Patient-Derived Ovarian Tumor Xenografts Recapitulate Human Clinicopathology and Genetic Alterations

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Abstract
Epithelial ovarian cancer (EOC) is the most lethal gynecologic malignancy. On the basis of its histopathology and molecular-genomic changes, ovarian cancer has been divided into subtypes, each with distinct biology and outcome. The aim of this study was to develop a panel of patient-derived EOC xenografts that recapitulate the molecular and biologic heterogeneity of human ovarian cancer. Thirty-four EOC xenografts were successfully established, either subcutaneously or intraperitoneally, in nude mice. The xenografts were histologically similar to the corresponding patient tumor and comprised all the major ovarian cancer subtypes. After orthotopic transplantation in the bursa of the mouse ovary, they disseminate into the organs of the peritoneal cavity and produce ascites, typical of ovarian cancer. Gene expression analysis and mutation status indicated a high degree of similarity with the original patient and discriminate different subsets of xenografts. They were very responsive, responsive, and resistant to cisplatin, resembling the clinical situation in ovarian cancer. This panel of patient-derived EOC xenografts that recapitulate the recently type I and type II classification serves to study the biology of ovarian cancer, identify tumor-specific molecular markers, and develop novel treatment modalities. Cancer Res; 74(23); 6980–90. ©2014 AACR.

Introduction
Epithelial ovarian cancer (EOC) accounts for 90% of ovarian cancer and is the most lethal gynecologic cancer in western countries accounting for more than 13,000 deaths/years (1). Even with optimal treatment, consisting of surgical cytoreduction (debulking) followed by platinum- and taxane-based chemotherapy, the 5-year survival for women with advanced stage disease is only 46% at best (2).

On the basis of histology and IHC analysis, five main subtypes of EOC can be recognized: high-grade serous carcinoma (70%), endometrioid carcinoma (10%), clear cell carcinoma (10%), mucinous carcinoma (3%), and low-grade serous carcinoma (<5%). The different histologic subtypes of EOC are unique entities as indicated by differences in epidemiologic and genetic risk factors, and each has its own distinct biologic behavior (precurrence lesions, pattern of tumor spread, response to chemotherapy and prognosis; refs. 3, 4). In recent years, the remarkable progress in understanding the molecular and cellular biology of ovarian cancer has brought to classify EOC in two main categories based on the pattern of tumor progression and molecular genetic alterations (3, 5–7). Type I EOCs include low-grade serous, low-grade endometrioid, mucinous, and a subset of clear cell carcinomas; they are genetically stable and relatively indolent. Most of the type II are high-grade serous and endometrioid carcinomas with an aggressive clinical course, genetically unstable, and frequently mutated in TP53 (8, 9).

The scarcity of in vivo preclinical models that closely reproduce the complexity and heterogeneity of ovarian cancer limits the development of new therapeutic strategies. Preclinical models of ovarian cancer rely on in vitro stabilized cancer cell lines, on tumor xenografts obtained from in vitro cell lines and, to a lesser extent, on patient-derived tumors (10, 11). Cancer cell lines are reproducible, easy to use, and useful for studying specific mechanisms, but their resemblance to the original tumor and thus their therapeutic predictive value is very limited (12). Two studies describing ovarian cancer patient-derived xenografts have been recently published (13, 14). However, in those studies little characterization was carried out in relation to the recently proposed origin and pathogenesis of ovarian cancer (i.e., type I or type II), that is now basis for novel target therapy. Recently, genetically engineered mouse...
(GEM) models of ovarian cancer have been obtained more closely resembling the origin and initiation of human ovarian cancer; however, we are still a long way from chemotherapeutic trials (8, 15, 16).

New preclinical ovarian cancer models are needed to foster and if possible to tumor-tailor drug development. Preclinical models based on xenografts obtained by engraftment of patient-derived tumor samples directly into animals rely on their limited dissimilarity from the patient’s tumor (17–19). Because ovarian tumors are known for their pathologic and biologic heterogeneity, with considerable differences in histology, genetics, and sensitivity to chemotherapy, the ideal preclinical model of ovarian cancer should consist of tumor xenografts that recapitulate this heterogeneity and preserve the characteristics of the original tumor. We report the establishment of transplantable patient-derived ovarian tumor grafts (EOC xenografts) that retain the original patients’ molecular and biologic features. Our investigation supports the use of this platform to develop novel treatment opportunities for ovarian cancer.

Materials and Methods

Specimen collection and clinical data

One hundred thirty-eight clinical specimens (primary ovarian tumors, metastasis, ascitic fluid) were obtained from patients undergoing surgery for ovarian tumor by laparotomy or paracentesis at the San Gerardo Hospital in Monza, Italy. Tumor specimens were engrafted in nude mice within 24 hours, as described below. The study protocol for tissue collection and clinical information was approved by the Institutional Review Board and patients provided written informed consent authorizing the collection and use of the tissue for study purposes. Detailed information is reported in the Supplementary Data section.

Animals

Female NCr-nu/nu mice obtained from Harlan Laboratories were used when 6 to 8 weeks old. Mice were maintained under specific pathogen-free conditions, housed in isolated ventilated cages, and handled using aseptic procedures. Procedures involving animals and their care were conducted in conformity with institutional guidelines at the IRCCS-Istituto di Ricerche Farmacologiche Mario Negri, in compliance with national and international laws and policies and in line with Guidelines for the Welfare and Use of Animals in Cancer Research (20).

Ovarian carcinoma xenografts

Routinely solid specimens from tumor masses (ovary and omentum) were engrafted subcutaneously (s.c.), whereas ascites were transplanted intraperitoneally (i.p.) as tumor suspension (Table 1). The ability of EOC xenografts to disseminate and metastasize was tested from intraperitoneal and intrabursal transplantations as detailed below.

Subcutis models. Primary tumors and metastases were dissected free of necrotic tissue repeatedly rinsed in HBSS and 2 to 4 mm of tissue was implanted subcutaneously in the flank of nude mice (11). Tumor growth was measured with a Vernier caliper, and weight (mg = mm³) calculated as follows: [length (mm) × width (mm²)]/2.

Intraperitoneal models. Ascites was centrifuged, washed repeatedly, resuspended in HBSS, and implanted intraperitoneally in nude mice at a dose of 10 to 20 × 10⁶ cells. Criteria for growing tumors were abdominal distention and palpable tumor masses in the peritoneal cavity (11). Mice were killed when they presented signs of discomfort (survival), ascites was harvested, and the volume recorded.

Intrabursal transplantation. Ovarian cancer cells from solid tumor enzymatic digestion or ascites (1 × 10⁶ cell suspension) were injected orthotopically under the bursa (i.b.) of the mouse ovary, as previously reported (21) and detailed in Supplementary Data. At necropsy, the ovary image was acquired with a macrodigital imaging system (MacroPATH; Milestone S.r.l); the two diameters were determined, the mean calculated and taken as measure of tumor mass. Ascites was harvested and the volume recorded.

For mice transplanted intraperitoneally or intrabursally, a complete necropsy was done on each mouse by two independent scientists. Tumor dissemination in representative organs of the peritoneal cavity (liver, diaphragm, omentum, pancreas, uterus/ovary, and enlarged lymph nodes) was rated using an arbitrary score for gross tumor dissemination: 0 = not infiltrated; 1 = small masses; 2 = evident masses; 3 = completely invaded, as previously described (22). EOC xenograft samples were snap frozen for genomic analysis, fixed in 10% formalin and embedded in paraffin (FFPE), or frozen in optimal cutting compound (OCT) for histologic and HIC analysis. Established EOC xenografts were transplanted serially in nude mice for further studies (i.e., therapy) and cryopreserved frozen in DMSO at different passages.

Hystopathological analysis

The morphology of patient’s tumor tissues was compared with their corresponding xenografts using paraffin-embedded sections and standard protocols (23), as detailed in Supplementary Data.

Molecular analysis

Mutational analysis. ARID1A (exons 1 to 20), BRAF (exons 11 and 15), CTNNB1 (exon 3), KRAS (exon 2), PIK3CA (exons 10 and 21), PPP2R1A (exons 5 and 6), and TP53 (exons 5 to 9) were sequenced to assess their mutational status. Genomic DNA was obtained from EOC xenografts (N = 34) and patient tumors (N = 23) and analyzed as described in Supplementary Data and Supplementary Table S1.

Gene copy number. The c-Met, c-Myc, PI3Ka, PTEN, FGFR1, ERBB2, RB1, and NFI gene copy number was assessed using the TaqMan Copy Number Assay (Applied Biosystems) using the ABI 7900, Applied Biosystems. RNase P copy number was used as reference gene.

Genome-wide gene expression. EOC xenografts collected from subcutis, abdominal masses, and ascites of mice engrafted with tumors at different passages (from 1 up to 13) and from patient specimens, underwent one-color microarray-based gene expression profiling. To assess the amount of
human- and mouse-derived cells in the xenograft tumors, total RNA was evaluated by species-specific qPCR assays for β-actin, as described in Supplementary Data. Only samples with a human RNA content >75% underwent gene expression analysis with SurePrint G3 Human GE V2 8 × 60 K microarrays (50,599 Biological Features/array; Agilent Technologies), as described in Supplementary Data. Microarray data analysis of nine patient specimens and 62 xenograft samples (representing

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Abbreviations: P, primary tumor; R, relapse. Treatment at relapse: 1CTX; CDDP-EPI; 2DDP; 3PAC; 4DDP/PTX; 5Caelyx; CBDCA/PTX; 6CBDCA; 7CBDCA/PTX; CBDCA/Caelyx; CBDCA/Topotecan; CBDCA/PTX/CBDCA/Gemcitabine; 8CBDCA; NA not available, pluri-treated but specific treatment not known. Ov, ovary; Om, omentum; A, ascites.

aThirty-four ovarian cancers were established as xenografts in nude mice.
bPatient treatment (first-line therapy): CBDCA (carboplatin); EPI (epirubicin); CDDP (cisplatin); CTX (cyclophosphamide); PAC (cisplatin- Adriamycin-cyclophosphamide); PTX (paclitaxel); Beva (bevacizumab); CP (CDDP-cyclophosphamide); TIP (paclitaxel-ifosfamide-cisplatin); PEC (cisplatin-epirubicin-cyclophosphamide); NA (not available).
cPatient response (adjuvant or neoadjuvant therapy): Y, sensitive tumor (relapsing after 12 months); PS, partially sensitive (relapsing in 6–12 months); N, resistant tumors (relapsing in 0–6 months).
dSource of the xenografts.
29 EOC xenograft models) was done with Bioconductor (24), using R statistical language, with MeV version 4.8 (25) and the functional annotation tool available in the DAVID bioinformatic resource (26), as detailed in Supplementary Data.

Microarray data are MIAME compliant and have been deposited into the NCBI (National Center for Biotechnical Information) database Gene Expression Omnibus (GEO accession no.GSE56920).

**Drugs and treatments**

Paclitaxel (PTX, Indena S.p.A.) was dissolved in 50% CremophorEL (Sigma-Aldrich) and 50% ethanol and further diluted with saline before use. Cisplatin (CDDP, Sigma-Aldrich) was dissolved in 0.9% NaCl. They were administered at their optimal dose and schedule as detailed in the Results.

For subcutaneous tumors, mice were randomized to treatment at approximately 150 mg of tumor weight (8–10 mice per group). Treatment efficacy was expressed as best tumor growth inhibition [\%T/C = (median weight of treated tumors/median weight of control tumors) × 100]. Animals were euthanized when primary tumor volume exceeded 15% of body weight.

For intraperitoneal tumors, mice (8 to 10 per group) were randomized to treatment at an advanced stage (i.e., 25% of expected median survival time, MST), regularly monitored, and killed at the first signs of discomfort (the day of death being considered the limit of survival). The increment of life span (ILS) was calculated as [(median survival day of treated group – median survival day of control group)/median survival day of control group] × 100.

Drug activity was interpreted as follows: subcutaneous tumors were considered resistant with T/C < 50%, responsive with 10%<T/C<50%, and very responsive with T/C<10%; intraperitoneal tumors were considered resistant with ILS<40%, responsive with 40%<ILS<100%, and very responsive with ILS ≥ 100%, according to published criteria (27, 28).

**Results**

**Generation of EOC xenografts from patients with ovarian cancer**

We collected 138 tumor samples from patients with ovarian cancer and xenotransplanted them in nude mice; 34 EOC xenografts could be established (25% tumor take) and successfully maintained through multiple rounds of serial transplantation. Approximately another 10% of the specimens engrafted in the mice receded and could not be transplanted further. Twenty-two EOC xenografts were established subcutaneously and twelve were obtained by transplanting ascites into the peritoneal cavity of the mice (Table 1 and Fig. 1). Table 1 and Supplementary Table S2 summarize the clinicopathological data of patients’ tumors from which the EOC xenografts were derived. Seventeen EOC xenografts came from chemotherapy-naive tumors, 17 from patients treated with a chemotherapy, two of them (MNHOC18 and MNHOC164) derived from patients who underwent neoadjuvant treatment. Tumor grade and tumor stage did not seem to predict engraftment in nude mice (Supplementary Table S3A); when only the serous histotype was considered, a correlation between residual tumor and tumor engraftment was found (P = 0.024; Supplementary Table S3B).

Figure 1 depicts the biologic behavior of the EOC xenografts established in nude mice as s.c. (Fig. 1A) or ascites (Fig. 1B–D). The growth rate of the EOC xenografts differed as suggested by the time to reach 1 g (1–15 months for subcutaneously transplanted xenografts; Fig. 1A), by the MST (1–4 months for intraperitoneally transplanted xenografts; Fig. 1B), and by the production of ascites and the level of tumor dissemination in the organs of the peritoneal cavity of the mice (Fig. IC and D).

**Morphologic and pathologic similarity of EOC xenografts to the original patient’s tumor**

To rule out any phenotypic drift that xenografted tumors might have acquired, the histology of tumors grown in mice was compared with the corresponding original patient tumor. In all the cases, the morphology and tissue architecture were similarly preserved (14 representative matched cases are reported in Fig. 2 and Supplementary Fig. S1).

The established xenografts (N = 34) were histologically similar to the patient tumors from which they were derived and 16 were classified as serous, three as mixed serous-endometrioid, five as endometrioid, one as mixed endometrioid-clear cell, two as clear cell, two as mucinous, two as mixed Mullerian tumors (carcinosarcoma), two as undifferentiated, and one as nonclassified.

All tumorgrafts retained positivity for a number of antibodies generally used for the diagnosis of ovarian cancer, including cytokeratin pool and CA-125 (Supplementary Fig. S2). After multiple passages in mice, the positivity for some markers decreased, but the tissue architecture of the tumor of origin was maintained (Supplementary Fig. S3).

**EOC xenografts reproduce the dissemination pattern of human ovarian cancer**

Ovarian carcinoma spreads into the peritoneal cavity with a clinical feature of disseminated carcinomatosis. The ability to recapitulate the dissemination pattern of human EOC was investigated in a subset of EOC xenografts transplanted in the bursa of the mouse ovary (Fig. 1E–G). The growth rate in the ovary seemed not to be influenced by the histotype (Fig. 1E). A diffuse tumor dissemination in the peritoneal cavity similar to that of the EOC xenograft engrafted intraperitoneally and involving liver, pancreas, ovary-uterus (controllateral), lymph nodes, diaphragm, and omentum was observed (Fig. IF and G). The growth rate in the bursa and their dissemination were not necessarily associated, with MNHOC10, MNHOC107, and MNHOC78 showing the greatest dissemination potential. Ascites did not always form, despite the ability of ovarian cancer cells to disseminate through the peritoneal cavity (11/17 EOC xenografts transplanted intrabursally formed ascites; Fig. IF). The ability to form abdominal effusion seemed to depend on the primary tumor source, not on the route of implantation, as almost all the xenografts from patients’ ascites gave rise to ascites in mice (except MNHOC8 and MNHOC84 when transplanted...
Figure 1. Biologic behavior of established EOC xenografts. A, subcutaneous growth. EOC xenografts were engrafted as tumor fragments in the subcutis of nude mice. Growth rate was expressed as time to reach 1 g. B–D, intraperitoneal tumor growth and dissemination. EOC xenografts were engrafted as tumor cell suspension in the peritoneal cavity of nude mice. Mice presenting abdominal distension and/or palpable tumor masses in the peritoneal cavity were killed when showing signs of discomfort. Tumor growth was expressed as MST (B); tumor burden was expressed as volume of ascites (C) and dissemination to the peritoneal organs (D). E–G, intrabursal tumor growth and dissemination. EOC xenografts were engrafted as tumor cell suspension in the bursa of the mouse ovary (1 × 10^6 cell suspension). Tumor burden is expressed as ovarian tumor mass (E), volume of ascites (F), and dissemination to the peritoneal organs (G). (Continued on the following page.)
Patient-Derived Ovarian Carcinoma Xenografts

EOC xenografts retain the molecular features of human ovarian cancer

Molecular characterization was undertaken in the original patient tumor and corresponding xenograft. We investigated the mutational status of genes involved in the pathogenesis of ovarian carcinoma. The detailed mutational spectrum is summarized in Supplementary Table S4. Nonsynonymous TP53 mutations were found in 76% of the EOC xenografts. Interestingly, clear cell, mucinous, and low-grade serous/endometrioid carcinomas harbored wt TP53, whereas the majority of high-grade serous carcinoma harbored a mutated TP53, in line with clinical data (29). No mutations were found in ARID1A, BRAF, CTNNB1, and PPP2R1A genes. MNHOC142 xenograft harbored a mutation in the catalytic subunit of PI3Ka and MNHOC84 xenograft and its corresponding patient tumor had a mutation affecting KRAS (G12A). The EOC xenografts and the corresponding patient tumors whose DNAs were available generally displayed the same mutational status (18/23 = 78%). Exceptions were one case showing a different TP53 missense mutation and four were the mutations that could not be detected in the patient tumor (Supplementary Table S4).

We then checked the copy number of different genes (cMet, cMyc, PI3Ka, PTEN, FGFR1, ERBB2, NF1, and RB1). Despite a similar gene copy number distribution (Supplementary Table S5), EOC xenografts tended to harbor higher gene copy numbers than patient tumors, suggesting greater tumor genomic instability upon in vivo selection; matching patient tumor and xenograft gene copy number is shown in Supplementary Fig. S4.

We carried out genome-wide gene expression analysis of EOC xenografts and patient tumors to evaluate their transcriptomic profiling. Unsupervised hierarchical clustering revealed a high correlation of global gene expression among the EOC regardless of their origin (patient or xenograft); EOC clustered far apart from other cancer types, tumor xenografts, and cell lines of different origin (Fig. 3A), obtained from public repositories and hybridized on the same Agilent platform. The high degree of similarity between EOC xenografts and patient tumors was confirmed by the Pearson correlation coefficient ranging from 0.84 to 0.99 (Supplementary Fig. S5). Two-class paired comparison between patient tumors and their paired EOC xenografts (9 cases) revealed 1,042 differentially expressed transcripts with log fold change greater than 1 or lower than −1 (P value <0.01). Interestingly, the main biologic processes represented in this dataset belonged to the immune response (Supplementary Table S6). Clustering based on these transcripts (Fig. 3B) showed a clear distinction between the patient tumors and the EOC xenografts, with most genes being down-regulated in the latter. Unsupervised hierarchical clustering of the EOC xenografts (based on the expression of all probes; Fig. 4) revealed a high reproducibility of global gene expression profiles among xenografts harvested from different in vivo passages or different site of implantation (i.e., subcutis or orthotopic-abdominal masses and ascitic effusion) of the same patient lesion. Interestingly, 85% (17 out of 20) of the high-grade/high-stage serous and endometrioid carcinomas EOC models clustered together, likewise the majority of clear cells, mucinous, low-grade/low-stage serous, and endometrioid ovarian cancer xenografts (83%; 5 out of 6; Fig. 4).

(Continued.) Tumor histotype are depicted as follows: serous [ ], serous/endometrioid [ ], endometrioid [ ], clear cell [ ], mucinous [ ], mixed Mullerian tumors [ ], undifferentiated [ ], and not classified [ ]. Each experimental group consisted of 5 to 6 mice. Data in A–G are expressed as median with the upper value limit. Histograms in D and G are the sum of the mean score of each organ evaluated as described in Materials and Methods. Each organ is depicted as follows: ovary/uterus [ ], liver [ ], diaphragm [ ], pancreas [ ], omentum [ ], lymph nodes [ ].
Global gene expression of EOC xenografts and patient tumors. Gene expression for 29 EOC xenograft models (62 xenograft samples) and 9 corresponding patient specimens was generated using SurePrint G3 Human GE V2.8 × 60 K microarrays (50,599 probes/array; Agilent Technologies).

Discussion

Collections of patient-derived tumor xenografts have been established for different tumor types (for review see ref. 19), including breast (30, 31), colorectal (32, 33), lung (34), pancreatic cancers (35, 36), and glioblastoma (37, 38). While we were preparing this manuscript, two papers were published on tumor grafts obtained from ovarian tumors, one focusing on a small series of high-grade serous type (13), whereas the other presenting a large tumor bank of ovarian cancer of different histotype (14).

The present work shows that (i) our panel of 34 EOC xenografts comprises all the main subtypes of ovarian carcinoma; (ii) the EOC xenografts in general maintain the key features of the original tumor, including histopathology, gene copy number, and mutational spectrum; (iii) the EOC xenografts reproduce the dissemination into the peritoneal cavity of mice typical of ovarian carcinoma; (iv) comprehensive genome-wide gene expression analysis confirms high-degree of similarity among the xenografts and between the xenografts and patient EOCs, and distinguishes different subsets of
EOC xenografts: (v) our EOC xenograft panel consists of tumors with different sensitivity to chemotherapy from very responsive, responsive, to resistant tumors, well reproducing the response to therapy in ovarian patients.

The EOC xenografts were obtained by transplanting subcutaneously or intraperitoneally in nude mice tumor samples freshly obtained after cytoreductive surgery for abdominal masses or paracentesis of ascites. In these experimental conditions, we obtained 25% xenografts, regardless of the transplantation route, a tumor take in line with earlier studies establishing patient-derived EOC xenografts in athymic nude mice (11, 39, 40). A better take could probably be obtained alternatively, by tumor genomic progression during xenograft establishment.

EOC xenografts showed consistency with the tumors they derived from, on the histopathological and molecular levels. The histotype and tissue architecture were fairly well maintained and, importantly, the xeno-panel reproduced the plethora of human ovarian carcinomas with all the different histotypes: serous, endometrioid, clear cell, mucinous, carcinosarcoma, and undifferentiated, similarly to what was recently reported by Weroha and colleagues (13, 14). Interestingly, we found the engraftment being correlated with residual tumor in high-serous ovarian carcinomas, further supporting residual tumor as a poor prognostic factor in this disease.

The EOC xenografts showed consistency with the patient tumors and their matching xenografts was above 0.88 for pancreatic cancers (41), from 0.78 to 0.95 for NSCLC (42), and lower for melanomas (43). The genes differentially expressed in the patient’s tumors and the xenografts belong to the human immune system and were mainly down-regulated in the xenografts. This is in accordance with previous reports (41, 42) and suggests a loss of human-infiltrating immune cells and a tumor stroma of murine origin. Although gene expression analysis on EOC xenografts and primary patient tumor was available for only nine cases, our findings are in agreement with previous studies showing molecular fidelity of tumor xenograft to its primary patient tumor (14, 30, 31). The overall gene expression profile of the individual EOC xenografts was conserved upon passaging and was not altered in relation to the site of tumor growth, so it appears that the process of engraftment and expansion does not largely change the molecular features of the cancer.

The general preservation of most of the patient tumor mutations in the EOC xenografts suggests that they are a valid model for functional and therapeutic studies. However, the altered mutations and higher gene copy number in four of the 34 xenografts call for caution in interpreting the results in those models. An enriched pattern of mutations in xenografts compared with primary tumors has been described (44, 45), and can be explained by the xenotransplantation selecting cells with a distinct subset of the primary tumor mutation repertoire or, alternatively, by tumor genomic progression during xenograft establishment.

The majority of our EOC xenografts derived from stage III/IV and grade 3 ovarian tumors and diffuse dissemination into the peritoneal cavity was observed when transplanting EOC xenografts intraperitoneally and intrabursally, with tumor masses...
growing and invading the visceral organs. In some cases, this was accompanied with the production of ascites. Our preclinical models mirror the clinical setting, where one third of women with ovarian cancer develop ascites during the course of their disease (46) and this seems not to be related to any specific histologic subtype.

The response to therapy is instrumental to validate the clinical predictive value of patient-derived xenografts. In 11 cases, we could compare the xenograft response with the clinical response to a CDDP-based therapy: it was completely different in two cases, while comparable in the remaining. More than in other tumors, ovarian cancer patient’s outcome depends on other factors, such as the disease dissemination and residual tumor (RT) after surgery (47). Our xenotrials were clearly not influenced by these factors as most of them were done in very different experimental conditions (i.e., “neoadjuvant like” setting with a limited tumor burden). For example, the MNHOCl24 xenograft responded very well; in contrast patient MNHOCl24, albeit achieving stable disease (SD) after adjuvant therapy, had a RT >10 mm that probably influenced her prognosis, as her overall survival was 9 months. Another case is MNHOCl19, a clear cell carcinoma with a RT = NED (nonevidence of disease) that likely determined the patient’s good response; however a poor prognosis with low response to chemotherapy was reported in advanced ovarian clear cell carcinomas (4), as in our corresponding xenograft. Response to platinum in ovarian cancer xenografts (eight cases) correlated well with patient’s clinical response in Weroha and colleagues study (14) and concordance was also reported by Topp and colleagues in their series of 12 high-grade serous ovarian patient-derived xenografts (13). Our panel of EOC xenografts fulfills all the different responses to platinum-based therapy observed in the clinic and this strengthens the possibility of this EOC xeno-bank being instrumental in understanding the mechanism of the resistance to CDDP and in testing novel therapeutic strategies to overcome it.

A dualistic model of ovarian carcinoma pathogenesis has been proposed that classifies them as type I and type II. Type I EOCs are low-grade, relatively indolent, and genetically stable; type II tumors include high-grade serous carcinoma, which is a highly aggressive cancer, genetically unstable, and frequently mutated in TP53 (7). The EOC xenografts we established are of different histologic types and span from grade 1 to 3; the integration of the clinical and preclinical data would allow to classify these xenografts as type I and type II on the basis of their pathologic and molecular characteristics (Fig. 4). This classification might help to a better understanding of ovarian cancers and enable us to tackle specific questions such as tailored therapy of high-grade serous ovarian carcinoma or isolation of tumor-initiating cells from type I and type II tumors (23).

The data reported reinforce the idea that EOC patient-derived xenografts largely retain the phenotypic and genomic characteristics of their original tumor. Our preclinical platform, along with the other two series of patient-derived ovarian cancer xenografts recently obtained (13, 14), offers an instructive framework for molecular target discovery/validation studies, for the identification of biomarker of...
platinum resistance and for testing new investigational therapeutic agents.

Disclosure of Potential Conflicts of Interest
No potential conflicts of interest were disclosed.

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