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Molecular Medicine in Practice

Molecular Cancer Therapeutics

BRCA Mutation Status and Determinant of Outcome in Women with Recurrent Epithelial Ovarian Cancer Treated with Pegylated Liposomal Doxorubicin

Tamar Safra[1,3], Lucia Borgato[5], Maria Ornella Nicoletto[5], Linda Rolnitzky[1], Sharon Pelles-Avraham[3], Ravit Geva[3], Martin Edward Donach[1], John Curtin[2], Akiva Novetsky[1], Tal Grenader[4], Wei-Chu V. Lai[2], Alberto Gabizon[4], Leslie Boyd[1], and Franco Muggia[1]

Abstract

Epithelial ovarian cancer (EOC) patients with *BRCA* mutations (*BRCA+*) benefit from platinum-based treatment more than noncarriers. Impaired ability to repair DNA by homologous recombination increases their chemosensitivity. We investigated whether *BRCA+* predicts for improved outcome following pegylated liposomal doxorubicin (PLD) for recurrence. Recurrent EOC patients receiving second- or third-line PLD from 1998 to 2009 in 4 institutions (Tel Aviv, New York, Padua, and Jerusalem) were subjected to retrospective comparisons between 40 (25.8%) patients who were *BRCA+*, and 115 (74.2%) deemed nonhereditary (NH). Median age was 59 years (range 31–83); 111 (72%) had a platinum-free interval more than 6 months [PLD alone ($n = 65$) and PLD plus platinum ($n = 90$)]; 104 received PLD in second-line and 51 in third-line. *BRCA+* versus NH comparisons: median time to treatment failure (TTF) 15.8 months [95% confidence interval (CI): 11.4–21.6] versus 8.1 months (95% CI: 6.1–10.3; $P = 0.009$); overall survival (OS) 56.8 months (95% CI: 32.5–indeterminate) versus 22.6 months (95% CI: 17.0–34.1; $P = 0.002$). In multivariate Cox models *BRCA* status was significantly associated with TTF (HR = 1.66; 95% CI: 1.08–2.55; $P = 0.02$) and OS (adjusted HR 2.07; 95% CI: 1.18–3.60; $P = 0.01$). Adjusted HR relating platinum sensitivity to OS was 1.58 (95% CI: 0.93–2.68; $P = 0.09$); no significant association found with age at diagnosis, line of PLD or combinations, or institution. In this retrospective analysis, recurrent EOC *BRCA* mutation carriers treated with PLD had an improved outcome, and this result seemed to be independent of platinum sensitivity. Tumors arising in a background of defective *BRCA* function are more sensitive than other EOCs to DNA-damaging agents such as PLD, even after acquiring platinum resistance. *Mol Cancer Ther; 10(10); 2000–7. ©2011 AACR.*

Introduction

Germline mutations in *BRCA1* or *BRCA2* (breast cancer susceptibility) tumor suppressor genes are found in approximately 10% of epithelial ovarian cancers (EOC; refs. 1, 2). *BRCA* genes preserve chromosomal stability, and influence transcription and cell-cycle control. Both *BRCA1* and *BRCA2* are critical for homologous recombination, the preferred pathway for repairing DNA double-strand breaks (DSB) that arise either spontaneously or are induced by exogenous agents such as chemotherapy or radiation (3–5). Platinum drugs are known to induce greater cytotoxicity against *BRCA−/−* cell lines, presumably because when it forms a covalent bond with DNA strands, repair cannot proceed by homologous recombination. Animal models further confirm that in the absence of *BRCA* function, tumors do not become resistant to platinums. With doxorubicin, a mechanism of DNA damage occurs by interaction with topoisomerase 2 resulting in DNA cleavage; other possible mechanisms have invoked DNA intercalation and free radical activation resulting in DNA strand breaks creating conditions where interference with DNA repair may enhance cytotoxicity (6–9).

Evidence supports a differential response to chemotherapy based on the presence or absence of the *BRCA* mutation. Preclinical models confirm the vulnerability of *BRCA−/−* tumors (4, 6–9). Increased sensitivity to cisplatin and other DNA-damaging agents was observed in ID8 murine ovarian cancer cells with *BRCA1* mutations

Authors' Affiliations: [1]New York University (NYU) Cancer Institute; [2]NYU School of Medicine, New York, New York; [3]Tel Aviv Sourasky Medical Center, Tel Aviv; [4]Shaare-Zedek Medical Center, Jerusalem, Israel; and [5]Instituto Oncologico Veneto (IOV) IRCCS, Padova, Italy

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Corresponding Author: Tamar Safra, NYU Cancer Institute, 160 34th St, New York, NY 10016. Phone: 347-598-420; Fax: 323-865-0061; E-mail: safrat@bezeqint.net

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(10). In contrast, increased resistance and decreased apoptotic response was observed when *BRCA1*-deficient murine cells were exposed to antimicrotubule agents (6). These findings have been confirmed in *in vitro* studies showing increased sensitivity to DNA-damaging agents (e.g., platinums, anthracyclines, mitomycin-C, etoposide, topotecan) and increased resistance to antimicrotubule agents (e.g., taxanes, vinca alkaloids) in the presence of *BRCA* mutations (3, 4, 6, 7, 11). Retrospective studies show a better prognosis, with more partial and complete responses to platinum-containing regimens, in *BRCA* mutation carriers compared with women with nonhereditary (NH) or sporadic EOC (12–14). These better outcomes with platinum-based therapy in *BRCA1*- and/or *BRCA2*-mutation carriers have been attributed to a failure of homologous recombination repair of DSBs that occurs in the absence of functional *BRCA1/2* genes. Thus, the impaired ability of *BRCA*-deficient cells to repair chemotherapy-induced damage of DNA may lead to improved chemosensitivity (4, 6, 15, 16).

The potential for disease sensitivity to platinum is perhaps the most important factor in planning subsequent treatment for women with EOC that recurs. Operationally, when platinum-free intervals (PFI) are less than 6 months from termination of the initial platinum-based regimen, tumors are defined as platinum resistant (17). If the PFI exceeds 6 months, patients are defined as platinum-sensitive and treated with a platinum-based therapy with either paclitaxel or docetaxel, gemcitabine, or pegylated liposomal doxorubicin (PLD; ref. 18). Eventually, as secondary platinum resistance supervenes, EOC patients are treated with noncross resistant single-agent chemotherapy, most commonly with PLD monotherapy (18–20). Doxorubicin, the core component of PLD, is a DNA intercalating anthracycline that inhibits topoisomerase II and leads to DSBs. Thus, the DNA-damaging PLD would hypothetically be a rational treatment choice for EOC in *BRCA* mutation carriers, whether platinum sensitive or platinum resistant, if the resistance is due to reasons other than repairing DNA-mediated platinum damage.

While reviewing our data on maintenance PLD alone or combined with oxaliplatin in patients with recurrent EOC, we noted overrepresentation of *BRCA* mutations or strong family history for breast or ovarian cancers among patients with long-term survival (21, 22). These findings led to our hypothesis that *BRCA* carriers would have a better outcome with PLD, providing a compelling rationale for developing a more personalized strategy for treating EOC based on *BRCA* status. We thus embarked on a larger-scale retrospective analysis of patients treated with PLD-containing regimens at 4 institutions. The aim of the study was to compare time to treatment failure (TTF) and overall survival (OS) following second- or third-line PLD (as monotherapy or combined with a platinum) in *BRCA* mutation carriers versus a NH population while taking into account other factors known to influence survival.

Materials and Methods

Study design

Investigators with previous collaboration on the use of PLD for patients with recurrent EOC participated in the study. Medical records of patients with recurrent EOC who were receiving second- or third-line treatment with PLD by the collaborating investigator were reviewed. Approval by Institutional Review Boards and Cancer Center Research Review committees and waivers of informed consent to use deidentified information were obtained from each of the 4 institutions. Genetic testing for *BRCA* mutations was done in most women diagnosed with ovarian cancer in Israel and in New York, who agreed to undergo testing, regardless of age and family history. At the Italian institution, however, the test was done by an academic research laboratory and confined to selected patients. The majority of patients, therefore, were *BRCA* mutation unknown. To minimize the probability of encountering *BRCA* mutations in this group, women with first-degree relatives diagnosed with breast or ovarian cancers were excluded. Clinical data retrieved included institution, patient age at diagnosis, ethnicity, comorbidities, stage of disease, tumor histology, tumor grade, family cancer history, presence of *BRCA* mutations, prior surgical and chemotherapy management, platinum sensitivity, time to first recurrence, line of PLD therapy (second or third), PLD regimen received, TTF, and OS. Treatment failure included any reason for introducing a treatment other than PLD maintenance or observation in addition to progression or death from any cause.

Patient eligibility

All patients with relapsed advanced stage primary ovarian cancer (stage III or IV) or histologically confirmed extrauterine Müllerian carcinoma (ovarian, tubal, and primary peritoneal) limited to high-grade papillary serous or endometrioid tumors who received second- or third-line treatment with PLD (alone or in combination with platinum) between 1998 and 2009 were included. Patients underwent treatment at New York University (NYU) Cancer Institute (New York, NY), Istituto Oncologico Veneto (IOV; Padua, Italy), Shaare Tzedek Hospital (Jerusalem, Israel), or Tel Aviv Sourasky Medical Center (Tel Aviv, Israel). The institutions followed similar protocols for initiating PLD. Oxaliplatin-PLD was the preferred regimen for both platinum-sensitive and -resistant patients at IOV. At NYU Cancer Institute, carboplatin-PLD was primarily used as second-line treatment for platinum-sensitive disease, and occasionally used in the third-line setting.

Study endpoints

The primary study endpoints were TTF and OS in *BRCA* mutation carriers and patients with NH recurrent EOC treated with PLD. Patients were considered to have NH disease if they were BRCA negative or untested.

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Patients not tested for *BRCA* mutation with heavy family history of breast and ovarian cancer were excluded from the analysis. The presence of the *BRCA* mutation was assessed using the BRCAnalysis (Myriad Genetics, Inc.) for germ-line *BRCA1* or *BRCA2* mutation.

Patients did not routinely have confirmatory imaging studies. Therefore, response to treatment by Response Evaluation Criteria in Solid Tumors (RECIST) criteria was not always possible, whereas rising CA125 often determined progression, as defined by Rustin (23, 24). In responding patients, there was no attempt to adjudicate discordance between RECIST and CA125 response; data were presented as best response by either method. Date of first relapse was defined as first instance of disease progression based on CT or PET-CT imaging by RECIST or rising CA125. TTF and OS were calculated from initiation of PLD to either progression or death or to the last known follow-up. Duration of primary PFI was defined as the time between the end of primary treatment and the date of first relapse.

Definition of platinum sensitivity

Disease was considered to be platinum sensitive if the patient had a documented response to platinum-based therapy without progression within 6 months. This included use of a second nonplatinum agent as maintenance in the extension of such response beyond 6 months.

Statistical methods

Patient characteristics, presented as percentages, were compared between groups using Fisher's exact test; age at diagnosis was compared between groups using Wilcoxon's rank sum test. Comparisons of OS and TTF between *BRCA1/2* mutation and NH patients were done using Kaplan–Meier analyses and both univariate and multivariate Cox regression models. Log-rank tests were used to compare Kaplan–Meier survival curves between the 2 groups. Age at diagnosis, primary PFI ($\leq$ 6 months or > 6 months), second- or third-line PLD therapy, PLD monotherapy or PLD combined with a platinum regimen, and institution were analyzed as covariates in the multivariate Cox regression models. Kaplan–Meier analyses, as described above, were also done separately for the platinum-resistant (PFI $\leq$ 6 months) and platinum-sensitive (PFI >6 months) populations. Because of the retrospective nature of the study and some incomplete data for individual patients, we did not attempt to use the PFI as a continuous rather than the dichomotous variable that was more easily assigned. All tests were 2-sided and carried out at a significance level of 0.05. Statistics were done using SAS version 9.2 statistical software.

Results

Patient population and treatment

After an initial screening process (Fig. 1), 165 medical records were reviewed. Ten were excluded because

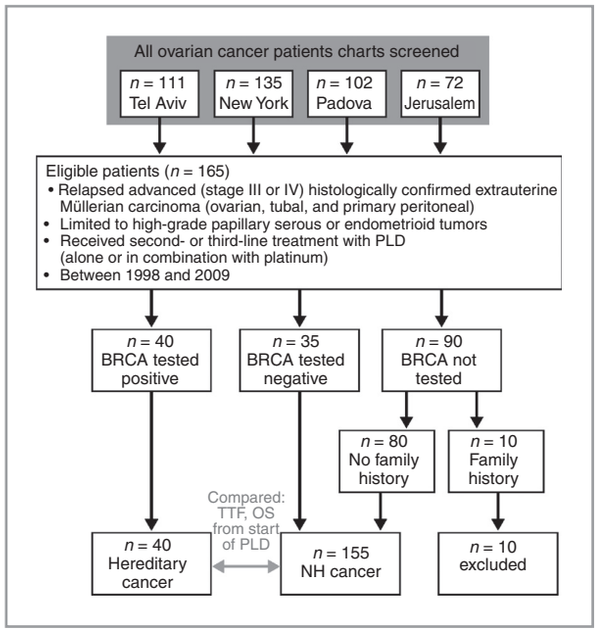

Figure 1. Study design and reviewed populations and *BRCA* status.

BRCA mutation testing was not done due to heavy family history. Data from 155 patients were analyzed. The median follow-up time was 19.1 months (range 1.63–144 months). Forty patients were carriers of the *BRCA1/2* mutation and 115 had NH disease (Table 1). Patients with NH disease were more likely than *BRCA* carriers to be older at diagnosis (53 vs. 60 years, $P < 0.004$). Differences in disease stage (III or IV) and treatments received were not significantly different between the 2 groups. Patients received either PLD as a single agent or combined with a platinum or bevacizumab as second- or third-line therapy for rEOC (Table 2). These different regimens reflect some variability in institutional policies. For example, the PLD + oxaliplatin regimen was almost entirely applied at IOV, whereas at NYU PLD + platinums was often applied, some as part of institutional phase I/II studies of these combinations; the PLD + Avastin cohort represented a later study at NYU. In all the institutions, PLD doses varied from 30 to 40 mg/m^2 and repeated every 3 to 4 weeks.

Clinical outcomes

Kaplan–Meier survival estimates of OS and TTF were compared between *BRCA1/2* mutation carriers and NH patients. OS was significantly prolonged: 56.84 months [95% confidence interval (CI): 32.49–indeterminate vs. 22.60 months (95% CI: 17.02–34.07)], respectively ($P = 0.002$; Fig. 2B). The median TTF was longer in *BRCA1/2* mutation carriers (15.84 months; 95% CI: 11.43–21.59) compared with patients with NH disease (8.05 months; 95% CI: 6.05–10.32; $P = 0.009$; Fig. 2A). Total response rate (CR + PR) also significantly favored *BRCA1/2* mutation carriers (68.4% vs. 49.0%; $P = 0.023$).

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Table 1. Patient characteristics

Category	Number of patients (%)			*P*
	BRCA-positive rEOC	NH rEOC	Total	
Total number of patients	40 (25.8)	115 (74.2)	155 (100)	
BRCA 1/2 mutation				
Total tested	40 (100.0)	35 (30.4)		
BRCA1 mutation	33 (82.5)			
BRCA2 mutation	7 (17.5)			
Not tested		80 (69.6)		
Median age at diagnosis, y (range)	53 (39–82)	60 (31–83)	59 (31–83)	<0.004
Stage of disease				0.12
III	36 (90)	90 (78.3)	126 (81.3)	
IV	4 (10)	25 (21.7)	29 (18.7)	
Histology				0.78
Serous	34 (85)	100 (87)	134 (86.5)	
Endometrial	6 (15)	15 (13)	21 (13.5)	
Platinum sensitivity				0.35
PFI $\leq$ 6 mo	9 (22.5)	35 (30.4)	44 (28.4)	
PFI > 6 mo	31 (77.5)	80 (69.6)	111 (71.6)	
PLD line of therapy				0.17
Second line	30 (75)	74 (64.3)	104 (67.1)	
Third line	10 (25)	41 (35.7)	51 (32.9)	
PLD regimen				0.85
PLD + platinum	22 (55)	68 (59.1)	90 (58.1)	
PLD alone	18 (45)	47 (40.9)	65 (41.9)	
Treatment status				1.00
PLD ongoing	4 (10)	13 (11.3)	17 (10.9)	
PLD discontinued	36 (90)	102 (88.7)	138 (89.0)	

Abbreviations: NH, nonhereditary; PFI, platinum-free interval; PLD, pegylated liposomal doxorubicin.

Kaplan–Meier survival estimates of TTF and OS from start of PLD therapy were compared separately for patients with platinum-resistant and platinum-sensitive tumors. The median TTF was longer in the platinum-sensitive group (11.43 months; 95% CI: 8.71–13.44) compared with the platinum-resistant group (7.06 months; 95% CI: 4.50–11.43); this difference did not reach statistical significance ($P = 0.08$; Fig. 2C). OS was significantly prolonged in the platinum-sensitive group (37.98 months, 95% CI: 22.61–48.03) compared with the platinum-resistant group (24.61 months, 95% CI: 12.03–36.30; $P = 0.041$; Fig. 2D).

Table 2. Type of PLD-containing regimens received

Regimen	Second-line setting	Third-line setting
PLD + carboplatin or cisplatin[a]	16	9
PLD + oxaliplatin	37	18
PLD + Avastin	5	9
PLD alone	40[b] (17 PR; 23 PS)	26 (16 PR; 10 PS)

Abbreviations: PLD, pegylated liposomal doxorubicin; PR, platinum resistant; PS, platinum sensitive.
[a]Only 1 patient received cisplatin.
[b]One patient received PLD with trabectedin.

On the basis of univariate Cox regression analyses, *BRCA* status (HR = 2.24; 95% CI: 1.33–3.79; $P = 0.003$) and platinum sensitivity (HR = 1.64; 95% CI: 1.02–2.64; $P = 0.04$) were significantly associated with OS. *BRCA* status was also significantly associated with TTF (HR = 1.70; 95% CI: 1.14–2.54; $P = 0.01$).

In the multivariate Cox models that included both *BRCA* status and platinum sensitivity, as well as age at diagnosis, PLD line of therapy, PLD-platinum combinations and institution, *BRCA* status was significantly and independently associated with OS (adjusted HR 2.07; 95% CI: 1.18–3.60; $P = 0.01$); the adjusted HR relating platinum sensitivity to OS was 1.58 (95% CI: 0.93–2.68; $P = 0.09$; Table 3). There was no significant association between OS and age at diagnosis, PLD line of therapy, PLD-platinum combinations, or institution. *BRCA* status was significantly and independently associated with TTF (HR = 1.66; 95% CI: 1.08–2.55; $P = 0.02$). There was no significant association between TTF and platinum sensitivity, age at diagnosis, PLD line of therapy, PLD-platinum

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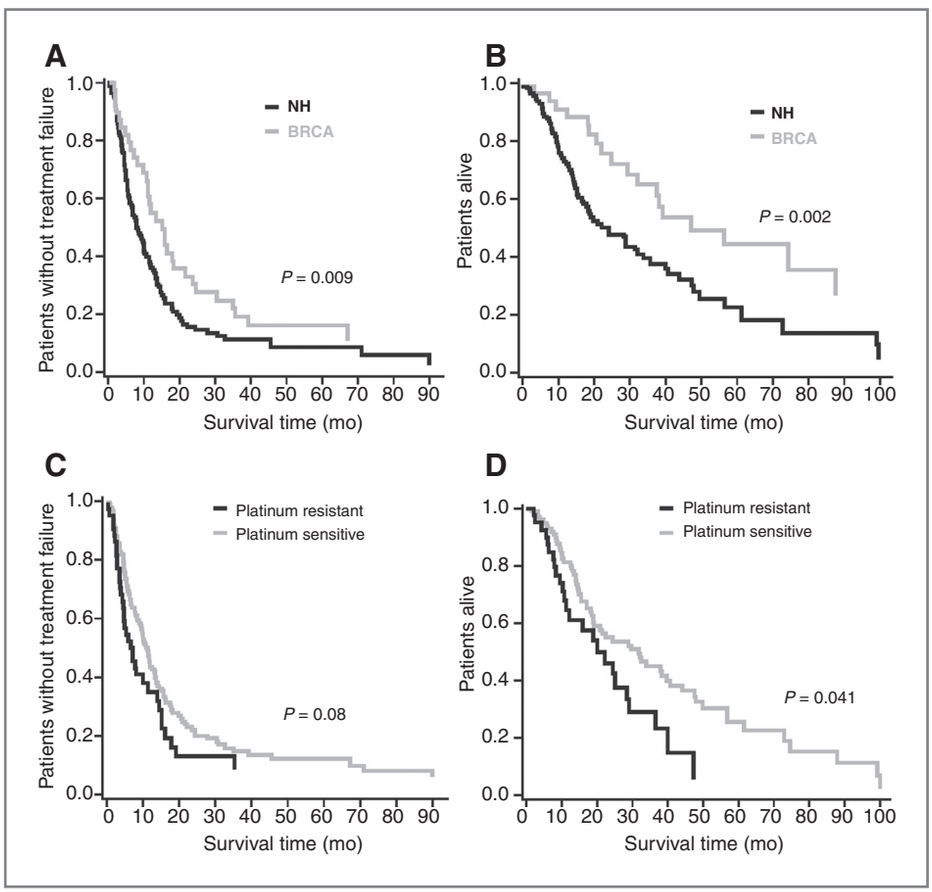

Figure 2. TTF (A) and OS (B) in carriers of the BRCA1/2 mutation versus patients with NH relapsed EOC. TTF (C) and OS (D) in all patients by platinum sensitivity.

combinations, or institution (Table 3). A Cox regression analysis of the different institutions showed no statistical difference between institutions.

In the Kaplan–Meier analyses restricted to the platinum-resistant population ($n = 44$), no significant differences between groups defined by *BRCA* status were found for OS or TTF (Table 4). In Kaplan–Meier analyses of the platinum-sensitive population, patients with *BRCA* 1/2 mutations had significantly longer TTF and OS compared with the NH patients ($P = 0.01$ and $P = 0.006$, respectively; Table 4).

Discussion

In today's medical practice, there is increasing emphasis on identifying unique patient or disease characteristics

Table 3. Results of multivariate Cox analyses ($n = 155$)

Variable	Parameter estimate	SE	*P*	HR
Overall survival				
BRCA vs. NH	0.725	0.284	0.01	2.07
Platinum sensitive vs. platinum resistant	0.457	0.269	0.09	1.58
Age at diagnosis (change per year)	0.006	0.011	0.60	1.01
PLD second line vs. third line	−0.190	0.236	0.42	0.83
PLD-platinum combination vs. PLD alone	−0.278	0.251	0.27	0.76
Time to treatment failure				
BRCA vs. NH	0.504	0.220	0.02	1.67
Platinum sensitive vs. platinum resistant	0.235	0.221	0.28	1.26
Age at diagnosis (change per year)	0.007	0.009	0.44	1.01
PLD second line vs. third line	−0.290	0.190	0.14	0.75
PLD-platinum combination vs. PLD alone	0.177	0.187	0.34	1.19

Abbreviations: HR, hazard ratio; NH, nonhereditary; PLD, pegylated liposomal doxorubicin.

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Table 4. Median TTF and OS by platinum sensitivity and *BRCA* mutation status (results of Kaplan–Meier analyses)

Outcome	BRCA-positive rEOC	NH rEOC	*P*
Platinum-resistant population, no. of patients.	$n = 9$	$n = 35$	
Median TTF, mo	11.4	5.5	
95% CI	3.6–35.4	3.9–10.0	0.46
Median OS, mo	25.1	20.2	
95% CI	9.7–indeterminate	11.0–36.3	0.21
Platinum-sensitive population, no. of patients.	$n = 31$	$n = 80$	
Median TTF, mo	16.4	9.4	
95% CI	11.4–24.5	6.7–11.6	0.01
Median OS, mo	74.7	24.5	
95% CI	32.4–indeterminate	17.0–44.2	0.006

Abbreviations: NH, nonhereditary; TTF, time to treatment failure; OS, overall survival.

associated with enhanced response to particular treatments. The evolution of systemic therapy for EOC has been particularly influenced by findings that suggest the presence of *BRCA* germline mutations influence outcome and responsiveness to platinum compounds (12, 13). Our converging interests for a similar role of *BRCA* mutations to influence outcome in EOC treated with PLD led to the current study.

In this analysis, 155 patients with recurrent EOC treated at 4 institutions (centers in United States, Israel, and Italy) with second- or third-line PLD monotherapy or combination with platinum were evaluated to compare outcomes between *BRCA* mutation carriers and the NH population. As evidenced by longer median TTF ($P = 0.009$), OS ($P = 0.002$), and response rate ($P = 0.023$), prognosis was improved in *BRCA* mutation carriers. A multivariate Cox analysis of baseline prognostic features and outcome confirmed a significant association between *BRCA* status and TTF and OS, a significant association between platinum sensitivity and OS, and no association with age, PLD line of therapy, PLD-platinum combinations, and institution. Results of the univariate Cox regression analysis also echo the results of the Kaplan–Meier analyses, providing additional information about the associations between *BRCA* status and both OS and TTP.

Studies to date reporting the relationship of *BRCA* mutation status and sensitivity to platinum treatment have only been in the setting of primary disease or first recurrence (6, 12–14). Our analysis—to the best of our knowledge—represents the first to assess this relationship in a post first-line setting of a nonplatinum agent, PLD. These results, however, have limitations: the study had to use retrospective response assessments, 4 institutions using different policies to treat patients with recurrent EOC in second or third line were included, and only dichomotous definition of platinum resistance was applied. Oxaliplatin was an additional platinum agent used with PLD, often in the presence of platinum resistance, both at the New York and Italian institutions. Although this unusual regimen is a potential confounder in considering the generalizability of our results, this practice was confined to a relatively small number of patients, and its use was not guided by *BRCA* status. On the other hand, common practices and collection of information because of participation in PLD trials, led to this collaboration. Our analysis does suggest, however, that results from PLD are superior in progression-free survival (PFS) and OS in patients who are known mutation carriers than in other patients identified as NH. Outcomes beyond first and subsequent lines of treatment, independent of assignation by platinum sensitivity, continue to be more favorable in *BRCA* mutation carriers. Indeed, these clinical findings provide evidence corroborating laboratory research showing hypersensitivity of tumors with major defects in homologous recombination repair to DNA-damaging agents (25).

In sporadic EOC, which represents the majority of EOCs (relative to hereditary EOC), reduced *BRCA* activity, induced by epigenetic hypermethylation or mechanisms other than somatic mutations may in turn alter chemosensitivity (26–29). Patients with recurrent EOC and *BRCA* mutations and possibly patients with sporadic EOC and reduced *BRCA* function represent a new subpopulation that might benefit significantly from the appropriate use of different chemotherapy agents. PLD seems more effective in *BRCA* mutation carriers, and, based on recent results of the CALYPSO trial showing improved efficacy and reduced toxicity with the combination of PLD and carboplatin (vs. paclitaxel-carboplatin), this combination warrants further evaluation in *BRCA* mutation carriers (30).

In the quest for treatment individualization, a better understanding of the complex function of *BRCA* genes has led to evaluation of a new group of targeted drugs. PARP-1 and -2 facilitate single-strand DNA break repair, and PARP inhibitors cause an increase and persistence of single-strand DNA breaks, which can lead to double-strand breaks on replication (31). In *BRCA* mutation carriers, double-strand DNA break repair is impaired and tumor cell damage significantly increased. Recent

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results from germline sequencing and a phase II trial indicate high activity of PARP1 inhibitors in ovarian cancer with *BRCA* mutations (32, 33). Results of a recently reported randomized phase II trial comparing the PARP inhibitor olaparib with PLD in *BRCA* mutation carriers with recurrent EOC who failed previous platinum therapy showed no significant benefit from olaparib (34). Surprisingly, the PLD-treated patients fared better (PFS of 7.1 months) compared with historical cohorts, further supporting the possibility that *BRCA*-related EOC responds better to PLD than sporadic EOC. Continued follow-up and results of ongoing trials will further elucidate the role of PARP inhibitors for recurrent EOC with *BRCA* mutations.

Yet another possibility for optimizing treatment of *BRCA* mutation carriers with recurrent EOC is to alternate therapy with DNA-damaging agents and anti-tubulin agents, such as taxanes. Preclinical findings suggest taxane resistance in *BRCA* mutation carriers with subsequent regaining of *BRCA* function (35). The DNA-damaging agent could be administered when *BRCA* function is low and anti-microtubulin agent administered when *BRCA* function is regained.

In conclusion, our study shows that tumors arising in a background of defective *BRCA* function are more sensitive than other EOCs to DNA-damaging agents such as PLD, even after operationally defined as platinum resistant. These improved outcomes suggest persistent defects in homologous recombination, perhaps even after the development of platinum resistance.

Disclosure of Potential Conflicts of Interest

A. Gabizon has a consultant or advisory relationship with Johnson & Johnson for which he was compensated, and he received other remuneration from Hadasit Co. F. Muggia has a consultant or advisory relationship with Johnson & Johnson for which he was compensated. He also received honoraria from Ortho Biotech.

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