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A high response rate to liposomal doxorubicin is seen among women with *BRCA* mutations treated for recurrent epithelial ovarian cancer

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Abstract

Objective—Ten percent of ovarian cancer is attributed to hereditary syndromes, most commonly to mutations in the *BRCA1* or *BRCA2* genes. These cancers are characterized by a prolonged sensitivity to platinum agents in spite of presentation at advanced stages. We hypothesized that women with *BRCA*-associated ovarian cancer would also show a high response rate to pegylated liposomal doxorubicin (Doxil).

Methods—A retrospective cohort study was conducted to compare the response rate, progression-free, and overall survival among women with *BRCA*-associated or sporadic ovarian cancer who were treated with Doxil.

Results—A response to Doxil was seen in 13 of 23 patients with *BRCA* mutations (56.5%; 3 by RECIST criteria and 10 by CA125 levels) compared with only 8 of 41 women with non-hereditary cancers (19.5%; 2 by RECIST criteria and 6 by CA125 levels; $p=0.004$). This was associated with an improved progression-free and overall survival as measured from the time of Doxil administration. Notably, platinum sensitivity did not directly correlate with a response to Doxil.

Conclusions—Women with *BRCA*-associated ovarian tumors demonstrate a greater sensitivity to cytotoxic therapy with Doxil than has previously been reported in unselected cases.

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Conflict of Interest Statement

The authors declare that there are no conflicts of interest.

Introduction

More than 20,000 women are diagnosed with ovarian cancer annually in the United States, and nearly 14,000 women succumbed to this disease in 2010. Approximately ten percent of all ovarian cancer cases are attributed to hereditary syndromes, most commonly the result of mutations in the breast cancer susceptibility genes, *BRCA1* and *BRCA2* (1). In addition to an elevated risk of breast cancer, *BRCA* mutation carriers have a 16–68% lifetime risk of developing epithelial ovarian carcinoma (2–4).

BRCA-associated ovarian tumors are characterized by presentation at advanced stages, with high grade serous histology and a propensity for visceral metastasis (5–6). Despite these aggressive features, women with *BRCA* mutations have an improved response to chemotherapy and prolonged treatment-free intervals when compared with cases of sporadic disease (5). In describing a “*BRCA*ness” phenotype, Tan *et al.* attribute this survival advantage to the persistent sensitivity of *BRCA*^{-/-} tumor cells to platinum-induced DNA damage (7). In support of this observation, acquired platinum resistance has been directly associated with reversions of germline *BRCA* mutations in both ovarian cancer cell lines and tumor specimens from patients with platinum-resistant disease (8–10).

Like platinum agents, one mechanism of action for the cytotoxic effect of anthracyclines is direct DNA damage. Doxorubicin has been shown to cause single- and double-stranded DNA breaks through several mechanisms, including direct intercalation and inhibition of DNA polymerase, interference with the function of topoisomerase II and the formation of intracellular free radicals.(11). Fortunately women who demonstrate resistance to platinum agents often do not demonstrate cross-resistance to doxorubicin. As a result, a well-tolerated liposomal formulation of doxorubicin (Doxil) has become the preferred second-line agent for platinum-resistant disease with reported response rates that range from 9–16% (12–13). Because *BRCA* mutations disable double-stranded DNA repair mechanisms, *BRCA*-associated cancers would be expected to demonstrate a preferential sensitivity to any DNA-damaging therapy, including Doxil.

We have observed an excellent response to Doxil among women with hereditary cancer treated in our clinical practice and hypothesized that the response to Doxil by women with *BRCA* mutations may more closely approximate the higher response rate seen in platinum-sensitive cases(12). Here we present a retrospective cohort study designed to determine whether the response rate and survival following Doxil therapy is significantly different in women with *BRCA*-deleterious or sporadic ovarian cancer undergoing treatment for advanced or recurrent disease.

Methods

Patient Selection

Patient information was retrieved from a pharmacy database at the University of Pennsylvania to identify women who underwent treatment with Doxil between 2001 and 2007. Ovarian cancer cases were confirmed using treatment records from the Division of Gynecologic Oncology and these patients were cross-referenced with data from the Cancer Genetics Program of the Abramson Cancer Center. At two collaborating institutions, Cedars-Sinai Medical Center in Los Angeles and Fox Chase Cancer Center in Philadelphia, women who had undergone testing for *BRCA* mutations were identified, and those with a diagnosis of ovarian cancer were included if they had received Doxil chemotherapy.

Patients who were confirmed to carry a *BRCA1* or *BRCA2* mutation were evaluated as the experimental (exposed) cohort. An unexposed group was comprised of women who were

tested and did not carry a *BRCA* gene mutation, and women with a thoroughly documented family history and no first- or second-degree relatives with breast or ovarian cancer who were unlikely to carry a genetic mutation, the “presumed negative” cases. Women were excluded from evaluation if they were still undergoing treatment with Doxil at the time of data collection, if family history or follow-up data was unavailable, or if they had a personal history of breast cancer or a concurrent diagnosis of a non-ovarian malignancy.

Patient data

After obtaining approval from each Institutional Review Board, charts were abstracted to collect basic demographic information, medical and oncologic history, and clinical course following Doxil therapy. Response rate was calculated based on either a decrease in tumor size or a fall in the CA125 during or following treatment (14–15). Progression-free survival was measured as the time from initiation of Doxil treatment until the first evidence of progression using either a rise in CA125 based on the Gynecologic Cancer Intergroup criteria or radiographic documentation of tumor growth based on RECIST criteria (Response Evaluation Criteria in Solid Tumors)(15–16). Overall survival was measured as the time from initiation of Doxil treatment until death or the date of last follow-up.

Data was collected regarding platinum sensitivity at the time that Doxil treatment was initiated: patients were considered to be platinum-sensitive if they had a 6 month treatment-free interval after their most recent course of platinum-based therapy. Patients who relapsed within 6 months of prior platinum therapy or who failed to respond to platinum therapy were considered platinum-resistant.

Statistical methods and sample size calculations

Our target sample size was calculated assuming a type I alpha error of 0.05, two-sided, and 80 percent power. We estimated that approximately 80–90% of all patients who received Doxil for recurrent disease would experience progression within one year (17). Furthermore, we anticipated that this fraction would be lower among patients with hereditary disease than among those with sporadic ovarian cancer. For these calculations we assumed that approximately 10% of all enrolled patients would be progression-free at one year, and that the ratio (*BRCA*^{-/-} vs. sporadic disease) would range from 1.4 to 2.9. Our target was to identify 25 women with a mutated *BRCA* genotype among 50 patients undergoing *BRCA* testing to detect a 60% relative improvement in progression-free survival over non-hereditary cases (i.e. a hazard ratio=0.40).

Initial bivariate comparisons between the groups were performed using Fisher’s exact test or Chi-square tests. Log-rank tests were used to evaluate differences in survival between sporadic disease and *BRCA*-associated cancers, and Cox proportional hazards models were used to identify predictors of overall and progression-free survival. Univariate Cox models were fit to determine initial associations between predictors of individual and progression-free survival, and multivariate models were fit with marginally significant predictors ($p < 0.10$) from univariate models. Statistical modeling was done using STATA version 11 (Stata Corp, College Station TX). All reported p-values are 2-sided with $p < 0.05$ considered to be statistically significant.

Results

Patient characteristics

One hundred and sixty-four cases of women undergoing treatment with Doxil were identified using pharmacy databases and 149 cases of women with ovarian cancer were identified from records of women undergoing genetic testing. Details of the patient selection

process are provided in Figure 1. From these, 23 women with epithelial ovarian cancer and a documented *BRCA1* or *BRCA2* mutation who received Doxil chemotherapy were identified and comprised the experimental cohort. An additional 31 patients who had no personal or family history of hereditary cancers, along with 10 women who underwent genetic testing but were found not to harbor a *BRCA* mutation, were considered the control group with sporadic disease.

Women in the *BRCA*-associated and sporadic disease cohorts did not differ significantly in age, stage at presentation, or grade or histologic subtypes of their tumors. The majority of patients in both groups had high grade tumors that presented at an advanced stage. All patients with *BRCA* mutations had papillary serous cancers except for one patient with a poorly differentiated tumor of mixed histology, and two for whom histology data was unavailable. Among the non-hereditary cases, 70% were papillary serous, 7% were poorly differentiated, and histology was unavailable for 7%. One patient in the sporadic cohort had an endometrioid cancer, and one had a clear cell cancer. The majority of patients were Caucasian (85% overall; 96% of the *BRCA* cohort and 80% of the sporadic cohort), with African American patients comprising 6% and Asian/Pacific Islanders comprising 3% of the total population. Ethnicity data was unavailable for two patients. Patient characteristics are compared in Table 1.

Response to Doxil treatment

A response to Doxil was seen in 56.5% of women with *BRCA*-associated cancer compared with only 19.5% of controls ($p=0.004$). Response was measured based on a decrease in the CA125 level by the third cycle of Doxil administration in 16 women, and by a reduction in the size of tumor implants on imaging studies using RECIST criteria in five cases (15). Women with stable disease were not counted among responders. In univariate logistic regression analysis, women with a *BRCA* mutation were 5.36 times more likely to experience a response than women with sporadic disease (95% CI [1.73, 16.59], $p=0.004$, Table 2).

Progression-free and overall survival

Progression-free and overall survival analyses were performed by measuring survival from the date of first Doxil administration, not from the time of diagnosis. Because women in the *BRCA* cohort received on average one additional course of chemotherapy, and because in general they would be expected to have longer treatment-free intervals between regimens, the survival data for these cases begins at a later time-point in the disease course. Despite this, both progression-free and overall survival was improved among *BRCA* mutation carriers (Figures 2, 3). The median disease-free interval for women with *BRCA*-associated cancer was 27.1 weeks compared with only 17 weeks among women with sporadic disease ($p=0.109$, Table 3). Overall survival in the *BRCA* cohort was significantly higher than that seen among controls, with a median survival of 89.1 weeks in women with *BRCA* mutations versus 48.3 weeks among women with non-hereditary cancers ($p=0.002$, Table 3).

Prior treatment with chemotherapy

Patients in both groups were heavily pre-treated, undergoing an average of 2.7 prior chemotherapeutic regimens before receiving Doxil (range 0–6). Women with *BRCA* mutations underwent a significantly higher number of prior regimens compared to women with sporadic disease (mean 3.4 vs 2.3 respectively, $p=0.004$).

Prior treatment was a negative confounding factor in calculating the response rate as well as both disease-free and overall survival. Because women with *BRCA* mutations underwent a higher number of prior chemotherapy regimens before receiving Doxil, a multivariable

logistic regression model controlling for the number of prior chemotherapy regimens was evaluated. In this model, the likelihood of response to Doxil was 12.85 times higher among the *BRCA* cohort compared to women with sporadic disease (95% CI [3.0, 54.92], $p=0.001$, Table 2).

As with the response rate, both progression-free and overall survival results were negatively affected by the number of prior chemotherapy regimens. After adjusting for *BRCA* mutation status, as the number of prior chemotherapy regimens increased, the relapse rate increased by 30.4% and the overall mortality rate increased by 22.5%. When controlling for prior treatment, the survival advantage associated with the presence of a *BRCA* mutation was statistically significant, with a hazard ratio of 0.508 (95% CI 0.28–0.93) for progression-free survival and 0.297 (95% CI 0.14, 0.59) for overall survival in the multivariate model ($p=0.027$ and 0.001, respectively, Table 3).

Specific data regarding platinum sensitivity at the time of Doxil treatment was available for 60 patients (93.7%). Among these, 14 of 19 (74%) *BRCA*-associated cases and 32 of 41 (78%) sporadic cases demonstrated platinum resistance prior to Doxil treatment. Women who were platinum-resistant received fewer prior courses of chemotherapy compared with those demonstrating persistent platinum-sensitivity (2.1 vs 3.27 prior courses, respectively). Although the response rate overall was higher among platinum-sensitive patients (41.6% versus 33% for platinum-resistant patients; $n=12$ and 48, respectively), the response to Doxil among patients in this study did not directly correlate with platinum sensitivity. Of the women with *BRCA*-associated cancers who demonstrated a response to Doxil, only 3 out of 13 were platinum-sensitive (23%), while 77% were considered platinum-resistant when they began treatment with Doxil (Table 3). Similarly, among 8 patients with sporadic disease who demonstrated a response to Doxil, 2 (25%) were platinum-sensitive and 6 (75%) were platinum-resistant.

Discussion

Our results demonstrate a significantly improved response rate of 56.5% among women with *BRCA* mutations treated with Doxil, which is markedly higher than has been reported in studies evaluating second-line therapies in unselected cases of ovarian cancer (12, 17–18). *BRCA* mutation status was also associated with an improved progression-free and overall survival as measured from the date of the first cycle of Doxil, despite the fact that patients in the *BRCA* cohort received more chemotherapy cycles prior to initiating Doxil treatment. When controlling for the number of prior regimens, both the progression-free and overall survival advantage associated with *BRCA* mutation status were statistically significant.

The 19.5% response rate we describe among the cohort of women with sporadic disease is consistent with previously reported rates of 17–26%, and we observed a median progression free survival of 17 weeks in this group, which is similar to the 16 week median survival in prior studies of platinum-resistant patients (12, 17–19). Based on this relatively high response rate for a second-line therapy and the low toxicity profile of Doxil compared with other agents, an expert panel of oncologists has recommended that Doxil be the first-choice agent for platinum-resistant disease, and that a role for Doxil as maintenance or combination therapy for primary adjuvant treatment be considered (18).

Although a minority of patients in either cohort remained platinum-sensitive at the start of Doxil therapy, a high proportion of platinum-sensitive patients demonstrated a response to Doxil (41.6% overall, 60% among *BRCA*-associated cases, $n=12$). A difference in response based on platinum sensitivity has been associated with long term survival in large prospective studies: a follow-up report of a phase III study evaluating Doxil therapy for

women with recurrent ovarian cancer documented significantly improved survival rates among platinum-sensitive patients treated with Doxil (74.1%, 51.2%, and 28.4% at 1, 3, and 5 years) compared with platinum refractory patients (41.5%, 21.1%, and 13.8 %, respectively) (17). Because both Doxil and platinum drugs induce direct DNA damage by intercalation, it is not surprising that platinum-sensitivity is associated with an improved response to Doxil. Similarly, the persistent platinum-sensitivity of *BRCA*^{-/-} tumors, resulting from an inability to repair double-stranded DNA breaks, would also be expected to render them vulnerable to Doxil-induced DNA damage (7–9).

It is notable, then, that our results do not show a direct correlation between platinum-sensitivity and response to Doxil among women with *BRCA* mutations. Among platinum-resistant patients, who have historically shown a response in only 9–16% of cases, the advantage associated with *BRCA* mutation status was more pronounced: 77% of platinum-resistant *BRCA* mutation carriers responded to Doxil treatment, compared with only 17% of women with platinum-resistant sporadic disease (n=48) (12–13). This would suggest that *BRCA* status cannot be used as a surrogate marker for platinum sensitivity to explain the high response rate in these cases.

Accumulating evidence has demonstrated that platinum resistance in some *BRCA*^{-/-} tumors may be the result of secondary mutations that restore wild-type *BRCA* function and the capacity to repair double-stranded DNA breaks (8–9). Although platinum-resistant *BRCA*^{-/-} tumors may lose their selective vulnerability to DNA damage, alternate mechanisms of action may be responsible for the enhanced cytotoxic effects of Doxil in these cases. Recently, an immunomodulatory role for Doxil has been demonstrated which may enhance host anti-tumor immune responses (20–23). In particular, Alagkiozidis *et al.* demonstrated that Doxil, in a dose-dependent fashion, alters the immunophenotype of non-apoptotic ovarian tumor cells, rendering them more susceptible to T cell-mediated tumor killing (20). Because *BRCA1*- cancers have been shown to recruit high numbers of T cells to tumor implants, these cancers may be especially vulnerable to therapies that enhance anti-tumor immunity (24), possibly contributing to the improved response rate even among platinum-resistant *BRCA*^{-/-} cases.

Limitations of this study include the retrospective design and the small sample size that lacked power to detect a significant difference in progression-free survival in univariate analysis. Although our results are comparable with historic response rates in unselected groups, a large proportion of patients were excluded due to a lack of sufficient data to consider them among the presumed negative group for analysis. Finally, because *BRCA* mutation carriers received more chemotherapy before beginning Doxil treatment, our decision to analyze survival outcomes starting at the time of Doxil administration may underestimate the survival benefit associated with *BRCA* mutation status and the potential benefit of Doxil for early recurrences in these patients. Prospective data evaluating Doxil therapy for *BRCA*-associated ovarian cancer are expected as results mature from a phase II trial comparing Doxil with PARP-inhibitors in women with hereditary ovarian cancer (Kaye *et al*, ESMO, 2010).

Overall, our results support the use of Doxil for advanced or recurrent ovarian cancer, particularly in women with hereditary disease. Together with data suggesting that Doxil treatment may prolong platinum-free intervals, and evidence that carboplatin combined with Doxil was not inferior to carboplatin/taxol therapy, our findings support a wider role for Doxil in the treatment of advanced ovarian cancer (25–26).

Research Highlights

- 56.5% of women with *BRCA*-associated ovarian cancer demonstrated a response to Doxil compared with 19.5% with sporadic disease
- *BRCA* mutation carriers had a longer progression-free and overall survival following Doxil treatment.
- The response to Doxil in these cases did not correlate with platinum sensitivity.

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Figure 1. Flow diagram for patient selection

Figure 2. Progression-Free Survival

Kaplan-Meier curves demonstrating the disease-free survival time from the first dose of Doxil until evidence of disease progression for women in the *BRCA* $-/-$ and sporadic disease cohorts.

Figure 3. Overall Survival

Kaplan-Meier curves demonstrating the overall survival time from the first dose of Doxil until death from any cause for women in the *BRCA* $-/-$ and sporadic disease cohorts.

Table 1
Patient Demographics

Demographic data for women in the sporadic disease or *BRCA*-associated cohorts. No significant difference was seen when comparing the age, stage or tumor grade in the two groups. Women with *BRCA* mutations underwent a greater number of chemotherapy regimens prior to starting Doxil (p=0.004)

	Sporadic Disease Number (%)	<i>BRCA-associated</i> Number (%)	p-value
Age <55	19 (46.3)	13 (56.5)	0.434
≥ 55	22 (53.6)	10(43.5)	
Stage I-II	4 (10)	3 (14.3)	0.683
III-IV	36 (90)	18 (85.7)	
Grade 1-2	4 (14.3)	1 (5.3)	0.635
Grade 3*	24 (85.7)	18 (94.7)	
Mean prior chemotherapy regimens	2.34	3.39	0.004

* Two patients were unstaged and grade was unknown for 17 cases

Response Rate

A comparison of the response rate using a univariate model or a multivariate model which controlled for the number of prior treatment regimens.

Table 2

Cases	Univariate Logistic Model				Multivariate Logistic Model		
	n	OR	p-value	95% CI	OR	p-value	95% CI
Sporadic disease	41	1			1		
BRCA-associated	23	5.36	0.004	[1.73, 16.59]	12.85	0.001	[3.0, 54.92]

Table 3

Platinum sensitivity at the time of Doxil administration

Demographic data, tumor histology, platinum-sensitivity at the time of Doxil administration, response rate and progression-free and overall survival of women with documented *BRCA* mutations treated with Doxil. PFS = progression-free survival; OS= overall survival; CR = complete response; PR= partial response; PD= progressive disease. Data regarding platinum sensitivity was unavailable for 4 cases.

Patient	Age	<i>BRCA</i> mutation	Stage	Histology	Number of prior regimens	Response to platinum prior to starting Doxil	Response to Doxil	Measure of Response	PFS (weeks)	OS (weeks)
1	24	1	4	Papillary serous	2	Resistant	PD	CA125	11.4	33.3
2	49	1	3	Papillary serous	5	Sensitive	PD	CA125	3.3	21.6
3	51	1	1	Papillary serous	4	Sensitive	CR	CA125	71.6	118.1
4	73	2	3	Papillary serous	1	Resistant	PR	CA125	30.3	83.2
5	32	2	3	Papillary serous	1	Resistant	CR	RECIST	131.1	316
6	64	2	3	Unknown	6	Sensitive	PD	RECIST	14	55.5
7	50	1	3	Papillary serous	2	Sensitive	CR	CA125	132.3	132.3
8	55	2	3	Papillary serous	6	Resistant	CR	CA125	28.7	309
9	44	2	4	Papillary serous	3	Resistant	PD	CA125	8	46.7
10	46	1	3	Papillary serous	1	Resistant	CR	RECIST	215.4	N/A
11	61	2	3	Papillary serous	2	Sensitive	PR	CA125	26	85
12	55	1	3	Papillary serous	2	Resistant	PR	CA125	120.9	N/A
13	62	1	3	Papillary serous	4	Resistant	CR	RECIST	24.7	51.8
14	52	1	3	Papillary serous	3	Resistant	PR	CA125	27.1	N/A
15	52	2	3	Papillary serous	4	Resistant	PD	CA125	8.4	11.3
16	64	1	1	Papillary serous	4	Resistant	PD	CA125	N/A	16.9
17	49	1	3	Papillary serous	1	Resistant	CR	CA125	70.1	291.9
18	33	1	3	Papillary serous	2	Resistant	PR	CA125	15.7	89.1
19	43	1	3	Papillary serous	4	Resistant	CR	RECIST	65.3	N/A
20	71	2	1	Papillary serous	7	Unknown	PD	RECIST	12	75
21	61	2	3	Mixed histology	4	Unknown	PD	CA125	5	95
22	43	1	3	Unknown	7	Unknown	PD	RECIST	11	69
23	66	2	U	Papillary serous	4	Unknown	PD	RECIST	9	78

Progression-Free and Overall Survival

Table 4

A comparison of the hazard ratios for progression-free and overall survival using a univariate model or a multivariate model controlling for the number of prior treatment regimens.

Factor	n	Median (weeks)	Range (weeks)	Univariate Cox Model			Multivariate Cox Model		
				HR	p-value	95% CI	HR	p-value	95% CI
Progression Free Survival									
Sporadic disease	41	17	(1.2 – 255.1)	1			1		
<i>BRCA</i> -associated	23	27.1	(3.3 – 215.4)	0.632	0.109	[0.36, 1.11]	0.508	0.027	[0.28–0.93]
Overall Survival									
Sporadic disease	41	48.3	(5.6 – 290.9)	1			1		
<i>BRCA</i> -associated	23	89.1	(11.3 – 445.9)	0.361	0.002	[0.36, 1.11]	0.297	0.001	[0.14, 0.59]