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Conservation of in vitro drug resistance patterns in epithelial ovarian carcinoma

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Abstract

Purpose. To compare the in vitro drug resistance profiles of advanced stage primary and recurrent epithelial ovarian cancer specimens using the tritiated thymidine uptake assay.

Methods. Extreme drug resistance (EDR) to cisplatin, paclitaxel, 4-hydroxycyclophosphamide, and topotecan was determined for an unselected population of primary and metastatic malignant ovarian tissues, synchronous tumors (primary and metastatic tissues obtained from the same patient at diagnosis), and metachronous lesions (specimens from the same patient before and after chemotherapy).

Results. For the large unselected population of malignant tissues (total, $N = 6990$; primary ovarian, $N = 2031$; metastatic ovarian, $N = 4959$), no statistically significant differences were discovered between primary tissues and metastatic lesions when a comparison was made between the percentage of tumors from each group that exhibited extreme drug resistance to the agents assayed. From the library of 6990 specimens, 119 synchronous pairings were identified. These synchronous lesions did not differ significantly in the %EDR between primary and metastatic sites in the same patient; approximately 10% shifted between low drug resistance and EDR. A total of 334 metachronous pairings were identified and the percentage of tissues that exhibited EDR also failed to show a significant difference when primary tumors were compared with matched recurrences in the same patient.

Conclusions. For the agents studied, acquired resistance was not a function of disease site. In vitro drug resistance observed at recurrence was not influenced significantly by intervening therapy. It is possible that assay results at diagnosis could be used to guide subsequent therapy at relapse, especially when recurrent tissue is not available for analysis.

Keywords: Chemoresistance assay; Acquired resistance; Tumor heterogeneity

Introduction

Epithelial ovarian cancer is the most lethal of gynecologic malignancies, and the fourth leading cause of cancer death in American women between the ages of 40 and 59 [1]. Approximately one woman dies from advanced disease every 45 min. With an incidence of 1 in 60, approximately 22,220 new cases will be diagnosed during 2004 [2]. The initial management of this insidious neoplasm involves

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optimal cytoreductive surgery whenever possible, thereby setting the stage for postoperative systemic chemotherapy. Based on Gynecologic Oncology Group studies, the combination of paclitaxel and a platinum compound (usually carboplatin or cisplatin) has emerged as the preferred regimen for chemotherapy-naïve disease, with response rates of up to 75% [1,3]. Unfortunately, overall cure rates have not improved, and most women suffer from recurrent disease. Although chemotherapy has increased survival duration, only 20% to 25% of women diagnosed with advanced disease are alive at 5 years.

While several modestly effective second-line agents are available for the treatment of recurrent ovarian cancer, no one agent stands out as the most effective. Response rates in the second-line setting are generally on the order of 15% to 20%. Thus, selection of second-line therapy has been based on various clinical factors, such as pre-existing toxicity and performance status [4,5]. Because of the equivalence of second-line agents and the significant heterogeneity in individual responses to chemotherapy, it is difficult to predict solely on the basis of clinical criteria who will benefit from a given agent. This dilemma has generated interest in the development of in vitro assays that could refine the treatment selection process by identifying individual patterns of drug response prior to chemotherapy administration. Currently available assays for use in oncology have relatively low positive predictive values (70%), but excellent negative predictive values (>90%) [6–8]. Thus, while in vitro drug response assays have not been as reliable at predicting drug sensitivity in the clinic, they may become useful in identifying those agents that have less than a 3% chance of clinical benefit [9]. Recently published data sets from non-randomized studies suggest that patients who receive agents to which their tumor is resistant to in vitro progress more rapidly and have shorter survival times [7,8,10–12].

In this study, we addressed the potential of tumor heterogeneity to confound the reliability of in vitro results to predict response. We compared in vitro drug response for a large population of patients where tumors were obtained from the primary ovarian site versus a non-ovarian metastatic site. We also evaluated differences in in vitro response for synchronous specimens obtained at two sites from the same patient at initial surgery as well as for metachronous specimens obtained from the same patient at two different times. We were interested in determining if in vitro drug resistance was a function of site or intervening therapy.

Materials and methods

Clinical material

From January 1990 to January 2000, a total of 6990 epithelial ovarian cancer specimens were submitted to Oncotech, Inc., from both regional and out-of-state institutions and evaluated by the tritiated thymidine uptake extreme drug resistance assay (EDRA). All tissue samples were obtained from women with advanced primary or recurrent International Federation of Gynecology and Obstetrics (FIGO) surgical stage III or IV disease (N = 6546 patients total). Metastatic sites from which malignant tissue was retrieved included the uterus and fallopian tubes, omental deposits, intestinal serosa and mesentery, bladder serosa, liver capsule and parenchyma, subdiaphragmatic surfaces, pelvic and aortocaval lymph node chains, splenic hilum, the peritoneal surfaces of the pelvic sidewall and cul-de-sac, and the rectum.

Institutional Review Board approval to analyze the existing pathologic material submitted to Oncotech, Inc. was granted under the Exempt classification. No subject identifiers were used.

Initially, data were arranged to separate the unselected population of tissues by site (i.e., primary tumors of the ovary versus metastatic sites). A sub-analysis was then made whenever multiple tumor specimens from the same patient were found; these data were classified as either *synchronous lesions* (tumor excised from the primary ovary and metastatic location in one operation) or *metachronous lesions* (i.e., tissues obtained from the same patient at diagnosis and at recurrence).

All metachronous cases were temporally separated by 5 years or less. No specimens with a pathologic diagnosis of primary peritoneal carcinoma, primary fallopian tube carcinoma, or ovarian carcinoma of low malignant potential were included. Due to the large number of referral centers, other clinical data such as age, performance status, systemic treatment regimens, cancer antigen 125 levels in serum, response rates, toxicity profiles, progression-free intervals, and survival were not uniformly obtainable.

Extreme drug resistance assay

The tritiated thymidine uptake EDRA procedures were performed according to previously published protocols [9–12]. Briefly, viable tumor specimens weighing 1–5 g were excised and submitted in transport media by overnight courier to Oncotech, Inc. Tissue specimens were minced with sharp surgical scissors and then subjected to enzymatic digestion using 0.14% collagenase type I and 0.01% DNAse. Following centrifugation, the pellet was suspended in 5 mL of tissue culture medium containing heat-inactivated fetal calf serum, 100 units of penicillin, 100 μg/mL of streptomycin, and 200 mM L-glutamine.

Soft agar matrix (0.4%) selective for tumor growth was placed in 24-well polystyrene culture dishes and 50 μL of chemotherapeutic drug was then added to appropriate wells. Quadruplicate “no-drug” negative control wells and duplicate positive controls (a suprapharmacological concentration of cisplatin) were assayed in parallel. Tumor cells

suspended in 0.5 mL tissue culture medium and 0.2% agarose were then added to each well. After 72 h incubation at 37°C in the presence of 5% CO_2, 5.0 μCi ^{3}H-thymidine was introduced into each well, and the plates were incubated for an additional 48 h to allow radioactive thymidine incorporation into the DNA of the surviving tumor cells. Cellular DNA was collected on Reeve Angel 9234 AH paper using a Brandel automatic harvester. The percent inhibition of cellular thymidine incorporation (PCI) as compared to the quadruplicate negative and duplicate positive controls was calculated for each drug using a liquid scintillation counter.

The laboratory was blinded to concurrent and pre-existing assay results when multiple tumor samples were submitted from the same patient.

Choice of antineoplastic agents

We studied the in vitro drug resistance profiles of ovarian cancer specimens exposed to cisplatin and paclitaxel, agents currently considered to be the standard initial treatment for newly diagnosed advanced stage epithelial ovarian carcinoma. We also evaluated the in vitro activity of the previous standard agent used in combination with platinum compounds, cyclophosphamide. Cyclophosphamide was added to the assay plates in the active form, 4-hydroxycyclophosphamide (4HC). We also studied the topoisomerase I inhibitor, topotecan, on the basis of its approval by the United States Food and Drug Administration for second-line treatment of epithelial ovarian cancer. Drug concentrations were as follows: cisplatin 1.67 μM [9]; paclitaxel 2.5 μM [12]; 4HC 4.35 μM [9]; topotecan 0.21 μM.

Classification of resistance

The degree of resistance of tumor specimens to each drug was stratified among three categories. Low drug resistance (LDR) occurred when the PCI result after exposure to a given agent was greater than the median PCI (i.e., when the tumor's cellular thymidine incorporation was inhibited by the drug to a degree that was greater than the median percent inhibition of thymidine incorporation determined prior to this study for a population of >14,000 ovarian cancer specimens, a subset of cases from a library containing over 30,000 tumor samples). Intermediate drug resistance (IDR) was observed when the PCI result was less than the median PCI but greater than the median PCI minus one standard deviation. Tumor specimens were classified as exhibiting extreme drug resistance (EDR) to an agent when the PCI result was more than 1 standard deviation below the median PCI for that drug.

The separation of cytotoxicity assay results at specified drug concentrations into low, intermediate, and extreme drug resistance categories using the median and median +1 standard deviation as the cutoff limits has been described previously by Larsson and Nygren [13]. Employing a statistical model based on Bayes theorem, the investigators correlated their assay results with clinical outcome and noted that LDR samples showed a higher response rate than expected, IDR a lower response rate, and EDR samples showed no response at all.

Statistical methods

Statistical differences between groups in the unselected population of primary and metastatic tumor specimens were determined using the Chi-square test run on the In-Stat (San Diego, CA) PC-based software program. The *kappa* hierarchical analysis of variances was employed to determine the statistical cohesivity of data sets generated from the synchronous primary and metastatic and metachronous pairings [14].

Conflicts of interest

There were no potential or actual conflicts of interest in the generation of the following data sets. Furthermore, the subsequent discussion represents an objective analysis of the information collected in the laboratory and in no manner reflects the position of ONCOTECH, Inc.

Results

Unselected population of primary and metastatic cases

As shown in Table 1, approximately one-third of the 6990 epithelial ovarian carcinoma specimens evaluated were submitted as the primary ovarian tumor tissue. These

Table 1
Drug resistance in ovarian carcinoma: primary and metastatic tumor specimens

Agent	Primary specimens (*N*)	Metastatic specimens (*N*)	% EDR			% IDR			% LDR		
			Primary	Metastatic	*P*	Primary	Metastatic	*P*	Primary	Metastatic	*P*
Cisplatin	2031	4959	7	11	NS	23	26	NS	70	63	NS
Paclitaxel	2032	4923	24	21	NS	29	29	NS	47	50	NS
4HC	1971	4757	17	17	NS	26	27	NS	57	56	NS
Topotecan	1217	3083	16	12	NS	24	25	NS	60	63	NS

EDR: Extreme drug resistance. IDR: Intermediate drug resistance. LDR: Low drug resistance. NS: Not statistically significant. 4HC: 4-hydroxycyclophosphamide.

2031 primary ovarian specimens were compared to the 4959 metastatic specimens to determine if there were differences in their EDRA results for cisplatin. A similar distribution and number of cases underwent EDRA for paclitaxel. With respect to 4HC, 1971 primary cancers and 4757 metastatic lesions were evaluated, while 1217 primary tumors and 3083 metastatic deposits were assayed with topotecan.

A comparison of the frequency of extreme drug resistance (i.e., EDR) for the agents studied fell into a range of 7% to 24% for primary cases and 11% to 21% for the metastatic cases. The highest frequency of EDR occurred for paclitaxel, which was seen in 24% and 21% of primary ovarian and metastatic tissues, respectively. For cisplatin, EDR was seen in 7% of the primary ovarian tissues versus 11% of the metastatic specimens. The EDR frequency for 4HC was 17% for both primary ovarian and metastatic tissues. Finally, 16% of primary specimens and 12% of metastatic specimens displayed EDR to topotecan. As shown in Table 1, none of the comparisons of EDR, IDR, or LDR frequencies between the primary and metastatic sites revealed a statistically significant difference for the agents examined.

Paired synchronous primary and metastatic cases

A total of 101 patients had synchronous specimens submitted at primary surgery from both the ovary and a metastatic site. For these matched synchronous pairings, 53 were incubated with topotecan, 98 with 4HC, and 101 were incubated with cisplatin as well as with paclitaxel (separately).

Table 2 depicts the percentages of synchronous primary and metastatic cases that fell into the Low, Intermediate, and Extreme Drug Resistance categories. Table 3 compares the distribution of the EDRA categories for the synchronous cases by site. EDR was observed in response to cisplatin in 6% of primary ovarian tumors and in 6% of synchronous metastases. LDR to cisplatin was seen in 67% of primary tissues, while 65% of metastases exhibited LDR to this agent. As shown in Table 3, 55% of cases exhibited LDR responses to cisplatin at both sites, while 3% of cases showed EDR at both sites. Overall, 74% of synchronous lesions retained their cisplatin EDRA response category, 24% exhibited a one-category shift, while 2% of cases showed a two-category difference between sites (Fig. 1).

Table 3
Drug resistance patterns for synchronous pairings

Primary ovary	Synchronous metastases		
	% EDR	% IDR	% LDR
Cisplatin (N = 101)			
% EDR	3	2	1
% IDR	2	16	9
% LDR	1	11	55
Paclitaxel (N = 101)			
% EDR	14	6	5
% IDR	4	9	12
% LDR	5	10	36
4HC (N = 98)			
% EDR	7	7	6
% IDR	4	4	10
% LDR	7	10	44
Topotecan (N = 53)			
% EDR	8	15	8
% IDR	6	19	4
% LDR	0	11	30

EDR: Extreme drug resistance. IDR: Intermediate drug resistance. LDR: Low drug resistance. NS: Not statistically significant. 4HC: 4-hydroxycyclophosphamide.

For the synchronous pairings incubated with paclitaxel, EDR was observed in 25% of primary specimens and in 23% of metastases (Table 2). EDR was seen at both sites in 14% of the cases (Table 3). Low drug resistance was noted in 50% of primary lesions and in 52% of metastases. LDR was seen at both sites in 36% of cases. Overall, 59% of cases (tissues from the same patient) showed no shift in their EDRA category for paclitaxel between sites, 32% of cases showed a one-category shift, and 10% of cases showed a two-category shift (Fig. 1).

Extreme drug resistance to 4HC was detected in 20% of primary cases and in 18% of metastases (Table 2). EDR to 4HC was seen at both sites in 7% of cases (Table 3). Sixty-one percent of primary cancers and 60% of metastatic deposits were LDR to 4HC, with 44% showing LDR at both sites. Overall, 55% of pairings (i.e., tissues from the same patient) showed no shift in EDR category for 4HC, 31% showed a one-category shift, and 13% showed a two-category shift (Fig. 1).

Finally, EDR to topotecan was demonstrated in 30% of primary tumors and in 13% of metastases (Table 2). LDR to

Table 2
In vitro drug resistance frequencies for synchronous cases by site

Agent	% EDR			% IDR			% LDR		
	Primary	Metastatic	P	Primary	Metastatic	P	Primary	Metastatic	P
Cisplatin, N = 101	6	6	NS	27	29	NS	67	65	NS
Paclitaxel, N = 101	25	23	NS	25	25	NS	50	52	NS
4HC, N = 98	20	18	NS	18	21	NS	61	60	NS
Topotecan, N = 53	30	13	NS	28	45	NS	42	42	NS

EDR: Extreme drug resistance. IDR: Intermediate drug resistance. LDR: Low drug resistance. NS: Not statistically significant. 4HC: 4-hydroxycyclophosphamide.

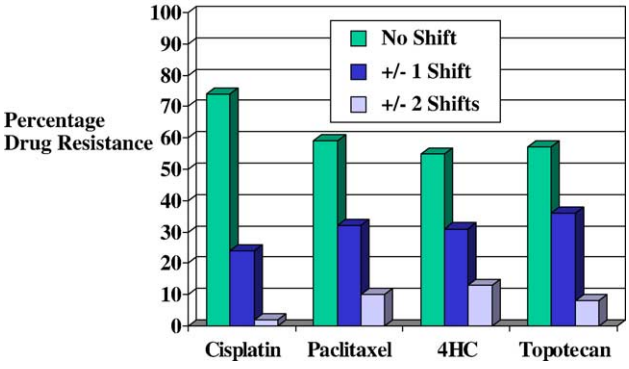

Fig. 1. Heterogeneity of in vitro drug response for paired synchronous primary and metastatic ovarian cancer specimens.

topotecan was seen in 42% of primary cases and 42% of metastases. EDR to topotecan at both sites was seen in 8% of cases, while LDR at both sites was seen in 30% of cases (Table 3). Overall, 57% of synchronous tissues showed the same EDRA response to topotecan between sites, 36% showed a one-category shift, and 8% showed a two-category shift (Fig. 1).

The shifts among the three drug resistance categories for the synchronous pairings are depicted in Fig. 1. In 52% to 71% of cases, the paired metastases from the same patient did not exhibit a different in vitro chemoresistance spectrum than the primary ovarian tumor to the four antineoplastic agents under study. When EDRA drug response categories differed between sites, they represented primarily a one-category shift (e.g., EDR to IDR or LDR to IDR). Only 2% to 13% of tumors exhibited a plus or minus 2-category shift (i.e., from LDR to EDR or EDR to LDR). The differences observed did not reach statistical significance by *kappa* hierarchical analysis of variances.

Paired metachronous cases

There were a total of 343 patients operated upon for whom specimens were obtained at primary surgery and at recurrence 1 month to 5 years after their primary operation. All metachronous tissues were tested against paclitaxel and 334 were assayed with cisplatin; 318 and 126 paired specimens were exposed to 4HC and topotecan, respectively (Table 4).

Table 5
Drug resistance patterns for metachronous pairings

Primary tissue	Recurrent tissue		
	% EDR	% IDR	% LDR
Cisplatin (N = 334)			
% EDR	3	4	2
% IDR	3	14	12
% LDR	3	16	43
Paclitaxel (N = 343)			
% EDR	10	7	9
% IDR	6	9	12
% LDR	5	12	30
4HC (N = 318)			
% EDR	7	6	5
% IDR	6	8	10
% LDR	13	13	33
Topotecan (N = 126)			
% EDR	8	10	12
% IDR	2	18	29
% LDR	2	3	16

EDR: Extreme drug resistance. IDR: Intermediate drug resistance. LDR: Low drug resistance. NS: Not statistically significant. 4HC: 4-hydroxycyclophosphamide.

As shown in Table 4, EDR to cisplatin was observed in 9% of primary ovarian tumors and in 10% of recurrences. Sixty-two percent of primary tissues and 56% of relapsing paired disease (i.e., specimen from the same patient) exhibited LDR to this agent, with 43% of specimens maintaining LDR at both sites. For the metachronous lesions exposed to paclitaxel, EDR was observed at diagnosis in 25% and in 21% of recurrences, with 10% maintaining EDR. Low drug resistance was noted at diagnosis in 48% and at recurrence in 51%; it was maintained in 30% of the metachronous cases. These data are recorded in Table 5.

Extreme drug resistance to 4HC was detected at diagnosis in 21% of cases and in 25% of cases at the time of relapse (Table 4). Fifty-eight percent of primary cancers and 52% of paired recurrences were LDR to 4HC, with maintenance of 33% (Table 5). Finally, the frequency of EDR to topotecan was 17% at diagnosis and 6% at recurrence (Table 4). Low drug resistance to topotecan was demonstrable in 51% of cases at the time of initial surgery and in 80% of cases at recurrence. LDR was maintained temporally in 16% of the metachronous pairings (Table 5).

Table 4
In vitro drug resistance frequencies for metachronous cases by site

Agent	% EDR			% IDR			% LDR		
	Primary	Recurrent	P	Primary	Recurrent	P	Primary	Recurrent	P
Cisplatin, N = 334	9	10	NS	29	34	NS	62	56	NS
Paclitaxel, N = 343	25	21	NS	27	28	NS	48	51	NS
4HC, N = 318	21	25	NS	21	23	NS	58	52	NS
Topotecan, N = 126	17	6	NS	33	14	NS	51	80	NS

EDR: Extreme drug resistance. IDR: Intermediate drug resistance. LDR: Low drug resistance. NS: Not statistically significant. 4HC: 4-hydroxycyclophosphamide.

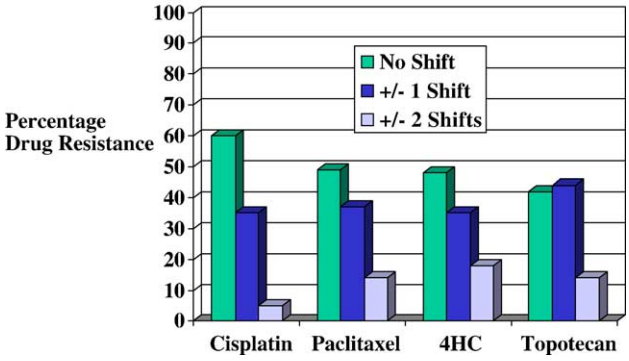

Fig. 2. Heterogeneity of in vitro drug response for paired metachronous ovarian cancer specimens.

The shifts among the three drug resistance categories for the metachronous pairings are depicted in Fig. 2. In 42% to 60% of cases, the recurrent cancers from the same patient did not exhibit a different in vitro chemoresistance spectrum than the primary ovarian tumor to the four antineoplastic agents under study. When spectral differences were manifest, a one-category shift occurred in approximately 35% to 44% of cases and a two-category shift was discernible less than 18% of the time. These differences also did not reach statistical significance by *kappa* hierarchical analysis of variances.

Discussion

Jacobs and colleagues at Duke University demonstrated the clonal origin of epithelial ovarian carcinoma [15]. Although clonal mutations in somatic cells may initiate the carcinogenesis process, it has become clear that ongoing genetic changes are involved in both disease progression and what has been called *clonal divergence*. This, in turn, leads to *tumor heterogeneity* [16]. Because extensive heterogeneity in cellular morphology, cell surface markers, and nuclear chromosomal content exist in human cancers, the end result can lead to differences in tumor growth rate, metabolic characteristics, immunogenicity, and sensitivity to and recovery from exposure to antineoplastic drugs.

Clonal divergence is linked to the *Goldie–Coldman* hypothesis, a mathematical model that has been advanced to explain the phenomenon of clonal divergence [17]. It holds that the biological and clinical characteristics of tumors may be the sequelae of spontaneous mutations, i.e., random mutations confer drug resistance to selected populations of cells. The de novo development of newly resistant clones may be a function of genomic plasticity [18]. The temporal nature of the random mutations is not precisely understood. It is still unclear if they occur early in tumorigenesis, continuously, or as later events. Defining and restricting the mutational events to a temporal window is a highly desirable goal, as it would suggest a pathway to intervention before acquired resistance has evolved. Several non-randomized studies have suggested that in vitro drug response testing may facilitate decision-making when selecting second-line therapy for ovarian carcinoma [6,7,11,12,18–20].

In this study, we applied a third-generation in vitro drug response assay to determine if drug resistance patterns for paired synchronous cases were related to the metastatic process and possibly subject to clonal divergence [9,11,12]. Separation of the large number of tumor specimens into primary ovarian and metastatic groups did not uncover any striking differences in chemoresistance profiles for the four agents studied. Indeed, not only did frequencies of in vitro drug resistance remain fairly constant between primary and metastatic sites (Table 1), but as shown in Table 2, the frequencies did not exhibit substantial divergence among synchronous lesions taken from the same patient. A second goal of this study was to determine if intervening therapy altered in vitro resistance patterns. Once again, the frequencies of in vitro drug resistance remained fairly constant in the metachronous pairings analysis from the same patient (Table 4).

While some variability was seen for paired cases, with a modestly greater variability for metachronous compared to synchronous cases, no dramatic increases in EDR rates were seen after intervening therapy (Table 4). Specifically, for each of the four agents tested in the metachronous setting, approximately 35% to 44% of lesions exhibited a 1-category shift (Fig. 2). These findings are especially noteworthy for cisplatin and for topotecan when compared to the synchronous pairings analysis. Importantly, for topotecan, we observe the development of acquired resistance with 44% metachronous lesions exhibiting a 1-category shift as compared to 42% which maintained their drug resistance profile (Fig. 2). However, a 1-category shift is not sufficient to move from an LDR phenotype to an EDR phenotype (or vice versa). Because the post-test probability of response is linear, a 1-category shift may not be clinically relevant. If an assayed specimen is close to the cusp of a category change, a few percentage point difference in the cell inhibition can shift the analysis to a lower category. Because clinical treatment-related data were not available, it is not known with certainty that patients from whom metachronous pairings were made indeed received the agents assayed in this study. However, given that the combination of platinum and paclitaxel emerged during the 1990s as the standard for first-line therapy, it is not unreasonable to have expected the majority of women from whom recurrent tissue was harvested to have been treated with these agents.

Our finding that metachronous lesions did not exhibit significant discordance for in vitro drug resistance was unexpected. One possible explanation for these results is that, after initial debulking surgery, patients harbor residual tumor in poorly vascularized areas. These tumor sites would be shielded from exposure to systemic chemotherapy, and might therefore make a greater contribution to tumor regrowth compared to well-vascularized tumor tissues that

would be exposed to treatment. The cryptic nature of poorly vascularized tumors would preclude their undergoing significant selective pressure by chemotherapy, increasing the likelihood that they would retain their initial drug response phenotype, and show a similar in vitro drug response profile at second biopsy.

Tumors which exhibit LDR to cisplatin may still recur following therapy due to growth beyond the vascular supply, thereby precluding their exposure to drug. This concept has profound implications for why intraperitoneal chemotherapy may be active in this disease and is supported by the observation that cisplatin-sensitive cases who recur more than 24 months after initial treatment generally have higher response rates than patients who recur within 6 months [4,5]. Thus, platinum-sensitive tumors that had an initial good response take longer to recur to detectable levels, and retain their sensitivity to cisplatin, while those cases that were initially resistant to cisplatin retain that phenotype and tend not to respond to retreatment.

There may be a trend towards *homogeneity* of tumors in their laboratory response to cisplatin with greater than 50% of primary and metastatic tissues maintaining an LDR profile (Table 3). Indeed, when considering all four agents tested, less than 40% of synchronous pairings demonstrated a 1-category shift (Table 3). The extrapolation of this homogeneity to the clinical arena would lend support to the inclusion of platinum and a second drug (e.g., paclitaxel or topotecan) as first-line therapy for advanced disease. While platinum with paclitaxel has emerged as the standard first-line regimen, the combination of platinum with topotecan recently underwent investigation by the Gynecologic Oncology Group in the Phase I/II Protocol 9906 (i.e., *sequential doublets* consisting of carboplatin and topotecan followed by carboplatin and paclitaxel) and in the 5-arm "octopus" Protocol 182.

These observations are also consistent with our previous study comparing biomarker expression (p53, HER2, EGFR, ploidy, s-phase) in primary versus metastatic sites, and for paired synchronous and metachronous cases [21]. We found no dramatic changes in biomarker profiles for these comparisons, suggesting that little clonal divergence for the factors examined occurs in these settings. Perhaps *intrinsic* drug resistance plays a larger role than had previously been considered since inherent mechanisms would be less labile to intervening therapy. In fact, a comparison of EDR frequencies versus time to second assay for the metachronous cases failed to show higher frequencies of cisplatin resistance at earlier time points (data forthcoming). This lack of significant acquired resistance suggests that a given patient's initial drug response profile is fairly robust in spite of intervening therapy, indicating that the patients who fail primary chemotherapy were resistant at the outset.

While the present study examined drug resistance in the laboratory setting, it is interesting to note that the majority of investigations concerning oncologic assays have had drug sensitivity as their focus. There have been three prospective, controlled, but *non-randomized* clinical trials which have employed laboratory assays to predict chemosensivity. Xu et al. compared assay-directed therapy in 73 patients with advanced breast cancer to 83 women who were given chemotherapy according to the clinican's direction (i.e., "physician's choice") [22]. The assay-directed group had a significantly higher response rate (77% vs. 44%) and a not statistically significant trend for improved overall survival. In an open-label prospective pilot trial for 25 women with recurrent ovarian cancer, Kurbacher et al. compared the results of the first 25 evaluable patients to a historical control group of 30 women and noted a 64% objective response rate in the assay-directed group as compared to a 37% objective response rate in the controls, although survival of responding patients was similar in both groups [23]. Finally, Fujita et al. conducted a trial using chemosensitivity testing to predict therapy in advanced gastric cancer; among those undergoing curative surgery ($N = 21$), the patients treated postoperatively with assay-directed therapy survived longer than those treated by "physician's choice" chemotherapy ($P < 0.05$) [24]. It is important to emphasize that none of the above trials were randomized and therefore there exists no concrete evidence to suggest that assay-directed therapy to select "sensitive" agents results in a superior clinical outcome to "physician's choice" of cytotoxic chemotherapy.

Chemo*resistance* and chemo*sensitivity* are not interchangeable. The accuracy to predict chemosensitivity is approximately 60%, whereas the ability to predict drug resistance is probably greater than 95%. An explanation for this difference is provided by an application of Bayes theorem which states that the predictive accuracy of any laboratory test is a function both of the characteristics of the technology and of the biology of the disease to which the test is applied [25]. Many solid tumors, including gynecologic malignancies, tend to be chemoresistant. In such clinical circumstances where chemosensitive disease is significantly less frequent than chemoresistant disease, Bayes theorem predicts that chemosensitivity will be more difficult to accurately predict than drug resistance.

Focusing on drug resistance, Holloway et al. correlated the clinical outcome of 79 evaluable chemotherapy naïve patients with advanced ovarian cancer to the EDR assay results for cisplatin and carboplatin [11]. In vitro platinum resistance remained an independent predictor of progression-free survival (6 months vs. 24 months) and overall survival (19% vs. 68%) in a multivariate analysis. The investigators concluded that patients with tumors demonstrating in vitro EDR to platinum were at significantly increased risk for progression and death when treated with standard platinum-based regimens. Currently, the EDR assay is a Medicare cover technology based on a Department of Health and Human Services Technology review.

In another investigation evaluating assay-based drug resistance, Orr, Orr, and Kern have presented preliminary data on 66 non-randomized patients with advanced ovarian cancer who received a platinum-based chemotherapy regimen (platinum plus paclitaxel for tissues demonstrating in vitro resistance to cyclophosphamide, and platinum plus cyclophosphamide for tissues demonstrating in vitro resistance to paclitaxel) [26]. There was no difference in 3-year survival between the two groups, and the cost effectiveness of each treatment option was determined. The investigators suggest that a consideration of costs avoided by the elimination of ineffective treatments, needless toxicity, and loss of quality of life is an advantage of assay-directed therapy (to weed out resistant agents) over conventional therapy.

Once again, it must be emphasized that none of the data sets discussed above have been generated through randomized trials. A recent Technology Assessment by the American Society of Clinical Oncology recommends that chemotherapy sensitivity and resistance assays should not be used to select chemotherapeutic agents outside of the clinical trial setting [27]. However, because the Technology Assessment Panel recognizes that in vitro analytic strategy has potential importance, the bulletin maintains that participation in clinical trials evaluating drug resistance assays remains a priority.

If verified prospectively, these data would suggest that assay results at diagnosis may be useful in guiding therapy at relapse, especially when managing a chemical recurrence or one in which tissue is not available for drug resistance testing. Due to a failure to demonstrate any survival benefit, reassessment laparotomy following primary therapy has, for the most part, been relegated to investigational protocols and secondary debulking remains controversial [28–30]. Thus, the likelihood of submitting tumor for chemosensitivity testing is highest at initial diagnosis since most patients undergo primary debulking and few undergo secondary and tertiary cytoreductive surgeries. Our data suggest that the molecular changes that lead to drug resistance may occur early in the carcinogenesis process, perhaps before metastases have been established. An analysis of metachronous tissues retrieved from less advanced patients who ultimately relapse following therapy may shed additional light onto this paradoxical subject.

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