ONCOLOGY

A chemoresponse assay for prediction of platinum resistance in primary ovarian cancer

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OBJECTIVE: Recurrence following primary platinum-based chemotherapy remains a challenge in the treatment of patients with advanced-stage epithelial ovarian cancer. This study examines whether a chemoresponse assay can identify patients who are platinum-resistant prior to treatment.

STUDY DESIGN: Women (n = 276) with International Federation of Gynecology and Obstetrics stage III-IV ovarian, fallopian, and peritoneal cancer were enrolled in an observational study, and the responsiveness of their tumors was evaluated using a chemoresponse assay. All patients were treated with a platinum/taxane regimen following cytoreductive surgery. Assay responses to carboplatin or paclitaxel were classified as sensitive, intermediate sensitive (IS), or resistant. Association of assay response with progression-free survival (PFS) was analyzed using the Kaplan-Meier method and a Cox regression model.

RESULTS: Patients whose tumors were resistant to carboplatin were at increased risk of disease progression compared to those with nonresistant (sensitive + IS) tumors (median PFS: 11.8 vs 16.6 months, respectively, $P < .001$), and the association was confirmed after adjusting for other clinical factors (hazard ratio, 1.71; 95% confidence interval, 1.12–2.62; $P = .013$). Association of assay response to paclitaxel with PFS trended in multivariate analysis (hazard ratio, 1.28; 95% confidence interval, 0.84–1.95; $P = .245$). For tumors resistant to carboplatin, 59% were sensitive or IS to at least 1 other commonly used agent, demonstrating the ability of the assay to inform treatment decisions beyond the standard platinum/taxane regimen.

CONCLUSION: Assay resistance to carboplatin is strongly associated with shortened PFS among advanced-stage epithelial ovarian cancer patients treated with carboplatin + paclitaxel therapy, supporting use of this assay to identify patients likely to experience early recurrence on standard platinum-based therapy.

Key words: chemoresponse assay, ovarian cancer, platinum resistance

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In 2013, it was estimated that there will be 22,240 new cases of ovarian cancer and 14,030 deaths due to this disease in the United States; epithelial ovarian cancer (EOC) represents the leading cause of death from gynecologic malignancies.[1] The poor prognosis observed with EOC is largely attributed to late detection of the disease (ie, once it has already advanced to late stages), as well as intrinsic drug refractory and/or emerging drug resistance to initial chemotherapy. Evidence from randomized clinical trials has established the platinum/taxane combination regimen as standard first-line chemotherapy for patients with advanced-stage EOC, yielding response rates of 60-70%.[2,3] However, most such patients experience relapse within 1-2 years, and only 30% live >5 years.[4] It is clear that EOC is

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a heterogeneous disease, and a platinum/taxane combination is not the optimal chemotherapy regimen for all patients.

Efforts have been taken to improve toxicities, response rates, and survival through the use of alternate chemotherapies, the use of different treatment schedules, or the incorporation of biologic agents, with encouraging data recently reported for the latter 2 approaches.[5-7] Over the last 2 decades, multiple clinical studies have attempted to identify chemotherapy regimens superior to platinum/taxane in the first-line treatment of advanced-stage EOC.[3,8-10] Although progression-free survival (PFS) and overall survival (OS) observed in these alternate regimens are no better (and, in many studies, are no worse) than those observed with the platinum/taxane standard, the alternate regimens may be considered to be equivalent in clinical practice.

In EOC, clinically useful markers that identify platinum-resistant tumors, among the overall high number of chemosensitive patients, remain a critical need. If identified early, platinum-resistant EOC patients could benefit from alternate and/or additional therapeutic options in first-line therapy. Moreover, reliable early identification of platinum resistance may allow the development of clinical trials specifically targeting this population with novel alternate therapies.

Chemoresponse assays have been investigated as a method for individualizing chemotherapy treatment decisions and improving outcomes in cancer patients. Recently, a prospective study demonstrated that women with persistent or recurrent EOC who were treated with an assay-sensitive therapy experienced significantly improved PFS and OS compared to those treated with assay-resistant therapies.[11] To further evaluate the clinical relevance of this assay in the primary setting, and in accordance with standards for the reporting of diagnostic accuracy criteria,[12] an observational study was conducted among women with stage III/IV EOC treated by standard-of-care chemotherapy. The primary objective of this study is to determine whether assay response to carboplatin or/and paclitaxel is associated with disease progression among patients with primary EOC following initial treatment with platinum/taxane regimen. Furthermore, this study will evaluate whether this assay can be used to identify patients who are resistant to platinum-based treatment and at high risk of early progression.

Materials and Methods

Study population

Participants were prospectively enrolled in an observational study of women with gynecologic cancers. Tumor samples from 54 institutions were submitted for chemoresponse testing from 2006 through 2010. Women with International Federation of Gynecology and Obstetrics stage III-IV EOC, fallopian tube cancer, and peritoneal cancer treated with carboplatin/paclitaxel-based chemotherapy following initial cytoreductive surgery were included in the study. Patients with a time interval of >2 months between surgery and initiation of chemotherapy, chemotherapy duration >6 months, and/or treatment consisting of >10 cycles of chemotherapy were excluded. The institutional review board at each participating center approved this study, and documented informed consent was obtained from all enrolled patients.

Chemoresponse assay

Details regarding the chemoresponse assay employed in this study (ChemoFx; Precision Therapeutics Inc, Pittsburgh, PA) have been described elsewhere.[13] Briefly, the inhibition of tumor growth was measured at different concentrations of each therapy. The survival fraction of tumor cells at each concentration was calculated as compared to a control (no drug). The summation of survival fraction values over 7 concentrations was computed as the drug response score, which represents the area under the dose-response curve (AUC). A smaller AUC score indicates greater sensitivity to the therapy. Chemoresponse is classified into 1 of 3 categories according to the AUC score: sensitive, intermediate sensitive (IS), or resistant. The classification criterion was defined based on the distribution of AUC scores among an external population of patients with primary EOC. Specifically, the distributions of AUC scores for carboplatin and paclitaxel were established based on referent specimens. Scores ranked at the 25th and 75th percentiles were obtained. A tumor with an AUC score <25th rank was classified as sensitive, between 25th-75th rank as IS, and >75th rank as resistant.

Statistical analysis

The primary endpoint of this study was PFS, calculated from the start of chemotherapy administration until the date of first documented disease recurrence, death, or most recent follow-up. Commonly utilized patient prognostic information was also collected, including: age, Eastern Cooperative Oncology Group performance status, histology, tumor grade, stage, debulking status, and type of chemotherapy administered. The physician(s) at each institution reported all clinical information, which was quality controlled according to a comprehensive monitoring plan. Disease progression was determined by clinical evidence, radiological examination, and/or cancer antigen 125. Optimal debulking was defined as residual tumor of ≤1 cm in maximal dimension at the end of surgery and was reported by enrolling physicians. PFS based on assay response was estimated using the Kaplan-Meier method, and the log rank test was used to compare the differences among sensitive, IS, and resistant patients. Since the primary objective of the current study was to identify platinum-resistant patients, sensitive and IS groups were combined for further analyses. The association of the assay and PFS was also assessed using Cox regression model adjusted for clinical covariates (age, performance status [1-3 vs 0], histology [high-grade serous vs non-high-grade serous], and stage/debulking status [III-suboptimal/IV vs III-optimal]). The hazard ratio (HR) of disease progression for patients treated with resistant vs nonresistant (sensitive + IS) therapy was estimated. Subgroup analyses stratified by age group, performance status, histology/tumor grade, or stage/debulking status were also conducted.

RESULTS

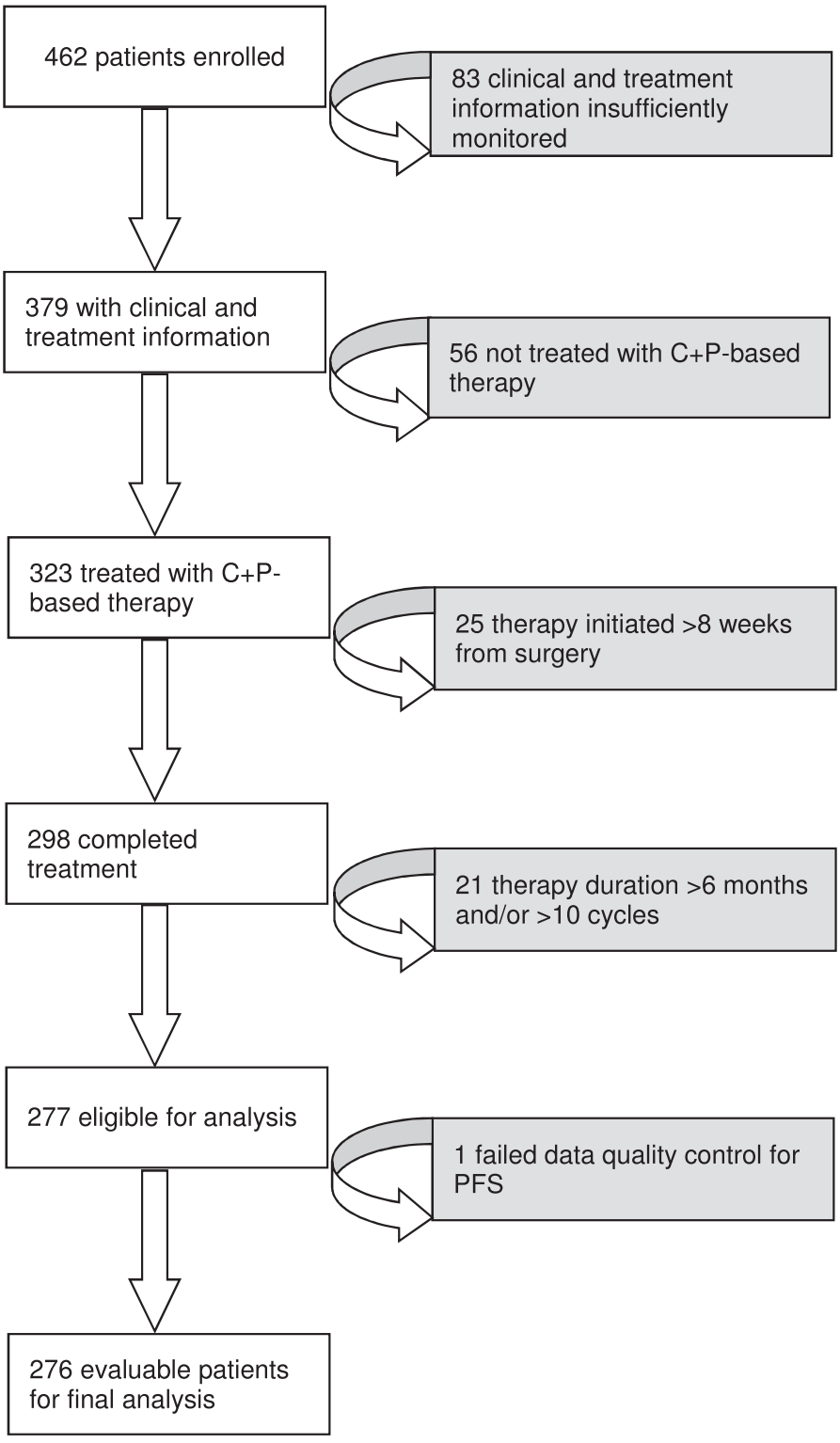
Patients

A total of 462 patients were enrolled in this study, with 276 evaluable for inclusion in the analysis (Figure 1). Patient characteristics are displayed in Table 1. The median age of the study population was 61 years, and most patients had tumors that were classified as papillary serous (84%), poorly differentiated (83%), stage III (85%), and optimally debulked (72%) (Table 1). The majority (94%) completed 4-8 cycles of chemotherapy. The median follow-up period was 23 months (range, 12−37 months), and 193 (70%) patients experienced disease progression within this time frame. The median PFS was estimated to be 15.9 months (95% confidence interval [CI], 14.3−17.1 months).

Chemoresponse assay and clinical outcomes

Assay results for carboplatin were available for 231 patients, with 44 (19.1%) patients identified as resistant to this therapy in the chemoresponse assay. Assay data for paclitaxel were available for 226 patients, 49 (21.7%) of whom were classified as resistant. Assay resistance by age, performance status, histology/grade, and stage/debulking status is summarized in Table 2. There is no evidence that assay result for either carboplatin or paclitaxel is correlated with patient characteristics. Assay result for carboplatin was significantly associated with clinical outcome (Figure 2). The median PFS was 16.6 and 11.8 months for assay nonresistant (sensitive + IS) and resistant tumors, respectively. Patients displaying assay resistance to carboplatin were at a higher risk of disease progression as compared to those who were nonresistant (HR, 1.87; 95% CI, 1.29−2.70; $P < .001$). These results were consistent in multivariate analysis after controlling for clinical covariates (HR, 1.71; 95% CI, 1.12−2.62; $P = .013$) (Table 3). Analysis of subgroups (age group, performance status, histology, stage/debulking status) was also conducted (Figure 3), and the association between PFS and assay result for carboplatin was suggested across all

FIGURE 1
CONSORT diagram



Flow diagram describing exclusion criteria and their effect on total number of evaluable patients in study.

C, carboplatin; *CONSORT*, Consolidated Standards of Reporting Trials; *P*, paclitaxel; *PFS*, progression-free survival.

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TABLE 1
Patient characteristics (n = 276)

Characteristic	No. patients	%
Age, y		
Median (range)	61 (30–85)	
<50	40	(14.5)
50–59	78	(28.3)
60–69	97	(35.1)
≥70	61	(22.1)
ECOG PS		
0	144	(52.2)
1	63	(23.8)
2	11	(4.0)
3	2	(0.7)
Unknown	56	(20.3)
Tumor site		
Ovarian	229	(83.0)
Peritoneal	32	(11.6)
Fallopian tube	15	(5.4)
Histology		
Serous	232	(84.1)
Endometrioid	8	(2.9)
Clear cell	6	(2.2)
Mucinous	3	(1.1)
Others	27	(9.8)
Tumor grade		
1	12	(4.4)
2	25	(9.1)
3	228	(82.6)
Unknown	11	(4.0)
Stage		
III	234	(84.8)
IV	42	(15.2)
Debulking		
Microscopic	64	(23.2)
Optimal	135	(48.9)
Suboptimal	59	(21.4)
Unknown	18	(6.5)

ECOG, Eastern Cooperative Oncology Group; *PS*, performance status.

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TABLE 2
Assay resistance by patient characteristics

	Carboplatin			Paclitaxel		
Characteristic	No. patients	No. resistant, n (%)	*P* value	No. patients	No. resistant, n (%)	*P* value
Age group, y			.987			.213
<60	100	19 (19.0)		96	17 (17.1)	
≥60	131	25 (19.1)		130	32 (24.6)	
ECOG PS			.636			.526
0	126	24 (19.1)		120	25 (20.8)	
≥1	59	13 (22.0)		60	15 (25.0)	
Tumor grade			.342			.438
1-2	32	8 (25.0)		30	5 (16.7)	
3	190	34 (17.9)		187	43 (23.0)	
HGS			.204			.270
Yes	165	28 (17.0)		161	38 (23.6)	
No	66	16 (24.2)		65	11 (16.9)	
Stage/debulking			.145			.136
III-Optimal	143	24 (16.8)		139	27 (19.4)	
III-Suboptimal/IV	76	19 (25.0)		74	21 (28.4)	
All	231	44 (19.1)		226	49 (21.7)	

ECOG, Eastern Cooperative Oncology Group; *HGS*, high-grade (grade-3) serous; *PS*, performance status.

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subgroups. The data also suggest that patients with assay resistance to paclitaxel would experience shortened PFS, but the association did not reach the level of statistical significance (Table 3).

Assay results for carboplatin and paclitaxel were highly correlated. For 220 patients with assay data available for both agents, 75.5% were nonresistant to both agents and 15.9% were resistant to both agents, while only 8.6% of patients were resistant to only 1 agent (5.9% to carboplatin and 2.7% to paclitaxel). Patients resistant to both agents experienced the worst outcomes (HR, 1.66; 95% CI, 1.10–2.52; $P = .017$, as compared to patients nonresistant to both agents). Multivariate analysis indicated the same tendency, although the association was not statistically significant (Table 3).

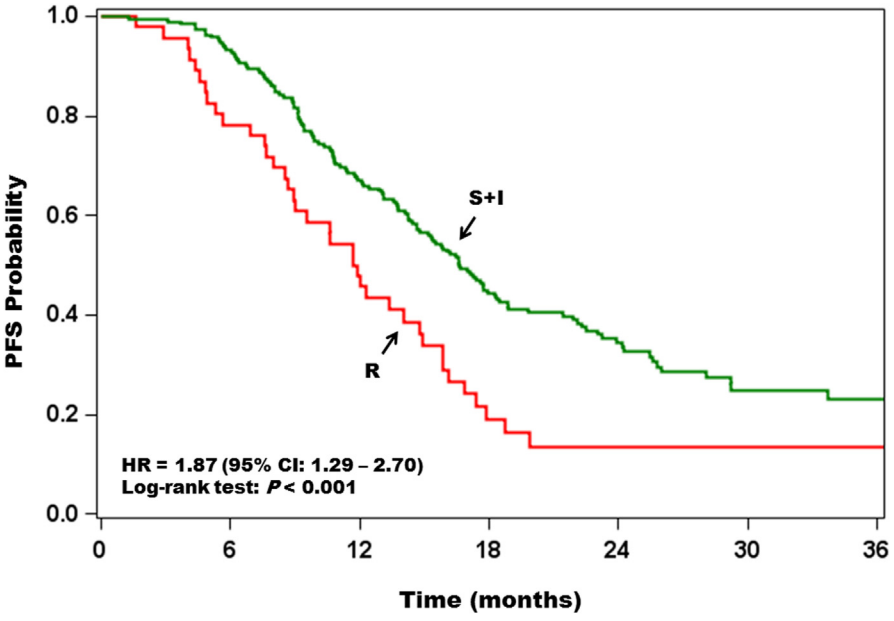
Pattern of assay response

To estimate the proportion of patients who may benefit from assay-informed therapy, the assay responses to 7 clinically relevant single agents (carboplatin, cisplatin, gemcitabine, pegylated liposomal doxorubicin, paclitaxel, docetaxel, and topotecan) were analyzed for 153 patients who had complete assay data for all 7 of these agents (Figure 4). Only 7% of the patients displayed assay resistance to all 7 agents, while 5% were sensitive to all 7 agents. Thus, 93% of the patients were nonresistant (sensitive or IS) to at least 1 agent. Specifically, 35% were IS to at least 1 agent, and 58% were sensitive to at least 1 agent. Of note, 18% of these tumors were resistant to carboplatin but, of those, 59% of them were nonresistant (sensitive or IS) to at least 1 other agent in the chemoresponse assay.

COMMENT

The standard of care for first-line treatment of patients with advanced-stage EOC consists of aggressive cytoreductive

FIGURE 2
Kaplan-Meier survival curves



Progression-free survival (PFS) in advanced-stage epithelial ovarian cancer patients receiving first-line carboplatin/paclitaxel-based chemotherapy and exhibiting resistant (R) vs nonresistant (sensitive [S] + intermediate sensitive [I]) chemoresponse assay results for carboplatin.

CI, confidence interval; *HR*, hazard ratio.

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TABLE 3
Univariate and multivariate analyses of factors affecting disease progression-free survival

	Univariate analysis		Multivariate analysis	
Assay result	**HR (95% CI)**	***P* value**	**HR (95% CI)[a]**	***P* value**
Assay for carboplatin				
Sensitive[b]	Referent		Referent	
Resistant	1.87 (1.29–2.70)	< .001	1.71 (1.12–2.62)	.013
Assay for paclitaxel				
Sensitive	Referent		Referent	
Resistant	1.43 (0.99–2.06)	.055	1.28 (0.84–1.95)	.245
Assay for carboplatin and paclitaxel				
Sensitive to both	Referent		Referent	
Resistant to one	1.42 (0.83–2.43)	.206	1.38 (0.76–2.51)	.297
Resistant to both	1.66 (1.10–2.52)	.017	1.51 (0.94–2.42)	.090

CI, confidence interval; *HR*, hazard ratio.

[a] HR for disease progression estimated from Cox model adjusted for age, Eastern Cooperative Oncology Group performance status, tumor grade/histology, and stage/debulking status; [b] Sensitive or intermediate sensitive assay result.

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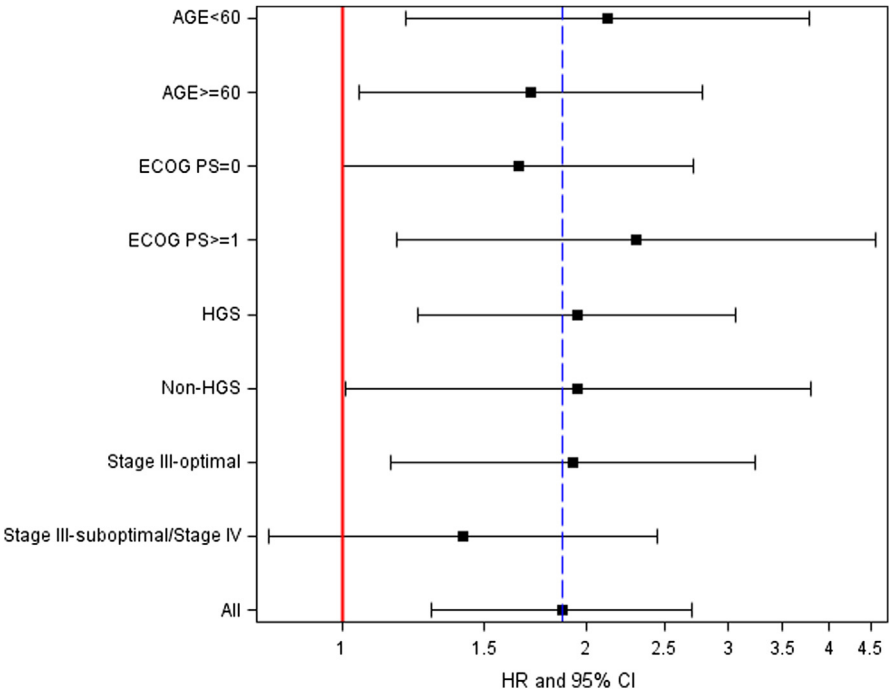
surgery followed by platinum/taxane-based chemotherapy[14]; however, in this treatment approach, approximately 20-30% of patients will have platinum-resistant disease.[15] If identified early, platinum-resistant EOC patients may benefit from alternate and/or additional therapeutic options in first-line therapy. At the time of recurrence, clinicians will classify patients as being platinum sensitive (EOC relapsing >6 months after the end of first-line chemotherapy) or platinum resistant (EOC relapsing within 6 months after the end of first-line chemotherapy).[16,17] This platinum status classification is the primary covariate used in determining future prognosis and subsequent treatment strategies. However, as with most clinical covariates, its accuracy is not absolute; additional measures of platinum responsiveness may be beneficial in further personalizing treatment strategies. Using the current standard clinical approach, identification of platinum-resistant disease is delayed until after the patient has already experienced the costs and toxicities associated with first-line therapy. Earlier identification of effective first-line treatment may improve the disease course in EOC patients, potentially allowing them to demonstrate response, avoid recurrence for a longer time, and delay the onset of decline in overall health, thereby allowing more therapies to be given that may further extend OS.

Unfortunately, molecular characterization of EOC has not yet been able to substitute for the clinically observed platinum status classification. The current study evaluates the potential utility of a chemoresponse assay in identifying platinum resistance in advanced-stage EOC patients undergoing standard first-line treatment. Determining platinum status earlier in the treatment of advanced-stage EOC may prevent this high-risk group of patients from being exposed to multiple cycles of ineffective therapy and allow for more effective alternate therapeutic options earlier in the disease, with the ultimate goal of improving patient outcomes.

The results reported herein suggest that the chemoresponse assay is an independent factor in identifying platinum

FIGURE 3
Subgroup analysis of association between assay results and progression-free survival



Hazard ratios (HR) (resistant vs nonresistant) estimated for each subgroup of patients stratified by major clinical covariates.

CI, confidence interval; *ECOG*, Eastern Cooperative Oncology Group; *HGS*, high-grade serous; *PS*, performance status.

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resistance in advanced-stage EOC patients treated with standard first-line therapy (carboplatin/paclitaxel). Patients whose tumors were assay-resistant to carboplatin had an increased risk of early disease progression, as compared to those whose assay results were nonresistant for carboplatin, recurring on average 5 months sooner. Furthermore, based on the Kaplan-Meier plot of the current study (Figure 2), within 6 months of the start of chemotherapy, 25% of assay-resistant patients had already recurred, while <10% of assay-sensitive (nonresistant) had recurred. Likewise, at 18 months after the start of chemotherapy, approximately 50% of assay-sensitive patients had been free of disease progression, while 80% of assay-resistant patients had recurred.

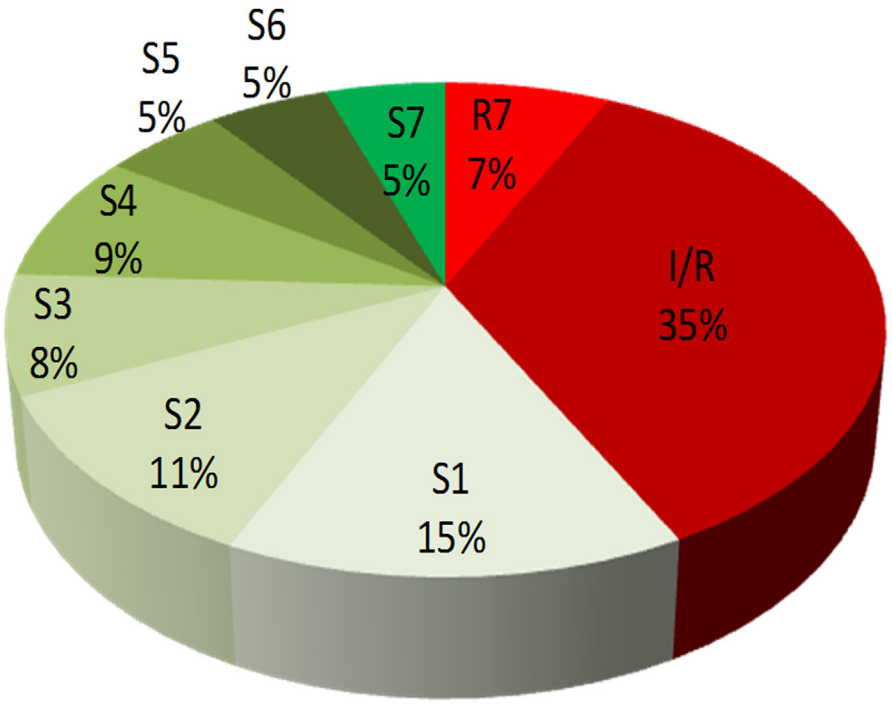
Multivariate analysis of assay results for paclitaxel demonstrated a positive trend, and, further, patients who were resistant to both agents demonstrated the worst outcomes, which was significantly different from patients nonresistant to both agents. These results are consistent with the notion that the platinum portion of the standard regimen for advanced-stage EOC plays the larger role in the clinical performance of that regimen.[18,19] As such, it is expected that assay results for paclitaxel are not as highly correlated with PFS as are those for carboplatin and carboplatin + paclitaxel. OS will be included in future analyses.

The ability of this assay to identify patients likely to be platinum resistant creates the opportunity to consider alternate treatments regimens for these patients earlier in the course of treatment. Alternate treatments may be considered either initially following surgery or upon first clinical indication of suboptimal performance during standard first-line treatment. Earlier intervention may allow for a reduction in toxicities incurred by the patient from ineffective therapy, as well as a reduction in the overall costs of treatment.[20] Most importantly, assay-informed treatment decisions may lead to earlier treatment with a more effective therapy, thereby delaying recurrence and potentially lengthening the overall expected survival duration for these high-risk patients. Identification of advanced-stage EOC patients as platinum resistant prior to treatment could inform first-line treatment decisions in a variety of ways, including substitution of alternate active agents, alteration of the planned first-line therapy to a dose-dense approach, or the addition of novel therapies that may overcome the resistance observed.[5-7,21-23] Results from various completed and ongoing studies investigating alternate treatment strategies to carboplatin + paclitaxel should be referenced when considering treatment different than carboplatin + paclitaxel.[3,5-7,8-10,21-23]

In addition to identifying platinum-resistant patients, the current study also demonstrates the ability of the chemoresponse assay to generally identify agents to which a patient's tumor is sensitive. The results presented herein show that >90% of patient tumors were sensitive or IS to at least 1 of the 7 most common agents utilized clinically to treat EOC. More importantly, for those tumors resistant to carboplatin, >50% of them were identified to be sensitive or IS to at least 1 other agent. These results exemplify the ability of the assay to inform treatment decisions beyond the carboplatin/paclitaxel standard of care. These findings are also consistent with those from a recent prospective study of patients with recurrent EOC who demonstrate an improvement in both PFS and OS when treated with an assay-sensitive therapy compared to those treated with a nonsensitive agent,[11] highlighting the clinical value of this assay for individualized treatment of EOC.

In summary, the chemoresponse assay evaluated herein is independently associated with PFS and may be used to

FIGURE 4
Distribution of assay results across 7 single-agent treatments



Patients (n = 153) were categorized as resistant to all 7 treatments (R7), intermediate or resistant to all 7 treatments (I/R), or sensitive to anywhere from 1 (S1) to 7 (S7) treatments. Single-agent treatments were: carboplatin, cisplatin, pegylated liposomal doxorubicin, gemcitabine, paclitaxel, docetaxel, topotecan.

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predict platinum resistance in patients with advanced-stage EOC prior to treatment. Patients predicted for poorer outcome (ie, platinum resistance) by the assay (and in conjunction with other clinical factors) may be considered for investigation of alternate treatment options. ■

REFERENCES

1. Siegel R, Naishadham D, Jemal A. Cancer statistics, 2013. CA Cancer J Clin 2013;63:11-30.

2. Ozols RF, Bundy BN, Greer BE, et al. Phase III trial of carboplatin and paclitaxel compared with cisplatin and paclitaxel in patients with optimally resected stage III ovarian cancer: a gynecologic oncology group study. J Clin Oncol 2003;21:194-200.

3. du Bois A, Luck HJ, Meier W, et al. A randomized clinical trial of cisplatin/paclitaxel versus carboplatin/paclitaxel as first-line treatment of ovarian cancer. J Natl Cancer Inst 2003;95:1320-9.

4. Coleman RL, Monk BJ, Sood AK, Herzog TJ. Latest research and treatment of advanced-stage epithelial ovarian cancer. Nat Rev Clin Oncol 2013;10:211-24.

5. Katsumata N, Yasuda M, Isonishi S, et al. Long-term results of dose-dense paclitaxel and carboplatin versus conventional paclitaxel and carboplatin for treatment of advanced epithelial ovarian, fallopian tube or primary peritoneal cancer (JGOG 3016): a randomized, controlled, open-label trial. Lancet Oncol 2013;14:1020-6.

6. Perren TJ, Swart AM, Pfisterer J, et al. A phase 3 trial of bevacizumab in ovarian cancer. N Engl J Med 2011;365:2484-96.

7. Burger RA, Brady MF, Bookman MA, et al. Incorporation of bevacizumab in the primary treatment of ovarian cancer. N Engl J Med 2011;365:2473-83.

8. Bookman MA, Brady MF, McGuire WP, et al. Evaluation of new platinum-based treatment regimens in advanced-stage ovarian cancer: a phase III trial of the gynecologic cancer intergroup. J Clin Oncol 2009;27:1419-25.

9. du Bois A, Weber B, Rochon J, et al. Addition of epirubicin as a third drug to carboplatin-paclitaxel in first-line treatment of advanced ovarian cancer: a prospectively randomized gynecologic cancer intergroup trial by the Arbeitsgemeinschaft Gynaekologische Onkologie Ovarian Cancer Study Group and the Groupe d'Investigateurs Nationaux pour l'Etude des Cancers Ovariens. J Clin Oncol 2006;24:1127-35.

10. Pfisterer J, Weber B, Reuss A, et al. Randomized phase III trial of topotecan following carboplatin and paclitaxel in first-line treatment of advanced ovarian cancer: a gynecologic cancer intergroup trial of the AGO-OVAR and GINECO. J Natl Cancer Inst 2006;98:1036-45.

11. Rutherford T, Orr J Jr, Grendys E Jr, et al. A prospective study evaluating the clinical relevance of a chemoresponse assay for treatment of patients with persistent or recurrent ovarian cancer. Gynecol Oncol 2013;131:362-7.

12. Bossuyt PM, Reitsma JB, Bruns DE, et al. The STARD statement for reporting studies of diagnostic accuracy: explanation and elaboration. Clin Chem 2003;49:7-18.

13. Brower SL, Fensterer JE, Bush JE. The ChemoFx assay: an ex vivo chemosensitivity and resistance assay for predicting patient response to cancer chemotherapy. In: Mor G, Alvero AB, eds. Methods in molecular biology. Vol. 414: apoptosis and cancer. Totowa, NJ: Humana Press Inc; 2008:57-78.

14. National Comprehensive Cancer Network. NCCN clinical practice guidelines in oncology. Ovarian cancer: including fallopian tube cancer and primary peritoneal cancer. Version 1.2014. Available at: http://www.nccn.org/professionals/physician_gls/pdf/ovarian.pdf. Accessed March 7, 2014.

15. Markman M, Bookman MA. Second-line treatment of ovarian cancer. Oncologist 2000;5:26-35.

16. Gore ME, Fryatt I, Wiltshaw E, Dawson T. Treatment of relapsed carcinoma of the ovary with cisplatin or carboplatin following initial treatment with these compounds. Gynecol Oncol 1990;36:207-11.

17. Markman M, Hoskins W. Responses to salvage chemotherapy in ovarian cancer: a critical need for precise definitions of the treated population. J Clin Oncol 1992;10:513-4.

18. Muggia FM, Braly PS, Brady MF, et al. Phase III randomized study of cisplatin versus paclitaxel versus cisplatin and paclitaxel in patients with suboptimal stage III or IV ovarian cancer: a gynecologic oncology group study. J Clin Oncol 2000;18:106-15.

19. International Collaborative Ovarian Neoplasm Group. Paclitaxel plus carboplatin versus standard chemotherapy with either single-agent carboplatin or cyclophosphamide, doxorubicin, and cisplatin in women with ovarian cancer: the ICON3 randomized trial. Lancet 2002;360:505-15.

20. Havrilesky LJ, Krivak TC, Mucensku JW, Myers ER. Impact of a chemoresponse assay on

treatment costs for recurrent ovarian cancer. Am J Obstet Gynecol 2010;203:160.e1-7.
21. Monk BJ, Herzog TJ, Kaye SB, et al. Trabectedin plus pegylated liposomal doxorubicin in recurrent ovarian cancer. J Clin Oncol 2010;28:3107-14.
22. Sehouli J, Stengel D, Oskay-Oezcelik G, et al. Nonplatinum topotecan combinations versus topotecan alone for recurrent ovarian cancer: results of a phase III study of the North-Eastern German Society of Gynecological Oncology Ovarian Cancer Study Group. J Clin Oncol 2008;26:3176-82.
23. Buda A, Floriani I, Rossi R, et al. Randomized controlled trial comparing single agent paclitaxel vs epidoxorubicin plus paclitaxel in patients with advanced ovarian cancer in early progression after platinum-based chemotherapy: an Italian collaborative study from the Mario Negri Institute, Milan, G. O.N.O. (Gruppo Oncologico Nord Ovest) group and I.O.R. (Istituto Oncologico Romangnolo) group. Br J Cancer 2004;90:2112-7.