Clinical Studies

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Clinicopathological Features of Homologous Recombination–Deficient Epithelial Ovarian Cancers: Sensitivity to PARP Inhibitors, Platinum, and Survival

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Abstract

Up to 50% of epithelial ovarian cancers (EOC) display defects in the homologous recombination (HR) pathway. We sought to determine the ramifications of the homologous recombination–deficient (HRD) status on the clinicopathologic features, chemotherapy response, and survival outcomes of patients with EOCs. HR status was determined in primary cultures from ascitic fluid in 50 chemotherapy-naïve patients by a functional RAD51 immunofluorescence assay and correlated with *in vitro* sensitivity to the PARP inhibitor (PARPi), rucaparib. All patients went on to receive platinum-based chemotherapy; platinum sensitivity, tumor progression, and overall survival were compared prospectively in HR-competent versus HRD patients. Compared with HR-competent patients, the HRD group was predominantly serous with a higher median CA125 at presentation. HRD was associated with higher *ex vivo* PARPi sensitivity and clinical platinum sensitivity. Median follow-up duration was 14 months; patients in the HRD group had lower tumor progression rates at 6 months, lower overall/disease-specific death rates at 12 months, and higher median survival. We therefore suggest that HRD as predicted by a functional RAD51 assay correlates with *in vitro* PARPi sensitivity, clinical platinum sensitivity, and improved survival outcome. *Cancer Res; 72(22); 5675–82. ©2012 AACR.*

Introduction

Approximately 10% of epithelial ovarian cancers (EOC) have germ line mutations in BRCA1/BRCA 2 rendering them deficient in the homologous recombination (HR) DNA repair pathway (1, 2). A majority of studies show an improved overall and progression-free survival (PFS) compared with non–BRCA-related sporadic EOCs (3–5). BRCAness is a term that has been coined to describe cancers that do not have mutations in BRCA genes but phenotypically behave like these tumors due to genetic/epigenetic events (6). These aberrations may render these tumors defective in HR and therefore HR deficiency (HRD) would appear to be a more appropriate terminology. It is estimated that up to 50% to 60% EOCs could be HRD; functional assays continue to be developed to identify this subset of EOCs (7). A recent study analyzing retrospective data showed that EOCs with BRCA-ness determined by gene expression profiling had improved disease-free survival compared with non–BRCA-like tumors (8). However, these data need to be confirmed in prospective studies.

There is now widespread agreement that a novel class of anti-cancer agents, PARP inhibitors (PARPi), have activity in BRCA-related cancers by using the inherent HR defectiveness of the tumors in a synthetically lethal manner (9, 10). Phase I and II clinical trials have shown good clinical benefit rates with minimal side effects in recurrent advanced-stage EOCs with *BRCA* mutations (11, 12). BRCA-related tumors have also been shown to have increased sensitivity to platinum-based chemotherapy compared with non–BRCA-related cancers (13).

It would appear therefore that EOCs with HRD are a subset of tumors that have distinct clinicopathologic features, chemosensitivity, and survival profiles. We developed a functional assay in primary cultures to classify EOCs based on HR status and correlated with *in vitro* sensitivity to PARPi (7). All these patients subsequently received platinum-based chemotherapy and were followed up. In this prospective study, we aimed to correlate HR status with chemoresponsiveness and clinical outcome.

Materials and Methods

Patients with advanced-stage ovarian/primary peritoneal cancer (PPC) were enrolled in this study between September 2008 and June 2010.

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Ascitic fluid was collected from chemotherapy-naïve patients, either those undergoing primary surgery or those undergoing paracentesis before receiving chemotherapy. Ethical approval and specific consent were obtained for collection of clinical material and clinical information. None of the patients had been screened for *BRCA* mutations or selected on the basis of a family history of breast or ovarian cancer.

Determination of HR status and *ex vivo* cytotoxicity to PARPi

Details of methods have been described in our previous publication (7). Primary cultures were developed successfully from ascitic fluid in more than 90% cases. HR function was determined by an immunofluorescence-based assay, which detects nuclear γH2AX foci as a marker of DNA double-strand breaks (DSB) and RAD51 foci as a marker for repair of DSB by HR. DNA DSBs were induced by 24-hour exposure to 10 mmol/L hydroxyurea (Sigma) or 10 μmol/L potent PARPi rucaparib (Clovis Oncology Boulder—formerly known as AG-014699, PF-01367338; Pfizer Oncology). On the basis of previous work, a 2-fold increase in nuclear RAD51 foci formation over untreated controls was taken as a cutoff to differentiate between HR-competent and HRD cultures (7). *Ex vivo* cytotoxicity following 24-hour exposure to rucaparib was determined by sulforhodamine B (SRB assay in response to 0.0, 0.3, 1.0, 10, 30, or 100 μmol/L of rucaparib) or clonogenic cell survival assay (in response to 0, 1, 10, or 50 μmol/L rucaparib). On the basis of our cell line work, cytotoxicity at 10 μmol/L, rucaparib distinguished between HR-competent and -deficient cell lines (14). Therefore, we used this cutoff to correlate cytotoxicity with the HR status determined by RAD51 foci in primary cultures. For categorical data, cell survival more than 70% compared with the untreated controls was taken as a cutoff to define *ex vivo* chemoresponse.

Clinical data collection

Patient data including age, family history, past history of cancer, pretreatment tumor markers, operative details and histologic subtype, stage, and grade were recorded from the clinical database. Histologic diagnosis of primary ovarian/PPC was confirmed by independent gynecologic-specific pathologists (15). Surgical stage, histologic grade, and cell type were classified according to the World Health Organization (WHO) and Federation Internationale des Gynaecologistes et Obstetristes (FIGO) standards.

BRCA1 immunostaining was conducted at Queen University (Belfast, N. Ireland, UK) on a tumor microarray created from formalin-fixed, paraffin-embedded (FFPE) tumor samples that had been characterized for HR status by the RAD51 assay. The scoring was done by 2 independent pathologists who were blinded to the HR status of the tumors. The original scoring estimated the percentage of cells stained, using a 6-point scale, (none, <10%, 10%–25%, 25%–50%, 50%–75%, and >75%), and also the intensity of staining (weak, moderate, strong). A modified *H*-score was generated by multiplying the area score with the intensity score giving a range of score from 1 to 18. A score of ≤ 4 was considered to be the cutoff point for a negative BRCA1 immunohistochemical (IHC) staining. This score was chosen as it correlates with the 10% cutoff used in previous work (16).

Patients underwent maximal effort primary cytoreductive surgery or neoadjuvant chemotherapy followed by interval debulking surgery. All surgeries were carried out by accredited Gynaecologic Oncologists. The following definitions were used for cytoreduction; complete, no visible residual disease; optimal, <1 cm residual disease maximum diameter; and suboptimal, >1 cm residual disease.

All patients received platinum-based chemotherapy with or without paclitaxel as first-line treatment. Analysis of patient survival and tumor response was limited to patients who completed at least 6-month follow-up since the last cycle of platinum-based chemotherapy. Response to chemotherapy was determined by serial clinical examinations, CA125 levels, and computed tomographic (CT) scans at predefined intervals and at completion of chemotherapy unless new onset symptoms warranted urgent evaluation. Comparison was made with pretreatment (chemotherapy) imaging and tumor markers. Tumor response was defined as radiologic evidence of complete/partial response or a CA125 response defined as more than 50% decline sustained for at least 4 weeks (17, 18). Tumor progression/recurrence was defined as the presence of progressive disease on CT scan, increasing CA125 levels, or clinical evidence. Chemosensitivity to platinum was defined as 1, platinum sensitive—no tumor progression within 6 months of completion of chemotherapy as 2, platinum-resistant—tumor progression within 6 months of completion of chemotherapy but after posttreatment evaluation as 3, platinum-refractory—tumor progression while on chemotherapy up to the date of their posttreatment evaluation (19).

Definitions used for survival data

Survival data were calculated using the date of diagnosis, defined as the date of histologic or cytologic confirmation of EOCs. For overall survival (OS), patients who died at follow-up (any cause) were considered uncensored, whereas patients alive at follow-up were censored (20).

Statistical analyses

Statistical Package for Social Sciences Software (SPSS version 15.0; SPSS Inc.) was used for analyses. The association between HR status and various clinicopathologic factors was assessed by the χ^2 and the Fisher exact tests. The Spearman test was applied for analyses of correlations. Univariate analyses for OS and PFS were generated by Kaplan–Meier survival curves and log-rank (Mantel–Cox) tests for statistical significance. Multivariate analyses to adjust for known prognostic factors were conducted by using a Cox proportional hazards regression model. For all statistical analyses, significant differences were set as $P < 0.05$ at the 2-sided test (SPSS version 15.0).

Results

Between September 2008 and June 2010, 75 primary cultures were generated from ascitic fluid with more than 90% success rate. HR status was determined in 62 patients. Histologic reports confirmed non-ovarian/peritoneal origin in 8 cultures including colon, endometrial, and metastatic breast cancers.

Table 1. Clinicopathologic characteristics in the HR-competent and HRD group

	HR-competent ($n = 24$)	HRD ($n = 26$)	*P*
Age (median), y	68.50 (52–85)	64.00 (45–87)	0.171
<65	9/24 (37.5%)	15/26 (57.7%)	
≥65	15/24	8/17	
Tumor site			
Ovarian	17/24 (70.8%)	20/26 (76.9%)	0.880
PPC	5/24 (20.8%)	7/26 (26.9%)	
Ovary/PPC	1/24	1/26	
Stage at diagnosis			
1	2/24	1/26	0.354
3	15/24 (62.5%)	21/26 (80.8%)	
4	7/24 (29.2%)	4/26 (15.4%)	
Histologic grade			
Grade 1/2	4/24	2/26	0.409
Grade 3	20/24 (83.3%)	24/26 (92.3%)	
Histologic subtype			
Serous	15/24 (62.5%)	24/26 (92.3%)	0.035[a]
Mucinous	2/24	0	
Endometrioid	1/24	2/26	
Clear cell	3/24	0	
Others (epithelial)	3/24	0	
CA125 at presentation, units			
Median (range)	427 (159–3639)	2,079.50 (102–16,852)	0.007[a]
Family history of cancer	2/24 (8.3%)	8/26 (30.8%)	0.098
Past history of cancer (breast)	4/24 (16.7%)	7/24 (28.1%)	
Treatment modality			
Primary surgery	15/24 (62.5%)	16/26 (61.5%)	
IDS	7/24	8/24	
Cytoreduction			
Optimal/complete	15/24 (62.5%)	21/26 (80.8%)	0.354
Suboptimal	7/24 (29.2%)	4/26 (15.4%)	
BRCA1 status on IHC			
Positive	17/22 (77.3%)	15/23 (65.2%)	0.372
Negative	5/22 (22.7%)	8/23 (34.8%)	

NOTE: Optimal cytoreduction is <1 cm residual disease.
Abbreviation: IDS, interval debulking surgery.

These patients were not followed up for clinicopathologic correlation but were assessed for HR status and had *ex vivo* cytotoxicity tests to correlate with sensitivity to PARPi. Of the remaining 54 ascitic fluid samples assessed for HR status and with confirmed EOCs, 50 had *ex vivo* cytotoxicity tests to correlate with sensitivity to PARPi. A flow diagram showing patients included in this study and the correlation of HR status and cytotoxicity to PARPi is presented in Supplementary Fig. S1. The data for RAD51 foci and cytotoxicity to 10 μmol/L rucaparib are presented in Supplementary Fig. S2.

Demographic characters

Demographic data were available for all patients. Fifty patients with EOC/PPC (HR-competent, $n = 24$; HR-deficient, $n = 26$), who completed follow-up for at least 6 months after completion of chemotherapy (till April 2011) were included in the present study for analysis of clinicopathologic variables (Table 1), platinum sensitivity (Table 2), and survival data (Table 3).

Histology

Twelve of 50 patients had PPC with a slightly higher proportion of PPCs seen in the HRD group (26.9% vs. 20.8%). The majority of patients had high-grade ($n = 44$ of 50), advanced-stage III/IV disease ($n = 47$ of 50), and serous ($n = 39$ of 50) carcinomas. The HRD group was predominantly serous compared with the HR-competent patients (92.3% vs. 62.5%, $P = 0.035$). Both the non-serous tumors seen in the HRD group were of high-grade endometrioid type. In keeping with the higher prevalence of serous tumors and PPCs, the median CA125 at presentation was also significantly higher in the HRD group (2,079 vs. 427 U/mL, $P = 0.007$). BRCA1 IHC was negative (H-score ≤ 4) in 8 of 23 (34.8%) of HRD tumors and negative in 5 of 22 (22.7%) of HR-competent tumors ($P = 0.37$; Table 1).

Table 2. Chemosensitivity to PARPi and platinum

	HR-competent ($n = 24$)	HRD ($n = 26$)	*P*
Sensitivity to PARPi (*ex vivo*)	0/24	24/26 (92.8%)	<0.001
Sensitivity to platinum			
Sensitive	4/24 (16.7%)	14/26 (53.8%)	0.063
Resistant	12/24 (50.0%)	9/26 (34.6%)	
Refractory	6/24 (25%)	3/26 (11.5%)	
Platinum-free interval			
Median, mo	4 (1–22)	6 (1–22)	

NOTE: Platinum sensitivity was defined as no disease recurrence within 6 months of completion of platinum-based chemotherapy.

Treatment modality

The majority of patients ($n = 31$ of 50) had primary surgery followed by chemotherapy, whereas the rest ($n = 18$ of 50) were treated with 3 cycles of neoadjuvant chemotherapy followed by interval debulking surgery and further 3 cycles of chemotherapy. One patient in the HRD group opted not to have any surgery and had 6 cycles of chemotherapy only. One patient in the HR-competent group died 2 months after primary surgery and therefore did not have any chemotherapy.

The median duration of follow up was 14 months in both groups. The majority of women received both platinum (carboplatin) and paclitaxel combination as first-line for the whole duration of chemotherapy ($n = 40$ of 50). Eight of 50 women developed toxicity/allergy to paclitaxel during therapy and therefore went on to receive platinum only and 2 patients with stage 1c disease received 6 cycles of carboplatin as single-agent therapy. Forty-five of 50 women completed 6 cycles (4 women died while on chemotherapy and 1 after primary surgery). At the time of final follow-up, 46% women had gone on to receive second-line chemotherapy and 25% of them further progressed to receive third-line chemotherapy.

Chemoresponse and sensitivity to platinum

Overall 39 of 50 (78%) women showed evidence of response to first-line chemotherapy as defined above. Women in the

Table 3. Tumor progression rate and OS in HR-competent versus -deficient group

	HR competent ($n = 24$)	HR deficient ($n = 26$)	*P*
Tumor progression at 6 months			
All histologic subtypes	8/24 (33.3%)	2/26 (7.7%)	0.024*
Serous only	5/15 (33.3%)	2/24 (8.3%)	0.048*
Tumor progression at 12 months			
	18/24 (75%)	14/26 (53.8%)	0.119
OS at 12 months (hazard/death rates)			
All patients	10/24 (41.7%)	3/26(11.5%)	0.015*
Histology			
Serous only	5/15 (33.3%)	3/24 (12.5%)	0.220
Nonserous	5/9	0/2	
Cytoreduction			
Optimal	6/15 (40.0%)	1/21 (4.8%)	0.008*
Suboptimal	4/7 (57.1%)	2/4 (50%)	
Treatment modality			
Primary surgery	6/15 (40.0%)	0/16	0.005*
Disease stage			
Stage 1/2	0/2	0/1	
Stage 3	6/15 (40.0%)	0/21 (0%)	0.001*
Stage 4	4/7 (57.1%)	3/4 (75%)	0.554
BRCA1 status on IHC			
Positive	6/17 (35.3%)	3/15 (20%)	0.337
Negative	2/5 (40.0%)	0/8 (0%)	0.052

HRD group had a higher proportion of platinum-sensitive disease than the HR-competent group (53.8% vs. 16.7%; $P = 0.063$) with an increased median platinum-free interval (6 vs. 4 months). Conversely, 75% of women in the HR-competent group had platinum-resistant or -refractory disease (Table 2).

Correlation between *in vivo* platinum sensitivity and *ex vivo* sensitivity to PARPi

Sensitivity to 10 μmol/L rucaparib was seen in 24 of 26 (92.8%) HRD cultures compared with none of the 24 HR-competent cultures ($P < 0.001$; Table 2). There was a correlation between *ex vivo* sensitivity to PARP inhibition and *in vivo* platinum sensitivity (Spearman's ρ, $P = 0.05$). Of 23 patients with *ex vivo* response to PARPi, 52% were platinum-sensitive. Conversely, of 23 patients showing no response to *ex vivo* PARP inhibition, 78.3% had platinum-resistant/refractory disease.

Survival

At 12 months, tumor progression was seen in 32 of 50 (64%) patients (Table 3). However, at 6 months, 7.7% women in the HRD group relapsed or progressed compared with 33.3% in the HR-competent group ($P = 0.024$). Median PFS was longer in women with HRD tumors (11 vs. 8 months) and was statistically significant (Fig. 1A, log-rank = 0.024).

Median OS was longer in HRD women (20 vs.14 months, Fig. 1B, log-rank $P = 0.08$). Minimum follow-up duration for all patients was 14 months (maximum 28 months); therefore, we looked into PFS (Fig. 2A) and OS (Fig. 2B) at 12 months. At 12 months since diagnosis, 13 of 50 (26%) patients died; 11.5% died in the HRD group compared with 41.7% in the HR-competent group [$P = 0.015$; univariate HR for death 0.23; 95% confidence interval (CI), 0.64–0.84; Fig. 2B]. The poor survival trend in the HR-competent group was evident even when stratified for treatment type and better prognostic factors including serous subtype, stage of disease (stage 3 or less), and optimal cytoreduction (Table 3). In univariate analysis of the clinical and pathologic factors studied, only stage of disease (stage 4 vs. lesser stage) was significantly associated (HR, 4.90; CI, 1.62–14.73; $P = 0.005$) with OS at 12 months and therefore was entered into Cox regression hazards model along with HR status (Table 4). HRD conferred a 70% reduction in relative risk of death at 12 months compared with HR-competent patients after adjusting for stage of disease, although this was not statistically significant. Although not reaching statistical significance, patients who had suboptimal cytoreduction had increased mortality at 12 months compared with optimal or complete cytoreduction (42.9% vs. 19.4%; $P = 0.090$) as did women with a nonserous histology compared with serous tumors (45.5% vs. 20.5%; $P = 0.096$).

Discussion

In line with our previous findings (7), approximately 50% of EOCs are HR-deficient and show *ex vivo* cytotoxicity to a PARPi in more than 90% cases. These findings are consistent with other published data from The Cancer Genome Atlas (TCGA) project, which used molecular pathway analysis of genetic aberrations to conclude that approximately 50% of high-grade serous cancers will have defective HRs (21).

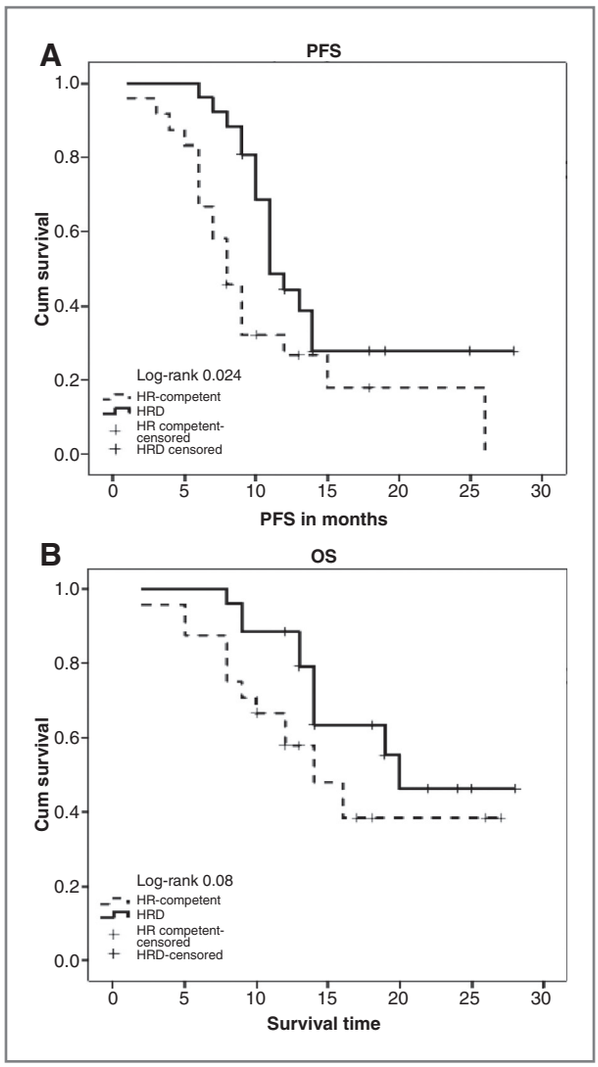

Figure 1. Kaplan–Meier survival curves for PFS and OS in HR-competent versus HR-deficient EOCs. A, median PFS was higher in HRD group (11 vs. 8 months, log-rank, $P = 0.024$). B, median OS was 19 months in HRD group compared with 14 months in HR-competent group (log-rank, $P = 0.08$).

These data further support the clinical use of PARPi in this group of patients, supporting data from the recently reported study of maintenance PARPi treatment with olaparib in this group (22). Conversely, we would predict that cancers that do not have HRD are unlikely to respond to this class of agents.

We also conclude that HRD EOC as a group has a distinct clinicopathologic phenotype compared with HR-competent EOCs; they are predominantly serous, show increased sensitivity to platinum, and tend to be associated with improved survival.

The results from our study are consistent with the prediction of the proportion of BRCAness phenotype expected in sporadic cancers as described by Turner and colleagues (6) and specific mechanisms have been identified in several studies (23–29). A limited number of studies have explored the

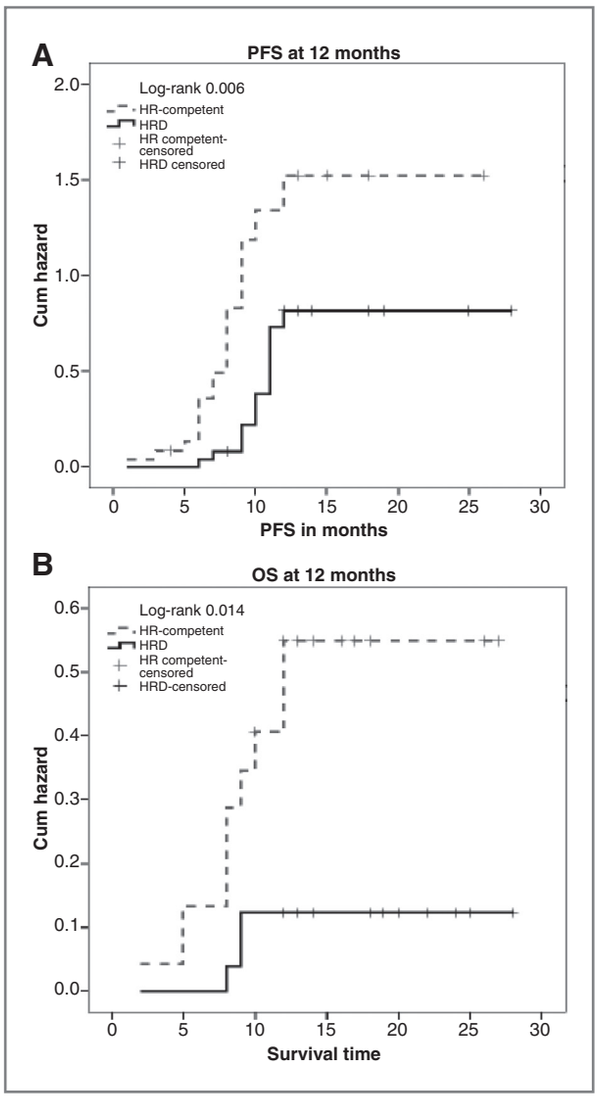

Figure 2. PFS (A) and OS (B) at 12 months in HR-competent versus HR-deficient EOCs.

relationship between HR and response to platinum or PARPi (26, 30–33). Our study is unique in that HR status was evaluated in clinical material from chemo-naïve patients with predominantly sporadic EOCs. Irrespective of the specific genetic aberration, our functional assay was able to predict accurately the group of women who could potentially benefit from PARPi (7). One interesting observation from our study was that even in non-EOCs or cancers from other sites, HR status was predictive of response to PARPi (Supplementary Fig. S1). This suggests a potential wider application of a functional HR assay and use of PARPi in other cancer types (34).

The other question that emerges from this study is whether these assays can be used as a surrogate response biomarker for other agents such as platinum. There is a correlation between chemosensitivity to platinum and PARPi, and BRCA-deficient EOCs have been shown to have increased platinum sensitivity (26, 31). In clinical studies, response to the oral PARPi olaparib seems to correlate with platinum-free interval, platinum-sensitive cancers showing a greater clinical benefit rate (30). In cell lines, reversion of open reading frames in the *BRCA* gene confers platinum resistance as well as resistance to PARPi (35, 36). Our study also shows a correlation between HRD status, *ex vivo* PARPi sensitivity, and clinical platinum sensitivity. An interesting group to study would appear to be women whose ascitic fluid cultures suggest that they would be sensitive to a PARPi but were shown to be platinum-resistant/refractory (14 of 32 women in our study). Whether in recurrent EOCs, administration of PARPi helps to regain chemosensitivity to platinum-based chemotherapy is currently being studied (37).

The strength of our study in terms of clinicopathologic and survival data is that it was a prospective study. Although not randomized, clinicians involved in managing the patients as regard to surgery or chemotherapy were not aware of the HR status. Also, blinding was maintained at time of clinical data collection and analysis. The HRD group was predominantly serous, associated with a higher proportion of PPCs and higher CA125 at presentation, which fits with our prediction that HRD phenotype is similar to BRCA-deficient tumors. Although patients were not screened for genetic testing, nor presented with known mutations except one woman in the HRD group who was subsequently found to have a germ line BRCA2 mutation, a higher proportion of women in the HRD group had a family history of breast/ovarian cancers in first-degree relatives (33%) as well as a past history of breast cancer. It is possible to postulate with these data that HR competence confers a biologically

Table 4. Univariate and multivariate analyses (Cox proportional hazard regression) of prognostic factors for OS at 12 months

Variable	HR (95% CI)	*P*
Univariate analysis		
Age <65 vs. ≥65 y	0.62 (0.20–1.90)	0.40
Stage 4 vs. 3 or less	4.90 (1.62–14.73)	0.005[a]
Nonserous vs. serous histology	2.84 (0.92–8.72)	0.067
Primary surgery vs. IDS/no surgery	0.49 (0.16–1.47)	0.20
Suboptimal vs. optimal cytoreduction	2.49 (0.83–7.43)	0.10
BRCA1 IHC negative vs. positive	0.57 (0.12–2.64)	0.47
Platinum-sensitive vs. resistance	0.02 (0.00–2.05)	0.09
HRD vs. HR-competent	0.23 (0.64–0.84)	0.026[a]
Multivariate analysis		
HRD status	0.31 (0.83–1.18)	0.088
Stage 4 disease	3.65 (1.18–11.29)	0.024

Abbreviation: IDS, interval debulking surgery.
[a]Stage of disease was an independent prognostic factor.

aggressive phenotype that leads to tumors presenting at higher stage, poor histologic subtypes, poor surgical resectability, and chemoresistance. There was a very poor correlation with the BRCA1 status on IHC and the HR status. Only 35% of HRD tumors were negative for BRCA1 immunostaining implying other defects in the HR pathway such as a BRCA2 defect and further validates the need for development of a functional assay for HRs.

Retrospective studies with long-term patient follow-up have indicated a significant OS benefit of 72 versus 41 months in patients with a BRCA-like profile on gene expression analysis (8). Our study will need long-term follow-up with the ongoing increase in patients' numbers to show a statistically relevant difference. However, in advanced EOCs, even short-term endpoints such as tumor progression at 6 months or OS at 12 months are clinically relevant especially taking into account that the majority of women (~75%) are likely to develop disease progression and die from the disease (38). At these intervals, our data suggest a trend toward a survival benefit in the HRD group. A higher proportion of women in the HRD group received second- and third-line chemotherapy as they survived longer despite disease progression.

To conclude, HR deficiency may identify a distinct group with favorable biologic features not only in the context of EOCs but also in other cancer types. There is a need to develop a functional assay to identify this group of tumors that is feasible and applicable in clinical settings. Clinical and translational data from ongoing clinical trials studying the role of PARPi in sporadic ovarian cancers might provide further insight into this group of cancers (39).

Disclosure of Potential Conflicts of Interest

N.J. Curtin has received a commercial research grant from Pfizer. No potential conflicts of interest were disclosed by the other authors.

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