Patient-derived ovarian tumor xenografts recapitulate human clinicopathology and genetic alterations

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ABSTRACT

Epithelial ovarian cancer (EOC) is the most lethal gynecological 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 biological 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.
INTRODUCTION

Epithelial ovarian cancer (EOC) accounts for 90% of ovarian cancer and is the most lethal gynecological 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 five-year survival for women with advanced stage disease is only 46% at best (2).

On the basis of histology and immunohistochemistry 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 histological subtypes of EOC are unique entities as indicated by differences in epidemiological and genetic risk factors, and each has its own distinct biological behavior (precursion lesions, pattern of tumor spread, response to chemotherapy and prognosis) (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 are based on their limited dissimilarity from the patient’s tumor (17-19). Since ovarian tumors are known for their pathological and biological 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 biological 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 24hr, 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 (Correzzana, Italy) were used when six to eight weeks old. Mice were maintained under specific pathogen-free conditions, housed in isolated vented 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 xenograft
Routinely solid specimens from tumor masses (ovary and omentum) were engrafted subcutaneously (s.c.), while ascites were transplanted intraperitoneally (i.p.) as tumor suspension (Table 1). The ability of EOC-xenografts to disseminate and metastasize was tested from i.p. and intrabursal transplantations as detailed below.

Subcutis models. Primary tumors and metastases were dissected free of necrotic tissue repeatedly rinsed in HBSS and 2-4 mm of tissue was implanted s.c. in the flank of nude mice (11). Tumor growth was measured with a Vernier caliper, and weight (mg = mm³) calculated as follows: (length [mm] × width² [mm²])/2.

Intraperitoneal models. Ascites was centrifuged, washed repeatedly, resuspended in HBSS and implanted i.p in nude mice at a dose of 10-20×10⁶ cells. Criteria for growing tumors were abdominal distension 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 enzymatic digestion from solid tumors 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, Sorisole, Italy); 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 i.p. or i.b. 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 histological and immuno-istochemistry 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 patients 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), *PI3KCA* (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 1.

*Gene copy number.* The c-Met, c-Myc, *PI3Kα*, *PTEN*, *FGFR1*, *ERBB2*, *RB1* and NFI gene copy number was assessed using the TaqMan Copy Number Assay (Applied Biosystems, Monza, Milan) 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 beta actin, as described in Supplementary data. Only samples with a human RNA content > 75% underwent gene expression analysis with SurePrint G3 Human GE V2 8x60K 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 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) x 100]. Animals were euthanized when primary tumor volume exceeded 15% of body weight.

For intraperitoneal tumors, mice were randomized 8-10 per group 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] x 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 ovarian cancer patients

We collected 138 tumor samples from ovarian cancer patients 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 2 summarizes the clinico-pathological data of patients’ tumors from which the EOC-xenografts were derived. Seventeen EOC-xenografts came from chemotherapy-naïve tumors, 17 from patients treated with a chemotherapy, two of them (MNHOC18 and MNHOC164) derived from patients who underwent neo-adjuvant treatment. Tumor grade and tumor stage seem not to predict engraftment in nude mice (Supplementary Table 3A); when only the serous histotype was considered, an inverse correlation between residual tumor and tumor engraftment was found (p=0.024) (Supplementary Table 3B).

Fig. 1 depicts the biological 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 1g (1 to 15 months for s.c transplanted xenografts; Fig. 1A), by the median survival time (MST, 1 to 4 months for i.p. 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. 1C,D).

Morphological and pathological 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. 1).

The established xenografts (N=34) were histologically similar to the patient tumors from which they derived and 16 were classified as serous, 3 as mixed serous-endometrioid, 5 as endometrioid, 1 as mixed endometrioid-clear cell, 2 as clear cell, 2 as mucinous, 2 as mixed-mullarian tumors (carcino-sarcoma), 2 as undifferentiated, and 1 as non classified.

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. 2). After multiple passages in mice the positivity for some markers decreased, but the tissue architecture of the tumor of origin was maintained (Supplementary Fig.3).
**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 i.p. and involving liver, pancreas, ovary-uterus (controlateral), lymph nodes, diaphragm, and omentum was observed (Fig. 1F 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 i.b. formed ascites) (Fig. 1F). 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 i.b.), while most of those from solid tumors did not.

**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 4. Non-synonymous TP53 mutations were found in 76% of the EOC-xenografts. Interestingly, clear cell, mucinous and low grade serous/endometrioid carcinomas harbored wt TP53, while 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 4).

We then checked the copy number of different genes (cMet, cMyc, PI3Kα, PTEN, FGFR1, ERBB2, NF1, and RB1). Despite a similar gene copy number distribution (Supplementary Table 5), 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. 4.
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’s correlation coefficient ranging from 0.84 to 0.99 (Supplementary Fig. 5). Two-class paired comparison between patient tumors and their paired EOC-xenografts (9 cases) revealed 1042 differentially expressed transcripts with log fold change greater that 1 or lower than -1 (p-value <0.01). Interestingly, the main biological processes represented in this data set belonged to the immune response (Supplementary Table 6). 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 endometroid carcinomas EOC models clustered together, likewise the majority of clear cells, mucinous, low grade/low stage serous and endometroid ovarian cancer xenografts (83%; 5 out of 6) (Fig. 4).

**EOC-xenografts and response to platinum-based therapy**

EOC-xenografts were pharmacologically characterized for their response to CDDP and PTX at optimal dose-schedules. CDDP antitumor activity was evaluated, not carboplatin, as previous data from our laboratory have shown similar antitumor activity of the two drugs in xenograft models. A heat-map representation of EOC-xenograft response to therapy is reported in Table 2. The spectrum of response to both drugs was wide, ranging from very good activity, in which tumor regressions could be observed, to no activity. The patient’s response to a platinum-based therapy for 11 cases could be compared to the response of the corresponding xenograft (Table 3). Only two cases showed a completely different response to a platinum-based therapy: the MNHOC124 xenograft was very responsive, while the patient’s clinical response was stable disease, and the MNHOC119 xenograft was resistant to CDDP, while the patient achieved a complete response. In all the other cases activity was similar with three cases that completely matched in patient and the corresponding xenograft (MNHOC8Y, MNHOC 10, MNHOC230).
DISCUSSION

Collections of patient-derived tumor xenografts have been established for different tumor types (for review see (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), while 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 EOC patients, 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 s.c. or i.p. 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 by transplanting ovarian tumor fragments in more immuno-deficient mice (SCID and NOD-SCID-IL2γR,) as recently reported (13,14).

Interestingly, we found the engraftment being inversely 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 tumors they derived from, on the histopathological and molecular levels. The histotype and tissue architecture were fairly well maintained and -importantly- the xeno-panel reproduce the plethora of human ovarian carcinomas with all the different histotypes: serous, endometrioid, clear cell, mucinous, carcinosarcoma and undifferentiated, similarly to what recently reported by Weroha et al (14).

Our genome-wide studies indicate a high degree of similarity among the ovarian cancers that clustered together far apart from clinical tumors of different types, xenograft models and cell types. The correlation coefficients (0.84-0.99) confirmed the high concordance of the clinical samples with the EOC-xenografts. In similar studies the correlation coefficient between the patient tumors and their matching xenografts was above 0.88 for pancreatic cancers (41), from 0.78 to 0.95 for NSCLC...
The genes differentially expressed in the patient’s tumor and the xenografts belong to the human immune system and were mainly down-regulated in the xenografts. This is in accordance with previous reports 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. 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 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 to primary tumors has been described, 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 i.p. and i.b., 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 and this seems not to be related to any specific histological 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 remainings. 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. Our xeno-trials 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 MNHOC124 xenograft responded very well; in contrast patient MNHOC124, albeit achieving stable disease (SD) after adjuvant therapy, had a RT >10 mm that probably influenced her prognosis, as her overall survival was nine months. Another case is MNHOC119, a clear-cell carcinoma with a RT=NED (non evidence of disease) that likely determined the patient’s good response; however a poor prognosis with low response to chemotherapy has been reported in advanced ovarian clear-cell carcinomas.
as in our corresponding xenograft. Response to platinum in ovarian cancer xenografts (eight cases) correlated well with patient’s clinical response in Weroha et al (14), study and concordance was also reported by Topp et al (13) in their series of 12 high-grade serous ovarian patient derived xenografts. Our panel of 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 are highly aggressive cancer genetically unstable and frequently mutated in TP53. The EOC-xenografts we established are of different histological 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 pathological and molecular characteristics (Figure 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 patients 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.

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REFERENCES


Table 1. Characteristics of the ovarian tumors from which EOC-xenografts derived

<table>
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<td>x</td>
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<td>x</td>
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</table>

* Thirtyfour ovarian cancers were established as xenografts in nude mice
° Source of the xenografts; Ov: ovary; om: omentum; A: ascites
* Patient treatment (first line therapy): CBDCA (carboplatin); EPI (epirubicin); CDDP (cisplatin); CTX (cyclophosphamide); PAC (cisplatin-adriamicin-cyclophosphamide); PTX (paclitaxel); Beva (bevacizumab); CP (CDDP-cyclophosphamide); TIP (paclitaxel-isofosfamide-cisplatin); PEC (cisplatin-epirubicin-cyclophosphamide); NA (not available).
§ Patient 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).
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, pluritreated but specific treatment not known.
Table 2. Heat-map of EOC-xenograft response to chemotherapy

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Mice bearing EOC xenografts were randomized as described in Materials and Methods. CDDP was given intravenously (i.v.) 4 mg/kg Q4x3 or 5 mg/kg Q7x3. PTX was given i.v. 20 mg/kg Q4x3 or Q7x3. All these schedules are reported to be effective in different experimental models (22,47).

*refers to s.c. transplanted xenografts - drug activity is expressed as T/C% as detailed in Material and Methods; 
$refers to i.p. transplanted xenografts- drug activity is expressed as ILS% as detailed in Material and Methods, 
very responsive, responsive, resistant.
Table 3. Heat-map of response to therapy in EOC-xenograft and corresponding patient tumor

<table>
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<th>Xenograft Response to CDDP</th>
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<td>EPI/CBDCA/VP16</td>
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<td>CBDCA</td>
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<td>$</td>
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<td></td>
<td>$</td>
</tr>
<tr>
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<tr>
<td>MNHOC88₇</td>
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</table>

°Relapsing tumor.

Patient treatment: CBDCA (carboplatin); EPI (epirubicin); CTX (cyclophosphamide); CDDP (cisplatin); VP16 (etoposide); PTX (paclitaxel). Patients response: □ relapsing after 12 months (sensitive); □ relapsing in 6-12 months (partially sensitive); ■ relapsing in 0-6 months (resistant).

Xenograft response: $refers to s.c. transplanted xenografts - drug activity is expressed as T/C% as detailed in Material and Methods; *refers to i.p. transplanted xenografts- drug activity is expressed as ILS% as detailed in Material and Methods, □ very responsive; □ responsive; ■ resistant.
LEGENDS TO FIGURES

Figure 1. Biological 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 1gr.

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 median survival time (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 mouse ovarian bursa (1x10^6 cell suspension). Tumor burden is expressed as ovarian tumor mass (E), volume of ascites (F) and dissemination to the peritoneal organs (G). 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 5-8 mice. Data in panels A, B, C, D, E, F, G are expressed as median with the upper value limit.

Histograms in panels 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 follow: ovary/uterus (■■■■■■■), liver (■■■■■■■), diaphragm (■■■■■■■), pancreas (■■■■■■■), omentum (■■■■■■■), lymph nodes (■■■■■■■).

Figure 2. Representative histologic characteristics of the original patient tumors and corresponding EOC-xenografts.

Sections from the patient tumor and the corresponding xenograft are shown (H&E). The EOC-xenograft identification number and the original clinical diagnosis are indicated.

Figure 3. 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 8x60K microarrays (50,599 probes/array; Agilent Technologies).

A. Unsupervised hierarchical clustering of EOC together with tumors of different origin. Clustering using Pearson correlation and complete linkage was based on 33,536 common probes across Agilent SurePrint G3 8x60K v1 and v2 platforms. To complement the analysis, external gene expression
datasets were retrieved from ArrayExpress (European Bioinformatic Institute – EBI) or GEO (NCBI database) and from experiments in G.C. laboratory).

**B. Clustering and heat map of EOC.** Two-class paired comparison analysis based on 50,599 probes indicated 1042 differentially expressed transcripts between EOC-xenografts and their corresponding patient tumors (9 cases). Clustering using Pearson correlation and average linkage was based on these transcripts. Data were divided by the mean of logIntensity values from patient tumors. A, ascites; am, abdominal masses; sc, subcutis of individual mice (1, 2, n)

**Figure 4. Transcriptomic profiling of EOC-xenografts.**
Unsupervised hierarchical clustering (based on 50,599 probes; Agilent SurePrint G3 Human GE V2 8x60K microarrays) of 62 EOC-xenograft replicates from different locations (A, ascites; am, abdominal masses; sc, subcutis) of individual mice (1, 2, n) representing 29 EOC-xenograft models. Colored circles depict mutations of the indicated genes (Supplementary Table 4). HGS: high-grade serous; LGS: low-grade serous; MUC: mucinous; LGE: low-grade endometroid; CC: clear cells; TMM: mixed mullerian tumor; HGE: high-grade endometroid.
Figure 1
Figure 2

EOC-XENO

MNHOC124
Serous

MNHOC154
Endometrioid

MNHOC119
Clear Cell

MNHOC164
Mucinous

MNHOC195
Mixed Mullerian Tumor

MNHOC213
Undifferentiated

PATIENT
A

B

Figure 3
Patient-derived ovarian tumor xenografts recapitulate human clinicopathology and genetic alterations

Francesca Ricci, Francesca Bizzaro, Marta Cesca, et al.

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