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# COX-2, c-KIT and HER-2/neu expression in uterine carcinosarcomas: prognostic factors or potential markers for targeted therapies?

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**Abstract**

*Objectives*. Uterine carcinosarcomas are uncommon, highly aggressive neoplasms that frequently recur after surgical treatment and adjuvant chemo-radiotherapy. Patients with recurrent disease respond poorly to salvage chemotherapy and irradiation. New therapeutic options are required for patients with metastatic disease. Clinical evidences showing the effect of a tyrosine kinase inhibitor, STI571, in c-KIT-positive gastrointestinal tumors, the role of COX-inhibitors chemotherapy-associated in colorectal cancer patients and the successful therapeutic possibility of anti-HER2 therapy in metastatic breast carcinoma, have encouraged us to study the expression of c-KIT, COX-2 and HER-2/neu in uterine carcinosarcomas.

*Methods*. We analyzed the expression of COX-2, c-KIT and HER-2/neu in 24 uterine carcinosarcomas and their correlation with clinical outcome. Disease-free interval and actuarial survival rates were the end points of the study.

*Results*. High staining intensity for COX-2 was observed in 8 cases (33.3%). C-KIT was expressed in 4 cases (16.7%) and HER-2/neu in 7 cases (29.2%). Patients with COX-2-positive tumors had a significantly poorer disease-free interval and survival ($P = 0.01$ and $P = 0.05$, respectively). All patients with c-KIT-positive tumors had early stage disease. In spite of this, their survival was not significantly better than that of c-KIT-negative cases. HER-2/neu expression did not show any correlation with clinical outcome.

*Conclusion*. c-KIT, COX-2, and HER-2/neu were expressed in different proportions of uterine carcinosarcomas. COX-2 expression was a strong indicator of unfavorable prognosis. These results warrant further study to evaluate the possible role of a new molecularly targeted cancer therapy with COX-2 inhibitors in patients with uterine carcinosarcomas. The role of c-KIT expression and consequently the hypothetical use of STI571 should be tested in a larger series.


*Keywords:* COX-2; c-KIT; HER-2/neu; Uterine carcinosarcomas; STI571; Nonsteroidal anti-inflammatory drugs; Trastuzumab; Platinum

## Introduction

Uterine carcinosarcomas are uncommon malignancies composed of carcinomatous and sarcomatous elements, in which the sarcomatous component may be either homologous or heterologous [1]. These highly aggressive tumors usually occur in advanced-age women [2]. Although the presence of the heterologous sarcomatous component was previously considered as an indicator of poor prognosis [3], currently tumor stage appears the only independent prognostic indicator of survival for these malignancies [2].

The 5-year survival rates of women with uterine carcinosarcomas is reported to range between 18% and 39% [4].

Total abdominal hysterectomy with bilateral salpingo-oophorectomy and pelvic lymph node dissection is the standard treatment. Adjuvant therapies are generally recommended in cases with extra-uterine spread. The chemo-

*Abbreviation:* COX-2, Cyclooxygenase-2.

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therapeutic regimen for uterine carcinosarcomas includes the same drugs also used for uterine malignant stromal tumors such as leiomyosarcomas or undifferentiated sarcomas [5]. However, the chemotherapies currently proposed are inadequate to control metastatic and unresectable diseases [6].

Although evidence from the literature of the last years is conflicting, recent studies have observed that the behavior of uterine carcinosarcomas depends more on the characteristics of the epithelial component than on the stromal component [7]. In addition, an in vitro study suggests that both the epithelial and the mesenchymal components of uterine carcinosarcomas derive from a common stem cell of epithelial origin [8]. A recent study showing the sensitivity of carcinosarcomas to cisplatin supports the hypothesis that these tumors could be high grade carcinomas with metaplastic sarcomatous elements [9].

The enzyme cyclooxygenase (COX) is responsible for the conversion of arachidonic acid to prostaglandins. The cyclooxigenase-1 (COX-1) is constitutively expressed in a wide range of tissue, while the cyclooxigenase-2 (COX-2) is inducible by pro-inflammatory cytokines and growth factors [10]. COX-2, which has a role in inflammatory processes and in control of cell growth, has also been associated with carcinogenesis [11]. Research over the last decade has established that nonsteroidal anti-inflammatory drugs are effective in both cancer prevention and as adjuvant therapy in the treatment of colorectal cancer [12].

The 145-kDa KIT tyrosine kinase transmembrane receptor is involved in cell signal transduction. The physiologic ligand is the cytokine stem cell factor (SFC), also called mast cell growth factor or Steel factor [13]. Gastrointestinal stromal tumor (GIST) cells express a growth factor receptor with tyrosine kinase activity termed c-kit [14]. Mutation of c-kit, detectable in most GISTs, cause uncontrolled cell proliferation [15]. According to initial clinical studies, the majority of GISTs respond to therapy with a competitive inhibitor of tyrosine kinases, such as STI571 [16].

Human epidermal growth factor receptor (HER-2/neu) is overexpressed in 25–30% of breast cancers, increasing the aggressiveness of the tumor [17]. HER-2/neu has a direct role in the pathogenesis of breast carcinomas [18]. There is evidence to encourage the use of chemotherapy plus a monoclonal antibody against HER-2/neu for metastatic breast cancer that overexpresses HER-2/neu [19].

Altogether, these evidences showing the effect of a tyrosine kinase inhibitor, STI571, in c-KIT-positive gastrointestinal tumors, the role of COX-inhibitors chemotherapy-associated in colorectal cancer patients and the successful therapeutic possibility of anti-HER2 therapy in metastatic breast carcinoma, have encouraged us to study the expression of c-KIT, COX-2 and HER-2/neu in uterine carcinosarcomas.

The aim of this study was to investigate the expression of COX-2, c-KIT and HER-2/neu as prognostic factors in a series of uterine carcinosarcoma patients and to evaluate the possible role of these markers in the selection of patients as putative candidates for new molecularly targeted cancer therapies.

## Patients and methods

### *Case selection*

The files of the Department of Human Pathology and Oncology of the University of Florence were searched from 1980 to 1999 for the diagnosis of uterine carcinosarcomas. The specimens come from 24 patients who had undergone surgery at the Department of Gynecology, Perinatology and Reproductive Medicine of the University of Florence. The mean age of the patients was 68.1 ($\pm$10.1) years of age (range, 41–86 years). All patients were staged retrospectively, according to a modified staging system of the International Federation of Gynecology and Obstetrics (FIGO) for corpus uteri carcinomas and mixed mesodermal tumors [20]. We derived staging information from operative notes and pathology reports. Follow-up information for all patients were available from the gynecologic oncologic database of the Department of Gynecology.

All microscopic slides were reviewed to confirm the histologic diagnosis, without knowledge of the clinical outcome. The diagnosis of uterine carcinosarcoma was based on previously published criteria [1].

### *Treatment and follow-up*

The patients underwent total abdominal hysterectomy, bilateral salpingo-oophorectomy, and debulking in cases with pelvic and/or abdominal spread. Peritoneal cytology and peritoneal biopsies were performed to rule out extrauterine disease when indicated.

After surgery, 6 patients (25%) received adjuvant chemotherapy. Because the study encompasses a relatively large period of time, different drug regimens were used, including cisplatin, cyclophosphamide and epirubicin in more recent years. The chemotherapy regimen more frequently used included: cisplatin (60 mg/m$^2$ body-surface area) in combination with cyclophosphamide (600 mg/m$^2$ body-surface area) and epirubicin (60 mg/m$^2$ body-surface area) every 21 days for six cycles. Whole pelvis adjuvant external irradiation (56 Gy) was given in 3 patients (12.5%).

All patients, at the time of diagnosis, were free of distant metastases. During the follow-up period, patients were seen regularly every 4 months in the first 2 years, every 6 months until the fifth year and once a year thereafter. Each time, a pelvic examination and a Pap smear from the vaginal vault were done. An abdomino-pelvic ultrasound scan was performed every 6 months, chest X-ray and CT scan were performed every year. Local recurrences, distant metastases and deaths were noted. The median follow-up time for the surviving patients was 66 months (range 23–114 months).

*Tissue specimens and immunohistochemistry*

The specimens were obtained by surgical resection in all cases and fixed in 10% formalin before being processed in paraffin. Hematoxylin–eosin stained sections from each histological specimen were reviewed by two pathologists to confirm the histological diagnosis. In addition, for each case, presence of tumor necrosis, depth of myometrial invasion, vessel infiltration, pattern of tumor growth and presence of heterologous component were evaluated. We considered tumor necrosis present when coagulative tumor cell necrosis was found [21]. Vessel infiltration was considered present when embolic tumor cells were documented in blood and/or lymphatic vessels of the tumor area. No quantitative criteria was adopted. The infiltrative pattern of growth was characterized by poorly demarcated margins, with glandular and mesenchymal elements irregularly scattered through the myometrium.

A representative section from each lesion was selected for immunohistochemical analysis. All sections were deparaffinized in Bio-Clear (Bio-Optica, Mi, Italy) and hydrated with grade ethanol concentration and finally with distilled water.

Antigen retrieval was routinely performed by microwave pretreatment (Microwave MicroMed T/T Mega, Milestone, Bg, Italy) in TEC buffer (TRIS-EDTA-Citrate), pH 7.8, for 30 min. We evaluated the immunohistochemical staining of uterine carcinosarcomas as follows.

Concerning COX-2, after blocking nonspecific antigen with normal horse serum (UltraVision, Lab-Vision, Fremont, CA), the sections were incubated overnight at 4°C with primary goat polyclonal anti-Cox-2 (sc-1745, Santa Cruz Biotechnology, Inc., Santa Cruz, CA) at 1:50 dilution. Staining was achieved using a biotin-conjugated anti-goat secondary antibody (Goat immunoglobulins, DAKO, A/S Denmark) and streptavidin–peroxidase (UltraVision). The bound antibodies were visualized using 3.3′ diaminobenzidine (BioGenex, San Ramon, CA) as chromogen. Nuclei were slightly counterstained with Mayer's hematoxylin. A section containing a COX-2 strongly positive colonic carcinoma, served as the positive control with each run. A negative control sample was included with each run by omitting the primary antibodies and yielded no signal.

Concerning c-KIT, we used a commercial rabbit polyclonal anti-c-KIT (Ventana Medical Systems, Tucson, AZ) raised against a peptide sequence mapping with the carboxy terminal domain of c-Kit with molecular weight of 145 kDa of human origin. All tissue sections were then placed on the Ventana NexES automated stainer using iVIEW DAB Detection Kit as a revelation system. The primary antibody to c-KIT was placed on the slides and incubated for 32 min according to the protocol suggested by Immunostainer Ventana NexES. When the staining run was complete, the tissue sections were removed from the stainer, counterstained with hematoxylin, dehydrated and mounted with Permount. A section containing a c-KIT strongly positive GIST served as the positive external control with each run. Mast cells within the tumor served as positive internal control. Each tumor was also stained with Giemsa to identify mast cells for comparison with the c-KIT results. A negative control sample was included with each run by omitting the primary antibodies and yielded no signal.

Concerning HER-2/neu, the samples were analyzed using the HercepTest TM Kit (DakoCytomation, Glostrup, Denmark A/S). All sections were subjected to an antigen retrieval technique by immersing the slides in thermostate bath at 97°C containing preheated Dako Epitope Retrieval Solution (0.1 mol/L citrate buffer, pH 6.0) for 40 min followed by cooling for 20 min r.t. After incubation with the polyclonal antibody anti-human HER-2 protein for 30 min, a ready-to-use Visualization Reagent based on dextran technology (dextran polymer conjugated with horseradish peroxidase and goat anti-rabbit immunoglobulins) was applied for 30 min. Bound antibody was detected using 3.3′ diaminobenzidine as chromogen. The sections were weakly counterstained with the Mayer's hematoxylin. In each staining run, a control slide containing three formalin-fixed, paraffin-embedded human breast cancer cell lines with staining intensity scores of 0, 1+ and 3+ was added. A negative control sample was included with each run by omitting the primary antibodies and yielded no signal.

*Evaluation of immunohistochemical staining*

Positive c-KIT stains appeared as cytoplasmic and/or membranous stains. The percentage of positive cells, and the degree and cellular distribution of the staining were scored. Only cases showing at least 20% moderate to strong membranous and/or cytoplasmic positivity were scored as positive, as previously described [22]. We evaluated both epithelial and mesenchymal component. No background or nonspecific staining of endometrial tissue and adjacent myometrial parenchyma was encountered, other than positivity in mast cells as expected.

The tumor sections showing brown staining of the antibody specific COX-2 of cytoplasm were scored as positive. The proportion of immunostained cells was scored at low magnification (5× objective lens) by evaluating the entire tumor area. When the tumor area with positive immunostaining was >10% of the total tumor area, the case was scored as positive. The intensity of staining was also evaluated subjectively using a range from 0 (none) to 1 (faint) to 2 (strong). Cases in which the intensity of staining was scored <2 were considered negative, as previously described [23]. We evaluated both epithelial and mesenchymal component.

HER-2/neu was evaluated as positive only when in the tumor sections the membranous staining was scored, with

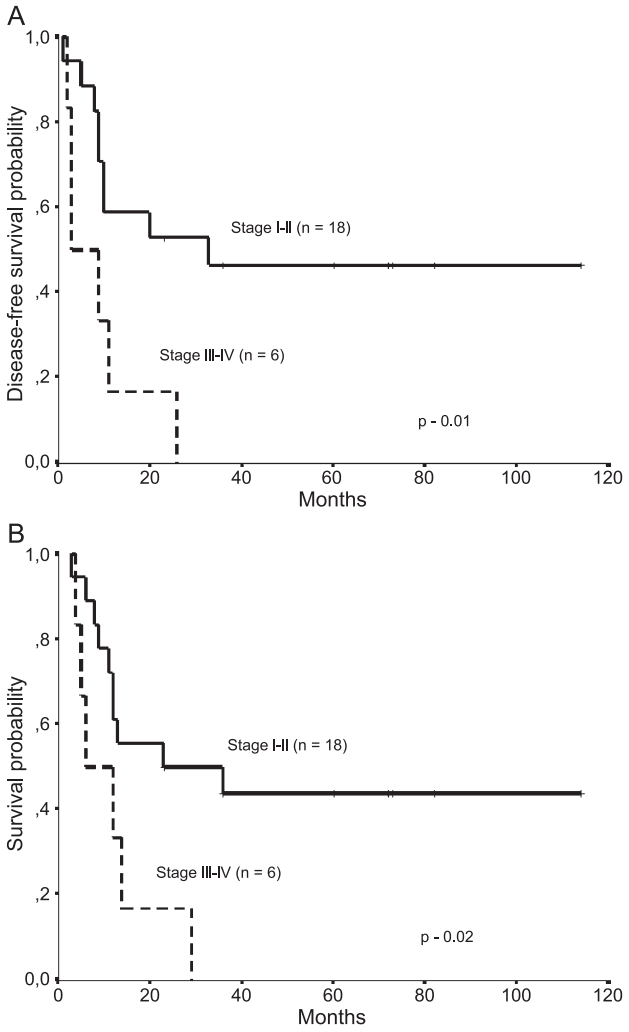


Fig. 1. Kaplan–Meier estimates for disease-free survival (A) and disease-specific survival (B) according to FIGO stage (I–II) vs. (III–IV) in 24 patients with uterine carcinosarcoma.

cytoplasmic staining being ignored. A four-point scale was used: 0 if there was no membranous staining; 1 if there was weak membrane staining in at least 10% of cells; 2 if there was moderate membrane staining in at least 10% of cells; 3 if there was strong membrane staining in at least 10% of cells. We have considered as positive only the cases that had weak-to-moderate staining of the entire tumor-cell membrane for HER-2/neu (referred to as a score of 2+) or more than moderate staining (referred to as a score of 3+) in more than 10% of tumor cells, as previously described [19]. We evaluated both epithelial and mesenchymal component.

To assess the interobserver variability in the evaluation of immunohistochemical stainings, the evaluations of one author (M.R.R.) were compared with those of one of the other authors (G.L.T.). Initially, the slides were evaluated independently, and those graded diversely were subsequently reevaluated by the two authors together and agreement was found. The immunohistochemical analysis was evaluated in a blinded fashion with regard to the clinical data.

### *Statistical analysis*

Data analysis was performed using the SPSS Version 8.0 (Chicago, IL) statistical package. Frequency data were evaluated by the Fisher's exact test or the Chi-square test. Disease-free interval and cause-specific survival rates were calculated according to the Kaplan–Meier method and differences were evaluated using the log rank test. A $P$ value $\leq 0.05$ was considered to be statistically significant.

## Results

Areas of tumor necrosis were observed in 11 cases (45.8%). Ten cases (41.7%) showed vessels infiltration. Myometrial invasion was >50% in 11 cases (45.8%). In 14 cases (58.3%), the tumor showed an infiltrating pattern of growth. An heterologous component was observed in 10 patients (41.7%), in particular, 3 cases (12.5%) had rhabdomyosarcomatous differentiation, 5 cases (20.8%)

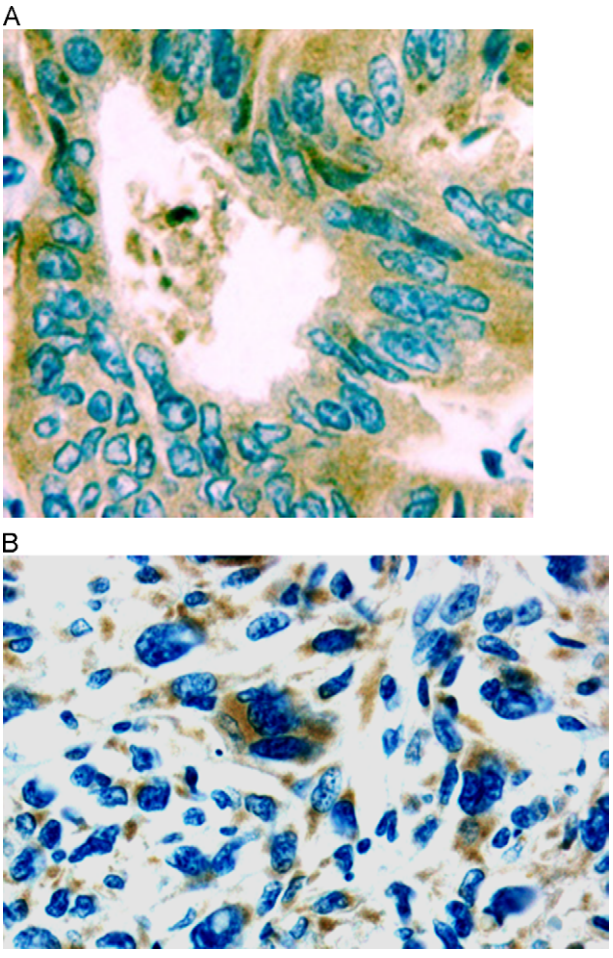


Fig. 2. COX-2-positive cytoplasmic immunostaining in the epithelial (A) and in the mesenchymal component (B) of uterine carcinosarcoma. Original magnification ×200.

Table 1
Distribution of COX-2, c-KIT and HER-2/neu positivity, according to clinico-pathological factors in 24 patients with uterine carcinosarcoma

| Characteristic | Total | COX-2+ | c-KIT+ | HER-2/neu+ |
|---|---|---|---|---|
| | | No. (%) | No. (%) | No. (%) |
| Necrosis | | | | |
| Present | 11 | 4 (36.3) | 1 (9.0) | 4 (36.3) |
| Absent | 13 | 4 (30.7) | 3 (23.0) | 3 (23.0) |
| Vessels infiltration | | | | |
| Present | 10 | 7 (70.0)* | 1 (10.0) | 4 (40.0) |
| Absent | 14 | 1 (7.1)* | 3 (21.4) | 3 (21.4) |
| Myometrial invasion | | | | |
| >50% | 11 | 6 (54.5) | 1 (9.0) | 2 (18.0) |
| ≤50% | 13 | 2 (15.3) | 3 (23.0) | 5 (38.4) |
| Pattern of growth | | | | |
| Infiltrating | 14 | 5 (35.7) | 1 (7.1) | 6 (42.8) |
| Pushing | 10 | 3 (30.0) | 3 (30.0) | 1 (10.0) |
| Mesenchymal component | | | | |
| Heterologous | 10 | 3 (30.0) | 1 (10.0) | 5 (50.0) |
| Homologous | 14 | 5 (35.7) | 3 (21.4) | 2 (14.2) |
| Age | | | | |
| <60 years | 4 | 1 (25.0) | – (–) | 1 (25.0) |
| ≥60 years | 20 | 7 (35.0) | 4 (20.0) | 6 (30.0) |
| FIGO stage | | | | |
| I–II | 18 | 5 (27.7) | 4 (22.2) | 6 (33.3) |
| III–IV | 6 | 3 (50.0) | – (–) | 1 (16.6) |

* $P = 0.002$, Fisher's exact test.

had chondrosarcomatous differentiation, and 2 cases (8.3%) had osteosarcomatous differentiation.

Distribution by FIGO stage was as follows: stage I, 17 patients (70.8%); stage II, 1 patient (4.1%); stage III, 4 patients (16.6%); stage IV, 2 patients (8.3%). For the purpose of survival evaluation, patients with disease limited to the uterus (stage I–II) were compared with those with extrauterine tumor spread (stage III–IV).

During the follow-up period, 15 patients (62.5%) recurred and 16 patients (66.7%) died of disease.

Among the conventional clinico-pathological parameters analyzed, only tumor stage (I–II vs. III–IV) was a significant prognostic factor for disease-free interval and survival ($P = 0.01$ and $P = 0.02$, respectively) (Fig. 1).

### *COX-2, c-KIT and HER-2/neu expression*

Positive staining for COX-2 was observed in 8 cases (33.3%). In the cases with positive immunostaining, the epithelial and mesenchymal components were stained, although in the mesenchymal component the percentage of stained cells was lower, (e.g., epithelial component 50% of the cells stained; mesenchymal component 30% of the stained cells) (Fig. 2). Distribution of COX-2 positivity was not significantly different according to FIGO stage (Fisher's exact test), with COX-2 positive tumors being 5/18 in stage I–II disease and 3/6 in stage III–IV disease, respectively. Conversely, COX-2 positivity was significantly more frequent in tumors showing vessels infiltration ($P = 0.002$, Fisher's exact test) (Table 1). Patients with COX-2 expression showed significantly poorer disease-free interval and survival ($P = 0.01$ and $P = 0.05$, respectively) (Fig. 3).

c-KIT expression was observed In 4 cases (16.7%). Positive cases displayed immunostaining in both glandular and mesenchymal component (Fig. 4). The ratio of epithelial stained cells was slightly higher than in the mesenchymal component. Also, the intensity of immunostaining tended to be stronger in the epithelial component. All the 4 patients with distinct c-KIT immunoreactivity had early stage disease (FIGO I–II) (difference not significant, Fisher's exact test). These four patients fared better than those with c-Kit-negative tumors, but the difference was not statistically significant (Fig. 5).

HER-2/neu expression was observed in 7 cases (29.2%). Again, the epithelial and mesenchymal component were both stained in positive cases, with a tendency towards more intense immunostaining in glandular pattern (score 3+) than in the mesenchymal areas (score 2+) (Fig. 6). Of the seven patients with distinct HER-2/neu positive staining, six had

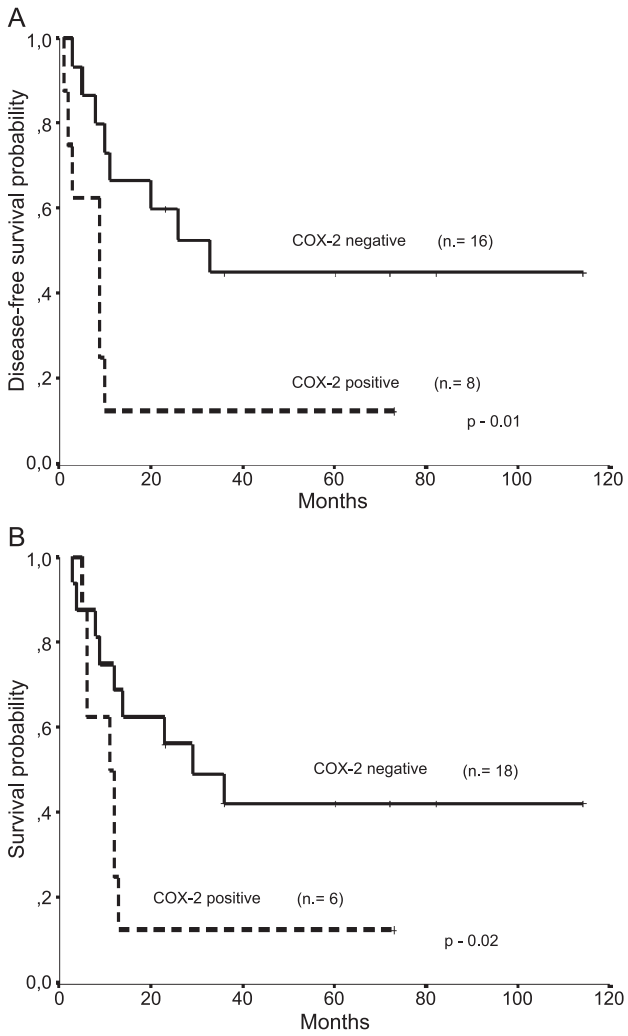


Fig. 3. Kaplan–Meier estimates for disease-free survival (A) and disease-specific survival (B) according to COX-2 expression in 24 patients with uterine carcinosarcoma.

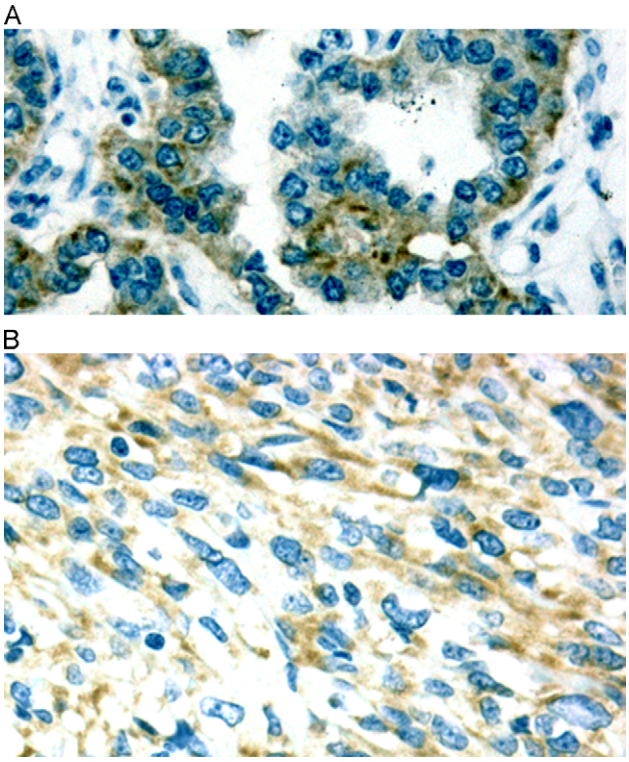


Fig. 4. c-KIT-positive cytoplasmic immunostaining in the epithelial (A) and in the mesenchymal component (B) of uterine carcinosarcoma. Original magnification ×200.

early stage disease and one had advanced stage disease (FIGO III–IV). The distribution of HER-2/neu positivity according to FIGO stage was not statistically significant (Fisher's exact test).

Disease free interval and survival rates were not significantly different according to HER-2/neu expression (Fig. 7).

The correlations between clinico-pathological factors and immunohistochemical data concerning COX-2, c-KIT, and HER-2/neu expression are summarized in Table 1.

## Discussion

This study tested the expression of potential markers for innovative molecularly targeted therapies such as COX-2, c-KIT and HER-2/neu in uterine carcinosarcomas. We found that COX-2, c-KIT and HER-2/neu are expressed in different proportions of patients with uterine carcinosarcomas. Tumor stage was the only traditional clinical-pathological factor with prognostic value in this series of 24 uterine carcinosarcomas, in agreement with previously published data [2].

COX-2 overexpression was noted in one third of the cases and was significantly correlated with vessels infiltration by tumor cells. Patients with COX-2 overexpression were found in both early and advanced stage disease. These patients fared significantly worse than those without COX-2 overexpression (Fig. 3). To the best of our knowledge, this is the first study that analyzes the prognostic role of COX-2 expression in uterine carcinosarcomas. There is only one previous study by Matsumoto et al. [24], which tested COX-2 expression in eight uterine carcinosarcomas, reporting a positive immunostaining in 88.9% of the cases, but no information nor on vessels infiltration neither on the clinical outcome was given. The different cutoff value for COX-2 positivity used by them can explain the higher positivity rate in that study. Overexpression of COX-2 has been reported in a number of other malignancies, including endometrial [25], ovarian [26] and cervical carcinomas [27]. In agreement with these previous studies on different gynecological tumors, we documented a more aggressive clinical behavior for the COX-2-positive carcinosarcomas, independently of tumor stage. Ferrandina et al. reported that in cervical [27] and ovarian carcinomas [23], COX-2 overexpression correlates with unfavorable clinical outcome and with platinum-based chemotherapy resistance. Unfortunately, only a minority of the patients (25%) in the current study

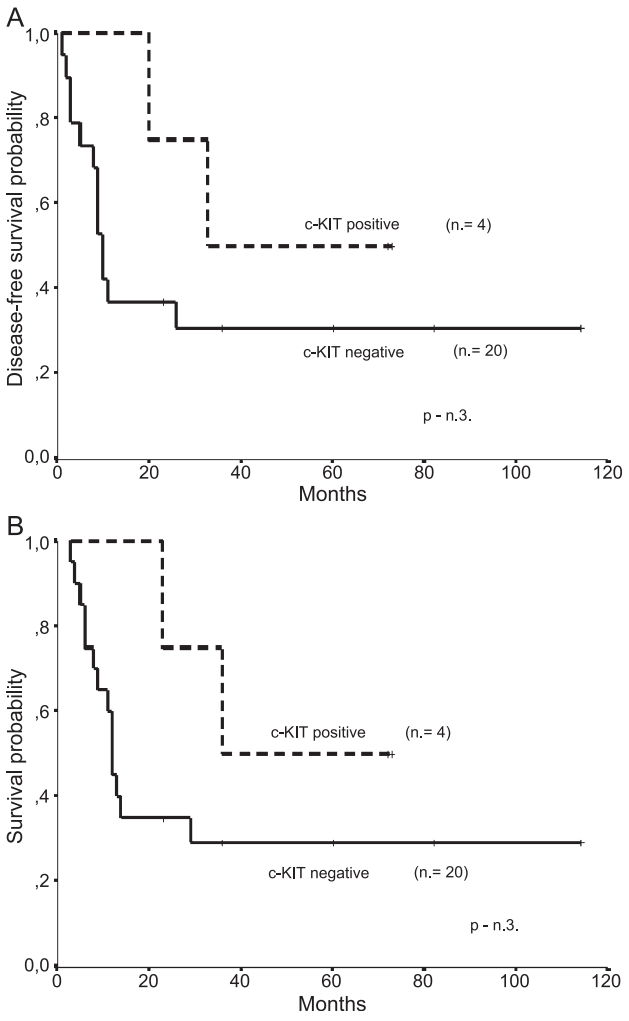


Fig. 5. Kaplan–Meier estimates for disease-free survival (A) and disease-specific survival (B) according to c-KIT expression in 24 patients with uterine carcinosarcoma.

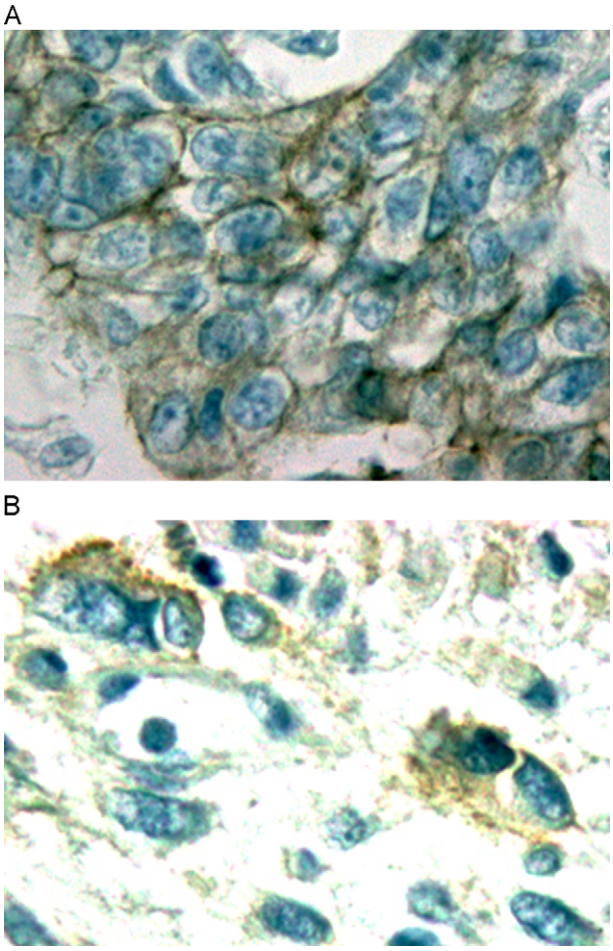


Fig. 6. HER-2/neu-positive membranous immunostaining in the epithelial (score 3+) (A) and in the mesenchymal component (B) of uterine carcinosarcoma (score 2+). Original magnification ×200.

received adjuvant chemotherapy and among them only three had regimens including platinum. This prevents us to test any relationship between COX-2 overexpression and platinum chemosensitivity in the current series.

At present, the use of platinum-based chemotherapy is under evaluation also in patients with uterine carcinosarcoma [28]. Therefore, information on platinum resistance could be clinically useful in these tumors. Furthermore, it has been shown that molecularly targeted therapies, such as COX-2 inhibitors, are indicated for colorectal cancer patients with COX-2 overexpression, in combination with traditional chemotherapy [29]. It has been suggested also that selective COX-2 inhibitors may have a role in the treatment of subgroup of patients with cervical and ovarian carcinomas [23,27]. In fact, COX-2 inhibitors have been demonstrated to enhance the cytotoxicity in vitro of several chemotherapeutic agents, including platinum [30]. Similarly, we could hypothesize that nonsteroidal anti-inflammatory drugs may have synergistic effects with platinum-based chemotherapy also in uterine carcinosarcoma patients who express COX-2. Our data, demonstrating overexpression of COX-2 in 33% of uterine carcinosarcomas, provide a rationale basis for further studies, to test the possible role of COX-2 inhibitors in the treatment of these aggressive malignancies.

Concerning c-KIT expression, we found positive immunostaining in 16.7% of the patients. All of them had early stage disease and did not display a significant correlation with clinical outcome (Fig. 5). However, it should be noted that c-KIT expression in GISTs is associated with unfavorable prognosis. Thus, this might explain why the outcome of the c-KIT-positive cases in this series was poorer than expected. In fact, the c-KIT-positive (early stage) cases did not fare significantly better of the c-KIT-negative group, which also included advanced stage cases. The finding of a relatively low percentage of-positive c-KIT immunostaining in the current series is in agreement with the study of Klein and Kurman [31], who reported that c-KIT expression was rare in uterine carcinosarcoma (0 of 6) and endometrial stromal sarcoma (1 of 12). Conversely, Winter et al. [32] and Sawada et al. [33] reported higher incidences of c-KIT positivity among 21 and 16 patients, respectively. The low

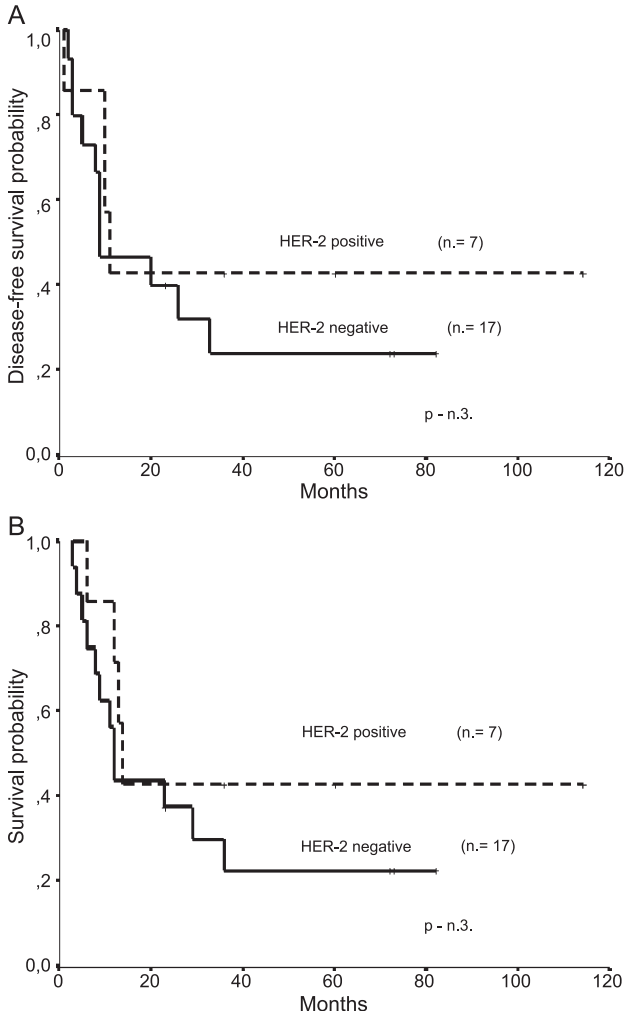


Fig. 7. Kaplan–Meier estimates for disease-free survival (A) and disease-specific survival (B) according to HER-2/neu expression in 24 patients with uterine carcinosarcoma.

number of cases in these series and differences in the type of c-KIT antibody used may account for this variability. In the current study, the low number of patients with c-KIT-positive tumors prevents any definitive statement concerning the prognostic significance of c-KIT expression in uterine carcinosarcomas. It is known that STI571, a competitive inhibitor of tyrosine kinases, has been shown to be of value in the treatment of hematologic malignances [34] and in metastatic GISTs that express immunohistochemicaly the c-KIT. Among GIST patients, the cases that respond better to STI571 are those with a mutation in exon 11 of the c-kit gene [35]. We provided further evidence that uterine carcinosarcomas express c-KIT. However, the possibility to use this marker to select patients for innovative targeted therapies using STI571 need to be demonstrated. The immunohistochemical overexpression of c-KIT has been reported also in other malignances such as small cell lung cancer [36] and uterine leiomyosarcomas [37]. Our previous study on c-KIT expression in uterine leiomyosarcomas failed to detect any mutation in exon 11 of the c-kit gene [37]. Therefore, the site of c-kit gene mutation and whether uterine carcinosarcoma patients may respond to KIT inhibition remain to be established and these are questions for future studies.

Concerning HER-2/neu expression, we found a positive immunostaining in approximately one third of our series. Most of the HER-2/neu-positive tumors were found among early stage disease patients, but this difference was not significant. No differences in disease-free and disease-specific survival rates were observed according to HER-2/neu expression (Fig. 7). To our knowledge, the current study is the first to evaluate the prognostic role of HER-2/neu in uterine carcinosarcomas. The only previous study that investigated HER-2/neu in this malignancy reported a positive immunostaining in 9 of 16 cases, but did not provide information on survival [33]. It is well known that approximately 25% of breast cancers overexpress HER-2/neu [38]. Anti-HER-2 (trastuzumab)-based combination therapy is currently used for patients with metastatic breast carcinoma that overexpress HER-2/neu and several studies demonstrated a survival advantage [39]. Although we did not found any prognostic role for HER-2/neu immunostaining in our series, we could not exclude a beneficial effect of trastuzumab in the treatment of recurrent or metastatic carcinosarcomas with HER-2/neu overexpression. Recently, a role for anti-HER-2 therapy has been hypothesized also in 5% of the patients with transitional cell carcinoma of the urinary bladder, which overexpress HER-2/neu [40]. Therefore, our results prompt further studies to clarify the issue of possible clinical utility of anti-HER2 treatment in metastatic uterine carcinosarcomas.

In conclusion, the most important outcome of the current study was the observation, for the first time, of a prognostic significance for COX-2 expression in uterine carcinosarcomas. The assessment of this biological tumor feature gave by far more information on tumor aggressiveness than classic histopathological parameters, such as presence of heterologous component or depth of myometrial infiltration. Hence, we believe that the relatively easy immunohistochemical evaluation of COX-2 could improve the identification of patients with more aggressive disease and possibly, candidate them to more individualized treatments. Because this is the first report of the prognostic role of COX-2 expression in uterine carcinosarcoma, our results should be interpreted with caution and validated in a larger series.

Another relevant outcome of this study was the documentation of the expression of COX-2, c-KIT and HER-2/neu in uterine carcinosarcomas, because we know that these same gene products are molecular targets for innovative treatments, such as COX-2 inhibitors, STI571 and trastuzumab, which had been already proven beneficial in colorectal cancer, GIST and breast carcinoma, respectively [16,41,42].

Indeed, our findings warrants further studies to verify if these specific molecularly targeted treatments may have a role in the treatment of uterine carcinosarcoma patients as well.

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