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ORIGINAL ARTICLE

Predicting platinum resistance in primary advanced ovarian cancer patients with an in vitro resistance index

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Abstract
Purpose We aimed to identify primary platinum resistance in epithelial ovarian cancer (OC) patients with FIGO stage III–IV disease by an in vitro drug-response assay and to correlate the findings with clinical response. We considered whether neoadjuvant chemotherapy or anatomic sample site and tumor heterogeneity would influence the results.
Methods We combined the ATP-based tumor-chemosensitivity and the extreme drug resistance assays for testing of 85 biopsies from 58 patients. Tumors were classified as sensitive or resistant by a resistance index (RI). We did separate analyses of primary tumors and metastases and compared chemo-naïve samples with samples obtained after neoadjuvant chemotherapy. Results were analyzed for association with clinical platinum resistance, progression-free survival (PFS), and overall survival (OS).
Results RI ≤ 250 predicted primary platinum resistance, without misclassification of sensitive patients. The test sensitivity for primary tumors was 15/15, specificity 3/10, negative predictive value 3/3, and positive predictive value 15/22. Patients with in vitro platinum-resistant samples had shorter PFS compared with patients with sensitive samples (3.4 vs. 10.0 months, $p = 0.02$). Comparing patient-matched primary and metastatic samples, there was about 1/3 mismatch in resistance. RI for platinum was lower in primary tumors exposed to neoadjuvant chemotherapy than in chemo-naïve tumors ($p < 0.01$).
Conclusions This in vitro assay predicted primary platinum resistance, without misclassification of sensitive OC patients, and the results were significantly associated with PFS. We suggest that samples from primary tumor and metastatic samples have different responses to chemotherapy and that exposure to chemotherapy might induce in vitro platinum resistance.

Keywords Ovarian carcinoma · Platinum resistance · Tumor heterogeneity · In vitro drug-response assay

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Introduction

Ovarian cancer (OC) is the most lethal gynecologic malignancy and one of the major causes of death from cancer among women in the western world. Two-thirds of the patients are diagnosed with advanced disease, and overall

survival is poor [1]. Since 1996, the gold standard of primary treatment has been maximal cytoreductive surgery and platinum/taxane combination therapy [2], with residual tumor volume after surgery being the most important factor for survival [3, 4]. Most patients respond to treatment initially; however, 15–20% of the patients have intrinsic resistance toward platinum and most patients develop resistance, often toward multiple drugs, after repeated lines of therapy [5]. Chemotherapy resistance in OC is poorly understood and at relapse of disease, patients are classified according to likelihood of response to re-treatment with platinum. Progression-free survival (PFS) of $\leq$6 months defines platinum-resistant disease, with a response rate less than 10% for repeated platinum treatment [6]. Patients with PFS >6 months are defined as platinum sensitive, with more than 30% response rate for platinum-based therapy [2, 7]. In resistant disease, most women will receive multiple alternative non-platinum agents such as weekly paclitaxel, liposomal doxorubicin, gemcitabine, and topotecan. So far, no study has shown survival benefit for any chemotherapy agent in this setting [8]. Despite that most patients develop a multi-drug-resistant tumor after repeated lines of chemotherapy, individual patients who do not respond to one regimen may respond to another drug [9]. Nevertheless, in recurrent disease, the selection of alternative drugs has remained mainly empirical based on clinical factors such as previous response to chemotherapy, toxicity, and performance status, and many patients receive ineffective chemotherapy [5]. In both primary and recurrent disease, identifying resistance toward platinum and alternative drugs before treatment would be of great value to spare patients of unnecessary side effects and guide the physician toward the selection of alternative treatment options. Previous studies of in vitro drug-response assays are inconsistent, but there are several promising results in predicting clinical outcome and improving the chance of chemotherapy response in OC patients [10–15].

The main goal of this study was to identify primary platinum resistance without misclassification of sensitive patients, as platinum is the most important drug to improve survival in OC. We applied a modified version of the ATP-based tumor-chemosensitivity (ATP-assay) [13] and the extreme drug resistance assays (EDR) [12] for in vitro drug-response testing of tumor material. The ability of the assay to predict lack of response to chemotherapy was assessed in clinical specimens consisting of 85 biopsies from 58 OC patients with epithelial histology and FIGO stage III/IV disease. We compared in vitro platinum response between patient-matched samples from primary tumor and metastatic site and considered whether exposure to chemotherapy would influence the in vitro platinum response by comparing chemo-naïve samples with samples obtained after neoadjuvant chemotherapy.

Materials and methods

Patient material

The patients represented a highly selected group, including only epithelial OC and FIGO stage III–IV disease. Mucinous and clear cell carcinomas were excluded as they respond poorly to chemotherapy. Biopsies were obtained after informed consent at primary surgery or after neoadjuvant chemotherapy at interval debulking surgery at the Dept. of Gynecological Oncology, the Norwegian Radium Hospital, Oslo University Hospital in 2006–2008. Study approval was given by the Regional Committee for Medical Research Ethics in Norway. Due to their similar phenotype, tubal carcinoma and peritoneal carcinoma are referred to as OC henceforth. Clinical data were registered in an unidentified manner in accordance with national data laws. End of follow-up was September 1, 2010. All biopsies were evaluated by a gyneco-pathologist after surgery, and samples lacking tumor cells were excluded. Only cases where untreated control cells demonstrated sufficient in vitro cell proliferation were included.

Eighty-five samples from 57 advanced serous and 1 endometrioid OC patients were included in the analyses. Clinicopathologic data are detailed in Table 1. The specimens were arranged in three groups; Group 1 (n = 58): all patients (33 primary, 16 omental, 4 peritoneal biopsies, and 5 biopsies not defined). Group 2 (n = 33): patients with primary tumor biopsies. Group 3 (n = 37): patients with metastatic biopsies (30 omental, 1 lymph node, and 6 peritoneal biopsies). In the first group, the primary tumor was chosen when patients had biopsies from more than one site (n = 16). Twenty-nine patients received adjuvant chemotherapy, and 29 patients received neoadjuvant chemotherapy. The chemotherapy regimes received by the patients are detailed in Table 1. The majority of patients received 6 cycles of chemotherapy. However, 2 patients received 5 cycles and 2 received 9 cycles. Twenty-nine patients received 2–4 cycles of chemotherapy before interval debulking.

Drug resistance assay

Fresh tumor tissue was mechanically disaggregated and treated with collagenase (700 U/ml) (Worthington Biochemical Corporation, Lakewood, NJ, USA) overnight and thereafter filtered through a 100-μm nylon Cell Strainer (BD Falcon, Franklin Lakes, NJ, USA) to remove debris and large cell clumps and washed in RPMI-1640 medium (BioWhittakerm Vervier, Belgium). If the cell pellet contained red blood cells, it was treated with ACK lysis buffer (Lonza, Walkersville, MD, Frederick) according to manufacturer's instructions. Cells were thereafter washed and

Table 1 Patient demographics

Subjects	58
Age (mean years ± SD)	62.6 ± 8.7
Type of cancer	
Ovarian cancer	49
Primary peritoneal cancer	8
Tubal cancer	1
FIGO stage	
IIIa	1
IIIb	0
IIIc	42
IV	15
Metastatic site in FIGO IV	
Pleura	11
Lung	1
Liver	1
Spleen	2
Grade	
I	4
II	19
III	31
NA[a]	4
Surgical treatment	
Primary surgery	29
Neoadjuvant chemotherapy	29
Residual tumor[b]	
≤1 cm	37 (10)
>1 cm	21 (19)
Primary chemotherapy treatment[b]	
Carboplatin/paclitaxel	48 (19)
Other platinol combinations	5 (6)
Carboplatin single	3 (3)
Paclitaxel	2 (1)
Primary treatment response[b]	
Complete response	20 (5)
Partial response	16 (11)
Stable disease	4 (3)
Progressive disease	6 (4)
NA[c]	12 (6)
PFS ≤/> 6 months[b]	
≤6 months	26 (20)
>6 months	28 (9)
NA[d]	4 (0)

[a] *NA* not available. Biopsy collected for diagnostic purposes during surgery was too small for grading

[b] Parentheses: number of patients who received neoadjuvant chemotherapy

[c] *NA* not available. Chemotherapy response could not be evaluated because of normalized CA-125 after surgery ($n = 10$) or missing CA-125 information ($n = 2$)

[d] Four patients were disease-free at last follow-up

resuspended in RPMI-1640 medium containing 10% Fetal bovine calf serum (FBS) (PAA Laboratories, Linz, Austria), 2 mM L-glutamine (GibcoBRL, Paisley, UK), 50 U/ml penicillin (Lonza), and streptomycin (BioWhittaker, Basel, CH, Switzerland). To analyze for drug response, approximately 20,000 viable tumor cells (as assessed by Trypan Blue exclusion), resuspended in RPMI-1640 medium containing 10% FBS and antibiotics, were plated in poly(2-hydroxyethyl methacrylate) (poly-hema) (Sigma-Aldrich, St. Louis, MO, USA) coated round-bottomed 96-well plates to avoid attachment and thereby growth of normal cells. The cells were cultured for 5 days, the last 24 h with addition of 3.7×10^4 Bq [^{3}H]-Thymidine (ARC, St. Louis, MO, USA). The cells were harvested using a Filtermate Harvester (Packard Instrument Co. Meriden, CT, USA), and [^{3}H]-Thymidine incorporation was assessed in a Packard Microplate Scintillation Counter. Samples were tested for in vitro response to platinum, paclitaxel, and the combination of the two. Six dilutions of drugs, corresponding to 200, 100, 50, 25, 12.5, and 6.25% of a standard test drug concentration (TDC), as recommended by DCS Innovative Diagnostic-system (Hamburg, Germany), were added to the wells in triplicate at the time of plating. Thus, for carboplatin, 100% TCD was 15.8 μg/ml (range 0.99–31.6 μg/ml) and for paclitaxel, 13.6 μg/ml (range 0.85–27.2 μg/ml). Percentage tumor growth inhibition (TGI) was calculated relative to untreated controls.

Classification of resistance

In vitro drug resistance was described by using a resistance index (RI), calculated by summarizing the percentage of TGI at each TDC tested: RI = ∑TGI at 200, 100, 50, 25, 12.5, and 6.25% TDC. Poor inhibition of cell growth provided a low RI that indicated resistance, and a high RI indicated sensitivity. Clinical platinum resistance was defined as PFS ≤6 months since last platinum-based treatment according to guidelines published by the gynecologic oncology group (GOG) [16]. Progressive disease or recurrence was evaluated by the RECIST criteria [17].

Statistical analyses

Statistical analyses were performed applying the SPSS-PC package (Version 16.0, Chicago, IL, USA). Differences between two independent groups were analyzed using the Mann–Whitney *U* test. Response to chemotherapy was grouped as complete response (CR) versus partial response (PR)/stable disease (SD)/progressive disease (PD) according to RECIST criteria. PFS and OS were calculated from the date of the last chemotherapy administration/diagnosis to documented progression/death or last follow-up (Sept. 01.2010), respectively. Univariate survival analyses of PFS

Table 2 In vitro results predicted in vivo platinum resistance

	RI[a]—Group 1[b,c,d]		RI[a]—Group 2[b,c]		RI[a]—Group 3[b,c]	
	>250	≤250	>250	≤250	>250	≤250
PFS[a]						
>6 months	24	1	15	0	11	0
≤6 months	14	5	7	3	11	4

[a] *PFS* progression-free survival in months. In vitro *resistance* is defined by a resistance index (RI); sensitive, RI > 250; resistant, RI ≤ 250

[b] Group 1, all patients (44/58 biopsies were available at all concentrations). Group 2, primary tumors (25/33 biopsies were available at all concentrations needed for the calculation of RI). Group 3, metastatic biopsies (26/37 biopsies were available at all concentrations)

[c] Group 1; Sensitivity: 24/25, specificity: 5/19, NPV: 5/6, and PPV: 24/38. Group 2; Sensitivity: 15/15, specificity: 3/10, NPV: 3/3, and PPV:15/22. Group 3; Sensitivity: 11/11, specificity: 4/15, NPV: 4/4, and PPV:11/22

[d] Group 1 consisted of 25 patients from group 2, 16 samples from group 3, and 3 patients with anatomic biopsy site not defined. One of the latter patients had PFS > 6 months and was misclassified as resistant in vitro

and OS were executed using the Kaplan–Meier method and log-rank test. Sensitivity, specificity, positive predictive value (PPV), and negative predictive value (NPV) of the RI were defined by applying two-by-two tables with positive and negative in vitro results placed against PFS ≤/> 6 months. Probability of <0.05 was considered statistically significant.

Results

Calculating a resistance index (RI) cutoff point to predict clinical platinum resistance

To examine the utility of RI to predict clinical chemoresistance (PFS ≤ 6 months), a Receiver Operating Characteristic curve (ROC curve) was plotted. The cutoff value that reached the highest score for test specificity without misclassification of chemo-sensitive patients was chosen. For platinum, RI ≤ 250 defined platinum resistance and RI > 250 defined platinum sensitivity. For carboplatin/paclitaxel (CP) combination and paclitaxel treatment, a cutoff value that met the criteria for sensitivity of the test could not be calculated.

Due to low cell yield, not all samples were tested at all concentrations needed to calculate the RI. In the first group, 44 of 58 samples were tested at all concentrations. Six patients were defined as platinum resistant, and 38 patients were defined as sensitive. The sensitivity of the test was 24/25, the specificity was 5/19, NPV was 5/6, and PPV was 24/38. In the second group, 25 of 33 samples were tested at all concentrations. Three patients were characterized as platinum resistant, and 22 patients were characterized as platinum sensitive (Table 2). The sensitivity of the test was 15/15, the specificity was 3/10, NPV was 3/3, and PPV was 15/22. In the third group, 26 of 37 samples were tested at all concentrations. Four samples were characterized as platinum resistant and 22 as sensitive. The sensitivity of the test was 11/11, the specificity was 4/15, NPV was 4/4, and PPV was 11/22.

In vitro platinum resistance and clinical response

As RI ≤ 250 defined the cutoff value for discrimination between in vitro platinum sensitive and resistant patients, it was of interest to evaluate how this parameter was associated with clinical response. Median PFS was 6.8 months for all patients (range 0–26 months). At last follow-up, 34 patients were dead of disease, 20 patients were alive with recurrent disease, and 4 patients were disease-free. Analyzing all patients together (Group 1), RI ≤ 250 was associated with poor PFS ($p = 0.03$). Median PFS was 3.9 (95% CI: 3.2–4.7) compared with 8.1 months (95% CI: 3.7–12.4) for patients with RI > 250 (Fig. 1). Patients with PR/SD/PD had lower RI than patients with CR ($p = 0.03$). Analyzing samples from primary tumors (Group 2), in vitro resistance to platinum was also significantly associated with poor PFS ($p = 0.02$). Patients with RI ≤ 250 had median PFS of 3.4 months (95% CI: 0.0–8.9 months) compared with 10.0 months (95% CI: 6.0–13.9) for patients with RI > 250 ($p = 0.02$) (Table 3). Patients with PR/SD/PD had lower RI than patients with CR ($p = 0.02$). In vitro response to platinum in metastatic samples (Group 3) was not associated with PFS or clinical response.

Response to paclitaxel or CP was not associated with PFS or clinical response in any of the groups. Median OS was 21 months for all patients (range 4–55 months), but in vitro response was not associated with OS in any of the groups.

In vitro resistance and sample site–related heterogeneity

To further evaluate dissimilar in vitro response to platinum between primary tumors and metastases, we studied

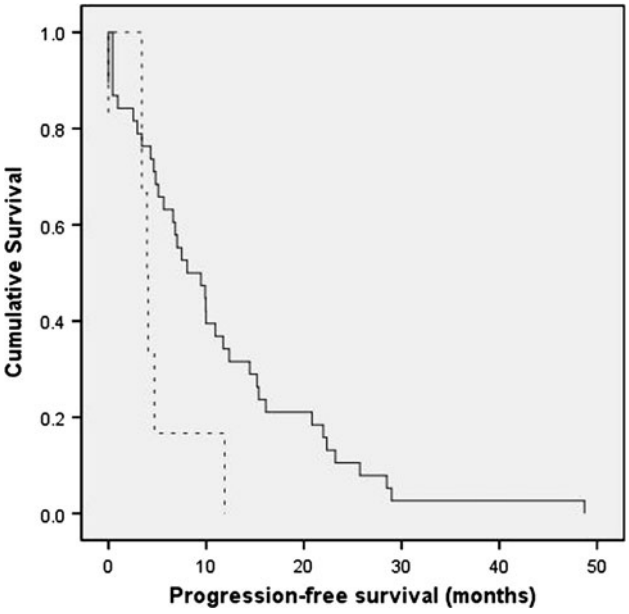

Fig. 1 In vitro platinum resistance is associated with poor progression-free survival. Kaplan–Meier survival curve showing the association between in vitro platinum resistance and progression-free survival (PFS), here demonstrated in Group 1 (all patients). Patients with in vitro platinum resistance (RI ≤ 250) ($n = 6$; *dashed line*) had median PFS of 3.9 months compared with 8.1 months in patients with in vitro platinum sensitivity (RI > 250) ($n = 38$; solid line; $p = 0.03$)

patient-matched samples from different sites, collected in 16 patients. There was a 3/9 mismatch between in vitro sensitivity and resistance in pairs of primary and metastatic samples (Table 4). This suggests a heterogeneity of chemotherapy response between primary tumor and metastasis in a substantial percentage of OC patients. However, sample size was too small for statistical evaluation.

Table 4 In vitro platinum response in patient-matched samples from primary OC tumors and metastases

	Primary tumor[a]	
	Sensitive[b]	Resistant[b]
Metastasis[a]		
Sensitive[b]	6	2
Resistant[b]	1	0

[a] Sixteen patients had matched samples from primary tumor and metastatic site at primary surgery. Nine samples were available at all concentrations needed for the calculation of RI

[b] Resistance index (RI); sensitive, RI > 250; resistant, RI ≤ 250

Significantly lower RI in primary tumors after neoadjuvant chemotherapy

Since it is suggested that platinum resistance increases with exposure to chemotherapy, it was of interest to compare the in vitro drug response between biopsies collected at primary surgery (chemo-naïve) and samples collected at interval debulking surgery (exposed to neoadjuvant chemotherapy). For primary tumors (Group 2), RI for platinum was significantly lower in tumors exposed to neoadjuvant chemotherapy compared with chemo-naïve tumors ($p < 0.01$: Fig. 2). Patients with poor clinical condition or patients unlikely to achieve optimal debulking in primary surgery were selected for neoadjuvant chemotherapy. PFS in the neoadjuvant group was significantly lower compared with the adjuvant group ($p < 0.01$). However, there was no difference in OS ($p = 0.84$), and residual tumor volume after surgery was comparable for the two groups ($p = 0.75$). To exclude residual tumor as a factor, the analysis was also performed in the subgroup of optimally debulked patients,

Table 3 In vitro platinum response and PFS/OS

	PFS (95% CI)[a]	*p* value[a]	OS (95% CI)[a]	*p* value[a]
Group 1[b]*: all patients*				
Sensitive[c] ($n = 38$)	8.1 (3.7–12.4)	**0.03**	31.8 (26.5–37.1)	0.4
Resistant[c] ($n = 6$)	3.9 (3.2– 4.7)		32.6 (17.7–47.5)	
Group 2[b]*: primary tumors*				
Sensitive[c] ($n = 22$)	10.0 (6.0–13.9)	**0.02**	30.8 (19.9–41.7)	0.1
Resistant[c] ($n = 3$)	3.4 (0.0–8.9)		20.3 (14.9–25.8)	
Group 3[b]*: metastasis*				
Sensitive[c] ($n = 22$)	5.6 (2.4–8.9)	0.1	26.7 (15.5–37.9)	1.0
Resistant[c] ($n = 4$)	4.1 (3.1–5.0)		21.9 (8.2–35.5)	

Bold values indicate a significant difference ($p < 0.05$) between sensitive and resistant patients

[a] Log-rank test of in vitro results with median progression-free survival (PFS) and overall survival (OS) in months

[b] Group 1: all patients (44/58 biopsies were available at all concentrations). Group 1 consisted of 25 patients from group 1, 16 samples from group 2 and 3 patients with anatomic biopsy site not defined. Group 2, primary tumors (25/33 biopsies were available at all concentrations needed for the calculation of RI). Group 3: metastatic/peritoneal biopsies (26/37 biopsies were available at all concentrations)

[c] Resistance Index (RI); sensitive, RI > 250; resistant, RI ≤ 250

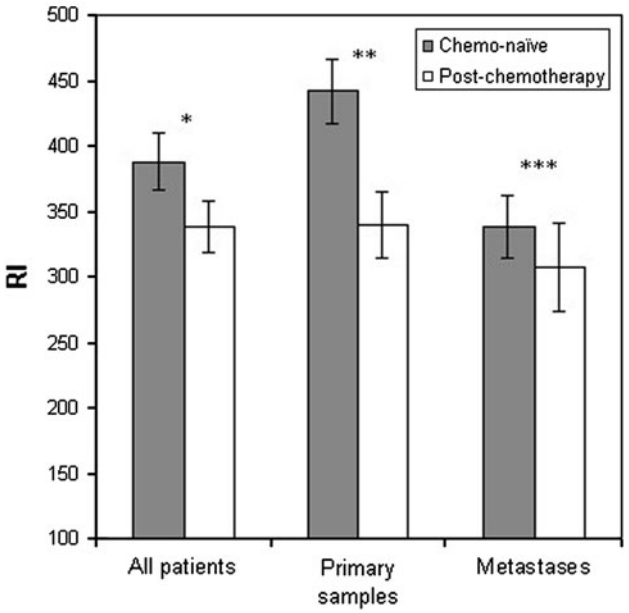

Fig. 2 RI in chemo-naïve and post-chemotherapy samples. Comparison of resistance index (RI: mean ± SE) between chemo-naïve samples (*gray bars*) and samples obtained after exposure neoadjuvant chemotherapy (*white bars*) in all patients, primary tumors, and metastatic samples. $*p = 0.1$, $**p < 0.01$, $***p = 0.62$

with similar result for PFS ($p = 0.01$).In the first and third group, there was no significant difference between RI in chemo-naïve samples and samples exposed to neoadjuvant chemotherapy ($p = 0.10$ and $p = 0.62$, respectively: Fig. 2).

Discussion

The aim of this study was using an in vitro drug-response assay to identify chemotherapy resistance in primary disease without misclassification of sensitive patients. We analyzed the samples in three groups: all patients together, primary tumor biopsies, and metastatic samples, to consider whether tumor heterogeneity between sample sites would influence the results. Furthermore, we considered whether in vitro resistance differed between chemo-naïve samples and samples exposed to neoadjuvant chemotherapy. The included patients formed a uniform group, with epithelial OC histology and FIGO stage III–IV disease.

For in vitro drug-response testing, we used a modified drug-response assay. The cells were cultured in poly-hema-coated round-bottomed wells, as previously described [10], to avoid growth of normal cells. Resembling the EDR assay, we determined the probability of drug response by measuring [^{3}H]-Thymidine incorporation in dividing cells. Previous trials using this method traditionally calculated the in vitro drug response relative to literature reported response rates for specific drugs [18]. In contrast, we calculated in vitro drug response relative to untreated controls as previously described [13] which provided reference values specific for our cohort. As tumor cells were cultured at several test drug concentrations, we could interpret the results by single drug concentrations and by calculating an index (RI) taking all concentrations into account. In accordance with other studies [13, 15, 19], RI was superior to evaluations by single concentrations. Measures at single concentrations achieved only NPV of 54–64% and sensitivity of 70% (data not shown) and were therefore not applied in the clinical analysis.

Generally, in vitro assays cannot account for individual pharmacodynamic aspects such as biotransformation, biodistribution, and tumor vascularity. However, in vitro drug concentrations are much higher than in vivo exposures, and if resistance is present under such conditions, it would probably result in clinical treatment failure. We tested both in vitro response to carboplatin, paclitaxel, and combination of the two. However, only results for platinum exposure showed associations with clinical outcome. In the clinic, resistant patients are defined by recurrence of disease ≤6 months since last platinum treatment, regardless of the last paclitaxel administration. Platinum is the most important drug to determine outcome in OC patients, and platinum resistance is often associated with cross-resistance toward other drugs. This might explain why only in vitro response toward platinum was associated with clinical outcome and why a RI for paclitaxel or CP could not be calculated to predict chemoresistance.

In accordance with other studies [9], careful selection of sample site by the surgeon and analyses of fresh tumor tissue at a pathology laboratory was of crucial importance, as several biopsies lacked tumor cells. These samples were not included in the study. Furthermore, samples with suboptimal in vitro performance, such as poor cell proliferation, were also excluded from the analysis. Exposure to neoadjuvant chemotherapy may have contributed to the latter and such selection of samples might have influenced the results. However, equal numbers of chemo-naïve samples and samples exposed to neoadjuvant chemotherapy were included in the final analysis.

RI ≤ 250 reached the highest accuracy for predicting platinum resistance without incorrect classification of clinically sensitive patients. Platinum is the most important drug to improve survival in OC. To deprive platinum-sensitive patients of platinum based on misclassification by the test would not be acceptable, which explains the choice of high sensitivity at the expense of low specificity in this study. The calculated cutoff point matched well to the value set by others [11, 13, 19]. We aimed for a test that was consistent with clinical practice [2, 8], and in vitro resistance therefore reflected PFS ≤6 months consistent with the definition set by GOG [16] and widely used in the clinic. In contrast,

other studies [13, 18] suggested longer PFS to define clinical resistance which would increase the sensitivity of the test.

We hypothesized that tumor heterogeneity between primary samples and metastases would influence our results and performed separate analysis for all patients together, primary tumors, and metastatic samples. Significant association with PFS was only observed in the groups including primary tumors, and analyses of primary tumors alone demonstrated the strongest association. In vitro response in metastatic samples was not associated with PFS. This suggests an influence on the results by tumor heterogeneity between sample sites. Furthermore, we suggest a mismatch between in vitro resistance and sensitivity in patient-matched primary and metastatic samples (Table 4); however, the number of samples was too small for final conclusions. Previous studies by our group demonstrated 25% variation in DNA ploidy between samples from primary tumor and metastases [20], and tumor heterogeneity has been demonstrated between primary tumor and metastatic samples [21] as well as between primary tumor and samples in recurrent OC [22]. In contrast, Tewari et al. [23] found no significant difference between in vitro drug resistance in primary and metastatic OC lesions, or primary tumors and recurrent samples. Some studies did not report biopsy sites and report inconsistent conclusions [13, 24, 25].

It is an ongoing discussion whether resistance is due to selection of resistant clones that exist within the tumor mass before treatment or that resistance evolves by molecular changes caused by selective pressure of chemotherapy [26]. Here, we demonstrate that RI was significantly lower in primary tumor biopsies obtained after neoadjuvant treatment compared with those obtained at primary surgery. This suggests that chemotherapy exposure induces in vitro platinum resistance. Previous studies comparing in vitro resistance between samples obtained before and after chemotherapy exposure show inconsistent results [23, 24]. In the present study, the amount of residual tumor after surgery may have influenced the results as patients unlikely to achieve optimal debulking was selected for neoadjuvant chemotherapy. However, residual disease was comparable between the groups, and there was no difference in RI between optimally debulked and suboptimally debulked patients. There is an assumption of "acquired" platinum resistance in patients with residual tumor after surgery [27] and to rule out residual tumor as a factor, we confirmed the findings in subanalyses of optimally debulked patients.

In conclusion, the applied in vitro drug-response assay predicted primary platinum resistance in OC, defined as PFS $\leq$6 months. We put emphasis on defining a cutoff value that would avoid misclassification of sensitive patients as resistant. In clinical practice, such analysis could potentially guide the physicians toward an alternative antineoplastic regime in patients classified as primary platinum resistant with minimal risk of depleting sensitive patient of platinum therapy. However, the sensitivity in this study needs to be confirmed in a larger trial.

Additionally, this study suggests that exposure to chemotherapy might induce resistance toward platinum and that samples from primary tumor and metastatic samples might have different responses to chemotherapy.

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