

Linköping University Medical Dissertations  
No. 583

Sarcoma  
of the female genital tract  
Histopathology, DNA cytometry,  
p53 and mdm-2 analysis related to prognosis

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Linköping 1999

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ISBN 91-7219-325-5

ISSN 0345-0082

Printed in Sweden by triva-tryck ab, Linköping 1999, Sweden



*Nothing comes from doing nothing  
(De nihilo nihil)*

Lucretius 95?-55 DC

*To my family*

## Abstract

Sarcomas of the female genital tract are rare tumors and account for less than 5% of gynecologic malignancies. Traditionally, gynecologic sarcomas have been divided into different tumor types according to their histopathological features. The most common are leiomyosarcoma (LMS), malignant mixed Müllerian tumors (MMMT), endometrial stromal sarcoma (ESS) and (Müllerian) adenosarcoma. The different tumor types are highly aggressive with early lymphatic and/or hematogenous spread. Treatment is difficult and it is believed that sarcomas have a low radio- and chemosensitivity, and the mainstay in treatment is surgical removal of the tumor. The most important prognostic feature has been tumor stage. Nevertheless, there are some early-stage tumors that run a biological course different from that expected and additional prognostic factors indicating high-risk tumors are desirable.

The study cohort consists of 49 uterine LMS, 44 uterine MMMTs, 17 uterine ESS, 11 uterine adenosarcomas and 26 ovarian MMMTs. The tumors were analyzed in a retrospective manner for DNA ploidy, S-phase fraction (SPF), p53 and mdm-2 expression, as well as traditional clinical and pathological prognostic factors, such as tumor stage, grade, atypia and mitotic index.

Of the 49 LMS, 36 (73%) were non-diploid and 13 (27%) were p53-positive. Among the 44 uterine MMMTs, 30 (68%) were non-diploid and 27 (61%) had an SPF>10%. Twenty-seven (61%) overexpressed p53 and 11 (25%) were mdm-2 positive. Furthermore, 40 (91%) of the uterine MMMTs had a high mitotic count and 42 (95%) had high grade cytologic atypia. All low-grade ESS were DNA diploid and had a low SPF. Among the four high-grade ESS, three (75%) were DNA aneuploid and three (75%) were p53-positive. Among 11 adenosarcomas, eight (73%) were non-diploid. All ovarian MMMT were non-diploid and all but two had an SPF>10%. 19 (73%) ovarian MMMTs were p53-positive.

The 5-year survival rate was 33% for LMS, 38% for uterine MMMT, 57% for ESS, 69% for adenosarcoma and 30% for ovarian MMMT.

Thirty-five (71%) patients with LMS died of disease and two of intercurrent disease. Stage was found to be the most important factor for survival ( $p=0.007$ ); in addition DNA ploidy ( $p=0.045$ ) and SPF ( $p=0.041$ ) had prognostic significance.

Twenty-seven (61%) patients with uterine MMMT died of disease and six (14%) died of intercurrent disease. Stage was the only prognostic factor for survival.

Nine (53%) patients with ESS died of disease. There was a significant correlation of survival to tumor grade ( $p=0.007$ ), DNA ploidy ( $p=0.026$ ), SPF ( $p=0.048$ ) and stage ( $p=0.026$ ).

Of the 11 patients with adenosarcoma, four (36%) patients died of disease and three (27%) patients died of intercurrent disease. There were no variables that correlated with survival.

Eighteen (69%) patients with ovarian MMMT died of disease and two (8%) patients died of intercurrent disease. In a multivariate analysis, only stage reached independent prognostic significance for survival ( $p=0.023$ ).

In summary, stage represents the most important prognostic factor for survival for uterine and ovarian sarcomas. DNA flow cytometry is useful in gaining additional prognostic information for LMS and ESS. P53- and mdm-2 overexpression had no prognostic value for survival rate. Most of the MMMT overexpressed p53 and were non-diploid. Treatment of sarcomatous neoplasms is difficult and the mainstay remains surgical removal of the tumor. For patients with early stage sarcoma there was a high recurrence rate, which suggests that a large proportion of patients may have systemic micrometastatic disease at the time of diagnosis. Recurrent and metastatic uterine sarcoma remains an incurable disease, and treatment must be considered palliative.

## **Preface**

This thesis is based on the following papers, which will be referred to in the text by their Roman numerals:

### **I.**

Blom R, Guerrieri C, Stål O, Malmström H, Simonsen E. Leiomyosarcoma of the uterus: A clinicopathologic, DNA flow cytometry, p53 and mdm-2 analysis of 49 cases. *Gynecol Oncol* 68:54-61, 1998.

### **II.**

Blom R, Guerrieri C, Stål O, Malmström H, Sullivan S, Simonsen E. Malignant mixed Müllerian tumors of the uterus: A clinicopathologic, DNA flow cytometric, p53 and mdm-2 analysis of 44 cases. *Gynecol Oncol* 68:18-24, 1998.

### **III.**

Blom R, Malmström H, Guerrieri C. Endometrial stromal sarcoma of the uterus: A clinicopathologic, DNA flow cytometric, p53 and mdm-2 analysis of 17 cases. Accepted for publication in *International Journal of Gynecological Cancer*.

### **IV.**

Blom R, Guerrieri C. Adenosarcoma of the uterus: A clinicopathologic, DNA flow cytometric, p53 and mdm-2 analysis of 11 cases. Accepted for publication in *International Journal of Gynecological Cancer*.

### **V.**

Guerrieri C, Blom R, Boeryd B, Simonsen E. Malignant mixed Müllerian tumors of the ovary: A clinicopathologic, DNA ploidy and p53 study of 26 cases. Submitted *Cancer*.

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## Abbreviations

AL	Atypical leiomyoma
AS	Adenosarcoma
AS-SO	Adenosarcoma with sarcomatous overgrowth
BL	Benign leiomyoma
BSO	Bilateral salpingo-oophorectomy
CR	Complete response
CV	Coefficient of variation
CY-VA-DIC	Cyclophosphamide, Vincristine, Adriamycin, Dacarbazine
DOD	Died of disease
DIC	Died of intercurrent disease
ED	Evidence of disease
EL	Exploratory laparotomy
ESS	Endometrial stromal sarcoma
FCM	Flow cytometry
FIGO	International Federation of Gynecology and Obstetrics
Gy	Gray
H&E	Hematoxylin & Eosin
hpf	High power fields
LIMA	Leiomyoma with increased mitotic activity
LMS	Leiomyosarcoma
mdm-2	Murine double minute 2
mf	Mitotic figures
MI	Mitotic index
MMMT	Malignant mixed Müllerian tumor
MV	Mega volt
NED	No evidence of disease
OT	Omentectomy
PAP	Peroxidase anti-peroxidase
PCR	Polymerase chain reaction
PR	Partial response
RaH	Radium hour
STLMP	Smooth muscle tumor of low malignant potential
SH	Subtotal hysterectomy
SPF	S-phase fraction
SSD	Source skin distance
TAH	Total abdominal hysterectomy
USO	Unilateral salpingo-oophorectomy
VAC	Vincristine, Actinomycin D and Cyclophosphamide
XRT	External radiation treatment

## Introduction

Uterine smooth muscles tumors are common neoplasms of the uterus. Many women over the age of 35 years develop uterine leiomyomas, the vast majority of which are benign despite symptomatic bleeding. On the other hand, sarcomas of the female genital tract are rare. Uterine sarcomas account for approximately 3% of all uterine malignancies and the incidence is 1/100 000 [1]. Traditionally, uterine sarcomas have been divided into different tumor types according to their histopathological features. The most common are leiomyosarcoma, malignant mixed Müllerian tumor (MMMT), endometrial stromal sarcoma (ESS) and Müllerian adenosarcoma.

Ovarian malignant mixed Müllerian tumors (MMMT) are highly aggressive and early spread beyond the ovary at the time of diagnosis is common. The survival rate is reported to be somewhat worse than that for usual surface epithelial ovarian carcinomas, although one recent study suggests that ovarian MMMTs and high-grade ovarian carcinomas may have a similar prognosis [2].

### *Tumor types*

*Uterine smooth muscle tumors* are a challenge for the pathologist to examine due to the wide spectrum that exists between clearly benign and clearly malignant tumors. This intermediate spectrum includes mitotically hyperactive leiomyomas (i.e., leiomyomas with increased mitotic activity or LIMA), leiomyomas that are mitotically hypoactive but have multiple bizarre and enlarged nuclei (i.e., atypical leiomyomas), and smooth muscle tumors of low malignant potential (STLMP) [1,3]. Even the presence of extrauterine disease does not establish the diagnosis of malignancy, as there are well-documented cases of benign ‘metastasizing’ leiomyomas, intravenous leiomyomatosis, and leiomyomatosis peritonealis disseminata [1,4]

*Uterine leiomyosarcomas* account for about 1% of all uterine malignancies and 30-40% of all uterine sarcomas. Bell et al. have emphasized the importance of analyzing multiple histological variables (i.e., necrosis, mitoses and atypia) in the separation of leiomyosarcomas from other much less aggressive variants of uterine smooth muscle tumors [3].

Even though the majority of patients present with early-stage disease, these tumors have a high recurrence rate and metastatic capacity. The most important prognostic feature has been tumor stage, and survival rates of 17%-37% are reported for all stages of disease and 20-67 % for stage I disease [5]. Other traditional prognostic factors have included grade of differentiation, tumor size, and mitotic index.

*Uterine malignant mixed Müllerian tumors (MMMT)* account for about 1% of all uterine malignancies and 30-40% of all uterine sarcomas. The terms malignant mixed Müllerian tumor, malignant mixed mesodermal tumor and carcinosarcoma have all been used for uterine neoplasms composed of malignant epithelial (carcinomatous) and malignant stromal (sarcomatous) elements. This tumor type is highly aggressive with early lymphatic and/or hematogenous spread beyond the uterine cavity at the time of diagnosis [6]. Clinical understaging often occurs because of unsuspected metastases in the parametria and lymph nodes [7-9]. The recurrence rate is high and metastases to the liver and lungs are not unusual.

Traditionally, MMMTs have been divided into homologous or heterologous types. The homologous type, also known as carcinosarcoma, contains mesenchymal tissue which is indigenous to the female genital tract; whereas the heterologous type (or the malignant mixed mesodermal tumor) contains sarcomatous tissue which is alien to the female genital tract, such as striated muscle, cartilage or fat [10].

**Endometrial stromal sarcomas (ESS)** account for approximately 8-15% of uterine sarcomas or 0.2% of all gynecologic malignancies [11]. Since the work of Norris and Taylor in 1966, ESS have been variably defined and graded according to the maximum number of mitoses per 10 high power fields (hpf), severity of nuclear atypia, or degree of endometrial stromal differentiation [12-15]. The two-grade classification systems have stressed that low-grade ESS demonstrates a less aggressive clinical course than their high-grade counterpart [12,14]. As for other uterine tumor types, stage is considered the strongest prognostic factor [13,16]. Nevertheless, there are some early-stage tumors that run a biological course different from that expected [13].

**Adenosarcoma (AS)** accounts for about 8-10% of the uterine sarcomas. Histopathologically it consists of an admixture of a benign epithelial, although occasionally atypical, epithelial component and a malignant stromal component. Most commonly, the epithelial component resembles inactive or proliferating endometrial glands whereas the malignant stromal component resembles endometrial stromal sarcoma.

AS has been regarded as a neoplasm of low malignancy. Since the late 1970s there have been reports describing a variant with a more aggressive clinical course. Kaku et al. described 17 cases and Clement 10 cases of adenosarcoma with sarcomatous overgrowth (AS-SO) [17,18]. Histopathologically, this tumor type is characterized by focal overgrowth of pure high-grade sarcoma. The presence of heterologous elements and deep myometrial invasion are also regarded as unfavorable prognostic factors [19,20].

**Ovarian malignant mixed Müllerian tumors (MMMT)** are rare tumors that account for less than 1% of all ovarian malignancies. The terminology of ovarian MMMT is the same as for the uterine MMMTs. It has been stated that 'even a small focus of sarcoma in a large tumor justifies the diagnosis of MMMT' [21].

#### *Histogenesis of malignant mixed Müllerian tumors*

The histogenesis of MMMTs has been an issue of great controversy. There are three classical theories [22].

- 1) Collision theory: in which two *independent* neoplasms, a carcinoma and sarcoma, converge;
- 2) Combination theory: in which a common, multipotent stem cell gives rise to both epithelial and mesenchymal components;
- 3) Composition theory: characterized by a malignant transformation of both glandular and mesenchymal cells in a single primary site.

Immunohistochemical studies and ultrastructural studies have recently suggested that MMMTs actually represent metaplastic carcinomas [22-29]. In this, *the conversion theory*, there is a transmutation (metaplasia) of one neoplastic (malignant) cell into another.

The separation of MMMTs from high-grade carcinomas with a sarcomatoid component has been based on histological (sharp separation of the two components *versus* obvious merging) and immunohistochemical features (inverse staining for epithelial and mesenchymal markers in the respective components). These criteria are not always easy to apply, leave ample space to subjective interpretation, are not universally accepted,

and weakened in their importance due to the alleged metaplastic origin of MMMTs. In fact, a transition between epithelial and mesenchymal components does not appear to be exclusive to the pseudosarcomatous carcinoma category. Indeed, de Brito et al. found that 76% of MMMTs demonstrated cells with ultrastructural features transitional between epithelial and stromal cells [25].

### ***Genetic changes in cancer and new (possible) prognosticators***

Genetic abnormalities in cancer cells affect genes that can be classified into two broad categories. (1) **oncogenes**, in which a genetic alteration leads to increased activity of a protein ordinarily involved in turning on cellular growth or proliferation (i.e., *mdm-2*); (2) **tumor suppressor genes**, in which a genetic change leads to decreased activity of a protein product that ordinarily plays a role in restraining cellular growth (i.e., *p53*) [30].

The *p53* tumor suppressor gene, located on the short arm of chromosome 17, encodes a protein with 393 aminoacids and a molecular weight of 53 kDa. P53 has been proven to participate in normal cell growth and division, genomic stability, DNA repair and apoptosis. Wild-type *p53* protein exerts its biological effects by binding to specific DNA sequences, thereby activating or inhibiting transcription of other genes. When function is impaired, cellular growth may become dysregulated, a change that often contributes to cancer development.

After DNA damage the intracellular levels of wild type *p53* rise, leading to cell cycle arrest at the G<sub>1</sub> checkpoint or apoptosis. This arrest allows the cell to repair the DNA damage [31,32]. Thus *p53* functions as a regulator of the cell cycle and a suppressor of tumor development [33].

The wild-type *p53* protein is present at low levels in normal tissue has a short half-life (20-30 minutes) and is not detectable by standard immunohistochemical methods [30]. Mutations of the *p53* gene often result in a different configuration of the *p53* protein, which will prevent the interaction between the *mdm-2* - and *p53* protein. This result in an accumulation of *p53* protein in the cell and therefore it becomes immunohistochemically detectable (see below).

Today there is ample evidence that mutation of *p53* is important in tumor development and about half of all human cancers contains abnormalities in the *p53* gene [30] (i.e., colon, breast, endometrial carcinoma and epithelial ovarian carcinoma) [33-37].

*mdm-2 (murine double minute-2)* is located on chromosome 12. It is a proto-oncogene that encodes a 90 kDa protein. Interactions between the *mdm-2* gene product and *p53* has been shown to block the activity of *p53*, presumably by preventing the binding of essential transcription factors binding to the N-terminal transcription activation domain of *p53*. In this way *mdm-2* negatively regulates wild-type *p53* mediated transcription by directly binding the *p53* protein. Biochemical and X-ray studies have shown how a small region of the N-terminus of the *p53* forms a tight protein-protein interaction with an N-terminal, hydrophobic pocket domain in *mdm-2* [38].

Overexpression of *mdm-2* has an effect that will be similar to that of mutations in the *p53* gene [39,40]. The *mdm-2* gene has been shown to be abnormally up-regulated in human tumors and tumor cell lines by gene amplification, increased transcript levels and enhanced translation. The *mdm-2* amplification appears to be more common in tumor cells of non-epithelial origin, especially those derived from the mesenchyme [41,42].

### ***The p53/mdm-2 interaction***

Lane and Hall describe a model explaining why p53 is stable in tumor cells. In this model the transcription of *mdm-2* gene is dependent on p53 itself. p53 drives the transcription of *mdm-2* that will target its own (p53) destruction. It is predicted that in tumor cells with mutant p53, *mdm-2* is absent, because the transcription factor (wild-type p53) necessary for the expression of *mdm-2* is missing (Fig 1). This leads to the conclusion that mutant p53 is only stable in tumor cells because the lack of *mdm-2* [38].

### ***Flow cytometry***

DNA flow cytometry (FCM) determines the cellular DNA content (DNA ploidy) in a tumor and the proportion of cells in division (S-phase fraction). Initially, FCM was only possible with fresh tissue, but Hedley in 1983 and Schutte in 1985 described a specific method for deparaffinization and tissue disintegration that allowed for FCM to be performed on formalin-fixed, paraffin-embedded specimens [43,44]. This technique implies that suspensions of single nuclei are prepared and the DNA content is measured at a speed of 100-1000 cells/sec. Thus the technique is fast and simple with widespread use. It is well known that DNA ploidy in a tumor adds to the prognostic information in many human malignancies. S-phase fraction (SPF) is also a well-known independent prognostic factor in some human malignancies such as breast and prostate cancer [45]. Flow cytometric studies indicate that DNA analysis also has prognostic value in gynecological malignancies [35,45-49]. Reports of the prognostic value of FCM in uterine sarcoma are few and their conclusions are ambiguous [50-52].

### ***Treatment***

#### ***Uterine sarcomas***

Treatment of sarcomatous neoplasms is difficult because of its low radio- and chemosensitivity, and the mainstay of therapy is surgical removal of the tumor with total hysterectomy, with or without bilateral salpingo-oophorectomy and lymphadenectomy [53].

Adjuvant treatment of early stage sarcoma is controversial. Pelvic radiation has not resulted in significantly improved survivals, but has in some studies demonstrated an improvement in local pelvic control rates [54,55]. The failure of pelvic radiation to improve survival is ascribable to the fact that over half of the patients treated with radiotherapy develop recurrences outside the treated area (i. e., upper abdomen and lungs) [56,57].

Over the past years several reports have been published on this issue. In most studies treatment has limited effect on survival rates, both in early stage tumors and in advanced tumors [53-76]. These studies have in common a small number of patients and only a few randomized studies have been performed [57,62,68,69].

### ***Ovarian sarcoma***

As for uterine sarcomas the mainstay in treatment is surgical removal of the tumor with total hysterectomy, bilateral salpingo-oophorectomy and omentectomy when possible. As their uterine counterpart, ovarian MMMTs have low radio- and chemosensitivity. However, one recent study suggests that ovarian MMMTs and similarly treated high-grade ovarian carcinomas may have an analogous behavior [2].

## **Aims of the Investigation**

1. To analyze p53 and mdm-2 expression, DNA ploidy and SPF in relation to traditional clinical and pathological prognostic factors, such as tumor stage, grade and mitotic index, in patients with uterine sarcomas and ovarian malignant mixed Müllerian tumors registered at the Department of Gynecologic Oncology, University Hospital, Linköping, Sweden.
2. To evaluate the prognostic significance of p53 and mdm-2 expression, DNA ploidy, SPF, and traditional clinical and pathological variables in relation to disease-free survival and cancer related survival for patients with uterine sarcomas and ovarian malignant mixed Müllerian tumors registered at the Department of Gynecologic Oncology, University Hospital, Linköping, Sweden.
3. To evaluate disease-free survival and cancer related survival in patients with uterine sarcomas and ovarian malignant mixed Müllerian tumors in relation to radio- and chemotherapy treatment.

## **Material**

### ***Uterine sarcoma***

Between January 1970 and December 1996, 186 patients with uterine sarcoma were registered at the Department of Gynecological Oncology of the University Hospital of Linköping. Histological slides and paraffin-embedded material were obtained from the referring hospitals in 180 cases. All of the original histopathological material (including uterine tumor, metastases and recurrences) was evaluated by one pathologist (Claudio Guerrieri). The histological distribution before and after reevaluation is shown in Table 1.

### ***Ovarian malignant mixed Müllerian tumors***

Twenty-six cases of MMT of the ovary diagnosed between 1982 and 1996 were obtained from the files of the Department of Gynecologic Oncology, University Hospital, Linköping, Sweden. The pathological material (including original tumor, second-look operation and recurrence) was obtained from the four major hospitals in the Southeast Health Care Region of Sweden (University Hospital of Linköping, and County Hospitals of Norrköping, Jönköping and Kalmar).

### ***Study cohort***

#### ***Uterine neoplasms***

#### ***Distribution of uterine smooth muscle tumors***

After reevaluation 80 tumors were classified as smooth muscle tumors. The distribution was as follows: 9 benign leiomyomas (BL), 6 atypical leiomyomas (AL), 3 smooth muscle tumors of low malignant potential (STLMP), 13 leiomyomas with increased mitotic activity (LIMA) and 49 leiomyosarcomas.

None of the patients with benign leiomyomas or LIMA had a recurrence or died of disease. Three of the six patients with atypical leiomyomas recurred, and two patients died of disease. Two of three smooth muscle tumors of low malignant potential recurred and died of disease. There was no tumor feature that could predict the outcome of patients with non-sarcomatous tumors and this tumor group will not be further described or discussed in this thesis.

#### ***Leiomyosarcoma***

Forty-six patients had surgery with at least subtotal hysterectomy. Three patients with leiomyosarcomas underwent local tumor excision without a subsequent hysterectomy. At the time of diagnosis they had lung metastases and were treated with chemotherapy. These three patients were included in the survival analysis as having FIGO surgical stage IV.

#### ***Malignant mixed Müllerian tumors***

Forty-two patients underwent surgical treatment with at least subtotal hysterectomy with removal of as much tumor as possible in cases with extrauterine spread. Two patients underwent endometrial curettage and exploratory laparotomy with excision of extrauterine tumor masses and omentum but without removal of the uterus.

We excluded three patients from further analysis because they had no operation. Two patients (one clinical stage III and one stage IV) had intracavitary brachyradiotherapy

treatment to the uterine cavity and vagina (3600 mg RaH and 5300 mg RaH, respectively), and one patient did not undergo any treatment because of poor general condition (clinical stage I). This leaves 44 patients eligible for analysis.

### ***Endometrial stromal sarcoma***

Fifteen patients underwent surgery with at least subtotal hysterectomy. Two had advanced tumor stage (clinical stage IVB) and were not operated but given chemotherapy, and included in the survival analysis as having FIGO surgical stage IV. Seventeen patients were eligible for analysis.

### ***Adenosarcoma***

All patients had surgery with at least subtotal hysterectomy. Eleven patients were eligible for analysis.

### ***Ovarian malignant mixed Müllerian tumors***

All twenty-six cases of MMMT of the ovary were eligible for analysis.

### ***Clinical data***

Clinical data was obtained from the patients' medical records and the tumors were clinically staged retrospectively according to the International Federation of Gynecology and Obstetrics (FIGO) staging system for endometrial carcinoma. Patients with uterine neoplasms who underwent at least explorative laparotomy were staged according to the FIGO surgical staging system for endometrial carcinoma. All clinical stage IV tumors were considered as surgical stage IV.

All patients with ovarian neoplasms underwent explorative laparotomy and were staged according to FIGO staging system for ovarian cancers.

### ***Treatment of the different tumor types (detailed description)***

#### ***Leiomyosarcoma***

##### ***Stage I-II (29 patients)***

Three patients underwent a total abdominal hysterectomy (TAH), 17 had a TAH and bilateral salpingo-oophorectomy (BSO), four had a TAH, BSO and omentectomy (OT), three had a TAH and unilateral salpingo-oophorectomy (USO), one had a subtotal hysterectomy (SH) with BSO, and one patient had only a SH.

Thirteen patients received postoperative radiation therapy to the lesser pelvis; eleven had external radiation (median dose 50 Gy, range 30.6-56 Gy) and two had external radiation treatment (30 and 45 Gy) and intracavitary brachyradiotherapy to the vagina (900 and 960 mg RaH).

Five patients were treated with chemotherapy. Melphalan was given to one patient with stage II disease. Cyclophosphamide/5-FU was used in three patients, two stage I and in one stage II, respectively. Doxorubicin was used in one patient with a stage I tumor. None of the early stage tumors had more than one line of chemotherapy in primary treatment.

##### ***Stage III-IV (20 patients)***

Ten patients underwent TAH with BSO, two a TAH with BSO and OT, one a TAH with USO, two a SH with BSO, one a SH and USO, and one underwent only a SH. Three patient did not undergo a hysterectomy.

Ten patients received postoperative radiation therapy to the lesser pelvis; all had external radiation (median dose 35 Gy, range 30-50 Gy). In addition two received intracavitary brachyradiotherapy to the vagina (1200 mg RaH each). Two patients who did not have a hysterectomy had intracavitary brachyradiotherapy to the vagina (1500 and 5800 mg RaH).

Fifteen patients were treated with chemotherapy; twelve postoperatively, two after intracavitary brachyradiotherapy and one had only chemotherapy. Several different regimens of chemotherapy were used. Five stage III tumors received chemotherapy: one had melphalan, one had cyclophosphamide/5-FU and 3 were given doxorubicin. Ten patients with stage IV tumors received chemotherapy; seven were treated with either cyclophosphamide, cyclophosphamide/5-FU, cisplatin/doxorubicin, doxorubicin/vincristine, methotrexate, CY-VA-DIC or ifosfamide/epirubicin/mesna; three were treated with vincristine/actinomycin D/cyclophosphamide (VAC).

Two patients had a second line chemotherapy, three had a third line and two had a fourth line treatment. All but one patient that had chemotherapy died of disease. One patient who had a second line treatment is alive and well 20 years after diagnosis.

### ***Uterine malignant mixed Müllerian tumors***

#### ***Stage I and II (23 patients)***

Nine patients had a TAH with BSO, twelve had a TAH with BSO and OT, one had a TAH and USO, and one had a TAH and OT.

Eight patients received preoperative radiation therapy to the lesser pelvis; one had external radiation (30 Gy) and intracavitary brachyradiotherapy to the uterus (4250 mg RaH). Seven had intracavitary brachyradiotherapy to the uterus (median 3800 mg RaH, range 1500-6700 mg RaH) and three had additional vaginal therapy (1100 mg RaH, 1200 mg RaH and 1300 mg RaH, respectively) and one had postoperative external radiation (7.5 Gy).

Eight patients received postoperative radiation therapy to the lesser pelvis; all had external radiation (median dose 45 Gy, range 40-50.4 Gy) and three had additional intracavitary brachyradiotherapy to the vagina (one had radium treatment 1600 mg RaH; and two had <sup>192</sup>Ir high-dose rate afterloading brachyradiotherapy 12.5 Gy and 16 Gy, respectively).

Six patients were treated with chemotherapy; all had stage I tumors. One received paraplatin and one cisplatin. Four patients were treated with cisplatin/epirubicin.

#### ***Stage III-IV (21 patients)***

Six patients underwent a TAH with BSO, 10 had a TAH with BSO and OT, one had a SH, one had a SH with BSO, and one had a SH and USO. Two patients had explorative laparotomy with removal of tumor from the abdominal cavity, but without hysterectomy.

Five patients received preoperative radiation therapy to the lesser pelvis; all had intracavitary brachyradiotherapy to the uterus (median 2000 mg RaH, range 1200-4000 mg RaH). One patient had additional vaginal therapy (1600 mg RaH). Two patients had preoperative (21 Gy and 30 Gy) and two had postoperative external radiation treatment (15 Gy and 30 Gy).

Four patients received postoperative radiation therapy to the lesser pelvis; four had external radiation (median dose 44.5 Gy, range 44-46 Gy) and two had additional intracavitary brachyradiotherapy to the vagina (1300 mg RaH and 2000 mg RaH).

Twelve patients were treated with chemotherapy. Similar to the patients with advanced leiomyosarcoma, several different modalities of chemotherapy were used. Seven patients with stage III tumors received chemotherapy; one had vincristine/actinomycin D/cyclophosphamide (VAC), one had actinomycin D/melphalan/methotrexate and 5 had ifosfamide/epirubicin/mesna. Five stage IV tumors received chemotherapy; one patient was treated postoperatively with intraperitoneal cisplatin. The other four patients were treated with either paraplatin, vincristine/actinomycin D/cyclophosphamide (VAC), cyclophosphamide/epirubicin/cisplatin or with ifosfamide/epirubicin/mesna. Three patients had second line treatment. Nine patients treated with chemotherapy died of disease; three are still alive and well at last follow-up (3.1, 4.9 and 6.6 years after diagnosis).

### ***Endometrial stromal sarcoma***

Seven patients with early stage (surgical stage I) tumors underwent TAH with BSO, while four had a TAH and one patient had a SH. Five of these patients received adjuvant external radiation therapy after surgery with a median dose of 30 Gy (range, 30.0-50.4 Gy) directed at the entire pelvis. Among the five patients with advanced tumor stage (surgical stage III-IV), surgery was performed in three patients with a TAH with BSO, while two patients underwent uterine curettage. Three patients received external radiotherapy and four were given chemotherapy.

### ***Adenosarcom***

Seven patients underwent a TAH with BSO, two had a SH with BSO, and two patients had a TAH. Two patients received preoperative radiation therapy; one had external radiation to the lesser pelvis (20 Gy) and the other had intracavitary brachyradiotherapy to the uterine cavity (3400 mg RaH). One patient had postoperative brachyradiotherapy to the vaginal surface (2000 mg RaH) and external radiation therapy (40 Gy) directed at the entire pelvis.

### ***Ovarian malignant mixed Müllerian tumors***

Optimal surgical cytoreduction (<1cm) was obtained in three of the six Stage II patients, but only in one of 17 women with Stage III-IV disease. The surgical interventions are summarized in Tables 3 and 4. Various chemotherapeutic regimens were administered to 22 patients. Two of these patients also received radiation therapy. Two patients received radiation therapy only. Two other patients died before initiation of treatment. Eleven patients underwent a second-look operation (3 Stage II, 6 Stage III, 2 Stage IV). Three of these had no remaining disease (2 Stage II and one Stage IV).

## Methods

### *Pathological evaluation*

#### *Leiomyosarcoma*

Tumor cell necrosis and degree of atypia were evaluated according to the descriptions by Bell et al. [3]. The mitotic index was recorded as the number of mitoses in 10 high power fields (hpf) of the most active area. A Zeiss microscope with a x40 objective and x10 ocular was used. The histologic grade of the leiomyosarcomas was calculated according to a scheme that incorporates mitotic activity, tumor necrosis and degree of atypia (see Table 2).

#### *Uterine malignant mixed Müllerian tumors*

The MMMTs were classified as homologous and heterologous types. The homologous sarcomatous component typically has the appearance of spindle cell sarcoma, leiomyosarcoma, fibrosarcoma, malignant fibrous histiocytoma, undifferentiated sarcoma or any combination thereof. Heterologous tumors contained one or more of the following stromal elements: rhabdomyoblasts, chondrosarcoma, osteosarcoma and liposarcoma [1]. The mitotic index (MI) was recorded by determining the number of mitotic figures (mf) per 10 high power fields (hpf) in the most active area. The tumors were graded according to the degree of nuclear pleomorphism, size of nucleoli and mitotic count.

#### *Endometrial stromal sarcoma*

The tumors were classified as low-grade and high-grade ESS, irrespective of their mitotic activity, in line with the criteria adopted by Silverberg and Kurman [14]. A low-grade ESS was characterized by a myoinvasive tumor composed of uniform small oval-spindle cells resembling proliferative-phase stromal cells and an arborizing vasculature. A high-grade ESS displayed cellular pleomorphism and nuclear atypia although maintaining some resemblance to endometrial stromal cells [14]. The mitotic index (MI) was recorded by determining the number of mitotic figures (mf) per 10 high power fields (hpf) in the most active area.

#### *Adenosarcoma*

This tumor type presents typically as a polypoid, broad-based tumor that fills the endometrial cavity. On microscopic examination, adenosarcoma has an epithelial component characterized by glands that are frequently dilated and are scattered regularly or irregularly throughout the mesenchymal component of the tumor. The glands may be lined by a variety of benign or atypical epithelia, most commonly of (proliferative) endometrial type, although other types may also be seen. The stromal component of the adenosarcoma is composed of round to spindle-shaped cells that most commonly resemble those of endometrial stromal sarcoma [1]. The diagnosis of adenosarcoma with sarcomatous overgrowth was made when the tumor contained areas of pure sarcoma devoid of epithelial elements that occupied a significant portion (>10%) of the tumor. The mitotic index (MI) was recorded by determining the number of mitotic figures (mf) per 10 high power fields (hpf) in the most active area. In cases of adenosarcoma with sarcomatous overgrowth, the mitotic activity was evaluated separately within areas of typical adenosarcoma and those with sarcomatous overgrowth. The tumors were graded ac-

ording to the degree of cellularity and nuclear pleomorphism. The sarcomatous component was classified as homologous and heterologous.

### ***Ovarian malignant mixed Müllerian tumors***

A MMT was diagnosed when an ovarian tumor was composed of intimately admixed carcinomatous and sarcomatous elements. The latter were either homologous (spindle or pleomorphic cell, or resembling malignant fibrous histiocytoma) or heterologous (chondrosarcoma, rhabdomyosarcoma, or osteosarcoma). Two tumors containing a minor component ( $\leq 5\%$  of the examined tumor) of spindle sarcomatous cells were also included in the study. Caution was exercised in order to exclude mimics of MMT such as malignant germ cell tumors, poorly differentiated carcinomas with solid pseudosarcomatous areas, and endometrioid carcinomas with a spindle cell component [21,77].

The histological examination involved subtyping of the epithelial and mesenchymal elements. Each histological component was then quantitated as a percentage of the entire tumor examined. The mitotic count and nuclear grade (based on criteria used for endometrial carcinomas [78]) were determined in both epithelial and mesenchymal parts. The carcinomatous areas were graded architecturally based on the proportion of solid growth (<10%, 11-50%, >50%).

### ***Flow cytometry***

#### ***Preparation and staining***

For DNA flow cytometry (FCM) two 50- $\mu\text{m}$  sections were taken from the paraffin block with the highest tumor content. Hematoxylin-eosin (H&E)-stained sections were cut before and after the sections for FCM. The tumor sample was cleared from necrotic and hemorrhagic tissue before DNA analysis. The method used for preparation, staining and analysis of paraffin-embedded tumors was a modification of the method described by Schutte et al. and has been described earlier by Wingren et al. [44,79]. Shortly, the samples were deparaffinized with xylene, rehydrated stepwise in ethanol and treated with 0.25% trypsin in citrate buffer in a waterbath over night at 37°C. After filtration through a nylon mesh the cell suspension was stained with propidium iodide as described by Vindelöv et al. [80].

#### ***DNA measurement***

The suspension was analyzed with a FACScan flow cytometer (Becton Dickinson, California, USA). DNA distribution histograms, including at least 15,000 cells, were recorded with the CellFit software (Becton Dickinson, California, USA).

#### ***Evaluation of the histograms***

The peak with the lowest DNA content was considered diploid as an internal reference. Classification of DNA histograms was done according to the recommendations of the International Society of Analytical Cytology and without knowledge of the clinical outcome [81,82]. The upper limit for coefficient of variation of the internal control was 8%. The mean coefficient of variation (CV) for the tumor  $G_{0/1}$  peak was 6.0%. Tumors with more than one peak were considered DNA aneuploid. Tumors with more than two DNA peaks were called DNA multiploid. The SPF was calculated using a rectangular model [79].

## ***Immunohistochemistry***

### ***Preparation and staining***

For immunohistochemistry 5µm sections were taken from the paraffin block with the highest tumor content. H&E sections were done before and after the sections for immunohistochemistry. The sections were deparaffinised in xylene and then rehydrated stepwise in ethanol [99.5% (twice), 95%, 80%] and distilled water. Endogenous peroxidase activity was blocked with 3% hydrogen peroxide (H<sub>2</sub>O<sub>2</sub>) in methanol for 15 minutes. After a short rinse in distilled water (5 minutes), sections were treated in a microwave oven 800 W for 15 minutes for p53 and in 10 minutes for mdm-2 in citrate buffer, pH 6.0. The sections were cooled in room temperature for 20 minutes and preincubated with 10% normal rabbit serum to block non-specific immunostaining for 20 minutes. After removal of the blocking solution, the sections were incubated with primary antibody. For p53 determination DAKO-p53 (DO-7 monoclonal mouse antibody; DAKO A/S, Glostrup, Denmark), which detect both wild and mutant form of p53 was applied in a 1/100 dilution for two hours at room temperature. For detection of mdm-2 overexpression, mdm-2 antibody (SMP-14; Santa Crus Biochemistry, Inc., USA) was applied in a 1/300 dilution for one hour in room temperature.

For **p53** detection we used the PAP technique. After removal of the primary antibody, the sections were incubated with secondary antibody, rabbit anti-mouse immunoglobulin in a 1/50 dilution (DAKO A/S, Glostrup, Denmark) for 30 minutes and then with mouse peroxidase antiperoxidase for 30 minutes with washing in phosphate buffered saline between the steps. The peroxidase reaction was allowed to proceed for 8 minutes, with 0.036% 3,3-diaminobenzidine tetrahydrochloride solution as substrate in phosphate buffered saline with 0.03 % hydrogen peroxide.

For **mdm-2** detection we used the biotin-streptavidin technique. After removal of the primary antibody, the sections were incubated with the biotinized rabbit-anti-mouse antibody (DAKO A/S, Glostrup, Denmark). After washing in phosphate buffered saline streptavidin with peroxidase was added for 30 minutes. The peroxidase reaction was performed as described for p53. Sections known to stain positively were included in each run, receiving either primary antibody or mouse immunoglobulin isotype MOPC-21 for IgG1 as positive and negative controls.

Finally, the sections were counterstained with hematoxylin for two minutes, dehydrated with ethanol and mounted under a coverslip.

### ***Evaluation of immunohistochemistry staining***

The slides were examined independently by myself and Claudio Guerrieri, unaware of the clinical outcome of the patients. p53 staining was scored in the following way: less than 10% positive tumor cells were classified as negative, between 10-25% were scored 1+, between 25-50% 2+, between 50-75% 3+ and finally more than 75% positive tumor cells 4+. For mdm-2 we only scored positive or negative, samples with more than 10% positive tumor cells were scored as positive.

### ***Description of external radiation technique***

Target volume was the small pelvis. The patient was in supine position. No immobilization devices were used. Simulation was done and the small pelvis was treated through parallel-opposed portals loaded equally (AP/PA technique). The target absorbed dose was prescribed on the central axis midway between the beam entrances. The external radiation treatment was accomplished by a linear accelerator (4-10 MV, SSD 80-100cm) with 1.8-2.0 Gy per fraction, 5 fractions per week aiming at a total dose of 30-45 Gy [83,84].

### ***Description of intracavitary brachyradiotherapy technique***

In total 29 patients had brachyradiotherapy. Therapy was performed with radium in 27 patients and high dose rate  $^{192}\text{Ir}$  in two patients.

### ***Radium treatment***

Intrauterine radium treatment was delivered according to the Heyman packing technique and vaginal intracavitary treatment was delivered with radium containing cylinders. In short the corpus was packed with as many individual cylindrical irradiators as possible. The size and number of the irradiators was chosen with respect to the uterine volume. The packing technique had one main objective: To bring irradiators in close contact with all parts of the inner uterine wall.

The treatment time was determined from experience and the dosage was quoted in milligram-hours of radium (mg RaH), which is a measure of the total energy emitted but not an absorbed dose [85].

### ***High dose rate $^{192}\text{Ir}$ treatment***

In  $^{192}\text{Ir}$  treatment the afterloading technique was used involving computerized doseplanning. Two patients treated had postoperative vaginal irradiation with  $^{192}\text{Ir}$  delivered by a Nucletron micro-Selectron-HDR system. Cylindrical applicators were used with diameters of 20-40 mm. The reference dose was defined at 5 mm from the applicator surface (vaginal mucosa) with a target volume of approximately half the length of vagina. The daily dose delivered was 2.5 – 4 Gy during 4 - 5 consecutive days for a total of 12.5 - 16 Gy.

### ***Statistical methods***

Correlation between two variables was evaluated with the Spearman rank order correlation and differences in proportions with Fisher's exact test, two tailed. Survival time was calculated from the day of diagnosis to the last date of contact or death. Patients who died of intercurrent disease were registered as censored responses. The Kaplan-Meier product limit method was used to generate survival curves [86] and in paper III-IV log rank test was used to test the prognostic effect of variables. In paper I, II and V the Cox proportional hazards regression model was used for both the univariate and the multivariate evaluation of event rates [87]. In paper III and IV multivariate analyses were not performed because of the small number of patients. A *P* value of < 0.05 was considered as statistically significant. Calculations were performed with software Statistica (Statsoft Inc., Tulsa, OK, USA)

## Results

### **Leiomyosarcoma of the uterus: A clinicopathologic, DNA flow cytometry, p53 and mdm-2 analysis of 49 cases. (I)**

The patients ranged in age from 35 to 82 years (median, 59 years). Eighteen patients were premenopausal. Median parity was two (range: 0-6). Twenty-five patients (51%) had FIGO stage I tumors, four had stage II (8%), eight had stage III (16%) and twelve (25%) had stage IV. Two tumors (4%) had mild atypia, twenty-eight (57%) had moderate atypia and 19 (39%) severe atypia. Forty-one (84%) tumors had tumor cells necrosis. Eight (16%) had tumor grade 1, twenty-one (43%) had grade 2 and 20 (41%) had grade 3. Finally, thirteen (27%) had a MI between 0-10, sixteen (32%) had a MI between 11-20 and twenty (41%) had a MI >20.

We found a significant correlation between S-phase fraction and stage ( $p=0.014$ ) and degree of atypia ( $p=0.04$ ).

#### ***DNA ploidy, SPF and p53-and mdm-2 expression***

With 43 tumors we could evaluate DNA ploidy and with 39 tumors also SPF. DNA ploidy, SPF, p53 and mdm-2 overexpression are shown in Table 5.

#### ***Recurrence pattern***

During the observation period 19 (66%) of stage I-II tumors relapsed. Seven in the lungs, three in the pelvis, two in the abdomen, two in the vagina, two in the pelvis and lungs, one in liver and lungs, one in the brain and lungs, and one in the abdomen/pelvis/pelvic wall.

#### ***Survival***

During the observation period 37 (76%) of the 49 patients with leiomyosarcoma died. Of these, 35 died of tumor and two of intercurrent disease. The 5-year survival rate for all stages was 33% (52% for stage I-II, 0% for stage III-IV). The median follow-up for patients alive at last contact (September 1996) was 57.3 months (range: 28-242 months). Among the patients with stage I-II tumors, 16 (55%) died of disease, two (7%) died of non-cancer-related disease and 11 (38%) patients are alive.

#### ***Analysis of prognostic factors for survival***

Prognostic factors for survival are shown in Table 6. When stage, DNA ploidy, SPF, tumor grade, atypia and mitotic index were included in a multivariate analysis, only 39 patients were assessable since ten patients had to be excluded due to missing data. Stage proved to be the most significant predictor of outcome ( $p=0.007$ ). DNA ploidy ( $p=0.045$ ) and SPF ( $p=0.041$ ) also had independent prognostic significance. Mitotic index, atypia and grade did not reach statistical significance.

In a subgroup univariate analysis of cancer-related death for FIGO stage I tumors, 22 patients could be evaluated (3 patients were excluded due to non-assessable DNA histograms). We found that DNA ploidy ( $p=0.04$ ) and tumor grade ( $p=0.01$ ) were statistically significant. In a multivariate analysis, only tumor grade had independent prognostic significance ( $p=0.01$ ).

### ***Analysis of prognostic factors for disease-free survival***

The analysis of disease-free survival included 29 patients with stage I-II leiomyosarcoma. When the same variables used in the univariate survival analysis were included we found that p53 overexpression ( $p=0.0016$ ), DNA ploidy ( $p=0.042$ ) and tumor grade ( $p=0.008$ ) were highly significant. On the other hand, S-phase fraction, mdm-2, grade, degree of atypia, menopausal status, age, necrosis and mitotic rate did not reach statistical significance.

When p53, DNA ploidy and tumor grade were included in a multivariate analysis, 25 of the stage I-II patients could be evaluated (4 were excluded due to lack of various data). Only p53 had independent statistical significance ( $p=0.01$ ). All seven patients with p53 overexpression had a recurrence within 28 months after diagnosis. Five of these seven patients died of disease and two are still alive

## **Malignant mixed Müllerian tumors of the uterus: A clinicopathologic, DNA flow cytometric, p53 and mdm-2 analysis of 44 cases. (II)**

The patients ranged in age from 46 to 85 years (median, 68 years). Only four patients were premenopausal. Median parity was two (range: 0-4).

Thirty-two patients (73%) had homologous and 12 (27%) had heterologous MMMTs. Twenty-one (48%) had FIGO stage I tumor, two (4%) had stage II, ten (23%) had stage III and 11 (25%) had stage IV tumors. Forty-two (95%) had high grade tumor and 25 (57%) had MI above 20/10 hpf. Thirty-three tumors could be evaluated for myometrial invasion; one (3%) had no invasion, fifteen (45%) had less than 50% invasion, and seventeen (52%) had greater or equal to 50% invasion.

There were no statistically significant correlations between tumor stage, tumor grade, mitotic index, DNA ploidy, S-phase fraction, p53 and mdm-2.

### ***DNA ploidy, SPF and p53-and mdm-2 expression***

A total of 44 tumors were accepted for DNA ploidy evaluation and 40 tumors for SPF evaluation. DNA ploidy, SPF, p53 and mdm-2 overexpression are shown in Table 5.

Simultaneous overexpression of p53 and mdm-2 was observed in seven homologous and one heterologous tumor. All p53-positive tumors showed a concordant staining pattern within the carcinomatous and sarcomatous areas.

### ***Recurrence pattern***

Seventeen (39%) patients had persistent disease after initial treatment and another nine (20 %) suffered tumor recurrence. Of the latter, four had recurrence in the lungs, one in the pelvis, one in the abdomen and pelvis, one in the lungs and vagina, one in the supraclavicular fossa, and one had brain metastases. All nine patients died of tumor.

### ***Survival***

During the observations period 27 (61%) patients died of disease and 6 (14%) died of inter-current disease. All nine patients whose tumor recurred died within three years after the first recurrence, and only two of these survived more than one year. Eleven (25%) patients are alive with no evidence of disease at last follow-up (December 1996). The median follow-up for the latter patients was 59 months (range, 28-178 months). The overall 5-year survival rate for all stages was 38%. The 2- and 5-year survival rates for stage I-II MMMTs were 65% and 54%, respectively, while for stage III-IV tumors they fell to 30% and 20%, respectively.

### ***Analysis of prognostic factors for survival***

Tumor grade was not included in this analysis because the great majority (95%) of the tumors were high grade. Prognostic factors for survival are shown in table 6.

In a subgroup analysis with stage I and II tumors alone (which included twenty-three patients), only mitotic index reached statistical significance ( $p=0.037$ ).

## **Endometrial Stromal Sarcoma of the uterus: A clinicopathologic, DNA flow cytometric, p53 and mdm-2 analysis of 17 cases. (III)**

The 17 patients ranged in age from 41 to 78 years (median, 57 years). Seven (41%) patients were premenopausal. Median parity was two (range: 0-6). No patient had received radiation treatment prior to diagnosis.

Thirteen patients (76%) had low-grade tumors and four had high-grade tumors. Twelve (70%) had a FIGO stage I tumor, two (12%) had a stage III, and three (18%) had a stage IV tumor. Among the low-grade tumors we found 12 (92%) with mitotic index less than 10/10 hpf, and for the high-grade tumors we found three (75%) with index above 10/10 hpf.

Three of four patients (75%) with high-grade tumors were over 60 years of age and eight of thirteen patients (62%) with low-grade tumors were younger than 60 years. One of 13 low-grade tumors and 3 of the 4 high-grade tumors had more than 10 mitoses/10 hpf.

We found a significant correlation between p53 and SPF ( $p=0.02$ ), grade ( $p=0.02$ ), MI ( $p=0.00001$ ), and stage ( $p=0.001$ ). There was also a significant correlation between DNA ploidy and mdm-2 ( $p=0.02$ ) and grade ( $p=0.00006$ ); between SPF and grade ( $p=0.006$ ), MI ( $p=0.006$ ), and stage ( $p=0.02$ ); between grade and MI ( $p=0.003$ ) and stage ( $p=0.02$ ); and between MI and stage ( $p=0.02$ ).

### ***DNA ploidy, SPF and p53-and mdm-2 expression***

Flow cytometric DNA histograms could be evaluated in 16 patients. DNA ploidy, SPF, p53 and mdm-2 overexpression are shown in Table 5.

### ***Recurrence***

Eight patients (47%) had recurrences and, of these, two (25%) are alive and six (75%) are dead of disease. Five (45%) low-grade, early stage (surgical stage I) tumors recurred, one in the lungs, one in the abdominal cavity and three at both local and distant sites. All three patients with both local and distant recurrences died of disease while the two patients with recurrence in the lungs and abdominal cavity are alive with no evidence of disease 8.9 and 21 years after diagnosis. Of the remaining three patients, one had local recurrence, one had local and distant recurrence, and one distant recurrence.

### ***Survival***

During the observation period, nine out of 17 (53%) patients (five with low-grade and four with high-grade tumors) died of disease. At last follow-up (February 1998), eight out of 17 (47%) patients are alive with no evidence of disease. The median follow-up for the living patients was 102 months (range, 15-252 months). The overall corrected 5-year survival rate for all stages was 57%. The 5-year survival rate for patients with low-grade ESS was 74%, while all four patients with high-grade ESS died of disease within 14 months of diagnosis. All patients with aneuploid tumors, SPF >10% or tumors that overexpressed mdm-2 died of disease. Four of five patients with p53-positive tumors died of disease.

Four tumors had a mitotic index above 10/10 hpf and, of these, three were high-grade tumors and one was a low-grade tumor. All three patients with high-grade tumors died of disease while the patient with the low-grade tumor had no recurrence and was alive with no evidence of disease 2.4 years after diagnosis.

***Analysis of prognostic factors for survival and disease-free survival***

Using log-rank test, we found a significant correlation between survival and tumor grade ( $p=0.007$ ), DNA ploidy ( $p=0.026$ ), SPF ( $p=0.048$ ) and FIGO surgical stage ( $p=0.026$ ) (table 6). There were no variables that correlated with disease-free survival.

## **Adenosarcoma of the uterus: A clinicopathologic, DNA flow cytometric, p53 and mdm-2 analysis of 11 cases. (IV)**

The 11 patients ranged in age from 31 to 90 years (median, 76 years). Only one patient was premenopausal at the time of diagnosis and five (45%) were nulliparous.

Nine tumors (82%) were in FIGO stage I and two (18%) in FIGO stage II. Four tumors (36%) showed no myometrial invasion, six (55%) <50% invasion and one (9%)  $\geq$  50% myometrial invasion.

Six of the tumors (55%) were pure adenosarcoma and five (45%) were adenosarcoma with sarcomatous overgrowth. Two of the adenosarcoma with sarcomatous overgrowth had heterologous elements with rhabdomyosarcoma differentiation. Three (60%) of tumors with sarcomatous overgrowth were high-grade tumors and four (80%) had more than 10 mitosis/10 hpf. Five (83%) of the pure adenosarcoma were low grade and had low mitotic index; only one was of medium grade and had high mitotic index.

### ***DNA ploidy, SPF and p53-and mdm-2 expression***

In all 11 tumors DNA ploidy could be evaluated and in 10 also SPF. DNA ploidy, SPF, p53 and mdm-2 overexpression are shown in Table 5.

Interestingly, we found p53 positivity and mdm-2 overexpression only within the mesenchymal component of the neoplasms.

### ***Recurrence***

Three (50%) of the adenosarcoma recurred; two in the lungs and one in uterine cervix. The two patients with lung metastases died of disease 2.2 and 20.5 months following the recurrence. Two tumors with sarcomatous overgrowth recurred, one in the pelvis and the other in the lungs and the patients died of disease 3.6 and 3.5 months following recurrence.

### ***Survival***

During the observation period four (36%) patients (two with adenosarcoma and two with sarcomatous overgrowth) died of disease. Another three (27%) patients died of intercurrent disease; one of these died of postoperative complications due to pulmonary embolus.

Four (36%) patients are alive with no evidence of disease at last follow up (March 1998). The median follow-up for the latter patients was 11.1 years (range, 4.2-13.9 years). The overall 5-year survival rate for all stages was 69%. The 5-year survival rate for pure adenosarcoma was 80%, and for tumors with sarcomatous overgrowth it was 50%.

### ***Analysis of prognostic factors for survival and disease-free survival***

There were no variables that correlated with survival or disease-free survival.

## **Malignant mixed Müllerian tumors of the ovary: A clinicopathologic, DNA ploidy and p53 study of 26 cases. (V)**

The 26 patients ranged in age from 42 to 79 years (median, 67 years), and all except two were postmenopausal. Nineteen (73%) women were parous, with a median of two (range; 0-5) and five of these had a parity of three or more. Seven (27%) patients were nulliparous. The FIGO stage distribution was as follows: three Stage I, six Stage II, 15 Stage III and two Stage IV. The ovarian tumors ranged in size from five to 23 cm (median, 10 cm). Fourteen (54%) were unilateral and 12 bilateral.

### ***Histopathologic findings***

Fourteen tumors were homologous and 12 heterologous. The epithelial component was composed of pure serous carcinoma in 11 tumors, mixed serous and another subtype (endometrioid, clear cell or squamous) in another 11 tumors, endometrioid in two, mixed clear cell and endometrioid in one, and undifferentiated carcinoma in one. The tumors contained from 2 to 75% of a sarcomatous component. The homologous tumors were composed of pure spindle cell sarcoma in seven cases, and spindle/pleomorphic cell or malignant fibrous histiocytoma in another seven. The proportion of the homologous sarcomatous areas in each tumor ranged from 2 to 75% (median, 40%). Notably, two tumors had 5% or less of a spindle cell sarcomatous component. The heterologous elements were chondroid in eight cases, rhabdoid in two, chondroid plus rhabdoid in one, and chondroid plus osteoid in one. The amount of heterologous sarcomatous areas in each tumor ranged from 1 to 50% (median, 10%). Focal merging between the carcinomatous and the sarcomatous components, although often difficult to appreciate, was noted in 16 cases.

Nuclear atypia in the epithelial component was grade II in three cases and grade III in 23; in the sarcomatous areas it was grade II in six cases and grade III in 20. Architectural grade of the epithelial component was grade I in four, grade II in 14, and grade III in eight tumors. The mitotic count ranged from 3 to 50/10 hpf (median, 20) in the epithelial components, and from 6 to 57/10 hpf (median, 20.5) in the sarcomatous areas. Seven tumors had a mitotic index < 20/10 hpf in both components, while 19 tumors had an index of > 20/10 hpf in at least one of the histological components.

Metastases were evaluated in 18 cases and were composed of pure carcinoma in 11, pure sarcoma in three, and mixed carcinoma/sarcoma in four cases. Of the seven sarcoma-containing metastases, four had heterologous elements. Only one of six (16%) patients with metastatic homologous MMMTs had sarcomatous elements present in the metastases, while six out of twelve metastatic heterologous tumors (50%) had sarcomatous metastatic deposits ( $p=0.3$ ). Recurrences were evaluated in two patients and were both composed of mixed carcinoma/sarcoma.

### ***DNA ploidy, SPF and p53-and mdm-2 expression***

With 24 tumors DNA ploidy could be evaluated and with 23 tumors also SPF. DNA ploidy, SPF, p53 and mdm-2 overexpression are shown in Table 5.

Seventeen (89%) of the p53-positive tumors showed concordant staining within both epithelial and sarcomatous areas whereas two cases showed p53-positivity in the sarcomatous areas but only rare positive cells in the epithelial component. Only two tumors were mdm-2-positive and both were also p53-positive.

### ***Recurrence pattern***

Eleven (42%) tumors recurred, seven in the pelvis, two in the abdomen, one in liver and lungs, and one in the liver and abdomen. All patients with recurrence are dead. Ten (91%) died of tumor, one (9%) of non-cancer-related disease.

### ***Survival***

Eighteen (69%) patients died of disease after one to 107 months (median, 11 months). Six patients were alive after 30 to 142 months (median, 71 months) of follow-up (August 1998). One patient died of post-operative complications and another died from uterine adenocarcinoma diagnosed after 13.4 years. The 5-year survival was 30% for all stages (67% for stage I, 80% for stage II and 17% for Stage III-IV).

The median survival in the group of patients with carcinoma-only metastases was 21 months, as opposed to 12 months in the group with sarcoma-containing metastases, but this difference was not significant ( $p=0.2$ ).

### ***Analysis of prognostic factors for survival***

Prognostic factors for survival are shown in table 6. In a multivariate analysis including stage and residual tumor in Stage II-IV tumors, only stage reached independent prognostic significance ( $p=0.023$ ).

## **Disease-free survival and survival in relation to treatment**

### ***Leiomyosarcoma***

In an univariate analysis only patients treated with surgery (46 patients) were included. Treatment was coded in the following way: 1, surgery; 2, surgery and radiation therapy; 3, surgery and chemotherapy; 4, surgery, radiation, and chemotherapy. With survival as the end-point and treatment as the independent variable, there was statistical significance for the variable 'treatment' (relative hazard 1.4,  $p=0.018$ ). In a subgroup analysis with only stage I tumors (25 patients), we found a statistical significance for 'treatment' (relative hazard 1.6,  $p=0.036$ , fig 2).

If the same variables were included in an univariate analysis for stage I-II tumors (29 patients) with disease-free survival as end-point, there was no statistical significance for the variable 'treatment' (relative hazard 1.06,  $p=0.77$ ).

### ***Uterine malignant mixed Müllerian tumors***

In univariate analysis with survival as the end-point and treatment as the independent variable, no statistical significance was found for treatment (relative hazard 0.92,  $p=0.66$ ). Likewise, in a subgroup analysis with only stage I tumors (21 patients) there was no statistical significance (relative hazard 0.71,  $p=0.38$ ).

If the same variables were included in a univariate analysis for stage I-II tumors (23 patients) with disease-free survival as the end-point, no statistical significance was found for the variable 'treatment' (relative hazard .65,  $p=0.29$ ).

### ***Endometrial Stromal Sarcoma and Adenosarcoma***

Additional treatment with radiotherapy and chemotherapy had no impact on survival or disease-free survival for these two sarcoma groups.

### ***Ovarian malignant mixed Müllerian tumor***

Various chemotherapeutic regimens were administered to 22 patients. With survival as the end-point, there were no statistical significance for treatment in a univariate analysis when platinum based regimens were compared to non-platinum based regimens ( $p=0.77$ ).

## Discussion

### *Leiomyosarcoma*

Uterine leiomyosarcomas are aggressive tumors with a high mortality rate and a 5-year survival rate of 30-40% [5,88]. Stage has been found to be a significant predictor of survival in both univariate and multivariate analyses [5,51,52,89]. We also found that stage is the most important predictor of survival. Furthermore, in stage I patients, grade was found to be the best prognostic factor in a multivariate analysis.

Only a few reports have analyzed DNA ploidy of leiomyosarcomas. Nordal et al. found no significance for DNA ploidy in a multivariate analysis of 58 patients with uterine leiomyosarcoma [5]. Similarly, Amada et al. found no significant correlation between DNA ploidy pattern and prognosis in 22 patients with uterine leiomyosarcoma [90]. However, Tsushima et al. did find increased survival for patients with DNA diploid tumors compared to those with non-diploid ones [91]. In a multivariate analysis Peters et al. demonstrated that only SPF and presence of extrauterine disease were adverse factors [4]. In our study we found that DNA ploidy and SPF were highly significant in both univariate and multivariate analyses. Diploid tumors and those with SPF <10% were less aggressive.

We found that 26% of uterine leiomyosarcomas overexpressed p53. Liu et al. demonstrated p53 overexpression in one of four (25%) uterine leiomyosarcomas [92], Amada et al. in eight of 24 (33%) [90], and Niemann et al. in 16 of 34 (47%) [93]. P53-positivity was found in 14 (40%) of 35 extrauterine leiomyosarcomas by O'Reilly et al. [94]. The importance of p53 in our study increased when stage IV was excluded. The number of p53 positive cases increased from stages I-II to stage III, whereas only one of 12 stage IV tumors overexpressed p53. For disease-free survival among early stage leiomyosarcomas we found that p53 was highly significant in both univariate and multivariate analysis. Tumors with overexpression of p53 all recurred within 28 months of diagnosis.

Only 8% of our leiomyosarcomas overexpressed mdm-2 and this had no significance in univariate analysis. Various studies have found an increased expression of mdm-2 in soft tissue sarcomas [95-97]. Using Southern blot analysis Patterson et al. found that only two (7%) of 29 leiomyosarcomas demonstrated overexpression of mdm-2 [98].

### *Uterine Malignant Mixed Müllerian Tumors*

Malignant mixed Müllerian tumors of the uterus are very aggressive tumors with a high mortality rate and a 5-year survival rate of 18-41% [8,9,99,100]. Stage has been found to be a significant predictor of survival [8,9,99-101]. To our knowledge no previous report has studied the prognostic implications of DNA ploidy in uterine MMMTs alone. In an univariate analysis of survival we found no statistically significant prognostic value for either DNA ploidy or SPF.

We found that 64% of the MMMTs overexpressed p53. Costa et al. demonstrated a positive reaction in 33/56 (58.9%) of uterine MMMTs [102]. Swisher et al. found p53 overexpression in six (30%) out of 20 patients [103]. Similarly, Mayall et al. found p53-positivity in five (30%) of 17 uterine MMMTs [29]. Both Liu et al. and Berchuck et al. found that 74% of MMMTs overexpressed p53 [92,104]. On the contrary, Iwasa et al. found p53 overexpression in only six out of 25 uterine MMMTs [105]. As other authors have seen, the MMMTs showed concordance in p53 staining between the carcinoma-

tous and the sarcomatous components [29,102]

In a recent study Seki et al. found with PCR technique three of eight (38%) uterine MMMTs with amplification of mdm-2 and 1/8 (13%) with both mdm-2 amplification and p53 overexpression [106]. In line with this, we found that 11 (26%) of the tumors overexpressed mdm-2, and eight (19%) of these simultaneously overexpressed p53. In a study of 211 adult soft tissue sarcomas, Cordon-Cardo et al. found that 36% (76/211) were mdm-2 positive and 27% (56/211) overexpressed p53. Twenty-two (10%) of these tumors overexpressed both p53 and mdm-2 [39].

The mitotic index reached statistical significance in the survival analysis only for early stage (stage I and II) tumors. Tumors with an index <20/10 hpf had a 5-year survival of 82% as opposed to 28% for those with ≥20/10 hpf. When higher (III and IV) stage tumors were included in the analysis this variable was not significant.

### ***Endometrial Stromal Sarcoma***

Endometrial stromal sarcoma is a heterogeneous histopathological entity and considered less aggressive compared to other uterine sarcomas, and a better outcome is often reported [107]. Our study showed a good correlation between histopathological features and DNA cytometric values and supports the conclusion that not only mitotic index but also tumor stage and grade influence the outcome [15,107-110].

We found, as did Mansi et al., that the distinction between high and low grade ESS is of major importance in determining the prognosis [11]. Most of the low-grade ESS were low-stage, had a low mitotic index, were DNA diploid with a low SPF, and were p53 and mdm-2 negative. Only two low-grade ESS metastasized; both were DNA diploid with a low SPF and had no mdm-2 overexpression although one was p53 positive. What surprised us was the fact that five (45%) of the early stage, low-grade ESS recurred, but there were no clinicopathological differences between the recurrent and non-recurrent ones.

For high-grade ESS we found that most were high stage, had a high mitotic index, were DNA aneuploid with a high SPF, and were p53-positive. All four patients with high-grade tumors died of disease within 14 months. This is in agreement with El-Naggar et al. [16], August et al. [111] and Hitchcock et al. [112].

Of the nine patients who died, only three overexpressed mdm-2 and four were p53-positive. To our knowledge there are no previous reports on mdm-2 overexpression in ESS. As for p53 expression in ESS, the reports are few. Liu et al. noted one patient with an ESS that was p53-negative [92]. The numbers are small but it appears that p53-and mdm-2 overexpression do not add prognostic information to grade.

As in other studies we found that FIGO surgical stage is an important prognostic factor [51,52,107,110]. In patients with advanced disease the median survival was only 13 months, whereas in patients with early stage tumors the median survival was not reached.

### ***Adenosarcoma***

Clement and Scully first described adenosarcoma of the uterus in 1974. This tumor type is composed of two intermixed neoplastic tissues with a benign epithelial component and a sarcomatous stromal component [113]. Like MMMT, the sarcomatous component sometimes harbors heterologous elements. Kaku et al. found that 10 of 17 (59%) tumors with sarcomatous overgrowth contained areas of rhabdomyosarcoma, and five of these patients died of disease [17]. In our study we found two adenosarcoma with sarcomatous overgrowth with heterologous elements composed of rhabdomyosarcomatous

tissue, and one patient is dead of disease while the other is alive with no evidence of disease at nine years after diagnosis.

In a literature review, Gollard et al. found 18 patients with distant recurrences and fifteen (83%) of these died of disease. The recurrences were almost exclusively sarcomatous [114]. Clement and Scully reported that recurrences develop in approximately 25% of cases and distant metastases occur in fewer than 5% [19]. In the series by Zaloudek et al., recurrences occurred in 10 of 25 cases (40%) [20]. This is in line with our study where five (45%) patients had recurrences. Three of these had distant recurrences with lung metastases, and all three died of disease. Two of the recurrences had pathological verification and both were composed of pure sarcoma.

Several reports have found that the risk of recurrence increases with myometrial invasion [17,19,20]. In our study three tumors that recurred had no myometrial invasion and two had < 50% myometrial invasion. In three of these cases, all pure adenosarcomas, recurrences appeared three or more years after diagnosis. This is in line with Clement et al. and Zaloudek et al. [19,20].

Generally, adenosarcoma has been regarded less aggressive compared to MMMT. In the present study, the 5-year survival rate was 80% compared to 38% for uterine MMMT seen in our department. Kaku et al. found that 5/16 (31%) patients with sarcomatous overgrowth died of disease compared to 1/14 (7%) of patients without sarcomatous overgrowth. They suggested that, sarcomatous overgrowth represents an important prognostic factor [17]. Clement has suggested that the overgrowth of pure sarcoma may represent a secondary tumor that arises in the adenosarcomatous stroma [18]. Clement and Scully concluded that sarcomatous overgrowth has a malignant potential similar to that of other high-grade uterine sarcomas such as MMMT and leiomyosarcomas [19]. Indeed, the 5-year survival rate was only 50% in our series.

In a recent study by Kerner et al., eleven adenosarcomas were evaluated for DNA ploidy and SPF. They found eight (73%) DNA diploid tumors and three (27%) DNA aneuploid tumors. All the DNA diploid tumors had a low SPF and two DNA aneuploid tumors had a high SPF [115]. In our study we found that all tumors with sarcomatous overgrowth were DNA aneuploid tumors and three of these had an SPF greater than 10%. On the other hand, three of six pure adenosarcomas were diploid; five had an SPF less than 10%, while one SPF was not assessable.

p53 expression in adenosarcoma has been recently investigated by a few authors. Swisher et al. found no p53 overexpression among six tumors [103]. In contrast Kerner et al. found that p53 expression was strongly positive in 9/11 tumors [115]. We found p53 positivity among two cases with sarcomatous overgrowth. We are not aware of reports on mdm-2 overexpression in adenosarcoma. We found overexpression of mdm-2 in one tumor with sarcomatous overgrowth and one pure adenosarcoma. Our material is small and it is not possible to draw any conclusions regarding the prognostic value of p53 positivity and mdm-2 overexpression.

The histogenesis of adenosarcoma is unclear. Most reports suggest a common origin for both tumor components [116]. Other authors suggest that the epithelial component may represent benign endometrial glands entrapped within the malignant stromal component [117]. The fact that overexpression of p53 was seen only in the mesenchymal component and not in the epithelial elements of two of our cases lends support to a biclonal nature of these tumors where the benign glands represent a separate and simultaneous clonal proliferation of glandular epithelium.

### ***Ovarian malignant mixed Müllerian tumor***

Ovarian MMMTs are aggressive tumors that are often diagnosed in the postmenopausal age and at an advanced stage of presentation. Survival rates have been very low, although recent studies have reported a favorable outcome for early stage tumors [118]. Several reports have concluded that stage is the only independent prognostic factor for survival [119,120].

Compared to homologous, we found that heterologous tumors were most likely to present at an advanced stage, were more often p53-positive and tended to have a more sarcomatous component with higher mitotic index. As seen in most reports, the distinction between homologous and heterologous ovarian MMMTs did not have prognostic significance [118,119]. Our patients with heterologous MMMTs tended to have decreased survival (15 months versus 57 months); however, this was not statistically significant ( $p=0.2$ ). Other investigators have noted that heterologous tumors tend to behave more aggressively [121,122]. Recently, Barakat et al. did find a trend ( $p=0.06$ ) toward improved survival in patients with homologous MMMTs while Iwasa et al. noted a significantly better survival for homologous tumors ( $p=0.04$ ) [105,119]. As noted also by Barakat et al., we found that heterologous MMMTs tended to metastasize with sarcomatous elements while homologous MMMTs did so more often with carcinomatous metastases. In addition, patients with sarcoma-containing metastases had a somewhat worse survival ( $p=0.2$ ) [120].

DNA flow cytometric analysis revealed that, not surprisingly, almost all ovarian MMMTs are non-diploid with a high SPF. Thus, this method could not be used in the prognostic assessment of such tumors.

Kounelis et al. analyzed p53 expression in 43 MMMTs of the female genital tract and found that 94% of uterine and 73% (8/11) of ovarian MMMTs were p53-positive. Most of the tumors showed similar staining in both carcinomatous and sarcomatous components. In addition, in nine of their cases they found identical p53 point mutations in each component. They concluded that such findings support the monoclonal multidirectional histogenesis of MMMTs [27]. Costa et al. found that 73% (8/11) of ovarian MMMTs were p53-positive. They also detected a concordant staining of both components in 75% (34/45) of the p53-positive female genital tract MMMTs. This observation led them to support the histogenetic theory of either a metaplastic origin of the sarcomatous component from a carcinoma or a derivation of both components from a single stem cell [102]. We also found a frequent (73%) overexpression of p53 in ovarian MMMTs, as well as positive staining for p53 in both components of most (89%) of the p53-positive tumors. However, analogous to uterine MMMTs, p53 overexpression in ovarian MMMTs did not appear to be useful as a prognostic indicator.

These findings lend support to the metaplastic theory of transformation of carcinomas to MMMTs. It appears that MMMTs represent carcinomas in which a number of cells initially invade its stroma in a pseudosarcomatous fashion. At this point the transition between the carcinoma and the spindle sarcomatoid cells is identifiable as foci where the two components merge. Thereafter, the sarcomatoid cells gradually lose their epithelial phenotype, travel throughout the tumor's stroma and surround epithelial glands and nests so that one sees a sharp demarcation between carcinoma and sarcoma. As the sarcomatous cells proliferate they may also undergo (neo)metaplasia and develop heterologous features.

One may presume that so-called 'pseudosarcomatous' or 'spindle cell' carcinomas, as well as MMMTs with a small (<5%) sarcomatous component, represent transitional forms within the progression of a poorly differentiated carcinoma towards a full blown MMMT. Whether such transitional groups of tumors have a behavior similar to classic

MMMTs or to high-grade carcinomas has yet to be investigated. However, this may be a futile task if future studies fail to witness any definite biological and clinical difference between comparably treated MMMTs and carcinomas of similar grade and stage.

Terada et al. found that the presence of chondrosarcomatous areas in heterologous MMMTs indicated a better prognosis when compared to those without this component [123]. We did not find any statistically significant difference between these two groups; however, among our patients with heterologous MMMT, the two patients devoid of chondrosarcomatous areas were the first to die of tumor. Boucher and Têtu found that MMMTs with a rhabdomyoblastic component had a less favorable outcome, a finding that was replicated in our study [124]. In fact, all three such patients were dead of disease within 17 months after diagnosis. Even though Boucher and Têtu found that tumors with a predominant mesenchymal component fared worse than those with a predominance of epithelial elements, we found no significant difference in survival between these two groups [124].

It has been reiterated that ovarian MMMTs occur mostly in nulliparous women; however, only 27% (7/26) of our patients were nulliparous. Indeed, the reported rates of nulliparity in patients with ovarian MMMTs has ranged from 11% to 61% [121,125]. Upon a review of the literature, it would appear that most patients with ovarian MMMT are parous, albeit often of low parity, and that the actual rate of nulliparity is about 33% [118,121-129].

### *Comments on pathological review of uterine sarcomas*

Out of 186 uterine sarcomas referred to the Department of Gynecologic Oncology during 1970-1996, 65 (35%) cases were excluded from the study. The majority of these (51 cases, 27%) were excluded because the sarcoma diagnosis could not be confirmed at the histopathological re-evaluation. Six cases were excluded from the study since the pathological material was not available for review (Table 1). In a histopathological review of a 10% sample (159 cases) of uterine sarcomas from the Swedish Cancer Registry, Larson et al. found that 18% of the cases could not be reclassified as sarcomas. Two-thirds of the over-diagnosed cases were benign leiomyomas originally judged as leiomyosarcomas, which means that 20% of the leiomyosarcomas were reinterpreted as leiomyomas [130]. In a report by Hart et al. 28 uterine tumors originally diagnosed as leiomyosarcomas were histologically reviewed, thirteen (46%) neoplasms were reinterpreted as leiomyomas. In 15 cases the diagnosis of leiomyosarcoma was confirmed [131]. In other reported series of leiomyosarcomas as much as 33% to 60% of the cases have been reclassified as leiomyomas [132,133].

Furthermore, Larson et al. found within the group of confirmed sarcomas in the sample from the Cancer Registry that there was a 23% discordance between the original and the review diagnosis with respect to histopathological subgroups. After reevaluation there was concordance in classification in 69/101 (68%) LMS, 23/29 (79%) MMMTs and 2/16 (13%) ESS [130]. These findings are in line with our study. In fact, we found that 31 (37%) out of 83 tumors originally diagnosed as leiomyosarcomas were reclassified as non-sarcomatous smooth muscle tumors. The numbers of concordantly classified cases were 44/83 (53%) for LMS, 45/63 (71%) for MMMTs, and 14/23 (61%) for ESS (Table 1).

The over-diagnosis of leiomyosarcomas reflects the difficulties in recognizing this rare tumor type. Hart et al. points out that the opportunities for a practicing pathologist to diagnose leiomyosarcomas of the uterus are limited. Overzealous diagnosis of cellular and pleomorphic leiomyomas as leiomyosarcomas appears to be a more common problem than underdiagnosis of sarcoma [131].

### *Treatment of uterine and ovarian sarcomas*

It is believed that uterine sarcomas have a low radio- and chemosensitivity and, thus, the main treatment is surgical extirpation of the tumor. Early stage uterine sarcoma has a high recurrence rate and metastatic capacity with a 5-years survival rate of 20-67%. In an attempt to improve survival it is desirable to give adjuvant therapy. Unfortunately, most studies have discouraging results.

Prospective studies of uterine sarcomas with radiotherapy and/or chemotherapy are sparse. Usually all subtypes are included in broad studies and the tumors are regarded to respond in a similar fashion to treatment.

In the present study we found statistical significance for treatment when including leiomyosarcoma of all stages as well as only early-stage (stage I) tumors. The interpretation of these findings should be made with caution, but it appears that patients with early-stage tumors do not benefit from adjuvant treatment (Fig. 2).

A summary of disease-free survival and overall survival rates after different treatment modalities in early-stage sarcomas are given in table 7. In most studies there is no or, at the most, a limited benefit of adjuvant treatment (radiation treatment as well as chemotherapy). In addition to the reports described in table 7, in a study including 64 stage I uterine sarcomas Rose et al. found a decreased recurrence rate for ESS but not for leiomyosarcomas treated with adjuvant radiation. Chemotherapy alone did not decrease recurrence rate in stage I sarcomas [56]. Ayhan et al. retrospectively evaluated 88 patients with uterine sarcoma. For patients with stage I tumors treated with and without adjuvant therapy there were no difference between survival rates. They concluded that patients with stage I disease should be followed without any form of adjuvant therapy until a clearly superior form of adjuvant therapy is developed [53].

Regarding advanced and metastatic uterine sarcomas the reports are confusing and contradicting. In most studies doxorubicin-containing combinations have been used. A summary is presented in table 8. In addition, Muss et al. treated 29 patients with metastatic leiomyosarcoma or MMMTs with mitoxantrone; no responses were seen with this treatment [64]. Seltzer et al. reported in a small study with six patients a 50% complete remission rate and 33% partial remissions in patients with advanced uterine MMMT treated with doxorubicin and cisplatin. This is the highest response reported in the literature for this tumor type [73]. In a French retrospective study of 203 patients with uterine sarcomas, George et al. found decreased 2-year recurrence and metastasis rates with addition of radiotherapy or chemotherapy, but no benefit on survival rates [54].

Katz et al. and other investigators have explored the role of estrogen and progesterone receptor measurements in ESS. Patients with low-grade ESS tend to have tumors positive for steroid receptors and usually respond to hormonal therapy [134]. The level of receptors is generally higher in this group of tumors than in the other sarcoma subtypes, such as leiomyosarcomas, MMMTs and high-grade ESS [135]. In line with Berchuck et al., we found that among patients with low-grade, early stage ESS there was a higher recurrence rate in those with residual ovarian tissue (3/5) than in those without residual ovarian tissue (2/6) [108]. Piver et al. evaluated the outcome of 52 low grade ESS. Fifty percent developed recurrence and among the stage I tumors, 47% recurred in the pelvis and 9% in distant sites after initial therapy. In total, of the 13 patients treated with hormonal therapy for recurrent disease, three had a complete response, and three had a partial response. Twelve patients were treated with chemotherapy and only 17% responded. The authors concluded that low grade ESS demonstrates hormonal sensitivity with therapeutic implications. The study further documents the limited response to systemic chemotherapy [71].

Sutton et al. conducted a prospective phase II study with ifosfamide treatment of recurrent or metastatic ESS. Twenty-one patients were evaluable for response, three patients had complete response and four had a partial response, yielding an overall response rate of 33.3% [72].

The effect on disease-free survival and survival rate of adjuvant progestin in early stage low-grade ESS is unknown; no studies have been reported.

To my knowledge there is no separate report regarding treatment of uterine adenosarcoma.

As in most studies of uterine sarcomas, the present study is hampered by the small study population and therefore definitive conclusions regarding treatment should be drawn with caution. A few studies have shown a decreased pelvic recurrence rate following adjuvant radiation therapy; however, the survival rate has not been improved with radiation therapy as patients succumb to metastatic disease outside the radiated area [55,56]. In MMMTs chemotherapy seems to have some activity (i.e., cisplatin, ifosfamide and doxorubicin), but for leiomyosarcomas the activity of chemotherapy is limited in most studies. In the present study, all stages of leiomyosarcoma as well as early stage leiomyosarcomas treated adjuvantly after surgery did worse. One possible explanation for this may be that the clinicians, on the basis of clinical experience, can select patients with higher risk of tumor recurrence and mortality.

In conclusion there is no definitive evidence that postoperative adjuvant pelvic radiotherapy is beneficial in preventing pelvic recurrence. There is one ongoing EORTC trial with adjuvant postoperative external radiation treatment to the entire pelvis and we will have to wait for the results of this trial. The efficacy of postoperative adjuvant chemotherapy in uterine sarcomas has not yet been demonstrated; thus, after a staging procedure patients with early stage uterine sarcomas may be followed without any adjuvant treatment (unless the patient is included in a randomized trial). One future task is to include early stage uterine sarcomas in multicenter, randomized trials with chemotherapy including cisplatin, ifosfamide and/or doxorubicin. The role of adjuvant progestin treatment in early stage low-grade ESS is unknown. Recurrent and metastatic uterine sarcoma remains an incurable disease, and treatment must be considered palliative. Cancer specific survival for uterine sarcoma in relation to histologic type is shown in table 3.

Concerning ovarian MMMTs, it has often been stated that this tumor type is more aggressive than high-grade carcinomas of similar stage. This has been recently challenged by Bicher et al. who reported on 36 patients with ovarian MMMTs treated with platinum-based chemotherapy and found that they had a clinical course similar to that of similarly treated patients with high-grade epithelial carcinoma of the ovary [2]. Among our eight cisplatin-treated Stage III-IV patients with MMMT the survival rate (12% at 2 and 5 years) and median survival (11 months) were somewhat worse than the survival rate (48% at 2 years; 17% at 5 years) and median survival (24 months) observed among 46 patients with Stage III-IV ovarian serous carcinomas treated with platinum-based chemotherapy at our department (log rank  $p=0.17$ ; unpublished data).

In the present study several different chemotherapy regimens were used. As for uterine sarcomas, the effect of chemotherapy on disease-free survival and survival is limited.

## Conclusions

For **uterine leiomyosarcoma** we found that stage represents the most important prognostic factor. DNA flow cytometry is useful in gaining additional prognostic information. In patients with stage I tumors, tumor grade gives significant information regarding clinical outcome. Interestingly, we found that p53 overexpression had prognostic value for disease-free survival in stage I-II tumors. This result indicates that early-stage leiomyosarcomas with p53 overexpression have a higher risk of recurrence.

For **uterine malignant mixed Müllerian tumors** we found that the majority had signs of aggressiveness such as high grade, high mitotic index, non-diploidy, high SPF and overexpression of p53. In univariate analysis, stage remains the most important prognostic factor. For early stage tumors mitotic index is an important prognostic factor.

For **endometrial stromal sarcomas** we found that tumor grade was a strong predictor of clinical outcome. In addition, advanced disease, DNA aneuploidy and high SPF correlated with a worse prognosis. All patients with high-grade tumors died of disease within 14 months of diagnosis, as compared to, only three of the ten patients with low-grade early-stage tumors.

We found that pure **adenosarcoma** presented at a lower stage, were of lower grade with a lower mitotic index, were less often DNA aneuploid and had a lower SPF than tumors with sarcomatous overgrowth. None of these variables correlated with survival.

For **ovarian malignant mixed Müllerian tumors** we found that the vast majority were aneuploid with a high SPF. Most were also p53-positive and mdm-2-negative. Stage and, to a lesser degree, residual tumor at primary surgery remain the most important prognostic indicators for these highly lethal tumors. Our findings also support the metaplastic histogenesis (conversion theory) of malignant mixed Müllerian tumors from high-grade carcinomas.

**Treatment** of sarcomatous neoplasms is difficult and the mainstay is surgical removal of the tumor. Patients with early stage sarcoma have a relatively high recurrence rate, which may be explained by the fact that a large proportion of patients must have systemic micrometastatic disease at the time of diagnosis. We found no benefit from postoperative adjuvant treatment of leiomyosarcomas with radiotherapy and/or chemotherapy. Recurrent and metastatic uterine sarcoma remains an incurable disease, and treatment must be considered palliative.

## Acknowledgment

During the years many have contributed and helped me in a variety of ways to complete this study. I wish to express my sincere gratitude to all those who have contributed and supported me during this period, and especially I would like to thank:

**Ernst Simonsen**, former Head of the Department of Gynecologic Oncology, my tutor and friend who took care of me like a father and introduced me to the art of gynecologic oncology and helped me getting started with medical research. I appreciate his support and help.

**Claudio Guerrieri**, MD, Pathologist, Department of Pathology of St. Vincent's Hospital, New York City, USA, my co-writer and pillar of support. His enormous skill, helpfulness and ability to cooperate have impressed me. His precision and knowledge have been outstanding. He has made this thesis possible.

**Henric Malmström**, my assistant tutor for introducing me with enthusiasm in the field of science.

**Olle Stål**, my assistant tutor who introduced and helped me with the DNA flow cytometry and immunohistochemistry, and guided me through the statistical jungle. His generous support and kindness are admirable.

**Bo Nordenskjöld**, for support and help throughout my work with the thesis.

**John Carstensen**, for advice and help concerning the statistics.

**Gun Johannesson** for assisting with the DNA flow cytometry, **Helén Richard** and **Birgitta Frånlund**, staff of the Department of Pathology, for help with the pathologic specimens and slides.

**Siw Sullivan** for teaching me with enthusiasm the art of immunohistochemistry (and how to act in a laboratory).

My **colleagues and friends** at the Department of Gynecologic Oncology and Department of Oncology for help and support and **Annika Håkansson**, the schedule manager and my room mate for making it possible for me to be off duty during the finale work of this thesis.

The **secretarial staff** at the Department of Gynecologic Oncology and, especially, **Heléne Karlsson** for support and help with the patients' file.

The **medical staff** at the Department of Gynecologic Oncology for good care.

**Chatarina Malm** for help with all 'practical' things.

The **pathology departments** of the county hospitals of Kalmar, Jönköping, Norrköping and Malmö and of the University Hospital of Lund for providing pathological material.

My family **Sofia, Christoffer** and **Isabella** for all their love, care and support.

My sister **Anni** for all your love, kindness and support.

Finally, to **all** not mentioned here who I owe thanks.

The study was supported by a grant from the Linköping Medical Association, the Research Foundation of the University of Linköping, and the Research Foundation of the Department of Gynecologic Oncology.

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**Table 1.** The histological distribution before and after reevaluation of uterine sarcomas.

<b>Histopathology after reevaluation</b>	No of patients	<b>Histopathology before reevaluation</b>				
		LMS (n=83)	MMMT (n=63)	ESS (n=23)	Adenosarcoma (n=4)	Unclassified (n=13)
Benign leiomyoma	9	8		1		
Atypical leiomyoma	6	6				
Smooth muscle tumor of low malignant potential	3	3				
Leiomyoma with increased mitotic activity	13	13				
Leiomyosarcoma	49	44	1	2		2
Malignant mixed Müllerian tumor	47	1	45			1
Endometrial stromal sarcoma	17	1		14		2
Adenosarcoma	11	1	4	1	4	1
Unclassified	3	2	1			
Unknown	13		6	3		4
Other malignancy	3	1		1		1
Benign tumor	2		1	1		
Endometrial carcinoma	2	1	1			
“Mixed leiomyosarcoma”	1					1
“Myxoid leiomyosarcoma”	1	1				
Not available	6	1	4			1

**Table 2.** Grading system of uterine leiomyosarcomas.<sup>a</sup>

<b>GRADE</b>	<b>NECROSIS</b>	<b>ATYPIA</b>	<b>MITOSES/HPF</b>
Grade 1:	No	Moderate-Severe	10 or more <sup>b</sup>
Grade 2:	Yes	Mild	10 or more
		<i>or</i>	
	Yes	Moderate-Severe	less than 20
Grade 3:	Yes	Moderate-Severe	20 or more

<sup>a</sup> the grading categories are adapted from ‘regions’ of the classification system for uterine smooth muscle tumors by Bell et al. [3].

<sup>b</sup> tumors with no necrosis, moderate-severe atypia but less than 10 mitoses are also included in the grade 1 category if they possess 2 or more atypical mitotic figures.

**Table 3.** Treatment of ovarian MMT, homologous type.

Patient no.	Age	Stage	Surgery	Radical surgery	Treatment***	Second look	Treatment after second look
1	79	3c	BSO	No	Postoperative intracavitary brachyradiotherapy (9.6 Gy) + Me		
2	79	3c	TAH+BSO	No	E		
3	76	2c	BSO	No	None		
4	73	2c	BSO	No	C-E	neg	C-E
5	74	3c	TAH+BSO	No	C		
6	67	2b	TAH+BSO+O	Yes	C-E		
7	70	2c	BSO+OT*	Yes	I-E-M	neg	None
8	58	3c	BSO	No	Mel-D	pos	None
9	62	1a	TAH+USO**	Yes	C-D		
10	60	1a	BSO	Yes	Postoperative xRT (30 Gy) to the small pelvis		
11	66	3c	TAH+BSO+O T	No	I-E-M	pos	I-E-M
12	61	1c	BSO*	Yes	E		
13	64	3b	TAH+BSO+O T	No	I-E-M		
14	42	2c	TAH+BSO+O	Yes	C		

**Table 4.** Treatment of ovarian MMT, heterologous type.

Patient no.	Age	Stage	Surgery	Radical surgery	Treatment***	Second look/ Second effort	Treatment after second look
15	70	3c	BSO	No	xRT (40 Gy) to pelvis + Cy-V-D	TAH (pos)	None
16	72	3c	TAH+USO+O T	No	None		
17	69	2b	BSO+OT	No	xRT (24 Gy) to pelvis	Second-look (pos)	
18	71	4b	BSO+OT	No	C-E	TAH (neg)	C-E
19	67	3c	TAH+BSO+O T	Yes	C-E		
20	65	4b	EL	No	C-E	TAH+BSO+O T	C-E
21	63	3c	TAH+BSO+O T	No	C-E	Second look (pos)	C-Et intraperitoneal
22	59	3c	EL	No	C-D	TAH+BSO+O T	Postoperative xRT <sup>o</sup>
23	69	3c	TAH+BSO	No	I-E-M		
24	63	3c	TAH+BSO+O T	No	C-E		
25	49	3c	EL	No	C-E	TAH+BSO+O T	C-E
26	56	3c	TAH+BSO+O T	No	I-E-M		

Abbreviations for tables 3 and 4.

TAH, Total abdominal hysterectomy; BSO, bilateral salpingo-oophorectomy; USO, unilateral salpingo-oophorectomy; OT, omentectomy; EL, exploratory laparotomy; \*previous TAH; \*\*previous USO; <sup>o</sup> Toronto field (40 Gy to the small pelvis and 20 Gy to abdomen); xRT, external radiation treatment  
 \*\*\* chemotherapy agents: C, cisplatin; D, doxorubicin; E, epirubicin; I, ifosfamide; M, mesna; Cy, cyclophosphamide; V, vincristine; Da, dactinomycin; Mel, melphalan; Me, methotrexate; Et, etoposide.

**Table 5.** Tumor characteristics for uterine sarcomas and ovarian malignant mixed Müllerian tumors in relation to DNA ploidy, SPF, p53 and mdm-2.

Characteristics	No of patients	DNA ploidy (%)			S-phase fraction (%)		p53 (%)		mdm-2 (%)	
		Diploid	Aneuploid	Multiploid	≤ 10	>10	Negative	Positive	Negative	Positive
<b>Leiomyosarcoma</b>	49	7 (16)	28 (65)	8 (19)	13 (33)	26 (67)	36 (73)	13 (27)	45 (92)	4 ( 8)
<b>Endometrial Stromal Sarcoma</b>										
Low grade	13	12			12		11 (85)	2 (15)	12 (92)	1 ( 8)
High grade	4	1 (25)	3 (75)		2 (50)	2 (50)	1 (25)	3 (25)	2 (50)	2 (50)
<b>Adenosarcoma</b>										
AS	6	3 (50)	2 (33)	1 (17)	5		6		5 (83)	1 (17)
AS-SO	5		5		2 (40)	3 (60)	3 (60)	2 (40)	4 (80)	1 (20)
<b>Uterine MMMT</b>										
Homologous	32	12 (38)	11 (34)	9 (28)	10 (34)	19 (66)	10 (31)	22 (69)	22 (71)	9 (29)
Heterologous	12	2 (17)	9 (75)	1 ( 8)	3 (27)	8 (73)	5 (50)	5 (50)	10 (83)	2 (17)
<b>Ovarian MMMT</b>										
Homologous	14		8 (67)	4 (33)	2 (18)	9 (82)	7 (50)	7 (50)	13 (93)	1 ( 7)
Heterologous	12		7 (58)	5 (42)		12		12	11 (92)	1 ( 8)

Abbreviations: AS, pure adenosarcoma; AS-SO, adenosarcoma with sarcomatous overgrowth. MMMT, malignant mixed Müllerian tumors.

**Table 6.** Prognostic factors for survival in uterine sarcomas and ovarian malignant mixed Müllerian tumors.

Characteristics	No of patients	FIGO surgical stage	DNA ploidy	S-Phase fraction	Nuclear atypia	Mitotic index	Tumor grade	Residual tumor size (<1cm)
<b>Leiomyosarcoma</b>	49	++	++	++	+	+	+	
<b>Endometrial stromal sarcoma</b>	17	+	+	+			+	
<b>Adenosarcoma</b>	11							
<b>Uterine malignant mixed Müllerian tumor</b>	44	+						
<b>Ovarian malignant mixed Müllerian tumor</b>	26	++						+

+ statistical significant in univariate analysis

++ statistical significant in multivariate analysis

**Table 7.** Treatment, recurrence- and survival rate for early stage uterine sarcomas.

Reference	No of patients	Study type	Histopathology#	Treatment (after surgery)*	Findings for adjuvant treatment
Hannigan et al.[58] (1983)	101	Retrospective	LMS/MMMT/ESS/other 21/70/9/1	Radiation (n=101). VAC (n=17). Adriamycin containing regimen (n=17)	No improvement in disease-free survival or survival rate after adjunctive chemotherapy
Omura et al.[57] (1985)	156	Prospective/ Randomized	LMS/MMMT/other 48/93/15	Adriamycin 60 mg/m <sup>2</sup> every 3 weeks for eight courses (n=75). Radiation therapy “optional”	No improvement in disease-free survival and survival rate for Adriamycin treatment
Sorbe [55] (1985)	59 (stage I-II)	Retrospective	LMS/MMMT/ESS/other 37/28/19/3 (stage I-IV)	“Chemotherapy” (n=17). Radiation therapy (n=18)	Adjuvant therapy reduce failure (recurrence) rate. No improvement in survival rate
Barter et al.[59] (1985) (1985)	18	Retrospective	LMS	Adriamycin containing regimen (n=8). VAC (n=2). Radiation and MAC (n=3)	No improvement in survival rate for patients treated with adjuvant chemotherapy
Hornback et al.[74] (1986)	157	Prospective/ Randomized	LMS/MMMT/ESS/other 48/95/10/4	Adriamycin 60 mg/m <sup>2</sup> every 3 weeks for eight courses (n=75). Radiation therapy “optional”	No improvement in progression-free interval or survival rate for Adriamycin treatment
Berchuck et al.[67] (1988)	34	Retrospective	LMS	“Chemotherapy” (n=7). Radiation therapy (n=4). Radiation and Adriamycin (n=1)	Recurrence rate-Adjuvant treatment: 10/12 (83.3%). No adjuvant treatment: 15/22 (68.2%)
Resnik et al.[75] (1995)	23	Prospective	MMMT	EPA	2 year progression-free survival 83%. 2-year overall survival 92%.
Hempling et al.[60] (1995)	20	Prospective	LMS/MMMT/ESS 10/7/3	CY-VA-DIC	No improvement in survival rate

**Table 8.** Treatment, recurrence- and survival rate in advanced or recurrent uterine sarcomas.

Reference	No of patients	Study type	Histopathology#	Chemotherapy regimen*	Findings (conclusion made by the authors)
Hannigan et al.[65] (1983)	74	Retrospective	LMS/MMMT/ESS/other 30/38/5/1	VAC	RR 28.9% (CR 13.3%, PR 15.6%). 2 and 5 years survival rate 23% and 15%, respectively
Hannigan et al.[66] (1983)	39	Retrospective	LMS/MMMT/other 17/19/3	Adriamycin or Adriamycin plus other chemotherapeutic drugs.	RR 10.3%. No responses in Adriamycin alone treatment group
Muss et al.[62] (1985)	104	Prospective/ Randomized	LMS/MMMT/ESS/other 38/51/11/4	Adriamycin 60 mg/m <sup>2</sup> every 3 weeks or Adriamycin plus Cyclophosphamide 500 mg/m <sup>2</sup>	RR 19%. No significant benefit of cyclophosphamide/Adriamycin versus Adriamycin for progression-free interval and survival
Thigpen et al.[69] (1986)	28	Prospective/ Phase II	MMMT	Cisplatin single agent, second line	RR 18% (CR 7%, PR 11%) Cisplatin appears to have activity as second line agent in MMTT
Sutton et al.[68] (1989)	28	Prospective/ Phase II	MMMT	Ifosfamide/Mesna	CR 17.9% (5/28), PR 14.3% (4/28). Ifosfamide is an unusually active drug in patients with MMTT
Peters III et al.[63] (1989)	11	Prospective	MMMT/ESS 8/3	Cisplatin 100 mg/m <sup>2</sup> and Adriamycin 45-60 mg/ m <sup>2</sup>	RR 73%. Combination chemotherapy with Adriamycin and Cisplatin has a high response rate
Hawkins et al.[61] (1990)	21	Prospective	LMS	Ifosfamide/Doxorubicin	RR 10% (2/21). Modest activity of Ifosfamide in LMS.
Thigpen et al.[70] (1991)	96	Prospective	LMS/MMMT 33/63	Cisplatin 50 mg/m <sup>2</sup>	RR 3%/19%. Cisplatin demonstrates activity in MMTT, but it appears to have no role in the treatment of LMS
Currie et al. [76] (1996)	38	Prospective/ Phase II	LMS	Hydroxurea/DTIC/Etoposide	RR 18.4%.

Abbreviations for table 7 and 8. # LMS, leiomyosarcoma. MMTT; malignant mixed Müllerian tumor. ESS, endometrial stromal sarcoma

\* MAC; methotrexate, actinomycin D, and cyclophosphamide. VAC; vincristine, actinomycin D, and cyclophosphamide.

EPA; etoposide, cisplatin, and doxorubicin. MAC; methotrexate, actinomycin D, cyclophosphamide. VAC; vincristine, actinomycin D, cyclophosphamide. DTIC, dacarbazine. CY-VA-DIC cyclophosphamide, vincristine, adriamycin, dacarbazine.