VOLUME 28 · NUMBER 22 · AUGUST 1 2010

JOURNAL OF CLINICAL ONCOLOGY

ORIGINAL REPORT

Gene Expression Profile of *BRCA*ness That Correlates With Responsiveness to Chemotherapy and With Outcome in Patients With Epithelial Ovarian Cancer

Panagiotis A. Konstantinopoulos, Dimitrios Spentzos, Beth Y. Karlan, Toshiyasu Taniguchi, Elena Fountzilas, Nancy Francoeur, Douglas A. Levine, and Stephen A. Cannistra

See accompanying article on page 3545

From the Beth Israel Deaconess Medical Center, Harvard Medical School, Boston, MA; Cedars-Sinai Medical Center, Los Angeles, CA; Howard Hughes Medical Institute, Chevy Chase, MD; Fred Hutchinson Cancer Research Center, Seattle, WA; and Memorial Sloan-Kettering Cancer Center, New York, NY.

Submitted December 16, 2009; accepted March 12, 2010; published online ahead of print at www.jco.org on June 14, 2010.

Supported in part through the Ovarian Cancer Specialized Program of Research Excellence (SPORE) Grant No. P50 CA105009 Career Development Award, the Bernice Shopkin Weisman Fund, the Ovarian Cancer Research Fund in memory of Amy Sachs Simon, LeAnn's Project, and the Sisters Against Ovarian Cancer.

Authors' disclosures of potential conflicts of interest and author contributions are found at the end of this article.

Corresponding author: Stephen A. Cannistra, MD, Program of Gynecologic Medical Oncology, Beth Israel Deaconess Medical Center, 330 Brookline Ave, Boston, MA 02215; e-mail: scannist@bidmc.harvard.edu.

0732-183X/10/2822-3555/$20.00

DOI: 10.1200/JCO.2009.27.5719

ABSTRACT

Purpose
To define a gene expression profile of *BRCA*ness that correlates with chemotherapy response and outcome in epithelial ovarian cancer (EOC).

Methods
A publicly available microarray data set including 61 patients with EOC with either sporadic disease or *BRCA1/2* germline mutations was used for development of the *BRCA*ness profile. Correlation with platinum responsiveness was assessed in platinum-sensitive and platinum-resistant tumor biopsy specimens from six patients with *BRCA* germline mutations. Association with poly-ADP ribose polymerase (PARP) inhibitor responsiveness and with radiation-induced RAD51 foci formation (a surrogate of homologous recombination) was assessed in Capan-1 cell line clones. The *BRCA*ness profile was validated in 70 patients enriched for sporadic disease to assess its association with outcome.

Results
The *BRCA*ness profile accurately predicted platinum responsiveness and mutation status in eight of 10 patient-derived tumor specimens and between PARP-inhibitor sensitivity and resistance in four of four Capan-1 clones. When applied to the 70 patients with sporadic disease, patients with the *BRCA*-like (BL) profile had improved disease-free survival (34 months *v* 15 months; log-rank $P = .013$) and overall survival (72 months *v* 41 months; log-rank $P = .006$) compared with patients with a non–*BRCA*-like (NBL) profile, respectively. The *BRCA*ness profile maintained independent prognostic value in multivariate analysis, which controlled for other known clinical prognostic factors.

Conclusion
The *BRCA*ness profile correlates with responsiveness to platinum and PARP inhibitors and identifies a subset of sporadic patients with improved outcome. Additional evaluation of this profile as a predictive tool in patients with sporadic EOC is warranted.

J Clin Oncol 28:3555-3561. © 2010 by American Society of Clinical Oncology

INTRODUCTION

Both BRCA1 and BRCA2 proteins are involved in the process of homologous recombination (HR), which mediates repair of double-stranded DNA breaks.[1] Patients with ovarian cancer with germline mutations in either *BRCA1* or *BRCA2* genes exhibit impaired ability to repair double-stranded DNA breaks via HR, which may partly explain the heightened sensitivity to platinum and the more favorable survival compared with wild-type counterparts.[2-4] Furthermore, in the setting of defective HR, it has been shown that inhibition of a second DNA repair pathway, such as base excision repair (BER), is often a lethal event.[5-7] On the basis of this observation, there has been great interest in developing inhibitors of the BER pathway for use as possible therapeutic agents in patients with ovarian cancer with germline *BRCA1/2* mutations.[8,9] Drugs that target BER typically inhibit poly-ADP ribose polymerase (PARP), an enzyme critical to BER, and have already shown promising activity in patients with recurrent ovarian cancer who harbor germline *BRCA1/2* mutations.[10,11]

The promise of PARP inhibitors in the management of epithelial ovarian cancer (EOC) is tempered by the presence of a germline mutation in *BRCA1* or *BRCA2* in only approximately 10% of

such patients.[12-14] At first glance, this might imply that 90% of patients with this highly lethal disease would not benefit from this novel class of drugs. However, it has been hypothesized that a subset of sporadic EOCs may harbor abnormalities in the HR pathway that could be associated with improved response rate and survival after treatment with platinum compounds in the absence of germline *BRCA1/2* mutation.[4,15] This *BRCA*ness phenotype may be due in part to defective HR related to several mechanisms, including epigenetic hypermethylation of the *BRCA1* promoter,[16-19] somatic mutation of *BRCA1/2*,[18,20-22] or loss of function mutations in other HR pathway genes.[23,24] At present, however, it has not been possible to reliably identify such patients on the basis of molecular- or protein-based biomarkers, and the concept of *BRCA*ness for patients with the sporadic form of the disease has remained elusive.

Given the heterogeneous mechanisms by which an ovarian cancer cell might develop defective HR, we reasoned that a broad-based approach that makes few assumptions about mechanism might have the highest chance of identifying patients with a *BRCA*ness phenotype. Microarray gene expression profiling lends itself to this goal, because it is not mechanism based and has already been demonstrated to have prognostic as well as predictive potential in EOC.[25-27] In this study, we show that it is possible to define a gene expression profile of *BRCA*ness, associated with responsiveness to platinum and PARP inhibitors, and we correlate this profile with important outcome measures in patients with the sporadic form of EOC.

METHODS

Development of a Gene Expression Profile of BRCA*ness*

For the purpose of profile development, we used a publicly available microarray data set that included tumor expression data from 61 patients with pathologically confirmed EOC, including 34 with *BRCA1/2* germline mutations (n= 18, *BRCA1*; n = 16, *BRCA2*), and 27 without either mutation (ie, sporadic cancers).[28] We used genome-wide hierarchical clustering to define *BRCA*-like (BL) and non–*BRCA*-like (NBL) tumors (Appendix Fig A1, online only; Data Supplement).

Patient Samples

Two patient cohorts were used in this study. The first included six patients with EOC with *BRCA1/2* germline mutations; both have been previously described.[29,30] Four patients had paired samples, before and after the development of platinum resistance, and two had samples obtained only at the time of platinum-sensitive disease.

The second patient cohort included 70 patients who were treated at Beth Israel Deaconess Medical Center, Memorial Sloan-Kettering Medical Center, and Cedars-Sinai Medical Center and who underwent exploratory laparotomy for diagnosis, staging, and debulking followed by first-line platinum-based chemotherapy. Standard post-chemotherapy surveillance included serial physical examination, serum CA-125 level, and computed tomography scanning as clinically indicated.

The study protocol for collection of tissue and clinical information for all patients was approved by the institutional review boards at all three institutions, and patients provided written informed consent authorizing the collection and use of the tissue for study purposes. Additional details are provided in the Data Supplement.

Cell Lines and RNA Isolation and Affymetrix GeneChip Hybridization

Twelve cisplatin-resistant clones of the *BRCA2*-mutated pancreatic cancer cell line Capan-1 have been previously described.[29] Total RNA isolation, microarray hybridization (U133 Plus 2.0 Array GeneChip; Affymetrix, Santa Clara, CA), and data processing were performed as previously described.[26,27,31]

Statistical Analysis

The association between the *BRCA*ness profile and various clinicopathologic factors was assessed by the Fisher's exact test. Overall survival (OS) and disease-free survival (DFS) curves were generated by the Kaplan-Meier method, and differences between survival curves were assessed for statistical significance with the log-rank test. Multivariate analyses to adjust for known prognostic factors were performed by using a Cox proportional hazards regression model that included grade (1 to 2 *v* 3), age ($<$ 65 years *v* $\geq$ 65 years), stage (2 or *v* 3 or 4), histology (clear-cell, papillary serous, endometrioid), debulking status (optimal [less than or equal to 1 cm] or suboptimal [greater than 1 cm] residual disease), and *BRCA*ness profile (BL *v* NBL).

RESULTS

Characteristics of the BRCA*ness Profile*

The strategy for developing the *BRCA*ness profile is described in detail in Appendix Figure A1. The optimal classifier was a 60-gene, diagonal linear discriminant predictor that distinguished BL from NBL tumors with 94% accuracy, as assessed by leave-one-out cross-validation and a 1,000 random permutations test (Fig 1; $P < .001$). Other predictive algorithms performed similarly, such as compound covariate predictor (92%), nearest centroid (92%), and support vector

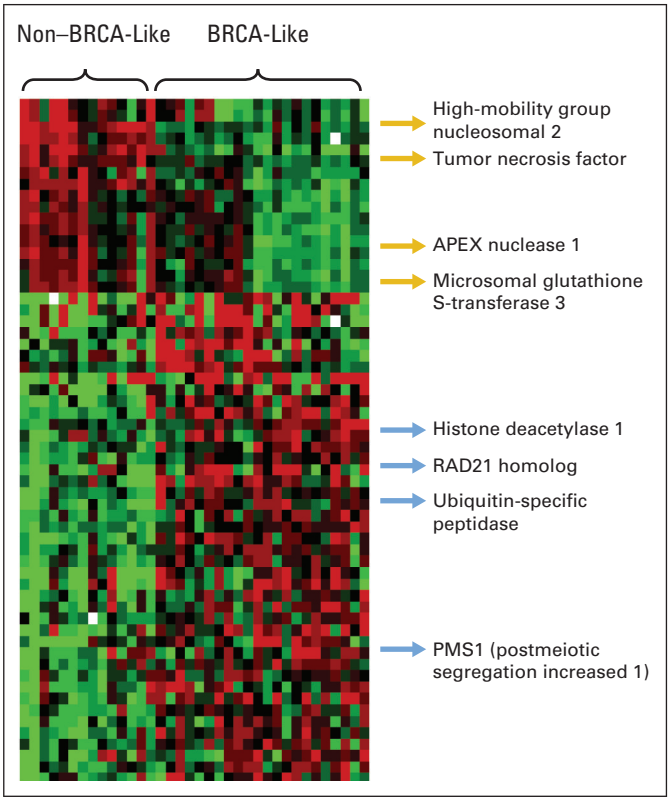
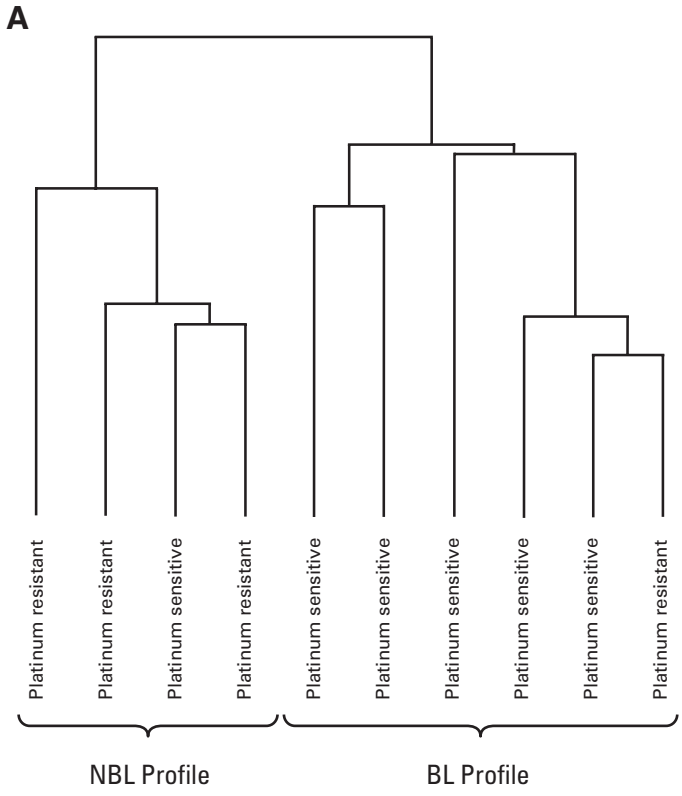


Fig 1. Expression plot of the 60 genes that comprise the *BRCA*ness profile. Columns represent set samples; rows, gene expression levels (normalized). Complete information regarding gene identity is provided in Appendix Table A1. Red indicates overexpressed genes; green, underexpressed genes. The gene expression signature that correlates with *BRCA*-like tumors is defined as the BL profile, and the signature that correlates with non–*BRCA*-like tumors is defined as the NBL profile.



B

Patient No.	BRCA Status	Platinum	Profile
1	BRCA-1 mutation	Sensitive	BL
	BRCA-1 revertant	Resistant	NBL
2	BRCA-1 mutation	Sensitive	BL
	BRCA-1 revertant	Resistant	NBL
3	BRCA-2 mutation	Sensitive	BL
	BRCA-2 revertant	Resistant	BL
4	BRCA-2 mutation	Sensitive	NBL
	BRCA-2 revertant	Resistant	NBL
5	BRCA-1 mutation	Sensitive	BL
6	BRCA-1 mutation	Sensitive	BL

Fig 2. *BRCA*ness profile distinguishes between platinum-sensitive and platinum-resistant tumor biopsy specimens in patients with known *BRCA* germline mutation status. (A) Hierarchical clustering that is based on the expression pattern of the 60 genes of the *BRCA*ness profile distinguished between platinum-resistant and platinum-sensitive tumor biopsy samples. Platinum sensitivity was defined as a complete response to treatment maintained without progression for at least 6 months after platinum therapy. Platinum resistance was defined as progressive disease on platinum therapy, or less than a complete response to platinum therapy, or progression within 6 months of completing platinum therapy. (B) Correlation of *BRCA*ness profile with platinum sensitivity and *BRCA* germline mutation status. The *BRCA*ness profile accurately distinguished between platinum sensitivity and platinum resistance in eight of 10 tumor specimens, which in turn correlated with the presence of mutated versus functional *BRCA* gene status, respectively. NBL, non–*BRCA*-like; BL, *BRCA*-like.

machines (92%).[32-35] For the analyses described in the Results section, the gene expression signature that correlates with BL tumors is defined as the BL profile, and the signature that correlates with NBL tumors is defined as the NBL profile. The identities of all *BRCA*ness profile genes are provided in Appendix Table A1 (online only).

BRCA*ness Profile Distinguishes Between Platinum-Sensitive and Platinum-Resistant Tumor Biopsy Samples*

We first investigated whether the *BRCA*ness profile could correlate with platinum responsiveness in patients with known *BRCA* germline mutation. For this purpose, we used 10 tumor biopsy specimens from six patients with either *BRCA1* or *BRCA2* germline mutation, four of whom were initially platinum sensitive but eventually developed platinum resistance (with pre- and post-biopsy pairs). These patients formed the basis of two previous reports, in which reversion of the *BRCA* genotype occurred (with re-establishment of *BRCA* function) on the development of platinum resistance.[29,30] A separate report that used the CAPAN-1 cell line demonstrated similar findings.[36] Thus, these samples afforded us with an opportunity to determine how the *BRCA*ness profile correlated with both platinum responsiveness and *BRCA* functional status (eg, mutant *v* revertant *BRCA* gene).

As shown in Figure 2, the *BRCA*ness profile could accurately distinguish between platinum sensitivity and platinum resistance in eight of 10 tumor specimens, which in turn correlated with the presence of mutated versus revertant (ie, functional) *BRCA* gene status, respectively. Specifically, five of six tumors with the BL signature were platinum sensitive (and were *BRCA1* or *BRCA2* mutated), whereas three of four tumors with the NBL signature were platinum resistant (and had reverted to functional *BRCA1* or *BRCA2*).[29,30] Furthermore, we observed two patients in whom the *BRCA*ness profile dynamically tracked the development of platinum resistance during the course of therapy (ie, the profile changed from BL to NBL after the development of platinum resistance, associated with reversion to functional *BRCA1*; Fig 2B).

BRCA*ness Profile Correlates With PARP Inhibitor Responsiveness and RAD51 Foci Formation*

As another surrogate of *BRCA*ness, we next evaluated whether the profile could correlate with the ability to form RAD51 foci after ionizing radiation, which is a surrogate of intact HR, as well as with responsiveness to PARP inhibitors. For this purpose, we used 12 clones of the *BRCA2*-mutated pancreatic cancer cell line Capan-1, previously characterized (by T.T.).[29] These clones were generated by exposing the parent Capan-1 cell line to platinum-selection pressure,

Table 1. Association of *BRCA*ness Profile With *BRCA2* Mutation Status, RAD51 Foci Formation After Radiation and PARP Inhibitor Responsiveness

Capan-1 Clone	*BRCA2* Status	RAD51 Foci Formation*	PARP Inhibitor Responsiveness†	Profile
C2-1	Revertant‡	Yes	ND	NBL
C2-2	Revertant	Yes	ND	NBL
C2-4	Revertant	Yes	ND	NBL
C2-14	Revertant	Yes	Resistant	NBL
C2-12	Revertant	Yes	Resistant	NBL
C2-5	Revertant	Yes	ND	BL
C2-16	Mutated§	Yes	ND	BL
C2-10	Mutated	No	Sensitive	BL
C2-13	Mutated	No	Sensitive	BL
C2-15	Mutated	No	ND	BL
C2-18	Mutated	No	ND	BL
C2-8	Mutated	No	ND	NBL

Abbreviations: PARP, poly-ADP ribose polymerase; ND, not determined; NBL, non–*BRCA*-like; BL, *BRCA*-like.

*Ability to form RAD51 formation after ionizing radiation, as previously described.[29]

†PARP inhibitor responsiveness in vitro, as previously described.[29]

‡Revertant refers to clones harboring secondary *BRCA2* mutations that cancel the effect of the inherited 6174delT *BRCA2* mutation and lead to functional *BRCA2* isoforms.

§Mutated refers to clones harboring only the original 6174delT *BRCA2* mutation without acquiring a secondary mutation that restored *BRCA2* function.

Table 2. Clinical and Pathologic Characteristics

Characteristic	Sequenced Cohort (n = 35)* No.	%	Nonsequenced Cohort (n = 35)† No.	%	Combined Cohort (n = 70)‡ No.	%
Age, years§						
Median	62.3		58.7		60.5	
Range	44-89		39-80		39-89	
Grade						
1-2	4	11.4	6	17.1	10	14.3
3	31	88.6	29	82.9	60	85.7
Histology						
Serous	34	97.1	31	88.6	65	92.9
Clear cell	0	0	2	5.7	2	2.9
Endometrioid	1	2.9	2	5.7	3	4.3
Stage						
2	2	5.7	1	2.9	3	4.3
3	28	80	29	82.9	57	81.4
4	5	14.3	5	14.3	10	14.3
Debulking status‖						
Optimal	27	79.4	22	62.9	49	71
Suboptimal	7	20.6	13	37.1	20	29

*All 35 patients underwent germline DNA sequencing and had wild-type *BRCA1* and *BRCA2*.

†Not sequenced but enriched for sporadic disease on the basis of the following: no family history of ovarian cancer, no family history of breast cancer younger than 50 years of age, no family history of more than one breast cancer at any age, and not of Ashkenazi Jewish ethnicity.

‡All patients received first-line platinum-based chemotherapy.

§There was no statistically significant difference in age, grade, histology, stage, or debulking status between sequenced and nonsequenced cohorts.

‖Debulking status was unknown for one patient. Optimal was defined as less than or equal to 1 cm; suboptimal, greater than 1 cm diameter residual disease.

eventually isolating 12 platinum-resistant clones. Seven of these clones formed intact RAD51 foci after ionizing radiation (six of these had reverted to functional *BRCA2* because of secondary *BRCA2* mutations, which canceled the effect of the inherited *BRCA2* mutation), and the remaining five exhibited deficient RAD51 foci formation (all of which contained the inherited, nonfunctional *BRCA2* mutation). PARP inhibitor sensitivity had been determined for four of these clones; two were PARP inhibitor sensitive, and two were PARP inhibitor resistant.[29] When applied to these cell lines, the *BRCA*ness profile correlated with RAD51 foci formation in nine of 12 Capan-1 clones and between presence of mutated versus revertant *BRCA2* gene status in 10 of 12 Capan-1 clones (Table 1). Importantly, the *BRCA*ness profile accurately distinguished between two PARP inhibitor–resistant clones (NBL signature) and two PARP inhibitor–sensitive clones (BL signature).

Relationship Between BRCA*ness Profile and Clinical Outcome in Patients With Sporadic EOC*

These data suggest that the *BRCA*ness profile may correlate with platinum and PARP-inhibitor responsiveness in the context of a known *BRCA* germline mutation, but they do not address whether the profile correlates with outcome in patients with sporadic disease. To test this, we applied the profile to tumor samples from 35 patients with invasive EOC who underwent sequencing for germline mutation (by using DNA obtained from peripheral-blood leukocytes) and did not harbor germline *BRCA1* or *BRCA2* mutations. We also studied an additional 35 patients who did not undergo genetic testing but who were enriched for sporadic disease on the basis of the following characteristics: no family history of ovarian cancer, no family history of breast cancer younger than 50 years of age, no family history of more than one breast cancer at any age, and not of Ashkenazi Jewish ethnicity.[37,38] The clinical and pathologic characteristics of all 70 patients are listed in Table 2. Overall, 20 (29%) of the 70-patient cohort demonstrated the BL profile (eight of 35 in the sequenced group, and 12 of 35 in the nonsequenced group; $P = .43$). Compared with the nonsequenced cohort, the sequenced cohort was enriched for patients with optimally debulked disease, although this did not reach statistical significance (two-sided Fisher's exact $P = .19$). As listed in Table 3, there were no differences in age, stage, grade, histology, or debulking status between the BL and the NBL signature groups. The ability to achieve a clinical remission for the BL and NBL groups was 90% compared with 74%, although this did not reach statistical significance (two-sided Fisher's exact $P = .2$).

For the entire 70-patient cohort, the *BRCA*ness profile was capable of discriminating between long and short median DFS; the patients with BL and NBL profiles had median DFS times of 34 months and 15 months, respectively (log-rank $P = .013$; Fig 3A). In addition, the percentages of patients who were disease free at 4 months for the BL and NBL groups were 90% and 64% ($P = .04$), respectively; at 6 months, percentages were 85% and 60%, respectively ($P = .053$); and at 18 months, percentages were 65% and 29%, respectively ($P = .007$). Finally, the *BRCA*ness profile distinguished between long and short median OS, as the patients in the BL and NBL groups had median OS times of 72 and 41 months, respectively (log-rank $P = .006$; Fig 3B). Similar findings were observed when applying the profile separately to the group of 35 sequenced patients who had undergone germline mutation testing and who were found to have wild-type *BRCA1* and *BRCA2* genes or to the group of the 35 nonsequenced

Table 3. Association of *BRCA*ness Profile With Clinical Characteristics and Remission Status After First-Line Therapy

Characteristic	NBL Profile (n = 50) No.	NBL Profile (n = 50) %	BL Profile (n = 20) No.	BL Profile (n = 20) %	*P*
Age, years					
Median	61		59.25		
Range	39-89		44-80		.55
Grade					
1-2	9	18	1	5	.477
3	41	82	19	95	
Histology					
Serous	46	92	19	95	.412
Clear cell	1	2	1	5	
Endometrioid	3	6	0	0	
Stage					
2	3	6	0	0	.479
3	41	82	16	80	
4	6	12	4	20	
Debulking status*					
Optimal	33	67.3	16	80	.386
Suboptimal	16	32.7	4	20	
Achievement of CR after first-line therapy	37	74	18	90	.2

Abbreviations: NBL, non–*BRCA*-like; BL, *BRCA*-like; CR, complete response.
*Debulking status was unknown for one patient. Optimal was defined as less than or equal to 1 cm; suboptimal, greater than 1 cm.

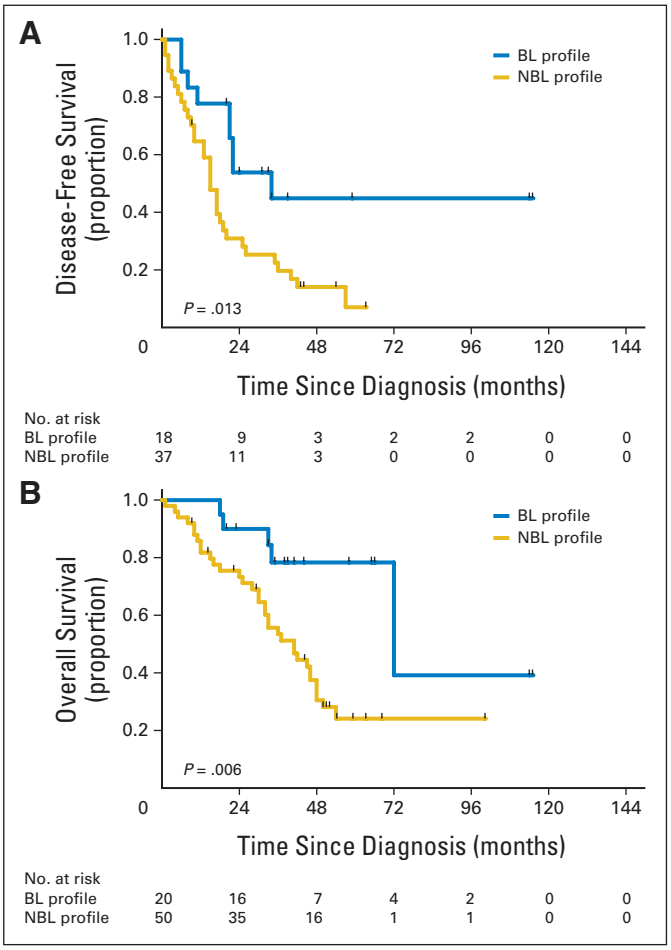


Fig 3. Association of *BRCA*ness profile with disease-free survival (DFS) and overall survival (OS) in the combined patient cohort (N = 70). (A) DFS in the combined patient cohort. The median DFS times for patients with either the *BRCA*-like (BL) or non–*BRCA*-like (NBL) profile were 34 months and 15 months, respectively (log-rank $P = .013$). (B) OS in the combined patient cohort. The median OS times for patients with either the BL or NBL profile were 72 months and 41 months, respectively (log-rank $P = .006$).

patients enriched for sporadic disease on the basis of clinical characteristics, as previously described (Appendix Figs A2 and A3, respectively, online only).

In univariate analysis, the hazard ratio for recurrence (NBL *v* BL group) was 2.47 ($P = .018$; 95% CI, 1.17 to 5.2), and the hazard ratio for death (NBL *v* BL group) was 3.29 ($P = .009$; 95% CI, 1.34 to 8.09; Table 4). Multivariate analysis, which included the *BRCA*ness profile, age, stage, grade, histology, and debulking status, demonstrated that the profile maintained an independent association with DFS and OS. The hazard ratio for recurrence (NBL *v* BL group) was 2.65 ($P = .016$; 95% CI, 1.2 to 5.86), and the hazard ratio for death (NBL *v* BL group) was 3.39 ($P = .009$; 95% CI, 1.35 to 8.5; Table 4). The lack of correlation of characteristics such as stage, grade, and histology with outcome in either univariate or multivariate analysis is likely because the vast majority of patients in this analysis had stage III disease (81%), grade 3 tumors (86%), and serous histology (93%).

DISCUSSION

PARP inhibitors have been evaluated in patients with germline *BRCA1/2* mutations with impressive results as single agents.[10,11] In addition to patients with germline *BRCA1/2* mutations, however, it has been suggested that PARP inhibition might be a useful therapeutic strategy for the treatment of patients with sporadic cancers that have a *BRCA*ness phenotype, characterized by defective HR.[15] In this regard, a number of mechanisms have been identified in sporadic ovarian cancer that might implicate the HR pathway in pathogenesis and in chemotherapy responsiveness. Such mechanisms include somatic *BRCA1/2* mutations in up to 20% of high-grade ovarian cancers[20] as well as mutations or epigenetic silencing in Fanconi anemia genes, intrinsic HR genes, or other DNA damage-response genes.[5,15,17,19,23] Amplification of genes that encode for proteins that inactivate *BRCA2* function, such as *EMSY*, has also been described.[24] *BRCA1* promoter methylation, *FANCF* promoter methylation, and *EMSY* amplification have been identified in 5% to 31%, 21%, and 17% of sporadic EOCs respectively,[15,17,19,23,24] which supports the notion that at least some patients with sporadic disease might harbor defects in HR, independent of the presence of a germline *BRCA1/2* mutation.

Although it is possible to identify individual molecular mechanisms by which the HR pathway might be disrupted in some patients with sporadic EOC, only a few studies have explored the relationship between HR and response to platinum or PARP inhibitors in this setting. D'Andrea et al[23] showed that inhibition of the *FANCF* gene in ovarian cancer cell lines through promoter methylation is associated with enhanced sensitivity to DNA damaging agents such as platinum, whereas demethylation of the *FANCF* promoter results in platinum resistance. Mccabe et al[5] showed that cells deficient in the expression of genes involved in HR (eg, *RAD51, ATR, ATM, CHK2*) are sensitive

Table 4. Predictive Value of *BRCA*ness Profile Adjusted for Standard Clinical Prognostic Factors

Factor	Univariate *P*		Multivariate *P*	
	DFS	OS	DFS	OS
Age, years	0.44	0.35	0.96	0.8
Grade	0.9	0.34	0.87	0.32
Histology	0.97	0.61	0.9	0.96
Stage	0.43	0.54	0.4	0.61
Debulking status	0.39	0.05*†	0.53	0.67
HR‡		1.84†		
NBL/BL profile	0.018†	0.009†	0.016†	0.009†
HR‡	2.47†	3.29†	2.65†	3.39†

Abbreviations: DFS, disease-free survival; OS, overall survival; HR, hazard ratio; NBL, non–*BRCA*-like; BL, *BRCA*-like.
*Debulking status was unknown for one patient.
†Statistically significant at $P \leq .05$.
‡HR for death represented as comparison of NBL *v* BL groups for statistically significant associations.

to PARP inhibitors. Teodoridis et al[16] used methylation-specific polymerase chain reaction and showed that *BRCA1* promoter hypermethylation is associated with improved response to platinum-based chemotherapy. In addition, Quinn et al[39] used siRNA knock-down to decrease the expression of the *BRCA1* gene in two separate ovarian cancer cell lines, which showed that lower levels of *BRCA1* mRNA correlated with enhanced in vitro sensitivity to cisplatin.

In this article, we have broadened the concept of *BRCA*ness by identifying a gene expression profile that is associated with platinum and PARP-inhibitor responsiveness, as well as RAD51 foci formation. The relatively small number of *BRCA1/2*-mutated tumors and Capan-1 clones used in this study precludes formal statistical analysis. Nonetheless, it is noteworthy that the *BRCA*ness profile was capable of tracking platinum response in eight of 10 tumor specimens and PARP-inhibitor response in four of four Capan-1 clones. Moreover, when applied to a population of patients enriched for sporadic disease, the profile correlated with clinical outcome, independent of standard prognostic factors such as age, grade, histology, stage, and debulking status. It is impossible to determine from our data whether the correlation between the BL signature and improved survival is indicative of enhanced platinum responsiveness or, conversely, might identify patients with a more indolent natural history. In this regard, it is intriguing that the proportion of patients rendered into a complete clinical remission at the end of first-line chemotherapy was higher in patients with a BL signature (90%) than in those with the NBL signature (74%), although this was not statistically significant ($P = .2$).

It is noteworthy that *BRCA*ness profile contained genes, such as *APEX1, MGST3*, and *PMS1*, that have been previously associated with platinum resistance or DNA repair (Fig 1).[40-45] Neither *BRCA1* nor *BRCA2* was part of our gene expression profile, which perhaps indirectly supported the notion that, at least for some patients, genes other than *BRCA1* or *BRCA2* may sometimes be responsible for *BRCA*ness in sporadic disease. However, it is possible that our profile is identifying a subset of patients with sporadic mutation in *BRCA1* or *BRCA2*, epigenetic silencing of the promoter for *BRCA1*, or as yet unknown defects in the HR (or related) pathway. Our future studies will be directed at better understanding the mechanisms underlying the association between the *BRCA*ness profile, chemotherapy response, and survival.

Although the *BRCA*ness profile was developed in ovarian tumors, it was also capable of predicting PARP-inhibitor sensitivity and RAD51 foci formation in the pancreatic cancer cell line Capan-1 (Table 1), which suggests that the profile may be detecting a pattern of gene expression that more globally reflects the status of HR, independent of cell lineage. Furthermore, we are currently investigating the predictive value of this profile in triple-negative breast cancer, which is thought to be enriched for *BRCA*ness and to have a high response to platinum-containing chemotherapy.[46] Ultimately, we intend to apply this profile in the context of a clinical trial involving patients with sporadic ovarian cancer treated with a PARP inhibitor to gain additional insight into the predictive value of this approach. Studies are currently being performed to explore the potential value of PARP inhibitors in patients with ovarian cancer independent of *BRCA* mutation status. In the future, it may be possible to use gene expression profiling as an eligibility criterion in such studies, to enrich for sporadic patients who may benefit the most from this novel class of agents. Although additional study is clearly needed, the identification of a gene expression profile that seems to correlate with *BRCA*ness may make it possible to eventually offer PARP inhibitors to a much larger number of patients with epithelial ovarian cancer, regardless of their *BRCA1* or *BRCA2* mutation status.

AUTHORS' DISCLOSURES OF POTENTIAL CONFLICTS OF INTEREST

Although all authors completed the disclosure declaration, the following author(s) indicated a financial or other interest that is relevant to the subject matter under consideration in this article. Certain relationships marked with a "U" are those for which no compensation was received; those relationships marked with a "C" were compensated. For a detailed description of the disclosure categories, or for more information about ASCO's conflict of interest policy, please refer to the Author Disclosure Declaration and the Disclosures of Potential Conflicts of Interest section in Information for Contributors.

Employment or Leadership Position: None **Consultant or Advisory Role:** None **Stock Ownership:** None **Honoraria:** None **Research Funding:** Beth Y. Karlan, AstraZeneca **Expert Testimony:** None **Other Remuneration:** None

AUTHOR CONTRIBUTIONS

Conception and design: Panagiotis A. Konstantinopoulos, Dimitrios Spentzos, Stephen A. Cannistra
Financial support: Stephen A. Cannistra
Administrative support: Stephen A. Cannistra
Provision of study materials or patients: Beth Y. Karlan, Toshiyasu Taniguchi, Douglas A. Levine, Stephen A. Cannistra
Collection and assembly of data: Panagiotis A. Konstantinopoulos, Dimitrios Spentzos, Beth Y. Karlan, Elena Fountzilas, Nancy Francoeur, Douglas A. Levine, Stephen A. Cannistra
Data analysis and interpretation: Panagiotis A. Konstantinopoulos, Dimitrios Spentzos, Beth Y. Karlan, Elena Fountzilas, Nancy Francoeur, Douglas A. Levine, Stephen A. Cannistra
Manuscript writing: Panagiotis A. Konstantinopoulos, Stephen A. Cannistra
Final approval of manuscript: Panagiotis A. Konstantinopoulos, Dimitrios Spentzos, Beth Y. Karlan, Toshiyasu Taniguchi, Elena Fountzilas, Nancy Francoeur, Douglas A. Levine, Stephen A. Cannistra

REFERENCES

1. D'Andrea AD, Grompe M: The Fanconi anaemia/*BRCA* pathway. Nat Rev Cancer 3:23-34, 2003

2. Cass I, Baldwin RL, Varkey T, et al: Improved survival in women with *BRCA*associated ovarian carcinoma. Cancer 97:2187-2195, 2003

3. Cannistra SA: Cancer of the ovary. N Engl J Med 351:2519-2529, 2004

4. Tan DS, Rothermundt C, Thomas K, et al: "*BRCA*ness" syndrome in ovarian cancer: A case-control study describing the clinical features and outcome of patients with epithelial ovarian cancer associated with *BRCA1* and *BRCA2* mutations. J Clin Oncol 26:5530-5536, 2008

5. McCabe N, Turner NC, Lord CJ, et al: Deficiency in the repair of DNA damage by homologous recombination and sensitivity to poly(ADP-ribose) polymerase inhibition. Cancer Res 66:8109-8115, 2006

6. Farmer H, McCabe N, Lord CJ, et al: Targeting the DNA repair defect in *BRCA* mutant cells as a therapeutic strategy. Nature 434:917-921, 2005

7. Brody LC: Treating cancer by targeting a weakness. N Engl J Med 353:949-950, 2005

8. Jagtap P, Szabó C: Poly(ADP-ribose) polymerase and the therapeutic effects of its inhibitors. Nat Rev Drug Discov 4:421-440, 2005

9. Ratnam K, Low JA: Current development of clinical inhibitors of poly(ADP-ribose) polymerase in oncology. Clin Cancer Res 13:1383-1388, 2007

10. Fong PC, Boss DS, Yap TA, et al: Inhibition of poly(ADP-ribose) polymerase in tumors from *BRCA* mutation carriers. N Engl J Med 361:123-134, 2009

11. Audeh MW, Penson RTF M, Friedlander M, et al: Phase II trial of the oral PARP inhibitor olaparib (AZD2281) in *BRCA*deficient advanced ovarian cancer. J Clin Oncol 27:277s, 2009 (suppl; abstr 5500)

12. Pal T, Permuth-Wey J, Betts JA, et al: *BRCA1* and *BRCA2* mutations account for a large proportion of ovarian carcinoma cases. Cancer 104:2807-2816, 2005

13. Boyd J: Specific keynote: Hereditary ovarian cancer—What we know. Gynecol Oncol 88:S8-S10, 2003; discussion S11-S13

14. Risch HA, McLaughlin JR, Cole DE, et al: Population *BRCA1* and *BRCA2* mutation frequencies and cancer penetrances: A kin-cohort study in Ontario, Canada. J Natl Cancer Inst 98:1694-1706, 2006

15. Turner N, Tutt A, Ashworth A: Hallmarks of '*BRCA*ness' in sporadic cancers. Nat Rev Cancer 4:814-819, 2004

16. Teodoridis JM, Hall J, Marsh S, et al: CpG island methylation of DNA damage response genes in advanced ovarian cancer. Cancer Res 65:8961-8967, 2005

17. Baldwin RL, Nemeth E, Tran H, et al: *BRCA1* promoter region hypermethylation in ovarian carcinoma: A population-based study. Cancer Res 60:5329-5333, 2000

18. Geisler JP, Hatterman-Zogg MA, Rathe JA, et al: Frequency of *BRCA1* dysfunction in ovarian cancer. J Natl Cancer Inst 94:61-67, 2002

19. Esteller M, Silva JM, Dominguez G, et al: Promoter hypermethylation and *BRCA1* inactivation in sporadic breast and ovarian tumors. J Natl Cancer Inst 92:564-569, 2000

20. Hennessy B, Timms K, Carey MS, et al: Somatic *BRCA* status in ovarian tumors. J Clin Oncol 27:284s, 2009 (suppl; abstr 5528)

21. Foster KA, Harrington P, Kerr J, et al: Somatic and germline mutations of the *BRCA2* gene in sporadic ovarian cancer. Cancer Res 56:3622-3625, 1996

22. Hilton JL, Geisler JP, Rathe JA, et al: Inactivation of *BRCA1* and *BRCA2* in ovarian cancer. J Natl Cancer Inst 94:1396-1406, 2002

23. Taniguchi T, Tischkowitz M, Ameziane N, et al: Disruption of the Fanconi anemia-*BRCA* pathway in cisplatin-sensitive ovarian tumors. Nat Med 9:568-574, 2003

24. Hughes-Davies L, Huntsman D, Ruas M, et al: EMSY links the *BRCA2* pathway to sporadic breast and ovarian cancer. Cell 115:523-535, 2003

25. Konstantinopoulos PA, Spentzos D, Cannistra SA: Gene-expression profiling in epithelial ovarian cancer. Nat Clin Pract Oncol 5:577-587, 2008

26. Spentzos D, Levine DA, Kolia S, et al: Unique gene expression profile based on pathologic response in epithelial ovarian cancer. J Clin Oncol 23:7911-7918, 2005

27. Spentzos D, Levine DA, Ramoni MF, et al: Gene expression signature with independent prognostic significance in epithelial ovarian cancer. J Clin Oncol 22:4700-4710, 2004

28. Jazaeri AA, Yee CJ, Sotiriou C, et al: Gene expression profiles of *BRCA1*-linked, *BRCA2*-linked, and sporadic ovarian cancers. J Natl Cancer Inst 94:990-1000, 2002

29. Sakai W, Swisher EM, Karlan BY, et al: Secondary mutations as a mechanism of cisplatin resistance in *BRCA2*-mutated cancers. Nature 451:1116-1120, 2008

30. Swisher EM, Sakai W, Karlan BY, et al: Secondary *BRCA1* mutations in *BRCA1*-mutated ovarian carcinomas with platinum resistance. Cancer Res 68:2581-2586, 2008

31. Konstantinopoulos PA, Fountzilas E, Pillay K, et al: Carboplatin-induced gene expression changes in vitro are prognostic of survival in epithelial ovarian cancer. BMC Med Genomics 1:59, 2008

32. Radmacher MD, McShane LM, Simon R: A paradigm for class prediction using gene expression profiles. J Comput Biol 9:505-511, 2002

33. Dabney AR: Classification of microarrays to nearest centroids. Bioinformatics 21:4148-4154, 2005

34. Dabney AR, Storey JD: Optimality driven nearest centroid classification from genomic data. PLoS One 2:e1002, 2007

35. Chang CC, Lin CJ: Training nu-support vector classifiers: Theory and algorithms. Neural Comput 13:2119-2147, 2001

36. Edwards SL, Brough R, Lord CJ, et al: Resistance to therapy caused by intragenic deletion in *BRCA2*. Nature 451:1111-1115, 2008

37. Frank TS, Deffenbaugh AM, Reid JE, et al: Clinical characteristics of individuals with germline mutations in *BRCA1* and *BRCA2*: Analysis of 10,000 individuals. J Clin Oncol 20:1480-1490, 2002

38. Shattuck-Eidens D, Oliphant A, McClure M, et al: *BRCA1* sequence analysis in women at high risk for susceptibility mutations: Risk factor analysis and implications for genetic testing. JAMA 278:1242-1250, 1997

39. Quinn JE, James CR, Stewart GE, et al: *BRCA1* mRNA expression levels predict for overall survival in ovarian cancer after chemotherapy. Clin Cancer Res 13:7413-7420, 2007

40. Freitas S, Moore DH, Michael H, et al: Studies of apurinic/apyrimidinic endonuclease/ref-1 expression in epithelial ovarian cancer: Correlations with tumor progression and platinum resistance. Clin Cancer Res 9:4689-4694, 2003

41. Mol CD, Hosfield DJ, Tainer JA: Abasic site recognition by two apurinic/apyrimidinic endonuclease families in DNA base excision repair: The 3′ ends justify the means. Mutat Res 460:211-229, 2000

42. Godwin AK, Meister A, O'Dwyer PJ, et al: High resistance to cisplatin in human ovarian cancer cell lines is associated with marked increase of glutathione synthesis. Proc Natl Acad Sci U S A 89:3070-3074, 1992

43. Johnson SW, Ozols RF, Hamilton TC: Mechanisms of drug resistance in ovarian cancer. Cancer 71:644-649, 1993

44. Aebi S, Kurdi-Haidar B, Gordon R, et al: Loss of DNA mismatch repair in acquired resistance to cisplatin. Cancer Res 56:3087-3090, 1996

45. Durant ST, Morris MM, Illand M, et al: Dependence on RAD52 and RAD1 for anticancer drug resistance mediated by inactivation of mismatch repair genes. Curr Biol 9:51-54, 1999

46. Ryan PD, Tung NM, Isakoff SJ, et al: Neoadjuvant cisplatin and bevacizumab in triple negative breast cancer (TNBC): Safety and efficacy. J Clin Oncol 27:18s, 2009 (suppl; abstr 551)

