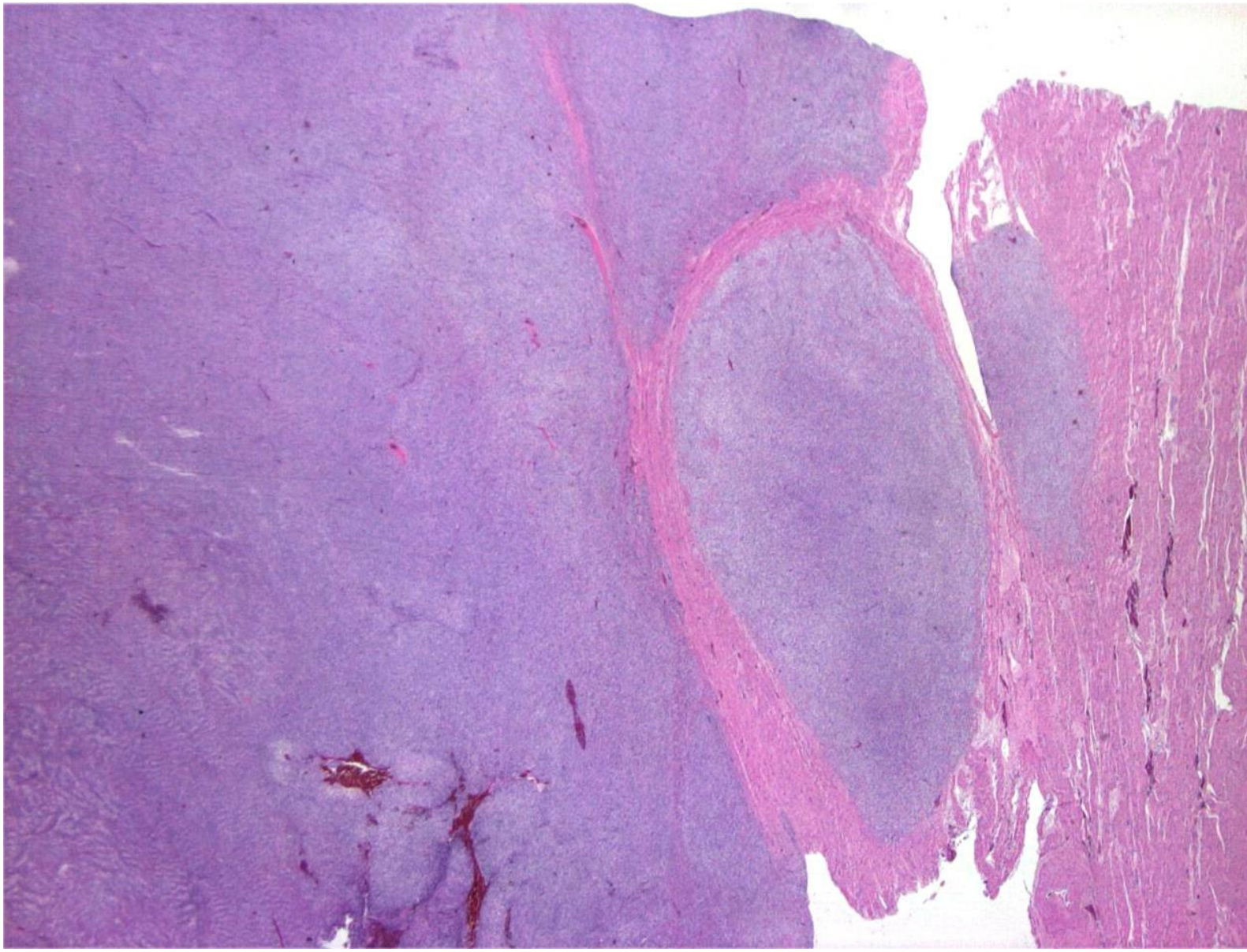
Endometrial stromal sarcoma and antihormonal therapy

Two case reports and review of literature



Stefanie Grüninger
Medical University of Graz
Department of Clinical Oncology
under the guidance of
ao. Univ.-Prof. Dr. Thomas Bauernhofer
2009

Diploma thesis

Endometrial stromal sarcoma and antihormonal therapy

Two case reports and review of literature

submitted by

Stefanie Grüninger

matriculation number: 0311673

for attainment the academic degree

Doktorin der gesamten Heilkunde

(Dr. med. univ.)

at the

Medical University of Graz

carried out at the

Department of Clinical Oncology

under the guidance of

ao. Univ.-Prof. Dr. Thomas Bauernhofer

Graz, ... Signature

Affidavit

I, hereby, declare that the following diploma thesis has been written only by the undersigned and without any assistance from third parties. Furthermore, I confirm that no sources have been used in preparation other than those indicated in the thesis.

Graz, ... *Signature*

Eidesstaatliche Erklärung

Ich erkläre ehrenwörtlich, dass ich die vorliegende Arbeit selbstständig und ohne fremde Hilfe verfasst habe, andere als die angegebenen Quellen nicht verwendet habe und die den benutzten Quellen wörtlich oder inhaltlich entnommenen Stellen als solche kenntlich gemacht habe.

Graz, am …… *Unterschrift*

Acknowledgement

First of all, I would like to thank Ao.Univ.-Prof. Dr.med.univ. Thomas Bauernhofer, Department of Clinical Oncology at the Medical University of Graz, for his support, advice, ideas, organization, documents, the (English-) correction and for his patience during the whole time.

I also want to thank Prim. Univ.-Prof. Dr.med.univ. Sigurd Lax and Sonja Urdl, BMA at the General Hospital Graz West, for their support, the excellent figures and the insight into immunohistochemistry, Dr.rer.nat. Andelko Hrzenjak, Department of Pathology at the Medical University of Graz, for his help, for the immunohistochemical documents and the figures, Ao.Univ.-Prof. Dr.med.univ. Farid Moinfar, Department of Pathology at the Medical University of Graz, for his support, Univ-Prof. Dr.med.univ. Gerhard Ranner, Department of Imaging Diagnostics at the private hospital of the “Kreuzschwestern” for the figures, Ass.-Prof. Dipl.-Ing. Dr.techn. Klaus-Martin Simonic, Department of Medical Informatics, Statistics and Documentation at the Medical University of Graz, for his formal ideas, Mag. Elisabeth Trattner for the English correction and all my friends for their support and understanding.

I would like to express my deepest gratitude to my parents Michaela and Ferdinand Grüninger. They made my studies possible and they supported me in every single way with a lot of patience and understanding. Thank you that you made it possible for me to study medicine. It was my greatest time and I am happily looking forward to carrying out my profession.

Stefanie Grüninger

List of Contents

List of Figures

List of Graphics

List of Tables

Abbreviations

AG	Aminoglutethimide
AGCT	Adult Granulosa Cell Tumor
AR	Androgen Receptor
bp	base pairs
BSA	Bovine Serum Albumin
BSO	Bilateral Salpingo-Oophorectomy
BT	Brachytherapy
CALLA	= CD10
CD	Cluster of Differentiation
CD10	= CALLA, Common Acute Lymphoblastic Leukemia Antigen
CD34	protein used to enrich stem cells from the peripheral blood for stem cell transplantation
CD44v3	glycoprotein for cell-cell and cell-matrix adhesion
CD45	leucocyte common antigen, a transmembrane protein on all hematopoietic cells except plasma cells and erythrocytes
CD99	non-specific marker for Ewing sarcoma and T-cell lymphoma
CD117	KIT, c-kit receptor, altered forms are associated with cancer
cf	confer, it means compare with
c-KIT	also known as CD117, a protooncogene
CL	Cellular Leiomyoma
CR	Complete Response
CS	Carcinosarcoma
CT	Computed Tomography
DAB	Diaminobenzidine
DIC	Died Due to Intercurrent Disease
DNA	Deoxyribonucleic Acid
DNAse	enzyme separating DNA
DOD	Died of Disease
EBT	External Beam Therapy
EGFR	Epidermal Growth Factor Receptor
EMA	Epithelial Membraneous Antigen
ER (α, β)	Estrogen Receptor (alpha, beta)

ERT	Estrogen Replacement Therapy
ESN	Endometrial Stromal Nodule
ESS	Endometrial Stromal Sarcoma
EST	Estrogen Sulfotransferase
ESTSCLE	Endometrial Stromal Tumors with Sex Cord-Like Elements
FDG	Fluor-Deoxyglucose
FFPE	Formalin-Fixed Paraffin-Embedded
FSH	Follicle Stimulating Hormone
GGO	Ground Glass Opacities
GIST	Gastrointestinal Stromal Tumor
GnRH	Gonadotropin Releasing Hormone
GnRHR	Gonadotropin Releasing Hormone Receptor
Gy	Gray
HE	Hematoxylin Eosin
HGESS	High-Grade Endometrial Stromal Sarcoma
HRP	Horseradish Peroxidase
HRT	Hormonal Replacement Therapy
HU	Hounsfield Units
IFADIC	ifosfamide, adriamycin, dacarbacine
IHC	Immunohistochemistry
kDa	kilo Dalton, unit of measurement used for describing the ability of elimination of a membrane
KI67	a proliferation marker
JJAZF1/JJAZ1	a chromosomal translocation between two zinc finger genes
LGESS	Low-Grade Endometrial Stromal Sarcoma
LH	Luteinizing Hormone
LMS	Leiomyosarcoma
LOH	Loss of Heterozygosity
MC	Mast Cell
Melan-A	melanoma specific antigen
MF/HPF	Mitotic Figures per High Power Field
MMP	Matrix Metalloproteinase
MMT	Mixed Müllerian Tumor
MPA	Medroxyprogesterone Acetat

MRI	Magnetic Resonance Imaging
mRNA	messenger Ribonucleic Acid
MSI	Microsatellite Instability
NED	No Evidence of Disease
NFDM	Non Fat Dry Milk
p53	a tumor supressor gene
PBMNC	Peripheral Blood Mononuclear Cells
PCR	Polymerase Chain Reaction
PCNA	Proliferating Cell Nuclear Antigen
PD	Progressive Disease
PDGFR	Platelet Derived Growth Factor Receptor
pH	pondus hydrogenii, dimension for hydrogen ion concentration
PET	Positron Emission Tomography
PR (A, B)	Progesterone Receptor (A, B)
PR	Partial Response
RNA	Ribonucleic Acid
RNAse	enzyme separating RNA
RT-PCR	Reverse Transcriptase Polymerase Chain Reaction
SD	Stable Disease
SDS-PAGE	Sodium Dodecyl Sulfate Polyacrylamide Gel Electrophoresis
SMA	Smooth Muscle Antigen
SFRP	Secreted Frizzled-Related Proteins
TAH	Total Abdominal Hysterectomy
TKI	Tyrosine Kinase Inhibitor
UES	Undifferentiated Endometrial Stromal Sarcoma
UES-P	UES with nuclear pleomorphism
UES-U	UES with nuclear uniformity
US	Ultrasonography
UTROSCT	Uterine Tumors Resembling Ovarian Sex-Cord Tumors
VEGF	Vascular Endothelial Growth Factor
W	Watt
Wnt	network of proteins playing role in embryogenesis and cancer

Abstract

Endometrial stromal sarcomas (ESS) are rare malignant uterine tumors predominantly occurring in pre- or perimenopausal women. ESS commonly express steroid hormone receptors as well as aromatase which renders them hormone-sensitive. Hyperestrogenism may play a role in carcinogenesis. However, the exact molecular pathogenesis is not clear. The JAZF1/JJAZ1 gene fusion is found in many ESS but a relationship with the estrogenic pathway is not well established.

This thesis focuses on the course of disease and the interdisciplinary treatment of two patients with ESS who have successfully been treated with aromatase inhibitors as adjuvant or palliative treatment strategy.

The ESS in the first case was treated with surgery and postoperative chemotherapy. Nine months after surgery, high-dose chemotherapy and autologous bone marrow transplantation was carried out due to peritoneal spread. A disease-free interval of seven years was observed. Thereafter, a local recurrence occurred and complete surgical resection was accomplished. Subsequent adjuvant radiotherapy was administered. Nevertheless, a second relapse was diagnosed one year later. Following renewed resection, the steroidal aromatase inhibitor exemestane was initiated due to the fact that immunohistochemistry showed progesterone receptor positivity (PR, 80%) and intratumoral aromatase expression. No evidence of disease was observed for five years subsequently. A third relapse was detected and again treated with surgical resection. A pseudoadjuvant therapy with the non-steroidal aromatase inhibitor anastrozole was started. The patient remained disease-free up to now.

In the second case report a woman with a history of total abdominal hysterectomy (TAH), bilateral salpingo-oophorectomy (BSO) and postoperative radiotherapy due to an ESS is presented. One year after surgery, metastases to the lung, the liver and the bones were detected. In opposition to the first case this neoplasm showed low immunohistochemical staining for estrogen receptors (ER, 5%) and negative staining for PR (<1%) and no intratumoral expression of aromatase. Nevertheless, therapy with the non- steroidal aromatase inhibitor letrozole was started. Within three months, partial remission of the lung and liver metastases and sclerosis of bone metastases was observed. Surprisingly, the patient developed a peripheral nerve sheath tumor as a second primary tumor definitely diagnosed four years

after initial diagnosis of ESS. The ESS however remained in stable remission for more than 4 years.

Despite differences in histological features and hormone expression level these two cases of ESS responded well to aromatase inhibitor therapy. Treatment of recurrent or metastatic ESS with aromatase inhibitor therapy is warranted even in cases with very low hormone receptor expression and no intratumoral aromatase expression.

Zusammenfassung

Endometriale Stromasarkome (ESS) sind seltene maligne Tumore des Uterus, die vorwiegend in prä- und perimenopausalen Frauen auftreten. Die meisten ESS exprimieren Steroidhormonrezeptoren und können intratumorale Aromataseaktivität aufweisen, die sie als hormonsensitive Tumoren kennzeichnet. Darüber hinaus scheint Hyperöstrogenismus in der Karzinogenese eine Rolle zu spielen. Der genaue molekulare Pathomechanismus ist jedoch noch nicht geklärt. Die JAZF1/JJAZ1 Gentranslokation wird in vielen Stromasarkomen gefunden, und ein Zusammenhang mit östrogenassoziierten Stoffwechselwegen wird diskutiert.

Fokus dieser Arbeit ist der Krankheitsverlauf und die interdisziplinäre Behandlung von zwei Patientinnen mit ESS, die erfolgreich mit Aromatasehemmern therapiert wurden.

Im ersten Fallbericht wird die Krankengeschichte einer Frau mit endometrialem Stromasarkom dokumentiert, das initial operativ und chemotherapeutisch behandelt wurde. Neun Monate danach wurden eine Hochdosischemotherapie und autologe Knochenmarkstransplantation aufgrund einer sequentiell aufgetretenen Peritonealkarzinose durchgeführt. Daraufhin konnte ein krankheitsfreies Intervall von sieben Jahren beobachtet werden. Danach wurde in der Kontrolluntersuchung ein Lokalrezidiv festgestellt. Nach kompletter chirurgischer Entfernung wurde eine adjuvante Strahlentherapie durchgeführt. Ein Jahr später kam es zum zweiten Rezidiv. Nach der neuerlichen Resektion folgte eine pseudoadjuvante Therapie mit dem steroidalen Aromatasehemmer Exemestan. Diese Therapie wurde auf Basis der in der Immunhistochemie festgestellten positiven Progesteronrezeptoren (PR, 80%) und dem intratumoralen Nachweis einer Aromataseaktivität eingeleitet. Ein krankheitsfreier Verlauf von fast fünf Jahren konnte erneut beobachtet werden. Das dritte Rezidiv wurde wieder operativ entfernt, gefolgt von pseudoadjuvanter Therapie mit dem nicht-stereoidalen Aromatasehemmer Anastrozol. Damit blieb die Patientin bis zum heutigen Zeitpunkt metastasenfrei.

Der zweite Fallbericht präsentiert eine Frau mit ESS, das initial mit einer totalen Hysterektomie und Salpingooophorektomie sowie postoperativer Strahlentherapie behandelt wurde. Ein Jahr nach der Operation wurden Lungen-, Leber-, und Knochenmetastasen entdeckt. In der immunhistochemischen Untersuchung zeigte das ESS eine schwache Positivität für Östrogenrezeptoren (ER, 5%) und eine Negati-

vität für PR (<1%) sowie fehlende intratumorale Aromataseaktivität. Aufgrund der weitgehenden Beschwerdefreiheit der Patientin und des höheren Alters wurde dennoch eine Therapie mit dem nicht-steroidalen Aromatasehemmer Letrozol eingeleitet. Innerhalb von drei Monaten konnte eine partielle Remission der Lungen- und Lebermetastasen und Sklerosierung der osteolytischen Knochenmetastasen nachgewiesen werden. Überraschenderweise entwickelte die Patientin nach vier Jahren einen zweiten Primärtumor in Form eines malignen peripheren Nervenscheidentumors, der aufgrund einer mediastinalen Einblutung akut operiert werden musste und somit erst vier Jahre nach der primären Diagnosestellung ESS definitiv diagnostiziert werden konnte. Das ESS blieb bis heute in stabiler Remission.

Die beiden Fallberichte bestätigen das hormonabhängige Wachstum von ESS. Die Therapie mit Aromataseinhibitoren kann zu langfristigen Remission auch bei ESS mit sehr geringer Hormonrezeptorexpression führen.

1 Motivation

I am very interested in internal medicine. I spent an elective placement within modul 16 at the Department of Oncology in October 2005. I liked it very much due to the fact that I had the opportunity to work with tumor patients as well as with general internal patients. I could learn and benefit a lot. Because of the great support by the tutors and because of the fact that this extensive subject has aroused and deepened my interest, I decided to write my thesis at the Department of Oncology. Prof. Bauernhofer suggested some topics. Beside internal medicine, I am also interested in gynecology. Therefore, the topic “endometrial stromal sarcoma” was suitable. First, I did not know anything about this type of sarcoma because when searching for this topic in books, nothing or just a few sentences could be found. Reviewing the literature on PubMed, however, enhanced my knowledge. After all it was also possible to discuss and to give advice to our patients. Although this issue is complex and although there are many controversial topics, it aroused my interest more and more. The follow-up of our patients was in particular exciting and informative.

During work for this thesis I learned a lot, not only with regard to the subject of my thesis but also about how to write scientific papers. Therefore, the idea of a thesis was beneficial because it contributed to my medical education.

2 Overview

ESS are a subgroup of uterine sarcomas. Uterine sarcomas account for about 3-5% of all malignant uterine neoplasms. The incidence is 17 per million inhabitants and year. In contrast to epithelial tumors of the uterus, which have a good prognosis, uterine sarcomas are associated with a poor prognosis. They have a high rate of local recurrences as well as a high rate of distant metastases. [1] The overall survival rate is less than 50% at 2 years even for early stage disease. Primary treatment for patients is radical surgery. Radiotherapy and chemotherapy are often employed in the adjuvant setting. However, due to the low incidence of these malignancies little is known about the risk factors for poor outcome and the optimal management. [2]

Uterine sarcomas are classified into three histological groups arising from two different uterine tissues: On the one hand, there is a neoplasm arising from the myometrium called “leiomyosarcoma” (LMS). On the other hand, there are two neoplasms which can arise from the endometrium. These tumors are called “carcinosarcoma” (CS) and “endometrial stromal sarcoma” (ESS). ESS represents 0.2%, LMS 1% and CS 2-3% of all uterine malignancies. [3] Investigations have shown that LMS and CS are more often found in black women than in white. [4,5]

3 Introduction

ESS are rare malignant neoplasms. They constitute about 10% of all uterine sarcomas and only 0.2% of all uterine malignancies. The incidence is 1-2 per million women and year. 400-700 new cases occur in Europe every year. [6]

In general, ESS are histologically characterized by homogenous spindle-shaped cells resembling normal endometrial stromal cells in the proliferative phase of a woman's cycle. [7]

ESS can be divided into the following subgroups: endometrial stromal nodule (ESN), low-grade endometrial stromal sarcoma (LGESS) and high-grade endometrial stromal sarcoma (HGESS). [6] The overall survival rate depends on the respective subtype.

Immunohistochemically, a positive reaction for CD10, ER and PR may be observed. [7]

Usually, abnormal vaginal bleeding is the primary symptom. Peri- and postmenopausal bleeding in combination with a polypoid tumor sticking out of the cervix is typical. Pelvic and abdominal pain as well as pelvic or abdominal mass are often associated with this fast growing neoplasm. Therefore, a fast growing “myoma” during menopause is suspicious for any kind of sarcoma including ESS. “Fast growing myomas” in young women are rarely uterine sarcomas (0.27%). Uterine sarcomas usually occur at an average age of 42-53 years. [8,9]

Diagnosis of most tumors is based on fractional curettage, hysteroscopy or endometrial biopsy and even by chance during hysterectomy. [8]

Sarcomas metastasize hematogenously and metastases are also found in lymph nodes in the pelvic and para-aortal region. The most affected sites include the lung, peritoneal cavity with omentum and upper abdomen. In bone and brain metastases are rarely seen. [8,9] Local recurrences may occur up to 20-30 years after initial treatment. [10]

Initial treatment consists of TAH and BSO. The benefit of radiotherapy, chemotherapy and hormonal therapy is poorly defined. [10]

3.1 Etiology

The exact etiology of ESS has not been elucidated to date but some risk factors have been attributed to the occurrence of ESS. In the following chapters the important influence of estrogen on the development of endometrial stromal neoplasms is highligthed:

3.1.1 Estrogen replacement therapy and preserved ovaries

An observation in which women recurred under estrogen replacement therapy (ERT) led to the study by Chu et al. [11] The conclusion was that ERT was contraindicated in the treatment of LGESS: 75% of stage I patients treated with ERT recurred. The stage III patient under ERT recurred, too, and then responded dramatically to progestin therapy.

Furthermore, after reviewing the literature, Chu [11] reported that about one half of stage I patients with preserved ovaries after surgery would recur possibly due to estrogen stimulation from the retained ovaries. Contrary, in his study, Chu [11] could show that 43% of patients whose ovaries had been removed at the time of initial surgery also developed recurrence. Therefore, the premenopausal progesterone production may be protective against any potential negative impact of a continued endogenous estrogen exposure. In summary, retention of normal functioning ovaries does not appear to be a remarkable improvement in outcome.

This is controversially discussed, however. [10,12]

3.1.2 Tamoxifen

Tamoxifen has been the standard treatment in hormone-sensitive breast cancer. On the one hand, it acts as an estrogen receptor antagonist in breast tissues. On the other hand, tamoxifen acts estrogen-agonistic in other tissues, for example in the endometrium. The latter feature leads to proliferation. The risk of hyperplasia, polyps and endometrial cancer is increased by 2-to 4-fold compared with patients treated without this selective estrogen receptor modulator. It has been reported that 10% of the patients treated with tamoxifen will develop endometrial pathology within 5 years. [13]

The spectrum of uterine malignancies associated with tamoxifen therapy in breast cancer is expanding. The most frequent lesion seen is the endometrial polyp. The

most frequent malignancy seen is the endometrial adenocarcinoma. Furthermore, sarcomas of the uterus such as the CS, the LMS and even the ESS have been reported. [14]

Saga et al. [15] described the development of an even HGESS due to therapy with tamoxifen.

Moreover, in a minority of cases, LGESS with sex cord-like features have been explored in association with tamoxifen. It has been suggested that it is tamoxifen, which would also be responsible for the development of stromal sarcomas with this specific histologic type. Therefore, continued surveillance of patients receiving this drug is needed. [14]

Concerning the treatment in breast cancer, the 'American Society of Clinical Oncology Technology Assessment' advises to switch to an aromatase inhibitor after 2 to 3 years with tamoxifen. With an aromatase inhibitor, the risk of tumor recurrence would be reduced as well as the incidence of life-threatening adverse events such as the reported malignancies. [16]

3.1.3 Endometriosis

Endometriosis is a common benign gynecologic condition. It is present in about 8-10% of women between 15 and 50 years. [17] Endometriosis means the presence of endometrial glands and endometrial stroma outside the uterine cavity. [18]

It was Sampson [19] who first described the development of ovarian carcinoma in endometriosis. Furthermore, he established the criteria which are necessary to diagnose the development of malignancies arising in endometriosis [18,19]:

(i) endometriosis has to be intimately associated with neoplasms,

(ii) the histologic type of the tumor has to be compatible with endometrial origin, and

(iii) no other primary site is allowed to be identified.

In 1953, Scott [20] suggested a further qualification. He felt it of great importance for the diagnosis that microscopic benign endometriosis was contiguous with the malignant tissue.

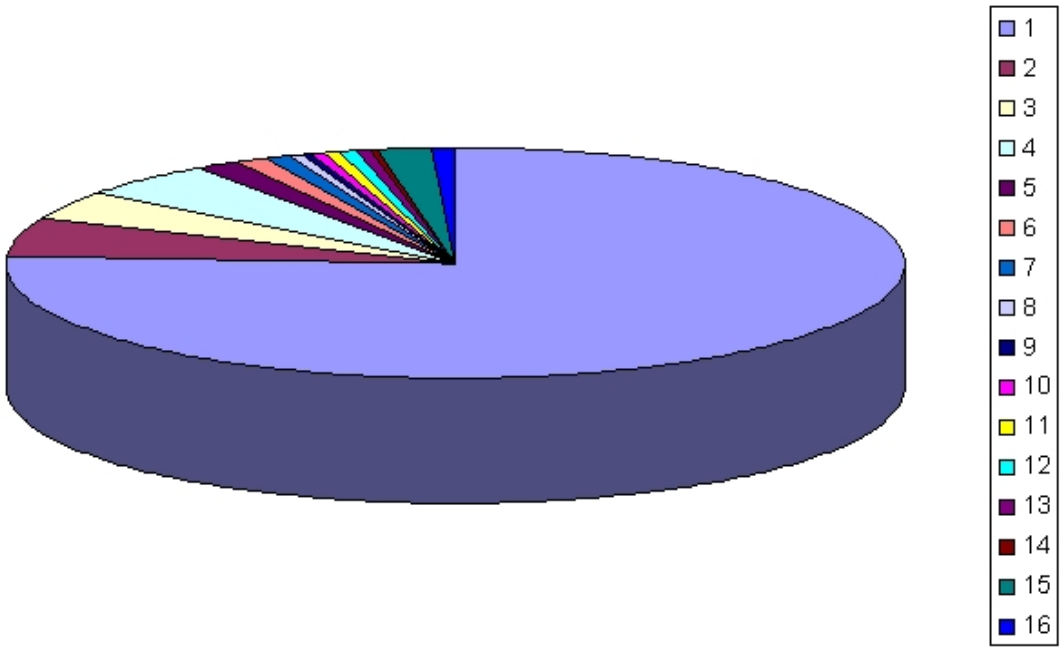
Following Sampson, many other reports have continued documenting malignancies which probably have arisen from endometriosis. Malignant transformation in endometriosis makes about 0.7-1.0%. [18]
Heaps et al. [21] reported about malignancies which have developed in foci of gonadal and extragonadal endometriosis. Irvin et al. [18] continued reviewing such reports: The most frequently affected site was the ovary with 76%. Extragonadal tumors were found in 24% of which 4% occurred in the rectovaginal septum, 5% in the colon, 1.8% in the vagina and 5.4% in the pelvic peritoneum. The most common neoplasm was the endometrioid adenocarcinoma with about 66.4% arising in endometriosis and 58.5% arising in endometriosis from extragonadal sites. The sarcomas made about 11.4% (arising in endometriosis) and 20.8% (arising in endometriosis from extragonadal sites) (see graphics 1-3).

When malignant transformation in endometriosis is present, primary surgery including complete resection of all disease should be performed. Biopsies of lymph nodes and tissues in the upper abdomen are suggested for staging evaluation when macroscopic disease is confined to the pelvis. Correlation is found between the prognosis for the malignant transformation and the stage. Patients with disease confined to the ovary have a 5-year survival rate of 67%. In patients with extragonadal disease confined to the site of origin, the 5-year survival rate is about 100%. However, disseminated intraperitoneal disease has a survival rate of only 12%. [18]

Chang et al. [22] explored 20 cases of primary extrauterine ESS and noted the observation that some neoplasms really develop within endometriosis. However, he also described that the presence of endometriosis would not always be the reason for ESS-development. Alternative explanations may exist, such as de novo formation.
Furthermore, no report can be found in any of the publications used which clearly demonstrates a direct transformation of ESS from endometriosis.

What is important here is that the association between exogenous hormone therapy and the development of malignancies in endometriosis is well established. [23]

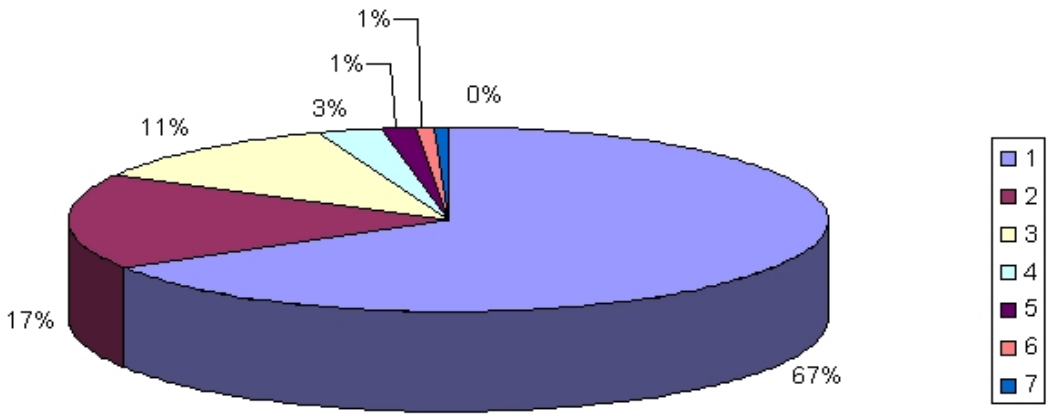
Graphic 1: Sites of tumors arising in endometriosis



1=Ovary, 2=Pelvis, 3=Rectovaginal Septum, 4=Colon/Rectum, 5=Vagina, 6=Omentum, 7=Umbilicus, 8= Node-obturator, 9=Broad ligament, 10=Ureter, 11=Bladder, 12=Vesicovaginal Septum, 13=Small Intestine, 14=Pleura, 15=Vulva, 16=Scar

Note: Review of world literature of malignancy arising in endometriosis 1990-1998 [18]: The ovary was the most frequently affected site. (Four patients had two different sites.)

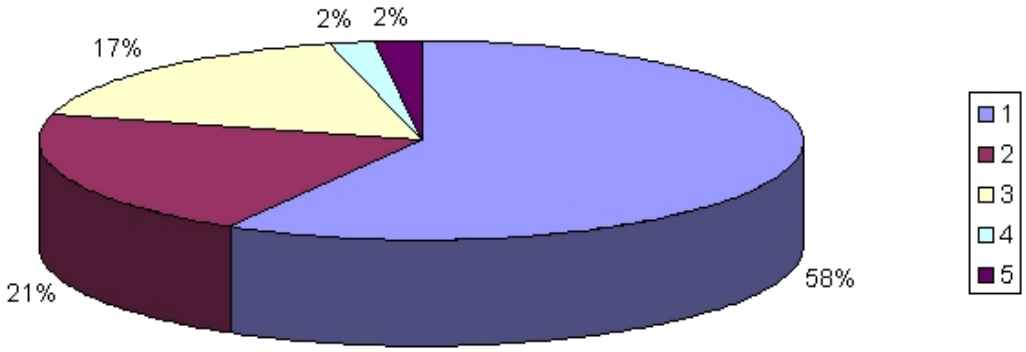
Graphic 2: Histology of tumors arising in endometriosis



1=Endometrioid Carcinoma, 2=Clear Cell Carcinoma, 3=Sarcoma including MMT, 4=Serous Cystadenocarcinoma, 5=Squamous Cell Carcinoma, 6=Mucinous Cystadenocarcinoma, 7=Mixed Germ Cell Tumor and Adenocarcinoma

Note: Review of world literature of malignancy arising in endometriosis 1990-1998 [18]: 195 carcinomas and 25 sarcomas were found. Endometrioid carcinoma was the most common malignancy and arised within the ovary. Percentage is rounded.

Graphic 3: Histology of tumors arising in endometriosis from extragonadal sites



1=Endometrioid Carcinoma, 2=Sarcoma including MMT, 3=Clear Cell Carcinoma, 4=Squamous Cell Carcinoma, 5=Mixed Germ Cell Tumor and Adenocarcinoma

Note: Review of world literature of malignancy arising in endometriosis 1990-1998 [18]: Extragonadal tumors were found in 53 cases (24%). Endometrioid adenocarcinoma was the most common malignancy. Pecentage is rounded.

3.2 Classification of endometrial stromal sarcomas

Norris and Taylor established the most widely accepted classification for ESS. They divide these neoplasms into endometrial stromal nodule (ESN), low-grade endometrial stromal sarcoma (LGESS) and high-grade endometrial stromal sarcoma (HGESS). [10] However, this classification is not valid any more. The term “endometrial stromal sarcoma” is now restricted to neoplasms which were formerly called low-grade endometrial stromal sarcomas. The formerly called high-grade endometrial stromal sarcomas with absence of recognizable evidence of a definite endometrial stromal phenotype are now termed endometrial sarcomas. [6] Benign stromal nodule, low-grade endometrial stromal sarcoma and undifferentiated endometrial sarcoma (UES) is the classification of the 'World Health Organization Classification of Tumors of the Breast and Female Genital Organs'. [24]

Because of ambiguous use of both classifications in the literature, LGESS and HGESS/UES are used to describe these neoplasms in this thesis.

3.2.1 Endometrial stomal nodule

ESN are clinically benign endometrial stromal neoplasms. They are well-circumscribed and they do not have any infiltrative or only minimal focal microscopic irregularities. Finger-like projections into the myometrium can be present which should not exceed 2 to 3 mm. The most important feature to distinguish them from LGESS is the status of margin. Examination of the periphery of the mass is recommended. Neither the size and number of the mass nor the mitotic figures (MF) are important in the differential diagnosis to ESS. However, it is sometimes difficult to distinguish marginal irregularities of ESN from the true invasion of ESS. Therefore, tumors showing limited infiltration have to be more carefully followed-up for possible metastatic spread. [25]

3.2.2 Low-grade endometrial stromal sarcoma

Doran and Lockyer were the first to describe endolymphatic stromal myosis in 1908 but this was not the only valid designation. “Uterine stromatosis, stromatoid mural sarcoma, stromal myosis, uterine stromal endometriosis, endometriosis interstitiale, endometrioid sarcoma, fibromyosis uteri-endometrial type, stromomyoma, uterine angiomatosis, and stromal endometriosis” were further terms de-

scribing this neoplasm. [26] Norris and Taylor established the common classification as LGESS. [10] LGESS represent about 80% of the stromal neoplasms. [11] They affect women at a median age range between 45 and 57 years. The women are younger than in other uterine sarcomas and they normally don't have the usual risk factors for endometrial cancer. [6]

ESS have a higher survival probability than the other uterine sarcomas. The 5-year survival rate is like that for patients with endometrial carcinomas and therefore about 60%. [27] However, general survival data as it can be seen in tables 4 and 5 have a wide range. This is due to inhomogenous reports. [8]
30% of women suffering from LGESS have an extrauterine disease at presentation. [6]

LGESS is histologically similar to the endometrial stroma in the proliferative phase of a woman's cycle including homogenous spindle-shaped cells [7] and thin-walled small arteriolar type vessels [28]. Absence of glands is typical. Neither atypia nor pleomorphism is demonstrated. The mitotic index is less than 10 mitotic figures (MF)/10 high-power field (HPF). A characteristic feature is the invasion into the myometrium and even into the lymphatics and veins. [10] However, one has to expect diagnostic difficulties, especially in the LGESS. The reason is that tumor cells usually show only slight or even no nuclear atypia. Moreover, the lack of any specific structural pattern on histological assessment complicates diagnosis. [29]
Satoh et al. [29] reported a case of pulmonary metastases from a LGESS. Asymptomatic nodules in both lungs were discovered 13 years after hysterectomy for a suspected leiomyoma. Initially, the nodules were diagnosed as pulmonary endometriosis.
Not only is the differential diagnosis of leiomyoma important. The broad differential diagnosis of pulmonary metastatic ESS consists of sclerosing hemangioma, carcinoid tumor, hemangiopericytoma, synovial sarcoma and solitary fibrous tumor. [30]
Several studies [31-34] showed that particularly the LGESS often express ER and PR. Therefore, a therapy with progestational agents would be an important adjunct to surgery in cases of LGESS. Recurrent or metastatic LGESS has been reported to be stabilized or suppressed with progestational agents in more than 50%. [28]

However, although tumors with high levels of receptors are more likely to respond to such kind of therapy, some tumors even with high receptor levels have not responded to this hormonal treatment. This is probably due to the heterogeneity of receptor expression in tumor cells. [28]

Local recurrences can be discovered in one-third to one-half of women and up to 30 years after initial treatment. [11] Authors agree that LGESS are able to spread lymphatically. Nevertheless, lymph node involvement and distant metastases are uncommon. [9]

3.2.3 High-grade endometrial stromal sarcoma

The formerly called HGESS is currently defined as a more undifferentiated stage of its low-grade counterpart. It is the more aggressive sarcoma and characterized by more than 10 MF/10 HPF [9] and severe cytological atypia [28]. However, the number of MF per HPF does no longer serve as differentiation between UES and LGESS. [24] Its growth is infiltrative and destructive into the myometrium and distant metastases are frequently observed. [9] These tumors lack the growth pattern and the vascularity that is typical of the LGESS. [28] Moreover, they may show areas of necrosis and hemorrhages. [35]
These UES were newly classified by Kurihara et al. [36] The reason for this new classification has been that it still is not clear whether these sarcomas are universally undifferentiated or not. Therefore, they are morphologically divided into “undifferentiated endometrial sarcoma with nuclear uniformity (UES-U)” and “undifferentiated endometrial sarcoma with nuclear pleomorphism (UES-P)”. It seems that immunohistochemically, UES-U are similar to LGESS whereas UES-P seem to considerably differ from the low-grade sarcomas (cf 3.4 and 3.6.2.3; Table 1). The question is if these undifferentiated sarcomas are just a dedifferentiation of the LGESS or if they constitute an own entity. Features of UES-U and UES-P are shown in Table 1.

The 5-year overall survival rate for HGESS is similar to that reported for patients with LMS or malignant mixed Mullerian tumor (MMT). It is less than 25%. [27]
Leath et al. [37] are the only researchers who reported a predilection for HGESS in African-American women.

In a case of mixed low-grade and high-grade sarcomas of the uterus, Cheung et al. [38] explored the hormone receptor status: In the low-grade tumors, immunoreactivity for ER and PR was positive. In the high-grade tumors both receptors were negative.
Sutton [39] showed that receptor levels are higher in LGESS than in HGESS. Thus, it is possible that ER and PR do exist in high-grade sarcomas, even though they are mostly present in the more differentiated areas. [35]

Dedifferentiation is well known in bone and soft tissue tumors. It means the coexistence of a high-grade tumor with a low-grade, well-differentiated malignant neoplasm of the same origin. This fact is a histological indicator of a more aggressive phenotype. However, only a few data about heterogeneity of differentiation status are available in uterine stromal tumors. Mixed areas of low and high grade have been rarely described in these tumors. [38]

Table 1: Features of the different types of ESS
cited from [36]

Histology	JAZF1-JJAZ1	ER/PR	ß-catenin	p53 Abnormality	Death from disease
ESS-LG	50%	94% for both	47%	ICH 0% Mutation 0%	0%
UES-U	33%	57% for both	85%	ICH 0% Mutation 0%	57%
UES-P	0%	0% for both	33%	ICH 50% Mutation 50%	60%

Abbreviations: ESS-LG=low-grade endometrial stromal sarcoma, UES-U=undifferentiated endometrial sarcoma with nuclear uniformity, UES-P=undifferentiated endometrial sarcoma with nuclear pleomorphism, IHC=immunohistochemistry, ER/PR=estrogen/progesterone receptor
This table shows the difference in receptor expression between UES-U and UES-P supporting the speculation that these two categories may be distinctive ones. Staining for ß-catenin differs to that reported in 3.6.2.4 but may be due to different antibodies. The ß-catenin expression in this study was higher in UES-U supporting the histogenetically difference and the possibility that a different pathway (p53) than the Wnt signalling pathway (see 3.6.2.4) may contribute to tumorigenesis in UES-P. [36]

3.2.4 Subtypes

As described above, ESS are divided into three histological groups which have or which lack the typical appearance of proliferative-phase endometrium.

- **ESS with sex cord-like elements**

Morehead and Bowman [40] were the first to describe uterine neoplasms which resembled ovarian sex cord-stromal tumors in 1945. In 1976, Clement and Scully [41] described 14 cases and classified them into two groups. The amount of sex cord-like elements defines the following groups:

The neoplasms in *group I* are endometrial stromal tumors. The epithelial-like sex-cord tissue is a minor component. It only involves 10-40 %. Therefore, the name of these tumors is "*endometrial stromal tumors with sex cord-like elements*", short *ESTSCLE*. These neoplasms focally differentiate as "plexiform cords one cell in width, small solid cell groups, anastomosing trabeculae, and well-formed tubules of epithelial-like cells in the background of poorly circumscribed mural masses". The flashy masses are of the color yellow to white. Involvement of the lymphatics and veins as well as infiltrating margins may be present. On the one hand, the tumors can be contiguous with the overlying endometrium. On the other hand, there may not necessarily exist any endometrial involvement. Group I tumors have a mitotic count ranging from 1 to 11 MF/10 HPF.

The neoplasms in *group II* are called "*uterine tumors resembling ovarian sex-cord tumors*", short *UTROSCT*. They are less common neoplasms and are characterized by diffuse or predominant sex cord-like differentiation. Usually, they behave in a benign fashion. They are surrounded by myometrial tissue and they don't involve the endometrium. In general, UTROSCT are seen in the uterine body or the fundus. They can even be present in the endometrial cavity or in the cervix. The tumors differentiate as "solid, round, well-circumscribed and myometrial tumors". The cut surface is yellow, grey or tan. On the one hand, pushing or infiltrating margins can be seen but on the other hand, involvement of lymphatics and veins is a rare event. Even mitotic activity is rare or absent. The "anastomosing trabeculae, nests, tubules and cords" of the ovarian sex-cord differentiation are predominantly or exclusively found in that mass where the minimal stromal tissue exists.

The sex cord tissue in these sex cord-stromal tumors resembles histologically granulosa cell or Sertoli cell neoplasms.
Concerning immunohistochemistry, a positivity for vimentin, alpha-inhibin and CD99 is common in most of the neoplasms resembling ovarian sex cord-stromal tumors. Inhibin would act as sensitive marker for sex cord differentiation. Some neoplasms even express smooth muscle antigen (SMA), myoglobulin and desmin. Cytokeratin coexpression has been seen in 50% and epithelial membraneous antigen (EMA) in 10%. Moreover, ER and PR have been found as well as melan-A.
Because group II tumors have a more clinically benign course, they can be treated in a more conservative approach. In contrast, group I tumors are treated by hysterectomy with or without BSO. They are followed up closely because they show a much higher recurrence rate than group II tumors. [41,42]

Further variants are:

- ESS with curvilinear calcification [35]
- ESS with smooth muscle and glandular differentiation [43]
- Myxoid ESS [44]

3.3 Immunohistochemical markers

The differential diagnosis between ESS and other tumors is difficult. Some markers may facilitate diagnosis.

3.3.1 CD10

CD 10 is called the common acute lymphoblastic leukemia antigen, short CALLA. It acts as a cell-surface neutral endopeptidase and is expressed by lymphoid precursor cells as well as by B lymphoid cells of germinal centre origin. Furthermore, expression has been explored in breast and salivary gland myoepithelial cells, in renal tubular and glomerular cells, in pulmonary alveolar lining cells and in prostatic glandular epithelium. Actually, CD10 antibodies are used in the diagnosis of leukemia and lymphoma. [45]
A recent study [46] has found that CD10 expression can also be present in non-hematopoietic neoplasms such as in cases of ESS but even in normal endometrial stroma.

During Mc Cluggage´s study about CD10 expression many cases of diagnosed ESS were reclassified as cellular leiomyoma because CD10 was negative and desmin was positive. [45]

Therefore, CD10 acts as a sensitive immunohistochemical marker of low-grade endometrial stromal neoplasms. In combination with the other marker desmin, the differential diagnosis of leiomyoma can be made much safer and much easier. Even the differential diagnosis of an adult granulosa cell tumor (AGCT) can be made with CD10 and alpha-inhibin. In most cases, AGCT is CD10 negative and ESS does not show positivity for alpha-inhibin. Malignant lymphomas can also exhibit CD10, but additionally CD45 and B or T lymphoid markers. UES had to be reclassified too. They were reclassified as undifferentiated endometrial carcinoma on the basis of morphology, the diffuse positive staining with anti-cytokeratin antibodies and the negative staining with CD10. Anti-cytokeratin antibodies are characteristic of endometrial carcinomas. However, positivity can also be present in endometrial stromal neoplasms.

LMS were focally CD10 positive too, but never strong and diffuse. Additionally, positivity for desmin and alpha-SMA pleads for the diagnosis of LMS.

In summary, CD10 may be a sensitive marker of normal and neoplastic endometrial stroma and may also be useful in establishing the diagnosis of extrauterine involvement or metastases.

3.3.2 Calponin h1 and h-caldesmon

The cytoskeletal proteins calponin h1 and high molecular weight caldesmon are limited to smooth muscle and myoepithelial cells. They interact with calmodulin, tropomyosin and actin and are therefore part of the regulation of smooth muscle contraction. Furthermore, they are useful in smooth muscle differentiation identification. [47]

Zhu et al. [47] found out that calponin h1 was very sensitive to stain smooth muscle but was not specific. H-caldesmon did not show any false-positive results and all the normal myometrium and the vascular smooth muscle as well as nearly all cases of cellular leiomyomas (CL) showed its expression. Positive staining was not seen in ESS excluding the expected staining of vessel walls. Therefore, h-caldesmon is a specific marker in distinguishing ESS from CL.

3.3.3 Desmin and Smooth muscle antigen

Desmin and SMA are markers for smooth muscle differentiation. Some authors noticed that diffuse desmin positivity would be more characteristic for uterine CL than for ESS. With this marker a differential diagnosis is possible. Although even ESS can be positive, it is only a small number of ESS which stains for desmin. Other authors found larger numbers of desmin and SMA positivity in ESS. Therefore, these two markers would not be such reliable and high discriminatory. On the one hand, ESS may have smooth muscle differentiation. On the other hand, CL may show dense cellularity, prominent vascularity and irregular margins. [47]

3.3.4 Additional markers

Other markers for diagnosis and differential diagosis may be

- Inhibin (cf 3.2.4, page 31)
- Proliferating cell nuclear antigen (PCNA) [47]
- CD44v3 [47]
- Mast cells (MC) [47]

3.4 Hormone receptors

Hormone receptors have been explored in ESS. They may play a role in pathogenesis and may offer treatment opportunities. As mentioned above, low-grade sarcomas usually express hormone receptors and are therefore candidates for antihormonal treatment.

3.4.1 Estrogen receptor

ER as well as PR are present in normal proliferative and secretory phase endometrium, in endometrial glands as well as in stromal cells. Even in epithelium and stromal cells of simple and complex hyperplasia such receptors have been explored. [28]

Two types of ER exist, ERα and ERβ. It is not only ERα which binds to estradiol. ERβ can do it as well. A ligand-dependent transcription stimulating activity via the estrogen regulatory regimes follows. However, ERβ has a different tissue distribution and even the ligand binding specifity differs from ERα. When estrogen binds

to ERα, cell proliferation is started. When ERβ is activated, cellular apoptosis or cell death is implicated. [11]

Both receptors have been found in endometrial stromal cells. However, in the study by Chu et al. [11], eight of ten LGESS were positive for ERα but none was positive for ERβ. This exploration suggests that ERT may act through the ERα as growth stimulus. Furthermore, it leads to the possibility of using immunohistochemical analysis of ERß in the differentiation between benign and malignant endometrial stromal proliferations.

Concerning Kurihara et al. [36], LGESS and UES-S showed staining for ER and PR (94% for both and 57% for both) whereas UES-P did not.

3.4.2 Progesterone receptor

Concerning PR, studies have shown that two isoforms exist: isoform A and B. Corresponding to ERα and ERβ, PRA and PRB may also have different functions. PRB acts as activator of progesterone-responsive genes. In contrast, PRA is transcriptionally inactive. Moreover, in the presence of PR agonists and antagonists, PRA is a strong and transdominant repressor of the transcriptional activity of PRB as well as of the ER. [48] As described in 3.5.1 and 3.5.4.2, progesterone may play an important role in protecting the endometrial tissue against the negative impact of estrogen exposure.

3.4.3 Androgen receptor

Androgen receptors (AR) in endometrial stromal neoplasms were first explored by Moinfar et al. [28]. In this study, 45% of the neoplasms stained positive for AR. The proportional distribution and concentration of these steroid receptors may influence the response to hormonal treatment. The biological significance of AR and the interaction with the other steroid receptors in ESS needs further investigation, however.

3.4.4 Gonadotropin-relasing hormone receptor

The gonadotropin-releasing hormone (GnRH) as well as its receptors have been explored in several human neoplasms. Two forms of GnRH receptors (GnRH-R) exist, GnRH-R I and GnRH-R II. In the series carried out by Reich et al. [49], these receptors were expressed in most primary and recurrent ESS. Staining was het-

erogenous showing diffuse pattern in the cytoplasm and a granular or vesicular pattern in the perinuclear region. Recurrent neoplasms showed stronger staining than the primary tumors.

GnRH plays a key role in estrogen biosynthesis which itself acts as growth stimulus in ESS. This feature leads to the treatment option of GnRH antagonists and GnRH agonists which have also been shown to decrease aromatase expression in lesions such as leiomyomas, endometriosis and adenomyosis.

3.5 Treatment of endometrial stromal sarcoma

Treatment options for ESS are surgery, radiotherapy, chemotherapy and hormonal therapy as well as treatment with tyrosine kinase inhibitors (TKI). In this chapter, all these options are discussed:

3.5.1 Surgery

Standard treatment of ESS consists of TAH and bilateral BSO. Additionally, debulking has to be carried out if extrauterine tumor is present. [27] However, there are different opinions about whether ovarian tissue should be extracted or conserved because of its hormone production. Some authors have documented poorer outcome among women without oophorectomy. Others couldn't find a worse outcome in patients with ovarian conservation [cf 10,11,49, Table 2]. As reported in 3.1.1, it was supposed that there is no remarkable difference between retention or extraction of functioning ovaries. If the ovaries are extracted on the one hand, estrogen can't be produced at that site any longer. Therefore, the growth stimulus in ESS decreases. If ovaries are extracted on the other hand, progesterone production is stopped, too. Therefore, the protective effect against any potential negative impact of continued estrogen exposure doesn't exist any longer.

Table 2: Recurrences in premenopausal patients in whom ovaries are retained
cited from [11]

Author	Number of Stage I patients	Number of retained ovaries	Recurrence when ovaries are preserved
Berchuck et al.	19	6	6 (100%)
Chang et al.	85	7	0 (0%)
Mansi et al.	6	5	2 (40%)
Young et al.	3	3	3 (100%)
Chu et al.	15	6	2 (33%)
Total	128	27	13 (48%)

As described in 3.6.3, lymphadenectomy has no role in the surgical treatment of ESS. Studies demonstrated that there is no difference in overall survival between patients with and without lymphadenectomy. The prognostic significance of such metastases and, therefore, for such surgical procedure is still unknown, however.
Besides debulking of metastases, thrombectomy of the inferior vena cava and, occasionally, even of the heart has been reported in some cases. [26,50-52]

3.5.2 Radiotherapy

Weitmann et al. [8] reported results of adjuvant radiation therapy and a review of the literature. They came to the conclusion that the combination of surgery and *adjuvant radiotherapy* is more effective in the treatment of ESS than surgery alone. This combination led to the lowest relapse rates and the highest 5-year survival rates (Table 3).
Concerning *primary irradiation* only few data exist which are shown in Table 4. If higher and adequate radiation doses might result in better tumor control is relatively unclear. Nevertheless, it is believed that primary irradiation may be a chance for patients who cannot undergo surgery. It may even be a curative treatment option.
With regard to *palliative radiotherapy*, there are also only few data available. However, complete remission has never been reported. Most patients just achieve partial response (PR) or stable disease (SD). Patients with metastatic high-grade sarcomas have a short overall survival, mostly a few months, whereas patients with low-grade sarcomas survive longer. Data from Weitmann et al. supports these obersavtion. Therefore, it is suggested that palliative radiotherapy should only be offered to patients with LGESS.

Little is known about the side effects of radiation therapy. One death due to small bowel fistulae after radiotherapy has been reported where autopsy did not reveal any tumor mass [53]. Furthermore, the development of an HGESS after irradiation for a LGESS, has been described. [54] Apart from that, side effects in the Weitmann's study were mild and acceptable. [8]

Despite considerable controversy, there seems to be an effect of radiation therapy on local recurrence-free survival. However, overall survival seems not to be significantly increased by any kind of radiotherapy. [55]

Table 3: Influence of adjuvant radiation therapy
cited from [8]

Author	n	Stage/Grade	Therapy	5-year-survival	Relapse rate	Remark
Berchuck et al.	18	I	S		56%	
	3	I	S&R		33%	1 distant failure
Echt et al.	5		S		60%	pelvic recurrence
	5		S&R		0%	
Evans et al.	3		S		100%	pelvic recurrence
	8		S&R		13%	
Gadducci et al.	16	low	S		31%	
	4	low	S&R		25%	
	6	high	S		50%	Stage I,II
	1	high	S&R		100%	Stage IV
Jereczek et al.	5		S		80%	1 distant failure 3 local failures
	4		S&R		25%	1 distant failure
Mansi et al.	3	I low	S		67%	
	3	I low	S&R		0%	
	6	I high	S		100%	5 died
Nola et al.	6		S		67%	
	20		S&R		45%	
Salazar et al.	15	I	S	47%		
	2	I	R	50%		
	7	I	S&R	88%		
	2	II-IV	S	0%		
	10	II-IV	R	0%		
	6	II-IV	S&R	33%		
Goss et al.	1	III	S			tumor persistance
	1	III	S&R&C			brain metastases
Vienna study	15	I-III	S&R	72%	20%	1 local failure distant failures

Abbreviations: S = surgery, R = radiotherapy, C = chemotherapy

Table 4: Primary irradiation
cited from [8]

Author	n	Stage/Grade	EBT&BT	Outcome
Berchuck et al.	1	low	no data	DIC 9 years
Jereczek et al.	2	no data	50-55 Gy EBT and different BT	1 local progression after 4 months 1 distant metastasis after 10 months
Larson et al.	2	III high III low	8 Gy and 54 Gy 10 Gy and 48 Gy	DOD 9 months local recurrence DOD 48 months local recurrence
Vienna study	2	III high	27 Gy and 48 Gy 42 Gy and 36 Gy	DIC 11 months DIC 6 months

Abbreviations: EBT = external beam therapy, BT = brachytherapy, DIC = died due to intercurrent disease, DOD = died of disease

3.5.3 Chemotherapy

Although antitumor activity has been shown in sporadic reports, the role of chemotherapy in ESS is limited. [56] In the study on uterine sarcomas, Muss et al. [57] compared doxorubicin plus cyclophosphamid with doxorubicin alone. One fifth of patients suffering from ESS attained an objective response in either arm of the study, combination did not show a significant benefit, however. Overall, 50% achieved at least stable disease.

Sutton et al. [58] preferred a combination chemotherapy containing doxorubicin too. Even Berchuck et al. [59] reported that 50% of patients responded well to doxorubicin alone or in combination in ten patients. Lehrner et al. [60] reported about a combination chemotherapy consisting of doxorubicin, vincristine and cyclophosphamide which was later combined with megestrol acetate and resulted in complete clinical remission of metastases.

In the case with intracardiac metastasis reported by Yokoyama et al. [61] ifosfamide-, epirubicin- and cisplatin-combining chemotherapy showed remarkable reduction of the tumor mass, except for the intracardiac site.

Ifosfamide is considered as an active drug with higher response rates than therapeutic regimes not containing ifosfamide. [for studies see 54]

Lin et al. [56] reported that prolonged oral etoposide therapy can be administered safely and conveniently for more than three years resulting in major palliation of the symptoms. Advantages of this therapy are the easy administration, good patient compliance and minor toxicity. It is better than doxorubicin, which can't be given in long-term use because of its cardiotoxicity. What has to be taken into

consideration in the prolonged use of etoposide is the possibility of a second, hematologic malignancy. This has been reported but patients suffering from that had childhood leukemia rather than an adult solid tumor. In conclusion, oral etoposide, especially used in hormone-refractory ESS, may be tolerable for a long time and may result in prolonged palliation of symptoms.
Moreover, every single aggressive chemotherapeutic agent should only be given to patients who progressed under hormonal therapy.

3.5.4 Hormonal treatment

Endometrial cells are sensitive to ovarian steroids. This can be proven by morphological changes occurring during the woman's menstrual cycle. The reason why steroid hormones are believed to play a role as a growth factor is as follows:

1. Most patients with the diagnosis of LGESS are premenopausal,
2. GNRH-R, ER, PR, AR and intratumoral aromatase activity have been reported in ESS (for details see chapter 3.4),
3. recurrences occur less frequently and later in patients treated with BSO,
4. control of metastatic disease after surgical- or radiation-induced castration is possible and
5. progesterone therapy is able to decrease tumor mass. [10]

The expression of all hormone receptors mentioned above can be used in the treatment of ESS and will be reviewed in the next chapters.

3.5.4.1 GnRH-agonists and antagonists

Gonadotropin-releasing hormones are secreted by the hypothalamus. The target organ is the pituitary gland, which itself produces and releases the luteinizing hormone (LH) as well as the follicle-stimulating hormone (FSH). In women, FSH is important for the maturation of the ovum and ovarial estrogen production. Under normal conditions, GnRH is released in a pulsatile way. However, if GnRH is applied as an agonistic analogue continuously and in an “overdose”, the release of gonadotropins by the pituitary gland is changed. First, many hormones are released. After approximately four weeks the secretion decreases due to desensitization and down-regulation. The secretion of the hormones for the ovary is stopped.

Many agonistic and antagonistic GnRH analogues have been explored. The efficacy to bind to the receptor is higher for agonistic analogues than for the natural GnRH. The GnRH antagonists inhibit the production of gonadotropin immediately in contrast to the agonists. [62]

The first who described response of LGESS to therapy with the GnRH-agonist leuprolide acetate was Mesia et al. [63].

The decapeptide gonadotropin-releasing hormone (GnRH-I) is not only known for hypophysiotropic actions. It may also be present in the brain and many other peripheral normal or tumoral organs. There, it is believed to act in an auto- or paracrine fashion. It has been shown that in endometrial, ovarial, breast and prostate cancer, agonistic as well as antagonistic analogues of GnRH-I are able to inhibit the in vitro proliferation. Not only do GnRH-I and its receptor exist. There is also a variant, the GnRH-II (called chicken GnRH-II) with its receptor. It has been shown that GnRH-I antagonists act as agonists on the type II receptor. [64]

3.5.4.2 Progestin

Progesterone is a steroid hormone. It is produced by the luteal corpus, the placenta and the adrenal cortex. Progesterone is important for the secretory transformation of the endometrium as well as for the whole preservation of a woman's pregnancy. [65]

In the treatment of cancer such as endometrial sarcoma, endometrial carcinoma and breast cancer, progesterone leads to tumor reduction in primary as well as in metastatic disease. It is effective because it causes stromal cell maturation as well as estrogen insensitivity. [10] Contrary to breast cancer, in which progestin may act through the inactivation of estrogen effects, the mechanism in uterine stromal tumors seems not to depend on estrogen: When progesterone binds to its receptor in high concentration, the cell starts to produce more of a binding protein which reacts with growth factors by inactivating them. The results are a stop or slower growth of tumor cells. [33]

On the one hand, progesterone lowers the frequency of the hypothalamic GnRH pulse, on the other hand, the LH pulse released by the adenohypophysis is increased. Concerning the pituitary gland, progesterone acts as an estrogen an-

tagonist by depressing the receptors and therefore blocking the LH release. The LH release is estradiol- dependent. [65]

Sutton [39] showed that LGESS have higher receptor levels than HGESS. Moreover, they respond much better to hormonal treatment. Sutton therefore hypothesized that the effectiveness of progesterone therapy may be correlated with the receptor level. Progestin therapy may not work when PR are absent. [11]

3.5.4.3 Aromatase inhibitors

Androstendione and testosterone from the adrenal gland are converted by the enzyme aromatase into estrone and estradiol. This process takes places in peripheral tissues such as the adipose tissue, the muscle and the liver. Drugs can prevent this process. They are called aromatase inhibitors. [66]
Aromatase inhibitors are divided into steroidal and non-steroidal subgroups:

- Steroidal aromatase inhibitors: This group binds to the active center of the aromatase in an irreversible way. Exemestane (Aromasin®) and formestane (Lentaron®) are representatives of this group.
- Non-steroidal aromatase inhibitors: This group reversibly binds to the iron atom of the porphyrin of the specific coenzyme CYP450.
  - Non-Selective: Aminoglutethimide (Orimeten®) is a non-selective, non-steroidal aromatase inhibitor. It is able to block other cytochrome-P450-enzymes which are themselves involved in steroid synthesis. Substitution of cortisol, for example, is needed.
  - Selective: Anastrozole (Arimidex®) and letrozole (Femara®) are selective, non-steroidal aromatase inhibitors. They have a higher selectivity as well as a higher potency compared with aminoglutethimide. Furthermore, in contrast to aminoglutethimide, they do not influence the corticoid- as well as the mineralocorticoid system. [67,68]

Side effects are osteoporosis, cardiovascular risks and vasomotoric problems. [68]

The following studies with letrozole have been carried out in patients with breast cancer:
In vivo studies with letrozole at a daily dose of 2.5 mg reduced levels of estradiol and estrone by 81 and 68%, respectively. Furthermore, the enzyme aromatase was suppressed in more than 98.9% of its activity. [69]
A randomized trial compared letrozole at a daily dose of 0.5 and 2.5 mg versus the progesterone megestrol acetate 160 mg in the treatment of metastatic or recurrent breast cancer. Letrozole 2.5 mg, 0.5 mg and megestrol acetate 160 mg resulted in response rates of 24%, 13% and 16%, respectively. The higher dose letrozole therapy was superior to the lower dose and to megestrol acetate. Moreover, the duration of response was higher in letrozole 2.5 mg compared with megestrol acetate and a significant impact on overall survival in favor of the high-dose letrozole arm was discovered. Adverse effects included fatigue, hot flashes, nausea, peripheral edema and weight gain. Compared with megestrol acetate, letrozole was better tolerated. [70]
Another trial was performed in breast cancer patients, comparing letrozole 0.5 mg and 2.5 mg with aminoglutethimide (AG) 250 mg. The response rate in the high-dose letrozole arm was 17.8%, in the low-dose letrozole arm 16.7%. In the aminoglutethimide-arm, the response rate was 11.2%. The median duration of response was longer in letrozole 2.5 mg than in the other treatment modalities. In summary, letrozole was superior to and better tolerated than AG. [71]

In LGESS, Maluf et al. [66] was the first to report a major objective response of a patient treated with letrozole. Furthermore, Spano [72] and Leunen [73] confirmed aromatase inhibitors as a successful treatment option for LGESS.

In summary, the review of the current literature revealed 9 patients treated with an aromatase inhibitor. Two patients achieved complete response (CR), 6 patients achieved partial response (PR), and 1 patient showed progressive disease (PD) (see discussion, Table 7). [55, 66,72,73]

3.5.4.4 Tyrosine kinase inhibitors

The immunohistochemical explored CD117 antigenic epitope identifies the KIT protein product which is a tyrosine kinase receptor of the c–KIT protooncogene.

This protooncogene is located on chromosome 4q11–q12. Mutations of c–KIT activate the receptor and therefore may play a role in tumorigenesis. Therapy with tyrosine kinase inhibitors is successful in cases of GIST. [74] Some authors searched for the expression in uterine sarcomas [74-76] and even response to imatinib mesylate is reported. [77-79]

3.6 Prognostic factors and metastasis

3.6.1 General prognostic factors

Several prognostic factors have an influence on recurrence rate and prognosis in patients with maligancies. The following are not tumor-specific, they are important in nearly every type of malignancy.

3.6.1.1 Age of patients

Concerning age at diagnosis of patients with ESS many controversial reports exist. Some authors report that most patients affected by these neoplasms are in a premenopausal stage. Other authors are of the opinion that mean age at diagnosis of ESS is in postmenpause. [8]

Furthermore, in the study by Li et al. [80] it was shown that median ages at diagnosis seem to be different for LGESS with 42 years and for HGESS with 51 years ($P<0.05$).

3.6.1.2 Tumor grade

LGESS and HGESS show big differences in treatment outcome. In HGESS, relapses are more often and much earlier seen, mostly within one year. Patients die within months because there is limited response to salvage therapy. Relapses in LGESS occur after years. With salvage therapy it is possible to achieve a good response possibly resulting in prolonged survival. [8]

Norris and Taylor [81] explored 5-year survival rates for patients whose tumors showed less than 10 MF/10 HPF and for patients whose tumors showed more than 10 MF/10 HPF. The group of patients with lower proliferation index showed higher 5-year survival rates and lower recurrence rates than the group of patients with higher proliferative index. Results are shown in Table 5.

Table 5: Influence of tumor grade
cited from [8]

Author	n	Grade	5-year-survival	Relapse rate	Remarks
Norris&Taylor	19	low	100%	37%	surgery alone
	15	high	55%	74%	surgery alone
Nordal et al.	25	low	92%		surgery, chemotherapy
	23	high	43%		surgery, chemotherapy
Berchuck et al.	22	low	73%		
	9	high	56%		1 DIC
Gadducci et al.	26	low		23%	
	40	high		70%	
Larson et al.	11	low		45%	surgery, irradiation
	14	high		50%	surgery, irradiation
Weitmann et al.	3	low	100%	0%	surgery, irradiation
	13	high	42%	23%	surgery, irradiation

Abbreviations: DIC = died due to intercurrent disease

3.6.1.3 Tumor stage

The survival rate as well as the relapse rate is dependend on tumor stage. The prognosis of stage I tumors is better than of tumors in higher stages. [8] For example, Salazar et al. [82] reported that patients with stage I ESS have a 5-year survival rate of 55% whereas in all other stages the survival rate is 12%.
More results are shown in Table 6.

Table 6: Influence of tumor stage
cited from [8]

Author	n	Stage	5-year-survival	Relapse rate	Remarks
Salazar et al.	23	I	55%		literature data
	42	II-IV	12%		
Berchuck et al.	6	I	83%		1 DIC
	3	IV	0%		all died within 8 months
Gadducci et al.	40	I&II		40%	
	26	III&IV		69%	
Goff et al.	8	I		25%	2 distant failures
	2	III		100%	1 local and 1 distant failure
Norris&Taylor	24	I&III		33%	
	10	III&IV		80%	
Weitmann et al.	12	I&II	74%	17%	1 DIC, 2 DOD (1 local failure)
	7	III&IV	29%	43%	2 DIC, 3 DOD

Abbreviations: DIC = died due to intercurrent disease, DOD = died of disease

3.6.1.4 Tumor size

Relapses are more frequently seen in the HGESS, which tends to be bigger than the LGESS. In the report by Norris and Taylor [81], the relapse rate of patients whose tumors were smaller than 5 cm was 14%. For tumors larger than 7.9 cm, the relapse rate was 50%. Patients who had a tumor which was smaller than 4 cm did not have any relapse.

3.6.1.5 Treatment

In the opinion of Nordal et al. [83] free resection margins have a significant influence on survival. They have a higher prognostic significance than the grade or the size of the tumor.

Hysterectomy with BSO may have a better outcome than hysterectomy alone. [8] However, there is controversy concerning extend of surgery and outcome (cf 3.5.1).

3.6.2 Tumor-specific prognostic factors

Some prognostic factors have been reported in the literature, which are specific to ESS. Again, the association with estrogen seems to play an important role.

3.6.2.1 Aromatase activity

Due to the fact that LGESS have been reported in patients with endogenous or exogenous hyperestrogenism, estrogen may act as a growth stimulus. Reich et al. [12] demonstrated that aromatase expression, previously only described in adenomyosis, stromal cells of endometriosis and endometrial cancer, can also be expressed in LGESS.

Aromatase is a key enzyme in estrogen biosynthesis and even after oophorectomy estrogen can be produced by the skin, muscle, fat, adrenal gland, and aromatase-positive tumor cells. As described in 3.5.4.3, aromatase is important in extraovarian estrogen biosynthesis because it converts androgen to estrogen. Moreover, it may play a role in a high recurrence rate.

3.6.2.2 Estrogen sulfotransferase

Estrogen sulfotransferase (EST) is an enzyme that plays a crucial role in hormone-dependent tumors of the prostate, the breast and the endometrium. This enzyme

is important in the regulation of the intracellular level of receptor-active estrogens. It sulfates estrogen which converts its biologically active into its inactive form. These sulfurylated steroids are no longer able to bind to their receptors with high affinity. Therefore, they are hormonally inactive. In contrast to the aromatase pathway, the estrogen sulfatase pathway is 40-500 times more active as it has been shown in breast cancer tissues. Estrogen which is produced via the steroid sulfatase pathway cannot be blocked by aromatase inhibitors. However, it can be inactivated by EST.

All in all, EST-negative (and aromatase positive) endometrial stromal sarcomas contain high levels of active estrogen. Therefore, these patients are at higher risk for recurrence and metastasis. Moreover, it has been shown that in ESS only a subset of these neoplasms posses the possibility of inactivating estrogens through the EST. [84]

3.6.2.3 JAZF1/JJAZ1

Genetic alterations and chromosomal abnormalities in ESS commonly include chromosome 1, 3, 6, 7, 13, 14, 15, 17, 19, 20 and 21. Moinfar et al. [85] examined the possibility of allelic imbalances such as the loss of heterozygosity (LOH) and microsatellite instability (MSI). However, the probably most important genetic alteration is the JAZF1/JJAZ1 gene fusion which has been explored in several studies. [86-89] The chromosomal translocation t(7;17)(p15;q21) results in a gene fusion joining the 5' end of one zinc finger gene called JAZF1 on chromosome 7 and the 3' end of the other zinc finger gene called JJAZ1 on chromosome 17.

Analyses of tumor RNA have shown that gene fusion may be present in all the types of ESS. However, it is less frequently seen in the HGESS. In the study by Kurihara et al. [36], JAZF1/JJAZ1 gene fusion was detected in 50% of LGESS, in only 33% of UES-U and in none of the UES-P.

These findings allow speculations concerning the role of JAZF1/JJAZ1 gene fusion in the pathogenesis of ESS: If there would be a relationship between the gene fusion and the estrogenic pathway is not clear until today. The fusion can be detected even in benign stromal nodules. Therefore, it may be a possible explanation that malignant stromal neoplasms develop from initially benign stromal proliferations. Due to the fact that only a minority of high-grade sarcomas have been tested

positive for this genetic alteration, in these neoplasms other pathogenic mechanism may play a role compared to LGESS. [87]
The JAZF1/JJAZ1 fusion may act as a specific marker for endometrial stromal tumors because it has not yet been seen in normal endometrium, leiomyomas, leiomyosarcomas or in hepatic, gastric or lung carcinomas. [89]
For molecular studies see the report by Lax [90].

3.6.2.4 Other relevant pathogenetic and risk factors

Other factors with an influence on pathogenesis and progression are:

- the KIT protein [74-79]
- the platelet derived growth factor receptor (PDGFR), [91]
- the epidermal growth factor receptor (EGFR), [92]
- the vascular endothelial growth factor (VEGF), [93]
- the matrix metalloproteinases (MMP) [94] and the
- secreted frizzled-related proteins (SFRP) [95].

Even higher parity seems to have a negative impact on survival concerning uterine sarcomas. [96]

All in all there are many factors that play a role in both prognosis and outcome. Whether one of these factors might be more important compared to others remains controversial.

3.6.3 Metastasis

Depending on these prognostic factors, ESS may have a more or less aggressive course of disease which is reflected in the rate of recurrence and metastases.
As described above, ESS may metastasize hematogenously as well as lymphatically. Evidence suggests that the hematogenous dissemination is the more important way to metastasize. This is underlined by the infrequent detection of lymph node metastases especially in LGESS. However, as the former name "endolymphatic stromal myosis" would suggest, LGESS have a strong tendency for local lymphatic invasion. Therefore, spreading to loco-regional lymph nodes would seem logical. Nevertheless lymph node involvement is very rare and therefore

surgical treatment rarely includes lymphadenectomy. It is believed that, on the one hand, this procedure would not result in relevant prognostic information or result in a survival benefit. [97] This is illustrated by a study of patients with uterine sarcomas, of which 24% were ESS, in which no significant difference in overall survival of patients with or without lymphadenectomy could be observed. [98] Interestingly, in the study by Riopel et al. [97], nodal metastases were found in 33% of patients with LGESS at some point in their course of disease. That was more than ever expected. But even in this study lymph node sampling was not carried out in 18 of 34 patients. It was speculated that more frequent lymph node sampling might lead to a higher rate of metastases. Thus, the potential prognostic significance of lymph node metastases is unknown. Larger investigations have to be carried out to find out their frequency and prognostic significance.

The most frequent metastatic sites in ESS are the pelvis, the peritoneal cavity and the vagina. Relapses have also been seen in the brain, liver, bladder, lungs, and bones. [61] Metastases in the spinal cord [60] and in the ovary [26] have also been reported. Intracardiac metastasis is a rare event and large vessels are rarely involved. [61] However, some cases of successful excision of cardiac metastases arising from recurrent LGESS have been reported in the literature. [99,100]
Involvement of the heart through the vena cava inferior by malignant neoplasms normally originates from renal cell carcinoma, nephroblastoma, colon adenocarcinoma, melanoma, hepatocellular carcinoma or bronchial carcinoma. It has been reported even in cases of leiomyosarcoma, of ovarian carcinoma, of uterine cervical carcinoma and benign uterine leiomyomatosis. CT scanning, echocardiography or transesophageal echography is needed to explore possible cardiac metastases in ESS. [61]

Authors [30,101] report about metastatic ESS to the lung. The incidence of pulmonary metastases may be 7-28%. The most common growth pattern appears as “well-circumscribed nodule with entrapped air spaces”. The nodules are lined by “non-neoplastic respiratory epithelium”. Uncommon growth patterns are composed of a “solitary nodule, of satellite with infiltrative margins, of lymphangitic pattern and of bilateral spontaneous pneumothoraces” which are associated with predominantly cystic lesions mimicking lymhangioleiomyomatosis. [reports in 101]

Kim et al. [101] reported a further unusual growth pattern consisting of small random micronodules and ground glass opacities (GGO). The micronodules were mainly seen in the intraalveolar spaces and they grew out from the alveolar wall suggesting hematogenous spread. They did not show entrapped respiratory epithelium. The nature of GGO was clinically considered as an alveolar hemorrhage or as intraalveolar fibroblastic polyps. All in all, pulmonary metastases of LGESS may have a very good prognosis and only little effect on survival.

Günhan-Bilgen et al. [102] was the first to describe breast metastases from a LGESS after a 17-year period. Metastases to the breast are very rare. The incidence is about 0.5-3%. Malignancies most commonly metastasizing to the breast include melanoma, lymphoma, lung, and ovarian carcinoma. Moreover, gastrointestinal carcinomas, cholangiocarcinomas, head and neck carcinomas, thyroid carcinomas, and urogenital carcinomas which include the cervix carcinoma and the prostate carcinoma can lead to breast metastases. They appear as well-circumscribed round masses and are therefore difficult in the differential diagnosis of benign lesions in mammography and ultrasonography.

Last but not least, it is important to know that ESS are morphologically heterogenous neoplasms with multipotential differentation. As reported by Dong et al. [103], metastases need not be similar to those of the primary tumor.

4 Case reports

4.1 Case 1

A 39-year-old nullipara woman was referred to the Department for Gynecology with a history of recurrent transvaginal bleeding episodes for three years and the detection of a fibroid lesion in the uterus in August 1994. Hysterectomy and left salpingo-oophorectomy were performed. During surgery the infracolic omentum showed peritoneal spread of the tumor and gross resection of the omentum was carried out additionally. Histological work-up revealed a high-grade endometrial stromal sarcoma Stage III. Therefore, a right salpingo-oophorectomy as well as pelvic lymphadenectomy and partial parametrectomy were performed two months after primary surgery. Involvement of the right adnexa and carcinomatosis of the peritoneum was diagnosed. Postoperatively, five courses of polychemotherapy were administered from November 1994 until April 1995. The patient received the IFADIC (ifosfamide, adriamycin and dacarbazine) regimen. Due to the young age and in the hope that high dose chemotherapy with autologous stem cell rescue might lead to long term tumor control the patient was presented at the Division of Oncology, Department of Internal Medicine.

In March 1995, stem cell priming was performed with 5 g/m² endoxan in order to collect CD34+ peripheral blood mononuclear cells (PBMNCs). Unfortunately, not enough CD34+ cells could be collected using this approach. Therefore, bone marrow was aspirated at the Department of Internal Medicine and Oncology of the Wilhelminen Spital, Vienna, in May 1995. A high-dose chemotherapy with autologous bone marrow transplantation was performed at the same institution between June and July 1995. The patient received 825 mg carboplatin on days -7, -6, -5 and -4, 1000 mg etoposide on days -6, -5 and -4 and 4100 mg ifosfamide on days -6, -5, -4 and -3. After 2 days of rest, the bone marrow was retransfused on the 27 of June 1995. The patient tolerated the treatment despite of diarrhea and mucositis for two days and febrile neutropenia for 14 days quite well. Hematopoiesis was fully reconstituted on day 21. As a result of treatment, the patient was free of disease for seven years.

A relapse was diagnosed by follow-up abdominal and pelvic CT scan examination in January 2002. A single peritoneal recurrence 6 cm in diameter was found in the abdominal cavity. The patient was subjected to surgery again. This time, the his-

tologic work-up of resected recurrence revealed a low-grade endometrial stromal sarcoma. Additional immunohistochemical staining for CD117 (standard automated procedures, autostainer from Dako Cytomation, Copenhagen, Denmark, monoclonal mouse antihuman antibody clone 104D2) remained negative. Upon review, the tumors resected in 1994 were reclassified as low-grade endometrial stromal sarcoma in concordance with the histology of the local recurrence resected in 2002. As next therapeutic step adjuvant radiotherapy was initiated in May 2002.

However, a second recurrence was diagnosed in October 2003. Resection of the tumor (8 cm in diameter) infiltrating the ascending colon necessitated additonal right hemicolectomy. Histologic examination revealed multiple peritoneal metastases of LGESS. Further immunohistochemical studies were carried out using standard automated procedures (compare with immunohistochemical analysis of case 2). Analyses showed that the primary neoplasm as well as both recurrences stained strongly positive for PR and positive for aromatase but negative for ER.

Thus, antihormonal therapy with exemestane (Aromasin® 25 mg) was started as adjuvant treatment to prevent local relapse.

The patient received the aromatase inhibitor exemestane from December 2003 until April 2008. During this relatively long period of time follow-up examination showed no signs of relapse and the patient tolerated the treatment well. Tinnitus was her only complaint which was a sequel of former chemotherapies.

Nevertheless, CT of the abdomen and the pelvis showed a suspicious 3.6x3.3x4 cm cystic expansion with irregular solid border in the right minor pelvis (Figure 1A, in Februray 2008). A PET-scan of the cystic lesion showed marginaliy increased FDG tracer uptake further substantiating the occurrence of the third relapse.

Consequently, the patient was submitted to surgery again and after relaparotomy as well as adhesiolysis, resection of that relapse in the pelvis including partial resection of the ileum with side-to-side anastomosis, partial resection of the urinary bladder and resection of suspected nodes of the intestine´s mesenterium was performed in May 2008 (Figures 1B). No neoplastic infiltration could be found in the urinary bladder. Nonetheless, peritoneal involvement of the intestine by low-grade endometrial stromal sarcoma was detected. Histological examination of the tumor showed vascularity, nuclear atypia as well as round, ovoid and spindle-shaped nuclei. Mitoses were found. Focally, cystic degeneration of the tumor mass was

discovered. Pseudoadjuvant therapy with letrozole (Femara® 2,5 mg), a non-steroidal aromatse inhibitor, was administered from July 2008 to September 2008. Because of cutaneous toxicity and alopecia, therapy was switched to another non-steroidal aromatase inhibitor anastrozole (Arimidex® 1mg 1x1 daily). This drug was better tolerated by the patient and she remained with no evidence of disease up to now.

Material and Methods

To better understand the possible mechanic action of the pseudo-adjuvant treatment with aromatase inhibitor exemestane, expression of aromatase as well as JAZF1/JJAZ1 was carried out. For the detection method of aromatase expression refer to materials and methods section of case 2 (pages 57-58).

In this case, aromatase was found to be positive (no figure available).

Detection of the JAZF1/JJAZ1 gene fusion was performed using the same method as described in materials and methods section of case 2 (pages 55-57).

Differently to case 2, JAZF1/JJAZ1 gene fusion was detectable in case 1 (Figures 2A and 2B).

4.2 Case 2

A 70-year old uniparous Caucasian woman was referred to the outpatient department of the Department of Clinical Oncology of the Medical University Graz due to newly diagnosed metastases of an ESS to the lung, liver and bones in April 2005.

One and a half years earlier, total abdominal hysterectomy, bilateral salpingo-oophorectomy and pelvic lymphadenectomy was performed after the detection of a tumor of the uterus. Histologic examination of the surgical specimen revealed an endometrial stromal sarcoma of the uterus without lymph node involvement. The primary tumor measured 9 cm in largest diameter and consisted of monomorphic round cells as well as of polymorphic spindle cells. Areas with a sex-cord like pattern and foci of necrosis and hemorrhages were present (Figures 3 and 4). Twenty five mitoses per ten high power fields were detected suggesting a high proliferative potential.

Immunohistochemical studies of the neoplasm were performed. For detection of ER and PR, formalin-fixed and paraffin-embedded tissues were analyzed with monoclonal mouse antihuman antibodies (SP1, Neomarker; PgR636, Dako) using standard procedures (Dako REAL™ En Vision™ Detection System, Peroxidase/DAB+, Rabbit/Mouse, Code K5007, Dako, Copenhagen, Denmark, DAKO autostainer). The tumor showed a low immunoreactivity for estrogen receptors (5-10% of tumor cells, Figure 5) and below 1% of the tumor cells showed immunoreactivity for progesterone receptors, which was considered as negative. Additional staining for the detection of CD10, caldesmon, as well as KI67 and p53 were carried out using again Dako REAL™ En Vision™ Detection System (Peroxidase/DAB+, Rabbit/Mouse, Code K5007, Dako, Copenhagen, Denmark, DAKO autostainer; monoclonal antibodies: 56C6, Novacastra; h-CD, Dako; MIB-1, Dako; DO-7, Dako). A high immunoreactivity for CD10 was present (about 60% of the tumor cells positive, Figure 6) whereas no immunoreactiviy was found for caldesmon (Figure 7). KI67 labelling-index was 10-20% and expression of p53 was positive in 10-20% of tumor cells. Staining for inhibin (R1, Dako) was found to be negative and desmin (DE-R-11, Dako) only stained positive in a few areas.

After surgery, the patient was admitted to adjuvant radiation therapy in pelvis box technique in January 2004. However, radiation treatment was terminated ahead of schedule due to side effects.

In a follow up examination in April 2005, multiple lung metastases up to one centimeter in both lobes (Figure 8A), six cystic liver metastases up to 3.2 cm (Figure 8C), and multiple bone metastases were detected by CT scans.

Considering the patient's age, the absence of symptoms and potential side effects caused by chemotherapy, treatment with the aromatase inhibitor letrozole (Femara® 2,5 mg daily) was started despite the very low expression of steroid hormone receptors in the tumor. In addition, the bisphosphonate zolendronic acid (Zometa® 4 mg) was administered intravenously every 4 weeks.

Within 3 months a partial response of lung and liver metastases as well as sclerosis of osteolytic bone metastases could be demonstrated in follow up CT scans. Specifically, the lesion in segment 1 of the liver regressed from 3.2 cm to 0.9 cm.

However, in November 2006, CT showed a liquid or semi liquid structure (80 Hounsfield Units (HU)) in the mediastinum with 4 cm in axial diameter (Figure 9). All other metastatic sites remained unchanged. To further evaluate the new lesion, a CT-guided biopsy was suggested. Nevertheless, the patient declined the diagnostic procedure at that time. She was free of symptoms and had a good quality of life.

In March 2006, a complete response of all lesions in the lung and continuous partial response of all liver lesions (Figures 8B and 8D) as well as stable disease in the bone could be demonstrated. The patient was free of symptoms. All relevant laboratory parameters were within the norm. Partial response of the lung and liver metastases and stable disease of bone metastases was the result of the antihormonal treatment.

In a series of follow up examinations (Figures 10A-10D) the cystic lesion in the mediastinum progressed continuously to size of 15 cm in craniocaudal, 7.5 cm in transversal and 11.5 cm in sagittal diameter ranging from the manubrium sterni to the diaphragm in May 2008. Furthermore, contrast medium extravasate into the proximal part of the cystic lesion was detected suggesting hemorrhage. After interdisciplinary discussion in a tumor board involving radiologists, thoracic surgeons and oncologists, insertion of a stent into the vena cava superior resulting in hemostasis was carried out in May 2008. Additionally, a biopsy of the cystic lesion was performed. However, the obtained specimens just showed myxoid necrotic connective tissue and no malignant tumor cells. Assuming tumor progression of the

ESS, second-line treatment with the aromatase inhibitor exemestane (Aromasin® 25 mg) was initiated.

In August 2008, the patient complained about chest pain and shortness of breath on exertion. CT scan of the thorax showed massive progression of the mediastinal lesion resulting in compression of the right lung. Due to further progression aromatase inhibitor treatment was terminated and switched to trofosfamid, an oral cytostatic drug (Ixoten® 50 mg two times daily).

In October 2008, the patient´s condition worsened dramatically. She complained about pain, dyspnea, and dizziness. After a collapse she was admitted to the ER in cardio-respiratory distress. CT scan with contrast enhancement showed acute bleeding into the mediastinal lesion with complete atelectase of the right lung and mediastinal shift hampering cardiac output. Emergency thoracic surgery was performed and the mediastinal mass was resected completely. The patient´s condition improved within a few days. Unexpectedly, the histological work-up of the cystic mediastinal expansion revealed a malignant peripheral nerve sheath tumor and not metastasis of the ESS. Due to the fact that this tumor was a second primary of a malignant soft tissue sarcoma, therapy with aromatase inhibitor Femara was reinitiated to further control metastatic disease of ESS.

Materials and Methods

Due to the good anti-tumor response of aromatase inhibitor treatment despite the weak estrogen receptor expression and negative progesterone receptor expression of the ESS presented we became more interested in the mechanism of action of aromatase inhibitor treatment in this tumor. For better understanding which additional hormone sensitive targets might be invoved in anti-tumor action, we performed retrospective analyses of paraffin-embedded and fresh frozen tumor tissue samples.

First, we looked for *JAZF1/JJAZ1* gene fusion. As noted above the *JAZF1/JJAZ1* gene fusion is present in most ESS and may be related to the estrogenic pathway. In our case, sections of paraffin-embedded tissue were cut on a MICROM HM 355S microtome and the RT-PCR was used for detection. Positive control was another ESS case. Negative control was the same approach, however without a target. In our case, the JAZF1/JJAZ1 gene fusion was found to be negative (Fig-

ure 11). We employed the following protocol for the detection of JAZF1/JJAZ1 gene fusion:

By using Trizol (Invitrogen Life Technologies, Los Angeles, CA, USA) RNA from frozen tissue was extracted, homogenized and incubated at room temperature for 15 minutes. Following chloroform extraction and precipitation with isopropanol we washed RNA with 80% ethanol. After washing RNA, the pellet was dissolved in water which was RNAse free.

By using the "Optimum™ FFPE RNA Isolation Kit" from Ambion (Ambion Diagnostics, Woodward, Austin TX, USA) RNA from the formalin-fixed and paraffin-embedded tissue was extracted. At room temperature paraffin was removed by extracting twice with xylene for ten minutes. After this extraction centrifugation at 13000 rpm for 3 minutes was started. Consequently, the tissue pellet was washed with 100%, 90% and 70% ethanol. Using aspiration, ethanol was then removed again. At room temperature, the tissue pellets had time to dry. Over night and at 60°C, the samples were incubated with 200 µl of Proteinase K working solution (100 µl preoteinase K Digestion Buffer and 10 µl proteinase K; 60 U/µl). Furthermore, RNA was isolated and with 20 µl at 75°C preheated Elution Solution it was eluted. Then DNAse I (2 U per sample) was used at 37°C for 30 minutes for treating the RNA samples. This DNAse I was inactivated with the DNAse I Inactivation Reagent which was supplied by the manufacturer. The RNA samples were now used for RT-PCR .

For reverse transcription the Superscript II reverse transcriptase (200 U/µl; Invitrogen) was used for one µg of total RNA. In a volume of 20µl reactions were performed. A buffer which was provided by the manufacturer was used and additionally 10 mmol/L dNTPs, 40 U/µl of Rnase inhibitor (Invitrogen), 0.3 µl (5nM) of random hexamers and 500 ng of RNA (for the cryopreserved tissue; for the paraffin–embedded samples 10µl of isolated RNA were used) were added. The conditions of reaction were 65°C and 5 minutes, 42°C and 90 minutes and 90°C and 15 minutes.

For detection of the JAZF1/JJAZ1 gene fusion either reverse transcriptase polymerase chain reaction (RT-PCR) or the nested PCR can be used. We used the RT-PCR.

For carrying out the RT-PCR of the control gene we had to mix 2 µl cDNA with 28 µl Mastermix (10 pM GAPDH–FW primer, 10 pmol GAPDH-RV primer 15 µl Uni-

versal Mastermix and ddH2O ad 30 µl). The cycling profile was as follows: One cycle of 15 seconds at 95°C and then 50 cycles of one minute at 60°C.
For JAZF1/JJAZ1 we had to mix 3 µl cDNA with 28µl Mastermix (6 mM dNTPs, 45 mM MgCl2, 3 µl 10xPCR buffer, 6 pM FW-primer, 6 pM RV-primer, ddH2O ad 30 µl and 0.12 µl Ampli Taq). The cycle profile was initial denaturation at 94°C for 30 seconds, then 40 cycles at 94°C for 30 seconds, at 57°C for 30 seconds, at 72°C for 20 seconds and finally 10 minutes at 72°C. [Hrzenjak A., Department of Pathology, Medical University of Graz]

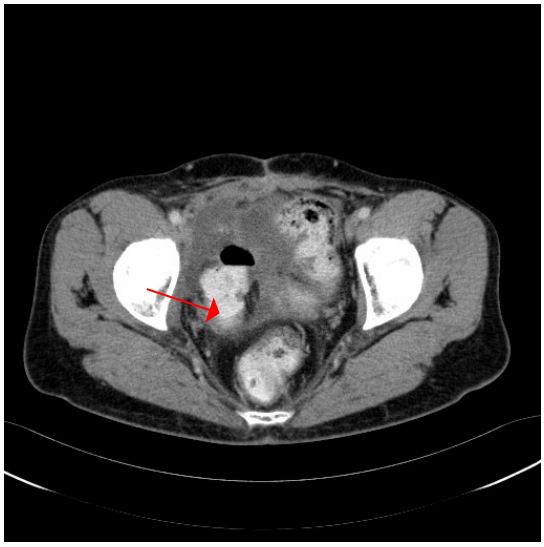
Next, the expression of *aromatase* was analized by immunohistochemistry using different dilutions of cytochrome P450 aromatase monoclonal antibody (SM2222P, clone H4; Acris Antibodies, Hiddenhausen, Germany). Surprisingly, the expression was found to be negative. To substantiate this finding and to exclude the possibility that the antibody used was not senitive enough, CYP450 aromatase expression was tested by immunoblotting: Proteins extracted from frozen tumor tissue (40 µg of total protein) were separated on 8% SDS-PAGE and blotted under standard condition using human placenta as a positive control. As a secondary antibody HRP-labelled rabbit anti mouse (1:2000) was used. Again no expression of aromatase could have been detected (Figure 12). Briefly, the immunoblotting procedure is described below:
Blotting is the technique to detect a target protein in a sample. Monoclonal or polyclonal antibodies can be used which are specific to that protein. To reduce non-specific protein interactions between the membrane and the antibody, the membrane is blocked. This means that the membrane is placed in a solution of non-fat dry milk (NFDM) as in our case or bovine serum albumin (BSA). The first antibody which has to be specific for the protein of interest is incubated with the membrane and should not bind to any other protein. To remove unbound primary antibody you have to rise the membrane and then the secondary antibody is incubated. The secondary antibody which is typically linked to an enzyme that produces fluorescence binds to the primary one. Unbound secondary antibody is washed away. The membrane is incubated with the enzyme substrate. The secondary membrane bound antibodies emit light so that the bands which correspond to the detected protein of interest appear as dark regions on the film.

SDS-PAGE means Sodium dodecyl sulfate polyacrylamide gel electophoresis. It is the most powerful technique for separating proteins according to their size. Before as well as during gel electrophoresis proteins are exposed to SDS which denatures secondary as well as non-disulfide-linked tertiary structures and which applies a negative charge to the proteins in proportion to the mass. Different proteins with similar molecular weights would migrate differently without SDS because of differences in mass-charge ratio. SDS treatment however eliminates the effects caused by differences in shape so that only the chain length reflecting the mass is the sole determinant of the migration rate. Therefore, to carry out a SDS-PAGE you have to make a gel and assemble the gel apparatures, you have to mix protein samples with sample buffer containing SDS and to heat the mixture at high temperature, you have to load samples and run the electrophoresis and then you have to fix and stain the separated proteins. [Hrzenjak A., Department of Pathology, Medical University of Graz]

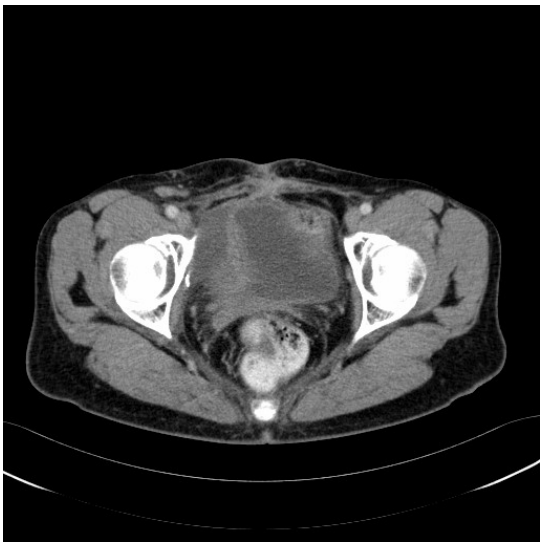
In conclusion, we could not explain the very good tumor response of the ESS to aromatase inhibitor treatment by the above mentioned examinations. Our interpretation of these results is that ESS tumor cells might be extremely sensitive to estrogens even with low estrogen receptor expression and inhibition of estrogen synthesis by at least peripheral blockage of aromatization may be sufficient to yield tumor regression.

5 Figures

5.1 Figures of case 1



A



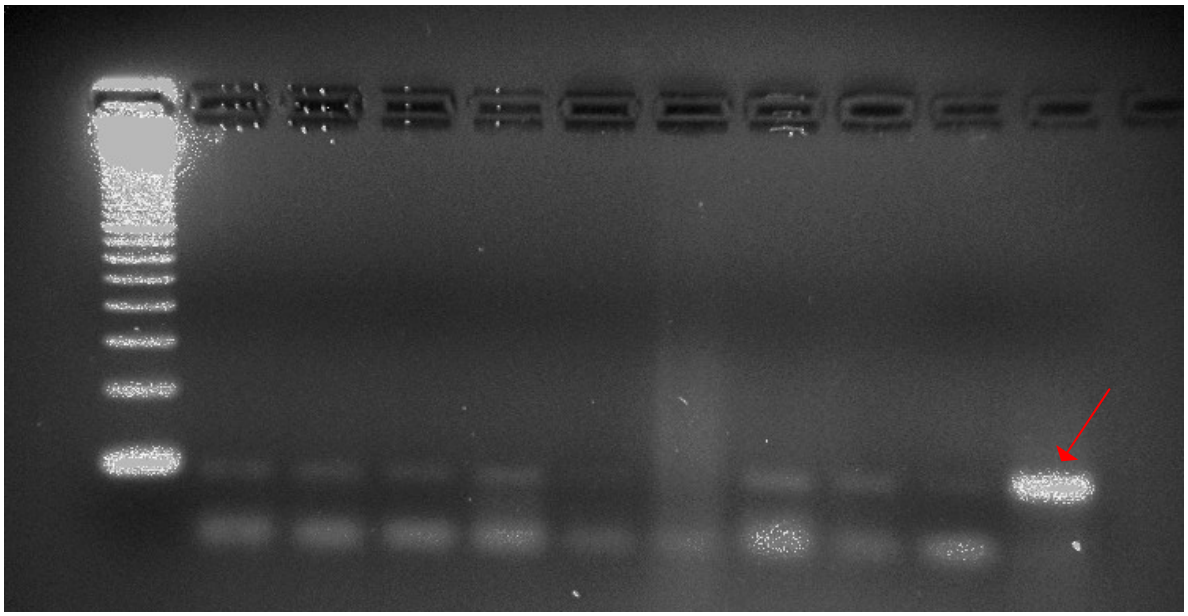
B

Figure 1: CT scan of the third relapse

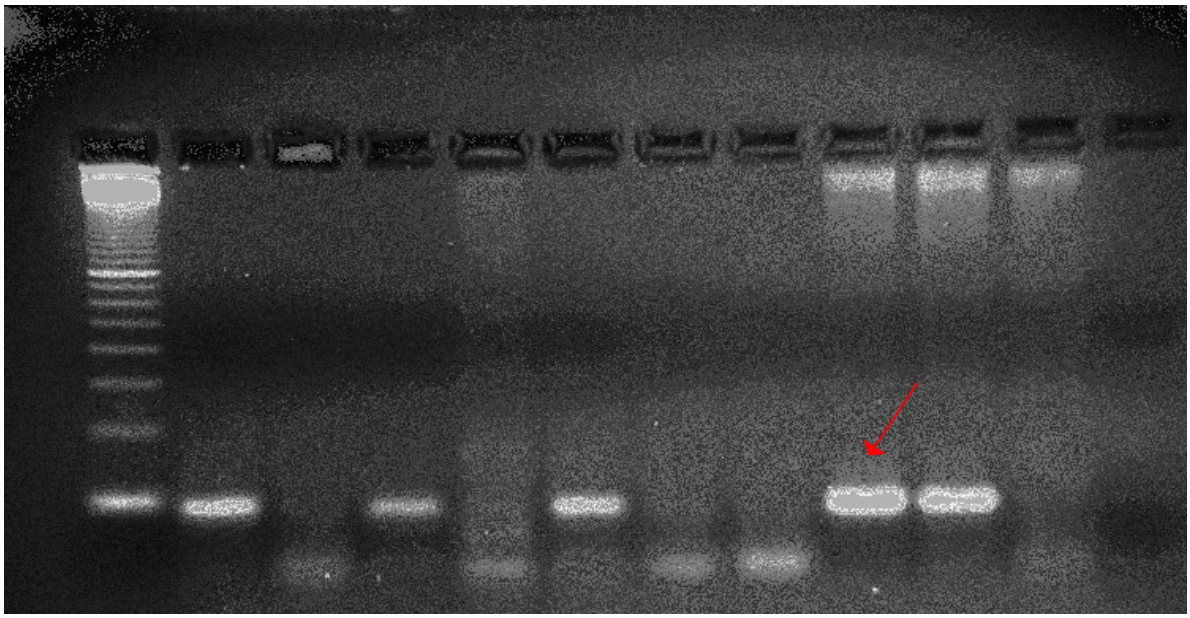
(A) CT scan of the pelvis on the 25th February 2008. The third relapse (arrow) in the pelvis is demonstrated.

(B) CT scan on the 2nd July 2008. showing the result of surgery.

Department of the private hospital of the “Kreuzschwestern”



A



B

Figure 2: RT-PCR for the detection of the JAZF1/JJAZ1 gene fusion

(A) Formalin fixed material (FFPE) of case 1: the red arrow indicates the JAZF1/JJAZ1 gene fusion positivity

(B) positive control=positive case of ESS, cryo=cryo-preserved material of case 1: the red arrow indicates again JAZF1/JJAZ1 gene fusion positivity;

Department of Pathology, Medical University of Graz

5.2 Figures of case 2

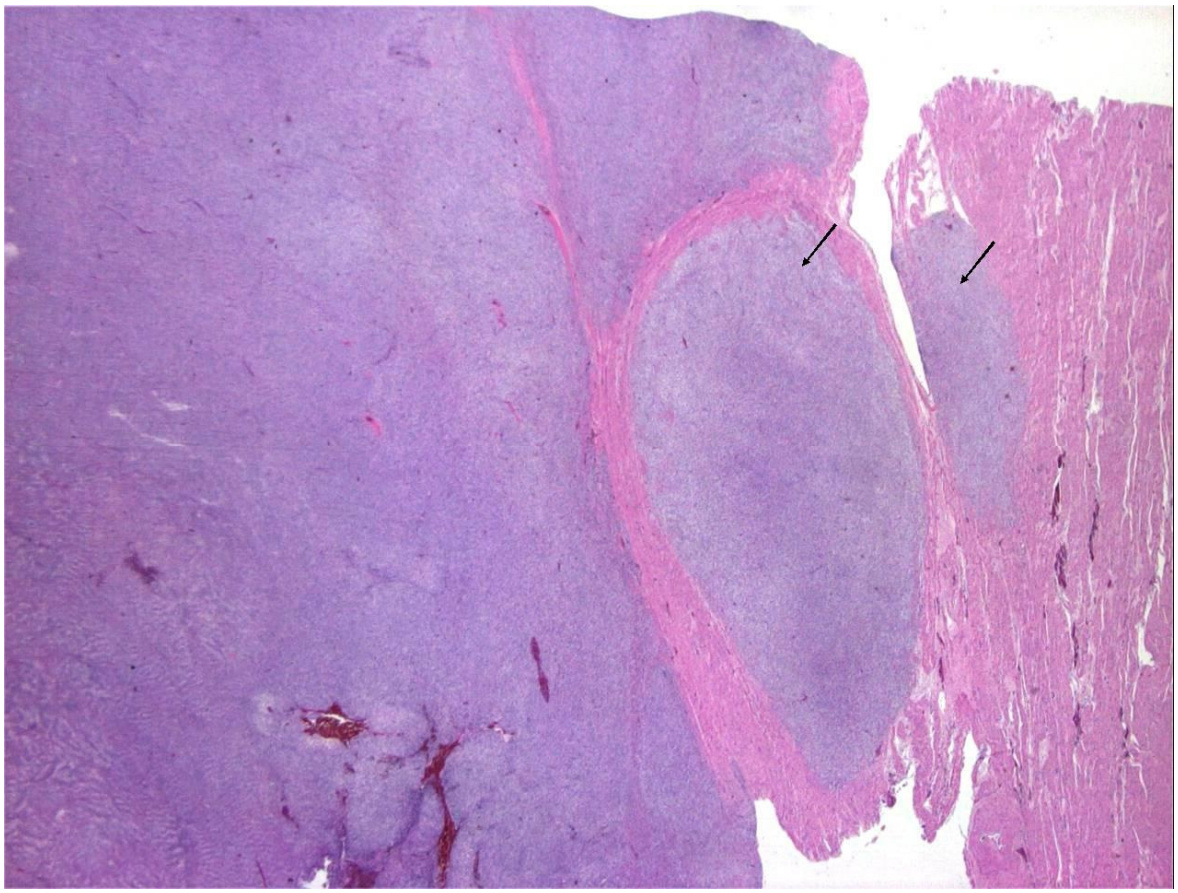


Figure 3: Primary tumor of the uterus, HE 10X

Tumor invasion is demonstrated by small nodules (arrows) within the adjacent myometrium.

Department of Pathology, General Hospital Graz West

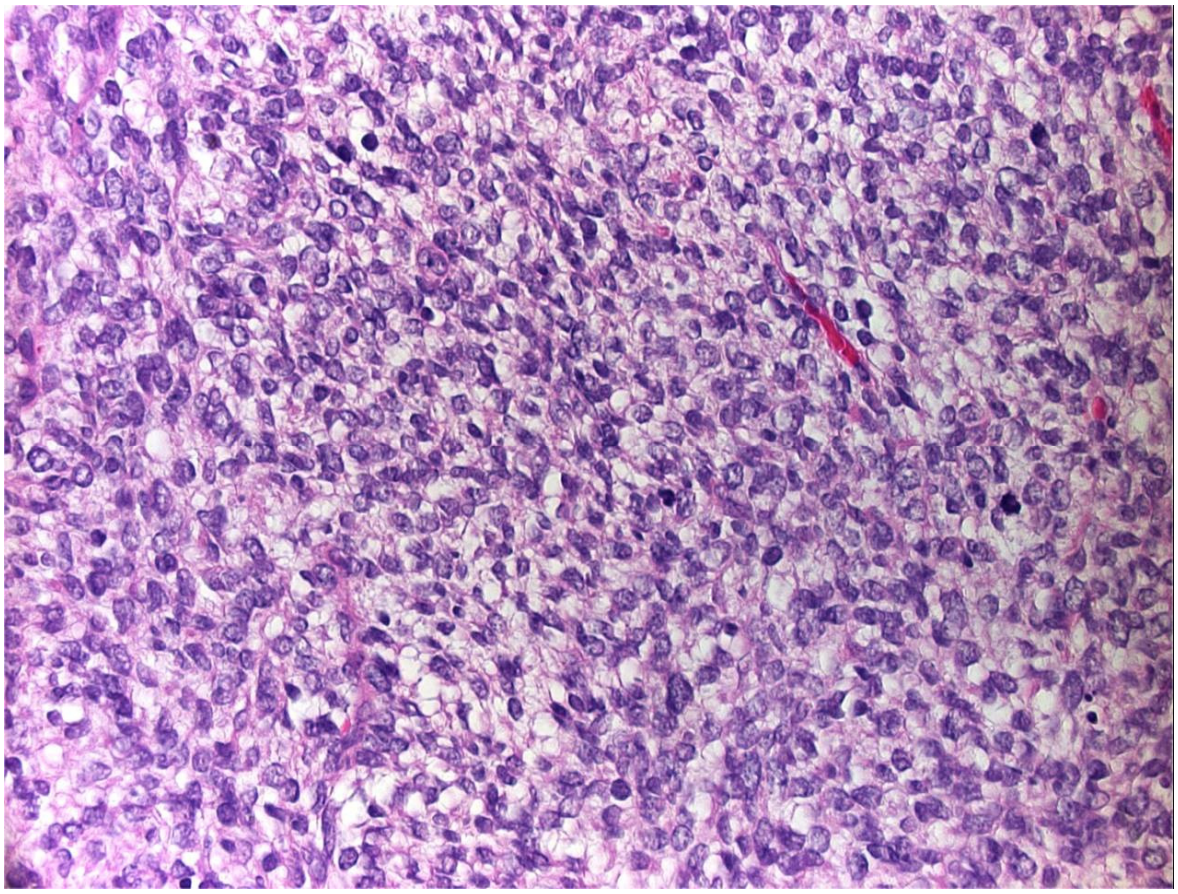


Figure 4: Tumor cells typical of ESS, HE 200X

At high magnification the tumor consists of small cells with mildly to moderately pleomorphic round to oval nuclei.

Department of Pathology, General Hospital Graz West

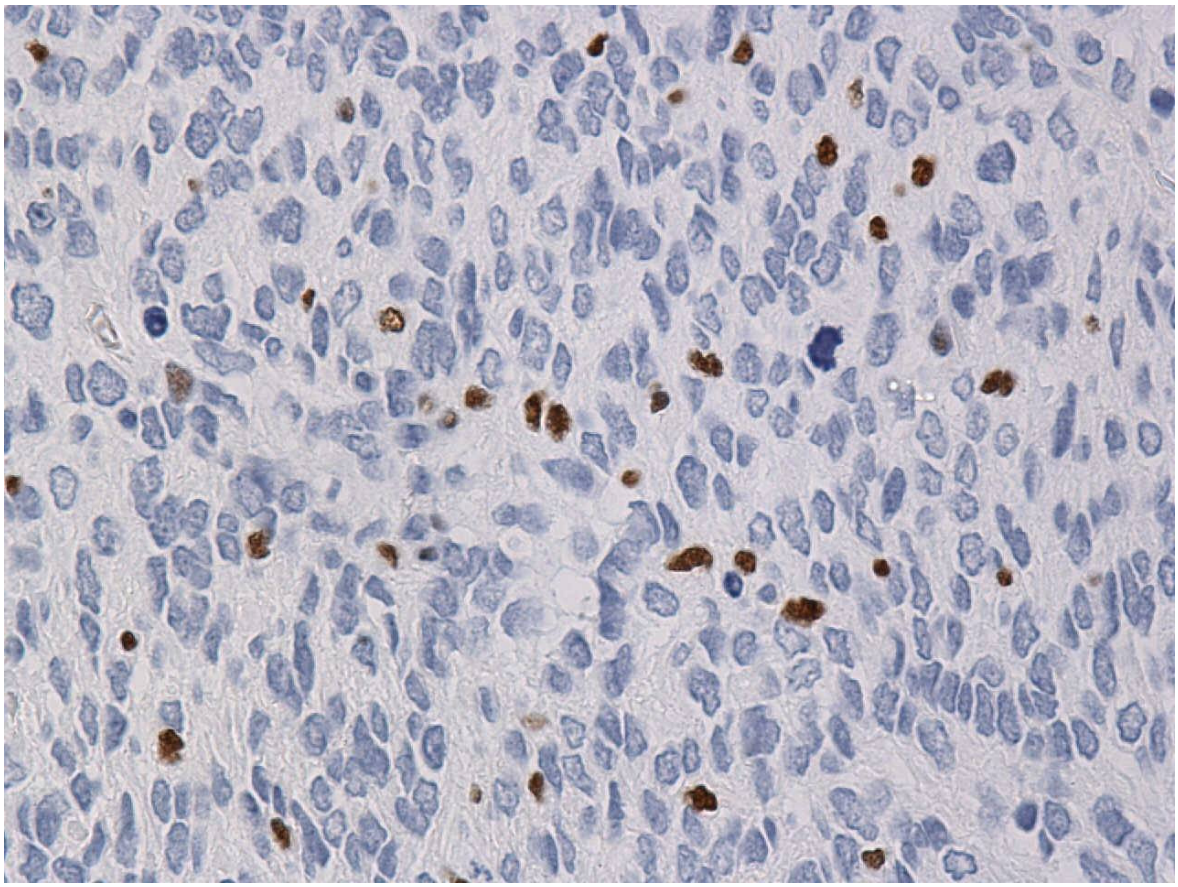


Figure 5: Estrogen receptor expression of ESS, DAB 400X

A subset of tumor cells (<1%) show immunoreactivity with antibodies against estrogen receptors.

Department of Pathology, General Hospital Graz West

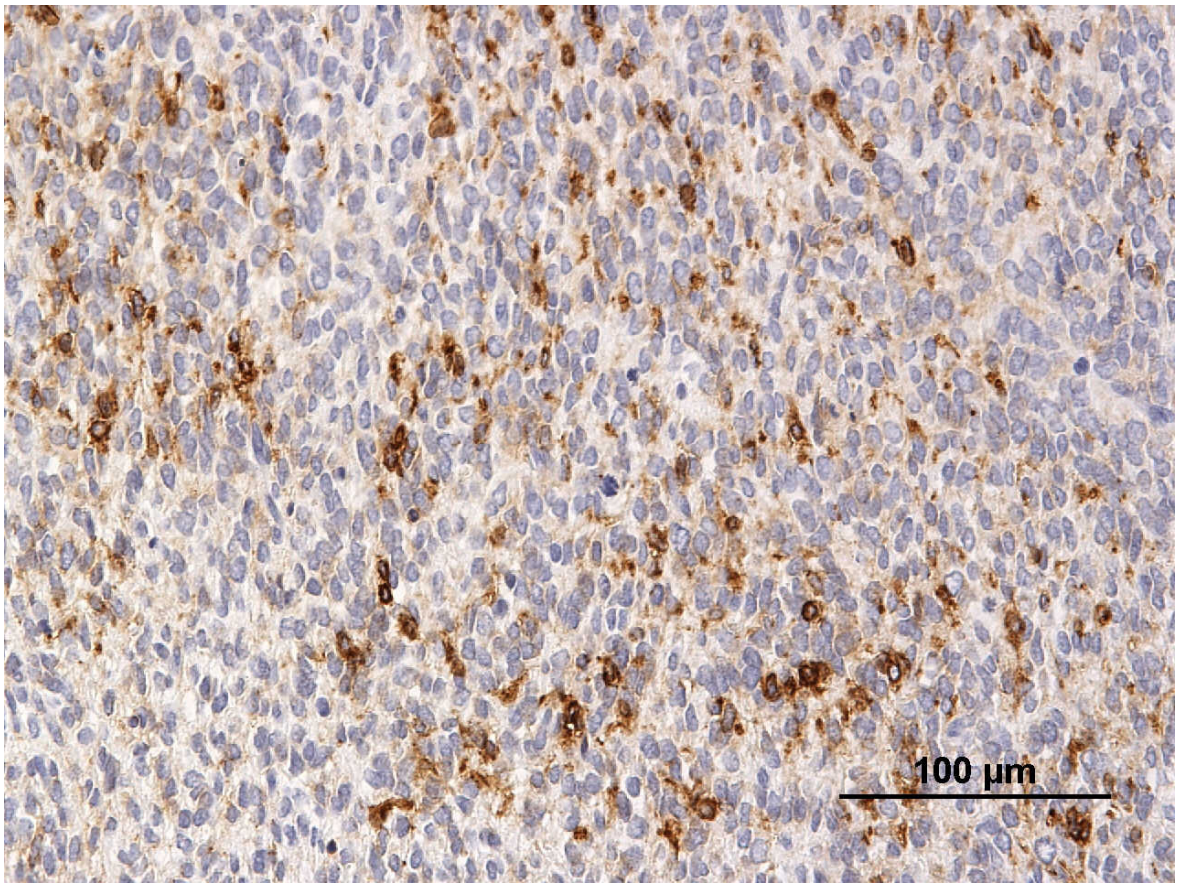


Figure 6: CD10 expression of ESS, DAB 200X

About 50-60% of the tumor cells are immunoreactive for CD10.

Department of Pathology, General Hospital Graz West

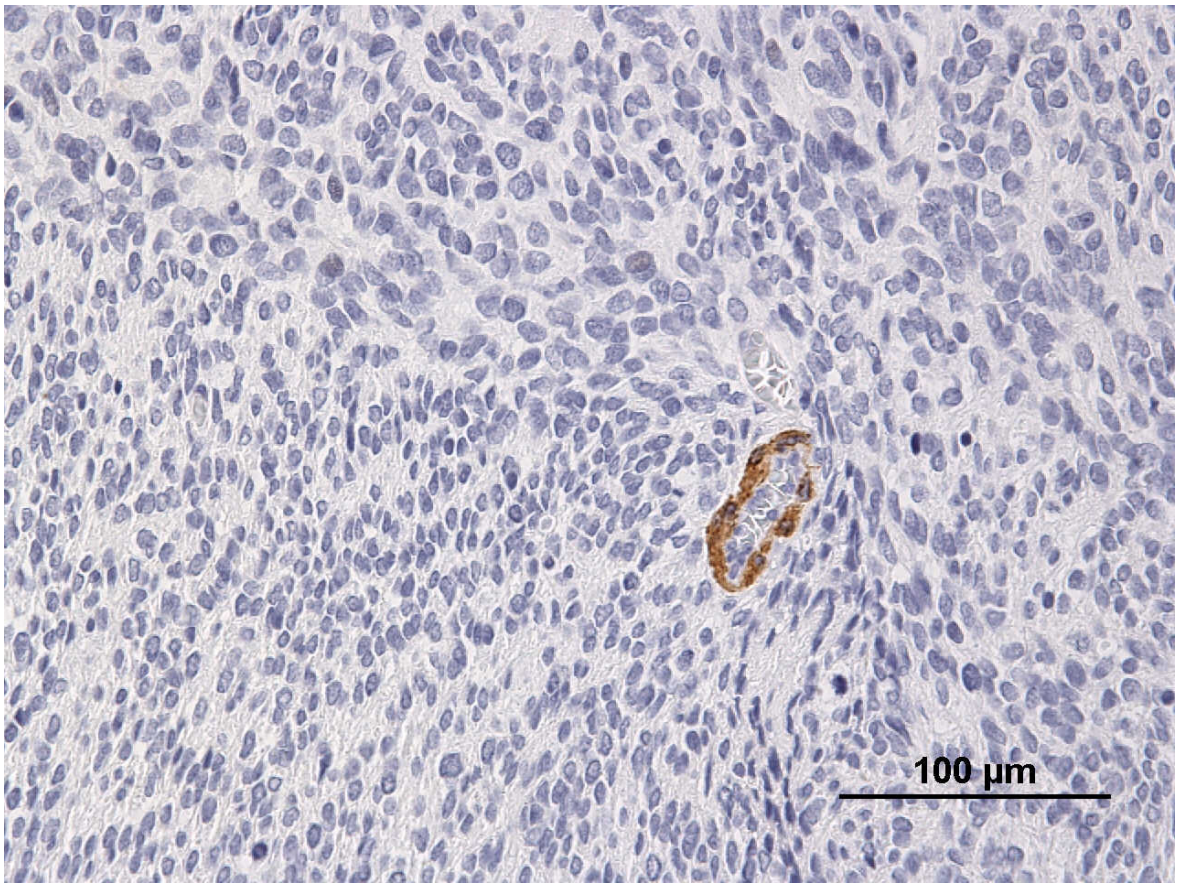
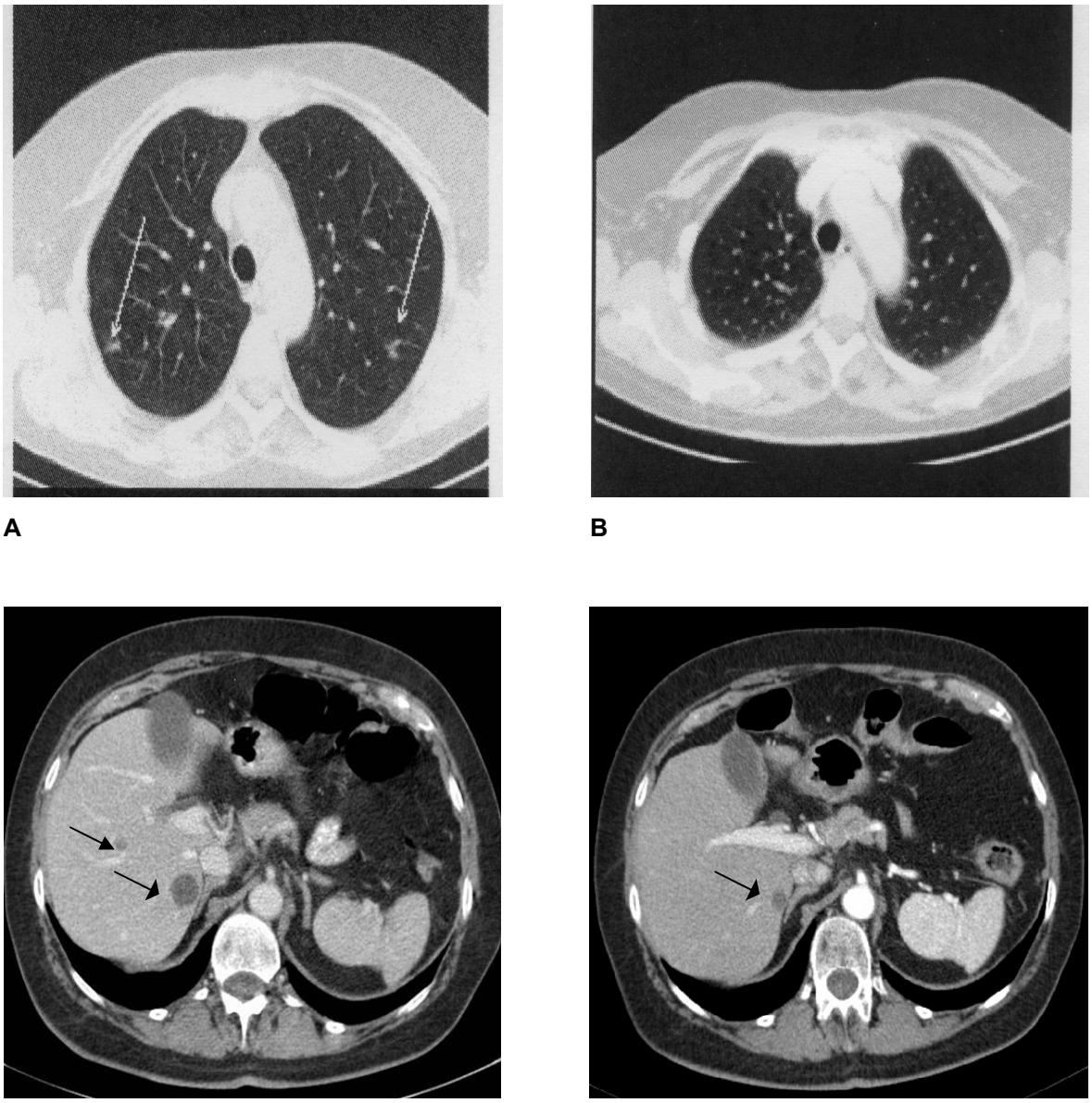


Figure 7: Caldesmon expression of ESS, DAB 200X

The tumor cells are negative for caldesmon, which is expressed by one small blood vessel (arrow). Department of Pathology, General Hospital Graz West



A **B**

C **D**

Figure 8: Lung and liver metastases

(A) CT scan of the lung showing multiple metastases (arrow) on the 15th April 2005.

(B) CT scan showing the remission of the lung metastases on the 30th November 2005.

(C) CT scan of the abdomen showing multiple metastases (arrow) on the 15th April 2005.

(D) CT scan showing the remission of the liver metastases on the 15th March 2006.

Department of Imaging Diagnostics, private hospital of the “Kreuzschwestern”

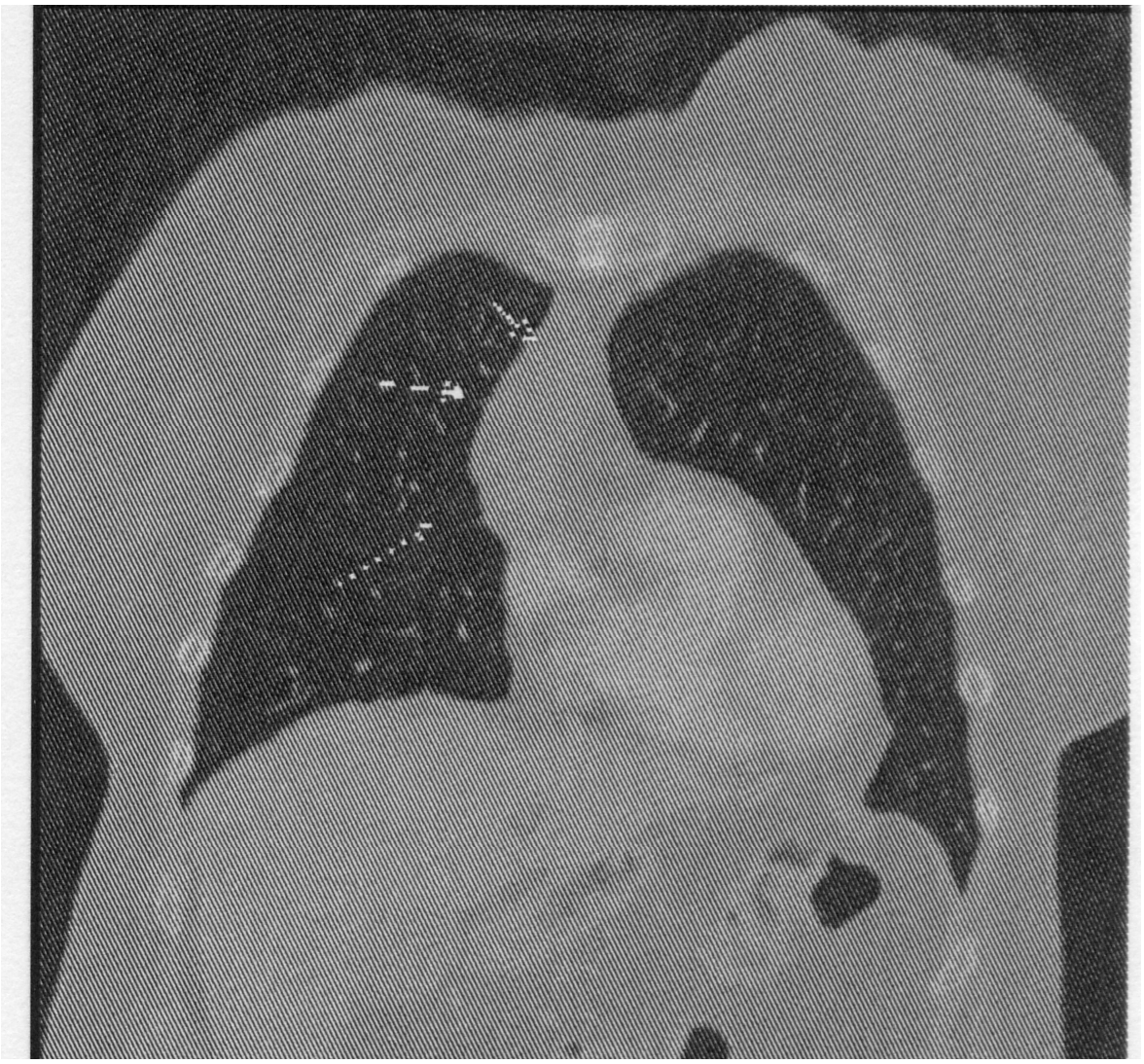


Figure 9: Mediastinal expansion

CT scan demonstrates the mediastinal expansion (arrows) for the first time on the 22 th November 2006. Because HU were 80, it was first suggested to be a chylothorax. However, because of the patient´s neoplasm and the possibility of metastases, follow-up CT scans were recommended.
Center of Diagnostics for CT and MRI, Graz-Kroisbach

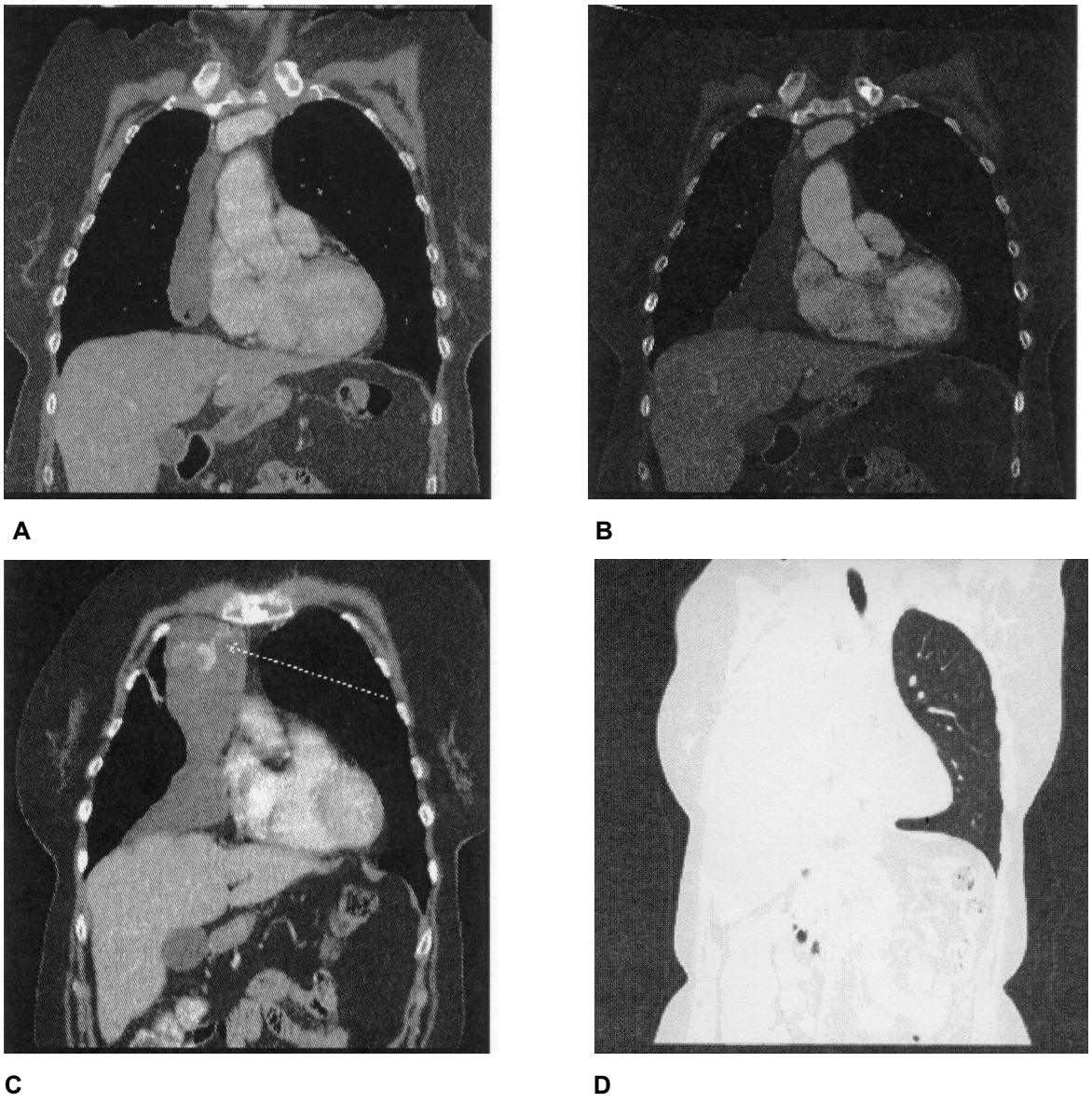


Figure 10: Follow-up examinations of the mediastinal expansion

(A) CT scan on the 6th June 2007 showing the cystic mediastinal expansion in near proximity of the right lung.

(B) CT scan on the 10th December 2007 showing the mediastinal expansion enlarged in size.

(C) CT scan on the 3rd April 2008. The contraste medium extravasate is demonstrated (arrow) suggesting hemmorrhage.

(D) CT scan on the 14th July 2008. It demonstates atelectasis of the right lung due to compression caused by renewed hemmorrhage.

Center of Diagnostics for CT and MRI, Graz-Kroisbach

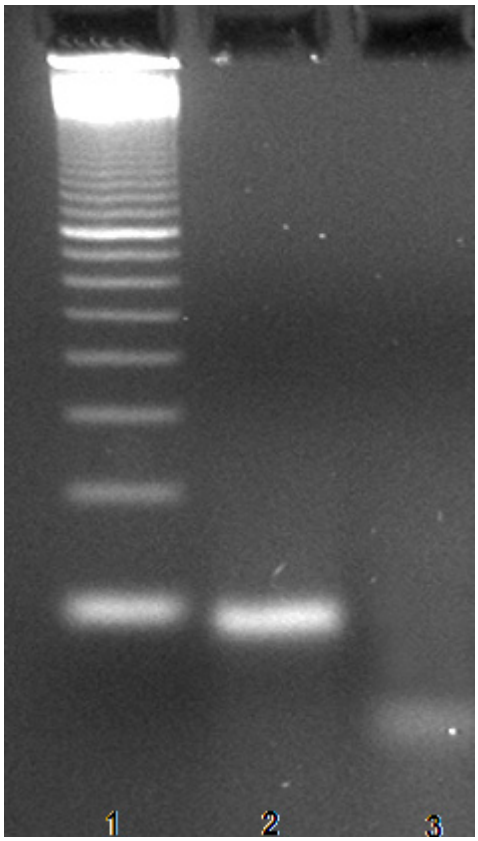


Figure 11: RT-PCR for the detection of the JAZF1/JJAZ1 gene fusion

1=DNS marker

2=positive control (positive case of ESS)

3=case 2

The DNS band has to be seen at 71 bp (compare with the positive control). It demonstrates that the gene fusion in case 2 is negative.

Department of Pathology, Medical University of Graz

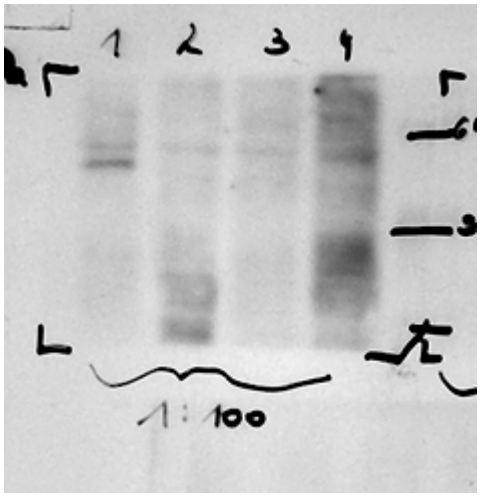


Figure 12: Western blot for the detection of aromatase expression

1= positive control (placenta)

2= ovary

3= testis

4= case 2

The protein band would normally has to be seen at 55 kDa. Case 2 is negative for aromatase expression.

Department of Pathology, Medical University of Graz

6 Discussion

We have reported two cases of ESS with differences in histological, molecular, and biological features. Especially hormone receptor expression, intratumoral aromatase activity, mitotic rate and presence of JAZF1/JJAZ1 gene fusion were dissimilar. Furthermore, the clinical course of disease showed distinct patterns of local as well as distant failure. However, the response to hormonal treatment strategies was very similar.

Histology and Immunohistochemistry

Diagnosis of ESS is based on histology, immunohistochemistry and molecular features. Our cases show that despite using state of the art immunohistochemistry and molecular staging, exact diagnosis and assignation to LGESS or HGESS may be difficult.

In the first case, the patient´s tumor was initially diagnosed as HGESS stage III. After resection of a relapse seven years later, the tumor had to be reclassified as LGESS taking the original histology of the primary tumor into account. Differentiation between those two entities might be further perturbed due to “intermediate” features like higher mitotic index, missing JAZF1/JJAZ1 gene fusion and low expression of hormonal receptor expression as demonstrated in case 2.

In case 1, ER were immunohistochemically negative. However, PR expression and aromatase activity were found to be positive which is a typical characteristic of LGESS. Retrospective analysis of the JAZF1/JJAZ1 gene fusion was performed with cryo-preserved tumor samples for better understanding the tumor´s molecular biology. In this case, the JAZF1/JJAZ1 gene fusion could be detected confirming the diagnosis of LGESS.

Focus of this thesis is the neoplasm of the second case and its response to antihormonal treatment. As mentioned above the neoplasm could not be assigned to a definite type of the classification system. On the one hand, the tumor showed several features which are characteristic for an UES: polymorphic spindle cells, tumor cell necrosis, hemorrhage, 25 MF/10 HPF suggesting a high proliferative potential and a more aggressive phenotype. The extremely low expression for ER and the negative staining for PR and aromatase expression point to an undifferentiated type of ESS. Higher KI67 expression and positivity for p53 reflected high prolifera-

tive potential. Again, retrospective analysis of the JAZF1/JJAZ1 gene fusion was carried out showing that this fusion was absent. Absence of the gene fusion substantiated the diagnosis of an HGESS or even of UES-P on molecular level. However, on the other hand, CD10 expression and negative staining for caldesmon was typical for ESS. The good and long-lasting response to antihormonal therapy despite the lack of hormone receptor expression did not fit well to the diagnosis of an UES-P or HGESS. Classification as an “intermediate type of endometrial stromal sarcoma” showing many features of undifferentiated sarcomas but responding very well to antihormonal treatment would better characterize this neoplasm specifically.

Whether intermediate grade ESS should be added new entity to the existing classification system remains to be determined and warrants further investigations.

Aromatase inhibitors in adjuvant setting

Due to the misinterpretation of HGESS in case 1 and the fact that treatment options for recurrent ESS were limited, it was decided to start conventional chemotherapy. As consolidation treatment even high-dose chemotherapy and autologous bone marrow transplantation were administered because of the young age of the patient, a tumor thought to be highly aggressive and due to peritoneal spread. At that time, high dose chemotherapy was a new hope which was believed to yielding higher response rates and longer response duration or even cure. In our case, a disease-fee interval of seven years was observed. After a local relapse, the treatment option of choice was resection and adjuvant radiotherapy. However, about one and a half years later, a second relapse was discovered and radical surgery was attempted. Reich and Regauer [12] were the first who explored intratumoral aromatase activity in these types of sarcomas. They could demonstrate that a subgroup of ESS show aromatase activity and suggested that aromatase inhibition might be a new treatment option for this tumor entity. Due to the fact that the tumor was found to be positive for aromatase activity in addition to high PR expression, we decided to treat the patient with the selective steroidal aromatase inhibitor exemestane as a pseudoadjuvant treatment strategy. Under exemestane therapy, the patient remained free of disease for nearly five years without appreciable side

effects. Compared to adjuvant radiation treatment after the first local relape, the disease free interval was significantly prolonged by aromatase inhibitor therapy. Nevertheless, a third relapse occured and after complete resection, pseudoadjuvant therapy with the selective non-steroidal letrozole was started. Because of cutaneous toxicity, therapy was switched to the selective non-steroidal aromatase inhibitor anastrozole. No further relapse has been observed up to now.
A retrospective analysis of the JAZF1/JJAZ1 gene fusion was found to be positive. If there is a relationship between the gene fusion and the estrogenic pathway is not clear until today. This case shows that aromatase inhibitors might be a new option in the adjuvant or pseudoadjuvant treatment of ESS

Aromatase inhibitors in palliative setting

Recent evidence suggests that metastatic ESS, especially ESS with high expression of ER and PR are sensitive to hormonal treatment including megestrol acetate, GNRH-agonists and aromatase inhibitors [for review see reference 8 in the supplement]. Furthermore, aromatase expression in LGESS has been described as mentioned in the case above. The extra-ovarian estrogen production depends on conversion of circulating androgens to estrogens via the aromatase enzyme complex. Aromatase inhibitors may have anti-tumor activity in ESS by blockage of peripheral as well as intratumoral aromatization. In the literature, the clinical response of 9 patients treated with aromatase inhibitors has been reported up to now demonstrating a complete response in 2 patients, partial response in 6 patients and progressive disease in one patient [55,66,72,73; Table 7). This review of literature shows that aromatase inhibitors are effective in the treatment of metastatic ESS. Compared to our case 2, ER and PR were positive in all the other studies (in one study the receptors were analysed biochemically instead of immunohistochemically). In addition, age and menopausal status of women presented varied. Different surgical and medical procedures were used as well as different types of aromatase inhibitors were employed (selectivebversus non-selective aromatase inhibitors). Furthermore, aromatase activity as well as JAZF1/JJAZ1 gene fusion status has not been explored in the other studies.
The absence of a JAZF1/JJAZ1 gene fusion in the tumor of case 2 which is typical for the vast majority of ESS suggests a more aggressive genotype. This concurs

with the very low expression of ER and absence of PR and aromatase. Treatment with the aromatase inhibitor letrozole was initiated due to the fact that the patient was asymptomatic and above 70 years of age not suitable for aggressive chemotherapy. A major objective response could be observed lasting for four years. It can be hypothesized that ESS tumor cells might be extremely sensitive to estrogens even with low ER expression and, that inhibition of estrogen synthesis by at least peripheral blockage of aromatization may be sufficient to yield tumor regression.

Table 7: Cases of successful aromatase inhibitor treatment

Author	Patient´s age	Metastases	Receptors	Pretreatment	Aromatase inhibitors	Response	Response Duration in Months
Maluf et al. [66]	51 years	pelvis, abdomen	ER+, PR+	TAH, BSO megestrol acetate 40 mg radiatio 50.4 Gy tamoxifen 20 mg	letrozole	PR	9 months until an experimental course with another treatment
Spano et al. [72]	44 and 37 years	lung	ER+, PR+	TAH in the first patient TAH, BSO, HRT in the second patient	AG	CR	follow up 14+ and 7+
Leunen et al. [73]	76 years	pelvis	ER+, PR+	TAH, BSO, HRT	letrozole	PR	follow up 36+
Pink et al. [55]	10 patients age range 31-69	lung, peritoneal spread, uterine tumor	ER+, PR+	ERT in 5 patients tamoxifen in 3 patients TAH in 9 patients	MPA	1 CR 1 PR	follow up 4+ to 164+
					letrozole	3 PR, 1st line 1 PR, 2nd line 1 PD, 2nd line	
					stopping ERT	1 NED 2 SD	

Abbreviations: ER=estrogen receptor, PR=progesterone receptor/ partial response, CR=complete response, PD=progressive disease, NED=no evidence of disease, SD=stable disease, TAH=total abdominal hysterectomy, BSO=bilateral salpingo-oophorectomy, HRT=hormonal replacement therapy, ERT=estrogen replacement therapy, AG=aminoglutethimide, MPA=medroxyprogesterone acetate

Notice: All the sarcomas were LGESS.

This table only gives a broad but not complete overview. For a detailed analysis you have to read the reports by the different authors.

7 CONCLUSION

The conclusion of this thesis is that aromatase inhibitors should be regarded as promising new treatment option for patients with ESS regardless of their aromatase activity status or level of hormonal receptors. As demonstrated in our study, response to aromatse inhibitior treatment might be observed even in tumors with low hormone receptor status and no intratumoral aromatase activity. Aromatase inhibitors might be employed in the adjuvant setting as well as in palliative setting. Further studies are warranted to determine the specific mode of action of anti-hormonal treatment in ESS. Furthermore, the question of the need of a new classification system for ESS including the possible existence of an “intermediate grade” ESS in addition to LGESS and HGESS has to be addressed.

.

8 References

1. Pautier P, Genestie C, Rey A, Morice P, Roche B, Lhommé C, et al. Analysis of clinicopathologic prognostic factors for 157 uterine sarcomas and evaluation of a grading score validated for soft tissue sarcoma. Cancer 2000;88(6):1425-31
2. Denschlag D, Masoud I, Stanimir G, Gilbert L. Prognostic factors and outcome in women with uterine sarcoma. Eur J Surg Oncol 2007;33(1):91-95
3. Kokawa K, Nishiyama K, Ikeuchi M, Ihara Y, Akamatsu N, Enomoto T, et al. Clinical outcomes of uterine sarcomas : results from 14 years worth of experience in the Kinki district in Japan (1990-2003). Int J Gynecol Cancer 2006;16(3):1358-1363
4. Harlow BL, Weiss NS, Lofton S. The epidemiology of sarcomas of the uterus. J Natl Cancer Inst 1986;76(3):399-402
5. Brooks SE, Zhan M, Cote T, Baquet CR. Surveillance, epidemiology, and end results analysis of 2677 cases of uterine sarcoma 1989-1999. Gynecol Oncol 2004;93(1):204-8
6. Ashraf-Ganjoei T, Behtash N, Shariat M, Mosavi A. Low grade endometrial stromal sarcoma of uterine corpus, a clinico-pathological and survey study in 14 cases. World J Surg Oncol 2006;4:50
7. Yamada K, Kano J, Tsunoda H, Yoshikawa H, Okubo C, Ishiyama T, et al. Phenotypic characterization of endometrial stromal sarcoma of the uterus. Cancer Sci 2006;97(2):106-112
8. Weitmann HD, Knocke TH, Kucera H, Pötter R. Radiation therapy in the treatment of endometrial stromal sarcoma. Int J Radiat Oncol Biol Phys 2001;49(3):739-748
9. Lenhard SM, Untch M, Himsl I, Ditsch N, Bittmann I, Friese K, et al. The high-grade endometrial sarcoma: a rare entity. Arch Gynecol Obstet 2006;274(1):56-59
10. Scribner DR, Walker JL. Low-grade endometrial stromal sarcoma preoperative treatment with Depo-Lupron and Megace. Gynecol Oncol 1998;71(3):458-60

11. Chu MC, Mor G, Lim C, Zheng W, Parkash V, Schwartz PE. Low-grade endometrial stromal sarcoma: hormonal aspects. Gynecol Oncol 2003;90(1)170-6
12. Reich O, Regauer S. Aromatase expression in low-grade endometrial stromal sarcomas: an immunohistochemical study. Mod Pathol 2004;17(1):104-8
13. Gerber B, Krause A, Reimer T, Mylonas I, Makovitzky J, Kundt G, et al. Anastrozole versus Tamoxifen treatment in postmenopausal women with endocrine-responsive breast cancer and tamoxifen-induced endometrial pathology. Clin Cancer Res 2006;12(4):1245-50
14. Eddy GL, Mazur MT. Endolymphatic stromal myosis associated with tamoxifen use. Gynecol Oncol 1997;64(2):262-4
15. Saga Y, Ohwada M, Kohno T, Takayashiki N, Suzuki M. High-grade endometrial stromal sarcoma after treatment with tamoxifen in a patient treated for breast cancer. Int J Gynecol Cancer 2003;13(5):690-2
16. Winer EP, Hudis C, Burstein HJ, Wolff AC, Pritchard KI, Ingle JN et al. American Society of Clinical Oncology technology assessment on the use of aromatase inhibitors as adjuvant therapy for postmenopausal women with hormone receptor-positive breast cancer: status report 2004. J Clin Oncol 2005;23(3):619-29
17. Stauber M, Weyerstahl T. Gynäkologie und Geburtshilfe, 2nd ed. Stuttgart: Georg Thieme; 2005. p. 310.
18. Irvin W, Pelkey T, Rice L, Andersen W. Endometrial stromal sarcoma of the vulva arising in extraovarian endometriosis: a case report and literature review. Gynecol Oncol 1998;71(2):313-6
19. Sampson JA. Endometrial carcinoma of the ovary, arising in endometrial tissue in that organ. Arch Surg 1925;10(1):1-72
20. Scott RB. Malignant changes in endometriosis. Obstet Gynecol 1953;2(3):283-9
21. Heaps JM, Nieberg RK, Berek JS. Malignant neoplasms arising in endometriosis. Obstet Gynecol 1990;75(6):1023-8
22. Chang KL, Crabtree GS, Lim-Tan SK, Kempson RL, Hendrickson MR. Primary extrauterine endometrial stromal neoplasm: a clinicopathologic study

of 20 cases and a review of the literature. Int J Gynecol Pathol 1993;12(4):282-96

23. Mourra N, Tiret E, Parc Y, de Saint-Maur P, Parc R, Flejou JF. Endometrial stromal sarcoma of the rectosigmoid colon arising in extragonadal endometriosis and revealed by portal vein thrombosis. Arch Pathol Lab Med 2001;125(8):1088-90
24. Tavassoli FA, Devilee P. Tumours of the breast and female genital organs. WHO classification of tumours. Lyon: IARC Press, 2003
25. Kim KR, Jun SY, Park IA, Ro JY, Nam JH. Endometrial stromal tumor with limited infiltration and probable extrauterine metastasis: report of a case. Ann Diagn Pathol 2005;9(1):57-60
26. Montag TW, Manart FD. Endolymphatic stromal myosis: surgical and hormonal therapy for extensive venous recurrence. Gynecol Oncol 1989;33(2):255-60
27. Bodner K, Bodner-Adler B, Obermair A, Windbichler G, Petru E, Mayerhofer S, et al. Prognostic parameters in endometrial stromal sarcoma: a clinicopathologic study in 31 patients. Gynecol Oncol 2001;81(2):160-5
28. Moinfar F, Regitnig P, Tabrizi AD, Denk H, Tavassoli FA. Expression of androgen receptors in benign and malignant endometrial stromal neoplasms. Virch Arch 2004;444(5):410-4
29. Satoh Y, Ishikawa Y, Miyoshi T, Mukai H, Okumura S, Nakagawa K. Pulmonary metastases from a low-grade endometrial stromal sarcoma confirmed by chromosome aberration and fluorescence in-situ hybridization approaches: a case of recurrence 13 years after hysterectomy. Virch Arch 2003;442(2):173-8
30. Aubry MC, Myers JL, Colby TV, Leslie KO, Tazelaar HD. Endometrial stromal sarcoma metastatic to the lung. A detailed analysis of 16 patients. Am J Surg Pathol 2002;26(4):440-9
31. Lantta M, Kahanpää K, Kärkkäinen J, Lehtovirta P, Wahlström T, Widholm O. Estradiol and progesterone receptors in two cases of endometrial stromal sarcoma. Gynecol Oncol 1984;18(2):233-9
32. Reich O, Regauer S, Urdl W, Lahousen M, Winter R. Expression of oestrogen and progesterone receptors in low-grade endometrial stromal sarcomas. Br J Cancer 2000;82(5):1030-4

33. Sabini G, Chumas JC, Mann WJ. Steroid hormone receptors in endometrial stromal sarcomas. A biochemical and immunohistochemical study. Am J Clin Pathol 1992;97(3):381-6
34. Tosi P, Sforza V, Santopietro R. Estrogen receptor content, immunohistochemically determined by monoclonal antibodies, in endometrial stromal sarcoma. Obstet Gynecol 1989;73(1):75-8
35. Pekindil G, Tuncyurek O, Orguc S, Inceboz U, Kandiloglu AR, Caglar H. A case of endometrial stromal sarcoma with curvilinear calcification. Gynecol Oncol 2005;98(2):318-21
36. Kurihara S, Oda Y, Ohishi Y, Iwasa A, Takahira T, Kaneki E, et al. Endometrial stromal sarcomas and related high-grade sarcomas: immunohistochemical and molecular genetic study of 31 cases. Am J Surg Pathol 2008;32(8):1228-38
37. Leath CA III, Huh WK, Hyde J Jr, Cohn DE, Resnick KE, Taylor NP, et al. A multi-institutional review of outcomes of endometrial stromal sarcoma. Gynecol Oncol 2007;105(3):630-4
38. Cheung ANY, Ng WF, Chung LP, Khoo US. Mixed low grade and high grade endometrial stromal sarcoma of uterus: differences on immunohistochemistry and chromosome in situ hybridisation. J Clin Pathol 1996;49(7):604-7
39. Sutton GP, Stehman FB, Michael H, Young PCM, Ehrlich CE: Estrogen and progesterone receptors in uterine sarcomas. Obstet Gynecol 1986;68(5):709-14
40. Morehead RP, Bowman MC. Heterologous mesodermal tumors of the uterus. Report of a neoplasm resembling a granulosa cell tumor. Am J Pathol 1945;21:53-61
41. Clement PB, Scully RE. Uterine tumors resembling ovarian sex-cord tumors. A clinicopathologic analysis of fourteen cases. Am J Clin Pathol 1976;66(3):512-25
42. Hillard JB, Malpica A, Ramirez PT. Conservative management of a uterine tumor resembling an ovarian sex cord-stromal tumor. Gynecol Oncol 2004;92(1):347-52

43. Mc Cluggage WG, Cromie AJ, Bryson C, Traub AI. Uterine endometrial stromal sarcomas with smooth muscle and glandular differentiation. J Clin Pathol 2001;54(6):481-3
44. Kasashima S, Kobayashi M, Yamada M, Oda Y. Myxoid endometrial stromal sarcoma of the uterus. Pathol Int 2003;53(9):637-41
45. Mc Cluggage WG, Sumathi VP, Maxwell P. CD10 is a sensitive and diagnostically useful immunohistochemical marker of normal endometrial stroma and of endometrial stromal neoplasms. Histopathology 2001;39(3):273-8
46. Chu P, Arber DA. Paraffin-section detection of CD10 in 505 nonhematopoietic neoplasms. Frequent expression in renal cell carcinoma and endometrial stromal sarcoma. Am J Clin Pathol 2000;113(3):374-82
47. Zhu XQ, Shi YF, Cheng XD, Zhao CL, Wu YZ. Immunohistochemical markers in differential diagnosis of endometrial stromal sarcoma and cellular leiomyoma. Gynecol Oncol 2004;92(1):71-9
48. Giangrande PH, Kimbrel EA, Edwards DP, Mc Donnell DP. The opposing transcriptional activities of the two isoforms of the human progesterone receptor are due to differential cofactor binding. Mol Cell Biol 2000;20(9):3102-15
49. Reich O, Nogales FF, Regauer S. Gonadotropin-releasing hormone receptor expression in endometrial stromal sarcomas: an immunohistochemical study. Mod Pathol 2005;18(4):573-6
50. Phillips MR, Bower TC, Orszulak TA, Hartmann LC. Intracardiac extension of an intracaval sarcoma of endometrial origin. Ann Thorac Surg 1995;59(3):742-4
51. Debing E, Van der Niepen P, Goossens A, Van den Brande P. Intracaval extension of a recurrent low-grade endometrial stromal sarcoma. Acta Chir Belg 1998;98(6):264-6
52. Veroux P, Veroux M, Nicosia A, Bonanno MG, Tumminelli MG, Milone P, et al. Thrombectomy of the inferior vena cava from recurrent low-grade endometrial stromal sarcoma: case report and review of the literature. J Surg Oncol 2000;74(1):45-8
53. Larson B, Silfverswärd C, Nilsson B, Pettersson F. Endometrial stromal sarcoma of the uterus. A clinical and histopathological study. The radium-

hemmet series 1936-1981. Eur J Obstet Gynecol Reprod Biol 1990;35(2-3):239-49

54. Gadducci A, Sartori E, Landoni F, Zola P, Maggino T, Urgesi A, et al. Endometrial stromal sarcoma: analysis of treatment failures and survival. Gynecol Oncol 1996;63(2):247-53
55. Pink D, Lindner T, Mrozek A, Kretzschmar A, Thuss-Patience PC, Dörken B, et al. Harm or benefit of hormonal treatment in metastatic low-grade endometrial stromal sarcoma: single center experience with 10 cases and review of literature. Gynecol Oncol 2006;101(3):464-9
56. Lin YC, Kudelka AP,Tresukosol D, Malpica A, Carrasco CH, Lawrence DD, et al. Prolonged stabilization of progressive endometrial stromal sarcoma with prolonged oral etoposide therapy. Gynecol Oncol 1995;58(2):262-5
57. Muss HB, Bundy B, DiSaia PJ, Homesley HD, Fowler WC, Creasman W, et al. Treatment of recurrent or advanced uterine sarcoma: a randomized trial of doxorubicin versus doxorubicin and cyclophosphamide (a phase III trial of the Gynecologic Oncology Group). Cancer 1985;55(8):1648-53
58. Sutton G, Blessing JA, Park R, DiSaia PJ, Rosenshein N. Ifosfamide treatment of recurrent or metastatic endometrial stromal sarcomas previously unexposed to chemotherapy: a study of the Gynecologic Oncology Group. Obstet Gynecol 1996;87(5):747-50
59. Berchuck A, Rubin SC, Hoskins WJ, Saigo PE, Pierce VK, Lewis JL Jr. Treatment of endometrial stromal tumors. Gynecol Oncol 1990;36(1):60-5
60. Lehrner LM, Miles PA, Enck RE. Complete remission of widely metastatic endometrial stromal sarcoma following combination chemotherapy. Cancer 1979;43(4):1189-94
61. Yokoyama Y, Ono Y, Sakamoto T, Fukuda I, Mizunuma H. Asymptomatic intracardiac metastasis from a low-grade endometrial stromal sarcoma with successful surgical resection. Gynecol Oncol 2004;92(3):999-1001
62. Stauber M, Weyerstahl T. Gynäkologie und Geburtshilfe. 2nd ed. Stuttgart: Georg Thieme; 2005. p. 79-83.
63. Mesia AF, Demopoulos RI. Effects of leuprolide acetate on low-grade endometrial stromal sarcoma. Am J Obstet Gynecol 2002;182(5):1140-1
64. Gründker C, Günthert AR, Millar RP, Emons G. Expression of gonadotropin-releasing hormone II (GnRH-II) receptor in human endometrial and

ovarian cancer cells and effects of GnRH-II on tumor cell proliferation. J Clin Endocrinol Metab 2002;87(3):1427-30

65. Stauber M, Weyerstahl T. Gynäkologie und Geburtshilfe. 2 nd ed. Stuttgart: Georg Thieme; 2005. p. 90-91
66. Maluf FC, Sabbatini P, Schwartz L, Xia J, Aghajanian C. Endometrial stromal sarcoma: objective response to letrozole. Gynecol Oncol 2001;82(2):384-8
67. Regierer AC, Possinger K. Mammakarzinom: Manual Diagnostik und Therapie. Köln: Deutscher Ärzte-Verlag GmbH; 2005. p. 96-97
68. Aktories K, Förstermann U, Hofmann F, Starke K. Allgemeine und spezielle Pharmakologie und Toxikologie. 9th ed. München: Urban&Fischer; 2005. p. 950.
69. Dowsett M, Jones A, Johnston SRD, Jacobs S, Trunet P, Smith IE. In vivo measurement of aromatase inhibition by letrozole (CGS 20267) in postmenopausal patients with breast cancer. Clin Cancer Res 1995;1(12):1511-15
70. Dombernowsky P, Smith I, Falkson G, Leonard R, Panasci L, Bellmunt J, et al. Letrozole, a new oral aromatase inhibitor for advanced breast cancer: double-blind randomized trial showing a dose effect and improved efficiacy and tolerability compared with megestrol acetate. J Clin Oncol 1998;16(2):453-61
71. Marty M, Gershanovich M, Campos B, Romieu G, Lurie H, Bonaventura T, et al. Letrozole, a new potent, selective aromatase inhibitor (AI) superior to aminoglutethimide (AG) in postmenopausal women with advanced breast cancer (ABC) previously treated with antiestrogens. Proc Am Soc Clin Oncol 1997;16:156
72. Spano JP, Soria JC, Kambouchner M, Piperno-Neuman S, Morin F, Morere JF, et al. Long-term survival of patients given hormonal therapy for metastatic endometrial stromal sarcoma. Med Oncol 2003;20(1):87-93
73. Leunen M, Breugelmans M, De Sutter P, Bourgain C, Amy JJ. Low-grade endometrial stromal sarcoma treated with the aromatase inhibitor letrozole. Gynecol Oncol 2004;95(3):769-71

74. Rushing RS, Shajahan S, Chendil D, Wilder JL, Pulliam J, Lee EY, et al. Uterine sarcomas express kit protein but lack mutation(s) in exon 11 or 17 of c-kit. Gynecol Oncol 2003;91(1):9-14
75. Geller MA, Argenta P, Bradley W, Dusenbery KE, Brooker D, Downs LS Jr, et al. Treatment and recurrence patterns in endometrial stromal sarcomas and the relation to c-kit expression. Gynecol Oncol 2004;95(3):632-6
76. Caudell JJ, Deavers MT, Slomovitz BM, Lu KH, Broaddus RR, Gershenson DM, et al. Imatinib Mesylate (Gleevec)-targeted kinases are expressed in uterine sarcomas. Appl Immunohistochem Mol Morphol 2005;13(2):167-70
77. Kalender ME, Sevinc A, Yilmaz M, Ozsarac C, Camci C. Detection of complete response to imatinib mesylate (Glivec/Gleevec) with 18F-FDG PET/CT for low-grade endometrial stromal sarcoma. Cancer Chemother Pharmacol 2009;63(3):555-9
78. Salvatierra A, Tarrats A, Gomez C, Sastre JM, Balaña C. A case of c-kit positive high-grade stromal endometrial sarcoma responding to Imatinib Mesylate. Gynecol Oncol 2006;101(3):545-7
79. Mitsuhashi T, Nakayama M, Sakurai S, Fujimura M, Shimizu Y, Ban S et al. KIT-negative undifferentiated endometrial sarcoma with the amplified epidermal growth factor receptor gene showing a temporary response to imatinib mesylate. Ann Diagn Pathol 2007;11(1):49-54
80. Li N, Wu LY, Zhang HT, An JS, Li XG, Ma SK. Treatment options in stage I endometrial stromal sarcoma: a retrospective analysis of 53 cases. Gynecol Oncol 2008;108(2):306-11
81. Norris HJ, Taylor HB. Mesenchymal tumors of the uterus. A clinical and pathological study of 53 endometrial stromal tumors. Cancer 1966;19(6):755-66
82. Salazar OM, Bonfiglio TA, Patten SF, Keller BE, Feldstein M, Dunne ME, et al. Uterine sarcomas: natural history, treatment and prognosis. Cancer 1978;42(3):1152-60
83. Nordal RR, Kristensen GB, Kaern J, Stenwig AE, Pettersen EO, Tropé CG. The prognostic significance of surgery, tumor size, malignancy grade, menopausal status, and DNA ploidy in endometrial stromal sarcoma. Gynecol Oncol 1996;62(2):254-9

84. Reich O, Singer C, Hudelist G, Regauer S. Estrogen sulfotransferase expression in endometrial stromal sarcomas: an immunohistochemical study. Pathol Res Pract 2007;203(2):85-7
85. Moinfar F, Kremser ML, Man YG, Zatloukal K, Tavassoli F, Denk H. Allelic imbalances in endometrial stromal neoplasms: frequent genetic alterations in the nontumorous normal-appearing endometrial and myometrial tissues. Gynecol Oncol 2004;95(3):662-71
86. Micci F, Walter CU, Teixeira MR, Panagopoulos I, Bjerkehagen B, Sæter G, et al. Cytogenetic and molecular genetic analyses of endometrial stromal sarcoma: nonrandom involvement of chromosome arms 6p and 7p and confimation of JAZF1/JJAZ1 gene fusion in t(7;17). Cancer Genet Cytogenet 2003;144(2):119-24
87. Koontz JI, Soreng AL, Nucci M, Kuo FC, Pauwels P, van den Berghe H, et al. Frequent fusion of the JAZF1 and JJAZ1 genes in endometrial stromal tumors. Proc Natl Acad Sci USA 2001;98(11):6348-53
88. Hrzenjak A, Moinfar F, Tavassoli FA, Strohmeier B, Kremser ML, Zatloukal K, et al. JAZF1/JJAZ1 gene fusion in endometrial stromal sarcomas. Molecular analysis by reverse transcriptase-polymerase chain reaction optimized for paraffin-embedded tissue. J Mol Diagn 2005;7(3):388-95
89. Huang HY, Ladanyi M, Soslow RA. Molecular detection of JAZF1-JJAZ1 gene fusion in endometrial stromal neoplasms with classic and variant histology. Evidence for genetic heterogeneity. Am J Surg Pathol 2004;28(2):224-32
90. Lax SF. Molecular genetic changes in epithelial, stromal and mixed neoplasms of the endometrium. Pathology 2007;39(1):46-54
91. Adams SF, Hickson JA, Hutto JY, Montag AG, Lengyel E, Yamada SD. PDGFR-α as a potential therapeutic target in uterine sarcomas. Gynecol Oncol 2007;104(3):524-8
92. Moinfar F, Gogg-Kamerer M, Sommersacher A, Regitnig P, Man YG, Zatloukal K, et al. Endometrial stromal sarcomas frequenly express epidermal growth factor receptor (EGFR, HER-1). Am J Surg Pathol 2005;29(4):485-9
93. Arita S, Kikkawa F, Kajiyama H, Shibata K, Kawai M, Mizuno K, et al. Prognostic importance of vascular endothelial growth factor and its receptors in the uterine sarcoma. Int J Gynecol Cancer 2005;15(2):329-36

94. Liokumovich P, Goldberg I, Davidson B, Gotlieb WH, Zahavi T, Ben-Baruch G, et al. Expression of metalloproteinases endometrial stromal sarcoma: immunohistochemical study using image analysis. J Clin Pathol 1999;52(3):198-202
95. Hrzenjak A, Tippl M, Kremser ML, Strohmeier B, Guelly C, Neumeister D, et al. Inverse correlation of secreted frizzled-related protein 4 and β-catenin expression in endometrial stromal sarcomas. J Pathol 2004;204(1):19-27
96. Koivisto-Korander R, Ralf B, Koivisto AM, Leminen A. Clinical outcome and prognostic factors in 100 cases of uterine sarcoma: experience in Helsinki University Central Hospital 1990-2001. Gynecol Oncol 2008;111(1):74-81
97. Riopel J, Plante M, Renaud MC, Roy M, Têtu B. Lymph node metastases in low-grade endometrial stromal sarcoma. Gynecol Oncol 2005;96(2):402-6
98. El Husseiny G, Al Bareedy N, Mourad WA, Mohamed G, Shoukri M, Subhi J, et al. Prognostic factors and treatment modalities in uterine sarcoma. Am J Clin Oncol 2002;25(3):256-60
99. Vargas-Barron J, Keirns C, Barragan-Garcia R, Beltran-Ortega A, Rotberg T, Santana-Gonzalez A, et al. Intracardiac extension of malignant uterine tumors. Echocardiographic detection and successful surgical resection. J Thorac Cardiovasc Surg 1990;99(6):1099-103
100. Mikami Y, Demopoulos RI, Boctor F, Febre EF, Harris M, Kronzen I, et al. Low-grade endometrial stromal sarcoma with intracardiac extension. Evolution of extensive smooth muscle differentiation and usefulness of immunohistochemistry for its recognition and distinction from intravenous leiomyomatosis. Pathol Res Pract 1999;195(7):501-8
101. Kim GY, Sung CO, Han J, Park JO, Lee KS. Pulmonary metastases of uterine endometrial stromal sarcoma: diffuse micronodular and ground glass opacities: a case report. J Korean Med Sci 2004;19(6):901-3
102. Günhan-Bilgen I, Memis A, Üstün EE, Özdemir N. Breast metastasis from low-grade endometrial stromal sarcoma after a 17-year period. Eur Radiol 2002;12(12):3023-5
103. Dong Y, Shi XJ, Li T, Zhang Y. The morphologic features of endometrial stromal sarcoma and its metastases. Zhonghua Bing Li Xue Za Zhi 2005;34(3):163-6

Supplement

Case report, submitted

Response of metastatic endometrial stromal sarcoma with low hormone receptor expression to aromatase inhibitor therapy

Stefanie Grüninger[1], Andelko Hrzenjak[2], Sigurd Lax[3], Farid Moinfar[2], Thomas Bauernhofer[1]

[1] Department of Clinical Oncology, University Clinic of Internal Medicine and [2] Departments of Pathology, Medical University of Graz and [3]General Hospital Graz West, Graz, Austria

Correspondence to:
Thomas Bauernhofer MD
Department of Clinical Oncology
University Clinic of Internal Medicine
Medical University Graz
Auenbruggerplatz 15
A-8036 Graz, Austria
Tel.: ++43 316 385 80274
Fax: ++43 316 385 4167
E-mail: **thomas.bauernhofer@meduni-graz.at**

Abstract

Introduction: Endometrial stromal sarcomas (ESS) are rare uterine malignancies usually expressing steroid hormone receptors and considered to be hormone-sensitive.

Case Report: A 70-year-old woman with a history of total abdominal hysterectomy and bilateral salpingo-oophorectomy and postoperative radiation therapy due to ESS presented with multiple metastases to lung, liver and bones. The primary tu-

mor showed low expression of estrogen receptors and anti-hormonal treatment using the aromatase inhibitor letrozole was initiated leading to a long-lasting partial remission of all metastatic sites. Retrospective analysis of the primary tumor did not reveal aromatase expression by immunohistochemistry or western blot.
Discussion: Even in ESS with a low expression of steroid hormone receptors and no aromatase expression anti-hormonal treatment using aromatase inhibitors may be highly effective leading to sustained disease control.

Keywords: Endometrial stromal sarcoma, aromatase inhibitors, estrogen receptor, aromatase expression

Introduction

Endometrial stromal sarcomas (ESS) are rare, accounting for about 0.2% of all uterine malignancies and occur predominantly in pre- or perimenopausal women [1]. Most ESS express estrogen (ER) and/or progesterone receptors (PR) [2] as well as androgen receptors [3] and aromatase [4] and are, therefore, regarded as hormone responsive. Under physiological conditions, proliferation and differentiation of endometrial stromal cells are regulated by steroid hormones after binding to their receptors. ESS often occurs in the setting of hyperestrogenism but little is known about molecular pathogenesis and putative precursor lesions [5]. Most ESS harbor specific translocations, in particular t(7;17) resulting in JAZF1/JJAZ1 gene fusion [6] but it remains so far unclear whether this molecular alterations interferes with the estrogenic pathway. The other type of sarcoma occurring in the endometrium is undifferentiated sarcoma (US) which is unrelated to estrogen and progesterone since it lacks ER and PR expression. US does not seem to develop from ESS since US does not harbor the JAZF1/JJAZ1 gene fusion [6].

Basic treatment is surgery including total abdominal hysterectomy and bilateral salpingo-oophorectomy. There might be a role for adjuvant chemotherapy, radiation therapy and hormonal treatment [7,8]. Nevertheless, in the metastatic setting there is growing evidence that hormonal therapy is an important option at least in patients with low grade ESS and expression of higher levels of ER and/or PR. Progestins and aromatase inhibitors have been successfully employed in this patient population [8,9]. However, very little information is available up to now about hormonal treatment strategies for patients with ESS and very low steroid receptor

expression as presented in this case. In our study aromatase inhibitor therapy resulted in long term partial remission in a patient with ESS and ER expression of 5% of tumor cells as well as no PR or aromatase expression.

Case Report

A 70-year-old uniparous Caucasian woman with metastatic ESS to the bone, liver and lung was referred to the Department of Clinical Oncology of the Medical University Graz, Austria, in April 2005. One and a half years earlier the patient had undergone total abdominal hysterectomy, bilateral salpingo-oophorectomy and pelvic lymphadenectomy due to the diagnosis of ESS. The tumor measured 9 cm in largest diameter and protruded as a polyp into the uterine cavity. Myometrial invasion showed a nodular pattern (Figure 1A). Histologically the tumor consisted in most parts of small round or spindly monomorphic cells with scant cytoplasm (Figure 1B) but there were also areas with moderately polymorphic spindle cells and areas with a sex cord like pattern. Mitoses were numerous (25 per 10 HPF) and tiny foci of tumor cell necrosis were present. Immunohistochemical analysis of the tumor demonstrated high immunoreactivity for CD10 (about 60% of the tumor cells positive) and low immunoreactivity for estrogen receptors (5-10% of tumor cells; Figure 1C). Only a few tumor cells were positive for progesterone receptors (< 1% of the tumor cells). Ki-67 labeling index was 10-20%. About 10-20% of the tumor cells were immunoreactive for p53. There was no immunoreactivity for caldesmon, calretinin and alpha-inhibin. None of 25 removed lymph nodes were metastatically involved. Post-operatively, adjuvant pelvic radiation therapy was carried out. After the detection of multiple lung metastases up to one centimeter in both lobes, liver metastases up to 3.2 cm, and multiple bone metastases by CT scan, treatment with the aromatase inhibitor letrozole was initiated. Within 3 months a partial response of lung and liver metastases as well as sclerosis of osteolytic bone metastases could be demonstrated in follow up CT scans. The response duration has been more than three years in all metastatic sites (Figures 2 A and 2B). However, a new cystic lesion in the mediastinum with slow progression was detected in July 2007. A hemorrhage into the mediastinal lesion leading to mediastinal shift and atelectasis of the right lung necessitated emergency surgery in October 2008. Total resection of the cystic tumor could be achieved. Surprisingly, histological analysis revealed a primitive neuroectodermal tumor (PNET) and

no metastatic lesion of ESS. After surgery aromatase inhibitor treatment was reinitiated and all metastatic sites are still in near complete remission up to now.

In an attempt to better understand the biology of the ESS retrospective analysis of the JAZF1/JJAZ1 gene fusion was performed on paraffin embedded and fresh frozen tumor tissue samples as published previously [10] and found to be negative. Immunohistochemical analysis of aromatase expression in tumor samples using different dilutions of cytochrome P450 aromatase monoclonal antibody (SM2222P, clone H4; Acris Antibodies, Hiddenhausen, Germany) was negative (full term placenta was used as positive control, data not shown). To further substantiate this finding, additional expression analysis of cytochrome P450 aromatase in tumor was tested by immunoblotting using proteins extracted from frozen tumor tissue (40 μg of total protein) separated on 8% SDS-PAGE and blotted under standard condition using human placenta as a positive control. Again no expression of aromatase could be demonstrated (data not shown).

Discussion

Recent evidence suggests that metastatic ESS, especially ESS with high expression of ER and PR are sensitive to hormonal treatment including megestrol acetate, GNRH-agonists and aromatase inhibitors [for review see 8]. Furthermore, aromatase expression in low grade ESS has been described. The extra-ovarian estrogen production depends on conversion of circulating androgens to estrogens via the aromatase enzyme complex. Aromatase inhibitors may have anti-tumor activity in ESS by blockage of peripheral as well as intra-tumoral aromatization. In the literature, the clinical response of 9 patients treated with aromatase inhibitors has been reported up to now demonstrating a complete response in two patients, partial response in 6 patients, and progressive disease in one patient [11–14]. The absence of a JAZF1/JJAZ1 gene fusion in our patient's tumor which is typical for the vast majority of ESS suggests a more aggressive genotype. This concurs with the very low expression of estrogen receptors and absence of progesterone receptors and aromatase. Treatment with the aromatase inhibitor letrozole was initiated due to the fact that the patient was asymptomatic and above 70 years of age not suitable for aggressive chemotherapy. A major objective response could be observed lasting for four years. It can be hypothesized that ESS tumor cells might be extremely sensitive to estrogens even with low estrogen receptor expression and,

that inhibition of estrogen synthesis by at least peripheral blockage of aromatization may be sufficient to yield tumor regression.

When we reviewed this case, we confirmed the diagnosis of ESS, most likely of an aggressive variant. Although our tumor did not harbor the JAZF1/JJAZ1 fusion gene and showed low ER and no PR expression we do not consider the diagnosis of undifferentiated sarcoma since the histological pattern is in favor of ESS. The high mitotic index and the presence of tumor cell necrosis concur with the diagnosis of ESS. Finally, undifferentiated sarcoma is characterized by a more aggressive clinical course.

In conclusion, this case suggests that aromatase inhibitor treatment is not only warranted in patients with ESS highly expressing ER and/or PR but may also be considered for ESS with very low hormone receptor and lack of aromatase expression.

Conflict of Interest statement

All authors declare that there are no conflicts of interest

Acknowledgments

This work was supported in part by Lore Saldow Research Fund (HA) and special immunohistochemical studies as well as analysis of JAZF1/JJAZ1 gene fusion were performed within the Lore Saldow Research Unit ‘Molecular Pathology of Gynecologic Tumors’.

.

References

[1] Ashraf-Ganjoei T, Behtash N, Shariat M, Mosavi A. Low grade endometrial stromal sarcoma of uterine corpus, a clinico-pathologic and survey study in 14 cases. World J Surg Oncol 2006;4:50.

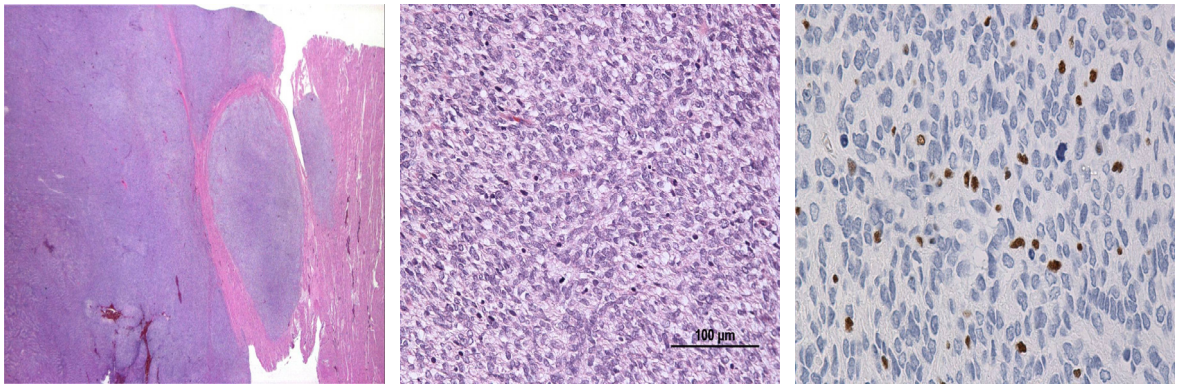
[2] Sabini G, Chumas JC, Mann WJ. Steroid hormone receptors in endometrial stromal sarcomas. A biochemical and immunohistochemical study. Am J Clin Pathol 1992;97(3):381–6.

[3] Moinfar F, Regitnig P, Tabrizi AD, Denk H, Tavassoli FA. Expression of androgen receptors in benign and malignant endometrial stromal neoplasms. Virch Arch 2004; 444(5):410–14.

[4] Reich O, Regauer S. Aromatase expression in low-grade endometrial stromal sarcomas: an immunohistochemical study. Mod Pathol 2004;17(1):104–8.
[5] Lax SF. Molecular genetic changes in epithelial, stromal and mixed neoplasms of the endometrium. Pathology 2007;39(1):46–54
[6] Koontz JI, Soreng AL, Nucci M, Kuo FC, Pauwels P, van den Berghe H, et al. Frequent fusion of the JAZF1 and JJAZ1 genes in endometrial stromal tumors. Proc Natl Acad Sci USA 2001;98(11):6348–53.
[7] Li N, Wu LY, Zhang HT, An JS, Li XG, Ma SK. Treatment options in stage I endometrial stromal sarcoma: A retrospective analysis of 53 cases. Gynecol Oncol 2008;108(2):306–311.
[8] Reich O, Regauer S. Hormonal therapy of endometrial stromal sarcoma. Curr Opin Oncol 2007;19:347–352.
[9] Chu MC, Mor G, Lim C, Zheng W, Parkash V, Schwartz PE. Low-grade endometrial stromal sarcoma: hormonal aspects. Gynecol Oncol 2003;90(1)170–6.
[10] Hrzenjak A, Moinfar F, Tavassoli FA, Strohmeier B, Kremser ML, Zatloukal K, et al. JAZF1/JJAZ1 gene fusion in endometrial stromal sarcomas. Molecular analysis by reverse transcriptase-polymerase chain reaction optimized for paraffin-embedded tissue. J Mol Diagn 2005;7(3):388–395.
[11] Spano JP, Soria JC, Kambouchner M, Piperno-Neuman S, Morin F, Morere JF, et al. Long term survival of patients given hormonal therapy for metastatic endometrial stromal sarcoma. Med Oncol 2003;20:87–93.
[12] Maluf FC, Sabbatini P, Schwartz L, Xia J, Aghanjanian C. Endometrial stromal sarcoma: objective response to letrozole. Gynecol Oncol 2001 Aug;82(2):384–8.
[13] Leunen M, Breugelmans M, De Sutter P, Bourgain C, Amy JJ. Low-grade endometrial stromal sarcoma treated with the aromatase inhibitor letrozole. Gynecol Oncol 2004;95(3):769–71.
[14] Pink D, Lindner T, Mrozek A, Kretzschmar A, Thuss-Patience PC, Dörken B, et al. Harm or benefit of hormonal treatment in metastatic low-grade endometrial stromal sarcoma: Single center experience with 10 cases and review of the literature. Gynecol Oncol 2006;101(3):464–69.

Figure legends

Figure 1:



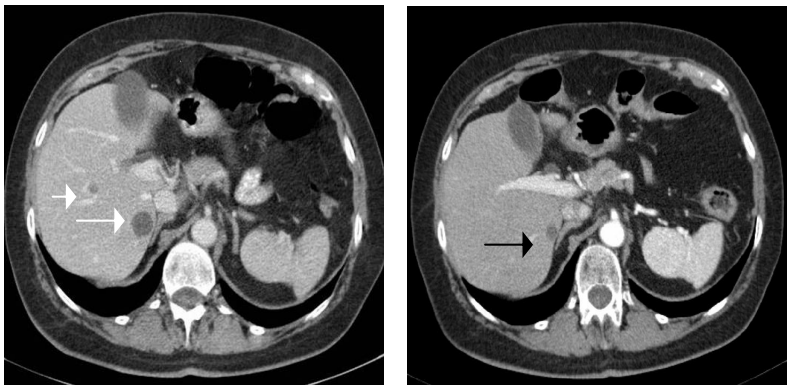
A B C

A: Nodular tumor masses infiltrating the myometrium. HE, 10X

B: The tumor is composed of small round and spindle monomorphic cells and contains numerous mitoses. HE, 200X.

C: Immunoreactivity for estrogen receptors is present in a subset of tumor cells. DAB, 400X.

Figure 2:



15.04.2005 15.03.2006

CT scans of cystic liver metastases of ESS (A) pretreatment (2 lesions 1 cm and 3,2 cm in diameter; white arrows) and (B) after one year of treatment with aromatase inhibitor letrozole (complete regression of one lesion and regression of second lesion to 1cm in diameter; black arrow)

CURRICULUM VITAE

Personal data:

Fisrt name, surname: Stefanie Grüninger

Date of birth: 30th November 1984

Place of birth: Villach

Nationality: Austrian

Family status: single

Address: 9702 Ferndorf 88 ; Am Rehgrund 4, 8043 Graz

E-mail-address: **steffi.grueninger@gmx.at**

stefanie.grueninger@stud.meduni-graz.at

School education:

06/2003: final examination at the grammar school Bundesgymnasium Porcia Spittal/Drau, graduated with distinction

1995-2003: grammar school Bundesgymnasium Porcia Spittal/Drau

1990-1995: elementary school in Ferndorf

Academic studies:

06/2008: second part of the studies completed

07/2004: first part of the studies completed

10/2003: beginning of the course of studies of human medicine at the Medical University of Graz

Elective placements:

07/2008 (4weeks): Department of Anesthesia and Intensive Care, LKH Villach

06/2008 (3 weeks): Department of Pediatrics, LKH-Univ. Klinikum Graz

09/2007 (4 weeks): Department of Trauma Surgery, KH Spittal/Drau

08/2008 (2.5 weeks): Office Pediatrician Dr. A Wutte, Spittal/Drau

02/2007 (3 weeks): Department of Neurology, LKH Villach

09/2006 (4 weeks): Department of Gynecology and Obstetrics, KH Spittal/Drau

07/2006 (4 weeks): Department of Internal Medicine, KH Spittal/Drau

02/2006 (3 weeks): Department of Internal Medicine, LKH Villach

08/2005 (4 weeks): Department of Anesthesia and Intensive Care, KH Spittal/Drau
08/2004 (4 weeks): Department of Trauma Surgery, UKH Klagenfurt

Further training:

02/2008: education for “Praxisanleitungssanitäter”, Cap Wörth,
Velden, Austrian Red Cross
09/2004: examination for paramedic, Cap Wörth, Velden

Further activities:

10/2008: attending the congress “Schuld und Gefühle”/Religiosität in Psychiatrie
und Psychologie, Graz
09/2008: practical course in the medical and chemical laboratory
Dr. Holzweber, Villach
09/2008: attending the congress “39. Jahrestagung der
Österreichischen Gesellschaft für Innere Medizin“, Graz
05/2008: participation/collaboration at the ninth interdisciplinary
workshop “Anatomie in der Regionalanästhesie/
Schmerztherapie“, MAG, Department of Anesthesiology
and Intensive Care and the Department of Anatomy,
Medical University of Graz
05/2008: teddy doc at the sixth teddy-hospital, LKH Univ. Klinikum Graz
02/2008: Common Invasive Procedures I, II
Department of Anatomy, Medical Unversity Graz
10/2007: attending the congress “Religiosität und Psychiatrie”, Graz

Hobbies/Interests: music, dancing, jogging, hiking, working at neighbor´s farm

Foreign languages: English, French