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MARKERS FOR CLINICAL OUTCOME AND THERAPY RESPONSE IN SOFT TISSUE SARCOMAS

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Stockholm 2013

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Published by Karolinska Institutet. Printed by Universitetsservice US-AB

ISBN 978-91-7549-117-2

To my wife Yvonne
and our children
Niklas, Hanna and Viktor

Thesis defense

Leksell Lecture Hall

Eugeniahemmet T3

Karolinska University Hospital Solna

Friday May 10th 2013

at 09.00

ABSTRACT

Soft-tissue sarcomas (STSs) constitute a heterogeneous group of rare but aggressive tumors that originate from mesenchymal cells in almost any part of the body. Gastrointestinal stromal tumor (GIST), located in the gastrointestinal tract, is the most common type of STS. The aim of this thesis was to evaluate markers for development of progressive disease in highly malignant STS; moreover to assess the impact of adverse drug reactions and surgical margins on outcome for patients with GIST.

Paper I. We evaluated 101 patients with high-grade STS for known and suggested prognostic markers, particularly the insulin-like growth factor type 1 receptor (IGF-1R). A significant association was seen between high expression of IGF-1R and favorable outcome. Furthermore, large tumor size, occurrence of necrosis, high mitotic count, intralesional surgery, deep location and microvessel density were all significantly associated with poor outcome, whereas no association with outcome was found for either malignancy grade 3 or 4, infiltrative growth pattern, vascular invasion or any of the remaining immunohistochemical markers Ki67, p53, p27 or Bcl-2.

Paper II. In 50 patients with highly malignant STS from the same series as *Paper I* we evaluated the prognostic role of ezrin, a protein involved in metastatic spread of cancer cells. Positive expression of ezrin by immunohistochemistry was found in half of the cases and this finding was significantly associated to death from or with disease as well as to development of metastasis.

Paper III. The application of surgery and the tyrosine kinase inhibitor imatinib in the treatment of GIST has led to dramatically prolonged survival. However, imatinib is associated with frequent side-effects of variable severity. In a retrospective review of medical records from 75 patients who had received imatinib, we correlate side-effects to outcome, and found that moderate to severe or life-threatening toxic reactions were registered in 30 patients. Most of the side-effects occurred early. For the 34 patients with metastatic or recurrent GIST, presence of side-effects and female gender were associated with longer recurrence-free survival.

Paper IV. Surgery is the main and only curative treatment for GIST. Complete surgical resection with microscopically negative margin (R0) is widely regarded as a prerequisite for intended curative treatment. We divided the patients on the basis of the surgical resection margin, as with other STSs, into wide, marginal or intralesional margin at surgery or referral and retrospectively correlated this to outcome. Local/peritoneal recurrence was diagnosed in 2 of 40 GISTs with wide margins, in 7 of 24 GISTs with marginal margins, and in 13 of 19 GISTs with intralesional surgery. Cox regression analysis showed that a wide surgical margin is of significant prognostic importance independently of size and site.
Furthermore, we analyzed the incidence of infiltrative growth pattern and its correlation to surgery and outcome. We found that 64% of GISTs had infiltrative growth and that this was significantly correlated to recurrent disease. However, there were no correlations to surgical margins.

LIST OF PUBLICATIONS

The thesis is based on the following papers, which will be referred to throughout the text by their Roman numerals.

I **Åhlén J***, Wejde J, Brosjö O, von Rosen A, Weng W-H, Girnita L, Larsson O, Larsson C: Insulin-like growth factor type 1 receptor expression correlates to good prognosis in highly malignant soft tissue sarcoma.
Clinical Cancer Research, 11(1):206-216, 2005.

II Weng W-H*, **Åhlén J**, Åström K, Lui W-O, Larsson C: Prognostic impact of immunohistochemical expression of ezrin in highly malignant soft tissue sarcomas.
Clinical Cancer Research, 11(17):6198-6204, 2005.

III **Åhlén J***, Westerdahl J, Zedenius J, Bränström R, Larsson C, Nilsson I-L: Side-effects from imatinib treatment of advanced GIST – associated with a better outcome.
Journal of Cancer Therapeutics and Research, 1: 11, 2012.

IV **Åhlén J***, Wejde J, Bränström R, Westerdahl J, Larsson O, Nilsson I-L, Larsson C: Influence of wide surgical margin and infiltrative growth pattern on patient outcome in gastrointestinal stromal tumors (GIST).
Submitted for publication

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LIST OF ABBREVIATIONS

bcl-2	B-cell lymphoma 2
CD117	Stem cell factor receptor (c-kit protein)
CT	Computed tomography
CTCAE	Common terminology criteria for adverse events
ERM	ezrin, radixin, moesin protein family
EUS	Endoscopic ultrasound
FDG	18F-fluoro-2-deoxy-D-glucose
GIST	Gastrointestinal stromal tumor
HPF	High power field
IGF-1	Insulin-like growth factor 1
IGF-2	Insulin-like growth factor 2
IGF-1R	Insulin-like growth factor type 1 receptor
Ki-67	Antigen identifying proliferation
KIT	v-kit Hardy Zuckerman 4 feline sarcoma viral oncogene homologue
p27	Cyclin-dependent kinase inhibitor 1B
p53	Tumor protein 53
PDGFRA	Platelet-derived growth factor receptor alpha
PET	Positron emission tomography
R0	No residual tumor after surgery
R1	Microscopic residual tumor left after surgery
R2	Macroscopic residual tumor left after surgery
SDH	Succinate dehydrogenase
SEER	Surveillance, Epidemiology, and End Results
SSG	Scandinavian Sarcoma Group
STS	Soft tissue sarcoma
TKI	Tyrosine kinase inhibitor
wtGIST	wild-type GIST

1 INTRODUCTION

The sarcoma entity constitutes a group of tumors that originate from mesenchymal cells in the connective tissue. The disease is rare and accounts for only approximately 1-2% of all malignant tumors (Ferrari, Sultan et al. 2011; Mastrangelo, Coindre et al. 2012; Stiller, Trama et al. 2013). Mesenchymal cells occur all over the body and therefore these tumors can occur in nearly all anatomical locations. Sarcomas are grossly divided into skeletal sarcomas and soft tissue sarcomas (STS); the latter group is more common than the former (Fletcher C.D.M. 2006; Stiller, Trama et al. 2013). The World Health Organization (WHO) classification recognizes more than 50 different entities of STS, all with more or less variable clinical, prognostic and therapeutic features (Fletcher C.D.M. 2006; Gatta, van der Zwan et al. 2011). The increased identification of specific molecular genetic alterations associated with certain types of sarcoma has improved diagnostic classifications and the understanding of factors influencing the success of conventional treatment. Targeted therapy based on the underlying molecular abnormality is presently applied to some types of sarcoma and such therapies are also in various phases of development for several other types (Cassier, Labidi-Galy et al. 2011). The introduction of targeted therapies has tremendously improved the outcome for certain groups of sarcoma patients and ongoing developments are expected to continuously improve the therapeutic options (Floris, Wozniak et al. 2013). Moreover, the need for lengthy, sometimes life-long, medical treatment calls for increased attention to secondary issues such as side-effects and development of drug resistance. Another key research issue is to define biomarkers that can identify the subset of patients who would benefit most from a particular treatment. There is also a demand for improved understanding of factors influencing the success of conventional treatment such as surgery.

1.1 SOFT TISSUE SARCOMA (STS)

1.1.1 Occurrence

Since sarcomas may occur in any anatomic site of the body and are differentiated into many subtypes, the true incidence of any one subtype is probably underestimated. Using large population-based registries, the annual incidence of only STS is estimated to 4.7-5.6 per 100,000 persons (Ferrari, Sultan et al. 2011; Mastrangelo, Coindre et al. 2012; Stiller, Trama et al. 2013). In the RARECARE compilation of rare cancers in Europe, 84% of sarcoma cases were STSs and 14% were bone sarcomas (Stiller, Trama et al. 2013). Annual incidence rates increase with age, starting from 0.9 per 100,000 in children under 10 years and rising more dramatically after the age of 30 years to 18.2 per 100,000 among individuals over 70 years (Ferrari, Sultan et al. 2011). Compared to other cancers, sarcomas occur more frequently in young adults and adolescents (Burningham, Hashibe et al. 2012). Overall, STSs account for approximately 7% of all pediatric malignancies and 20% of all pediatric solid malignant tumors but only constitute 1.5 % of all malignancies in adults (Clark, Fisher et al. 2005; Fletcher C.D.M. 2006; Stiller, Trama et al. 2013).

Based on available data, an intra-abdominal location is most common (incidence of about 1.1-1.5 per 100,000), followed by the lower extremities (0.8-1.2), the trunk wall (0.54-0.77), the upper extremities (0.37-0.54) and retroperitoneum (0.2-0.76) (Nilsson, Bumming et al. 2005; Tryggvason, Gislason et al. 2005; Stiller, Trama et al. 2013). Except for a female predominance for leiomyosarcoma and soft tissue alveolar sarcoma, the incidence is similar or slightly higher among men. One third of tumors located in the trunk wall and extremities are superficial, with an average size of 5 cm; the remaining two thirds are deep-seated with an average size of 9 cm (Fletcher C.D.M. 2006).

The overall survival for all persons diagnosed with any type of STS was reported to be 51% at 5 years, 38% at 10 years, and 30% at 15 years. Relative survival estimates were 57-62%, 55% and 51% at 5, 10, and 15 years, respectively. Patients under 50 years of age at diagnosis had a better cancer-specific survival (about 88.8%) than older patients (40%). Overall and relative survival in gastrointestinal stromal tumor (GIST) were 59% and 68%, respectively, while patients diagnosed with

retroperitoneal sarcoma had relatively poor outcome with 5-year overall survival of 36-58% (Porter, Baxter et al. 2006; Ferrari, Sultan et al. 2011; Stiller, Trama et al. 2013).

The most common STS is GIST, followed by liposarcoma, leiomyosarcoma and synovial sarcoma. The age-related incidence varies between the entities. Leiomyosarcoma and liposarcoma are the most common histopathological entities in the elderly, whereas synovial sarcoma mostly occurs in young adults and embryonal rhabdomyosarcoma almost only in children. Lipoma is the most common benign form of soft tissue tumor (Fletcher C.D.M. 2006; Ferrari, Sultan et al. 2011; Mastrangelo, Coindre et al. 2012).

1.1.2 Signs and symptoms

As a rule, patients with STS have no or very few symptoms from the tumor. Symptoms such as pain, tenderness or loss of function are rarely present and the person typically feels perfectly healthy. The only signs of a possibly malignant tumor are bleeding or the presence of a lump accidentally noted by the patient. That is why these tumors are often misinterpreted as benign lesions and left without further notice by both patients and physicians. Consequently, a long interval may elapse before the patient is referred to a clinical center. In a study by Saithna *et al,* the median delay before referral was 70 weeks; however, no significant difference in outcome was noted, except for patients with metastasis at diagnosis, who were referred earlier but had a worse prognosis from the start (Saithna, Pynsent et al. 2008).

1.1.3 Etiology

Only a few STS have an identifiable cause. Many studies have analyzed various potential risk factors for sarcoma development, for example radiation, infection, occupation, genetic predisposition and chemical exposure (e.g. herbicides) (Li and Fraumeni 1969; Serraino, Franceschi et al. 1992; Smith and Christophers 1992; Kogevinas, Kauppinen et al. 1995; Pollack and Mulvihill 1997; Hum, Kreiger et al. 1998; Pearce, Hammal et al. 2007; Burningham, Hashibe et al. 2012). However, most of these studies have not found sufficient evidence for definite conclusions. Nevertheless, some definite risk factors have been identified. Acquired immunodeficiency resulting from treatment in transplant recipients or from HIV-infection is associated with an increased risk of Kaposis sarcoma, where EBV and

HHV8 are the viruses of central importance (Kedes, Operskalski et al. 1996; Grulich, van Leeuwen et al. 2007). Radiation exposure in connection with radiotherapy is strongly associated with development of secondary sarcoma (Rubino, Shamsaldin et al. 2005; Virtanen, Pukkala et al. 2006). (Burningham, Hashibe et al. 2012).

Genetic predisposition has been identified in some situations, although less than might be expected from the relatively higher occurrence in children and adolescents. The Li-Fraumeni's syndrome was first recognized as associated with sarcoma predisposition (Li and Fraumeni 1969). This syndrome is associated with constitutional mutations of the *TP53* gene and a high risk of osteosarcoma, STS, breast cancer and various other cancers (Burningham, Hashibe et al. 2012). More recently, constitutional mutations have been identified in a few patients with GIST in *KIT* and *PDGFRA,* which also show frequent somatic mutations in this disease. In addition, constitutional mutations occur in the neurofibromatosis type 1 gene (*NF1*) and the members of the succinate dehydrogenase complex (*SDH*) genes (Miettinen, Fetsch et al. 2006; Agaimy, Vassos et al. 2012; Miettinen, Killian et al. 2013; Oudijk, Gaal et al. 2013), suggesting a link to syndromes with other neuro-endocrine tumors.

1.1.4 Histopathology and grading

The histopathological diagnosis is made in accordance with the WHO Classification (Fletcher C.D.M. 2006). The most important variables in histopathologic malignancy grading include tumor size, depth, necrosis, and vascular invasion, as well as cellularity and cellular atypia (Engellau 2004; 2012). Malignancy grading is of importance in the treatment of all types of cancer, including sarcoma, in order to predict outcome and improve the treatment of each patient. Several grading systems of STS have been described, including the three-tier National Cancer Institute (NCI) system, the three-tier French Federation of Cancer Centers (FNCLCC) system, the two-tier Memorial Sloan-Kettering Cancer Center (MSKCC) system and the four-tier system modified from Broder *et al.* The histological malignancy grading of STS used to date in Scandinavia is based on a four-tiered scale modified from Broder's system (Goyanna, Torres et al. 1951; Angervall, Kindblom et al. 1986). Grade 1 and 2 are considered as low malignancy and grades 3 and 4 as high malignancy. The assessment is based on histopathological assessments of tumor cellularity, differentiation, mitotic rate, cellular atypia and amount of necrosis (Angervall, Kindblom et al. 1986). Although no grading system is universally accepted, high-grade histology, characterized by poor

differentiation, cellular pleomorphism, necrosis, and mitoses, has consistently emerged as a negative prognostic factor for patients with STS, irrespective of which grading system is used.

Prognostic factors used for STSs are currently based on clinical and histopathological characteristics such as morphological malignancy grade, size, site, depth, necrosis, vascular invasion and growth pattern, all of which are significant factors for outcome. Vascular invasion, growth pattern and tumor necrosis are also strong prognostic factors for metastases (Mandard, Petiot et al. 1989; Engellau, Bendahl et al. 2005). The TNM classification system is commonly applied to STS, as detailed in Table 1.

Table 1. TNM classification of sarcomas

Parameter / Classification	Sub-classification	Criteria
Primary tumor (T)		
TX		primary tumor cannot be assessed
T0		no evidence of primary tumor
T1		tumor < 5 cm in greatest dimension
	T1a	superficial tumor*
	T1b	deep tumor
T2		tumor > 5 cm in greatest dimension
	T2a	superficial tumor
	T2b	deep tumor
Regional lymph nodes (N)		
NX		regional lymph nodes cannot be assessed
N0		no regional lymph node metastasis
N1		regional lymph node metastasis
Distant metastasis		
M0		no distant metastasis
M1		distant metastasis

Modified from NCCN Clinical Practice Guidelines in Oncology: Soft Tissue Sarcoma at http://www.nccn.org/professionals/physician_gls/pdf/sarcoma.pdf.

1.1.5 Prognosis

The 5-year overall survival for STS patients is about 57% to 62% (Ferrari, Sultan et al. 2011; Stiller, Trama et al. 2013). The prognosis for patients with STS is influenced by

so many factors that no grading system has been universally accepted. Irrespective of which grading system is used, patients with high-grade tumors are at significant risk of distant recurrence, and as many as 50% of them die of their disease. Conversely, patients with low-grade STS have an excellent prognosis, with 5-year survival rates of 85% or more (Kattan, Leung et al. 2002; Canter, Qin et al. 2008). Retroperitoneal tumors are generally much larger at diagnosis and this group has a poorer prognosis (36-58%). Metastases are detected in 36% of STS patients at diagnosis or during follow-up (Engellau, Bendahl et al. 2005). Approximately 11% of all STS patients have detectable metastasis already at diagnosis, most commonly in the lungs, and the 5-year survival in this group is only 14%. The occurrence of local recurrence depend on factors such as the local treatment. In a report by Engellau *et al.* local recurrence occurred in 32% of patients with inadequate local treatment as compared to 15% of those with adequate local treatment (Engellau, Bendahl et al. 2005).

1.1.6 Treatment

Treatment of STS follows guidelines from the Scandinavian Sarcoma Group (http://www.ssg-org.net/treatment-protocols-and-recommendations, SSG XVII, SSG XX and SSG XIX), and the European Society for Medical Oncology (ESMO; Guidelines for soft tissue sarcoma ESMO) (2012). In order to provide adequate expertise in surgical and adjuvant therapy, patients with STS are best treated in specialized multidisciplinary centers, and a histopathological review should always be done by an expert on STS (Clasby, Tilling et al. 1997). Surgery and radiotherapy are classical treatments for sarcoma patients. Surgery with a wide margin is the main treatment of STS, as has been known for a long time (Bowden and Booher 1958). STSs grow spherically along tissue planes and are surrounded by a false capsule of compressed surrounding tissue. Malignant cells penetrate this capsule and simple removal leaves microscopic disease and a high risk of recurrence (Bowden and Booher 1958; Gerrand, Wunder et al. 2001). For patients with low-grade sarcoma, where the prognosis is fairly good, surgery alone is the standard treatment, whereas in high-grade sarcoma, where the prognosis is poor, a decision has to be made on whether or not the patient should receive adjuvant therapy. Based on a study from Roswell Park Cancer Institute of sarcoma patients treated between 1979 and 1998, radiotherapy was recommended for all patients with a margin smaller than 10 mm. In that study, the smallest microscopic margins were divided into three categories: at least 10 mm, 1–9

mm and 0 mm, which yielded 5-year local control rates of 84, 58 and 58%, respectively (McKee, Liu et al. 2004).

In addition, chemotherapy and therapies directed at molecular tumor features are increasingly applied. Chemosensitivity varies between the types of STS and is influenced by tumor grade, timing of metastatic disease and patient performance. Neoadjuvant therapy is increasingly used and may improve prognosis in high-risk cases (Grobmyer, Maki et al. 2004). Trabectidin (Yondelis, Swedish Orphan Biovitrum), a natural product from the marine tunicate Ecteinascidia turbinate, is a new agent that has been found to have an effect in advanced disease resistant to conventional cytotoxic drugs (Yovine, Riofrio et al. 2004).

1.1.7 Surgical margin

A definition of surgical margin has been proposed by the Scandinavian Sarcoma Group. The margin should be assessed at surgery and by macroscopic and microscopic pathological examination. The poorest margin is most important, i.e. the part of the specimen where the tissue coverage is poorest (qualitatively and quantitatively). This is the area where the pathologist should record the type of tissue (e.g. fat, connective tissue) and the thickness (mm) of tissues covering the tumor. Positive margin refer to situations where the surgery was not radical and all tumor tissue was not removed. Two types of positive margin are described:
Gross tumor left: The tumor is transected during the operation and macroscopic tumor tissue is left. This situation is reported by the surgeon.
Intralesional: Microscopic tumor tissue is seen at the resection border (reported by the pathologist) or leakage of fluid/tissue from the tumor into the wound occurs during surgery (reported by the surgeon).

When the surgical procedure successfully removes the entire tumor mass, this is referred to as negative margin. Two types of negative margin are defined: marginal and wide. The decision as to whether or not the margin is negative, i.e. tumor-free, lies with the pathologist. In the case of a negative margin, the pathologist reports the shortest distance (in mm) between the tumor and the resection border in fat, muscle or loose areolar tissue in an area where there is no fascia between the tumor and the resection border. A fascia unengaged by the tumor is considered to be sufficient for a wide margin irrespective of the distance between tumor and fascia. A total myectomy with

the tumor completely surrounded by unengaged fascia needs no measurements and is classified by the surgeon as a wide margin. The distinction between a marginal and a wide margin is made by the surgeon and is based on the combined information from surgery and histopathological examination.

Marginal margin: The closest margin is outside but near the tumor in one or more places (irrespective of how much healthy tissue is included elsewhere) or all round the tumor (shelling out). Microscopically, the margin is negative all round the tumor (otherwise the margin is intralesional), but tumor cells may be present only millimeters from the margin.

Wide margin: There is a cuff of healthy tissue all round the tumor. Unengaged fascia is considered to constitute a cuff regardless of the thickness of tissue between the tumor and the fascia. A cuff of fatty or muscular or loose areolar tissue must be at least 10 mm thick as measured at the histopathological examination to qualify as a wide margin. Definitions as recommended by the Scandinavian Sarcoma Group (http://www.ssg-org.net/treatment-protocols-and-recommendations; Centralized Registration of Sarcoma Patients in Scandinavia, SSG VII:4).

1.2 GASTROINTESTINAL STROMAL TUMOR (GIST)

Gastrointestinal stromal tumor (GIST) was shown in the 1980s to be a separate entity (Mazur and Clark 1983; Miettinen 1988). These tumors had previously been diagnosed as leiomyoma, leiomyoblastoma, schwannoma or leiomyosarcoma. In 1998, Hirota *et al*. found gain-of-function mutations of the *KIT* gene in GISTs and proposed that GISTs may originate from the interstitial cells of Cajal. The c-kit receptor encoded by the *KIT* gene was suggested to be a target for diagnosis and treatment (Hirota, Isozaki et al. 1998; Kindblom, Remotti et al. 1998; Nakahara, Isozaki et al. 1998). The breakthrough in the knowledge of molecular mechanisms and new molecular targeted therapies have resulted in effective systemic treatment with tyrosine kinase inhibitors (TKIs) and a dramatically improved outcome for patients with recurrent or metastatic disease, as well as prolonged the time to recurrence and overall survival in highly malignant GIST (Verweij, Casali et al. 2004; Blanke, Demetri et al. 2008). Many novel agents are now being tested in the treatment of GIST. Nevertheless, the majority of patients are treated with surgery alone, giving a 15-year survival rate of 60% for patients with primarily operable tumors.

1.2.1 Occurrence

GIST is by far the most common mesenchymal tumor of the gastrointestinal tract and is assumed to be the most common mesenchymal tumor whatsoever (Mastrangelo, Coindre et al. 2012; Stiller, Trama et al. 2013). The annual incidence of clinically detected GIST in the Western region of Sweden was estimated to be 15 cases per million and the prevalence to be 129 per million (Figure 1) (Nilsson, Bumming et al. 2005). In two population-based series from Iceland and Italy, the annual incidence of GIST was 11 and 7 per million, respectively (Tryggvason, Gislason et al. 2005; Mucciarini, Rossi et al. 2007). GIST is slightly more frequent in males except for pediatric GIST, where females predominate (Janeway and Pappo 2012; Joensuu, Vehtari et al. 2012). The median age at diagnosis is 63 to 65 years, but GIST occurs at all ages. GIST can arise throughout the gastrointestinal tract from esophagus to rectum, most commonly in the stomach (55%), followed by the small intestine (35%) and rectum (5%). Colon and esophagus are rare locations (less than 5%). Rarely, GIST arises outside the gastrointestinal tract but inside the abdominal cavity (Janeway and Weldon 2012; Joensuu and DeMatteo 2012).

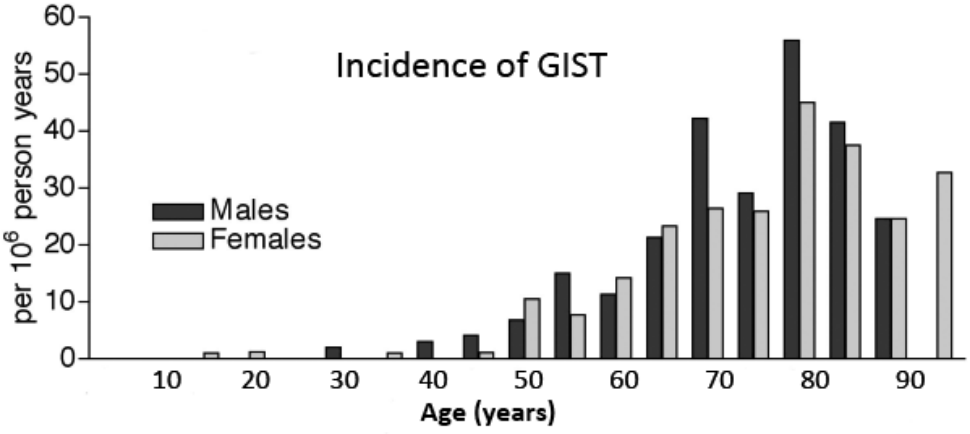

Figure 1. Incidence of GIST in a Western Swedish population (n = 259). Modified from Nilsson *et al.* (Nilsson, Bumming et al. 2005).

1.2.2 Signs and symptoms

As in other sarcomas, GIST usually gives sparse and/or late symptoms. Bleeding from the GI-tract, due to ulcer in the mucosa at the site of the tumor, is the most common symptom, seen in about 50%. Other symptoms are mostly dependent on the site of origin; abdominal pain/discomfort represents about 20% and obstruction as initial symptom is seen in about 10% of the patients. Fatigue is rare but may occur in cases with large tumors. The median size of the tumor at diagnosis is 5-7 cm but can be up to 30-40 cm (Joensuu, Fletcher et al. 2002; Nilsson, Bumming et al. 2005; Joensuu and DeMatteo 2012).

1.2.3 Histopathology, grading and prognosis

The diagnosis is based on morphology and immunophenotyping, and in difficult cases also on mutation analysis. Morphologically GIST is typically characterized by a cellular tumor with either spindle cells (70%), epitheloid cells (15%) or mixed cell forms (15%), however pleomorphic cells also occur (Miettinen 1988). Immunohistochemistry usually show positivity for CD117, DOG-1 and CD34, and sometimes also for smooth-muscle actin (SMA) (Table 2) (Sarlomo-Rikala, Kovatich et al. 1998; Miettinen, Sobin et al. 2000; West, Corless et al. 2004; Kang, Jung et al. 2010). Mitotic count determined as mitotic figures per 50 high power fields (HPFs) and the size of the tumor are both important for prognostic classification of GIST (Fletcher,

Berman et al. 2002; Miettinen, Majidi et al. 2002; Joensuu 2008; Miettinen and Lasota 2011).

Table 2. Markers for immunohistochemical characterization of GIST.

Target protein		
Symbol	Name	Finding in GIST
c-kit	Stem cell factor receptor	88-97% positive cases
DOG1	Discovered on GIST-1	87-97% positive cases
CD34	Hematopoietic progenitor cell antigen	60-70% positive cases
α-SMA	Alpha smooth muscle actin	30-40% positive cases
S-100	S100 protein	5% positive cases
Desmin	Desmin	<5% positive cases
Ki-67	Antigen identified by the antibody Ki-67	MIB-1 proliferation index <1-80%

No GIST can be regarded as truly benign, although patients with small tumors of low proliferation index have the same survival as the normal population. Based on pooled cohorts of patients with surgically completely removed GISTs who had not received neoadjuvant or adjuvant treatment, recurrence-free survival after surgery was estimated to 60%. Few recurrences occurred after 10 years of follow-up. Tumor size, high mitotic count, non-gastric location, ruptured tumor and male gender were found to be significant, independently adverse risk factors (Joensuu, Vehtari et al. 2012). In contrast, for patients with high-risk GIST, the 3-year survival was only about 20-30% and the median survival for patients with advanced disease was 18 months (Dematteo, Heinrich et al. 2002). Miettinen *et al.* have published several studies on the risk of progressive disease for patients with GIST at different sites (Table 3). Interestingly, the risk for malignant behavior was lower in the stomach but differed markedly depending on where in the stomach the tumor was located. The highest risk of progressive disease was seen for GIST in the cardia region (53%), somewhat lower in fundus (36%) and markedly lower in antrum (8%) (Miettinen, Sobin et al. 2005; Miettinen and Lasota 2006; Miettinen, Makhlouf et al. 2006).

Table 3. Risk of progressive disease in operable GIST based on follow-up data

Tumor size	Mitotic count	Risk of progressive disease			
(cm)	per 50 HPF	Stomach	Duodenum	Jejunum/Ileum	Rectum
≤ 2.0	≤ 5	0%	-	-	-
2.1 - 5.0	≤ 5	2%	4%	8%	9%
5.1 - 10.0	≤ 5	4%	24%	25%	-
> 10.0	≤ 5	12%	52%	52%	57%
≤ 2.0	> 5	0%	-	50%	54%
2.1 - 5.0	> 5	16%	73%	75%	52%
5.1 - 10.0	> 5	55%	85%	86%	-
> 10.0	> 5	86%	90%	90%	71%

Modified from Miettinen M, Lasota and Sobin 2005-2006

HPF = High power field

Following the introduction of imatinib, the prognosis of advanced GIST has changed dramatically. The response rates for patients with unresectable, metastatic or recurrent GIST are 50-70%. The survival for this group has been prolonged to a median progression-free survival of 18-20 months (Miettinen, Makhlouf et al. 2006; Rajendra, Pollack et al. 2013). In a follow-up study of a randomized phase II B2222 trial, in which patients with recurrent or unresectable GIST were followed for a median of 40 months (range 2.5-103) under imatinib treatment, the median progression-free survival was 89 months and the overall survival for 8 years was 67%. In one subgroup (17.7%), the patients survived with continuous imatinib treatment for more than 9 years (Saito, Nakata et al. 2013).

Several systems for risk stratification have been developed to achieve individualized treatment and follow-up by assessing the prognosis of the individual patient (Fletcher, Berman et al. 2002; Nilsson, Bumming et al. 2005; Miettinen and Lasota 2006). In Scandinavia we mainly use the NIH risk categories modified by Joensuu *et al.* to include tumor rupture (Joensuu 2008) (Table 4).

Table 4. Definition of risk categories proposed by Joensuu et al.

Risk Category	Tumor size (cm)	Mitotic index (per 50 HPF)	Tumor site
Very low risk	≤ 2.0	≤ 5	Any
Low risk	2.1 - 5.0	≤ 5	Any
Intermediate risk	2.1 - 5.0	≤ 5	Gastric
	≤ 5.0	6-10	Any
	5.1 - 10.0	≤ 5	Gastric
High risk	Any	Any	Tumor rupture
	> 10.0	Any	Any
	Any	> 10	Any
	> 5.0	> 5	Any
	≤ 5.0	> 5	Non-gastric
	5.1 - 10.0	≤ 5	Non-gastric

(Joensuu 2008)

Size and proliferation are the most important prognostic factors in GIST. In general, large tumors and tumors with high mitotic count (mitosis per 50 HPF) have a high risk of recurrence (Fletcher et al., 2002). However, follow-up data show that the site is also of importance. For tumors in the stomach the prognosis appears to be prognosis and proliferation is more important than size, while tumors in the small intestine and rectum mostly have a worse outcome (Miettinen and Lasota, 2006). Tumor rupture has also been shown to be significantly correlated to recurrence and overall survival (Rutkowski, Nowecki et al. 2007; Hohenberger, Ronellenfitsch et al. 2010). Tumor rupture has therefore been included in the modified NIH criteria proposed by Joensuu *et al.* (Table 4)(Joensuu 2008).

1.2.4 Molecular Pathology

GISTs usually express mutations in the tyrosine kinase receptor genes *KIT* or *PDGFRA*. These oncogenes encode the transmembrane glycoproteins c-kit (CD117, stem cell factor receptor) and platelet-derived growth factor receptor alpha (PDGFRA), both members of the type III tyrosine kinase receptor family. c-kit and PFGFRA are activated by binding of their respective ligands, the stem cell factor and PDGFA, to the extracellular region. These serve as binding sites for various signaling proteins, leading to phosphorylation cascades with activation of signaling substrates that regulate cell

proliferation, adhesion, motility, survival and differentiation of hematopoietic stem cells, mast cells, melanocytes, germ cells and interstitial cells of Cajal. Expression of c-kit can be detected in GIST, but also in other tumors such as small cell lung carcinoma, melanoma and seminoma.

Mutations of *KIT* and *PDGFRA* in GIST are activating (so-called gain-of-function mutations), leading to ligand-independent dimerization, autophosphorylation and activation of downstream signaling pathways (Hirota et al., 1998). The majority of *KIT* mutations are located within exon 11 (Hirota, Isozaki et al. 1998; Tabone, Theou et al. 2005; Lasota and Miettinen 2008; Liegl-Atzwanger, Fletcher et al. 2010). *KIT* exon 11 mutations are found in approximately 60-70% of all GISTs, and exon 9 mutations are found in 10%, whereas other mutations are rare; exon 13 mutations in 1%, and exon 17 mutations in <1% of cases. Mutant *KIT* supports growth by activation of downstream signaling pathways, e.g. the MAPK P13K-AKT and STAT3 pathways. The P13K-AKT pathway seems to be the most important, since inhibitors of P13K have a better effect on reducing proliferation and inducing apoptosis. About 8% of GISTs exhibit mutations in *PDGFRA*, exon 12, 14 or 18 and there is an extensive functional overlap between c-kit and PDGFRA. As in c-kit has mutated *PDGFRA* a kinase activity despite absence of its ligand. The activated downstream pathways are overlapping in *PDGFRA* and *KIT* mutant GIST (Heinrich, Corless et al. 2003; Corless, Barnett et al. 2011).

Almost all GISTs express mutated *KIT*, causing a ligand independent activation of c-kit. Mutation analysis is a useful tool in the management of GIST patients and should be done whenever possible. The results may be needed in the diagnostic setting and may be used as prognostic markers, sometimes influencing the choice of treatment. The location of mutations has an impact on the outcome in untreated patients and different mutations are also associated with the response to imatinib treatment. GISTs with *KIT* exon 11 deletions have a higher rate of progressive disease compared to those with point mutations (22% vs. 0%). In addition, *KIT* exon 13 or 17 mutations are often detected in recurrences and cases with resistance to imatinib treatment (Corless, Barnett et al. 2011). *PDGFRA* mutations are not significantly correlated to prognosis (Heinrich, Corless et al. 2003; Andersson, Bumming et al. 2006; Corless, Barnett et al. 2011).

The 5 to 10% of GISTs that lack mutations in either *KIT* or *PDGFRA* have been named wild-type GIST (wtGIST) (Heinrich, Corless et al. 2003; Corless, Fletcher et al. 2004). These wtGIST are otherwise clinically identical and express high levels of c-kit. However, recent studies have described that wtGISTs are a heterogeneous group displaying various oncogenic mutations, e.g. in *BRAF* (Corless, Barnett et al. 2011). Furthermore, defects in the succinate dehydrogenase (SDH) complex have been reported in wtGIST. IGF-1R expression was detected in 89% of SDH-deficient GISTs but in less than 1% of SDH-positive GISTs. The latter group was characterized by mutations in *KIT* or *PDGFRA* and occurred in older patients. SDH-deficient tumors had lost the expression of SDH subunit B, resulting in inactivation of the SDH-complex. This subtype of GISTs has only been identified in the stomach and mostly in children or young adults (Lasota, Wang et al. 2013; Miettinen, Killian et al. 2013).

1.2.5 Treatment

Generally, treatment of GIST should follow guidelines issued by SSG (http://www.ssg-org.net/treatment-protocols-and-recommendations; SSG XVII) and ESMO guidelines for GIST (2012).

Presurgical evaluation

A reliable *Diagnosis before treatment* is mandatory, in particular if the tumor is large and the surgical procedure is expected to be complex or if neoadjuvant treatment is considered. Fine-needle aspiration biopsy is the method of choice for preoperative diagnosis and, since about 60% of all GISTs are located in the stomach, endoscopic ultrasound (EUS) is preferable if possible. With EUS, both fine-needle aspiration biopsy and core biopsy could be performed. Moreover, EUS has by itself a high diagnostic value, based on its ability to determine the localization within the ventricular wall (Fu, Eloubeidi et al. 2002; Kataoka, Kawai et al. 2012; Layfield and Wallander 2012). Core-needle biopsy is rarely indicated and should be used only before neoadjuvant treatment, when mutation analysis is needed or if fine-needle aspiration gives insufficient information. Open biopsy should never be performed as this procedure will mimic tumor rupture and significantly influence the prognosis.

For *Anatomical evaluation* of GIST, computed tomography (CT) is regarded as the standard modality that may be used for primary detection of a tumor, treatment decisions, planning for surgery, assessment of response and follow-up, and for

distinguishing the lesion from other malignancies (Buckley and Fishman 1998; Chourmouzi, Sinakos et al. 2009). Most commonly, the appearance is that of an exophytic tumor growing from the wall of the gastrointestinal tract, but GISTs may also appear as intramural masses or intraluminal polyps. The majority of GISTs appear to be well-defined lesions with varying attenuation. Larger lesions are often less distinctly demarcated, with invasion of adjacent structures. Areas of necrosis or hemorrhage are often seen (Levy, Remotti et al. 2003; Chourmouzi, Sinakos et al. 2009).

For assessment of response to neoadjuvant treatment with TKIs, contrast-enhanced CT is routinely used. Response is usually seen as a rapid transition from a heterogeneously hyperattenuating pattern to a more homogeneously hypoattenuating pattern, often followed by a reduction of tumor size and extensive cystic changes. In the follow-up, detection of a focal or a new solid lesion is often an early sign of progressive disease (Choi, Charnsangavej et al. 2004; Werewka-Maczuga, Osinski et al. 2011).

Positron emission tomography combined with CT (PET/CT), using the radiotracer 18F-fluoro-2-deoxy-D-glucose (FDG), is superior to conventional CT in some situations. PET is especially valuable when an early assessment is needed during neoadjuvant imatinib treatment. Like most malignant tumor cells, GIST cells normally have a high uptake of glucose. In response treatment with imatinib, a change in glucose uptake is detected early. FDG-PET/CT is also useful for distinguishing active tumor tissue from necrosing tissue, as well as for detecting metastatic or recurrent disease (Van den Abbeele and Badawi 2002; Stroobants, Goeminne et al. 2003; Yoshikawa, Shimada et al. 2012).

Surgery

Despite the introduction of effective molecular targeted therapy, which has dramatically changed the course for patients with highly malignant tumors, surgery is still the main treatment for non-metastatic GIST and the only curative option. Joensuu *et al.* conducted an observational cohort study based on pooled data from several published population-based series of patients with operable GIST without metastasis who did not receive adjuvant systemic therapy; only patients with GISTs that macroscopically were completely removed at surgery were included. In this study, the estimated 15-year recurrence-free survival after surgery alone was 60% (95% CI 56–64). Most of the recurrences occurred within 5 years from diagnosis, a few recurrences

occurred after the first 10 years of follow-up and occasional cases were seen later (Joensuu, Vehtari et al. 2012).

A complete surgical resection with histopathologically negative margin (R0) is commonly regarded as a prerequisite for potentially curative treatment (Ng, Pollock et al. 1992; Bucher, Egger et al. 2006; Rutkowski, Nowecki et al. 2007; Catena, Di Battista et al. 2012; Joensuu, Vehtari et al. 2012). A study by Ng *et al*. found that complete resection without tumor rupture was the only significant factor for overall survival, using multivariate analysis (Ng, Pollock et al. 1992). This is also partly supported by reports showing that tumor rupture is associated with a worse outcome (Ng, Pollock et al. 1992; Rutkowski, Nowecki et al. 2007; Hohenberger, Ronellenfitsch et al. 2010; Joensuu, Vehtari et al. 2012). Most recurrences occur in patients with ruptured tumor or within the high-risk group. In a study on patients treated for rectal GIST, Jakob *et al*. found that none of the patients with local recurrences during follow-up had received preoperative imatinib and all had undergone local resection with positive resection margins (R1)(Jakob, Mussi et al. 2013). There is, however, still some uncertainty about surgical margins since some studies have shown that outcome does not differ significantly between R0 and R1 resection (DeMatteo, Lewis et al. 2000; McCarter, Antonescu et al. 2012).

For STSs other than GIST, surgery is classified on the basis of resection margins as *intralesional* (equivalent to R1), and *marginal* or *wide* (equivalent to R1 resection) modified from Enneking et al. (Enneking, Spanier et al. 2003). This subgrouping of R0 surgery is well established and has been shown to have prognostic significance in several studies where a resection margin of 2-3 cm has been suggested to provide good local control (Trovik, Bauer et al. 2000; Sampo, Tarkkanen et al. 2008). Although this classification has not been shown to be applicable in GIST, *Paper IV* in this thesis supports the necessity of complete resection margins, equal to R0, and indicates that wide surgical resection margins provide the best outcome.

In conclusion, the main treatment for non-metastatic GIST is surgery, which should be planned and performed in accordance with surgery for other kinds of visceral and retroperitoneal sarcomas aiming to achieve R0 resection (Bumming, Ahlman et al. 2006). Surgery may be performed by either traditional open or laparoscopic surgery as long as the oncological precautions are strictly observed and should if necessary

include adjacent organs that are adherent to the tumor. Since GIST almost never metastasizes through the lymphatics, lymph node resection is not indicated (Everett and Gutman 2008).

Surgery in advanced, recurrent and metastatic disease

Increasing evidence supports surgery in cases of advanced, metastatic or recurrent disease (Maehara, Chijiiwa et al. 2008; Zaydfudim, Okuno et al. 2012; Tielen, Verhoef et al. 2013). Surgery improves the overall survival compared to systemic therapy alone but 1- and 5-year overall survival rates are strongly associated with both preoperative response to TKI and R0 resection (Maehara, Chijiiwa et al. 2008; Zaydfudim, Okuno et al. 2012). Using neoadjuvant treatment of primary advanced GIST, Tielen *et al.* reported that downsizing of tumor was achieved in 52 of 57 patients who could be operated on with R0 resection without tumor rupture (Tielen, Verhoef et al. 2013). Six months of neoadjuvant treatment is recommended to avoid the occurrence of secondary mutations (Gold and Dematteo 2007).

Treatment of advanced GIST

In 2001 Joensuu *et al*. reported a patient who had been treated with the TKI STI571 (Imatinib mesylate, Glivec) and had a complete metabolic response within one month (Joensuu, Roberts et al. 2001). This initial report lead to an amazing era of TKIs in the treatment of GIST. The Phase II study of imatinib [CSTI571B 2222] treatment of 147 patients with recurrent or metastatic GIST resulted in partial response rates of 67% and 66% for treatment with 400 mg/d and 600 mg/d, respectively. For patients with unresectable or metastatic GIST, the introduction of imatinib treatment has extended the median overall survival from 19 to 57 months. A follow-up study of the randomized phase II trial B2222 found that a subset of patients (almost 18%) had survived for more than 9 years with continuous imatinib treatment (Blanke, Demetri et al. 2008; Patel 2013). Saito *et al*. reported even longer progression-free survival, 89 months, and overall survival at 8 years was 67%. The authors attribute this mainly to the intensive management of adverse events (Saito, Nakata et al. 2013).

Imatinib is currently considered as the first-line systemic therapy in patients with metastatic or primarily non-operable disease. A daily dose of 400 mg is the standard in most cases (Demetri, von Mehren et al. 2002; Joensuu, Fletcher et al. 2002; Blanke, Rankin et al. 2008; Joensuu and DeMatteo 2012). The response to imatinib treatment is

best in patients with *KIT* exon 11 mutations (Heinrich, Corless et al. 2003), but patients with other mutations or wtGIST may also respond. A higher dose (800 mg imatinib) is recommended for patients with tumors harboring exon 9 mutations. This is based on the phase III European Organization for Research and Treatment of Cancer trial, where patients with exon 9 mutations had a superior progression-free survival when initially treated with a higher dose of imatinib (Debiec-Rychter, Sciot et al. 2006). Approximately 80% of GIST patients respond to treatment with imatinib; tumor volume decreases in about 50% and the remainder has stable disease.

In most patients with advanced GIST who initially respond to imatinib, disease progression eventually occurs. The median time to progression exceeds 2 years. The maximum duration of response to imatinib is not yet known, but in some patients it is much longer than 5 years. The most common cause of acquired imatinib resistance is a second *KIT* mutation (Joensuu and DeMatteo 2012). Furthermore, about 15% of the patients have primary resistant tumors. In progressive disease, a fraction of patients respond to an increase in the dose to 600-800 mg daily or even higher (Blanke, Rankin et al. 2008).

For patients with tumors who do not respond to imatinib, the approved second-line treatment is currently sunitinib. Here the median time to progression is 6.3 months compared to 1.4 months in untreated patients (Demetri, van Oosterom et al. 2006). Today there are several other TKIs with activity in advanced GIST that can be used as third-line treatment, including sorafenib, vatalanib, nilotinib and masatinib (Joensuu, De Braud et al. 2008; Montemurro, Schoffski et al. 2009; Le Cesne, Blay et al. 2010). There are also new TKIs undergoing trails, for instance pasopanib (SSG XXI, PAGIST, Scandinavian Sarcoma Group).

Imatinib

One could say that the imatinib story started in 1960 when Nowell and Hungerford reported their observation of a small abnormal chromosome in patients with chronic myeloid leukemia (CML)(Nowell and Hungerford 1960) (Figure 2). Ten years later the possibility of chromosome identification after banding was described by Caspersson and colleges. Using this technique Rowley *et al*. could show that this so-called Philadelphia chromosome resulted from of a translocation between chromosomes 9 and 22 (Rowley 1973). The translocation was subsequently clarified on the molecular level

as generating the *BCR-ABL* fusiongene (Heisterkamp, Stam et al. 1985). The *BCR-ABL* oncogene which is detected in 95% of patients with CML, is the causative molecular abnormality in CML. In 1988 Yaish *et al.* reported he synthesis of tyrphostins (Yaish, Gazit et al. 1988), with specificity for individual tyrosine kinases. One of these tyrphostins was found to inhibit the bcr-abl kinase and could kill bcr-abl expressing cells *in vitro* (Kaur, Gazit et al. 1994). In parallel, Druker, Lydon and Bushdunger identified the kinase inhibitor CGP 57148 (now STI571, imatinib) that specifically inhibited the tyrosine kinase domain in abl, c-kit and PDGF-R. STI571 acts by blocking the ATP binding site of bcr-abl, and thus inhibit the phosphorylation of substrate needed for bcr-abl function (Buchdunger, Zimmermann et al. 1996; Druker, Tamura et al. 1996). All reviewed by Mauro et al. In the clinical trial published by Druker and collegues in 2001 imatinib was found to be well tolerated and remission was achieved in a large proportion of CML patients (Mauro and Druker 2001) Imatinib was also found to be effective in GIST and the function in GIST is similar to CML. The c-kit receptor is normally activated by binding of the stem-cell factor ligand, followed by receptor autophosphorylation and triggering of cell-signaling cascades involved in apoptosis and proliferation. *KIT* mutations affecting the juxtamembrane domain are oncogenic leading to ligand-independent c-kit dimerization and activation of the kinase enzymatic domain. Imatinib treatment will block the ATP binding site of c-kit and inhibit enzyme activity leading to normalization of cellular functions(Miettinen and Lasota 2006; Corless, Barnett et al. 2011).

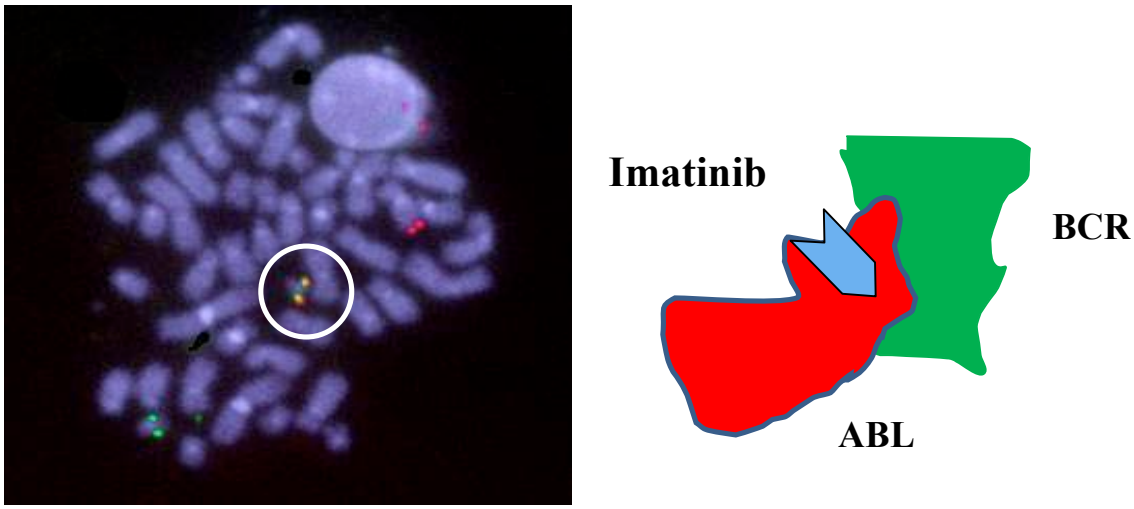

Figure 2. Illustration of the BCR-ABL fusion (marked by circle) by fluorescent *in situ* hybridization and targeting of abl by imatinib.

Adjuvant treatment

There is evolving evidence to support adjuvant treatment, at least in "high-risk" patients. The Scandinavian Sarcoma Group (SSG XVIII) trial, which compared 12 and 36 months of imatinib treatment in high risk GISTs, showed prolonged progression-free survival and, somewhat unexpectedly, also prolonged overall survival for the 36 month group (Joensuu, Eriksson et al. 2012). The ACOSOG Z9001 trial demonstrated significantly improved progression-free survival in patients who received one year of imatinib compared to placebo; however, the follow-up was short because the trial was discontinued prematurely when interim analysis showed significantly fewer recurrences in the treated group. In a study where patients with high-risk tumors were treated with adjuvant imatinib after radical surgery, the results were compared with historical controls; only one of 23 patients (4%) had a recurrence in the adjuvant treatment group compared to 32/48 (67%) in the control group (Nilsson, Sjolund et al. 2007). Estimation of the risk of recurrence after surgery for a GIST is of utmost importance when selecting patients who could possibly benefit from adjuvant treatment.

Surgery is the standard treatment for patients with a primary resectable GIST, and the aim is to achieve complete resection with negative microscopic margins. Complete resection is possible in the majority of localized GISTs, but only about 60% remain recurrence-free for five years or more. Several risk stratification models have been proposed, such as the original NIH consensus criteria, to rank the risk of recurrent disease in resected GIST. However, the NIH criteria do not take into account tumor rupture, incomplete or intralesional surgical resection or tumor site. Joensuu *et al*. has proposed a modification of the NIH consensus criteria, to include tumor site and rupture as prognostic variables (Joensuu, Vehtari et al. 2012).

In patients with tumors that are classified as "high risk", surgical removal with microscopic free margins is followed by three years of adjuvant treatment with imatinib (standard treatment). An exception, depending on conferred resistance to imatinib, is patients whose GIST harbors the *PDGFRA* gene exon 18 substitution mutation D842V (Asp842Val) (Corless, Barnett et al. 2011) or NF-1 associated wtGIST (Mussi, Schildhaus et al. 2008); neither should be treated with adjuvant imatinib. Mutation analysis should always be carried out before adjuvant treatment, as an aid in treatment decisions (Joensuu 2012).

Neoadjuvant treatment

Selected patients with primary inoperable tumors may benefit from the use of down-staging treatment with pre-operative imatinib followed by surgery. Surgery for focal progressive lesions or metastasis (Maehara, Chijiiwa et al. 2008) could also be considered as part of the second- or third-line armamentarium in selected cases (DeMatteo, Maki et al. 2007; Zaydfudim, Okuno et al. 2012; Tielen, Verhoef et al. 2013). Surgery of residual disease at best clinical response seems to be associated with survival benefit compared with historical controls in similar patient, treated with imatinib alone (Mussi, Ronellenfitsch et al. 2010; Doyon, Sideris et al. 2012; Roggin and Posner 2012). The suggested duration of treatment is within 6 months to avoid development of secondary mutations (Gold and Dematteo 2007).

1.3 THE INSULIN-LIKE GROWTH FACTOR RECEPTOR (IGF-1R)

The insulin-like growth factor (IGF) system is composed of three growth factors (IGF-I, IGF-II and insulin), three membrane receptors (IGF-IR, IGF-IIR and insulin receptor (IR)), six circulating IGF binding proteins (IGFBP 1–6) and proteases that modulate ligand availability. IGFs are evolutionary conserved factors that regulate energy metabolism and growth. IGF-I acts through the type 1 IGF receptor (IGF-1R); signaling leads to downstream activation of pathways involved in proliferation, apoptosis, invasion/metastasis and angiogenesis (Figure 3). IGF signaling also influences hypoxia signaling, protease secretion, tumor cell motility and adhesion (Djiogue, Nwabo Kamdje et al. 2013; Walenkamp, Losekoot et al. 2013).

Normally, IGF-I and IGF-1R are involved in pre- and postnatal growth; a deficiency leads to impaired pre- and postnatal growth, impaired brain development, sensorial hearing loss and failure to thrive (Walenkamp, Losekoot et al. 2013; Wit and Walenkamp 2013).

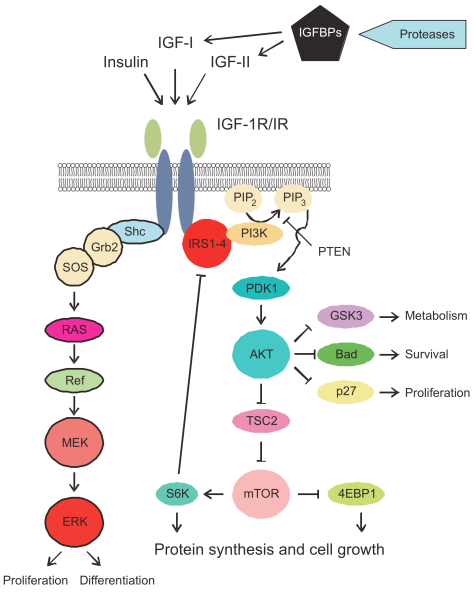

Figure 3. The IGF-1R signaling cascade.

IGF-1R, a member of the insulin receptor family of the tyrosine kinase membrane receptor, is a

heterotetramer consisting of two ligand-binding extracellular α-subunits and two β-subunits involving a transmembrane domain, an intracellular tyrosine kinase domain and a C-terminal domain. The subunits are linked together by disulphide bridges. Conformational changes occur when a ligand (IGFs or insulin) binds to the alpha subunits which enables autophosphorylation of the intrinsic tyrosine kinase domains subsequently initiating an intracellular signaling cascade. IGFs have 100-fold higher affinity than insulin to IGF-1R but insulin and IGFs can otherwise interact with each other's receptors. The mediated effect is similar but the general opinion is that insulin mediates mainly metabolic responses, whereas IGFs and the IGF-1R mediate growth-promoting effects (Pollak 2012; Varewijck and Janssen 2012).

IGF-1R is frequently over-expressed in cancer and has been reported to be one of the major mediators of the growth and survival of cancer cells (Jernberg-Wiklund and Nilsson 2012), including sarcomas (Rikhof, de Jong et al. 2009). High expression of IGF-1R has been shown to be associated with poor prognosis and metastasis in a broad spectrum of cancer and sarcoma subtypes (Xie, Skytting et al. 1999; All-Ericsson, Girnita et al. 2002; Rikhof, de Jong et al. 2009; Asmane, Watkin et al. 2012; Mountzios, Kostopoulos et al. 2013).

Recently, expression of IGF-1R was also detected in pediatric and wtGIST, where it seems to be linked to succinate dehydrogenase (SDH) deficient gastric GIST, whereas all small intestinal GISTs were negative (Lasota, Wang et al. 2013; Miettinen, Killian et al. 2013; Nannini, Astolfi et al. 2013).

Insulin and IGFs are potent mitogens; their receptors have received considerable attention as therapeutic targets in oncology. Indeed, in sarcomas, activity has been observed clinically in e.g. Ewing sarcoma, rhabdomyosarcoma, osteosarcoma, solitary fibrous tumor and synovial sarcoma (Sun, Gao et al. 2006; Hajdu, Singer et al. 2010) (Olmos, Martins et al. 2011), as well as in non-small-cell lung cancer, neuroendocrine tumors and prostate cancer. The encouraging early data on treatment of tumors via the IGF receptor family, have led to trials of drug candidates that target these receptors. However, the results of several recently reported trials have unfortunately been disappointing, mostly due to toxicity (the main side effects have been hyperglycemia, fatigue, and thrombocytopenia) but also because the benefit was limited to a small subset of patients, only 8-15% in Ewing sarcoma, and the duration of the response was

rather short in many patients due to the development of acquired resistance. Furthermore, biomarkers are not yet available to identify patients who could respond (Kim, Wan et al. 2009; Pollak 2012; O'Neill, Shah et al. 2013).

1.4 EZRIN

Ezrin is a member of the ezrin, radixin, moesin protein family (ERM) that has important functions in the organization and maintenance of the cell cortex, organization of the cytoskeleton, cellular morphology, migration and coordination of cell-to-cell signals (Crepaldi, Gautreau et al. 1997; Khanna, Khan et al. 2001; Ng and Streilein 2001; Saotome, Curto et al. 2004). They also function as links between the extracellular environment, cell membrane and the underlying cytoskeleton and cytoplasm by interacting with the plasma membrane and the actin cytoskeleton. ERM proteins thereby regulate signaling pathways by linking transmembrane receptors to downstream signaling components, such as activation of T-cell and other lymphocytes. Ezrin has also been shown to be involved in the release of insulin granule in beta-cells (Arpin, Chirivino et al. 2011; Neisch and Fehon 2011).

Khanna *et al.* (Khanna, Wan et al. 2004) and Yu et al. (Yu, Khan et al. 2004) initially reported that ezrin had high expression in highly metastatic osteosarcoma and rhabdomyosarcoma cell lines and that inhibition of ezrin significantly reduced the metastatic capability (Khanna, Khan et al. 2001; Hunter 2004; Khanna, Wan et al. 2004; Park, Jung et al. 2006; Bulut, Hong et al. 2012). Over-expression of ezrin and subsequent uptake of the negative regulatory molecules might result in amplification of metastasis-associated signaling from the plasma membrane through the Rho-associated signal transduction pathways (Hunter 2004). (Bretscher, Edwards et al. 2002; Hunter 2004).

Our findings, presented in *Paper II*, showed that ezrin expression in the primary tumor of initially metastasis-free STS patients was strongly associated with development of metastases during follow-up and consequently also with poor survival. The relationship between ezrin and tumor progression, metastasis and poor clinical outcome has also been shown in other tumors, including breast (Mak, Naba et al. 2012), uterus (Ohtani, Sakamoto et al. 2002), melanoma (Federici, Brambilla et al. 2009), and gastric (Jin, Jin et al. 2012). Similar findings have been reported for different types of STS in studies of malignant fibrous histiocytoma (Li, Akbari et al. 2008), STS (Carneiro, Bendahl et al.

2011) and GIST (Koon, Schneider-Stock et al. 2004), in which overexpression was significantly correlated with poorer disease-free survival (Wei, Li et al. 2009).

Using ezrin as a target for cancer therapy is of great interest. Among others, Bulut *et al.* have reported the identification of two small molecules that interact directly with ezrin and inhibit its function in multiple assays, both *in vitro* and *in vivo*. Both molecules inhibited lung metastasis of ezrin-sensitive cells but not ezrin-resistant cells. These small molecule inhibitors are proposed as a novel targeted therapy that directly inhibits ezrin, as an approach to prevent tumor metastasis, and their development is suggested as novel anti-ezrin compounds for clinical use.(Bulut, Hong et al. 2012).

2 AIMS OF THE STUDY

The purpose of this study was to identify molecular, pathophysiological and clinical characteristics associated with prognosis, in order to improve the management of patients with STS, including GIST, and ultimately affect the prognosis for the better.

The natural course of highly malignant STSs cannot be predicted reliably at the time of diagnosis, and the question arises whether the patient should receive adjuvant therapy in addition to surgery. The aim of ***Paper I*** was to evaluate the prognostic impact of known and suggested prognostic markers, particularly IGF-1R, within a cohort of primary highly malignant STS.

Ezrin is a cytoskeleton linker protein, involved in the growth and metastatic capacity of cancer cells. The aim of ***Paper II*** was to investigate the value of ezrin expression as a prognostic marker in clinical use, using immunohistochemical staining in primary highly malignant STSs.

Imatinib is considered to be first-line therapy in patients with unresectable and/or metastatic GISTs and has dramatically changed the course of this disease, but side-effects are common. The aim of ***Paper III*** was to evaluate the occurrence and prognostic significance of side-effects from imatinib treatment.

Surgical resection with microscopically negative margin (R0) is widely considered to be a prerequisite for intended curative treatment of GIST. In STSs other than GIST, surgery is classified on the basis of resection margins into “intralesional”, equivalent to R1, and R0, which is subclassified into marginal and wide surgical margin, respectively. This classification has not been proven to be applicable to GIST.
It has recently been reported that microGISTs mainly show an infiltrative growth pattern. To our knowledge this has not been investigated in other GISTs. The aim of ***Paper IV*** was to analyze the prognostic significance of resection margins defined as for other STSs, as well as to investigate the presence of infiltrative growth and pushing border and their correlation to surgical margins and prognosis in GISTs.

3 MATERIALS AND METHODS

3.1 PATIENTS AND CLINICAL MATERIAL

3.1.1 Highly malignant STS

In *Papers I* and *II*, all highly malignant STS patients primarily operated at the Orthopedic Department, Karolinska University Hospital, during the period 1985 to 1993 were included. Only patients treated for highly malignant STS (grade 3 or 4), without metastases at the time of diagnosis, who had not received any preoperative or postoperative adjuvant treatment, were selected. All patients were operated with a curative intent. An experienced histopathologist, without knowledge of the clinical course or the primary histology, re-evaluated all cases (in total 128 patients). After exclusion of patients with Ewing sarcoma, osteosarcoma, low malignancy grade or non-STS, 101 cases remained for evaluation.

In *Paper I*, all 101 patients were included. There were 47 males (47%) and 54 females (53%); the mean age at diagnosis was 62 years (range 5-90 years). The primary tumors were located in either the lower extremities (n = 66), the upper extremities (n = 16), the pelvic area (n = 9), the trunk or the abdominal wall (n = 10). The histopathological entities included malignant fibrous histiocytoma (n = 65), liposarcoma (n = 16), malignant peripheral nerve sheath tumor (n = 4), synovial sarcoma (n = 3), fibrosarcoma (n = 3), alveolar STS (n = 3), leiomyosarcoma (n = 2), mesenchymoma (n = 1), rhabdomyosarcoma (n = 1), spindle cell sarcoma (n = 1), angiosarcoma (n = 1), and high-grade sarcoma NOS (n = 1).

A subset of the cases from *Paper I* were included in *Paper II*. They comprised 24 men (48%) and 26 females (52%) with a mean age of 60 years at diagnosis (range 19-81years). The primary tumors were localized in the lower extremities (n = 33), the upper extremities (n = 6), the pelvic area (n = 6), or the trunk or abdominal wall (n = 5). The different histopathological entities included malignant fibrous histiocytoma (n = 34), liposarcoma (n = 7), malignant peripheral nerve sheath tumor (n = 2), synovial sarcoma (n = 1), fibrosarcoma (n = 1), alveolar STS (n = 2), mesenchymoma (n = 1), angiosarcoma (n = 1), and high-grade sarcoma NOS (n = 1).

3.1.2 GIST

In *Paper III*, 75 patients diagnosed with GIST who had received imatinib treatment at the Karolinska University Hospital, Sweden, were included. Patient records were reviewed retrospectively. All patients had been treated with surgery and imatinib between 2002 and 2010. The size of the tumor ranged from 2.5 to 28 cm. Totally 37 tumors were >10 cm, 24 were 5-10 cm, 9 were <5 cm, while in one case the size was not available. All but 6 patients were classified as high risk.

The indication for imatinib treatment was metastatic or recurrent disease, diagnosed before initiation of imatinib therapy in 31 cases; another 3 cases were defined as having metastatic disease as a consequence of earlier invasive procedures (exploratory laparotomy with open biopsy or external drainage). These patients received imatinib as neoadjuvant (n = 10), palliative (n = 12), recurrence (n = 10) or adjuvant (n = 2) treatment. The remaining 41 patients received imatinib treatment as adjuvant treatment (n = 31) or neoadjuvant treatment (n = 10).

In *Paper IV*, a cohort of 83 patients diagnosed with GIST, admitted to Karolinska University Hospital 1997-2010 and with available data on surgical margins, was analyzed. The cohort was selected from an initial cohort of 129 patients after exclusion of patients who had received neo or adjuvant treatment with imatinib (n = 31) or had metastasis at diagnosis (n = 15). The 83 patients comprised 46 females (55%) and 37 males (45%). Most of the tumors were located in the ventricle (n = 46), followed by small intestine (n = 24), duodenum (n = 5), colon (n = 3), rectum (n = 3) and esophagus (n = 2). Thirty-two were classified as high risk, 17 as intermediate risk and 34 as low or very low risk tumors according to NIH risk criteria. No micro GIST, defined as tumor smaller than 1 cm, was included. There were 19 tumors with a largest diameter >10 cm, 20 with >5-≤10 cm, 35 with 2-5 cm and 6 with <2 cm.

For 67 of the 83 patients (36 females (54%) and 31 males (46%)), hematoxylin slides were available with evaluable tumor border for analysis of growth pattern. Thirty-two of these tumors were localized in the ventricle, 7 in duodenum, 20 in the small intestine, 4 in rectum, 2 in colon and 2 in esophagus. Thirty-one were classified as high risk, 12 as intermediate risk and 24 as low or very low risk tumors.

3.2 CLINICAL ASESSMENTS

3.2.1 Follow-up in in STS and GIST

In *Papers I* and *II,* including highly malignant STSs, the patients were followed at the Orthopedic Department, Karolinska University Hospital, until either October 2001 or the patients' death, and the occurrence of metastasis and/or local recurrence was recorded. The patients were divided into the following groups depending on the type of recurrent disease:

1. Patients who had no evidence of disease during follow-up (DFS). This group was used as a reference in calculations of local recurrence and metastasis.
2. Patients who developed local recurrence but no metastases during follow-up (Only Lrec).
3. Patients with metastases but no local recurrence during follow-up (Only Met).
4. Patients who had developed both local recurrence and metastases during follow-up (Lrec + Met). For this group, the date of the first event was used in the statistical analyses.
5. Separate calculations were also done for the group of patients who developed local recurrence irrespective of metastases during follow-up (All Lrec) and the patients with metastases irrespective of local recurrence during follow-up (All Met).
6. All patients who were alive, without evidence of disease at the end of follow-up (NED), were used as reference in calculations of death from or with disease, irrespective of whether they had either local recurrence or metastasis during follow-up.

In *Paper II*, time to metastasis and local recurrence were used in the analyses. For patients with both local recurrence and metastasis, time to metastasis was used in the calculations. As in *Paper I*, all patients who were alive without evidence of disease at the end of follow-up, regardless of whether they had recurrence or metastasis during follow-up, were used as reference in the calculations of survival concerning death from or with disease.

In *Paper III*, all patients were followed-up routinely for tumor recurrence. Tumor response was evaluated by FDG-PET (11 cases in the neoadjuvant group) and/or contrast-enhanced CT (n = 75) and histopathological examination of the excised tumor (n = 75). Follow-up was defined as time from initiation of imatinib treatment to last

documentation of state, i.e. no evidence of disease (NED), evidence of disease (ED) or death.

In *Paper IV*, all patients were followed-up routinely for recurrent or metastatic disease with contrast-enhanced CT every 6 months or in the event of symptoms. The patients were followed-up until death or March 2012. Recurrence, site of recurrence, metastasis and cause of death were registered. The 83 patients included in analyses for surgical margins had a median follow-up of 61 months (range 8-154 months) and the 67 patients included in the analyses of growth pattern had a median follow-up of 61 months (range 8-154 months)

3.2.2 Tumor size

In all Papers, tumor size was defined as the largest diameter, assessed by examination of the resected specimen; the figures were taken from the histopathological report.

In *Paper I*, the patients were divided into three groups according to size: ≤6 cm, 7-11 cm and >10 cm.

In *Paper II,* the median size was 11 cm (range 1-23 cm) and the actual diameter was used in the calculations.

In *Paper III*, the observed tumor size ranged from 2.5 cm to 28 cm and was subgrouped as <5 cm, 5-10 cm, or >10 cm.

In *Paper IV*, the median diameter was 6.8 (range 1.2 -28 cm); in the calculations, all cases were subgrouped as <2 cm, 2-5 cm, >5-≤10 cm or >10 cm.

3.2.3 Imatinib side effects in GIST

In *Paper III*, records from all patients were carefully reviewed for side-effects and the history of moderate to severe or life-threatening side-effects was classified according to Common Terminology Criteria for Adverse Events v4.0 (CTCAE).

3.2.4 Surgical margin

The definition of surgical margin used in this thesis comes from the Centralized Registration of Sarcoma Patients in Scandinavia (SSG VII http://www.ssg-org.net/treatment-protocols-and-recommendations/ongoing). The definition is modified from Enneking *et al.* (Enneking, Spanier et al. 2003).

Surgical margins in STS

In *Paper I* surgical margins were assessed at surgery and upon pathological macroscopic and microscopic examination. The tissue coverage of the specimen with the poorest margin was recorded.

Cases with positive margin included:

Gross tumor left: When macroscopic tumor tissue is left behind, as reported by the surgeon. This is equivalent to R2 resection.

Intralesional: When microscopic tumor is reported at the resection border,as judged by the pathologist. This also includes situations when leakage of fluid/tissue occurs during surgery from the tumor into the wound, as when the tumor is ruptured or the pseudocapsula is injured. This is equivalent to R1 resection.

A negative microscopic margin is equivalent to R0 resection. The classification is based on the shortest distance (mm) between the tumor and resection border in an area where there is no fascia between the tumor and resection border. The distinction between a marginal and a wide margin is made by the surgeon, based on the combined information from surgery and histopathological examination.

Two types of negative margin are defined:

Marginal margin: There is microscopically negative margin but with a close margin in at least one portion of the tumor, regardless of how much healthy tissue there is elsewhere around the tumor.

Wide margin: There is either a cuff of healthy tissue of at least 20 mm that must be present all round the tumor, or an unengaged fascia. An intact fascia is considered sufficient for wide margin, irrespective of the distance between tumor and fascia.

Surgical margin in GIST

In *Paper IV*, surgical margins were assessed during surgery, complemented by subsequent histopathological examination. The definition used is the same as for

surgical margins of STS in other locations, modified to be applicable to STS in the abdominal cavity, including GIST, and the retroperitoneal space.

Intralesional margin: Defined as R1 resection (microscopically positive margins), which includes tumor enucleation or when the surrounding peritoneal surface is damaged.
Marginal margin: Defined as R0 resection (microscopically negative margins, i.e. tumor-free (Wittekind, Compton et al. 2002)), with less than 2 cm in the organ of origin or in tissue adherent to the tumor.
Wide margin: Defined as R0 resection with >2 cm surgical margin in the organ of origin with a completely intact peritoneal surface covering the tumor and "un-bloc" resection of surrounding organs and structures that are adherent to the tumor.

3.2.5 Peripheral growth pattern in GIST

The peripheral tumor growth pattern was classified as pushing border or infiltrative growth. The definitions applied are in accordance with the classification applied by Engellau *et al.* for soft tissue sarcomas (Engellau, Bendahl et al. 2005; Rossi, Gasparotto et al. 2010; Wu, Hou et al. 2011).

Pushing border: The tumor border was free from growth of tumor cells outside the tumor restriction in the surrounding normal tissue.
Infiltrative growth: There were findings of tumor growth outside the tumor pseudo-capsule or tumor cells infiltrating the surrounding normal tissue outside the tumor restrictions.

3.3 EXPERIMENTAL METHODS

3.3.1 Immunohistochemistry

Immunohistochemistry was used to evaluate protein expression and localization in *Paper I* and *II*. Paraffin-embedded tissue from the primary tumor was available for all cases; the single most representative tumor tissue block was used for immunohistochemical analyses.

Paraffin sections of 4 μm were deparaffinized, rehydrated, and pretreated with citrate buffer at pH 6 in a microwave for 20 minutes. After rinsing, the endogenous peroxidase

activity was blocked by treatment with 0.5% hydrogen peroxide for 30 minutes. The sections were then rinsed and incubated with blocking serum for 20 minutes.

The primary antibody was applied and sections were incubated overnight in a moist chamber at +8 °C (Ezrin, +4 °C). A biotinylated anti-mouse immunoglobulin G, used as secondary antibody, was incubated for 30 minutes, followed by rinsing in the avidin-biotin complex for another 30 minutes. The peroxidase reaction was developed using 3,3-diaminobenzidine for 6 minutes and nuclear counterstaining. TBS (pH 7.4) was used for rinsing between the steps.

In *Paper I*, the following antibodies were used for antigen detection: anti human-Bcl-2 oncoprotein, DO-1 for p53, Mib-1 for Ki-67, Clone F8/86 for anti-human von Willebrand Factor, Kip-1 for p27, and a polyclonal antibody to the IGF-1R a-subunit (N20 Santa Cruz Biotechnology). In control experiments, slides from 10 of the cases were incubated with another polyclonal antibody against the IGF-1R h-subunit (H60 Santa Cruz). The immunohistochemical staining was done according to the standard ABC technique. For IGF-1R, the same method for immunostaining was done as for the other antibodies, except that the 20-minute pretreatment with citrate buffer was omitted and biotinylated anti rabbit immunoglobulin G was used as a secondary antibody. Paraffin sections from tissues known to be negative or positive for the respective markers were analyzed in parallel as negative and positive controls.

In *Paper II*, a primary mouse monoclonal antibody against ezrin was applied. Paraffin sections from placenta collected after birth and normal mesenchymal tissues from liposarcoma patients were analyzed in parallel as positive and negative controls, respectively.

Scoring of immunohistochemical slides

In *Paper I*, for Bcl-2, p53, Ki-67, and p27, the degree of staining was calculated as the proportion of positive cells using a 10 x 10 grid in 10 HPFs (200 x). For each HPF, the total numbers of positive and negative cells were determined by counting all positively stained cells in two or more representative rows and multiplying to 10 x 10. The scoring was done by one independent observer for all cases and by two independent observers for randomly selected cases. In the evaluation of vessel density, 10 fields

(200x) with the most intensive neovascularization (hotspot) were evaluated, and all vessels stained by Factor VIII were counted.

The IGF-1R staining was evaluated by three independent observers using a semiquantitative approach by which the proportion of positive cells was scored in 25% intervals, giving either negative tumors (0% positive cells) or positive tumors (1-25%, 26-50%, 51-75%, or 76-100% positive cells). In addition, the STSs with positive IGF-1R expression were also evaluated for relative staining intensity on the cellular level.

In *Paper II*, the immunostaining for ezrin was scored for all cases by two observers in an open discussion without knowledge of the clinical details. First, the ezrin expression was scored as positive or negative. Negative cases included those where no tumor cells showed cytoplasmic immunoreactivity or where only single tumor cells showed immunoreactivity. All cases scored as positive showed ezrin immunoreactivity in the cytoplasm of a subset or all tumor cells. Positive cases were also evaluated concerning the proportion of positively stained cells. A semiquantitative approach was used whereby the tumors were grouped into four classes with 1% to 25%, 26% to 50%, 51% to 75%, or 76% to 100% positively stained cells. For these quantitative analyses, positive cells were only counted in areas with a high proportion of tumor cell representation, whereas areas with necrosis and/or lymphocyte infiltration were excluded to rule out an incorrectly high proportion of positively stained cells.

3.4 STATISTICAL METHODS

Correlations between clinical, immunohistochemical and histopathologic variables and outcome during follow-up were analyzed. Recurrence-free survival was measured from date of diagnosis (start of imatinib treatment in *Paper III*) to the time of first recurrence, most recent follow-up or death. The results were calculated using the log-rank test and illustrated by Kaplan-Meier plotting. Prognostic factors were compared using Cox proportional hazards model; correlation analyses were done with Spearman rank order test. The calculations were done with Statistica 6.0 software for *Papers I* and *II*, Statistica 10.0 software for *Paper IV,* and the PASW for Windows statistical package 18.0 for *Paper III*. $P < 0.05$ was considered to be statistically significant.

In *Paper I*, each variable was evaluated in a series of cut-off values. The cut-off that gave the best P-value is presented in the tables and used in the statistical analyses.

In *Paper II*, associations between ezrin expression and recurrent disease, irrespective of time, were calculated by Chi-square test in addition to other analyses.

In *Paper III*, Pearson's Chi-square test was used to compare the distribution of patient and tumor characteristics between groups.

In *Paper IV*, time from diagnosis to recurrence, metastasis and tumor free survival were compered to outcome in the different groups by calculating with Kaplan-Meier lot, Chi-square test as well as cox regression analysis and correlation analysis.

4 RESULTS AND DISCUSSION

4.1 IGF-1R EXPRESSION IN HIGHLY MALIGNANT STS (*PAPER I*)

The purpose of this study was to evaluate known and suggested prognostic markers, in particular the insulin growth factor type 1 receptor (IGF1R), in highly malignant soft tissue sarcoma (STS). In this patient group which is known to entail a high risk of recurrence, it can be difficult to identify which patients will develop a recurrence or metastases and thus, could be considered for additional adjuvant treatment.

The study includes 101 patients with orthopedic STSs of high malignancy grade that were evaluated with respect to previously known prognostic factors such as gender, location (deep / subcutaneously), tumor size, necrosis, vascular ingrowth, infiltrative growth pattern, mitotic index, surgical margin and grade 3 or 4. We also determined immunohistochemical expression of cell cycle factors (p53 and p27), apoptosis marker (Bcl-2), MIB-1 proliferation index (Ki-67), and a growth factor (IGF-1R). Neovascularization was assessed as vessel density by Factor VIII labeling of the endothelium. Sections from paraffin-embedded material were incubated with the current antibody and the proportion of positive cells was counted in a grid (10x10 lines). In the assessment of vascular density, the number of vessels was counted in the same grid pattern.

Tumor size was found to be the most prominent prognostic factor among which 11 cm and 6 cm, were most significant. Thus, patients with tumors smaller than 6 cm had a good prognosis, while those with tumors larger than 11 cm had a generally poor prognosis. Other clinical and histopathological parameters with prognostic significance were deep vs. subcutaneous localization, necrosis, mitotic rate and to some degree surgical margin. Although necrosis and mitotic index are included as part of the malignancy grading, no difference in prognosis was found between malignancy grades 3 and 4. These data suggest that a two-grade (low malignant/high malignant) or three-grade scale may be sufficient for an adequate assessment of malignancy grade and risk of recurrence. Moreover, the individual components necrosis and mitotic index are more relevant than the overall assessment.

No prognostic value could be demonstrated for the parameters gender, infiltrative growth pattern and vascular invasion. Infiltrative growth pattern was seen in 93% of the cases, indicating that this characteristic is common in highly malignant STS but lacks prognostic significance in this group of highly malignant tumors.

Among the immunohistochemical markers, only IGF-1R expression and vascular density were significantly associated with prognosis. Vascular density showed correlation to death from or with disease as well as to survival. However, results in other studies are divergent and further studies are needed to determine the prognostic importance of vascular density in STS.

Insulin-like growth factors (IGFs) are normally involved in the intrauterine and postnatal growth and development of tissues and organs. IGF-1 receptor (IGF-1R) is a cell membrane receptor activated by its ligands IGF-1 and IGF-2. Activation effects on proliferation, differentiation and apoptosis, as well as deficiency, lead to impaired pre- and postnatal growth, impaired brain development, sensorial hearing loss and failure to thrive. We have found that IGF-1R is expressed in approximately half of the tumors, which indicates that this system is frequently activated in STS. Furthermore, our study has shown that high expression of IGF-1R is associated with a reduced risk of death in or with disease, as well as with improved overall survival, compared with tumors showing a low number of IGF-1R-positive cells. Therefore, IGF-1R may be a factor of potential prognostic significance.

4.2 PROGNOSTIC VALUE OF EZRIN EXPRESSION IN HIGHLY MALIGNANT STS (*PAPER II*)

Earlier findings in other sarcomas, such as osteosarcoma and rhabdomyosarcoma, indicated that ezrin were of interest and might be a key component in tumor progression and metastasis. In the study of Khanna *et al.* the role of ezrin expression in development of metastasis was demonstrated in a mouse model, in a natural dog model as well as in pediatric osteosarcoma patients (Khanna, Wan et al. 2004). The aim of *Paper II* was to investigate ezrin, using immunohistochemical expression, as a prognostic marker for clinical use in highly malignant STS.

The study includes 50 cases with primary STS of malignancy grade 3 or 4, which were also part of *Paper I*. None of the patients had local or distant metastases at the time of the initial surgery, and none had received pre-or post-operative adjuvant treatment. Follow-up was done retrospectively for at least 4 years or until death. Information about survival, metastasis and local recurrence were collected and analysed separately against ezrin expression. The results show that 26 of the 50 patients developed metastasis (52%), 16 patients developed local recurrence (32%) and 16 remained without any recurrent disease (32%); thus, 8 patients had both metastasis and local recurrence. Of the 34 patients who developed recurrent disease, 28 (82%) died and the other 6 (18%) were alive without any sign of disease at the end of the follow-up.

The expression of ezrin was determined by immunohistochemistry using a monoclonal antibody. Placenta was used as a positive control and normal mesenchymal tissue from a liposarcoma patient served as negative control. The results were evaluated concerning positive/negative cases as well as concerning the proportion of ezrin expressing cells in positive cases. Half of the cases were found positive showing immunoreactivity in the membrane and cytoplasm in 1 to 100% of the tumor cells, as exemplified in Figure 4.

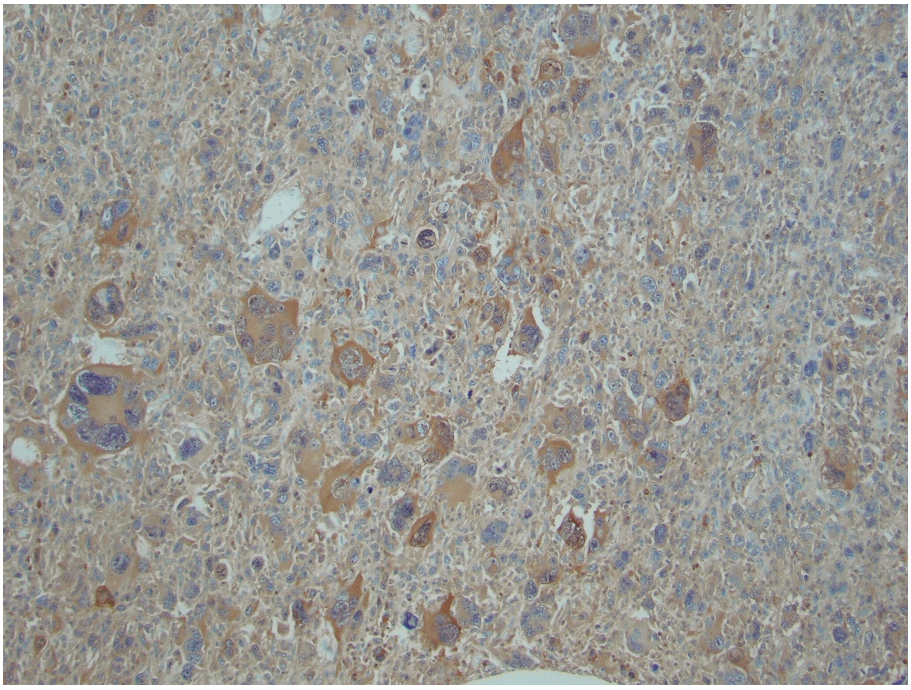

Figure 4. Photomicrograph showing immunohistochemical expression of ezrin in a highly malignant STS.

Positive ezrin expression was associated with development of metastasis and with short survival (Figure 5). Of the 25 positive cases, 17 developed metastasis, compared with only 9 of the 25 negative cases ($P = 0.023$). Distant metastases were significantly more frequently in patients with ezrin-positive as compared to ezrin-negative tumors ($P = 0.031$). No association was found between ezrin expression and local recurrence. Ezrin expression was also shown to be an independent prognostic marker for development of metastatic disease in multivariate analyses and consequently also associated with death from disease ($P = 0.014$) as well as with overall survival ($P = 0.007$).

Interestingly, a comparison of results from *Paper I* showed that ezrin expression was significantly correlated to infiltrative growth pattern ($P = 0.03$). None of the other tested parameters was significantly correlated to ezrin. Tested parameters were: clinical (sex, size, site, and depth), histopathological (malignancy grade, necrosis, and mitoses), and immunohistochemical expression of Ki-67, p53, p27, Bcl-2, IGF-IR, or Factor VIII (i.e. antigens involved in proliferation, differentiation, angiogenesis, and apoptotic processes). Furthermore, in a subset of cases previously analyzed for DNA copy number alterations using comparative genomic hybridization, a strong correlation was found between ezrin expression and copy number gain of chromosomal region 9cen-

q22 (R = 0.47, *P* = 0.02). However no association was observed between ezrin expression and alterations of the region harboring the ezrin coding gene in the long arm of chromosome 6.

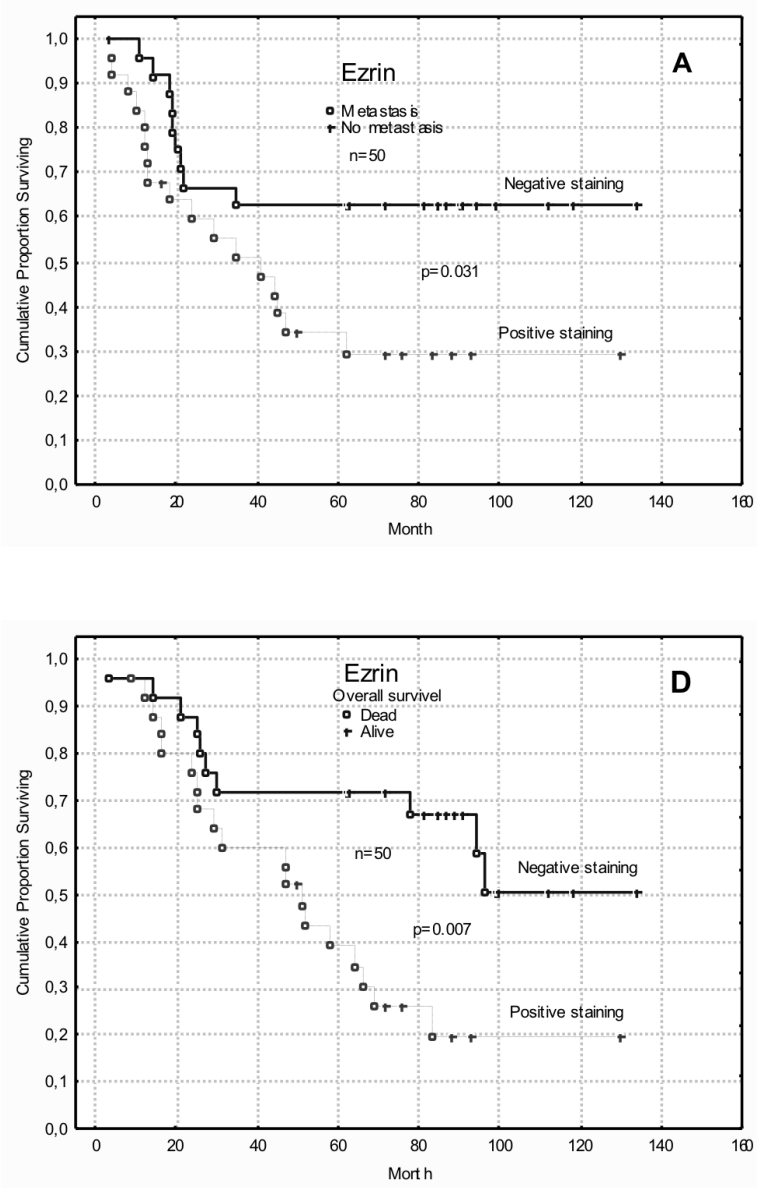

Figure 5. Kaplan-Meier plots showing associations between positive ezrin expression and development of metastases (top) and shorter overall survival (bottom).

In conclusion, our findings show that high ezrin expression in the primary tumor of initially metastasis-free STS patients is strongly associated with development of metastases during follow-up and consequently also with poor survival. A relationship between ezrin and tumor progression, metastasis and poor clinical outcome has also been shown in other tumors, including various types of STS, malignant fibrous histiocytoma (MFH) (Li, Akbari et al. 2008), STS generally (Carneiro, Bendahl et al.

2011) and GIST (Koon, Schneider-Stock et al. 2004), where over-expression significantly correlated with poorer disease-free survival (Wei, Li et al. 2009).

4.3 SIDE-EFFECTS AND OUTCOME IN IMATINIB TREATED GIST (*PAPER III*)

GIST is commonly treated by surgery alone or in combination with a TKI such as imatinib. The standard treatment is surgery with negative tumor margins (Casali, Jost et al. 2008; Joensuu, Vehtari et al. 2012). However, only about 50% of the tumors are localized at the time of diagnosis (Nilsson, Bumming et al. 2005; Tryggvason, Gislason et al. 2005). Therefore curative surgery is often not possible and many patients relapse (DeMatteo, Lewis et al. 2000). While GISTs are resistant to conventional chemo- and radiation therapy (Joensuu, Roberts et al. 2001) the use of TKIs have proven highly successful. Indeed, the c-kit tyrosine kinase inhibitor imatinib has significantly improved the outcome of advanced GIST. Imatinib mesylate is now considered the standard first-line treatment for advanced and recurrent GIST and also for adjuvant treatment (Demetri, von Mehren et al. 2002; Dematteo, Ballman et al. 2009). Neoadjuvant treatment in primarily unresectable GISTs may permit complete resection (R0) and also organ-saving surgery (Eisenberg, Harris et al. 2009; McAuliffe, Hunt et al. 2009). Adjuvant treatment offers improvement in progression-free survival (Dematteo, Ballman et al. 2009; Joensuu, Eriksson et al. 2012).

The medication is relatively well tolerated, but side effects are encountered in almost all patients (Joensuu, Trent et al. 2011), which may be more severe for higher doses (Blanke, Rankin et al. 2008; Dematteo, Ballman et al. 2009). However, the relationship between side effects and imatinib plasma concentrations is not clear (Cortes, Egorin et al. 2009) (Yoo, Ryu et al. 2010). Interestingly, low plasma concentrations of imatinib have been associated with a shortened time to disease progression (Demetri, Wang et al. 2009). Given these circumstances we here aimed to monitor the development of imatinib side-effects and their possible influence on patient outcome in GIST patients treated at our institution. For this purpose, a cohort of patients was identified and retrospectively reviewed concerning tumor characteristics, treatment, side effects and follow-up.

The study includes 75 GIST patients who had been operated and treated with imatinib. The tumor size ranged from 2.5 to 28 cm and cases were sub-grouped as <5 cm, 5-10 cm, or >10 cm. The tumors were located in esophagus (n = 2), stomach (n = 34), duodenum (n = 7), small intestine (n = 24), colorectal (n = 7) or uncertain (n = 1). The risk of recurrence was defined using the modified consensus classification of NIH, based on primary tumor size, location, rupture and mitotic index (Joensuu 2008). The classification criteria gave 69 patients at high risk and 6 patients at intermediate risk. The median follow-up time from the initiation of imatinib treatment until the last examination or death was 48 months (4–107 months).

Totally 41 patients without known metastatic disease at diagnosis had received neoadjuvant or adjuvant treatment with imatinib due to high risk, intermediate risk or primarily inoperable tumor. Metastatic or recurrent disease was diagnosed in 31 patients and an additional 3 patients were treated as if they had metastasis due to ruptured disease. These patients received imatinib in a neoadjuvant, palliative, or adjuvant setting.

The side-effects were classified according to the Common Terminology Criteria for Adverse Events v4.0 (CTCAE) as grade 1 (mild), grade 2 (moderate), grade 3 (severe), grade 4 (life-threatening) or grade 5 (death related to adverse effects).
Grade 2-4 side-effects were observed in 30 patients. Life-threatening complications occurred in two cases in the form of agranulocytosis and tumor rupture, respectively. The other 28 patients showed dermatitis/rash (n=15), gastrointestinal symptoms (n = 9), edema (n = 7), muscle cramps (n = 4),fatigue (n = 2) and several other side effects in single cases.

Overall, the side-effects occurred already after a short period of treatment. In 9 cases this lead to interruption of the treatment because of symptoms such as agranulocytosis (n=1), dermatitis, rash (n = 6), pruritus (n = 2), edema (n = 2), nausea, diarrhea (n = 3), and myalgia and muscle cramps (n = 1).

Among patients with metastatic or recurrent disease a significant association between presence of side-effects and favorable recurrence-free survival was observed ($P = 0.01$) (Figure 6). However, side effects were not associated with other clinical parameters.

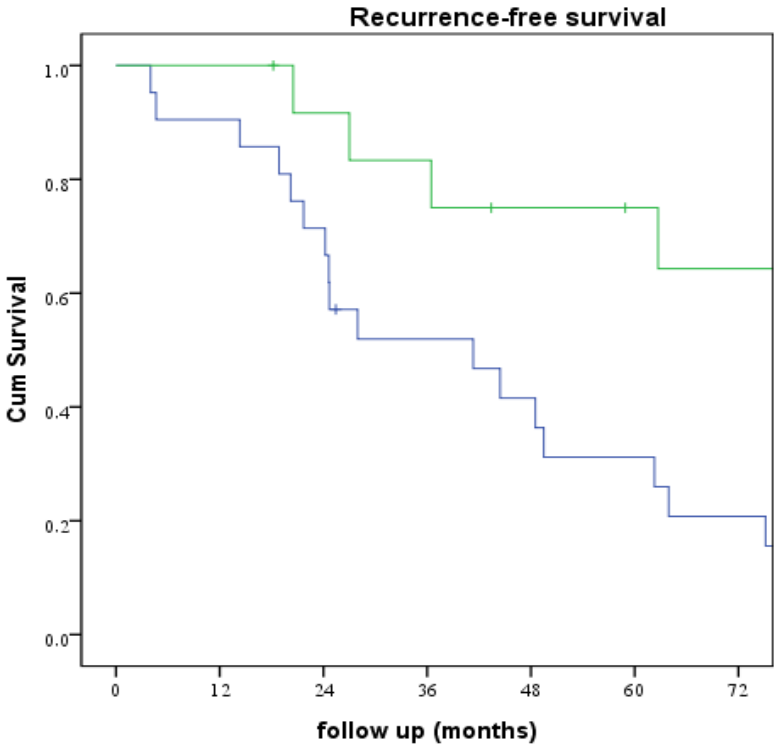

Figure 6. Kaplan-Meier plot showing better recurrence-free survival in patients with metastatic/recurrent GIST and side-effects from imatinib treatment.

The observation of improved survival for GIST patients with imatinib side-effects is of interest and raises several questions about the underlying mechanism and possible implications. First, it is possible that side effects from imatinib treatment reflect higher concentrations of the drug and unwanted effects on e.g. the abl target. This could be related to several factors such as drug uptake, metabolism, and tumor cell uptake. A natural continuation of this study would be comparison of side-effects with plasma concentration of imatinib. However, such analyses are not used routinely in clinical practice, while such information is not available for the present series. Prospective analysis of imatinib concentrations in comparison to side-effects and outcome are encouraged based on the present data. Furthermore, the occurrence of adverse effects may reduce patient compliance and discontinuation of imatinib treatment has been reported to result in rapid tumor progression in patients with advanced GIST (Le Cesne, Ray-Coquard et al. 2010). Based on the results in *Paper III* the first clinical translation is suggested to be further support of patients to continue imatinib treatment without any interruptions also in the presence of tolerable side-effects.

4.4 SURGICAL MARGIN, GROWTH PATTERN AND OUTCOME IN GIST (*PAPER IV*)

GIST patients are presently treated by surgery and / or a TKI which has dramatically improved results during the last decade. While the surgical intervention is the only possible curative option, TKI virtually requires life-long treatment which is associated with development of drug resistance at some point of the treatment. Therefore further development of the surgical intervention to achieve curative or delay recurrence is desired. . In operable patients treated with surgery alone, the estimated 15-year recurrence-free survival rate is presently approximately 60% (Joensuu, Vehtari et al. 2012). An example of the surgical intervention is shown in Figure 7.

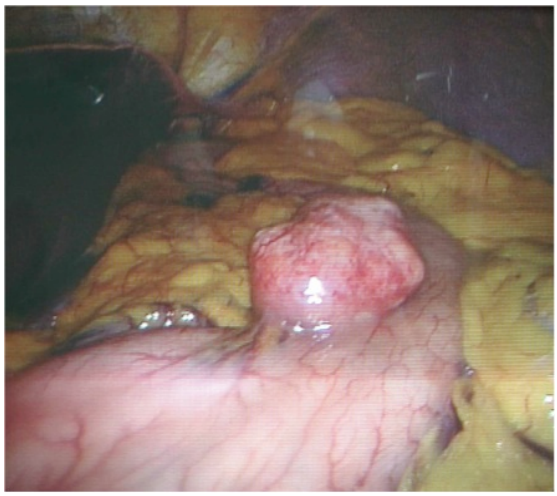

Figure 7. Photograph showing an GIST in the stomach during surgery.

A resection with microscopically negative margin (R0) is a prerequisite to achieve curative treatment in GIST (Catena, Di Battista et al. 2012). This notion is partly supported by reports showing that tumor rupture is associated with a worse prognosis (Rutkowski, Nowecki et al. 2007; Joensuu, Vehtari et al. 2012). In STS other than GIST, surgery is classified on the basis of resection margins into intralesional (equivalent to R1 resection), marginal (equivalent to R0 resection but with < 2 cm normal tissue in the organ of origin and/or intact fascia), or wide(also R0 but with >2 cm margin and/or intact fascia), modified from Enneking *et al.*(Enneking, Spanier et al. 2003). This has been shown to have prognostic significance in STS (Trovik, Bauer et al. 2000; Sampo, Tarkkanen et al. 2008), however the classification has not yet been

proven to be applicable to GIST. The aim of this study was therefore to analyze the prognostic significance of surgical resection margins in surgery for GISTs.
In addition we aimed to examine the peripheral growth pattern and its relation to patient outcome and surgical margins. Micro-GISTs (less than 1 cm) have recently been reported to show a mainly infiltrative growth pattern (Rossi, Gasparotto et al. 2010); to our knowledge this has not been investigated in large GIST.

The GIST cohort studied was identified from all patients admitted to our institution. In the entire cohort, established prognostic criteria were associated with time to first event (local/peritoneal or metastatic recurrence) as expected including: size ($P = 0.003$), mitotic index ($P = 0.00006$) and tumor site, calculated as gastric or non-gastric ($P = 0.003$).

Surgical margin

A total of 83 patients without metastasis at diagnosis who had not received any neoadjuvant or adjuvant treatment were included in the study of surgical margin, 37 males and 46 females (Table 5). Tumor size was based on the largest diameter and subgrouped as <2 cm (n = 6), 2-5 cm (n = 35), >5-10 cm (n = 20) or >10 cm (n = 19). Mitotic index was defined as the number of mitoses in 50 HPF and subgrouped as low (<2, n = 39), intermediate (2-10, n = 23) or high (>10, n = 12). Recurrence was defined as local/peritoneal if it was at the site of origin or in the peritoneum. Metastatic disease was defined as recurrence at a distant location, most commonly the liver. Risk stratification was classified as High (n =32), Intermediate (n = 17) or Low or very low (n = 34). The median follow-up time for patients with non-recurrent disease was 61 months. Thirty of the patients developed recurrent disease, distributed as local/peritoneal recurrence in 22 cases and metastasis in 16. Eleven patients died from disease during follow-up. The median time to any event was 41 months (range 2-274 months).

A wide surgical margin was observed in 40 cases, 24 cases had marginal margin and 19 case intralesional margin. Local/peritoneal recurrence was diagnosed in 2/40 cases with wide margins, in 7/24 cases with marginal margins, and in 13/19 cases with intralesional surgery. The risk was thereby significantly higher for patients with marginal [hazard ratio HR 6.3 (1.3-30.4)] and intralesional [HR 11.3 (2.5-51)] margins as compared to wide surgical margins ($P = 0.002$) (Figure 8). Surgical margin remained

a significant independent risk factor for local/peritoneal recurrence after adjustment for tumor size and site; (marginal HR 6.5 (1.3-31.9) and HR 6.2 (1.3-30.1); intralesional HR 9.8 (2-48.2) and HR 9.6 (2.1-44.7)). No differences in outcome were observed between patients operated with marginal compared with intralesional margins. Wide surgical margin was significantly associated with prolonged time to distant metastases ($P = 0.005$) and inversely correlated to death from disease ($P = 0.02$).

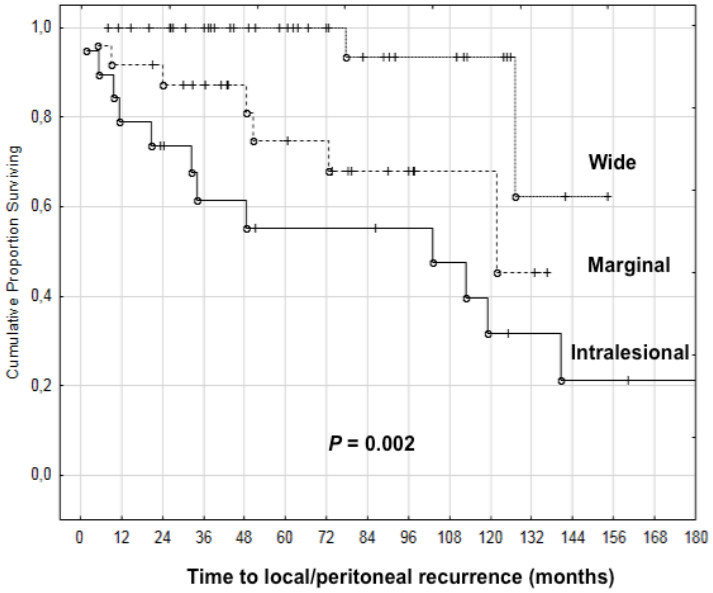

Figure 8. Kaplan-Meier plots showing longer time to recurrence in GIST patients operated with wide surgical margin.

In summary, a wide surgical margin was strongly associated with a reduced risk of local recurrence or metastasis and with longer tumor-specific survival, compared with narrow or intralesional surgical margins. The results show that precautions must be taken to obtain true free microscopic margins.

Peripheral tumor growth pattern

A total of 67 patients, 31 males and 36 females, were included in the analysis of growth pattern. Median age at diagnosis was 61 years (range 10-86 years) and median follow-up was 61 months (range 8-154 months). The correlation analysis between surgical margins and peripheral growth pattern included only the 56 patients who were without metastasis at diagnosis.

An infiltrative growth pattern was observed in more than half of the GISTs, 43 out of 67 (64%), while 24 cases (36%) had a pushing border (Figure 9); which is almost similar to findings in micro-GISTs (63%).

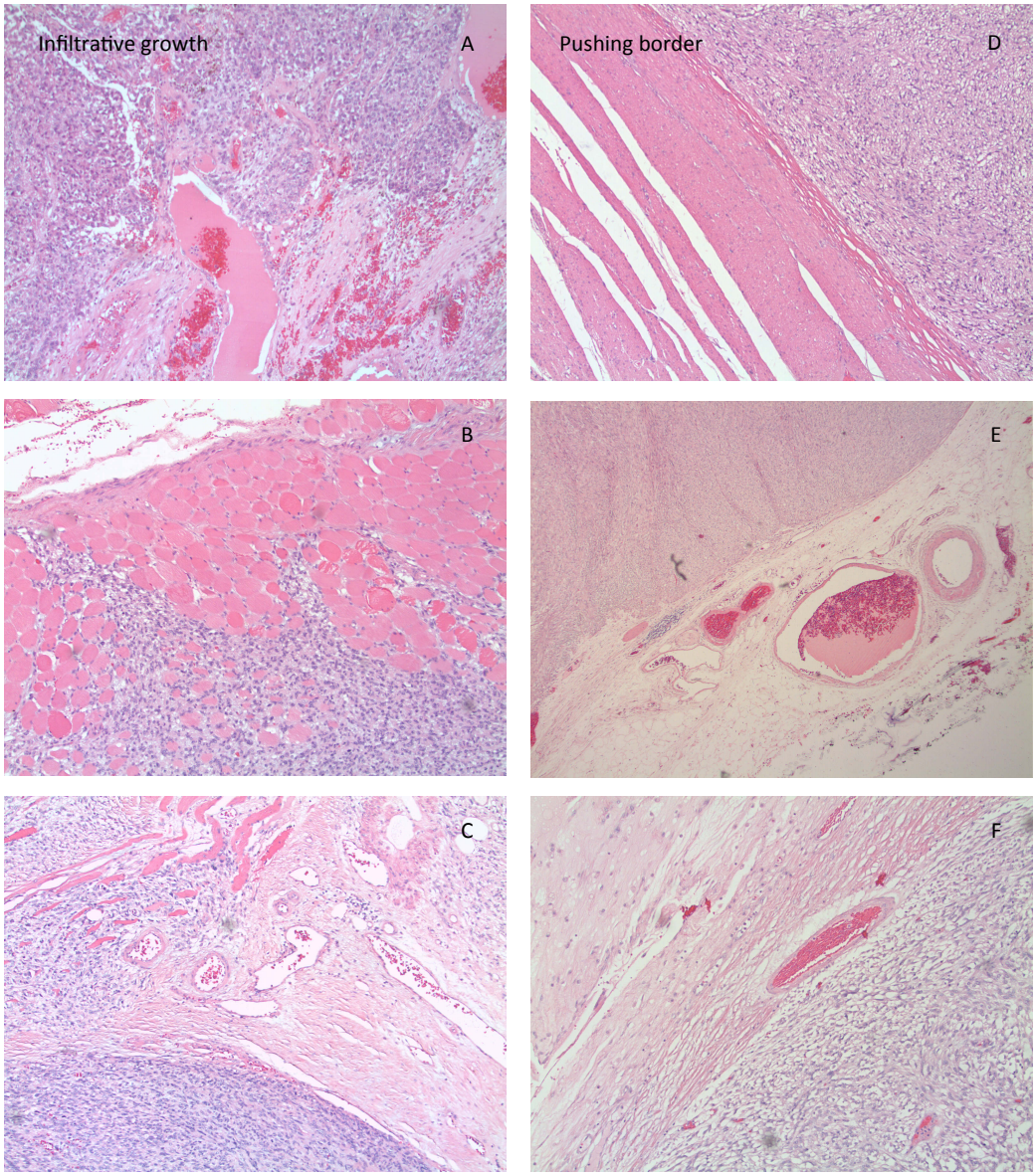

Figure 1. A-C Infiltrative growth, D-F Pushing border

Figure 9. Hematoxylin slides of GISTs with infiltrative growth (left) or pushing border (right).

An infiltrative growth pattern was significantly associated with a shorter time to first event, compared to pushing border ($P = 0.027$ (Figure 10). However, when analyzed separately, infiltrative growth was not associated with local/peritoneal recurrence, metastatic disease or survival. Contrary to our expectations, no correlation was observed between growth pattern, surgical margin and outcome. This was the case even when patients resected with marginal margins were analyzed separately; in view of the narrow surgical margin, one might expect that tumors with infiltrative growth would recur more often within this group.

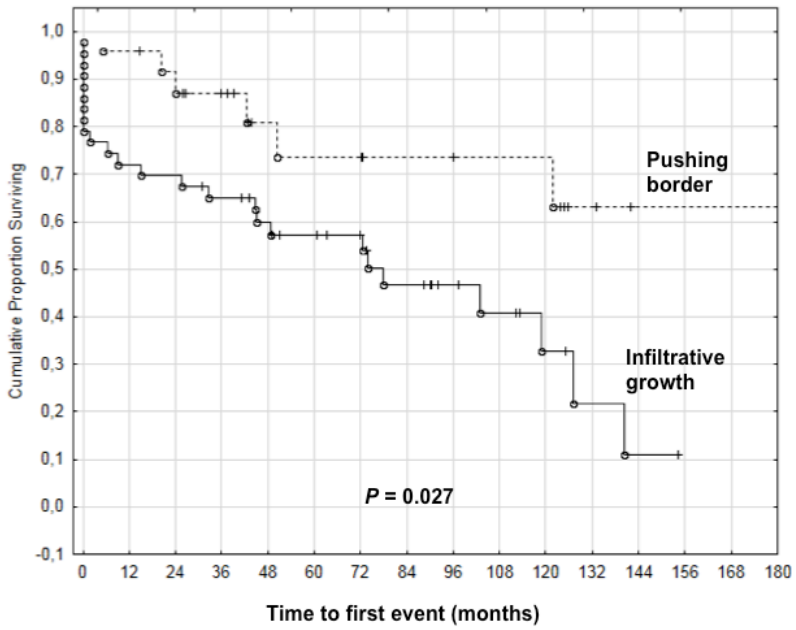

Figure 10. Kaplan-Meier plot showing shorter time to first event in GISTs with infiltrative growth.

Infiltrative growth tended to be more common (close to significant, $P = 0.0503$) among cases with metastasis at diagnosis: 10 out of 11 (91%) compared to 48 out of 79 (60%) without metastasis at diagnosis, Furthermore, a similar tendency was seen in a comparison between patients with infiltratively growing tumors with metastasis at diagnosis (10 of 11, 91%) and those who developed distant metastasis later during follow-up, 8 out of 14 (57%) ($P = 0.062$). This observation may indicate differences in mechanisms underlying early tumor progression but this needs further investigation.

Table 5. Details of the 83 GIST cases in Paper IV. Case 1-28

Case	Sex	Age	Growth	Location	Margin	Risk	Size group	Mitosis	L-rec	Met	DOD	Time (month) L-rec	Met	DOD	end
1	F	56	inf	S-int	Marg	High	>10	≥5<10	L-rec	-	DOD	9		13	13
2	F	10	inf	Sto	wide	Inter	≥5<10	≥5<10	L-rec	-	-	78			117
3	M	54		Sto	Marg	Inter	>2<5	≥5<10	-	Met	DOD		29	79	79
4	F	50	pu	Sto	Marg	Low	>2<5	-	-	-	-				133
5	F	67	inf	Sto	wide	Low	<2	<5	-	-	-				113
6	F	58	inf	S-int	wide	Inter	≥5<10	<5	-	-	-				112
7	F	54	inf	Sto	Marg	High	>2<5	<5	-	Met	DOD		74	137	137
8	F	75	inf	Sto	wide	Low	<2	≥5<10	-	-	-				90
9	M	56		S-int	wide	High	>10	-	-	-	-				83
10	F	53	pu	Eso	Marg	Inter	≥5<10	<5	-	-	-				96
11	M	67	inf	Sto	wide	Low	<2	<5	-	-	-				89
12	F	74	pu	Sto	wide	Inter	≥5<10	<5	-	-	-				15
13	M	74		Sto	Marg	High	≥5<10	>10	-	-	-				98
14	F	48	inf	Sto	Intra	High	>2<5	<5	L-rec	-	-	140			193
15	F	80	inf	Eso	Intra	High	≥5<10	>10	L-rec	Met	-	21	15		115
16	F	40	inf	S-int	wide	Inter	≥5<10	-	-	-	-				154
17	M	64	inf	S-int	Marg	Inter	≥5<10	<5	-	-	-				90
18	F	68	inf	S-int	wide	Low	>2<5	≥5<10	-	-	-			72	72
19	M	67		Sto	wide	Low	>2<5	≥5<10	-	-	-				91
20	F	19		S-int	Intra	High	>2<5	-	L-rec	-	-	10			208
21	M	59		Sto	Marg	Inter	>2<5	<5	-	-	-				61
22	M	36		S-int	Marg	High	>10	<5	-	Met	-		46		78
23	M	61	inf	Sto	wide	Low	>2<5	≥5<10	-	-	-				92
24	M	59	inf	Duo	Marg	Low	>2<5	<5	-	-	-				97
25	F	82	inf	S-int	Intra	Low	>10	<5	L-rec	-	DOD	49		72	72
26	F	78		Sto	wide	Inter	≥5<10	<5	-	-	-				67
27	F	78	inf	S-int	Marg	Low	<2	<5	-	-	-			74	74
28	F	80	pu	Sto	wide	High	>2<5	>10	-	-	-				73

pu = pushing border; inf = infiltrative growth;

Sto = Stomach; Eso = Esophagus; S-int = Small intestine; Col = Colon; Rec = Rectum

L-rec = Local recurrence; Met = Metastasis; DOD = Dead from disease

Table 5. Details of the 83 GIST cases in Paper IV. Case 29-56

												Time (month)			
Case	Sex	Age	Growth	Location	Margin	Risk	Size group	Mitosis	L-rec	Met	DOD	L-rec	Met	DOD	end
29	F	57	inf	S-int	Intra	High	>2<5	≥5<10	L-rec	-	-	103			172
30	M	85	pu	S-int	Marg	Low	<2	<5	L-rec	Met	DOD	51	51	51	33
31	M	54	inf	Sto	Intra	Low	≥5<10	<5	-	-	-				125
32	M	41	pu	Col	Intra	High	>10	>10	L-rec	Met	-	274			365
33	M	60		Sto	wide	Low	>2<5	<5	-	-	-				38
34	F	70		Sto	Intra	High		>10	L-rec	-	-	34			68
35	F	56	pu	Col	Marg	High	>10	<5	L-rec	Met	DOD	49	43	167	167
36	F	58	inf	Sto	wide	Inter	>2<5	≥5<10	-	-	-				61
37	M	73	inf	Sto	wide	Low	>2<5	<5	-	-	-				63
38	M	64	inf	Rec	Intra	Low	>2<5	<5	L-rec	-	-	32			120
39	M	77	pu	Sto	Marg	High	≥5<10	>10	L-rec	-	-	5		13	13
40	M	48	inf	Duo	Marg	Low	>2<5	<5	-	-	-				41
41	M	29	inf	S-int	wide	Inter	>2<5	≥5<10	-	Met	-		45		45
42	F	67	pu	Sto	wide	Inter	>2<5	<5	-	-	-				44
43	F	63	inf	S-int	wide	Inter	>2<5	≥5<10	-	-	-				51
44	F	49		S-int	Intra	Low	>2<5	≥5<10	-	-	-				24
45	M	77	inf	Sto	Marg	High	≥5<10	≥5<10	-	-	-				43
46	F	79	pu	Sto	wide	Low	>2<5	<5	-	-	-				26
47	F	83		S-int	wide	Low	>2<5	<5	-	-	-			10	10
48	F	66	inf	Duo	Intra	High	>10	<5	L-rec	Met	-	119	133		171
49	F	57	inf	S-int	wide	High	>10	>10	L-rec	-	-	127			130
50	M	77	inf	Rec	Marg	High	>10	≥5<10	-	Met	-		6	21	21
51	F	74	pu	Sto	wide	Low	>2<5	≥5<10	-	-	-				37
52	M	40	pu	Sto	Marg	Low	≥5<10	<5	-	-	-				43
53	M	73	pu	S-int	wide	High	>10	≥5<10	-	-	-				39
54	F	81	pu	Sto	Marg	High	>10	≥5<10	L-rec	Met	-	24	26		141
55	M	54		Sto	Intra	High	>10	-	L-rec	Met	DOD	11	27	31	31
56	F	86	pu	S-int	wide	Low	≥5<10	<5	-	-	-				38

pu = pushing border; inf = infiltrative growth;

Sto = Stomach; Eso = Esophagus; S-int = Small intestine; Col = Colon; Rec = Rectum

L-rec = Local recurrence; Met = Metastasis; DOD = Dead from disease

Table 5. Details of the 83 GIST cases in Paper IV. Case 57-83

												Time (month)			
Case	Sex	Age	Growth	Location	Margin	Risk	Size group	Mitosis	L-rec	Met	DOD	L-rec	Met	DOD	end
57	M	80		Sto	Marg	Inter	≥5<10	≥5<10	-	-	-				37
58	F	75		S-int	Intra	High	>10	-	-	Met	-		40		86
59	F	73		Sto	wide	Low	>2<5	≥5<10	-	-	-				58
60	F	65		Sto	wide	Low	>2<5	<5	-	-	-			62	62
61	M	61	inf	Sto	wide	Low	>2<5	<5	-	-	-				49
62	F	59		Sto	Marg	Low	>2<5	<5	-	-	-				33
63	F	75	inf	Sto	wide	Low	>2<5	≥5<10	-	-	-				31
64	M	46	inf	S-int	Marg	High	>2<5	>10	L-rec	Met	-	73	73		105
65	F	58	pu	Sto	Marg	Inter	≥5<10	≥5<10	L-rec	-	-	122			153
66	M	64	inf	S-int	Intra	High	>10	>10	L-rec	-	DOD	2		5	5
67	F	51		S-int	wide	High	>10	-	-	-	-				11
68	M	73		Col	Intra	High	>10	>10	L-rec	-	DOD	5		7	7
69	M	60		Sto	Marg	High	>10	<5	-	-	-				30
70	F	78	pu	Sto	wide	Low	>2<5	<5	-	-	-				27
71	F	64		Sto	wide	High	≥5<10	≥5<10	-	-	-				27
72	M	53		Sto	Intra	Low	>2<5	<5	-	-	-				23
73	F	43		Sto	wide	Low		-	-	-	-				15
74	M	58	pu	Sto	wide	Low	>2<5	-	-	-	-				36
75	F	65		Sto	wide	Low	≥5<10	<5	-	-	-			110	110
76	F	56	inf	Duo	Intra	High	>10	<5	-	Met	-		26		160
77	F	61	pu	S-int	Intra	High		>10	-	Met	DOD		20	51	51
78	M	64	pu	Sto	wide	Inter	≥5<10	<5	-	-	-				125
79	M	68	pu	Rec	wide	High	>10	<5	-	-	-				142
80	F	50		Duo	Intra	High	>2<5	>10	L-rec	-	DOD	113		194	194
81	M	58	pu	Sto	wide	Low	<2	<5	-	-	-				124
82	M	69	pu	Sto	wide	Low	≥5<10	<5	-	-	-				126
83	M	79		Sto	wide	Inter	>2<5	≥5<10	-	-	-			20	20

pu = pushing border; inf = infiltrative growth;

Sto = Stomach; Eso = Esophagus; S-int = Small intestine; Col = Colon; Rec = Rectum

L-rec = Local recurrence; Met = Metastasis; DOD = Dead from disease

5 CONCLUSIONS

In the studies included in this thesis we aimed to identify specific molecular alterations associated with progressive disease and clinical evaluation has been done of the incidence and prognostic significance of adverse drug reactions, surgical margins and tumor growth pattern.

Paper I

Using immunohistochemical techniques and pathophysiological evaluation, we assessed 101 patients with highly malignant STS for known and suggested prognostic markers, particularly insulin-like growth factor type 1 receptor (IGF-1R).

We concluded that in patients with highly malignant tumors (grade 3 or 4 on a four-tailed scale), many of the already known prognostic markers, such as tumor size, necrosis, mitotic count, intralesional surgery, deep location and micro vascular density, were useful prognostic tools. However, malignancy grade 3 or 4 could not predict prognosis and neither could infiltrative growth pattern, vascular invasion, or any of the remaining immunohistochemical markers Ki67, p53, p27 and Bcl-2.

Furthermore, we concluded that IGF-1R expression was a common feature in highly malignant STS, in our study associated with better survival and a low risk of developing metastases. These findings may suggest that IGF-1R and the IGF system are involved in malignant behavior of STS and may also provide a new prognostic tool in STS.

Paper II

Ezrin is a cytoskeleton protein involved in the growth and metastatic spread of cancer cells. We used immunohistochemical techniques to evaluate the prognostic role of the expression of ezrin in 50 patients with highly malignant STS from the same series as in *Paper I.*

We found that ezrin expression was significantly associated with poor survival and the risk of developing metastatic disease. Ezrin may therefore be useful as an additional prognostic marker in highly malignant STS.

Paper III

Imatinib, currently the first-line tyrosine kinase inhibitor (TKI) in advanced GIST, has led to a dramatically prolonged survival in advanced GIST. However, imatinib is associated with a lot of side effects. In a retrospective review of medical records from 75 patients who had received imatinib, we analyzed the association between the occurrence of side effects and the outcome in patients with GIST.

We found that moderate to severe or life-threatening side effects from imatinib were quite common and that the occurrence of adverse reactions might be associated with a better outcome. This indicates the importance of caring for patients with side effects so that they can manage them. Further investigation is needed into the correlation with plasma and intracellular concentrations.

Paper IV

In the last study, we analyzed the prognostic significance of surgical margins for GISTs. The extent of the resection margin was prospectively registered at the time of surgery or referral and the relation between resection margins and outcome was analyzed retrospectively. Margins were classified into wide, marginal or intralesional, as in other type of STSs.

We found that a wide surgical margin is of significant prognostic importance, independent of both size and site of the tumor, suggesting its value as a prognostic marker and, most importantly, showing that precautions must be taken to obtain true free microscopic margins.

Within the same cohort, we found the presence of a peripheral infiltrative growth pattern in more than half of the GISTs. Contrary to our expectations, we found no correlation between peripheral growth pattern and surgical margins. On the other hand, we did find that infiltrative growth was correlated to a higher risk of a first recurrent event, defined as local/peritoneal recurrence or metastasis, suggesting that the growth pattern has a role in the malignant behavior of GISTs.

6 ACKNOWLEDGEMENTS

I want to give many thanks to all of you who has helped, supported and pushed me over the years that I have spent with the studies presented within this thesis, and there have been many!! Actually, I think everyone at the surgical department as well as at the department of breast and endocrine surgery have been more or less engaged and supporting over the years, not to forget all that works at other departments as well as other people I have met in relation to this work. I can not name you all, and even if I wanted to and tried I would forget too many. There is so many that I actually would like to thank. What I want to say again is that I am really, really thankful to all of you, and no one is forgotten even though not mentioned here!!

A special thanks to my supervisors, Catharina Larsson, Inga-Lena Nilsson, Olle Larsson, Otte Brosjö, Anette von Rosen and my mentor Henrik Bauer.
Moreover, I can not thank enough to, especially Catharina Larsson who has really, really helped and supported me with her vast knowledge and her insistent, not to mention the great work she has done over the years, but also to Inga-Lena Nilsson for her insistent efforts, dedication and belief in me to complete this thesis.
I will also give a special thanks to Anette von Rosen who has greatly supported me.

I will also thank my coauthors, not mentioned above, Wendy Weng, Weng-Onn Lui, Jan Zedenius, Johan Westerdahl, Johan Wejde, Leonard Girnita, Kristina Åström, and Robert Bränström for the great help they have given in the respective studies.
I want to especially thank Jan Zedenius, my chief, colleague and friend for so many years that has supported and engaged in me, my studies and my ideas about sarcoma, which actually is the reason that we are where we are today.
In this context I also must mention Robert Bränström and thank him for being a friend, and colleague, always enthusiastic, who have engaged in me, my work and the sarcoma patients as well as being eagerly engage in sarcoma research.
I do have to thank Sofia Grindberg for her support and engagement, both in me to finish this but also in the sarcoma patients.
I also really want to thank Johan Westerdahl for his support in me, the sarcoma patients and for being a friend as well as a part in our sarcoma team.
I want also to mention Fredrik Karlsson for his engagement and support.

Others that must be mentioned is my former chief and colleague Martin Bäckdahl for his support in me and the sarcoma patients and a very special thanks to Harald Blegen for his engagement and support in me as well as for being a friend.

I also want to thank my mother, Inga-Britt for being simply the best mother and she has always been so enthusiastic, and I also want to thank my siblings Gunilla and Torbjörn.

However, absolutely most thanks is given to my wife Yvonne and our children Niklas, Hanna and Viktor for everything, I love you.

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