>
<td>0.99 (0.62–1.59), 0.97</td>
</tr>
<tr>
<td>1</td>
<td>137</td>
<td>1.00</td>
<td>1.00</td>
</tr>
</tbody>
</table>

BRCA1, breast cancer 1; PFS, progression-free survival; CI, confidence interval; RD, residual disease; FIGO, International Federation of Gynecology and Obstetrics; ECOG, Eastern Cooperative Oncology Group.

### Table 3. Multivariate analysis based on BRCA1 expression as a continuous variable for all with BRCA1 expression data with no macroscopic RD or with macroscopic RD (<1 cm) after debulking

<table>
<thead>
<tr>
<th>Factor</th>
<th>n</th>
<th>Adjusted HR for PFS (95% CI), P</th>
<th>Adjusted HR for response (95% CI), P</th>
</tr>
</thead>
<tbody>
<tr>
<td>Square root of BRCA1 expression</td>
<td>116</td>
<td>1.40 (1.04–1.89), 0.03</td>
<td>0.91 (0.55–1.52), 0.73</td>
</tr>
<tr>
<td>FIGO stage</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>II</td>
<td>20</td>
<td>0.30 (0.14–0.63), 0.001</td>
<td>0.18 (0.02–1.36), 0.10</td>
</tr>
<tr>
<td>III or IV</td>
<td>96</td>
<td>1.0</td>
<td>1.00</td>
</tr>
<tr>
<td>ECOG performance status</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>0</td>
<td>32</td>
<td>1.16 (0.71–1.91), 0.56</td>
<td>0.64 (0.70–3.92), 0.27</td>
</tr>
<tr>
<td>1</td>
<td>84</td>
<td>1.0</td>
<td>1.00</td>
</tr>
</tbody>
</table>

BRCA1, breast cancer 1; RD, residual disease; PFS, progression-free survival; CI, confidence interval; FIGO, International Federation of Gynecology and Obstetrics; ECOG, Eastern Cooperative Oncology Group.

### Table 4. Multivariate analysis based on minimum $P$ value cut-off for 116 patients with BRCA1 expression data with no macroscopic RD or with macroscopic RD (<1 cm) after debulking

<table>
<thead>
<tr>
<th>Factor</th>
<th>n (%)</th>
<th>Adjusted HR for PFS (95% CI), P</th>
</tr>
</thead>
<tbody>
<tr>
<td>BRCA1 expression</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Low ($\leq 2.5$)</td>
<td>75 (65)</td>
<td>0.56 (0.35–0.01), 0.01</td>
</tr>
<tr>
<td>High ($&gt;2.5$)</td>
<td>41 (35)</td>
<td>1.0</td>
</tr>
<tr>
<td>FIGO stage</td>
<td></td>
<td></td>
</tr>
<tr>
<td>II</td>
<td>20 (17)</td>
<td>0.30 (0.15–0.64), 0.002</td>
</tr>
<tr>
<td>III or IV</td>
<td>96 (83)</td>
<td>1.0</td>
</tr>
<tr>
<td>ECOG performance status</td>
<td></td>
<td></td>
</tr>
<tr>
<td>0</td>
<td>32 (28)</td>
<td>1.10 (0.66–1.83), 0.71</td>
</tr>
<tr>
<td>1</td>
<td>84 (72)</td>
<td>1.0</td>
</tr>
</tbody>
</table>

BRCA1, breast cancer 1; RD, residual disease; PFS, progression-free survival; CI, confidence interval; FIGO, International Federation of Gynecology and Obstetrics; ECOG, Eastern Cooperative Oncology Group.
While protein analysis has several limitations, other potential assays also have challenges to consider. RNA expression analysis has the advantage of quantitative rather than qualitative analysis, but frozen tissue is rarely available and RNA quality from paraffin tissue renders severe limitations on interpretable data. The characterization of a molecular biomarker requires that the test not only must be reproducible and have acceptable sensitivity and specificity but also must be relatively inexpensive. Other groups have suggested that gene expression signatures can be used to stratify patients according to survival and response to chemotherapy [25], but the inherent cost associated with such studies is a significant drawback.

A number of well-established adverse prognostic factors for advanced OC that are based on clinicopathologic criteria are increasing age, advanced stage, mucinous and clear cell histology, performance status 1 or 2 and residual tumor volume >1 cm [2]. A plethora of biomarker studies in OC has also addressed early detection of disease as well as patient prognosis with the majority of markers having little clinical relevance. The results of mainly retrospective studies have concluded that CA125 levels at the time of diagnosis are of limited prognostic significance, but decreasing levels of CA125 after cytoreductive surgery and during chemotherapy correlate with response and survival [26]. The role of CA125 as a predictive marker of OS in advanced OC remains unclear. HE4 is expressed in one-third of OC without CA125 expression, and serum HE4 has been shown to improve the prediction of malignancy in ovarian masses [27]. Other potential markers of patient prognosis have been studied extensively, such as p53, HER-2/neu and cyclin E. Only high cyclin E protein (≥40% cyclin E-positive tumor cells) by immunohistochemistry has been associated with a worse OS in patients with suboptimally debulked advanced OC [28]. The findings in the current study support our previous report that emphasized the importance of RD status in biomarker studies in OC [12]. The prognostic value of BRCA1, which is lost in the presence of RD >1 cm, suggests that significant RD is an overriding prognostic factor that needs to be accounted for in future biomarkers studies in OC.

Currently, there is no suggestion that denying a patient standard platinum-based treatment is valid based on any molecular biomarker since there is no alternate first-line therapy that is superior to platinum. However, moving forward in the era of molecular inhibitors, as more targeted agents are being tested in phase II/III clinical trials in OC, BRCA1 should be further evaluated as a potential predictive marker. Whether BRCA1 protein expression may be a strong enough predictive biomarker to associate with response or lack of response to a particular therapy remains to be determined. OV.16 was a negative trial, with no additional efficacy in the addition of topotecan to platinum/taxol chemotherapy. Although the current study was not designed to evaluate BRCA1 expression as a predictive marker of topotecan benefit, the mechanism of action of a topoisomerase I (Top1) inhibitor, which ultimately leads to double strand breaks, suggests that BRCA1 function may be of relevance for topotecan activity. This may warrant investigation in second-line use of the single-agent topotecan in a selected group of patients. In the current study, the group of patients with low BRCA1 who received triple therapy with topotecan had a significantly worse PFS compared with those who did not receive topotecan. One hypothesis to explain this finding is that patients in the topotecan arm received a lower platinum dose (50 mg/m2) than in the standard treatment arm and experienced more treatment delays, thus leading to less platinum exposure. Furthermore, the group by Zander et al. [29], which studied a genetically engineered mouse model for BRCA1-deficient breast cancer, found that tumors in all 20 xenografts had an initial response to topotecan. However, all xenografts soon acquired drug resistance in association with overexpression of Abcg2/Bcrp and markedly reduced protein levels of the drug target Top1, a further mechanism that may explain the inferior response in the topotecan group.

PARP1 is an enzyme that involved in the repair of DNA single-strand breaks through the base excision repair pathway, and BRCA1-deficient cells which have defective homologous recombination repair, are known to be sensitive to PARP inhibitors [30, 31]. A number of clinical trials have demonstrated that PARP inhibitors may be particularly useful for the treatment of patients with an inherited mutation in BRCA1/BRCA2 [32, 33]. Given the significant proportion of sporadic OCs that demonstrate BRCA1 deficiency, a reliable assay to detect BRCA1 expression is required to identify those patients who may be the most susceptible to this class of targeted agent. The current report supports the immunohistochemical technique as a potential assay to predict response to PARP inhibitors and further study in this area is urgently needed.

There are limitations to this study which merit consideration. First, the patients who are BRCA1 mutation carriers have not been identified in this study. It is anticipated that the

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**Figure 2.** Kaplan–Meier curves between low and high breast cancer 1 (BRCA1) expression defined by minimum P value cut-off for 116 patients with no macroscopic residual disease (RD) or with macroscopic RD <1 cm after debulking. The median progression-free survival (PFS) was 24.7 and 16.6 months for patients with respective low (BRCA1 scores ≤5.25) and high BRCA1 expression. The adjusted HR between patients with low and high BRCA1 expression defined by this cut-off was 0.56, 95% confidence interval (CI) 0.35–0.89, P = 0.01, in the multivariate analysis adjusting for International Federation of Gynecology and Obstetrics (FIGO) stage and Eastern Cooperative Oncology Group (ECOG) performance status.
prevalence of women who carry a BRCA1 mutation is comparable to that of the general population, but the slightly younger median age at presentation in the study population could imply a higher rate of gene mutation. The MS110 antibody demonstrates BRCA1 immunoreactivity in tumors from patients with most BRCA1 mutations except for those who are very proximal to the N-terminal, such as 185delAg [34]. As a result, the MS110 antibody cannot reliably differentiate between tumors that are associated with hereditary versus sporadic carcinoma. BRCA1 staining may be positive in BRCA1-mutated tumors since BRCA1 mRNA expression is related to the retention of wild-type alleles or to heterozygosity among cancer cells [18, 35]. However, previous reports have shown that the expression of BRCA1 mRNA was low, regardless of the type of mutation. Complete loss of nuclear BRCA1 staining in familial BRCA1-associated breast carcinomas was found in ~40% of patients [18, 34]. In the study by Yoshikawa et al. [18], of the 19 breast cancer patients with germline mutations of the BRCA1 gene detected by MS110, BRCA1 protein expression level was reduced (1+ or 0) in 7 of 11 tumors associated with truncating mutations, and in all eight carcinomas with intronic or missense mutations, including five with complete loss of BRCA1 protein expression. Given these findings, the proportion of patients who are BRCA1 mutation carriers that demonstrate high BRCA1 protein expression by immunohistochemistry in our study is expected to be low. Nonetheless, the use of the MS110 antibody as a biomarker may be best applied to the majority of patients with sporadic OC.

Prognostic and predictive studies using immunohistochemistry which adhere to standardized criteria remain valid in clinical application and future efforts should focus on the discovery of a panel of biomarkers in OC, including BRCA1, which may guide patient care in selected populations. RD status appears to be an overlapping prognostic marker, such that the potential benefit of knowing biomarker status may only be applicable in patients with optimal surgical debulking. This study supports BRCA1 as one of the most important prognostic markers in OC and further studies are needed to explore its therapeutic potential.

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Disclosure
The authors declare no conflicts of interest.

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