Ex vivo assessment of cancer drug sensitivity in epithelial ovarian cancer and its association with histopathological type, treatment history and clinical outcome

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Abstract. Epithelial ovarian cancer (EOC) is divided into type I and type II based on histopathological features. Type I is clinically more indolent, but also less sensitive to chemotherapy, compared with type II. The basis for this difference is not fully clarified. The present study investigated the pattern of drug activity in type I and type II EOC for standard cytotoxic drugs and recently introduced tyrosine kinase inhibitors (TKIs), and assessed the association with treatment history and clinical outcome. Isolated EOC tumor cells obtained at surgery were investigated for their sensitivity to seven standard cytotoxic drugs and nine TKIs using a short-term fluorescent microculture cytotoxicity assay (FMCA). Drug activity was compared with respect to EOC subtype, preoperative chemotherapy, cross-resistance and association with progression-free survival (PFS). Out of 128 EOC samples, 120 samples, including 21 type I and 99 type II, were successfully analyzed using FMCA. Patients with EOC type I had a significantly longer PFS time than patients with EOC type II (P=0.01). In line with clinical experience, EOC type I samples were generally more resistant than type II samples to both standard cytotoxic drugs and the TKIs, reaching statistical significance for cisplatin (P=0.03) and dasatinib (P=0.002). A similar pattern was noted in samples from patients treated with chemotherapy prior to surgery compared with treatment-naive samples, reaching statistical significance for fluorouracil, irinotecan, dasatinib and nintedanib (all P<0.05). PFS time gradually shortened with increasing degree of drug resistance. Cross-resistance between drugs was in most cases statistically significant yet moderate in degree (r<0.5). The clinically observed relative drug resistance of EOC type I, as well as in patients previously treated, is at least partly due to mechanisms in the tumor cells. These mechanisms seemingly also encompass kinase inhibitors. *Ex vivo* assessment of drug activity is suggested to have a role in the optimization of drug therapy in EOC.

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Abbreviations: 5-FU, 5-fluorouracil; ASA, American Society of Anesthesiology; CC, completeness of cytoreduction; CT, computed tomography; EDR, extreme drug resistance; EOC, epithelial ovarian cancer; FMCA, fluorometric microculture cytotoxicity assay; LDR, low drug resistance; PCI, Peritoneal Cancer Index; PFS, progression-free survival; SD, standard deviation; TKI, tyrosine kinase inhibitor

Key words: ovarian cancer, type I and II, drug sensitivity, *ex vivo*

Introduction

Ovarian cancer is the most lethal gynecological malignancy, responsible for >200,000 deaths globally in 2020 (1). The most common form is epithelial ovarian cancer (EOC). However, despite major research efforts, the exact origin and early pathogenesis of EOC are still not fully understood. EOC can include cells of origin from both the ovarian surface epithelium and the fallopian tube (2). Moreover, EOC is not a single disease, but a heterogenic group of tumors that can be classified by their genetic and histological features. In 2004, Shih and Kurman (3) suggested a dualistic model, with type I and type II EOC, and this model has been helpful in understanding EOC development and tumor biology. Thus, type I (low-grade serous G1, low-grade endometrioid G1/G2, mucinous or clear cell) tumors are associated with corresponding benign ovarian cystic neoplasms, often developing through an intermediate borderline step and have a better prognosis. Type II (high-grade serous G2/G3, high-grade endometrioid G3 or carcinosarcoma) tumors are highly aggressive and genetically unstable tumors that most often present at advanced stages and are responsible for the majority of EOC-associated deaths (3,4).

In most cases, modern treatment of ovarian cancer includes radical cytoreductive surgery, followed by platinum- and paclitaxel-based systemic chemotherapy (5-7). However, current treatment regimens are far from optimal (8). The histopathology of ovarian cancer is heterogeneous, and each subtype harbors specific genetic mutations that can be used for diagnostics and targeted treatment. New drugs target some of the stepwise genetic mutations the neoplastic cells gain to become masters of their growth and proliferation (9). For example, tyrosine kinases play a critical role in growth factor signaling and sustained proliferation, and all histopathological subtypes of EOC seem to have modifications in growth factor signaling. For instance, EOC type I tumors typically are chemoresistant tumors that harbor mutations in BRAF, KRAS and PIK3CA (10).

Tyrosine kinase inhibitors (TKIs) have been trialled in EOC, and one review has summarized the results of 75 completed and ongoing clinical trials (11). While there is still some promise for a few TKIs, the overall findings point to low efficacy (12-15). Furthermore, with the exception of poly(ADP-ribose) polymerase-inhibitors and BRCA1/2 mutations, current systemic treatments are usually based on group-level clinical trial data and do not consider histopathology, molecular characterization or individual drug sensitivity, even though it is well known that response rates to cancer drugs vary (16). As a result, individuals are at risk of side effects, while the tumor may be unresponsive to therapy (17). As clinicopathological parameters are insufficient for the prediction of response to chemotherapy, additional methods are needed to individualize treatment.

The present study used a short-term culture chemotherapy sensitivity assay to evaluate *ex vivo* EOC tumor cell sensitivity to established cytotoxic drugs and TKIs. The aim was to explore sensitivity patterns in the two EOC subtypes and in samples with or without previous exposure to cytotoxic drugs. Furthermore, the study aimed to explore cross-resistance between drugs and evaluate the association between drug sensitivity and progression-free survival (PFS) in the patients.

Materials and methods

Patients and tumor samples. In total, 128 patients scheduled for ovarian cancer surgery between May 2006 and December 2016 at Uppsala University Hospital (Uppsala, Sweden), Örebro University Hospital (Örebro, Sweden), Falun hospital (Falun, Sweden) and the private Uppsala Cancer Clinic (Uppsala, Sweden) were included in the study, with the majority included during the last 5 years. A successful chemotherapy sensitivity assay was obtained in 120 patients, and these were included for further analysis. Of these, 93 patients were scheduled for potentially curative cytoreductive surgery, whereas 18 underwent laparotomy, but were too advanced for cytoreductive surgery and underwent debulking surgery for symptom relief or only diagnostic laparotomy. As the private clinic closed during the study, information about surgery could not be obtained for the remaining 9 patients that were included at this site. However, at all sites, surgery was performed by gynecological surgeons and patient tumor burden was assessed according to the Peritoneal Cancer Index (PCI) (18) at the start of surgery. Residual disease after surgery was quantified according to the completeness of cytoreduction (CC) score (19,20), where a CC score of 0 (no macroscopic tumor left) and 1 (residual tumor <0.25 cm) were considered as complete cytoreduction. Preoperative performance status was classified according to the American Society of Anesthesiologists (ASA) Physical Status Classification System (21). Tumor samples were collected during surgery and immediately sent for *ex vivo* drug activity assessment.

Tumor sample classifications of type I (low-grade serous G1, low-grade endometrioid G1/G2, mucinous or clear cell) or type II (high-grade serous G2/G3, high-grade endometroid G3 or carcinosarcoma) made by an experienced pathologist at a Swedish tertiary care hospital were collected from the patient medical records (3).

Following surgery, patients started chemotherapy within 4 to 6 weeks, most commonly with paclitaxel 175 mg/m^2 and carboplatin (area under the curve, 5). After completing treatment, patients were followed up with computed tomography (CT) scans, and then clinical examination, a transvaginal ultrasound and cancer antigen 125 assessment every 3 months for 2 years, every 6 months for another 3 years, and every 12 months up to 10 years. Findings at the clinical examination and/or increased tumor marker levels would trigger a CT scan for the verification of relapse (22). Characteristics of the patients included are detailed in Table I. Information on histopathological subtype, clinical characteristics, chemotherapy, surgery, disease status and survival were obtained from the medical records of Uppsala University Hospital and the other participating centers. Among patients in which complete cytoreduction (n=74) was achieved, data for PFS were collected until February 2017. All tumor sampling and data collection was performed once written informed consent had been obtained, and the study was approved by the Regional Ethical Committee in Uppsala (approval no. Dnr 2007/237).

Ex vivo assessment of drug sensitivity. The tumor specimens were kept in a transport culture medium at room temperature until cell preparation, which mostly started within 3 h of tumor sampling. Tumor cells were prepared by collagenase digestion as described previously (23). The cells obtained were mostly single cells or small cell clusters, in cell suspension, with ≥90% viability and <30% contaminating non-malignant cells, as judged by viability staining, using toluidine blue, and morphological examinations of May-Grünwald-Giemsa-stained cytocentrifuge preparations, respectively (Fig. S1). Cytocentrifuge glasses (100 μl, 700 g; 5 min at room temperature) were stained using May-Grünwald for 5 min followed by Giemsa stain for 10 min. The glasses were then left to air dry, in room temperature, before examination using light microscopy.

Seven standard solid tumor cytotoxic drugs and nine recently introduced TKIs with different indications were tested *ex vivo*. The drugs were commercially available clinical preparations (cisplatin) or obtained from Selleck Chemicals (oxaliplatin and crizotinib), MilliporeSigma (irinotecan and 5-fluorouracil) or LC Laboratories (gemcitabine, dasatinib, docetaxel, doxorubicin, erlotinib, lapatinib, nintedanib, regorafenib, sorafenib and sunitinib). From 2006 until mid-2013, the drugs were tested at three 10-fold dilutions from the maximal concentration of 1,000 μM for 5-fluorouracil (5-FU), gemcitabine and irinotecan, and 100 μM for oxaliplatin,

Table I. Clinical characteristics of the ovarian cancer samples successfully analyzed *ex vivo* (n=120).

Characteristic	Value
Mean age (range), years	59 (19-81)
Mean BMI (range), kg/m^2	25 (16-44)
ASA, n (%)	
1	16 (13.3)
2	62 (51.7)
3	21 (17.5)
Unknown	21 (17.5)
Histopathology, n (%)	
Type I	21 (17.5)
Low-grade serous	13 (10.8)
Low-grade endometroid	3 (2.5)
Mucinous	2 (1.7)
Clear cell	3 (2.5)
Type II	99 (82.5)
High-grade serous	93 (77.5)
High-grade endometroid	3 (2.5)
Carcinosarcoma	3 (2.5)
Prior chemotherapy, n (%)	52 (43.3)
Peritoneal cancer index, n (%)	
1-10	11 (9.2)
11-20	30 (25.0)
21-39	44 (36.7)
Unknown	35 (29.2)
Operable, n (%)	
Yes	93 (77.5)
No	18 (15.0)
Unknown	9 (7.5)
Complete cytoreductive surgery, n (%)[a]	
Yes	74 (79.6)
No	18 (19.4)
Not detailed	1 (1.1)

[a]In patients in which curative surgery was attempted (n=93). ASA, American Society of Anesthesiology; BMI, body mass index.

cisplatin, docetaxel, doxorubicin, erlotinib, lapatinib, sorafenib and sunitinib. From mid-2013, five concentrations were tested with four three-fold dilutions from a lowered maximal concentration, including some recently introduced TKIs: 180 μM for 5-FU and irinotecan, 90 μM for oxaliplatin, docetaxel, gemcitabine, crizotinib, dasatinib, erlotinib, lapatinib, nintedanib, regorafenib, sorafenib and sunitinib, 45 μM for doxorubicin and vemurafenib, and finally 30 μM for cisplatin. The drug concentrations used *ex vivo* were chosen empirically to produce concentration-response curves allowing estimation of the half maximal inhibitory concentrations (IC_{50}), i.e., the drug concentration producing a cell survival rate of 50% compared with the unexposed control. From 2006 until mid-2013, 384-well microplates (Nalge Nunc International) were prepared with 5 μl drug solution at 10 times the final drug concentration using the pipetting robot BioMek 2000 (Beckman Coulter, Inc.). The plates were prepared freshly every 3 months, tested for stability, and then stored at -70˚C until further use.

The semiautomated fluorometric microculture cytotoxicity assay (FMCA) assessed drug sensitivity (24,25). Briefly, tumor cells from patient samples (5,000 cells/well) in 45 μl RPMI 1640 culture medium [supplemented with 10% fetal calf serum, glutamine and penicillin-streptomycin (all from MilliporeSigma)] were seeded in the drug-prepared 384-well plates using the pipetting robot Precision 2000 (Bio-Tek Instruments, Inc.). From mid-2013, the drugs were added immediately after cell seeding using the liquid handling system ECHO® 550 (Labcyte, Inc.). This allowed for fast transfer of volumes ≥2.5 nl from source plates into destination wells. In ECHO® experiments, source plates were prepared with appropriate concentrations of drugs in DMSO (except cisplatin, where the clinical preparation was used) and stored in the oxygen and moisture free MiniPod™ system (Roylan Developments Ltd.) until further use. The method for drug addition did not affect the assay results. Three columns without drugs served as negative controls, and one column with medium only served as a blank control.

The culture plates were incubated at 37˚C in a humidified atmosphere containing 95% air and 5% CO_2. After 72 h of incubation, the culture medium was washed away and 50 μl/well of a physiological buffer containing 10 μg/ml of the vital dye fluorescein diacetate (FDA) was added to the negative control, experimental and blank control wells. After incubation for 30-45 min at 37˚C, the fluorescence from each well was read in a FluosStar Optima (BMG Labtech GmbH).

Quality criteria for a successful assay were: ≥70% tumor cells in the cell preparation before incubation and/or on the assay day, a fluorescence signal in control cultures of ≥5 times the mean blank values and a coefficient of variation of cell survival in control cultures of ≤30%. A total of 8 out of the 128 samples (6%) did not fulfill these quality criteria and were not included in the results presentation. The results obtained by the viability indicator FDA were calculated as the survival index (SI), defined as the fluorescence of the drug-exposed wells as a percentage of control cultures, with blank values subtracted: $SI\ (\%)=100x[(F_{experimental}-F_{blank\ control})/(F_{negative\ control}-F_{blank\ control})]$, where F_i corresponds to the average fluorescence signal in i=experimental, negative control and blank control wells, respectively.

Data evaluation and statistical analysis. IC_{50} calculations and statistical analyses thereof were performed using GraphPad Prism version 5.0 for Mac (GraphPad Software, Inc.). Drug IC_{50} was calculated using non-linear regression to a standard sigmoidal dose-response model. Sample sensitivity for regression analysis was categorized as follows: Low drug resistance (LDR), IC_{50} below the median; intermediate drug resistance (IDR), IC_{50} between the median and the median plus one standard deviation (SD); or extreme drug resistance (EDR), IC_{50} above the median plus one SD, based on all samples investigated *ex vivo* (24-26). Drug sensitivity correlations for assessment of cross-resistance were calculated at the drug concentration where the tumor samples showed the greatest scatter of SI-values and evaluated using the Pearson correlation test in GraphPad Prism (Graphpad Software, Inc.).

Table II. Half maximal inhibitory concentration values for standard drugs in ovarian cancer samples (n=120), according to preoperative cytotoxic drug treatment and histopathological subtype.

		Preoperative cytotoxic drug treatment			Histopathological subtype		
Drug	Total patients, n	Yes (n=52)	No (n=68)	P-value	Type I (n=21)	Type II (n=99)	P-value
Cytotoxic							
5-FU, μM	119	309±328	171±181	0.015[a]	267±313	224±254	0.806
Oxaliplatin, μM	118	32.9±32.1	22.8±24.2	0.055	35.3±37.0	25.4±25.9	0.557
Cisplatin, μM	106	11.9±15.4	10.0±14.2	0.126	16.5±22.5	9.81±12.6	0.030[a]
Docetaxel, μM	105	45.9±46.7	42.0±38.0	0.895	65.7±66.5	39.2±34.6	0.321
Irinotecan, μM	119	90.8±79.9	66.7±62.2	0.021[a]	85.4±75.1	75.5±70.6	0.378
Doxorubicin, μM	107	1.77±3.37	1.10±1.53	0.085	1.66±1.64	1.37±2.73	0.081
Gemcitabine, μM	92	396±386	240±335	0.058	253±330	314±370	0.651
TKI							
Crizotinib, μM	69	16.7±23.6	9.44±16.1	0.053	20.2±27.1	11.0±18.0	0.064
Dasatinib, μM	67	11.3±11.2	6.64±9.04	0.013[a]	18.3±13.6	6.71±8.35	0.002[a]
Erlotinib, μM	92	61.3±35.6	62.0±36.8	0.874	57.3±37.0	62.6±36.0	0.612
Lapatinib, μM	75	15.9±24.2	14.2±18.5	0.877	16.0±19.9	14.6±20.9	0.686
Nintedanib, μM	44	23.8±29.5	11.5±21.7	0.008[a]	26.4±36.0	14.0±23.3	0.171
Regorafenib, μM	71	15.4±7.91	12.4±9.05	0.054	14.0±7.68	13.6±8.88	0.607
Sorafenib, μM	99	13.8±9.89	15.4±18.4	0.560	12.5±7.44	15.1±16.3	0.879
Sunitinib, μM	104	5.14±3.72	6.42±6.89	0.559	6.93±6.52	5.62±5.49	0.735
Vemurafenib, μM	62	32.3±11.9	29.7±12.3	0.362	37.7±11.9	29.5±11.8	0.066

[a]P<0.05. Comparisons made by Mann-Whitney U-test. Data are presented as the mean ± standard deviation. 5-FU, 5-fluorouracil; tyrosine kinase inhibitor.

As the IC_{50} values for the drugs did not follow a normal distribution as evaluated by Shapiro-Wilk and Kolmogorov-Smirnov tests, comparisons between histopathological subtypes and those who had or had not received preoperative cytotoxic drug treatment were made by Mann-Whitney U test. The prognostic importance of *ex vivo* drug sensitivity on PFS was evaluated using the Cox proportional hazard model in SPSS version 28.0 (IBM Corp.). Several confounders were tested in these analyses, but the only one with significant influence was the EOC tumor type. Due to the prognostic value of the EOC tumor type, subsequent analysis of the importance of *ex vivo* drug sensitivity was performed in patients with type II tumors only (n=61), with adjustment for ASA class and PCI. The significance level for all statistical tests was set to P<0.05. Data are presented as the mean ± SD unless otherwise stated.

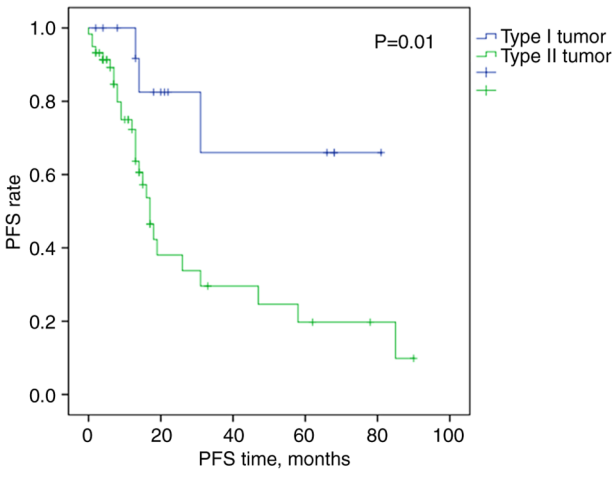

Figure 1. Progression-free survival of patients with complete cytoreductive surgery (n=74) divided into those with type I and type II epithelial ovarian cancer.

Results

A successful *ex vivo* assay was obtained in 120 out of 128 samples (94%). The remaining 8 samples did not pass technical quality control (see *Materials and methods* section for details). A total of 99 patients had type II tumors, of which 93 had high-grade serous histology (Table I). Among the patients with type I tumors (n=21), low-grade serous histology was the most common type. A total of 52 patients (43%) had received chemotherapy prior to surgery, 50 of these with paclitaxel and carboplatin. According to the ASA classification, most patients had no or mild functional limitation (Table I). Curative cytoreductive surgery was attempted in 93 patients and was achieved in 74 (80%), of which 13 had type I and 61 type II tumors, respectively.

As expected, patients with complete cytoreduction and type I tumors had longer PFS times than patients with type II tumors, at 60.9 months (95% CI, 41.7-80.2) vs. 32.0 months (95% CI, 20.9-43.0) (Fig. 1).

Table III. Multivariable Cox regression model for progression-free survival according to drug sensitivity in patients with type II ovarian cancer who underwent complete cytoreductive surgery (n=61)[a].

Drug	n	Adjusted HR	95% CI	P-value
5-FU				
Low drug resistance	29	1.00		
Intermediate drug resistance	26	1.27	0.55-2.92	0.575
Extreme drug resistance	4	1.67	0.52-5.42	0.391
Oxaliplatin				
Low drug resistance	28	1.00		
Intermediate drug resistance	22	1.81	0.73-4.47	0.198
Extreme drug resistance	8	2.44	0.73-8.17	0.148
Cisplatin				
Low drug resistance	28	1.00		
Intermediate drug resistance	26	0.92	0.41-2.07	0.841
Extreme drug resistance	2	3.65	0.76-17.21	0.102
Docetaxel				
Low drug resistance	27	1.00		
Intermediate drug resistance	24	1.18	0.48-2.93	0.715
Extreme drug resistance	5	2.69	0.64-11.34	0.179
Irinotecan				
Low drug resistance	29	1.00		
Intermediate drug resistance	26	1.56	0.67-3.62	0.305
Extreme drug resistance	4	1.40	0.37-5.22	0.617
Doxorubicin				
Low drug resistance	28	1.00		
Intermediate drug resistance	27	1.63	0.69-3.89	0.268
Extreme drug resistance	2	1.57	0.17-14.43	0.693
Gemcitabin				
Low drug resistance	25	1.00		
Intermediate drug resistance	17	1.60	0.58-4.42	0.367
Extreme drug resistance	9	1.71	0.55-5.32	0.358
Crizotinib				
Low drug resistance	22	1.00		
Intermediate drug resistance	19	3.49	1.08-11.25	0.037[b]
Extreme drug resistance	3	-	-	0.988[c]
Dasatinib				
Low drug resistance	22	1.00		
Intermediate drug resistance	19	3.34	0.90-12.39	0.072
Extreme drug resistance	2	16.37	1.25-213	0.033[b]
Erlotinib				
Low drug resistance	26	1.00		
Intermediate drug resistance	25	3.83	1.42-10.35	0.008[b]
Extreme drug resistance	0			
Lapatinib				
Low drug resistance	24	1.00		
Intermediate drug resistance	20	1.39	0.52-3.77	0.514
Extreme drug resistance	3	1.09	0.23-5.30	0.913
Nintedanib				
Low drug resistance	17	1.00		
Intermediate drug resistance	14	1.28	0.17-9.39	0.812
Extreme drug resistance	3	4.72	0.59-37.87	0.144

Table III. Continued.

Drug	n	Adjusted HR	95% CI	P-value
Regorafenib				
Low drug resistance	22	1.00		
Intermediate drug resistance	20	3.07	0.89-15.38	0.071
Extreme drug resistance	3	28.31	4.95-161	0.001[b]
Sorafenib				
Low drug resistance	26	1.00		
Intermediate drug resistance	23	2.28	0.85-6.13	0.102
Extreme drug resistance	4	11.22	2.96-42.55	0.001[b]
Sunitinib				
Low drug resistance	28	1.00		
Intermediate drug resistance	26	1.21	0.53-2.78	0.646
Extreme drug resistance	2	1.88	0.22-15.79	0.563
Vemurafenib				
Low drug resistance	22	1.00		
Intermediate drug resistance	18	1.51	0.49-4.65	0.472
Extreme drug resistance	0			

[a]Adjusted for Peritoneal Cancer Index group and American Society of Anesthesiology. [b]P<0.05. [c]No adjusted HR estimates obtained (all patients censored). CI, confidence interval; HR, hazard ratio; 5-FU, 5-fluorouracil.

Cytotoxic drug sensitivity varied considerably between patient samples, as indicated by the high SDs in the IC_{50} values for the tested drugs (Table II). Tumors previously exposed to chemotherapy were less sensitive, i.e., had higher IC_{50}, to all cytotoxic drugs and to three out of the nine kinase inhibitors, reaching statistical significance for 5-FU, irinotecan, dasatinib and nintedanib. Notably, for cisplatin, the difference in sensitivity with respect to treatment status was minimal, and for erlotinib, sorafenib and sunitinib, samples from previously treated patients were slightly more, although not statistically significantly, sensitive compared with treatment naïve samples.

Compared with type I tumors, type II tumors were more sensitive to all drugs except gemcitabine, reaching statistical significance for cisplatin (Table II). The pattern was similar for the TKIs, with type II tumors being more sensitive to all TKIs except for erlotinib and sorafenib, with statistical significance for dasatinib.

To remove the prognostic influence of tumor type, the analysis of PFS was performed separately for patients with type II tumors with complete cytoreduction (n=61). For all drugs either IDR and/or EDR were associated with a higher risk of progression compared with those with LDR (adjusted HR >1), reaching statistical significance for the kinase inhibitors crizotinib, dasatinib, erlotinib, regorafenib and sorafenib (Table III), although the resistance classification was not significant overall for crizotinib and dasatinib (Fig. 2).

Cross-resistance between the key ovarian cancer drug cisplatin and certain selected cytotoxic drugs and TKIs was modest or statistically significant in most cases, except for between cisplatin and nintedanib, where there was an absence of cross-resistance (Table IV).

Table IV. Correlations of survival index (%) and linear regression slope between the pairs of drugs indicated[a].

Drug pair	r	Slope	P-value
Cisplatin/5-FU	0.499	0.398±0.120	0.0023[b]
Cisplatin/oxaliplatin	0.307	0.243±0.088	0.0075[b]
Cisplatin/docetaxel	0.270	0.349±0.137	0.0130[b]
Cisplatin/irinotecan	0.224	0.249±0.189	0.1962
Cisplatin/doxorubicin	0.301	0.276±0.152	0.0792
Cisplatin/lapatinib	0.344	0.0335±0.116	0.0051[b]
Cisplatin/nintedanib	0.026	0.040±0.216	0.8539
Cisplatin/sorafenib	0.336	0.309±0.101	0.0030[b]
Cisplatin/sunitinib	0.385	0.354±0.099	0.0006[b]
Sorafenib/regorafenib	0.357	0.325±0.100	0.0018[b]

[a]Concentrations selected for analyses were 10 μM cisplatin, 1 μM doxorubicin, 20 μM docetaxel, 1,000 μM 5-FU, 100 μM irinotecan, 30 μM oxaliplatin, 10 μM sorafenib, 10 μM sunitinib, 10 μM nintedanib and 10 μM regorafenib. The number of data points for correlations ranged from 35-113. [b]P<0.05. 5-FU, 5-fluorouracil.

Discussion

Type I epithelial ovarian tumors are reported to have a better prognosis than the highly aggressive and genetically more unstable type II tumors (4). This assumption was also confirmed in the present study. Type II tumors are characterized by initial sensitivity to cytotoxic agents that often affect DNA repair pathways. By contrast, type I tumors show more indolent behavior and are less sensitive to conventional

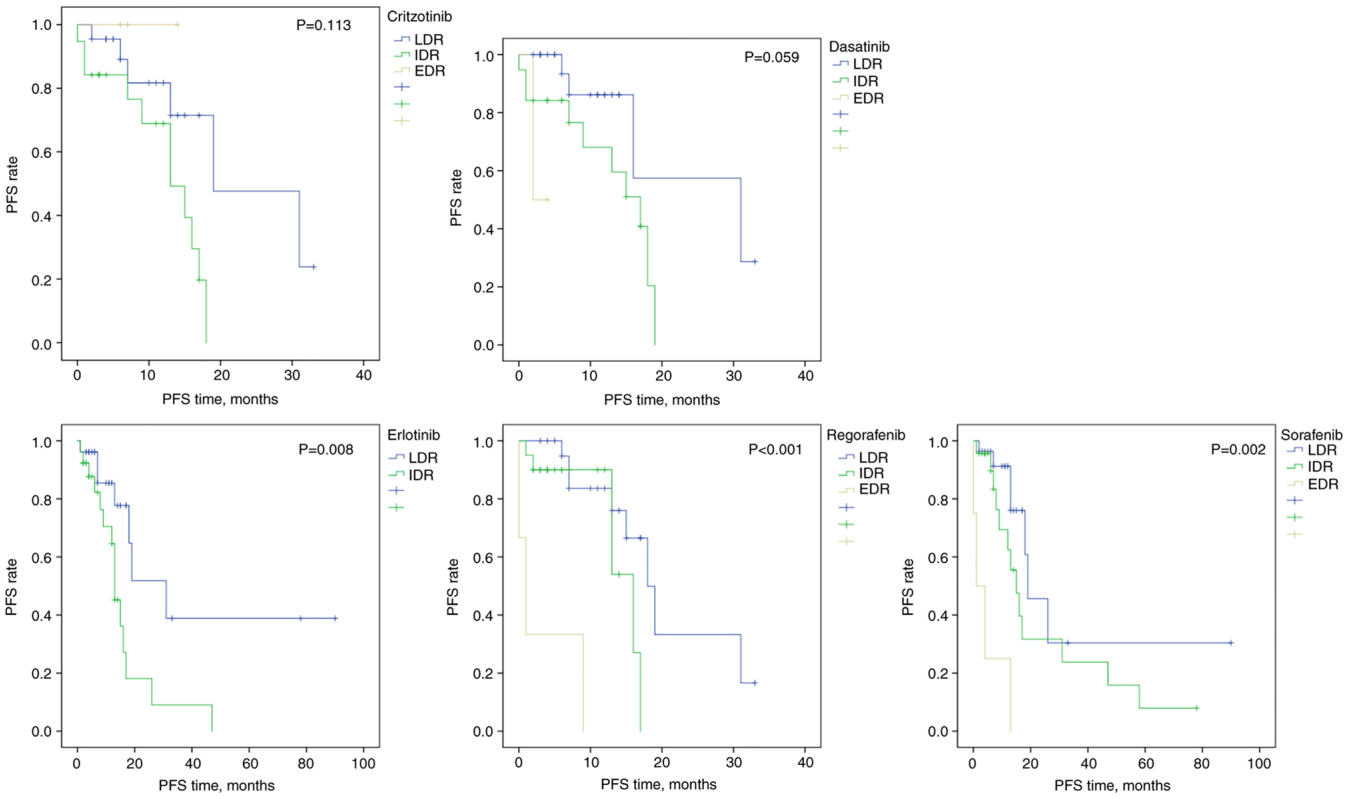

Figure 2. Progression-free survival of patients with type II epithelial ovarian cancer and complete cytoreductive surgery (n=61) based on *ex vivo* activity of the tyrosine kinase inhibitors indicated and found to provide statistically significant prognostic information, as detailed in Table III. Drug activity was classified into LDR, IDR and EDR as detailed in the *Materials and methods* section. Overall P-values for the activity classification, as obtained in the Cox proportional hazard regression, is indicated for each drug. All samples were not investigated for all drugs and, therefore, the number of data points does not necessarily add up to 61 in each panel. LDR, low drug resistance; IDR, intermediate drug resistance; EDR, extreme drug resistance.

treatment than type II tumors (27,28). The *ex vivo* results reported in the present study are in line with this clinical experience. The type I tumors were generally less sensitive to cytotoxic agents than the type II tumors, typically illustrated by the difference for cisplatin. Hence, the difference in PFS in favor of the type I histology combined with the reduced cytotoxic drug sensitivity in type I tumors suggests that the overall improved prognosis is due to their more indolent tumor biology. To improve the prognosis in type I tumors further, the present study points to erlotinib and sorafenib as drugs with some promise to be relatively active in this subgroup. However, the limited clinical experience with erlotinib and sorafenib in EOC points to very low activity, but available studies have not reported on histopathological subgroups (12-15).

Furthermore, the present study observed a stepwise increase in risk for disease progression or death with decreasing drug sensitivity. This finding, that the clinical pattern of drug activity is reflected in an *ex vivo* total cell kill assay like the FMCA, lends support for a clinical role for such assays in clinical treatment decision-making for cancer drug therapy in EOC, much in line with the findings by von Heideman *et al* (29). This pattern of drug sensitivity also applied to most TKIs and was also observed when comparing samples from previously untreated and treated patients, indicating that cancer drug resistance is somewhat of a general phenomenon and not isolated to one or a few individual drugs. This means that once drug resistance has been observed in the clinic, the probability that another drug, irrespective of mechanistic class, will work decreases. However, given the considerable variability and modest cross-resistance between drugs, response in later line therapy is not excluded.

Samples from patients previously exposed to cytotoxic drugs generally tended to be more resistant to most drugs than samples from unexposed patients. This observation is in line with clinical experience and findings, supporting the notion that exposure to cytotoxic treatments contributes to development of more or less general resistance mechanisms (30). Induced chemoresistance seems to be less or absent for cisplatin, supporting the fact that platinum is often active even in treating relapses (31). On the other hand, resistance to the TKIs after exposure varied, but was seemingly less pronounced than for standard cytotoxic drugs. Sorafenib and sunitinib seemingly lack development of resistance after prior cytotoxic drug exposure, and they may be notable drugs for further investigation in the treatment of resistant disease (32); however, as aforementioned, the results from limited clinical experience with these drugs is not very promising (12-15).

A limitation of the present study was that the genetic constitution of the tumors was not available for use as a covariate. Such data would enable an integrated precision medicine study in which novel genetic markers for effect could be identified.

Drug sensitivity varied considerably between patient samples, indicating that *ex vivo* drug sensitivity testing may be helpful prior to the treatment of patients with EOC. Previous studies with FMCA and similar assays have proven useful in providing prognostic information (29,33,34). On the

other hand, assay-based drug selection for EOC treatment has shown variable results. A randomized controlled trial with 180 patients suggested a trend towards improved responses and more prolonged PFS time from assay-guided therapy. Still, no significant impact on overall survival could be demonstrated (35). In another comparative yet non-randomized trial in patients with EOC relapse, a cell-based assay was useful and revealed longer PFS and overall survival times in patients with platinum-sensitive disease (36).

In the present study, the cross-resistance *in vitro* between the platinum drugs cisplatin and oxaliplatin was modest and significant, in line with previous findings in preclinical and clinical settings (37-40) Oxaliplatin differs somewhat from cisplatin concerning mechanism of action and resistance (30). Oxaliplatin is effective in EOC, but cisplatin is the platinum drug established in first-line treatment of EOC (41,42). Previously published FMCA EOC results suggested no cross-resistance between docetaxel and cisplatin, supporting different pathways of action and clinical benefits with combinations of platinum and docetaxel in EOC treatment (29,43-45). Cross-resistance between cisplatin and docetaxel was modest to low in the present study and supported the suitability of clinical use of this combination.

In conclusion, *ex vivo* assessment of drug activity based on total cell kill reveals that EOC type I and II are differently sensitive to standard cytotoxic drugs and recently introduced TKIs, and that none of these seem very promising for the treatment of drug-resistant type I disease. *Ex vivo* reported tumor cell drug sensitivity in EOC is in line with clinical experience and outcome, pointing towards a role for such assays to optimize drug therapy in EOC.

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Availability of data and materials

The datasets used and/or analyzed during the current study are available from the corresponding author on reasonable request.

Authors' contributions

KBj, ISP, RL and PN were responsible for study conception and design. Drug sensitivity assays were performed by KBl. KBj, KBl, ISP, KS, AML, FB, ÅN, CA, RL and PN were responsible for data acquisition, analysis and interpretation. KBj and PN drafted the manuscript followed by its review and revision by all authors. All authors approved the final manuscript and take responsibility for its content. KBj, KBl and PN confirm the authenticity of all the raw data.

Ethics approval and consent to participate

All tumor sampling and data collection was performed once written informed consent had been obtained, and the study was approved by the Regional Ethical Committee in Uppsala (approval no. Dnr 2007/237).

Patient consent for publication

Not applicable.

Competing interests

The authors declare that they have no competing interests.

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