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## Gynecologic Oncology

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## A prospective study evaluating the clinical relevance of a chemoresponse assay for treatment of patients with persistent or recurrent ovarian cancer

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### HIGHLIGHTS

- A prospective clinical trial examines the ability of a chemoresponse assay to improve clinical outcomes in recurrent ovarian cancer.
- Patients treated with an assay-sensitive treatment had significantly improved clinical outcomes over those treated with a non-sensitive treatment.
- Although only 25% of patients were empirically treated with a sensitive treatment, over 50% had at least one sensitive result.

### ARTICLE INFO

#### Article history:

Received 9 May 2013

Accepted 8 August 2013

Available online 13 August 2013

#### Keywords:

Chemoresponse assay

Ovarian cancer

Prospective clinical trial

### ABSTRACT

**Objective.** Use of in vitro chemoresponse assays for informing effective treatment selection is a compelling clinical question and a topic of debate among oncologists. A prospective study was conducted evaluating the use of a chemoresponse assay in recurrent ovarian cancer patients.

**Methods.** Women with persistent or recurrent ovarian cancer were enrolled under an IRB-approved protocol, and fresh tissue samples were collected for chemoresponse testing. Patients were treated with one of 15 protocol-designated treatments empirically selected by the oncologist, blinded to the assay results. Each treatment was classified by the assay as: sensitive (S), intermediate (I), or resistant (R). Patients were prospectively monitored for progression-free survival (PFS) and overall survival (OS). Associations of assay response for the physician-selected treatment with PFS and OS were analyzed.

**Results.** A total of 262 evaluable patients were enrolled. Patients treated with an assay-sensitive regimen demonstrated significantly improved PFS and OS while there was no difference in clinical outcomes between I and R groups. Median PFS was 8.8 months for S vs. 5.9 months for I + R (hazard ratio [HR] = 0.67,  $p = 0.009$ ). The association with assay response was consistent in both platinum-sensitive and platinum-resistant tumors (HR: 0.71 vs. 0.66) and was independent of other covariates in multivariate analysis (HR = 0.66,  $p = 0.020$ ). A statistically significant 14-month improvement in mean OS (37.5 months for S vs. 23.9 months for I + R, HR = 0.61,  $p = 0.010$ ) was demonstrated.

**Conclusions.** This prospective study demonstrated improved PFS and OS for patients with either platinum-sensitive or platinum-resistant recurrent ovarian cancer treated with assay-sensitive agents.

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## Introduction

Epithelial ovarian cancer (EOC) is the leading cause of gynecologic cancer mortality in the United States [1]. Despite the achievement of high response rates, improvements in survival with aggressive surgical debulking and use of platinum/taxane combination chemotherapy, the disease recurs in the majority of the patients [2]. Recurrent EOC has many treatment options depending on specific aspects of its presentation, including secondary cyto-reductive surgery and numerous second-line chemotherapy treatment options. While most patients eventually succumb to progression of recurrent disease, many will benefit from therapy and experience prolonged remissions and symptom-free survival [2,3]. Patients experiencing a relapse greater than six months following first-line platinum-based treatment have been defined “platinum-sensitive” and have significant responses to further platinum-based combination therapies [4,5]. The response rate increases in relation to the platinum-free interval such that those relapsing greater than 24 months from initial treatment are similar to chemo-naïve patients with respect to response [4,5]. Patients experiencing disease progression within 6 months from the completion of first-line chemotherapy are defined as platinum-resistant [4,5] and are typically treated with multiple single agent therapies. Expected progression-free survival (PFS) and overall survivals (OS) are significantly compromised for this group of patients.

Due to the inherent challenges of multiple treatment variables, costs and time necessary to conduct large prospective clinical trials in recurrent EOC [6], prior studies evaluating chemoresponse assays have been largely retrospective. Furthermore, findings from several retrospective studies have been inconsistent in the demonstration of correlation between assay results and treatment outcomes in EOC [7–11], triggering debate over the use of assay-informed treatment in EOC [12]. Many of these studies had small sample size cohorts, lacked detailed information and were missing the necessary patient follow-up that clinical trials require. Even those studies that were of a larger scale did not always yield the level of evidence necessary to validate the use of chemoresponse assays in ovarian cancer [9,13,14]. Still, several large, retrospective clinical studies have demonstrated that chemoresponse assays are clinically feasible, correlate with treatment outcomes, and may have the potential to aid in the prioritization of drug therapies [15]. Specifically, Gallion et al. [10] reported that patients treated with an assay-sensitive regimen had progression-free intervals three times longer than those who received an assay-resistant treatment, and OS was more than double for assay-sensitive patients in a subsequent analysis [11]. For these reasons, a prospective, multi-site, non-interventional (blinded to avoid physician treatment bias) clinical trial was conducted to evaluate the ability of a chemoresponse assay to identify treatments that may lead to improvement in PFS and OS. The study results reported herein are in compliance with REMARK [16] and STARD [17] guidelines.

## Patients and methods

### Patient eligibility

Patients with histologically confirmed EOC, fallopian tube (FTC), or primary peritoneal cancer (PPC) were enrolled at 35 sites across the United States. Eligible patients received  $\leq 2$  prior chemotherapy regimens and experienced persistent, recurrent or progressive disease as documented by imaging or by an increased level of CA-125. Both platinum-sensitive (platinum-free interval [PFI]  $\geq 6$  months) and platinum-resistant (PFI  $< 6$  months) diseases, based on clinical outcomes following treatment in the primary setting, were included. Other eligibility criteria included  $\geq 18$  years of age, ECOG performance status  $\leq 2$ , adequate bone marrow, renal and hepatic function, and viable tumor tissue available for chemoresponse assay from surgical excision or drainage of ascites/effusions.

### Treatment and follow-up

This study was designed to be non-interventional in order to assess the assay-outcome correlation in an unbiased manner. Patients were treated with one of 15 prospectively-specified protocol treatments, based on the medical judgment of the oncologist (i.e. patients and physicians were blinded to the assay results for the initial protocol treatment). Treatment was required to begin within 8 weeks of sending tissue for in vitro testing. Once disease progression was demonstrated with the treatment selected for the study, there were no exclusions from further therapy, and the physician could optionally gain access to the assay results (physicians requested access to assay results, post progression, for 32% of the evaluable patients). Supportive care was also allowed at the discretion of the treating physician. Disease progression was measured by radiologic examination (CT scan as the primary imaging method), physical examination, and CA-125 measurements using RECIST or GCIC criteria, and the assessment was performed every other cycle during the treatment, every 3 months for the first 2 years, every 6 months for the next 3 years and annually thereafter.

### Chemoresponse assay

Fresh tissue samples were collected from each patient at the time of recurrence for in vitro testing (ChemoFx®, Precision Therapeutics, Inc., Pittsburgh, PA). Details regarding the assay procedure have been described elsewhere [18]. In brief, primary cultures were initiated by mincing each tissue sample into 1 mm<sup>3</sup> explants, which were then seeded into culture flasks. Upon near confluency, primary cultures were trypsinized and seeded into 384-well microtiter plates (Corning, Lowell, MA) at 8000 cells/mL and used immediately for in vitro testing. Ten concentrations of each treatment were prepared by serial dilution. Each concentration was added to three replicate wells on the microtiter plate; three replicates of control (no treatment) wells were associated with each treatment also. Culture seeding into microtiter plates, as well as serial treatment dilution and application, were completed using highly automated liquid handling robotics. After 72 h of incubation with treatment, surviving adherent cells were stained with DAPI (Molecular Probes, Carlsbad, CA) and counted using proprietary, automated computer-assisted microscopy (Precision Therapeutics, Inc., Pittsburgh, PA) [19]. The inhibition of tumor growth was measured for each concentration (average of cell counts in three replicates) of a given treatment. The survival fraction (SF) of tumor cells at each concentration was calculated as compared to control (no treatment). The summation of SF values over concentrations 1 through 7 was computed as the drug response score, which represents the area under the dose response curve (defined as AUC7 score hereafter). A smaller AUC7 score indicates that a tumor is more sensitive to a treatment in vitro; a larger score indicates greater resistance to a treatment. For each treatment, in vitro tumor response was classified into one of three categories according to the AUC7 score: sensitive (S), intermediate (I), and resistant (R). The cut-point thresholds for the classifications were previously and independently established based on the 25th and 75th AUC7 percentiles in external and independent recurrent EOC patients.

### Statistical analysis

The primary endpoint of this study was PFS, defined as the length of time from the start of recurrence chemotherapy selected by the physician for this study until the date of first documented disease progression or death; OS was the secondary endpoint. The study was designed to detect a hazard ratio (HR) of 0.6 at a power of 0.80 ( $\alpha = 0.05$ ), requiring 256 patients with 80% of the patients experiencing disease progression or death. PFS was estimated by the Kaplan–Meier procedure, and the difference between in vitro response categories was compared using the log-rank test. The association of in vitro assay results and PFS was also assessed using the Cox regression model adjusted for clinical

covariates (age, performance status [PS 1–2 vs. PS 0], histology [serous vs. others], tumor grade [grade 3 vs. grades 1–2] and platinum sensitivity status [platinum-sensitive vs. platinum-resistant]) [20]. The HR of disease progression for S vs. I + R was estimated. Planned, non-powered subgroup analyses for platinum-sensitive and platinum-resistant patients were also conducted. The analyses for OS were employed using the same approaches. All of the analyses were conducted using the assay treatment that was an exact match of the patient (clinical) treatment.

## Results

### Patients

Between 2004 and 2011, 335 eligible patients were enrolled, and 262 (78%) had both a successful assay and complete clinical data, making them evaluable for this study. Seventy-three (22%) patients were excluded, primarily due to lack of growth of a sufficient number of malignant cells in culture ( $n = 34$ ) and quality control failure ( $n = 21$ ). The majority of tissue was submitted as biopsies (93%), with the remaining 7% being in the form of ascites fluid. Patient characteristics are summarized in Table 1 and Table S2. The majority of the tumors were high-grade papillary serous, and 55% of patients had platinum-sensitive recurrent EOC. A broad range of chemotherapies was administered based on the medical judgment of the treating physicians, covering 12 distinct treatments (encompassing both single agents and 2 agent combinations); docetaxel, cisplatin/docetaxel, and cisplatin/topotecan were available for use in this study but were not administered clinically to any of the evaluable patients (Table 2). All patients were treated with at least one cycle of the physician selected treatment, with half of the patients in the cohort completing at least 4 treatment cycles (range: 1–13). For platinum-sensitive tumors, carboplatin/paclitaxel (31%), PLD (15%) and carboplatin/gemcitabine (12%) were most frequently used, while carboplatin/paclitaxel (29%), PLD (28%) and topotecan (13%) were usually offered to persistent or

platinum-resistant recurrent tumors (Table S1). It is interesting to note that no single treatment accounted for more than 30% of the treatments assessed in this study, demonstrating the lack of a standard of care in this indication. Furthermore, the distribution of treatments across S and I + R patient cohorts is not materially different from one another (Table 2), supporting the notion that different patients respond differently to various therapies and that no one treatment generally outperforms the others.

Across the entire study cohort, the median follow-up time was 29 months (range: 1–71 months), based on achievement of the primary endpoint, PFS. At study closure, 33 (12.6%) patients were alive without disease progression, 67 (25.6%) were alive with progression, and 162 (61.8%) were deceased. The median PFS for the total study population was 6.7 months and the median OS was 26.5 months. The median PFS for platinum-sensitive and platinum-resistant patients were 9.3 months and 3.8 months ( $p < 0.001$ ), respectively; and the median OS were 33.6 months and 21.8 months ( $p < 0.001$ ), respectively.

### Chemoresponse assay and clinical outcomes

For the 262 patients, the assay result for the treatment received clinically was S (28.6%), I (45.8%) or R (25.6%). There were no significant differences in patient characteristics across the three groups, except for age, with the S group being younger than I + R patients (57 vs. 63 years,  $p < 0.001$ ).

From univariate analysis, patients with tumor response defined as S for their clinical treatment demonstrated significantly improved PFS [median PFS 8.8 months for S vs. 5.9 months for I + R (HR = 0.67, 95% CI = 0.50–0.91,  $p = 0.009$ )] (Fig. 1A). There was no difference in PFS between the I and R groups (HR = 0.92, 95% CI = 0.67–1.26,  $p = 0.591$ ). The association with in vitro response was consistent within platinum-sensitive and platinum-resistant subpopulations as well (HR: 0.71 vs. 0.66, respectively;  $p = 0.690$  for interaction test), showing improved outcomes when using S regimens in either cohort (Fig. 2). In multivariate analysis, platinum sensitivity status and in vitro assay results remained the only two independent factors significantly associated with PFS (Table 3). Age did not remain significant in multivariate analysis. Specifically, patients defined as S had a 34% reduced risk of disease progression compared to those defined as I or R, when controlling for age, performance status, histology, tumor grade and prior platinum sensitivity status (HR = 0.66, 95% CI = 0.47–0.94,  $p = 0.020$ ). A similar correlation was identified for OS (median OS: 37.5 months for S vs. 23.9 months for I + R, HR = 0.61, 95% CI = 0.41–0.89,  $p = 0.010$ ; Fig. 1B) and the relationship was consistent in multivariate analysis (HR = 0.59, 95% CI = 0.38–0.93,  $p = 0.023$ ; Table 3).

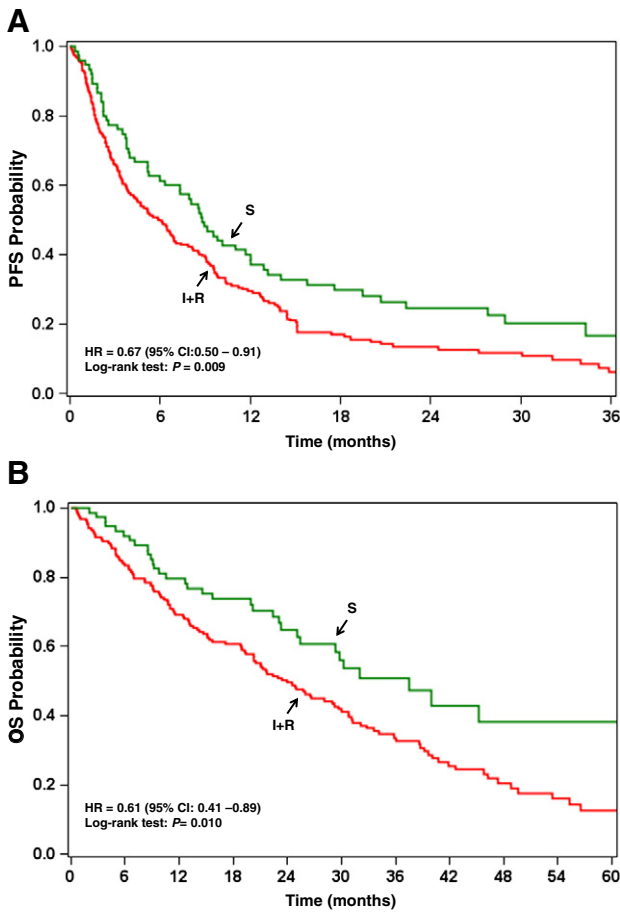
**Table 1**  
Patient characteristics.

	No. of patients	%
<i>Age (years)</i>		
<50	38	(14.5)
50–59	79	(30.2)
60–69	80	(30.5)
≥70	65	(24.8)
Median (range)	61 (24–93)	
<i>Race</i>		
Caucasian	234	(89.3)
African American	14	(5.3)
Others	14	(5.3)
<i>ECOG performance status</i>		
0	183	(69.9)
1	66	(25.2)
2	13	(5.0)
<i>Histology</i>		
Serous	178	(67.9)
Endometrioid	19	(7.3)
Clear cell	19	(7.3)
Mucinous	3	(1.3)
Adenocarcinoma, NOS	30	(11.5)
Others	13	(5.0)
<i>Tumor grade</i>		
1	12	(4.6)
2	33	(12.6)
3	168	(64.1)
Unknown	49	(18.7)
<i>Platinum sensitivity status</i>		
Platinum-sensitive	145	(55.3)
Platinum-resistant	117	(44.7)

**Table 2**  
Distribution of treatment selection by assay result.

Therapy <sup>a</sup>	No. of patients (%)		
	S	I + R	All
Carboplatin/paclitaxel	27 (36.0)	52 (27.8)	79 (30.2)
PLD	11 (14.7)	44 (23.5)	55 (21.0)
Carboplatin/gemcitabine	13 (17.3)	12 (6.4)	25 (9.5)
Topotecan	5 (6.7)	17 (9.1)	22 (8.4)
Carboplatin	6 (8.0)	9 (4.8)	15 (5.7)
Carboplatin/docetaxel	2 (2.7)	13 (7.0)	15 (5.7)
Cisplatin/gemcitabine	2 (2.7)	13 (7.0)	15 (5.7)
Cisplatin/paclitaxel	2 (2.7)	11 (5.9)	13 (5.0)
Gemcitabine	2 (2.7)	7 (3.7)	9 (3.4)
Carboplatin/topotecan	3 (4.0)	2 (1.1)	5 (1.9)
Paclitaxel	1 (1.3)	4 (2.1)	5 (1.9)
Cisplatin	1 (1.3)	3 (1.6)	4 (1.5)

<sup>a</sup> Docetaxel, cisplatin/docetaxel and cisplatin/topotecan were also available for use in this study but were not clinically administered to any of the evaluable patients.



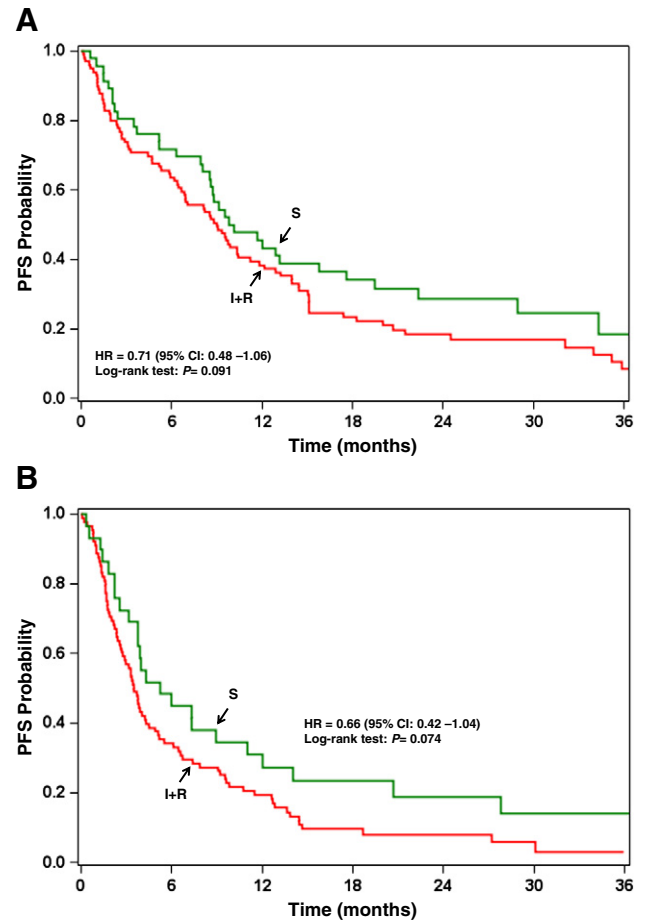
**Fig. 1.** PFS (A) and OS (B) in recurrent ovarian cancer patients treated with assay S versus I + R treatments. Patients treated with assay S treatments (n = 75) experienced a median PFS of 8.8 months and median OS of 37.5 months, while those treated with assay I or R treatments (n = 187) experienced a median PFS of 5.9 months and median OS of 23.9 months.

*Pattern of in vitro tumor response*

To estimate the proportion of patients that may benefit from an S treatment chosen prospectively, the in vitro drug responses to seven single agents (carboplatin, cisplatin, gemcitabine, PLD, paclitaxel, docetaxel and topotecan) were analyzed (Fig. 3). These seven agents, alone or in combination, encompass the 15 treatments included in this study. The current analysis was conducted on the single agents in order to elucidate the mechanism of action of each drug individually. For patients with complete assay data for all seven of these agents (n = 208), 48% of tumors were either I or R to all seven agents, whereas 10% were S to all. The remainder of the patients (42%) were S to between 1 and 6 agents, suggesting that although cross-resistance is considered to be common in persistent or recurrent EOC, a relatively large number of patients (more than half) may benefit from assay-informed individualized chemotherapy.

**Discussion**

Currently, empiric treatment of recurrent EOC, FTC, and PPC is based primarily on the patient’s treatment free interval, anticipated toxicities, the availability of clinical trials using notice agents, and population response rates from phase II and III clinical trials, as the response rates are relatively modest across the various treatment options. However, as many as 20 different clinically-acceptable and equivalent treatment choices are identified in current treatment guidelines [21], with insufficient evidence to indicate that any one



**Fig. 2.** PFS in platinum-sensitive (A) and platinum-resistant (B) recurrent ovarian cancer patients treated with assay S versus I + R treatments. Platinum-sensitive patients treated with assay S treatments (n = 46) experienced a median PFS of 10.0 months, while those treated with assay I or R treatments (n = 99) experienced a median PFS of 9.0 months. Platinum-resistant patients treated with assay S treatments (n = 29) experienced a median PFS of 5.2 months, while those treated with assay I or R treatments (n = 88) experienced a median PFS of 3.5 months.

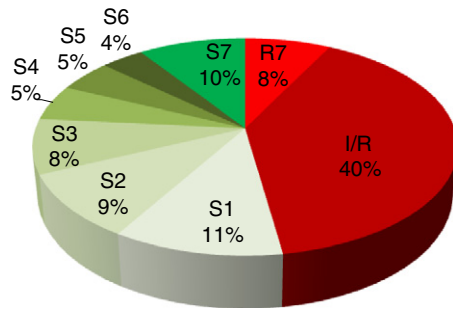
agent is superior to any other. Additionally, in EOC, unlike some other solid tumors, there is a lack of validated biomarkers that stratify patients for individualized treatment choices, and population-based studies continue to be the primary source of information for physicians’ empiric treatment decisions. Although a prospective, randomized trial has

**Table 3**  
Multivariate analysis of factors affecting PFS and OS.

	PFS		OS	
	HR <sup>a</sup> (95% CI)	p value	HR <sup>a</sup> (95% CI)	p value
Age				
Inc. per 10 years	1.07 (0.93–1.23)	0.360	1.03 (0.87–1.22)	0.744
ECOG PS				
1 or 2 vs. 0	1.12 (0.80–1.57)	0.437	1.30 (0.86–1.95)	0.209
Histology				
Serous vs. others	1.36 (0.96–1.93)	0.082	1.09 (0.73–1.63)	0.684
Tumor grade				
3 vs. 0 or 1	1.13 (0.78–1.63)	0.530	1.20 (0.75–1.90)	0.444
Platinum sensitivity status				
Plat sensitive vs. plat resistant	0.64 (0.47–0.87)	0.004	0.66 (0.45–0.96)	0.029
Chemoresponse assay results				
S vs. I or R	0.66 (0.47–0.94)	0.020	0.59 (0.38–0.93)	0.023

<sup>a</sup> Hazard ratio (HR) estimated from proportional hazards model adjusted for covariates.





**Fig. 3.** Distribution of assay results across seven single agent treatments (carboplatin, cisplatin, gemcitabine, PLD, paclitaxel, docetaxel, topotecan). Patients ( $n = 208$ ) 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.

been previously reported, neither the PFS nor the OS results achieved a level of significance, possibly due, in part, to a physician “learning effect” associated with a randomized trial design where one arm of the study is treated empirically [22]. Several investigators have commented on proposals for prospective clinical trial designs evaluating biomarkers and concluded that a 2-arm (empiric vs. marker) trial design, such as that attempted by Cree et al. [22] is largely impractical due to the large sample size demanded, cross-arm treatment overlap (i.e. inefficiency), potential for physician treatment bias, and other logistical and pragmatic concerns [23–26].

The current study evaluates the correlation of chemoresponse assay results to treatment outcomes, with therapies chosen by oncologists that were blinded to the assay results. This study is the first clinical trial of this design conducted to our knowledge.

This study demonstrates that patients who were treated with an assay-sensitive regimen had an improvement in both PFS and OS compared to patients who were treated with assay-resistant regimens (Table 3). This significant ( $\geq 50\%$ ) improvement in both PFS and OS represents an OS increase of 14 months. Importantly, these observed improvements in PFS and OS were evident in both the platinum-sensitive and platinum-resistant subgroups. Ideally, the follow-up time would be further extended for enhanced assessment of OS; this may be undertaken in a future analysis. In addition, the contribution of further treatments (subsequent to the treatment selected for this study) towards OS is unknown. However, if a sensitive treatment had followed a resistant study treatment, or vice versa, then the difference in OS (and associated HR) between S and I + R arms would have been smaller than reported herein. So, while this study focused on patient progression on the study treatment, and information regarding further treatments (including whether or not results from the chemoresponse assay were used to inform those subsequent treatment decisions) was not collected, the 14 month improvement in OS may be further increased with utilization of assay-informed treatments at future recurrence(s).

It is important to emphasize that, consistent with clinical studies in a mixed population of platinum-sensitive and -resistant patients [27,28], approximately 25 to 30% of the patients were clinically responsive to their empirically selected treatment (Table 2). Another 25% of the patients tested S to at least one of the 15 protocol therapies in the assay, but were treated with an I or R therapy empirically. Therefore, more than 50% of the tumors were found to be S to at least one drug tested in vitro (Fig. 3), suggesting that although resistance is common in recurrent EOC, a majority of patients may benefit from assay-informed individualized chemotherapy. In other words, if the oncologists had not been blinded to the results of the chemoresponse assay in this study, the number of patients who could have benefitted from an S treatment would have more than doubled. In clinical practice this scenario is frequently encountered when oncologists must choose between seemingly equivalent therapies in platinum-sensitive recurrent EOC (e.g. carboplatin in combination with PLD vs. gemcitabine vs. paclitaxel).

Furthermore, current chemoresponse assays are not compatible with directly assaying the efficacy of anti-angiogenic therapy due to the focus on epithelial (malignant) cell response to chemotherapy and the associated lack of (intact) endothelial cells in culture on which anti-angiogenic therapies act. Thus, given the recently demonstrated benefits of adding anti-angiogenic therapy (bevacizumab) to standard cytotoxic chemotherapy in ovarian cancer [29–32], chemoresponse assays may be used to inform decisions on which cytotoxic therapy to couple with anti-angiogenic therapy.

ChemoFx®, the chemoresponse assay employed in the current study, has been previously evaluated in retrospective studies inclusive of both primary and recurrent EOC [10,11]. These promising results warranted further evaluation in the form of this current prospective, multi-site, non-interventional trial. Moreover, a subgroup analysis conducted herein demonstrated an association between assay results and clinical outcome in both platinum-sensitive and -resistant patients; assay association with clinical response was also independent of platinum-sensitive or -resistant status (as well as all other clinical covariates) in multivariate analysis. A previous study using a different chemoresponse assay showed a correlation between PFS and assay results in platinum-sensitive patients but not in patients with platinum-resistant disease [33]. Because recommended treatment options differ between these two subgroups, demonstrated performance of a chemoresponse assay in both groups of patients with recurrent EOC is clinically useful.

In conclusion, this multi-institutional prospective study demonstrates that recurrent ovarian cancer patients who were treated with a regimen identified as sensitive by a chemoresponse assay experienced significantly improved PFS and OS of 3 and 14 months, respectively. These results are in notable contrast to multiple, randomized drug studies in this indication that have repeatedly shown little or no difference between various treatment regimens. Results from this study indicate that a chemoresponse assay may be a very useful tool for optimizing treatment selection when there are multiple clinically-acceptable and -equivalent treatments available, and few, if any, biological markers that can reliably assist in a more individualized treatment plan. When treatments are individualized, even though the same regimens are used clinically, patients experience marked improvements in outcome. Furthermore, the results suggest that effective (sensitive) treatment options could be available for many more patients than is currently achieved by empiric treatment. These compelling data suggest that it may be reasonable to prospectively utilize chemoresponse assays to assist clinicians in the optimal prioritization of therapy for both platinum-sensitive and platinum-resistant patients with recurrent EOC.

#### Conflict of interest statement

Some authors (SLB, CT) are paid employees of Precision Therapeutics, Inc., which sponsored this work. Other authors received compensation from Precision Therapeutics, Inc. for their participation in this clinical trial (TR, JO, EG, RE, TCK, RH, RGM, LP, TT, JCS) or as a member of an advisory board supporting this trial (TJH).

#### Acknowledgments

The authors wish to acknowledge the valuable contributions of all sites and their associated investigators that participated in this study. These include: Abington Memorial Hospital, Bayfront Medical Center, Cancer Center of the Carolinas, Cleveland Clinic, Cooper Health System, Florida Hospital, Kaiser Permanente, Lankenau Hospital, Lee Memorial Hospital, Magee Womens Hospital – UPMC, Midtown Gynecologic Oncology, Northwestern University, Rush University Medical Center, Schwartz Gynecologic Oncology, Southeastern Gynecologic Oncology, St. Barnabas, St. Elizabeth Medical Center, St. Louis University, St. Vincent Gyn Onc, St. Vincent's Hospital, The Methodist Hospital, The West Clinic, University Hospitals Case Medical Center, University of California – Irvine, University of Cincinnati, University of Minnesota, University of Oklahoma, University of Toledo, University of Virginia, University of

Wisconsin, UT Southwestern Medical Center at Dallas, Vanderbilt University, Washington University, Women & Infant's Hospital, and Yale University.

## Appendix A. Supplementary data

Supplementary data to this article can be found online at <http://dx.doi.org/10.1016/j.ygyno.2013.08.009>.

## References

- [1] Siegel R, Naishadham D, Jemal A. Cancer statistics, 2013. *CA Cancer J Clin* 2013;63:11–30.
- [2] Thigpen T. A rational approach to the management of recurrent or persistent ovarian carcinoma. *Clin Obstet Gynecol* 2012;55:114–30.
- [3] Cooke SL, Brenton JD. Evolution of platinum resistance in high-grade serous ovarian cancer. *Lancet Oncol* 2011;12:1169–74.
- [4] 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.
- [5] 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.
- [6] Kris MG, Meropol NJ, Winer EP. Accelerating progress against cancer: ASCO's blueprint for transforming clinical and translational cancer research. <http://www.asco.org/sites/default/files/blueprint.pdf>; 2011.
- [7] Holloway RW, Mehta RS, Finkler NJ, Li KT, McLaren CE, Parker RJ, et al. Association between in vitro platinum resistance in the EDR assay and clinical outcomes for ovarian cancer patients. *Gynecol Oncol* 2002;87:8–16.
- [8] Pant AC, Diaz-Montes T, Tanner E, Ahmad S, Giuntoli RL, Holloway RW, et al. Correlation of extreme drug resistant assay results and progression-free survival following intraperitoneal chemotherapy for advanced ovarian cancer. *J Chemother* 2010;22:270–4.
- [9] Matsuo K, Eno ML, Im DD, Rosenshein NB, Sood AK. Clinical relevance of extent of extreme drug resistance in epithelial ovarian carcinoma. *Gynecol Oncol* 2010;116:61–5.
- [10] Gallion H, Christopherson WA, Coleman RL, Demars L, Herzog T, Hosford S, et al. Progression-free interval in ovarian cancer and predictive value of an ex vivo chemoresponse assay. *Int J Gynecol Cancer* 2006;16:194–201.
- [11] Herzog TJ, Krivak TC, Nickles Fader A, Coleman RL. Chemosensitivity testing with ChemoFx and overall survival in primary ovarian cancer. *Am J Obstet Gynecol* 2010;203(68):e1–6.
- [12] Holloway RW. Extreme drug resistance assay does not influence survival in women with epithelial ovarian cancer. *Gynecol Oncol* 2010;116:147–8.
- [13] Matsuo K, Bond VK, Eno ML, Im DD, Rosenshein NB. Low drug resistance to both platinum and taxane chemotherapy on an in vitro drug resistance assay predicts improved survival in patients with advanced epithelial ovarian, fallopian and peritoneal cancer. *Int J Cancer* 2009;125:2721–7.
- [14] Cree IA. Chemosensitivity and chemoresistance testing in ovarian cancer. *Curr Opin Obstet Gynecol* 2009;21:39–43.
- [15] Schink JC, Copeland IJ. Point: chemosensitivity assays have a role in the management of recurrent ovarian cancer. *J Natl Compr Canc Netw* 2011;9:115–20.
- [16] McShane LM, Altman DG, Sauerbrei W, Taube SE, Gion M, Clark GM. Reporting recommendations for tumor marker prognostic studies. *J Clin Oncol* 2005;23:9067–72.
- [17] Bossuyt PM, Reitsma JB, Bruns DE, Gatsonis CA, Glasziou PP, Irwig LM, et al. The STARD statement for reporting studies of diagnostic accuracy: explanation and elaboration. *Clin Chem* 2003;49:7–18.
- [18] 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, editors. *Methods in molecular biology, apoptosis and cancer*. Totowa, NJ: Humana Press Inc.; 2008. p. 57–78.
- [19] Heinzman JH, Rice SD, Corkan LA. Robotic liquid handlers and semiautomated cell quantification systems increase consistency and reproducibility in high-throughput, cell-based assay. *JALA* 2010;15:7–15.
- [20] Winter III WE, Maxwell GL, Tian C, Carlson JW, Ozols RF, Rose PG, et al. Prognostic factors for stage III epithelial ovarian cancer: a Gynecologic Oncology Group Study. *J Clin Oncol* 2007;25:3621–7.
- [21] Comprehensive Cancer Network National. NCCN clinical practice guidelines in oncology. Ovarian cancer: including fallopian tube cancer and primary peritoneal cancer. Version 1.2013; 2013.
- [22] Cree IA, Kurbacher CM, Lamont A, Hindley AC, Love S. A prospective randomized controlled trial of tumour chemosensitivity assay directed chemotherapy versus physician's choice in patients with recurrent platinum-resistant ovarian cancer. *Anticancer Drugs* 2007;18:1093–101.
- [23] Simon R, Maitoumam A. Evaluating the efficiency of targeted designs for randomized clinical trials. *Clin Cancer Res* 2004;10:6759–63.
- [24] Sargent DJ, Conley BA, Allegra C, Collette L. Clinical trial designs for predictive marker validation in cancer treatment trials. *J Clin Oncol* 2005;23:2020–7.
- [25] Freidlin B, McShane LM, Korn EL. Randomized clinical trials with biomarkers: design issues. *J Natl Cancer Inst* 2010;102:152–60.
- [26] Deverka P, Messner D, Dutta T. Evaluation of clinical validity and clinical utility of actionable molecular diagnostic tests in adult oncology. Center for Medical Technology Policy. [http://www.cmpnet.org/wp-content/uploads/downloads/2013/07/CMTP\\_MDx\\_EGD07-17-2013.pdf](http://www.cmpnet.org/wp-content/uploads/downloads/2013/07/CMTP_MDx_EGD07-17-2013.pdf); 2013.
- [27] 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.
- [28] Herzog TJ. Recurrent ovarian cancer: how important is it to treat to disease progression? *Clin Cancer Res* 2004;10:7439–49.
- [29] Perren TJ, Swart AM, Pfisterer J, et al. A phase III trial of bevacizumab in ovarian cancer. *N Engl J Med* 2011;365:2484–96.
- [30] Aghajanian C, Blank SV, Goff BA, Judson PL, Teneriello MG, Husain A, et al. OCEANS: a randomized, double-blind, placebo-controlled phase III trial of chemotherapy with or without bevacizumab in patients with platinum-sensitive recurrent epithelial ovarian, primary peritoneal, or fallopian tube cancer. *J Clin Oncol* 2012;30:2039–45.
- [31] Pujade-Lauraine E, Hilpert F, Weber B, et al. AURELIA: a randomized phase III trial evaluating bevacizumab (BEV) plus chemotherapy (CT) for platinum (PT)-resistant recurrent ovarian cancer (OC). *J Clin Oncol* 2012;30(15 Suppl.) [LBA5002].
- [32] 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.
- [33] Loizzi V, Chan JK, Osann K, Cappuccini F, DiSaia PJ, Berman ML. Survival outcomes in patients with recurrent ovarian cancer who were treated with chemoresistance assay-guided chemotherapy. *Am J Obstet Gynecol* 2003;189:1301–7.