From Department of Molecular Medicine and Surgery
Karolinska Institutet, Stockholm, Sweden

EPIDEMIOLOGICAL AND EXPERIMENTAL STUDIES OF SARCOMA WITH FOCUS ON GASTROINTESTINAL STROMAL TUMORS

Fredrik Karlsson

Karolinska Institutet

Stockholm 2020

Published by Karolinska Institutet
Printed by US-AB, Stockholm

ISBN 978-91-7831-982-4

Epidemiological and experimental studies of sarcoma with focus on gastrointestinal stromal tumors

THESIS FOR DOCTORAL DEGREE (Ph.D.)

By

Fredrik Karlsson

Principal Supervisor:
Associate professor Robert Bränström
Karolinska Institutet
Department of Molecular Medicine and Surgery
Section of Endocrine and sarcoma surgery

Co-supervisors:
Associate professor Inga-Lena Nilsson
Karolinska Institutet
Department of Molecular Medicine and Surgery
Section of Endocrine and sarcoma surgery

Professor Jan Zedenius
Karolinska Institutet
Department of Molecular Medicine and Surgery
Section of Endocrine and sarcoma surgery

Opponent:
Associate professor Andreas Muth
University of Gothenburg
Sahlgrenska Academy
Department of Surgery
Institute of Clinical Sciences

Examination Board:
Associate professor Jonas Fuxe
Karolinska Institutet
Department of Medical Biochemistry and Biophysics

Associate professor Hanna Dahlstrand
Uppsala University
Department of Immunology, Genetics and Pathology

Professor emerita Eva Haglind
University of Gothenburg
Sahlgrenska Academy
Department of Surgery
Institute of Clinical Sciences

Time is the Enemy
Quantic

ABSTRACT

Sarcoma is the common denominator for malignant tumors of mesenchymal origin. Sarcomas encompass almost 100 diagnoses with different histology, molecular features and natural history, and may present in any part of the body. The most common sarcoma in the abdomen is the gastrointestinal stromal tumor (GIST). GIST is most frequent in the stomach followed by the small intestine, yet can occur throughout the gastrointestinal tract. The first line of treatment for GIST is complete surgical resection, if feasible. Since the discovery of targeted small-molecule therapy with imatinib in 1998 and the successful treatment of the first GIST patient two years later, this therapy has attracted much attention and GIST has become a model system for modern oncological treatment. This thesis is based on translational research in the field of sarcoma with the main focus on GIST. In **paper I,** a *proof-of-concept* study of intracellular imatinib measurements is presented. Cell-cultures of imatinib-sensitive and resistant cells were exposed to imatinib in different concentrations. The analysis was performed with liquid chromatography mass spectrometry and *time-of-flight* detection (LC/MS-TOF). The imatinib-resistant cell-line had significantly lower imatinib concentrations. Clinical samples from three patients were analyzed using the same protocol and showed imatinib accumulation in tissue and a large variability between patients. In **paper II,** the importance of surgical technique and surgical margins was studied. Resecting GIST with a wide margin of >2 cm normal tissue and intact covering peritoneum, lead to improved recurrence-free survival. The impact of the margin was independent when adjusting for other known risk factors such as size, site and mitotic index. **Paper III** analyzed an expanded cohort of imatinib treated and resected GIST using an improved, updated protocol for mass spectrometry and drug transporter expression analysis. The previous finding of large intra- and interpatient variability of imatinib concentrations was confirmed. Plasma and tissue concentrations were not correlated to the response. Low expression of drug transport proteins was correlated to the improved histological response. Finally, **paper IV** is a nested case-control study and describes trends in breast sarcoma incidence in Sweden during the period of 1993-2003, showing a 4-fold increase of angiosarcoma. The angiosarcoma patients were overrepresented as carrying a history of breast cancer with the highest risk 5-10 years after their breast cancer diagnosis (OR 167, CI 95% 35.1-791; $p<0.001$). This points to the possibility that the increased use of radiotherapy could be a reason for a rise in incidence of angiosarcoma.

LIST OF SCIENTIFIC PAPERS

This thesis is based on the following papers, hereby denoted with Roman numbers:

I. Berglund E, Ubhayasekera SJ, **Karlsson F**, Akcakaya P, Aluthgedara W, Åhlen J, Fröbom R, Nilsson IL, Lui WO, Larsson C, Zedenius J, Bergquist J, Bränström R. Intracellular concentration of the tyrosine kinase inhibitor imatinib in gastrointestinal stromal tumor cells. *Anticancer Drugs.* 2014; 25(4):415-422.

II. Åhlen, J, **Karlsson F**, Wejde J, Nilsson IL, Larsson C, Bränstrom R. Wide Surgical Margin Improves the Outcome for Patients with Gastrointestinal Stromal Tumors (GISTs). *World J Surg* 2018;42(8): 2512-2521.

III. **Karlsson F**, Ubhayasekera SJ, Haglund F, Fröbom R, Åhlén J, Nilsson IL, Zedenius J, Bergquist J, Bränström R. Intracellular concentration of imatinib and drug transporter expression in gastrointestinal stromal cell tumors (GIST) *Submitted*

IV. **Karlsson F**, Granath F, Smedby KE, Zedenius J, Bränström R, Nilsson IL.Sarcoma of the breast: breast cancer history as etiologic and prognostic factor-A population-based case-control study. *Breast Cancer Res. Treat.* 2020;183(3):669-675.

OTHER PUBLICATIONS

Berglund E, Akcakaya P, Berglund D, **Karlsson F**, Vukojević V, Lee L, Bogdanović D, Lui WO, Larsson C, Zedenius J, Fröbom R, Bränström R. Functional role of the Ca^{2+}-activated Cl^- channel DOG1/TMEM16A in gastrointestinal stromal tumor cells. *Exp. Cell Res.* 2014;326(2):315-325.

Juhlin CC, **Karlsson F,** Bränström R. A rare case of giant amyloid goiter: A case report and review of literature. *Med. Case Rep.* 2019;5(2):100.

CONTENTS

LIST OF ABBREVIATIONS

ACOSOG	American College of Surgeons Oncology Group
AJCC	American Joint Committee on Cancer
ARR	Absolute risk reduction
AS	Angiosarcoma
BCS	Breast conserving surgery
BS	Bone sarcoma
CT	Computed tomography
DCIS	Ductal breast cancer in situ
DOG1	Discovered on GIST 1 (synonyms TMEM16A, ANO1)
DSS	Disease specific survival
ES	Extremity sarcoma
FDA	The US Food and Drug Administration
FDG	Fluorodeoxyglucose
FNCLCC	Fédération Nationale des Centres de Lutte Contre le Cancer
GI	Gastrointestinal
GIST	Gastrointestinal stromal tumor
HPF	High power field
KIT	Stem cell growth factor receptor, proto-oncogene *c-kit*
MDT	Multidisciplinary team
MFH	Malignant fibrous histiocytoma
MSKCC	Memorial Sloan Kettering Cancer Center
NCI	National Cancer Institute
OS	Overall survival
PDGFRA	Platelet-derived growth factor alpha
PET/CT	Positron emission tomography/computed tomography
RCT	Randomized controlled trial
RET	Proto-oncogene RET, rearranged during transfection
RFS	Recurrence free survival

RPS	Retroperitoneal sarcoma
RT	Radiotherapy
RTK	Receptor tyrosine kinase
SEER	Surveillance epidemiology and end results
SSG	Scandinavian Sarcoma Group
STS	Soft tissue sarcoma
TKI	Tyrosine kinase inhibitor
TNM	Tumor, Node, Metastases
VEGFR	Vascular endothelial growth factor receptor
VS	Visceral sarcoma
WHO	World Health Organization

1 INTRODUCTION

Sarcoma is the general description of malignant tumors arising from mesenchymal cells and can develop in any part of the body. The mesenchymal origin separates sarcomas from carcinomas, which originate from epithelial cells. Sarcoma is a heterogeneous group of diseases with low incidence in the population. It contains several different diagnoses, traditionally categorized by their tissue of origin, such as lipocytes being the precursor of liposarcoma, smooth- or skeletal muscle cells transforming into leiomyosarcoma and rhabdomyosarcoma, respectively.

The first known reference of sarcoma dates back to 1500 BC when a text on a papyrus roll describes a "fatty tumor" and recommends that it should be treated with the knife [1]. The term sarcoma was coined by the Greek physician, Galen, who was active in Rome in the late 2nd century where he, among other things, acted as a surgeon for gladiators. The term is derived from the Greek word *sarkos* (σαρκός) meaning meat, based on the appearance of soft tissue tumors [1, 2]. The meaning of sarcoma as well as other terms describing soft tissue tumors was, however, ill-defined and varied among physicians through the centuries. In the mid 19th century the use of microscopical examination of tissue, including tumors, grew in popularity. In 1858-1859 three authors, Virchow, Gross and Wilks, almost simultaneously published features that distinguish sarcoma from carcinoma [1]. The first classification system for sarcomas was published in 1902 by the German pathologist Borst [3]. During the 20th century, following numerous case reports and studies, new and expanded classifications of sarcomas were proposed by Ewing in 1919 [4] and Stout in 1953 [5]. The World Health Organization (WHO) publishes and regularly updates a classification system for soft tissue and bone tumors, i.e. sarcomas. The first edition was published in 1969 [6] and was originally based on histology; however, with emerging knowledge, the 4th edition, which was released in 2013, specifically emphasizes the increased use of molecular genetics in the sarcoma diagnosis [7, 8]. The latest version, the 5th edition, released in May 2020, further expands classifications of sarcomas and based on genetic rearrangement and mutation, introduces new subgroups and diagnoses into the sarcoma group [9]. Currently, the group of diseases known as sarcomas, comprises around 100 histologically and/or molecularly different tumors and with the increased use of

molecular pathology and understanding of different entities, new subtypes are continuously added while others are reclassified [10].

The nomenclature in sarcoma literature may be confusing and there are still several different classifications and grading systems in use. Sarcomas are most often categorized by location into extremity sarcoma (ES) or sarcoma of the trunk, including visceral sarcoma (VS) and retroperitoneal sarcoma (RPS). Based on the site (tissue) of origin, the tumors can also be classified as bone sarcoma (BS) or soft tissue sarcoma (STS). Historically, soft tissue sarcomas were often clustered, without regard for histology, in order to facilitate inclusion in clinical trials, therein producing doubtful results [11, 12].

In lack of a commonly accepted nomenclature and registration praxis, available reports differ in subdivision of tumor sites and types. This makes pooling of data from different studies of this heterogeneous group of tumors and comparisons of populations rather challenging [13].

1.1 EPIDEMIOLOGY OF SARCOMA

Since sarcomas are a heterogeneous group of rare tumors and definitions are still evolving, conclusive and comparative epidemiological data is scarce. Different health care databases have been studied with varying results.

Perhaps the most comprehensive epidemiological data comes from the European RARECARE project that accumulates data on cancers with incidence of less than 6/100 000/year from European cancer registries. Sarcoma cases are classified based on tumor site and to some extent, morphology. In a recent overview with data from 94 cancer registries within RARECARE, trends in incidence rate and survival were analyzed for 73 795 patients with soft tissue sarcoma in the period 2000-2007. The crude incidence for soft tissue sarcoma was 4.7/100 000 person-years (male 4.4, female 5.0). The annual incidence for STS of viscera was 0.4 per 100 000. GIST was reported separately with an incidence of 0.3/100 000/year. The incidence for RPS was 0.3/100 000/year. Standardized incidence rates were calculated compared to the European population [14]. In an earlier analysis of the RARECARE cohort covering 45 568 incident sarcoma cases from 76 population-based cancer registries between 1995 and 2002, higher incidence in northern Europe and lower incidence in eastern Europe were observed. This study also reported

survival analysis data for STS based on tumor site. Overall, five-year survival was estimated to 57.8%, with the worst prognosis for sarcoma of the heart (10.7%) and mediastinum (15.3%). For VS five-year survival was 45.6% and for GIST, which was analyzed separately, it was 67.7%. Sarcoma of the pelvis had an estimated five-year survival of 42.2% and RPS 42.0%. Trend analysis indicated improved survival over time, especially for VS and sarcoma of the trunk [15].

Trautman *et al.* studied health insurance data for the population in Saxony, Germany between 2005 and 2012 comprising 2 615 865 individuals and reported an annual age standardized incidence of STS to be 4.5/100 000 [16]. A French-Italian study of sarcoma incidence over a two-year period in three regions with a total of 26 million inhabitants, distinguishes between STS in extremities, trunk wall and retroperitoneum as well as malignant mesenchymal tumors in internal organs, VS. The overall annual incidence in this study was reported to be 5.76/100 000 consisting of 3.58 STS/100 000 and 2.18 VS/100 000, while the age standardized incidence adjusting for the 19 age groups in the 2000 US standard population (ASR-US) was 5.12/100 000 [17, 18].

Another study that highlights the difficulties in identifying sarcoma cases was conducted in France where central histopathological review was used to prospectively classify cases of sarcoma. The study was performed in a population-based manner with a population of 6 million during the period March 2005 to February 2007. The overall yearly incidence was 3.6/100 000 for STS and 2.0 for VS. The world age standardized incidence was 2.8/100 000 for STS and 1.4 for VS. In this study, the most common histological type was GIST, which comprised 18% of cases with a yearly incidence of 1.1/100 000 followed by unclassified sarcoma (16%; 1/100 000), liposarcoma (15%; 0.9/100 000) and leiomyosarcoma (11%; 0.7/100 000) [19].

In a population-based study from Denmark during 1979-2008, the age standardized incidence for STS was found to be 1.4/100 000 [20]. In addition, a study from the Osaka Cancer registry, covering 8.7 million people, identified 6 998 incident cases of STS during the period 1978-2007. The age-standardized incidence rate of STS (ASR-US) was 2.7/100 000 (male 2.8, female 2.6). The trend during the second 10-year period showed increased incidence in both genders, however non-

significant amongst males. The most common subtype was leiomyosarcoma in digestive organs and GIST followed by leiomyosarcoma in other locations and liposarcoma. As an indication of the diagnostic difficulties while studying sarcoma, the authors found that after registration of the first GIST-case in 1988 there was an increase of this diagnosis and a simultaneous decrease in diagnosis of gastrointestinal leiomyosarcoma [21].

In a study from two regions in Spain between 1981-2005, the annual incidence of sarcoma in the gastrointestinal (GI)-tract was found to be 0.42/100 000 persons in the early study period (1981-1985) and 1.18 in the late period (2001-2005). In the second studied region no data was presented for the early period; however, in the later period, authors found 1.36 cases/100 000 persons/year. Cases were analyzed with immunohistochemistry for KIT to confirm cases of GIST and the result was an incidence of 1.24/100 000/year [22]. Analysis of the SEER registry covering about 26% of the US population during the period 1973-2006 reported an incidence for STS of 5.9/100 000 persons/year. The incidence was lowest in the pediatric population younger than 10 years, 0.9/100 000/year and highest for persons older than 70 years, 18.2/100 000/year. Notably, this study included Kaposi´s sarcoma [23]. In another study from the US, Porter *et al*. reported data from the SEER registry during the period 1973-2001 and analyzed the incidence of retroperitoneal sarcoma (RPS). A total of 2 348 cases were identified and the annual incidence was calculated to 2.7 per 1 000 000. There was no significant difference in incidence over time according to this study [24]. Interestingly, a Finnish study examined previous cancer and treatment with chemotherapy, radiation or a combination of both and the risk for development of sarcoma. Patients with primary cancer of the breast, uterus, lung, ovary, prostate, rectum or lymphoma diagnosed in 1953-2000, were included and followed longitudinally. Outcomes were compared to the expected number of cases based upon current incidence figures and the standardized incidence ratios (SIR) were calculated. After ten years of follow-up, SIR for sarcoma was increased in all study groups. Among patients who had received neither radiotherapy nor chemotherapy, SIR was 2.0 (95% CI 1.3–3.0), for radiotherapy alone SIR was 3.2, (95% CI 2.3–4.3), for chemotherapy alone SIR was 4.9, (95% CI 1.0–14.4) and for combined radio/chemotherapy SIR was 3.4, (95% CI 0.4–12.5)

[25]. The reason for elevated risk among patients receiving neither chemo- nor radiotherapy might be underlying susceptivity to developing malignant tumors, such as p53 mutations [26, 27], but it is more likely that the incidence figures used to calculate SIR underestimate the true incidence in the reference population. Another possible explanation could be surveillance bias during follow-up of patients with previous malignancies. A weighted case control study might have provided a truer estimation of the risk.

Finally, a French study of the period 2000-2013 using centralized histopathological review, based on the 4th edition of the WHO classification, reports a crude annual incidence of 7.4/100 000. In this material, sarcomas constitute 1.3% of all diagnosed malignancies. Recalculated into age-standardized incidence rates compared to the world population, the overall sarcoma incidence was 5.0/100 000/year including 2.68 STS, 0.81 gastrointestinal and 0.82 in the female genital tract. The authors encourage future investigators to use similar inclusion criteria in order to facilitate comparison between studies [13].

In summary, the reported annual incidence for STS ranges from 1.15 to 5.9/100 000, where the higher incidences include Kaposi´s sarcoma. The incidence for VS or intraabdominal sarcoma was reported to be between 1.18 and 2.18/100 000/year. The incidence of RPS was reported to be 0.27-0.3/100 000/year.

There was high heterogeneity between these studies that explained some of the differences but there was also, in many studies, a trend of increasing incidence over time. This may reflect the fact that sarcomas were being under-diagnosed or under-reported. Another explanation could be that general health is improving and malignant tumors develop more often in an ageing population. Furthermore, advances in genomics and molecular testing have brought about changes in classification of sarcomas. Some subgroups seem to increase in number, e.g. GIST and solitary fibrous tumors, while others, like fibrosarcoma, seem to decrease. The evolution of subgroup classification complicates analyses of changes over time.

1.2 DIAGNOSIS

Due to the rarity and heterogeneity of these tumors, referral to expert centers is recommended if there is suspicion of sarcoma [28, 29]. For the same reasons, no clear-cut recommendations on diagnostic work-up can be given for the entire group. Usually, suspicion of sarcoma is raised based on radiological findings or a palpable mass. Complete radiological examination with different modalities is important in order to assess tumor growth in vicinity to vital organs and the presence of metastatic disease. The most important investigation for optimizing treatment is to obtain a biopsy from the tumor. Different techniques can be used including fine needle aspiration, core needle or in selected cases, open biopsy. Care should be taken to pass the needle through a channel that can be excised in a later operation or in cases of abdominal/retroperitoneal sarcoma, not to pass the peritoneal cavity [28]. The risk of tumor seeding in the needle channel is expected to be low, although several case reports describe this phenomenon. A meta-analysis pooling data from four studies including 547 patients, records 2 cases of presumed needle tract seeding (0.37%) [30].

Recently, a retrospective study of more than 12 500 patients diagnosed with sarcoma between 2010-2014 showed improved recurrence-free survival (RFS), more R0 resections and less reoperations for patients presented at multi-disciplinary tumor board at an expert sarcoma center prior to surgery as to compared to those patients who were not. Notably, 87.7% of patients managed at a sarcoma center had a pre-operative biopsy, compared to 41.9% of patients treated in other settings [31]. This finding is in accordance with previous reports emphasizing the multidisciplinary and centralized management of these rare tumors [28, 29, 32, 33].

1.3 GRADING AND STAGING

Different systems have been established in order to predict prognosis. Tumor “grade” characterizes the tumor as such, whereas “stage” describes the disease burden in terms of severity of the primary tumor, yet also describes the presence of metastases. There are several systems for assessment of tumor grade and prognosis. One of the most frequently used in sarcoma is the three-tier system proposed by the French Fédération Nationale des Centres de Lutte Contre le Cancer (FNCLCC). This system is based upon tumor differentiation, mitotic activity and necrosis [34] (Table

1). All factors are assessed and the resulting scores are added: Score 2-3 equals Grade 1, score 4-5 Grade 2 and score 6-8 Grade 3. It is the most validated in use, however, other grading systems are also used [35]. The National Cancer Institute (NCI) Grading system outlines several histological subtypes of sarcoma and assigns them to different grades. For example, according to this system, a well-differentiated liposarcoma is always grade 1 and a synovial sarcoma is always grade 3, whereas a leiomyosarcoma can be grade 1-3. The latter is distinguished by using a combination of number of mitoses, pleomorphism, cellularity, matrix and necrosis. The weight assigned to each factor also differs for the histologic subtypes; for instance in malignant fibrous histiocytoma (MFH), pleomorphism is used to separate grade 2 from grade 3 although, in leiomyosarcoma, mitotic count is of greater importance for separating grades [36]. For subtypes of sarcomas, especially GIST, separate grading systems have been developed, see section 2.6.

Score	*Tumor differentiation*	*Mitoses per 10 hpf*	*Necrosis*
0			No
1	Resembling normal adult mesenchymal tissue (well-differentiated liposarcoma or leiomyosarcoma)	0-9	<50%
2	Certain histological typing (biphasic synoviosarcoma, alveolar soft-part sarcoma, myxoid liposarcoma)	10-19	>50%
3	Undifferentiated sarcomas, embryonal sarcomas and sarcomas of doubtful type	>20	

Table 1. FNCLCC grading for sarcoma, adapted from Trojani *et al.* 1984 [34].

The American Joint Committee on Cancer (AJCC) has recently updated its TNM based staging system for sarcoma [37]. T staging of sarcoma is based on tumor size, grade according to FNCLCC and location. The updated version has been validated and compared to earlier versions. Although it has a wider span of tumor sizes for T

stage, the correlation with overall survival is comparable to the earlier version [38, 39]. For sarcomas in extremities, trunk and retroperitoneum T stages are:

pT1: Tumor ≤ 5 cm in greatest dimension
pT2: Tumor >5 cm and ≤10 cm in greatest dimension
pT3: Tumor >10 cm and ≤15 cm in greatest dimension
pT4: Tumor >15 cm in greatest dimension

There is also a separate TNM staging for abdominal and thoracic visceral organs, where the T stage is defined according to involved organs, rather than tumor size:

pT1: Organ confined tumor
pT2: Tumor extension into tissue beyond organ
pT2a: Invades serosa or visceral peritoneum
pT2b: Extension beyond serosa
pT3: Invades another organ
pT4: Multifocal involvement
pT4a: Multifocal (2 sites)
pT4b: Multifocal (3 - 5 sites)
pT4c: Multifocal (> 5 sites)

Lymph node metastases are generally rare in sarcoma, although some subtypes may be more prone to involve lymph nodes [40].

To further assess prognosis in sarcoma and aid clinicians in decision-making, different nomograms may be used and are often more specific to the underlying histology. The nomograms try to predict risk of recurrence and proportion of overall survival based on known risk factor such as the patients age, tumor size, grade and histology, accounting for the fact that some risk factors are continuous variables. Some of these nomograms for risk assessment of retroperitoneal sarcoma (primary or recurrent) and extremity soft tissue sarcoma have been included in an app (Sarculator) [41].

2 GIST

The most common intraabdominal sarcoma, or sarcoma of the GI-tract, is gastrointestinal stromal tumor (hereafter referred to as GIST) [17, 19, 42]. GIST originates from Cajal´s interstitial cells or its progenitor and can arise in any part of the GI tract. The most common site is the stomach (55-65%) followed by the small intestine (25-32%). More unusual locations are esophagus (about 2-3.5%), duodenum (about 3%) and colorectal (about 3-6 %) [43-46]. In Sweden, the annual incidence of GIST has been reported to be 1.45 per 100 000 inhabitants and the prevalence 12.9 / 100 000 [47]. About two-thirds of GIST patients present with symptoms, most commonly GI bleeding (overt or microscopical causing anemia) or a palpable mass; however, symptoms may also include vague abdominal discomfort, dysphagia, early satiety and in rare cases, bowel obstruction and intestinal perforation. The remaining cases are mainly discovered as incidental findings on radiology, endoscopy or during surgery for other conditions [48]. The clinical course in GIST ranges from small indolent tumors to aggressive metastasized sarcomas and the treatment protocols must be personalized. More than 70% of patients can be cured with surgical resection alone. In cases with a high risk of recurrence, adjuvant therapy should be recommended after surgery [49]. The prognosis of advanced GIST has changed dramatically after introducing molecularly targeted treatment with imatinib and other Tyrosine kinase inhibitors (TKIs). In the pre-imatinib era, GIST was a disease with high mortality (Figure 1).

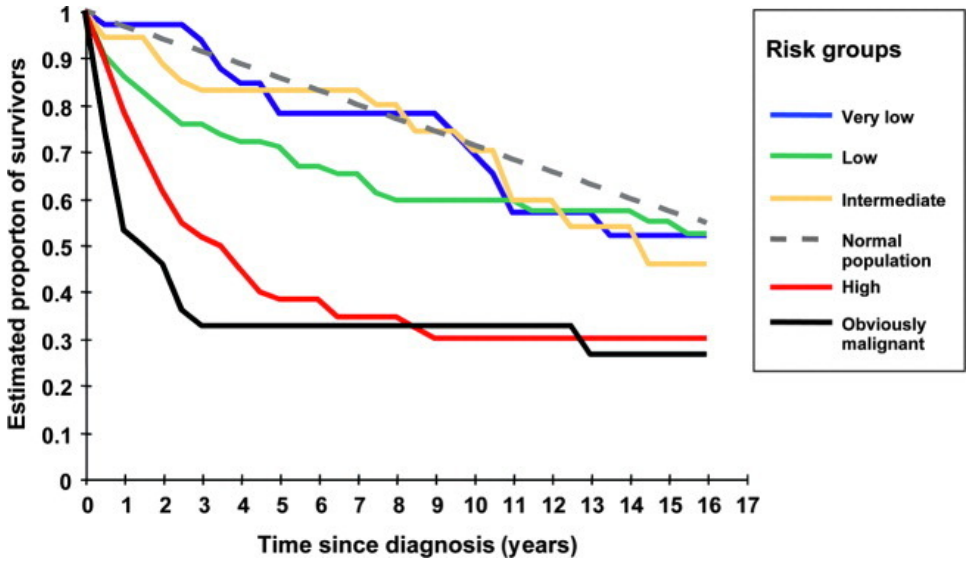

Figure 1. Estimated overall survival in 259 patients with GIST 1983-2000 compared to age and gender matched controls. Risk groups according to NIH criteria [47]. Reprinted with permission.

2.1 MOLECULAR PATHOLOGY

GIST was first described as a separate pathologic entity in the 1980s and the tumors were previously often classified as schwannomas, leiomyomas or leiomyosarcomas [50-52]. GIST morphology can be spindle cell or epithelioid and may be difficult to distinguish from other potential diagnoses. Using immunohistochemistry, several markers are available for diagnostic purposes. The most often used markers are KIT (CD117) and DOG-1, which are mutually over-expressed in almost all GISTs [53]. However, 2.6% of GISTs are reported to be negative for both these markers [54]. Other markers can be useful in difficult cases and rare GIST with doubtful staining of the commonly used antibodies (Table 3).

Protein marker	Proportion positive in GIST
KIT (CD117)	95%
DOG1	≈98%
PKC theta	85%
CD34	60-70%
Smooth-muscle actin	30-40%
S100	5%

Table 3. Immunohistochemical profile of GIST [55-57].

KIT is a receptor tyrosine kinase present on the surface of many different cells. In normally functioning cells, the receptor is activated by its ligand, stem cell factor. Binding of stem cell factor to the receptor causes dimerization and ATP mediated activation of downstream signaling pathways, thus promoting cell proliferation [58]. The *c-kit* gene was cloned in 1998 by Hirota, who proposed Cajal´s interstitial cells as the cell of origin for GIST [59]. The most common underlying mutations in GIST involve the *c-kit* gene and account for about 75% of cases. Notably, KIT expression, detected with the CD117-antibody, is present in a larger proportion of GIST and is not diagnostic for mutations in the *c-kit* gene. About 10% of GISTs have a mutation in the *PDGFRA* (platelet-derived growth factor alpha) gene, encoding a transmembrane tyrosine kinase similar to KIT (Figure 2). The remaining 15% of GIST (often called wild type) can harbor mutations in genes encoding succinyl dehydrogenase (SDHA/B/C/D) (about 6%), *BRAF 600E* (about 2%), or extremely

rare mutations such as *HRAS, NRAS*, phosphoinositide 3-kinase (*PIK3CA*), or neurofibromin-1 (*NF-1*) [45].

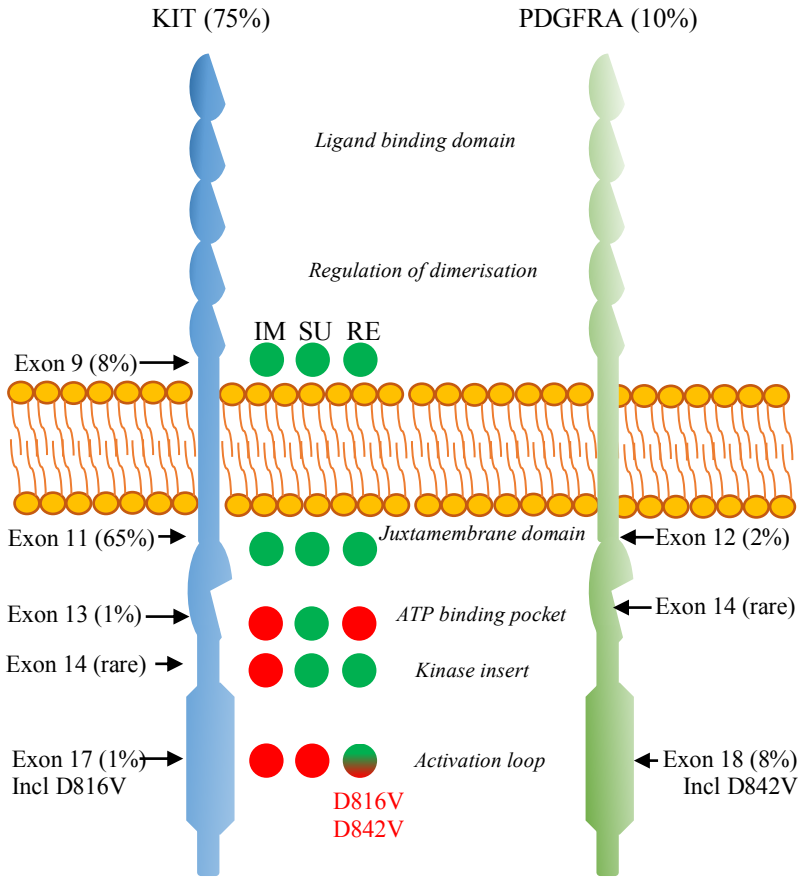

Figure 2. Schematic distribution of KIT and PDGFRA mutations in GIST and potential sensitivity to first-, second- and third-line TKI (IM - imatinib, SU - sunitinib and RE - regorafinib). Note that KIT D816V and PDGFRA D842V mutations are resistant to all approved lines of TKI. Adapted from Corless *et al.* 2014 [45], Serrano *et al.* 2019 [60], and Mazzocca *et al*. 2019 [61].

Mutations in *c-kit* predict the clinical course as well as the response to pharmacological therapy. The most common mutation is found in exon 11 and causes conformation changes of the protein, which allows for activation in the absence of the ligand. These mutations are typically sensitive to treatment with imatinib. The second most common mutation is in exon 9. This mutation is almost exclusively seen in intestinal GIST and very seldom in gastric GIST. Exon 9 mutated GIST has a lower sensitivity to imatinib and a double dose of 800 mg daily is recommended to treat these tumors [45]. Mutational analysis is also essential in order to identify genotypes resistant to TKI, like in *PDGFRA* on codon 842 (D842V) [62] (Figure 2).

DOG1 (Discovered On GIST-1), also known as TMEM16A or ANO1 is a Ca^{2+}-activated Cl^- channel (CaCC), that is overexpressed in more than 95% of all GISTs and is a reliable marker for immunohistochemical diagnosis in combination with KIT [54, 63]. This Cl^- channel is present in many different cell types throughout the body. Its function is not fully understood but in mouse models, knockdown experiments have shown that it is essential since its absence caused severe tracheomalacia and death within the first month [64]. The ion channel is activated when the intracellular concentration of free Ca^{2+} increases due to release from intracellular stores or influx through other plasma membrane channels [65]. Studies on GIST cell lines have shown that DOG1 can be activated and inhibited by pharmacological and electric stimulation. Activation of DOG1 by Ca^{2+} or membrane depolarization induces Cl^- currents, whereas blocking DOG1 might shift early apoptotic cells to late apoptosis [66]. Further studies have shown that blocking DOG1 may have anti-tumoral effects *in vitro* by inducing G_1 cell cycle arrest and reduce colony formation. DOG1 might be a potential future target for GIST treatment [67].

2.2 THE TYROSINE KINASE INHIBITOR IMATINIB

Imatinib was one of the first targeted therapies against cancer and its discovery was the result of a large drug-screening project. The target for imatinib was the Bcr-Abl fusion transcript protein that has tyrosine kinase activity and is expressed in the majority of chronic myeloid leukemias (CML). The Bcr-Abl fusion gene is formed by the translocation between chromosomes 9 and 22, consequently leading to formation of the so-called Philadelphia chromosome [68]. Since the structure of the Bcr-Abl fusion protein was not known, the development was made by testing a large drug library *in vitro*. After finding a lead compound that was a weak inhibitor of protein kinase C (PKC) and PDGFR tyrosine kinases, it was optimized for inhibition of PDGFR. The resulting compound, known as experimental drug STI-571 (later Glivec®, Gleevec® or imatinib), proved to be a potent inhibitor of auto-phosphorylation of v-Abl, PDGF receptors and KIT receptors. Clinical trials treating Philadelphia chromosome positive CML commenced in June 1998 [69]. That same year, the *c-kit* gene was cloned [59] and the first patient with progressing metastasized GIST was treated successfully in 2000 [70].

Imatinib acts on the intracellular domain of the KIT receptor where it has affinity to the ATP-binding motif, thereby blocking it and preventing auto-phosphorylation and activation of downstream signaling. The underlying mutation in *c-kit* or *PDGFRA* genes and resulting conformation changes in the receptor aids in the prediction of sensitivity to imatinib treatment (Figure 2).

Imatinib is still the mainstay treatment of inoperable or metastasized GISTs. It is also used for neoadjuvant and adjuvant therapy [71].

2.3 DIAGNOSIS AND TREATMENT

While many GISTs are asymptomatic and are accidentally discovered while performing investigations for other conditions, approximately 2/3 of all GISTs present with symptoms such as GI bleeding, bowel obstruction or a palpable mass [48]. The tumor is often first detected either by endoscopy or computed tomography (CT) scan. Both investigations should be included in the primary evaluation of GIST. CT gives information on tumor site, size, over-growth to other organs and presence of metastases. In selected cases (notably rectal GIST), MRI is useful for detailed anatomical assessment. Endoscopy with biopsy gives the morphological diagnosis. Since tumor growth is submucosal, forceps biopsy is often non-diagnostic and fine needle biopsy guided by endoscopic ultrasound is the investigation of choice. In metastatic disease, percutaneous biopsy can be utilized but is otherwise normally discouraged. Therefore, when the tumor is located in the small bowel, pre-operative biopsy can often not be performed without risk of dissemination. In this situation surgery can be planned without an additional diagnostic work-up. This may also be the case in other anatomical locations where it is not possible to perform a pre-operative diagnostic biopsy yet the tumor is otherwise primarily resectable [48, 72]. As mentioned above, immunohistochemical diagnosis is typically based on the expression of KIT (CD117) and DOG1. Typical histopathological pictures of GIST are shown in Figure 3.

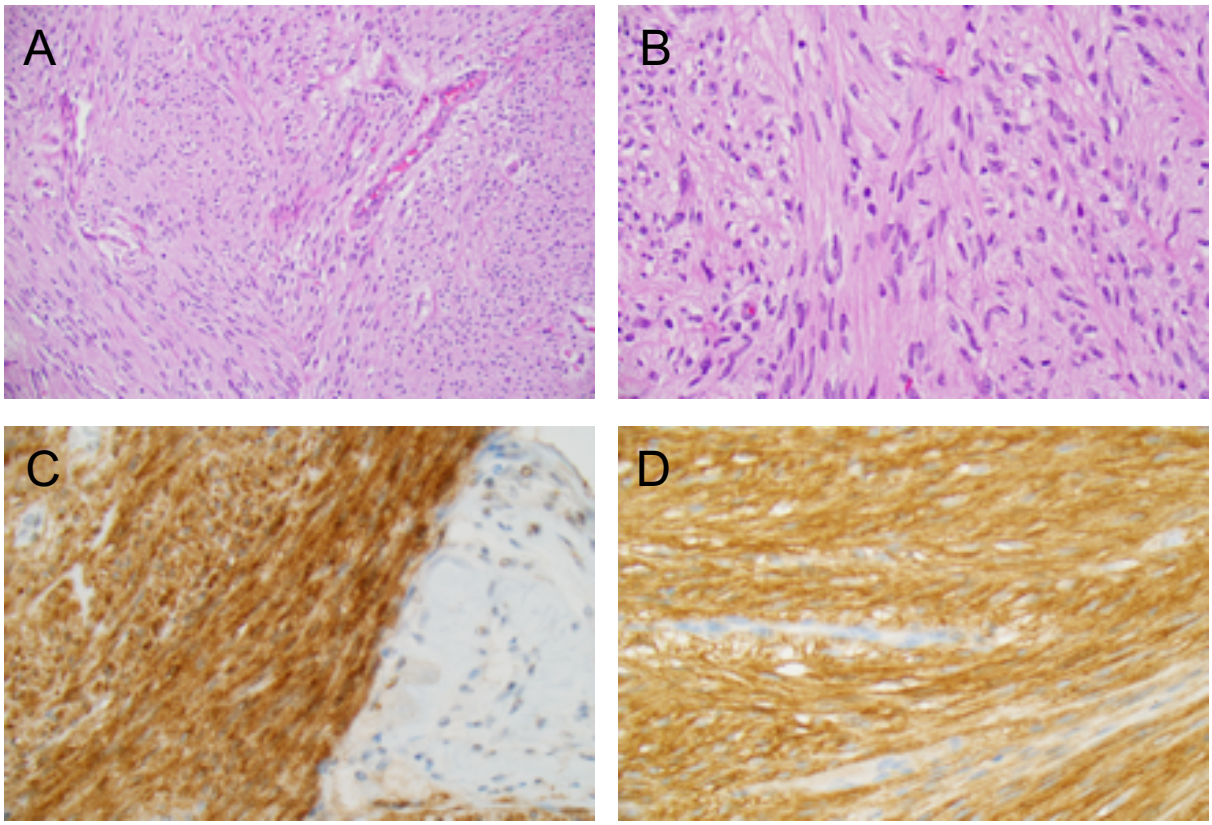

Figure 3. Histologic images of GIST. Hematoxylin eosin (H&E) stain at x200 magnification (A), H&E stain at x400 magnification (B), CD117 (KIT) stain (C), and DOG1 stain (D).

2.4 NEOADJUVANT THERAPY

In cases with advanced tumors, in which primary resection is not possible without severe morbidity due to anatomical site of the tumor or tumor size/overgrowth on adjacent organs, neoadjuvant therapy may be considered. The decision to start neoadjuvant therapy should be multidisciplinary. Mutational analysis to assess potential TKI sensitivity should be performed. In order to evaluate efficacy of adjuvant therapy, ^{18}fluoro-deoxy glucose positron emission tomography/computed tomography (FDG-PET/CT) is helpful. Typically, an FDG-PET/CT scan is performed before starting neoadjuvant therapy and is then repeated after a few weeks of therapy. If effective, the glucose uptake in the tumor should decrease markedly. An example of this is illustrated in Figure 4.

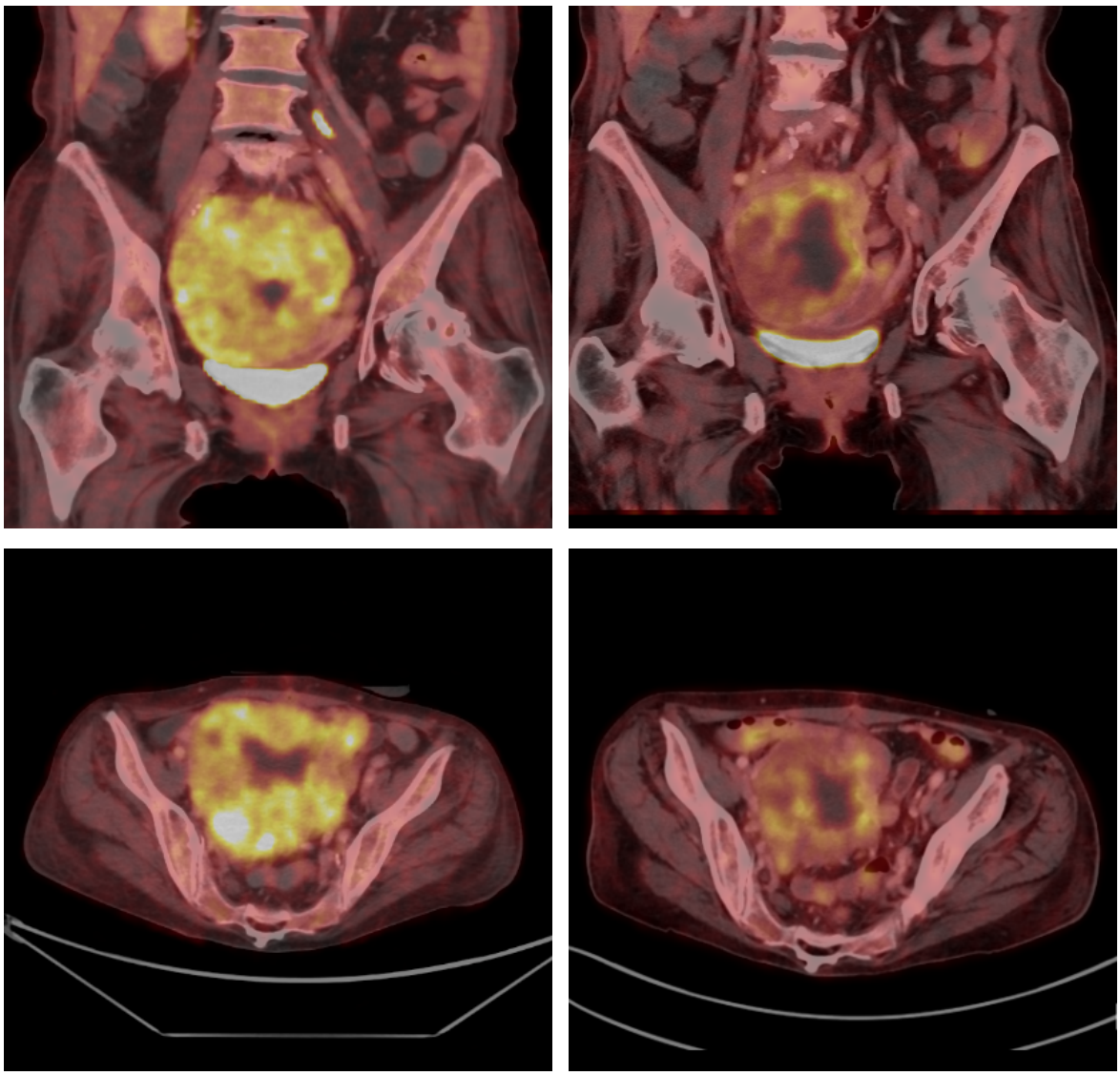

Figure 4. Small-intestinal GIST in the pelvis during neoadjuvant treatment with imatinib, 400 mg daily. Left panel FDG-PET/CT before start of treatment, right panel FDG-PET/CT four weeks after start of treatment, showing decreased metabolic activity and decreased size of the tumor. Mutation analysis showed an insertion in *c-kit* exon 11.

Further follow-ups with conventional CT or MRI after, e.g. 3 and 6 months, are encouraged in order to detect failure of down-staging [73, 74]. Regression of tumor size during neoadjuvant therapy with imatinib can usually be expected within the first six months, after which maximum response is achieved and the patient should be reevaluated for surgery within 6-12 months from start of treatment [71, 75].

2.5 SURGERY

Surgery is the cornerstone in treating GIST. For non-metastatic GIST the treatment of choice is complete resection of the tumor without lymph node clearance and surgery alone could be curative [71, 76, 77]. Small GISTs can be locally resected,

whereas larger tumors may require resection of adjacent organs in order to achieve clear margins. The tumors are often soft and may partially only be covered by a peritoneal surface; hence, a careful surgical approach is mandatory. Special care should be taken not to damage the tumor, since perioperative tumor rupture puts the patient at the same risk for recurrence as preoperative rupture or highly malignant tumors [78]. Margins can be assessed by the R system [79] or in accordance with other soft tissue sarcomas by the system proposed by Enneking [80]. The R system is based on the residual tumor after resection and comprises the following categories:

RX not assessable
R0 no microscopic residual tumor at the resection margin
R1-microscopic residual tumor at the resection margin
R2 macroscopic residual tumor at the resection margin

The original Enneking definition is cited below:

"1. Intralesional. An intralesional margin is accomplished by a procedure in which the dissection passes within the lesion. Macroscopic or microscopic tumor is left at the margins of the wound, and there is contamination of all the exposed tissue planes. Most commonly, local intralesional procedures are performed as a diagnostic incisional biopsy, by curettage of a presumably benign lesion, or by subtotal removal of a lesion to be managed by other means. An intralesional amputation is sometimes intended as a palliative procedure, but more commonly is done inadvertently because of occult microextensions of the lesion.

***2.** Marginal. A marginal margin is achieved by a procedure in which the lesion is removed in one piece. The plane of dissection is through the pseudocapsule or reactive tissue about the lesion, and when performed for malignant lesions, leaves microscopic disease at the margin of the wound in a high percentage of the cases. As a local procedure, marginal excision is usually described as excisional biopsy or "shell 'em out" of a presumed benign lesion. Marginal amputation is usually done as either a palliative procedure, an attempted definitive procedure constrained by anatomic inaccessibility, or as an adjunctive procedure.*

***3.** Wide. A wide margin is accomplished by a procedure in which the lesion, its pseudocapsule and/or reactive zone, and a surrounding cuff of normal tissue are taken as a single block. The plane of dissection is entirely through normal tissue but within the involved compartment. No effort is made to remove the entire length of involved muscle from origin to insertion or bone from joint to joint. The local wide procedure probably corresponds to what is referred to as "wide local excision," "en bloc excision," and "radical en bloc excision." A wide margin is definitive surgical management for Stage I lesions and can usually be accomplished by a local procedure for IA lesion Because Stage IB lesions usually involve some combination of bone, soft parts, and neurovascular structures, amputation is more likely to be required.*

4. *Radical. A radical margin is achieved by a procedure in which the lesion, pseudocapsule, reactive zone, and the entire muscles or bone involved are removed as one block. Longitudinally, the plane of dissection goes through or beyond the joint proximally and distally to the bone involved and through the tendinous origin and insertion of involved muscles. Transversely, the dissection passes beyond the major fascial septa of the involved soft tissue compartments or beyond the periosteum of intraosseous lesions. A radical margin does not necessarily imply a greater distance from the lesion to the margin of the wound than a wide margin. A margin on the other side of the intermuscular septum of a lesion in the vastus lateralis will constitute a radical margin but may be considerably closer than a wide margin achieved by amputation. A radical margin is definitive for Stage I1 lesions. A radical local resection can often be done for a Stage IIA lesion. If a lesion involves more than one compartment, or extends into or arises in the extracompartmental planes or spaces, compartmental containment is lost, and a radical margin is usually not attainable with a local procedure. Thus, radical amputation is usually carried out to achieve a radical margin in Stage IIB lesions, and it often requires a disarticulation or amputation proximal to the joint in question."* [81]

The Enneking principles of margins are based on extremity sarcoma and reflects compartmental excision and amputation. In the abdomen, compartments are not clearly defined; however, covering intact peritoneum is considered to be a sufficient anatomical barrier [82].

The impact of margin status on GIST prognosis is under debate and some studies show no difference for patients operated with microscopically positive margins (R1 resection) compared to R0 resection. However, in **paper II** we show a favorable outcome when obtaining wide surgical margins [83]. In this context, a wide margin is defined as ≥2 cm margin and/or intact peritoneal coverage of the tumor (Figure 5). This may involve resection of organs adherent to or in close proximity of the tumor.

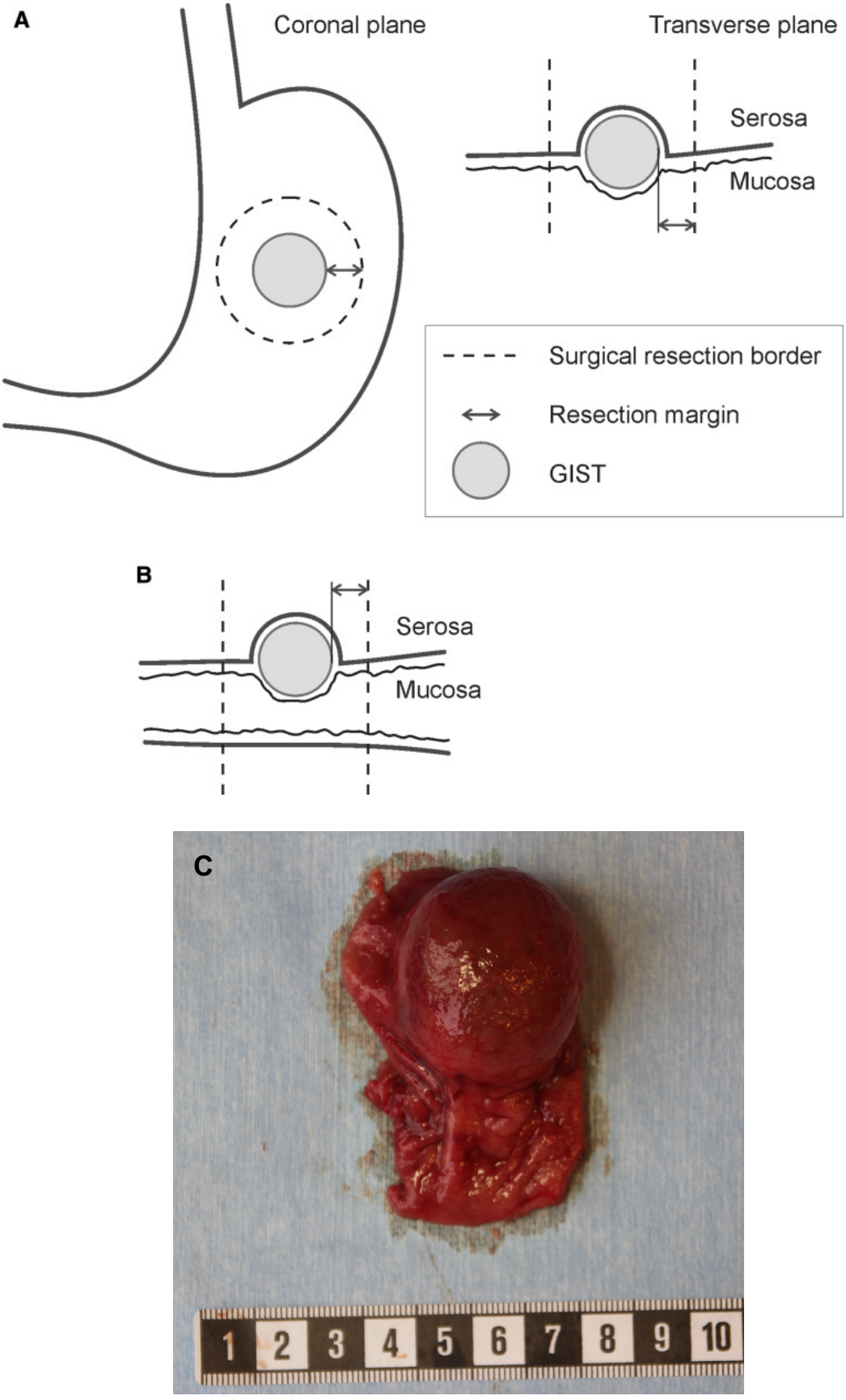

Figure 5. Schematic drawing of ideal resection of GIST with wide surgical margin ≥2 cm, located in stomach (A) and small intestine (B) [83]. Photo of resected gastric GIST at pathological grossing (C).

The surgical technique should command minimal contact with the tumor. Typically, a circular gastric resection or a small bowel resection with ≥2 cm margin is performed. Laparoscopic technique can be considered for selected cases, yet it is essential that damage to the tumor be avoided. Unlike epithelial-origin cancer, such as colorectal cancer, sarcoma originates from wall layers with significantly less protective membranes. Not infrequently is there only a thin layer of peritoneum or serosa surrounding the tumor tissue and damage to this barrier significantly increases local recurrence risk.

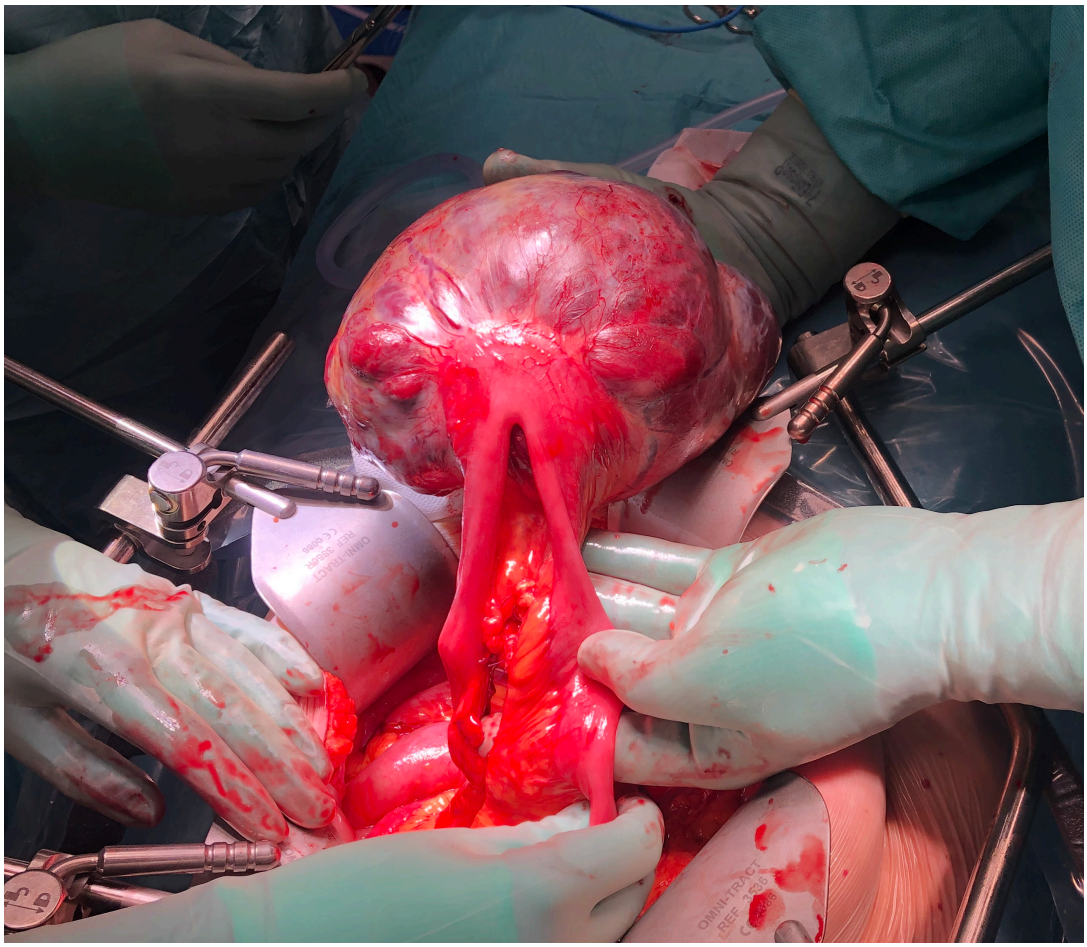

Figure 6. Resection of small bowel GIST. Open laparotomy is the most common approach due to large tumor, tumor site, and resection technique. Laparoscopic resection may be feasible in selected cases.

2.6 FOLLOW-UP AND ADJUVANT THERAPY

There is still a risk for recurrent disease even after complete resection of GIST, either locoregionally or metastatically, mainly to the liver. Prognostic assessment of risk for recurrence, metastasis and/or death is mandatory upon GIST resection. Several grading systems have been proposed and evaluated. With small differences, all commonly used evaluation methods predict tumors with high risk for recurrence yet there are differences in the intermediate risk-groups [84, 85]. Still, there is no consensus on which grading system provides the most accurate risk estimation [86]. There are currently three main categorical grading systems that divide risk categories in different tiers. The National Institutes of Health (NIH) system proposed by Fletcher *et al*. is based on tumor size and mitotic index [87]. This system later evolved into the AFIP (Armed Forces Institute of Pathology) criteria that, based on long term follow-up studies, also accounts for the observation that gastric GIST generally has a better prognosis and incorporates the primary tumor's anatomic site as a risk factor[52, 88, 89]. At present, one of the most established grading systems, currently in wide use for clinical assessment of risk for recurrence, is the modified NIH criteria (Table 2), based upon tumor site, size, presence of mitoses and tumor rupture as an individual factor for high risk of recurrent disease [78, 90].

Risk category	*Tumor size (cm)*	*Mitotic index (per 50 HPFs)*	*Primary tumor site*
Very low	<2.0	≤5	Any
Low	2.1-5.0	≤5	Any
Intermediate	2.1-5.0	>5	Gastric
	<5.0	6-10	Any
	5.1-10.0	≤5	Gastric
High	Any	Any	Tumor rupture
	>10	Any	Any
	Any	>10	Any
	>5.0	>5	Any
	2.1-5.0	>5	Nongastric
	5.1-10.0	≤5	Nongastric

Table 2. Modified NIH consensus criteria for risk of recurrence after R0 resected GIST. Adapted from Joensuu 2008[90]. HPF - high power field.

Some authors have also proposed the use of continuous risk stratification systems such as nomograms [91-93] or contour heat-maps that account for a non-linear relationship between tumor size and recurrence risk [85]. An example of a nomogram for estimation of recurrence-free survival after resection of GIST is the Memorial Sloan Kettering (MSKCC) nomogram, proposed by Gold *et al.* [91]. In this nomogram prognostic factors such as size, mitotic index and site are considered and each are assigned a point which is then summarized and read as probability for 2- and 5-year recurrence-free survival (Figure 7).

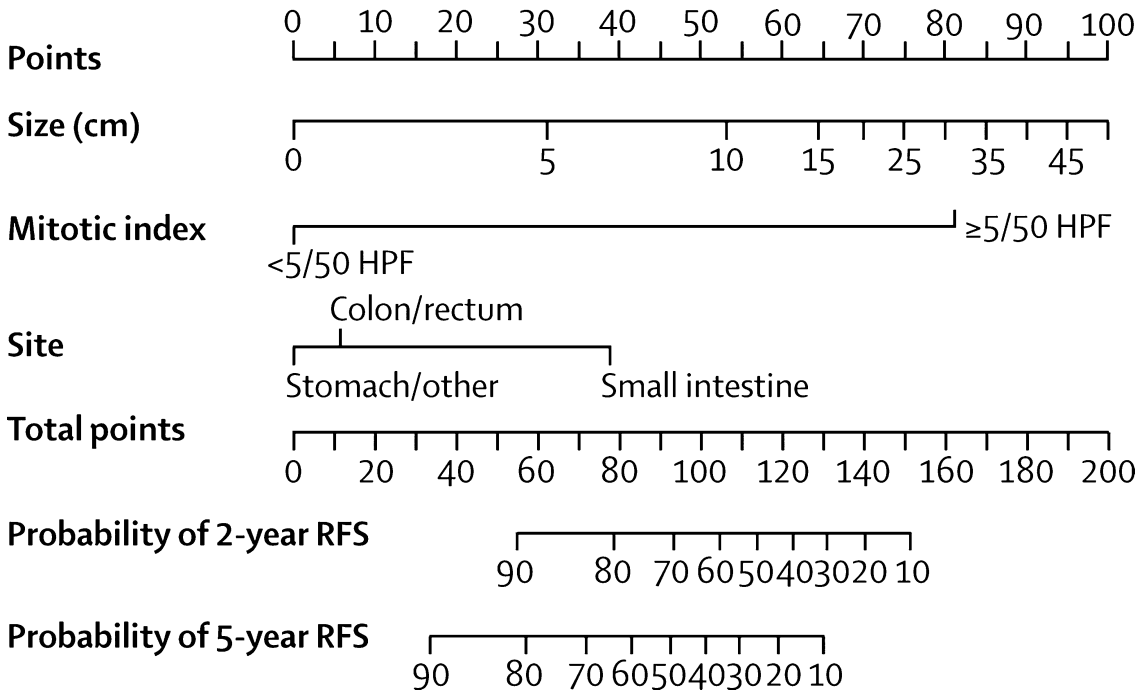

Figure 7. The MSKCC nomogram for estimation of recurrence risk after resection of GIST [91]. Reprinted with permission from the publisher.

Cases with a high risk for recurrence should be offered adjuvant therapy with TKI after discussion with a multidisciplinary board. Notably, in patients treated with neoadjuvant imatinib, the mitotic count is not assessable for risk evaluation since the treatment effect down-regulates proliferation. This is often not a problem in clinical practice since the tumors eligible for neoadjuvant therapy have other features placing them in the high-risk group. The caveat are esophageal and rectal GISTs where the anatomical location often mandates neoadjuvant imatinib. In these cases, the MDT suggestion of adjuvant imatinib need to be based upon pragmatic

principles and if the treatment has not caused severe side effects, it is usually completed. If imatinib is discontinued, close follow-ups during the first years may be warranted. The duration of adjuvant therapy is still debated. Adjuvant treatment started following the ACOSOG Z9001 phase III double-blinded, randomized clinical trial showing improved progression-free survival (PFS) in patients receiving one-year adjuvant imatinib as compared to placebo [94]. No difference in overall survival (OS) was observed in this study. The Scandinavian Sarcoma Group (SSG) conducted a study comparing 1 versus 3 years of imatinib (SSG study no. 18, abbreviated SSGXVIII), showing further improvements in PFS but also OS [95]. Based on these results the generally accepted treatment time is now three years. A recently published long-term follow-up study of the SSGXVIII cohort further emphasizes this strategy and shows a clear benefit in terms of OS in the group treated 3 years with adjuvant imatinib as compared to one year [96]. The ongoing SSGXXII trial evaluates potential benefits with prolonged adjuvant treatment of up to five years after surgery (ClinicalTrials.gov Identifier: NCT02413736). This study is a complement to a phase 2 safety study (PERCIST-5), which showed no recurrences during 5 years with imatinib treatment of resected GIST patients, however, the study had a high degree of early drop-out (49%) [97].

There is no clear consensus on duration and intervals for follow-up after resection of GIST with or without adjuvant therapy. However, it is well known that even “low-risk” GISTs may recur and often later than high-risk tumors [52, 98]. This must be considered when planning follow-ups. Further, a higher risk of recurrences is observed after cessation of adjuvant treatment, why these patients may benefit from closer follow-ups after ending imatinib medication [71].

D’Ambrosio *et al.* have evaluated a protocol where 233 patients without TKI treatment were followed for a median of 68.3 months (95% CI=59.8–76.8). During follow-up, recurrent disease was detected in 40.3% of the patients and in 26.6% other cancers were detected. The protocol comprised clinical examinations and CTs every 3–4 months during the first 3 years, every 6 months year 4-5 and annually thereafter for up to ten years in intermediate/moderate- and high-risk patients. For patients with low- and very low-risk, clinical examinations and CTs were performed every 6 months for 5 years and thereafter annually up to ten years [99]. This

protocol is in accordance with the follow-up routines at our institution where we generally perform a CT or MRI of the abdomen every six months for five years and then annually for up to ten years. In selected cases with a high risk of recurrence according to risk stratification or uncertain margins, shorter intervals during the first years may be recommended after the MDT conference.

2.7 IMATINIB RESISTANCE

While most *c-kit* mutated GISTs are sensitive to imatinib treatment, there are certain mutations that are primarily resistant. In Exon 11 mutated GIST, 95% are primarily sensitive. Exon 9 mutated tumors are sensitive but higher doses of imatinib are proposed for patients with these mutations and those cases treated with standard doses may be perceived as primarily resistant [72]. The most common *PDGFRA* mutation in GIST is D842V on Exon 18 and is primarily completely resistant to imatinib [45, 62].

A common problem is the development of secondary resistance, which is typically seen after about two to two and a half years of imatinib treatment of primarily sensitive tumors. In many cases resistance is caused by secondary mutations that alter the configuration of the receptor, i.e. *c-kit* exon 13,14, 17 or 18 [48]. These mutations are seen in about 67% of secondary resistant GIST [62]. It is not known if secondary mutations develop during the course of treatment or are present at start of treatment and clonally expanded under selection pressure from the treatment. Interestingly, secondary mutations do not seem to explain all cases of secondary resistance and there is data on the positive effect of dose escalation in cases of secondary progression during imatinib treatment [48]. There is also data, primarily from CML, on the effect of altered drug transporter expression. For example, cell-lines transfected with a mutated variant of the transport protein ABCB1 showed lower intracellular uptake of imatinib and lower antiproliferative effect [100]. Polymorphism in the drug transporter ABCG2/BCRP is also implicated in differences in the uptake of many drugs, including imatinib [101]. In **Paper III,** we explore this possible mechanism of resistance.

2.8 OTHER TKI'S

After discovering imatinib, a plethora of tyrosine kinase inhibitors have been added to the family and still more are being developed. The U.S. Food and Drug Administration (FDA) approved Sunitinib in 2006 as second-line treatment of GIST when progression occurs during treatment with imatinib. Sunitinib blocks KIT, PDGFR, vascular endothelial growth factor receptor (VEGFR) and RET. It is administered orally in doses of 37.5 to 50 mg and a regimen of 4 weeks treatment followed by a two-week pause is recommended. As third-line treatment regorafenib was approved by the FDA in 2013 [102]. Many other TKIs have been and are still undergoing trials and the current situation for advanced GIST after imatinib failure is summarized below (Table 4).

TKI	*Affinity*	*Clinical trial*	*Phase*	*Line*	*ORR*	*mPFS (months)*
Sunitinib	KIT, PDGFR, VEGFR, RET	Demetri 2006 [103]	III	2	7%	6.4
Regorafenib	KIT, PDGFR, RET, RAF1, BRAF, FGFR VEGFR1-3, TIE-2,	Demetri 2013 [104]	III	3	4.5%	4.8
Pazopanib	KIT, PDGFR, VEGFR	Mir 2016 [105]	II	3	0%	3.4
Avapritinib (BLU-285)	KIT, PDGFRA (including D842V)	Heinrich 2017 [106]	I	≥2	28%	n/a
		Heinrich 2020* [107]	I	≥1	88%	not met
		NCT03465722	III	≥2		active
Ripretinib (DCC2618)	KIT, PDGFRA (including D842V)	Serrano 2018 [108]	Ib	≥3	0%	2.1
		Von Mehren 2019 [109]	III	≥4	9.4%	1.5
		NCT03673501	III	2		recruiting
Nilotinib	ABL1/BCR-ABL1, KIT, PDGFR.	Montemurro 2009 [110]	II	≥3	10%	2.8
		Sawaki 2011 [111]	II	3	3%	3.7
		Cauchi 2012 [112]	II	≥3	0%	2.0
		Reichardt 2012 [113]	III	3	<1%	3.6
Masitinib	KIT (exon 9 & 11) highly selective	Adenis 2014 [114]	II	2	n/a	3.7
		NCT01694277	III	2		recruiting
Ponatinib	BCR-ABL, KIT	NCT03171389	II	≥2		recruiting
Dovitinib	VEGFR, FGFR, KIT, PDGFR-β	Kang 2013 [115]	II	≥3	3%	3.6
		Joensuu 2017 [116]	II	2	2.6%	4.6
Sorafinib	CRAF, V600E BRAF, KIT, FLT-3 VEGFR, PDGFR-β	Kindler 2011 [117]	II	≥2	13%	5.2
		Park 2012 [118]	II	≥3	13%	4.9
Crenolanib	PDGFRA	NCT02847429	III	any		recruiting D842V

Table 4. TKI with KIT inbititory activity in trials with advanced/metastatic GIST after imatinib failure. mPFS – median progression-free survival. * Subset analysis of D842V mutated patients (n=56). Adapted from Serrano *et al.* 2017 [119] and Mazzocca *et al.* 2019 [61].

2.9 TKI SIDE EFFECTS

Target treatment with TKI is generally well tolerated as compared to conventional cytotoxic drugs. Still, more than 95% of TKI treated patients experience side-effects [120]. Most side effects are mild and tolerable but serious adverse effects need to be considered and monitoring of patients on TKI treatment is recommended. In some cases, adverse effects can be managed by dose reduction, whereas in other cases discontinuance of medication or changing to a different line of TKI is necessary [71].

Imatinib commonly causes nausea, fatigue, fluid retention/edema and in some cases skin rashes. These side effects are often manageable on the standard dose of 400 mg daily [121]. Hematological side effects with myelosuppression causing mild anemia are common. In rare cases, severe anemia or agranulocytosis is observed. More infrequently, liver failure/hepatitis may occur [122]. There are a few case reports on fatal liver failure caused by imatinib [123-125]. Often early signs of liver failure are detected during monitoring and may be reversible through dose reduction, yet may infer cessation of imatinib therapy [126]. The relationship of liver failure during imatinib treatment and concomitant treatment with acetaminophen (paracetamol) has been investigated and in a mouse model co-administration of the two drugs induced irreversible liver damage [127]. In clinical practice, we advise patients on imatinib to avoid acetaminophen. Imatinib has, in most studies, not been shown to cause hypothyroidism but interacts with levothyroxine and in patients with hypothyroidism, an escalation of levothyroxine dose may be required to maintain euthyroid levels [128, 129].

Second-line TKI sunitinib has a similar side effect profile to imatinib and yet additionally, stomatitis, hand-foot syndrome, hypertension and hypothyroidism are more common in patients treated with sunitinib [120]. Third-line TKI regorafenib shares the same panorama of side effects as sunitinib [104]. Novel TKIs, such as avapritinib and ripritinib, have in studies, shown side effect profiles comparable to those of imatinib with nausea, vomiting and periorbital edema. Adverse effects were in most cases mild and tolerable, however, 9-11% of study patients dropped out from the study due to side effects [61].

3 SARCOMA OF THE BREAST

Like sarcomas in other parts of the body, breast sarcomas consist of many different histopathological subtypes. Sarcoma accounts for about 1% of all breast malignancies, the most common histopathological subtypes being angiosarcoma and malignant phyllodes tumors but a large variety of more uncommon diagnoses are present. The annual incidence of breast sarcoma is reported to be 0.4/100 000 persons [15, 130, 131]. Often breast sarcomas are detected as a palpable mass, though smaller in size than abdominal/retro-peritoneal sarcomas. Surgical resection can be curative, although recurrence rates are high even following surgery with clear margins [132, 133].

3.1 ANGIOSARCOMA

Angiosarcoma is derived from endothelial cells of blood or lymphatic vessels. Like most other sarcomas, it can develop in all parts of the body but the most common site is head/neck, including scalp. Angiosarcoma is one of the most common sarcomas of the breast and is usually divided into primary and secondary angiosarcomas where the primary presents in younger women without known exposure to risk factors [134]. Secondary angiosarcoma presents in older women, often 5-15 years after radiotherapy to the breast. Previous irradiation is a known risk factor for its development [135, 136]. Causal links to individual susceptibility dependent upon gene-environment interactions have been suggested [137]. Stewart Treves syndrome, the term coined after the authors of a report of six cases of angiosarcoma in 1948, i.e. chronic lymphoedema following a mastectomy with lymph node dissection in combination with irradiation, is another reported risk factor for lymphangiosarcoma [138, 139].

Angiosarcomas are aggressive tumors with a high risk of local recurrences and dissemination, most commonly lung metastases. The 5-year over-all or disease specific survival is reported to be between 31-43% [133, 140, 141]. The median over-all survival for patients with local angiosarcoma in the breast has been reported to be 3.6 ± 1 year, but for patients who were diagnosed with metastatic disease median over-all survival was 0.7 ± 0.2 years [140].

Primary surgery with negative margins is recommended and in combination with tumor grade are shown to be the primary factors influencing prognosis [142]. In fact, some authors recommend excision of the entire radiated area in cases of radiation-associated angiosarcoma, due to the tumors' propensity to multi-focal and diffuse growth [133, 143, 144].

3.2 PHYLLODES TUMORS

Phyllodes tumors are fibroepithelial tumors, first described by the German pathologist Johannes Müller 1831. The original term describing these tumors was cystosarcoma phyllodes, derived from the Greek word *phullṓdēs,* meaning, "resembling a leaf", based upon their leaf-like features in the microscope. However, these tumors are rarely cystic [145].

The annual incidence is reported to be 2.1 per million, and the tumor can affect patients of all ages with a peak at 41-50 years (range 8-89) [146, 147]. Phyllodes tumors are composed of both epithelial cells and connective tissue stroma and range from benign (grade 1) over to borderline (grade 2) and to overtly malignant (grade 3). Grading is based upon several histopathological features, including stromal cellularity, overgrowth and atypia, mitoses and tumor border (well defined or permeative). The three tier classification was first introduced by Azzopardi in 1979, subsequently refined by Salvadori in 1989 [147] and has later been included in the WHO classification of bone and soft tissue tumors [148]. The malignant phyllodes tumors have a higher proportion of mesenchymal proliferation and a genetic profile similar to other breast sarcomas, though not to angiosarcoma [149].

Owing to the spectrum of behavior in the different grades of differentiation of phyllodes tumors, surgical treatment should be individualized. In a review, Tan *et al.* summarized the literature on surgical margins and concluded that firm evidence on appropriate margins in Phyllodes tumors are lacking. Based upon available data, the recommendation in this study was to excise recurrent or malignant phyllodes tumors with negative margins of >1 mm, whereas benign phyllodes tumors may be treated expectantly. Lymph node dissection is not recommended [150]. Zhou *et al.* also reviewed the literature and found that surgical margins were reported to have an impact on recurrence rates in 15 of 24 reviewed articles, yet did not define any recommendations on the extent of margin [151]. In a Canadian study, margin status

has been addressed. No significant difference in local recurrence rates was observed when margins of >2 mm, 2-10 mm and >10 mm were compared but positive margins implied an elevated risk for local recurrence. Of 49 patients with malignant phyllodes, 24 were treated with mastectomy and none of these had local recurrence. In the 25 patients treated with breast conserving surgery, 7 local recurrences (28%) were observed during a median follow-up of 65 months (range 0.5-197) [152].

4 AIMS OF THE THESIS

This thesis aims to increase the knowledge of sarcoma management and especially GIST, the most common intraabdominal sarcoma. The project envisioned a translational approach, spanning from cell studies through surgical techniques and to epidemiological methods.

Specific aims:

- To explore a possible mechanism for imatinib resistance in GIST
- To asses the importance of surgical technique in treatment of GIST
- To explore possible underlying factors for the increasing incidence of angiosarcoma in the breast

5 PATIENTS AND METHODS

5.1 PAPER I

Cell cultures with known imatinib-sensitivity (GIST882) and resistance (GIST48) were cultivated for 4-5 days until near-total confluence. The cultures were then exposed to imatinib in concentrations of 1100 to 3300 ng/ml for three hours at 37°C in 5% CO_2 atmosphere. After washing, cells were detached with trypsin and spun down. The pellet was snap-frozen to -80°C pending analysis. We also collected tissue (tumor and adipose) and blood plasma from three patients undergoing surgery with ongoing imatinib treatment. All patients in this study were taking 400 mg imatinib orally per day. Imatinib concentrations were measured using liquid chromatography with coupled mass-spectrometry and time of flight detection. This method was first described by Elhamili and Bergquist [153] and this paper is a *proof-of-concept* study on applying the method for intra-cellular concentration measurements in GIST-cells from cell culture and tumor tissue.

5.2 PAPER II

One hundred twenty-nine patients undergoing surgery for GIST at Karolinska University hospital, in whom surgical margins were assessed at the time of resection, were included. Patients were classified as non-metastatic at diagnosis and without TKI treatment (nonMET/nonTKI n=79), as patients with metastatic disease at diagnosis (n=14) and patients without metastases at diagnosis, yet with TKI treatment pre- or post-surgery (nonMET n=36), (Figure 8).

Patient data including age, gender, tumor size, site, risk group, according to modified NIH criteria [90], and treatment were recorded. Patients were followed regarding recurrence, distant metastasis and survival. Median follow-up time was 76 months (range 10–179).

Tumor size was recorded based on histopathological reports. Tumors were grouped based on greatest diameter and anatomic location. All 115 patients who were without metastasis at diagnosis, regardless of TKI treatment (GIST-nonMet) were analyzed, but in order to avoid confounding as to the effects of TKI, the subgroup of 79 patients with non-metastatic disease and no TKI treatment was analyzed separately.

Statistical analysis

The Kaplan–Meier method and log-rank test were used to compare time from diagnosis to local/peritoneal recurrence or metastasis, time to death from any cause and time to death from disease. Multivariate analysis with Cox proportional hazards model was used to compare prognostic factors. All tests were performed two-tailed and p values <0.05 were considered to be statistically significant.

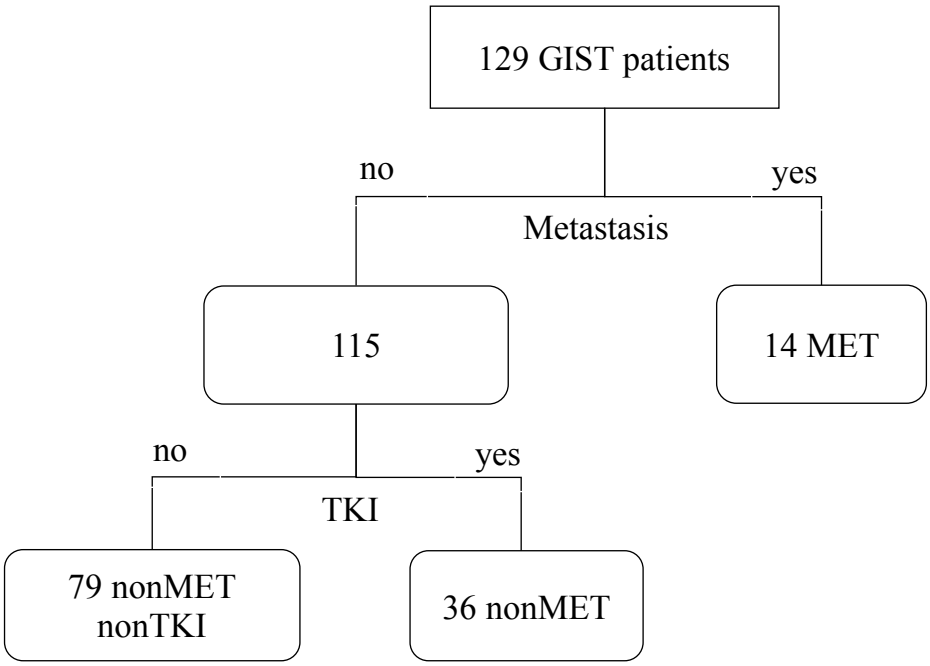

Figure 8. Subgroups of patients in paper II.

5.3 PAPER III

A series of consecutive patients with GISTs pre-treated with imatinib and undergoing tumor resection at our department from 2013 to 2017 were included after informed consent. The included patients had either metastatic disease or locally advanced and primarily non-resectable tumors. At the time of surgery, samples from tumor, normal tissue and blood plasma were collected. After resection, the tumor was incised and samples were collected from peripheral and central parts. In some cases, with larger tumors, additional samples were collected from the intermediate part of the tumor (Figure 9). In cases with multiple tumor locations samples could be obtained from different tumors.

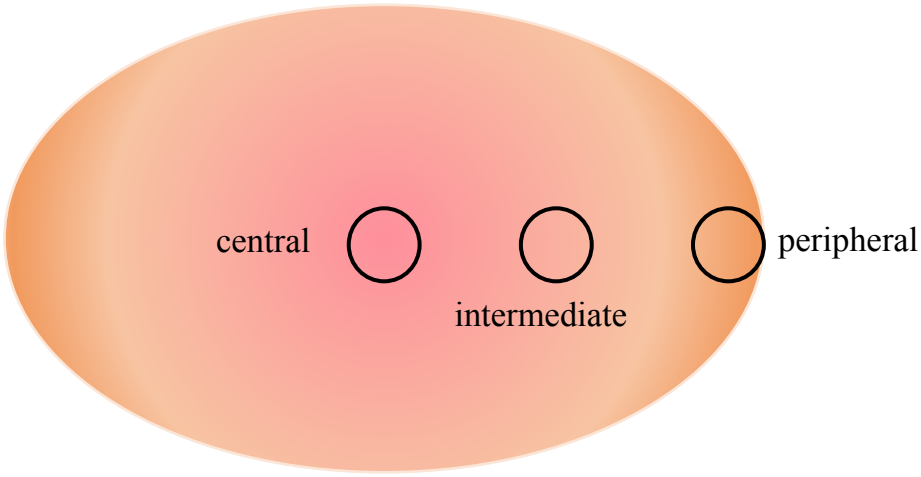

Figure 9. Acquisition of samples from different parts of resected tumors.

All samples were instantly frozen on dry ice and transported to -80°C freezer. Clinical data was retrieved from patient medical records. In relevant cases, the radiological response was evaluated during the pre-treatment period and CT scans performed prior to treatment were compared to the latest pre-operative CTs according to Choi criteria. The histopathological response was assessed and graded based on the proportion of viable tumor cells. Mutation analyses were performed in order to determine mutational status of *KIT* and *PDGFRA* genes, using bidirectional sequencing with Genetic Analyzer 3500 or Genetic Analyzer 3130xl (Applied Biosystems, Foster city USA). Analytic software Mutation Surveyor SoftGenetics LLC v4.0.4 was used. The imatinib concentration in plasma and tissue samples was analyzed using ultra-performed supercritical fluid chromatography-tandem mass spectrometry. The analysis protocol was an improved and validated version of that described in **Paper I**, where the internal standard for these analyses was deuterium-marked imatinib (imatinib D_8). All analyses were run in duplicates.

Tissue was collected in connection with surgery and was analyzed on Formalin-fixed paraffin-embedded (FFPE) samples. Using conventional immunohistochemistry, these were stained with antibodies for drug transporters (rabbit monoclonal anti-MDR1/ABCB1 (clone E1Y7S) at 1:400, rabbit monoclonal anti-ABCG2 (clone D5V2K) at 1:350; both from Cell Signaling Technology, Danvers, MA, USA and mouse monoclonal anti-OCT1/Pou2f1 (clone 12F11) at 1:100 from Santa Cruz Biotechnology, Dallas, TX, USA). An experienced

pathologist reviewed the slides and the expression was graded as low, intermediate or high. The same pathologist, who was blinded from concentration analysis results, also graded histological response in terms of percentage of viable cells and mitotic index.

Statistical analysis

Since data was not normally distributed, non-parametrical tests, Mann-Whitney, Wilcoxon signed ranks and Kruskal-Wallis, were used to calculate correlations. For correlations between histological features, Fisher´s exact test was used. A level of $p<0.05$ was considered statistically significant.

5.4 PAPER IV

In this case-control study, Swedish national registries were used to identify 344 patients with mesenchymal tumors in the breast diagnosed between 1993-2013. These were classified according to reported histopathology and each case was matched according to age and sex to up to ten controls. Incidence was analyzed and compared for 5-year periods during the study time. The cases were compared to controls regarding previous cancer history, where breast cancer and other cancers were analyzed separately. We also analyzed socioeconomic factors and survival.

Statistical analysis

Incidence was calculated using Poisson regression. Kaplan-Meier and Cox regression analyses were used for survival analyses and conditional logistic regression models were used for comparing cases and controls regarding cancer history.

6 RESULTS

6.1 PAPER I

Significantly differing concentrations were observed in cell cultures exposed to imatinib at high or low concentration. There was also a significant difference in concentrations between the two cell lines used, where the imatinib-sensitive cell line GIST882 had higher intracellular imatinib concentrations than the imatinib-resistant GIST48. Repeated experiments showed high reproducibility of the measurements. Negative controls were performed and, as expected, showed no intracellular imatinib. In patient samples, large inter- and intra-tumor variability regarding imatinib concentrations were observed. Generally, a higher imatinib concentration was observed in tumor tissue than in normal adipose tissue.

6.2 PAPER II

In the group of 79 patients without TKI treatment, 31 (39%) developed local/peritoneal recurrence and/or metastases. Thirteen patients (16%) died from disease during follow-up, of which 5 died from local/peritoneal disease without distant metastasis. The 5-year OS was 86%. Thirty-nine patients had surgery with a wide margin, 2 of these died during follow-up, but none from GIST. Of 22 patients operated with marginal margin, 5 died, of whom two from the disease. In 18 patients who had intralesional surgery, 4 patients died within 5 years, all from recurrent/metastatic GIST.

The risk for local/peritoneal recurrence was higher for patients with marginal margin HR 6.8 (1.4–32.7) and intralesional margin HR 13.5 (3–61) as compared to wide margin (p=0.003). The surgical margin remained an independent risk factor for local recurrence in multivariate analysis after adjusting for tumor size, mitotic index and site. The results were similar when the entire group of 115 patients with non-metastatic GIST was analyzed, i.e. including the group of 36 patients who had TKI treatment.

6.3 PAPER III

Twenty-one patients with imatinib-treated advanced and/or metastatic GISTs were included. The imatinib concentration in tumor tissue, normal tissue and plasma showed great variability and no significant correlation between these was found. Also, imatinib concentrations did not correlate to BMI, duration of treatment or *c-kit/PDGFRA* mutational status. The histopathological response was correlated with absent expression of efflux drug transporters ABCG2 and MDR1 and not to imatinib concentrations in plasma or tumor tissue. Absent immunostaining for ABCG2 was significantly associated with a lower mitotic activity (Fisher´s exact test $p < 0.001$). Positive staining for MDR1 ($p<0.001$, Fisher's exact test) or ABCG2 ($p=0.11$, Fisher's exact test) was associated with a higher proportion of viable tumor.

6.4 PAPER IV

Annual breast sarcoma incidence increased from a mean of 1.52 cases per million, during the first half of the study period to 2.04 during the second half and the incidence of angiosarcoma increased 4-fold from 0.09 per million per year during the first period, 1993-1998, to 0.42 in 2009-2013 (trend of increase in the incidence: 1.10, 95% CI 1.05-1.16; $p<0.001$). Angiosarcoma patients were heavily overrepresented, having a history of breast cancer compared with controls. The highest risk association was observed 5-10 years after breast cancer diagnosis (OR 167, CI 95% 35.1-791; $p<0.001$). Survival analyses showed a median survival time for angiosarcoma patients of 4.4 years (95% CI 3.13-6.17) and unspecified sarcoma 5.7 years (95% CI 0-11.6). Interestingly, borderline phyllodes tumors had no significant differences in survival when compared to benign phyllodes or controls, whereas survival for patients with malignant phyllodes tumors was more similar to those with other sarcomas in the breast.

7 DISCUSSION

This thesis work spans from the basic lab-bench methods to epidemiological registry studies. The translational approach is appropriate for studies on sarcoma and indeed, applicable in daily clinical work. Understanding of different tumor´s growth pattern, prevalence in the population, molecular aberrations and potential treatment targets, helps clinical decision-making. Collaboration in multidisciplinary teams not only facilitates this, but has also been shown to improve patient outcomes [31]. Since the knowledge about sarcomas is rapidly evolving, clinicians must have insight into current research methods and results.

In **paper I,** we used an established analytical method, LC/MS-TOF, and validated a new protocol for measuring plasma- and intracellular imatinib concentrations in the nanomolar range. The results were robust and reproducible and the protocol has later been used in other studies[154, 155]. In our series, intracellular imatinib concentrations in cultured cells were dependent upon exposure to different doses of the drug, yet also on the imatinib resistance of the cell-line, where imatinib-resistant cells had a lower intracellular concentration when exposed to an equal dose. Based on these findings, a mechanism of imatinib uptake into the cell or efflux from the cell was suspected and studied further in **paper III**. In patient samples, we noticed large variability in intra- and intertumoral imatinib concentrations. Imatinib concentrations were higher in tumor tissue than in plasma.

Key findings:

- **Intracellular concentration of imatinib in GIST cells is measurable and measurements are robust and reproducible**
- **Imatinib-resistant cells (GIST48) had significantly lower imatinib concentrations as compared to imatinib-sensitive cells (GIST882)**

In **paper II** we demonstrate the importance of wide surgical margin and careful technique when resecting a GIST. The reason for conducting this study was a clinical observation of higher rates of recurrences in GIST patients operated with small or no margins. It is also well established that pre- or intraoperative tumor rupture is a risk factor and this has been included in the modified NIH risk assessment system. The strength of this study was that the surgeon assessed margins at the time of resection. When the pathologist assesses margins the specimens may have been damaged in transport and the thin peritoneal coverage may be fractured, resulting in the microscopic finding of tumor-growth in the resection border, i.e. R1. Another effect on the specimen after resection is the contraction of especially the gastric wall, which may cause separation of the mucosa and the muscle-layers, thus exposing the tumor. Thirdly, not all GISTs are rapidly growing and in case of an R1 resection it may take many years for the microscopic residual tissue to grow back into a clinically detectable tumor. Although rare, recurrent GIST has been reported up to 20 years after resection of the primary tumor [98].

The findings in this study are not uncontroversial and there are several contradictory reports in which R1 resected patients show a prognosis comparable to those with negative resection margins. A recent report from Oslo showed that tumor rupture is an adverse prognostic factor, though minor damage to the peritoneal coverage or R1 resection did not carry a significantly worse prognosis. The authors conclude that the recurrence risk associated to R1 resection is in fact confounded by tumor rupture. Four hundred and ten patients were followed for a median of 45 months (range 0-175). Fifty-two patients had tumor rupture and 39 of these had recurrent disease. Forty-seven patients had R1 resection, of whom 17 developed recurrent disease. No recurrences were recorded in 24 patients with non-high-risk tumors who received an R1 resection, while recurrence was recorded in two out of seven patients with high-risk tumors [156]. It is not surprising to see the high recurrence risk after tumor rupture. The relatively low number of recurrences in R1 resected patients might be explained by a relatively short follow-up time, or possibly the use of adjuvant imatinib treatment.

In contrast, a study by Rutkowski *et al.* identified risk factors for recurrence in 335 GIST patients with a median follow-up of 31 months (range 4–292 months).

The significant predictors for recurrence were size, site, high mitotic count, male gender and R1 resection. Notably, only 6% had a known GIST diagnosis prior to surgery [157]. Ahmed *et al.* reported their experience of GIST in the mid-Trent region, UK. In total, 185 patients where studied of whom 155 had surgical resection of GIST. Of these, 79 had documented R0, 11 had R1 and 15 had R2 resections. For the remaining 48 patients, no data on the resection margin was available. No statistical analysis is presented in this study on margins *versus* recurrence, but in the R1 resected patients 4 had intermediate risk according to NIH criteria and 50% (2/4) developed recurrence. Five patients had high risk and of these 100% (5/5) had recurrent disease. No recurrences were recorded in the low-risk group. Median follow up was 4.2 years (range 0.5-15). This study is difficult to interpret since conclusive data on risk factors for recurrent disease is lacking. Also, data on surgical margin is missing in more than 25% of the study population. However, the authors conclude that R1 resection is a risk factor for recurrence in intermediate and high-risk tumors [158].

A meta-analysis of twelve studies including 1 905 patients with resected GIST show negative impact on disease-free survival in patients who had R1 resection compared to R0 (HR 1.596, 95% CI 1.128–2.258), but not on overall survival (HR 1.430, 95% CI 0.608–3.363). The negative effect of microscopically positive margins was mitigated by imatinib treatment [159].

Although our study (**Paper II**) showed beneficial effects of R0 compared with R1 resection, the primary end point was to investigate the effects of wide margins. Both marginal and wide margins are R0. When excluding the patients operated with wide margins and analyzing intralesional (R1) versus marginal (R0) margins, the differences between the groups were no longer significant, which may help in understanding the findings in some of the previous studies that fail to show a positive effect of R0 versus R1 surgery in GIST, since the difference is, at least in our material, explained by the wide margins. Adjusting for tumor size, site and mitotic index, wide surgical margin was still an independent positive prognostic factor for recurrence-free survival. According to our definition of margin, all ruptured tumors are considered to have intralesional margins, regardless of the time of rupture (pre- or intra operatively).

Key findings:

- **Excision of GIST with a wide surgical margin improves progression-free and disease-specific survival**
- **Margin status is an independent risk factor regardless of tumor size mitotic index, and site**
- **When excluding patients operated with a wide margin, the prognostic impact of R0 versus R1 resection was no longer significant**

In **paper III,** we used an improved protocol of the analytical methods described in **paper I** to measure drug concentration in GIST cells from patients treated with imatinib prior to surgery. In this expanded cohort of patients, we could confirm the previous findings of great variability in imatinib concentrations, not only between different subjects, but also between different parts of the same tumor. The plasma levels were generally in line with expected results. Previous data has shown beneficial effect of imatinib plasma concentrations above 1100 ng/ml (2.23 μM) [160]. In the current study, mean plasma concentration was 2.16 μM (range 0.60-3.68 μM). However, concentrations above or below the cut-off proposed by Demetri *et al.* could not be correlated to either radiological or histological response. In fact, no correlations were found between plasma or tissue concentrations and response. This finding was somewhat surprising. Since all analyses were performed at the same time and using the same protocol for preparation and run in duplicates with small differences, the risk for methodological error as an explanation for this finding is low. However, considering the limited and relatively heterogeneous patient cohort, a type II error cannot be ruled out. On the other hand, the measurements of intracellular, or tumor tissue, imatinib is considered to reflect the total imatinib content, not accounting for sequestration in subcellular compartments or protein binding. Imatinib is bound to α1-acid glycoprotein to a large extent (>96%) [161]. This may imply that the total imatinib concentration is not predictive for response,

since only a minor and individually variable portion is free and available to reach its target site.

The hypothesis for this study was that acquired imatinib resistance could in part be explained by pharmacodynamic changes during tumor development, leading to decreased imatinib levels intracellularly. The finding that efflux protein expression was inversely correlated to response indicates that there might be some substance to this hypothesis, albeit not explained by changes in whole-cell imatinib concentration measurements. Interestingly, a recently published study on CML cell lines, that were rendered imatinib resistant by prolonged imatinib exposure, shows increased nilotinib effect when simultaneously blocking efflux transporter BRCP/ABCG2 [162]. To further explore TKI resistance mechanisms, studies of imatinib bioavailability and localization in subcellular compartments could be of interest but first, one would want to confirm the findings of MDR1 and ABCG2 efflux transporters as prognostic markers in GIST. This could preferably be performed in a larger patient cohort and possibly with quantitative PCR.

Key findings:

- **Efflux drug transporters ABCG2 and MDR1 expression is correlated to histologic response in GIST where absent expression is associated with better response**
- **Intracellular imatinib concentration in GIST shows large variability and is not correlated to plasma levels or histopathological response**

The epidemiological project, **paper IV**, was initially intended to assess GIST incidence in Sweden and the potential risk factors for developing GIST. However, it became evident that GIST diagnosis was extremely difficult to identify accurately in the registers used and the aim of the study was shifted to sarcoma in general. As it turned out, the major finding of this registry study was the increasing incidence of angiosarcoma of the breast and a correlation to a previous history of breast carcinoma. This finding is also in line with clinical observations of an increasing

number of patients presenting with angiosarcoma approximately five to ten years after breast cancer diagnosis and treatment. This observation led us to undertake the current study. The association to breast cancer may not be an entirely novel finding and concern has been raised in previous studies that increased use of radiotherapy following breast conserving surgery may lead to more patients developing secondary angiosarcoma [163-166], however, our results confirm other studies. The strength of our study is the population-based approach and the adequacy of Swedish registries. A weakness is that radiation data was only available for a subset of the included patients. When considering the implications of our findings, the well documented positive effect of breast conserving surgery and subsequent radiotherapy must be taken into account. For example, a meta-analysis of 17 studies on BCS ± RT, comprising 10 801 women, showed a reduced risk of recurrence (ARR 15.7%, 95% CI 13.7–17.7, p<0.00001) and an all-cause mortality (ARR 300.6–5.4, p=0.03). In this material there are however, subgroups with a higher risk of recurrence and therefore differences in benefit from RT are identified. The high-risk features include lymph node metastasis, lumpectomy (compared to more extensive surgery), young age at diagnosis and high-grade tumors as well as older women with ER positive tumors not treated with anti-estrogens. The authors assigned the study population retrospectively into three risk groups based on these factors and found an ARR for recurrence in the high-risk group (1 924 patients) of 24.3% (95% CI 19.6-29.0), in the intermediate group (3 763 patients) ARR was 12.4% (95% CI 9.7-15.1), whereas in the low risk group (1 600 patients) ARR was 6.9% (95% CI 2.2-11.6) [167].

Interestingly, a Swedish RCT by Wickberg *et al.* showed a protective effect by radiotherapy after breast-conserving surgery during the first five years after resection of the primary breast cancer, but no significant differences in survival at 20 year follow-ups [168]. With the growing evidence for an increasing incidence of radiation-associated angiosarcoma there might be a reason for prospective risk assessment when recommending breast cancer treatment. For patients with high-risk features, radiotherapy is beneficial following breast cancer, however in the low-risk groups, the possible side effects may outweigh the small risk reduction added by RT. Moreover, radiotherapy is currently recommended for the treatment of ductal

breast cancer *in situ* (DCIS). While this entity is not invasive cancer, DCIS may progress in 36% of patients and is treated with a combination of surgery, hormone therapy and RT. No survival benefits have been demonstrated with these treatments and currently, trials are underway to evaluate watchful waiting [169].

Key findings:

- **The incidence of angiosarcoma of the breast increased 4-fold during a 21-year period**
- **Compared with the control population, patients with angiosarcoma were heavily over-represented with a history of breast cancer**
- **Benign and borderline phyllodes tumors did not have a negative effect on survival but malignant phyllodes tumors had a poor prognosis, similar to that of angiosarcoma**

8 CONCLUSIONS

- Imatinib is measurable intracellularly (*proof-of-concept*, **paper I**) and is accumulated in tissue, leading to higher tumor tissue levels than those in plasma. The tissue concentrations show large variability between and within different individuals and are not correlated to plasma concentration or response (**paper III**).

- Expresion of cell membrane efflux transporters ABCG2 and MDR1 is associated with inferior response in imatinib treated GIST (**paper III**).

- Adequate surgical technique with wide surgical margins and careful handling of the tumor in order to prevent tumor rupture is of prognostic importance in GIST (**paper II**).

- The incidence of angiosarcoma of the breast is increasing in Sweden and previous breast cancer is a risk factor, possibly related to radiotherapy (**paper IV**).

9 SUMMARY IN SWEDISH

Sarkom är samlingsnamnet för en grupp olikartade och ovanliga tumörer som utgår från så kallade mesenkymala celler. Denna celltyp finns i hela kroppen och är ursprung för t ex bindväv, blodkärl, fett, muskler och skelett. De flesta sarkom är elakartade och de kan uppstå var som helst i kroppen. Sarkom beräknas utgöra cirka 1–2% av all cancer, och i dagsläget finns ett 100-tal olika varianter beskrivna. Det vanligaste sarkomet i bukhålan är gastrointestinal stromacellstumör (GIST), som årligen drabbar cirka 15 personer/miljon invånare. GIST uppkommer oftast i magsäcken (cirka 60%), följt av tunntarm (cirka 25–30%), men kan förekomma i hela magtarmkanalen. GIST i matstrupe, tolvfingertarm och ändtarm utgör cirka 3–5% respektive och tumören är mycket ovanlig i tjocktarmen. GIST kan uppstå i alla åldrar, men är vanligast hos personer över 60 år och drabbar män och kvinnor i samma utsträckning. Symtom på GIST inkluderar blödning, tidig mättnadskänsla eller en kännbar knöl. Ungefär hälften av patienterna har inga symtom och tumören upptäcks då av en slump i samband med utredning eller operation av annan orsak. Behandlingen av GIST är i första hand kirurgi, men i de fall där tumören är avancerad eller om det finns tecken till spridning (metastaser) ges medicinsk onkologisk behandling, antingen som enda behandling, eller i syfte att krympa tumören och möjliggöra kirurgi. Onkologisk behandling kan också bli aktuell för att minska återfallsrisk efter operation, i synnerhet om ursprungstumören är stor, har hög celldelningstakt eller tecken på skadad tumörkapsel, s k perforation.

Den cellulära mekanism som driver GIST är i de flesta fall en mutation i en tillväxtreceptor på cellytan; vanligast är KIT (75%) och PDGFRA (10%). Båda är så kallade tyrosinkinasreceptorer, och punktmutationer i genen som kodar för receptorn gör att “signal” ständigt är påslagen och stimulerar bl a celltillväxt och cellöverlevnad. Onkologisk behandling av GIST revolutionerades kring millennieskiftet då imatinib, en nyligen framtagen behandling mot kronisk lymfatisk leukemi, även visade sig vara mycket effektiv mot GIST. Vad imatinib gör är att selektivt blockera dessa receptorer, och slår på så vis av tillväxtsignalen. Införandet av imatinib förändrade dramatiskt utsikterna för patienter med GIST, och i många avseenden har GIST och imatinib blivit ett modellsystem för modern onkologisk

behandling. Gruppen av läkemedel, så kallade tyrosinkinashämmare, ofta förkortat TKI, har sedan vuxit stadigt och innefattar idag ett 10-tal registrerade varianter, och fler är på väg. Ett återkommande problem med TKI-behandling är att tumörcellerna efter hand ofta utvecklar motståndskraft, s k resistens, mot behandlingen vilket leder till återväxt av tumören.

I **delarbete I** beskrivs en ny metod för att mäta koncentration av läkemedlet imatinib i GIST-celler, både från cellodling och från tumörer som avlägsnats kirurgiskt. I odlade celler uppmättes högre koncentrationer i en imatinibkänslig cellinje, jämfört med en imatinibresistent cellinje. I prover från patienter uppmättes ansamling av imatinib i tumörceller jämfört med nivåerna i plasma. Koncentrationerna i tumörceller och plasma skiljde sig mycket mellan olika individer.

I **delarbete II** undersöktes betydelsen av kirurgisk marginal av frisk vävnad kring tumören vid resektion av GIST. De patienter som opererats med en så kallad ”vid marginal” med ≥2 cm frisk vävnad kring tumören uppvisade lägre risk för återfall jämfört med de patienter som opererats med mindre marginal (Hazard ratio 6.8 (1.4–32.7) för marginell marginal respektive 13.5 (3–61) för intralesionell marginal ($p = 0.003$)).

Delarbete III är en fortsättningsstudie av **delarbete I**, och här studerades en större grupp av GIST-patienter vilka förbehandlats med imatinib inför kirurgi. Med en vidareutveckling av metoden från delarbete I mättes imatinibkoncentrationer i tumörceller och plasma. Dessutom studerades uttrycket av läkemedelstransport-proteiner på tumörcellerna, eftersom imatinib aktivt behöver transporteras över cellmembranet för att utöva sin effekt. Resultaten visade stor variation avseende imatinibkoncentrationer och ingen signifikant koppling mellan nivåer i plasma och tumörceller, eller till effekt av behandlingen. Uttrycket av transportproteiner var däremot kopplat till behandlingseffekt, och ett lägre uttryck av utflödesproteinet ABCG2 var associerat till bättre effekt i form av låg celldelningstakt i tumören. Tumörceller som uttryckte antingen ABCG2 eller MDR1, båda utflödesproteiner, hade högre andel opåverkade (viabla) celler.

Sarkom kan, som nämnts ovan, uppstå i hela kroppen och i **delarbete IV** studerades förekomst av och prognos vid bröstsarkom. Patienter med sarkom i bröstet under perioden 1993–2013 jämfördes med en matchad kontrollgrupp som inte hade sarkom. Insjuknande i den ovanliga sjukdomen angiosarkom ökade fyrfaldigt under studieperioden. Angiosarkom är en ofta snabbt och diffust tillväxande tumör som utgår ifrån blod- eller lymfkärl, och spridning (metastasering) till lungorna är vanlig. Den förväntade överlevnaden är kort och aggressiv kirurgisk behandling kan krävas för att avlägsna tumören. Det har tidigare beskrivits att stålning är en riskfaktor för att utveckla angiosarkom. Flertalet av patienterna med angiosarkom i den aktuella studien hade tidigare behandlats för bröstcancer, en behandling som ofta innefattar strålning. Det fanns en tydlig skillnad mot kontrollgruppen avseende genomgången bröstcancer. Patienter med andra sarkomtyper i bröstet hade inte i lika hög grad en historia av bröstcancer.

Sammanfattningsvis studeras i denna avhandling några av de ovanliga sjukdomar som ingår i gruppen sarkom. Resultaten visar en möjlig delförklaring till resistensmekanism mot läkemedelsbehandling av GIST. Fortsatta studier av detta är av värde, och det finns data från andra forskargrupper där farmakologisk påverkan av transportproteiner kan ha betydelse för effekt av TKI behandling. Vidare belyses vikten av korrekt kirurgisk teknik vid resektion av GIST, vilket är högaktuellt i diskussioner om nationell nivåstrukturering som pågår i Sverige. Slutligen visas att insjuknande i den ovanliga tumörsjukdomen angiosarkom i bröst ökar, och överrepresentation av tidigare bröstcancer tyder på en koppling till tidigare strålbehandling. Fortsatt vaksamhet på denna utveckling är motiverad. Samtliga studier har gjorts med utgångspunkt från kliniskt mycket relevanta problem, och resultaten har bitvis redan satt avtryck i den kliniska vardagen vid utredning, behandling och uppföljning av patienter med sarkom.

10 ACKNOWLEDGEMENTS

I would like to express my gratitude to all the all the good and helpful people who have aided me in different ways during the work leading up to this thesis. Some of you are mentioned below;

Robert Bränström - huvudhandledare, för ditt stora engagemang och din outsinliga energi. För att du tror på mig och alltid ger en liten knuff i rätt riktning när det behövs. För din vänskap och för att du aldrig nöjer dig med det näst bästa. För att du alltid tar dig tid, även då den inte finns. Och inte minst för att du orkat med detta långa projekt, där du hastigt fick rycka in under olyckliga omständigheter.

Inga-Lena Nilsson - bihandledare, för din noggrannhet och ditt sinne för detaljer. För du trots (eller på grund av?) ditt gedigna kunnande om statistiska metoder inte lämnar något åt slumpen.

Jan Zedenius - bihandledare, för din träffsäkra humor och för att du generöst delar med dig av dina erfarenheter. För att du kritiskt granskar och värderar tillvaron och tar rollen som den tionde personen.

Lisa Prahl Wittberg - mentor, för många trevliga diskussioner som ibland även berört forskningen.

Johan Westerdahl postumt - tidigare handledare, för det vi hann med, vilket var alldeles för lite. Du är fortfarande saknad.

Jan Åhlén - medförfattare, för ditt stora kirurgiska mod och ditt engagemang för sarkom. För alla timmar på operation och för allt du lärt mig om sarkomkirurgi.

Fredrik Granath - medförfattare och statistisk stöttepelare, för ditt tålamod och din förmåga att hålla tråden över tid.

Robin Fröbom - medförfattare, för hjälp med provhantering och kloka inspel i skrivarbetet. Och inte minst för alla inhopp på operation. Du har räddat schemaläggaren många gånger.

Erik Berglund - medförfattare, för gott samarbete i början av min forskarutbildning.

Felix Haglund - medförfattare, patolog, för gott och smidigt samarbete samt fina bilder.

Johan Wejde - medförfattare, patolog, för ditt engagemang inom sarkompatologin och för att jag kan ringa och diskutera knepiga fall.

Catharina Larsson - medförfattare, för alla GIST-gruppmöten du organiserat och därigenom hjälpt till att skapa förutsättningar för en multidisciplinär sarkomforskning.

Kumari Ubhayasekera - coauthor, for fruitful collaboration and for letting a surgeon into your analytical lab.

Foteini Triantafyllopoulou - for assisting me while preparing tissue samples for analysis.

All personal på analytisk kemi, BMC i Uppsala, för trevligt bemötande på och kring labbet.

Nuvarande och tidigare endokrinkirurgkollegor på BEK/BES: **Anna Koman, Cia Ihre-Lundgren, David Thorsteinsson, Harald Blegen, Henrik Andersson, Ivan Shabo, Karin Lind, Magnus Kjellman, Bertil Hamberger, Jörgen Nordenström, Eva Reihner, Lars-Ove Farnebo, Martin Bäckdahl** och **Pelle Mattsson**, för att ni tillsammans skapar den bästa arbetsplatsen där vi har patienten i fokus. Högt kunnande och effektivitet samt öppen attityd för att hjälpa till där det behövs. Det är aldrig långtråkigt på BES.

Bröstkirurgerna på BES: **Amelia Chiorescu, Basel Abo Alniaj, Fredrik Lohmander, Hanna Fredholm, Helena Ikonomidis Sackey, Irma Fredriksson, Jan Frisell, Kerstin Sandelin** och **Maria Kouvaraki** för trevlig stämning och bra samarbete, inte minst kring bröstsarkompatienterna.

Nuvarande och tidigare onkologkollegor vid BES/sarkomcentrum Karolinska: **Andri Papakonstantinou, Christina Linder-Stragliotto, Elisabeth Lidbrink, Li Jalmsell** och **Maja Zemmler**, för gott och nära samarbete och för att jag nästan varje dag får lära mig mer om onkologisk behandling av sarkom.

Tumörortopedkollegor vid sarkomcentrum Karolinska: **Asle Hesla, Henrik Bauer, Otte Brosjö** och **Panos Tsagkozis**, för gott samarbete och för att vi nu faktiskt är mer än ett virtuellt centrum

Linus Blohmé, Ulrika Palmer Kazen, **Alireza Daryapeyma**, kärlkirurger, för gott och nära samarbete som kan göra det kirurgiskt omöjliga möjligt.

Tobias Lekberg - sektionschefkollega, rumskamrat, för trevligt småprat i vårt tjusiga rum och gott samarbete. Lycka till med din fortsatta forskning!

All personal på mottagningen och operationsplaneringen Endokrina tumörer och sarkom för att ni håller reda på tillvaron. Ingen glömd, men särskilt tack till **Liselotte Karlsson**, **Malin Kalliamvakou**, **Rebecka Lindvall**, **Annika Bungerfeldt**, **Maria Götesson** och **Rebecka Hallbeck**.

Toni Meeks för språkgranskning och fina formuleringar.

Petra Severin, Sofie Fredriksson, Tina Ahlbäck och **Violetta Fahlgren** för det administrativa stöd som gör att jag hinner ägna en stund åt forskningen emellanåt.

Ann-Britt Wikström för all administrativ hjälp under doktorandtiden.

Chatrin Lindahl, för gott samarbete under lång tid i dina olika roller inom BEK/BES/MMK.

Lunchgänget på forskarskolan: **Petri Rantanen, Peter Alpkvist, Janne Westerbacka**, för vänskap och alla samtal med perspektiv på tillvaron som forskande kliniker med småbarn.

Erik Sundström för en välorganiserad och givande forskarskola med inriktning molekylär medicin.

Min familj; mamma **Karin** för allt du gjort för mig både tidigt i livet och på senare år. Du är enastående.

Björn Jonsson, för ovärderligt barnvaktsstöd och mycket annat.

Pappa **Sven-Bertil** och **Gunilla** för många trevliga middagar och annan samvaro.

Min syster **Anna** för glada tillrop och för att du alltid ställer upp när det behövs.

Tore Eriksson och **Astrid Mäkitalo**, mina svärföräldrar, för alla trevliga stunder både nära och långt borta och för att ni tar så väl hand om våra barn.

Min kära hustru **Elin** för att du finns och för allt stöd, inte bara i samband med det här projektet, utan genomgående i livet. Tillsammans är vi det bästa teamet!

Tor, Edwin och **Iris**, för att ni ser till att upprätthålla en hög och konstant entropi. För att ni stått ut med att jag skrivit den här boken, fast jag stundtals varit en tråkig pappa. För att ni alltid kan få mig att tänka på annat än jobb och forskning, vilket kan vara en nödvändig avslappning och för att ni är det finaste jag har.

11 REFERENCES

1. Hajdu SI: **Soft tissue sarcomas**. *Cancer* 2007, **109**(9):1697-1704.
2. Hajdu SI: **Greco-Roman thought about cancer**. *Cancer* 2004, **100**(10):2048-2051.
3. Borst M: **Die Lehre von den Geschwulsten : mit einem mikroskopischen Atlas**. Wiesbaden; 1902.
4. Ewing J: **NEOPLASTIC DISEASES**. *Annals of Surgery* 1919, **69**(3).
5. Stout AP, Pathology AFIo: **Tumors of the Soft Tissues**: Armed Forces Institute of Pathology; 1953.
6. Enzinger FM, Lattes R, Torloni H, World Health O: **Histological typing of soft tissue tumours / F. M. Enzinger, in collaboration with R. Lattes, H. Torloni and pathologists in fourteen countries**. In. Geneva: World Health Organization; 1969.
7. Jo VY, Fletcher CD: **WHO classification of soft tissue tumours: an update based on the 2013 (4th) edition**. *Pathology* 2014, **46**(2):95-104.
8. Fletcher CD: **The evolving classification of soft tissue tumours - an update based on the new 2013 WHO classification**. *Histopathology* 2014, **64**(1):2-11.
9. Kallen ME, Hornick JL: **The 2020 WHO Classification: What's New in Soft Tissue Tumor Pathology?** *The American journal of surgical pathology* 2020.
10. Gamboa AC, Gronchi A, Cardona K: **Soft-tissue sarcoma in adults: An update on the current state of histiotype-specific management in an era of personalized medicine**. *CA: a cancer journal for clinicians* 2020, **70**(3):200-229.
11. Katz D, Palmerini E, Pollack SM: **More Than 50 Subtypes of Soft Tissue Sarcoma: Paving the Path for Histology-Driven Treatments**. *American Society of Clinical Oncology Educational Book* 2018(38):925-938.
12. Demetri GD: **Evolution of the International Sarcoma Community: A Personal Perspective**. *Oncology* 2018, **95 Suppl 1**:1-4.
13. Amadeo B, Penel N, Coindre JM, Ray-Coquard I, Ligier K, Delafosse P, Bouvier AM, Plouvier S, Gallet J, Lacourt A *et al*: **Incidence and time trends of sarcoma (2000-2013): results from the French network of cancer registries (FRANCIM)**. *BMC Cancer* 2020, **20**(1):190.
14. Gatta G, Capocaccia R, Botta L, Mallone S, De Angelis R, Ardanaz E, Comber H, Dimitrova N, Leinonen MK, Siesling S *et al*: **Burden and centralised treatment in Europe of rare tumours: results of RARECAREnet-a population-based study**. *Lancet Oncol* 2017, **18**(8):1022-1039.
15. Stiller CA, Trama A, Serraino D, Rossi S, Navarro C, Chirlaque MD, Casali PG: **Descriptive epidemiology of sarcomas in Europe: report from the RARECARE project**. *Eur J Cancer* 2013, **49**(3):684-695.
16. Trautmann F, Schuler M, Schmitt J: **Burden of soft-tissue and bone sarcoma in routine care: Estimation of incidence, prevalence and survival for health services research**. *Cancer epidemiology* 2015.
17. Mastrangelo G, Coindre JM, Ducimetiere F, Dei Tos AP, Fadda E, Blay JY, Buja A, Fedeli U, Cegolon L, Frasson A *et al*: **Incidence of soft tissue sarcoma and beyond: a population-based prospective study in 3 European regions**. *Cancer* 2012, **118**(21):5339-5348.
18. Mastrangelo G, Fadda E, Cegolon L, Montesco MC, Ray-Coquard I, Buja A, Fedeli U, Frasson A, Spolaore P, Rossi CR: **A European project on incidence, treatment, and outcome of sarcoma**. *BMC public health* 2010, **10**:188.
19. Ducimetiere F, Lurkin A, Ranchere-Vince D, Decouvelaere AV, Peoc'h M, Istier L, Chalabreysse P, Muller C, Alberti L, Bringuier PP *et al*: **Incidence of sarcoma histotypes and molecular subtypes in a prospective epidemiological study with central pathology review and molecular testing**. *PloS one* 2011, **6**(8):e20294.

20. Maretty-Nielsen K, Aggerholm-Pedersen N, Keller J, Safwat A, Baerentzen S, Pedersen AB: **Population-based Aarhus Sarcoma Registry: validity, completeness of registration, and incidence of bone and soft tissue sarcomas in western Denmark**. *Clinical epidemiology* 2013, **5**:45-56.
21. Nomura E, Ioka A, Tsukuma H: **Incidence of soft tissue sarcoma focusing on gastrointestinal stromal sarcoma in Osaka, Japan, during 1978-2007**. *Japanese journal of clinical oncology* 2013, **43**(8):841-845.
22. Rubio-Casadevall J, Borras JL, Carmona C, Ameijide A, Osca G, Vilardell L, Izquierdo A, Galceran J, Marcos-Gragera R: **Temporal trends of incidence and survival of sarcoma of digestive tract including Gastrointestinal Stromal Tumours (GIST) in two areas of the north-east of Spain in the period 1981-2005: a population-based study**. *Clinical & translational oncology : official publication of the Federation of Spanish Oncology Societies and of the National Cancer Institute of Mexico* 2014, **16**(7):660-667.
23. Ferrari A, Sultan I, Huang TT, Rodriguez-Galindo C, Shehadeh A, Meazza C, Ness KK, Casanova M, Spunt SL: **Soft tissue sarcoma across the age spectrum: a population-based study from the Surveillance Epidemiology and End Results database**. *Pediatric blood & cancer* 2011, **57**(6):943-949.
24. Porter GA, Baxter NN, Pisters PW: **Retroperitoneal sarcoma: a population-based analysis of epidemiology, surgery, and radiotherapy**. *Cancer* 2006, **106**(7):1610-1616.
25. Virtanen A, Pukkala E, Auvinen A: **Incidence of bone and soft tissue sarcoma after radiotherapy: a cohort study of 295,712 Finnish cancer patients**. *International journal of cancer Journal international du cancer* 2006, **118**(4):1017-1021.
26. Vogelstein B, Lane D, Levine AJ: **Surfing the p53 network**. *Nature* 2000, **408**(6810):307-310.
27. Gonin-Laurent N, Gibaud A, Huygue M, Lefevre SH, Le Bras M, Chauveinc L, Sastre-Garau X, Doz F, Lumbroso L, Chevillard S *et al*: **Specific TP53 mutation pattern in radiation-induced sarcomas**. *Carcinogenesis* 2006, **27**(6):1266-1272.
28. Bourcier K, Le Cesne A, Tselikas L, Adam J, Mir O, Honore C, de Baere T: **Basic Knowledge in Soft Tissue Sarcoma**. *Cardiovasc Intervent Radiol* 2019, **42**(9):1255-1261.
29. Skubitz KM, D'Adamo DR: **Sarcoma**. *Mayo Clin Proc* 2007, **82**(11):1409-1432.
30. Berger-Richardson D, Swallow CJ: **Needle tract seeding after percutaneous biopsy of sarcoma: Risk/benefit considerations**. *Cancer* 2017, **123**(4):560-567.
31. Blay JY, Soibinet P, Penel N, Bompas E, Duffaud F, Stoeckle E, Mir O, Adam J, Chevreau C, Bonvalot S *et al*: **Improved survival using specialized multidisciplinary board in sarcoma patients**. *Annals of oncology : official journal of the European Society for Medical Oncology / ESMO* 2017, **28**(11):2852-2859.
32. Ray-Coquard I, Thiesse P, Ranchere-Vince D, Chauvin F, Bobin JY, Sunyach MP, Carret JP, Mongodin B, Marec-Berard P, Philip T *et al*: **Conformity to clinical practice guidelines, multidisciplinary management and outcome of treatment for soft tissue sarcomas**. *Annals of oncology : official journal of the European Society for Medical Oncology / ESMO* 2004, **15**(2):307-315.
33. van Houdt WJ, Zaidi S, Messiou C, Thway K, Strauss DC, Jones RL: **Treatment of retroperitoneal sarcoma: current standards and new developments**. *Current Opinion in Oncology* 2017, **29**(4):260-267.
34. Trojani M, Contesso G, Coindre JM, Rouesse J, Bui NB, de Mascarel A, Goussot JF, David M, Bonichon F, Lagarde C: **Soft-tissue sarcomas of adults; study of pathological prognostic variables and definition of a histopathological grading**

system. *International journal of cancer Journal international du cancer* 1984, **33**(1):37-42.
35. Baig M: **Evaluation of performance of various histological grading systems of soft tissue sarcomas and the prognosis (metastatic risk and survival rate)**. *International Journal of Research in Medical Sciences* 2015, **3**(9):2394-2401.
36. Costa J, Wesley RA, Glatstein E, Rosenberg SA: **The grading of soft tissue sarcomas. Results of a clinicohistopathologic correlation in a series of 163 cases**. *Cancer* 1984, **53**(3):530-541.
37. Amin M, Edge S, Greene F: **AJCC Cancer Staging Manual**: Springer International Publishing; 2017.
38. Fisher SB, Chiang YJ, Feig BW, Cormier JN, Hunt KK, Torres KE, Roland CL: **Comparative Performance of the 7th and 8th Editions of the American Joint Committee on Cancer Staging Systems for Soft Tissue Sarcoma of the Trunk and Extremities**. *Ann Surg Oncol* 2018, **25**(5):1126-1132.
39. Fisher SB, Chiang YJ, Feig BW, Cormier JN, Hunt KK, Torres KE, Roland CL: **An Evaluation of the Eighth Edition of the American Joint Committee on Cancer (AJCC) Staging System for Retroperitoneal Sarcomas Using the National Cancer Data Base (NCDB): Does Size Matter?** *American journal of clinical oncology* 2019, **42**(2):160-165.
40. Fong Y, Coit DG, Woodruff JM, Brennan MF: **Lymph node metastasis from soft tissue sarcoma in adults. Analysis of data from a prospective database of 1772 sarcoma patients**. *Ann Surg* 1993, **217**(1):72-77.
41. Spolverato G, Callegaro D, Gronchi A: **Defining Which Patients Are at High Risk for Recurrence of Soft Tissue Sarcoma**. *Current Treatment Options in Oncology* 2020, **21**(7):56.
42. Chiang NJ, Chen LT, Tsai CR, Chang JS: **The epidemiology of gastrointestinal stromal tumors in Taiwan, 1998-2008: a nation-wide cancer registry-based study**. *BMC Cancer* 2014, **14**:9.
43. Mucciarini C, Rossi G, Bertolini F, Valli R, Cirilli C, Rashid I, Marcheselli L, Luppi G, Federico M: **Incidence and clinicopathologic features of gastrointestinal stromal tumors. A population-based study**. *BMC Cancer* 2007, **7**:7.
44. Tryggvason G, Gislason HG, Magnusson MK, Jonasson JG: **Gastrointestinal stromal tumors in Iceland, 1990-2003: The Icelandic GIST study, a population-based incidence and pathologic risk stratification study**. *Int J Cancer* 2005, **117**(2):289-293.
45. Corless CL: **Gastrointestinal stromal tumors: what do we know now?** *Mod Pathol* 2014, **27 Suppl 1**:S1-16.
46. Soreide K, Sandvik OM, Soreide JA, Giljaca V, Jureckova A, Bulusu VR: **Global epidemiology of gastrointestinal stromal tumours (GIST): A systematic review of population-based cohort studies**. *Cancer epidemiology* 2016, **40**:39-46.
47. Nilsson B, Bumming P, Meis-Kindblom JM, Oden A, Dortok A, Gustavsson B, Sablinska K, Kindblom LG: **Gastrointestinal stromal tumors: the incidence, prevalence, clinical course, and prognostication in the preimatinib mesylate era--a population-based study in western Sweden**. *Cancer* 2005, **103**(4):821-829.
48. Keung EZ, Raut CP: **Management of Gastrointestinal Stromal Tumors**. *The Surgical clinics of North America* 2017, **97**(2):437-452.
49. Etherington MS, DeMatteo RP: **Tailored management of primary gastrointestinal stromal tumors**. *Cancer* 2019, **125**(13):2164-2171.
50. Mazur MT, Clark HB: **Gastric stromal tumors. Reappraisal of histogenesis**. *The American journal of surgical pathology* 1983, **7**(6):507-519.

51. Miettinen M: **Gastrointestinal stromal tumors. An immunohistochemical study of cellular differentiation**. *American journal of clinical pathology* 1988, **89**(5):601-610.
52. Miettinen M, Makhlouf H, Sobin LH, Lasota J: **Gastrointestinal stromal tumors of the jejunum and ileum: a clinicopathologic, immunohistochemical, and molecular genetic study of 906 cases before imatinib with long-term follow-up**. *The American journal of surgical pathology* 2006, **30**(4):477-489.
53. Novelli M, Rossi S, Rodriguez-Justo M, Taniere P, Seddon B, Toffolatti L, Sartor C, Hogendoorn PC, Sciot R, Van Glabbeke M *et al*: **DOG1 and CD117 are the antibodies of choice in the diagnosis of gastrointestinal stromal tumours**. *Histopathology* 2010, **57**(2):259-270.
54. Miettinen M, Wang ZF, Lasota J: **DOG1 antibody in the differential diagnosis of gastrointestinal stromal tumors: a study of 1840 cases**. *The American journal of surgical pathology* 2009, **33**(9):1401-1408.
55. Fletcher CD, Berman JJ, Corless C, Gorstein F, Lasota J, Longley BJ, Miettinen M, O'Leary TJ, Remotti H, Rubin BP *et al*: **Diagnosis of gastrointestinal stromal tumors: A consensus approach**. *Hum Pathol* 2002, **33**(5):459-465.
56. Motegi A, Sakurai S, Nakayama H, Sano T, Oyama T, Nakajima T: **PKC theta, a novel immunohistochemical marker for gastrointestinal stromal tumors (GIST), especially useful for identifying KIT-negative tumors**. *Pathol Int* 2005, **55**(3):106-112.
57. West RB, Corless CL, Chen X, Rubin BP, Subramanian S, Montgomery K, Zhu S, Ball CA, Nielsen TO, Patel R *et al*: **The novel marker, DOG1, is expressed ubiquitously in gastrointestinal stromal tumors irrespective of KIT or PDGFRA mutation status**. *Am J Pathol* 2004, **165**(1):107-113.
58. Blume-Jensen P, Claesson-Welsh L, Siegbahn A, Zsebo KM, Westermark B, Heldin CH: **Activation of the human c-kit product by ligand-induced dimerization mediates circular actin reorganization and chemotaxis**. *EMBO J* 1991, **10**(13):4121-4128.
59. Hirota S, Isozaki K, Moriyama Y, Hashimoto K, Nishida T, Ishiguro S, Kawano K, Hanada M, Kurata A, Takeda M *et al*: **Gain-of-function mutations of c-kit in human gastrointestinal stromal tumors**. *Science* 1998, **279**(5350):577-580.
60. Serrano C, Mariño-Enríquez A, Tao DL, Ketzer J, Eilers G, Zhu M, Yu C, Mannan AM, Rubin BP, Demetri GD *et al*: **Complementary activity of tyrosine kinase inhibitors against secondary kit mutations in imatinib-resistant gastrointestinal stromal tumours**. *British journal of cancer* 2019, **120**(6):612-620.
61. Mazzocca A, Napolitano A, Silletta M, Spalato Ceruso M, Santini D, Tonini G, Vincenzi B: **New frontiers in the medical management of gastrointestinal stromal tumours**. *Ther Adv Med Oncol* 2019, **11**:1758835919841946.
62. Heinrich MC, Corless CL, Blanke CD, Demetri GD, Joensuu H, Roberts PJ, Eisenberg BL, von Mehren M, Fletcher CD, Sandau K *et al*: **Molecular correlates of imatinib resistance in gastrointestinal stromal tumors**. *Journal of clinical oncology : official journal of the American Society of Clinical Oncology* 2006, **24**(29):4764-4774.
63. Liegl B, Hornick JL, Corless CL, Fletcher CD: **Monoclonal antibody DOG1.1 shows higher sensitivity than KIT in the diagnosis of gastrointestinal stromal tumors, including unusual subtypes**. *The American journal of surgical pathology* 2009, **33**(3):437-446.
64. Rock JR, Futtner CR, Harfe BD: **The transmembrane protein TMEM16A is required for normal development of the murine trachea**. *Dev Biol* 2008, **321**(1):141-149.

65. Ferrera L, Caputo A, Galietta LJ: **TMEM16A protein: a new identity for Ca(2+)-dependent Cl(-) channels**. *Physiology (Bethesda, Md)* 2010, **25**(6):357-363.
66. Berglund E, Akcakaya P, Berglund D, Karlsson F, Vukojević V, Lee L, Bogdanović D, Lui WO, Larsson C, Zedenius J *et al*: **Functional role of the Ca^{2+}-activated Cl^- channel DOG1/TMEM16A in gastrointestinal stromal tumor cells**. *Exp Cell Res* 2014, **326**(2):315-325.
67. Fröbom R, Sellberg F, Xu C, Zhao A, Larsson C, Lui WO, Nilsson IL, Berglund E, Bränström R: **Biochemical Inhibition of DOG1/TMEM16A Achieves Antitumoral Effects in Human Gastrointestinal Stromal Tumor Cells In Vitro**. *Anticancer Res* 2019, **39**(7):3433-3442.
68. Melo JV, Gordon DE, Tuszynski A, Dhut S, Young BD, Goldman JM: **Expression of the ABL-BCR fusion gene in Philadelphia-positive acute lymphoblastic leukemia**. *Blood* 1993, **81**(10):2488-2491.
69. Druker BJ, Lydon NB: **Lessons learned from the development of an Abl tyrosine kinase inhibitor for chronic myelogenous leukemia**. *Journal of Clinical Investigation* 2000, **105**(1):3-7.
70. Joensuu H, Roberts PJ, Sarlomo-Rikala M, Andersson LC, Tervahartiala P, Tuveson D, Silberman SL, Capdeville R, Dimitrijevic S, Druker B *et al*: **Effect of the Tyrosine Kinase Inhibitor STI571 in a Patient with a Metastatic Gastrointestinal Stromal Tumor**. *New England Journal of Medicine* 2001, **344**(14):1052-1056.
71. Casali PG, Abecassis N, Aro HT, Bauer S, Biagini R, Bielack S, Bonvalot S, Boukovinas I, Bovee J, Brodowicz T *et al*: **Gastrointestinal stromal tumours: ESMO-EURACAN Clinical Practice Guidelines for diagnosis, treatment and follow-up**. *Annals of oncology : official journal of the European Society for Medical Oncology / ESMO* 2018, **29**(Suppl 4):iv68-iv78.
72. **Soft tissue and visceral sarcomas: ESMO Clinical Practice Guidelines for diagnosis, treatment and follow-up**. *Annals of oncology : official journal of the European Society for Medical Oncology / ESMO* 2014, **25 Suppl 3**:iii102-112.
73. Eisenberg BL, Harris J, Blanke CD, Demetri GD, Heinrich MC, Watson JC, Hoffman JP, Okuno S, Kane JM, von Mehren M: **Phase II trial of neoadjuvant/adjuvant imatinib mesylate (IM) for advanced primary and metastatic/recurrent operable gastrointestinal stromal tumor (GIST): early results of RTOG 0132/ACRIN 6665**. *J Surg Oncol* 2009, **99**(1):42-47.
74. Blesius A, Cassier PA, Bertucci F, Fayette J, Ray-Coquard I, Bui B, Adenis A, Rios M, Cupissol D, Perol D *et al*: **Neoadjuvant imatinib in patients with locally advanced non metastatic GIST in the prospective BFR14 trial**. *BMC Cancer* 2011, **11**:72.
75. Tirumani SH, Shinagare AB, Jagannathan JP, Krajewski KM, Ramaiya NH, Raut CP: **Radiologic assessment of earliest, best, and plateau response of gastrointestinal stromal tumors to neoadjuvant imatinib prior to successful surgical resection**. *Eur J Surg Oncol* 2014, **40**(4):420-428.
76. Landi B, Blay J-Y, Bonvalot S, Brasseur M, Coindre JM, Emile JF, Hautefeuille V, Honore C, Lartigau E, Mantion G *et al*: **Gastrointestinal stromal tumours (GISTs): French Intergroup Clinical Practice Guidelines for diagnosis, treatments and follow-up (SNFGE, FFCD, GERCOR, UNICANCER, SFCD, SFED, SFRO)**. *Digestive and Liver Disease* 2019, **51**(9):1223-1231.
77. Norton JA, Kim T, Kim J, McCarter MD, Kelly KJ, Wong J, Sicklick JK: **SSAT State-of-the-Art Conference: Current Surgical Management of Gastric Tumors**. *Journal of Gastrointestinal Surgery* 2018, **22**(1):32-42.
78. Joensuu H: **Gastrointestinal stromal tumors: risk assessment and adjuvant therapy**. *Hematol Oncol Clin North Am* 2013, **27**(5):889-904.

79. Wittekind C, Compton CC, Greene FL, Sobin LH: **TNM residual tumor classification revisited**. *Cancer* 2002, **94**(9):2511-2516.
80. Rydholm A: **Surgical margins for soft tissue sarcoma**. *Acta orthopaedica Scandinavica Supplementum* 1997, **273**:81-85.
81. Enneking WF, Spanier SS, Goodman MA: **A system for the surgical staging of musculoskeletal sarcoma**. *Clin Orthop Relat Res* 1980(153):106-120.
82. Rutkowski P, Skoczylas J, Wisniewski P: **Is the Surgical Margin in Gastrointestinal Stromal Tumors Different**. *Visceral Medicine* 2018, **34**(5):347-352.
83. Ahlen J, Karlsson F, Wejde J, Nilsson IL, Larsson C, Branstrom R: **Wide Surgical Margin Improves the Outcome for Patients with Gastrointestinal Stromal Tumors (GISTs)**. *World J Surg* 2018, **42**(8):2512-2521.
84. Schmieder M, Henne-Bruns D, Mayer B, Knippschild U, Rolke C, Schwab M, Kramer K: **Comparison of Different Risk Classification Systems in 558 Patients with Gastrointestinal Stromal Tumors after R0-Resection**. *Frontiers in Pharmacology* 2016, **7**:504.
85. Joensuu H, Vehtari A, Riihimaki J, Nishida T, Steigen SE, Brabec P, Plank L, Nilsson B, Cirilli C, Braconi C *et al*: **Risk of recurrence of gastrointestinal stromal tumour after surgery: an analysis of pooled population-based cohorts**. *Lancet Oncol* 2012, **13**(3):265-274.
86. Khoo CY, Chai X, Quek R, Teo MCC, Goh BKP: **Systematic review of current prognostication systems for primary gastrointestinal stromal tumors**. *European Journal of Surgical Oncology* 2018, **44**(4):388-394.
87. Fletcher CD, Berman JJ, Corless C, Gorstein F, Lasota J, Longley BJ, Miettinen M, O'Leary TJ, Remotti H, Rubin BP *et al*: **Diagnosis of gastrointestinal stromal tumors: a consensus approach**. *International journal of surgical pathology* 2002, **10**(2):81-89.
88. Miettinen M, Lasota J: **Gastrointestinal stromal tumors: pathology and prognosis at different sites**. *Semin Diagn Pathol* 2006, **23**(2):70-83.
89. Miettinen M, Lasota J: **Gastrointestinal stromal tumors: review on morphology, molecular pathology, prognosis, and differential diagnosis**. *Arch Pathol Lab Med* 2006, **130**(10):1466-1478.
90. Joensuu H: **Risk stratification of patients diagnosed with gastrointestinal stromal tumor**. *Hum Pathol* 2008, **39**(10):1411-1419.
91. Gold JS, Gönen M, Gutiérrez A, Broto JM, García-del-Muro X, Smyrk TC, Maki RG, Singer S, Brennan MF, Antonescu CR *et al*: **Development and validation of a prognostic nomogram for recurrence-free survival after complete surgical resection of localised primary gastrointestinal stromal tumour: a retrospective analysis**. *The Lancet Oncology* 2009, **10**(11):1045-1052.
92. Rossi S, Miceli R, Messerini L, Bearzi I, Mazzoleni G, Capella C, Arrigoni G, Sonzogni A, Sidoni A, Toffolatti L *et al*: **Natural History of Imatinib-naive GISTs: A Retrospective Analysis of 929 Cases With Long-term Follow-up and Development of a Survival Nomogram Based on Mitotic Index and Size as Continuous Variables**. *The American journal of surgical pathology* 2011, **35**(11).
93. Bischof DA, Kim Y, Behman R, Karanicolas PJ, Quereshy FA, Blazer DG, Maithel SK, Gamblin TC, Bauer TW, Pawlik TM: **A Nomogram to Predict Disease-Free Survival After Surgical Resection of GIST**. *Journal of Gastrointestinal Surgery* 2014, **18**(12):2123-2129.
94. Dematteo RP, Ballman KV, Antonescu CR, Maki RG, Pisters PW, Demetri GD, Blackstein ME, Blanke CD, von Mehren M, Brennan MF *et al*: **Adjuvant imatinib mesylate after resection of localised, primary gastrointestinal stromal tumour:**

a randomised, double-blind, placebo-controlled trial. *Lancet* 2009, **373**(9669):1097-1104.
95. Joensuu H, Eriksson M, Sundby Hall K, Hartmann JT, Pink D, Schütte J, Ramadori G, Hohenberger P, Duyster J, Al-Batran SE *et al*: **One vs three years of adjuvant imatinib for operable gastrointestinal stromal tumor: a randomized trial**. *Jama* 2012, **307**(12):1265-1272.
96. Joensuu H, Eriksson M, Sundby Hall K, Reichardt A, Hermes B, Schütte J, Cameron S, Hohenberger P, Jost PJ, Al-Batran S-E *et al*: **Survival Outcomes Associated With 3 Years vs 1 Year of Adjuvant Imatinib for Patients With High-Risk Gastrointestinal Stromal Tumors: An Analysis of a Randomized Clinical Trial After 10-Year Follow-up**. *JAMA Oncology* 2020, **6**(8):1241-1246.
97. Raut CP, Espat NJ, Maki RG, Araujo DM, Trent J, Williams TF, Purkayastha DD, DeMatteo RP: **Efficacy and Tolerability of 5-Year Adjuvant Imatinib Treatment for Patients With Resected Intermediate- or High-Risk Primary Gastrointestinal Stromal Tumor: The PERSIST-5 Clinical Trial**. *JAMA Oncology* 2018, **4**(12):e184060-e184060.
98. Miettinen M, Sobin LH, Lasota J: **Gastrointestinal Stromal Tumors of the Stomach: A Clinicopathologic, Immunohistochemical, and Molecular Genetic Study of 1765 Cases With Long-term Follow-up**. *The American journal of surgical pathology* 2005, **29**(1).
99. D'Ambrosio L, Palesandro E, Boccone P, Tolomeo F, Miano S, Galizia D, Manca A, Chiara G, Bertotto I, Russo F *et al*: **Impact of a risk-based follow-up in patients affected by gastrointestinal stromal tumour**. *Eur J Cancer* 2017, **78**:122-132.
100. Dessilly G, Elens L, Panin N, Karmani L, Demoulin JB, Haufroid V: **ABCB1 1199G>A polymorphism (rs2229109) affects the transport of imatinib, nilotinib and dasatinib**. *Pharmacogenomics* 2016, **17**(8):883-890.
101. Noguchi K, Katayama K, Sugimoto Y: **Human ABC transporter ABCG2/BCRP expression in chemoresistance: basic and clinical perspectives for molecular cancer therapeutics**. *Pharmacogenomics and personalized medicine* 2014, **7**:53-64.
102. Lim KT, Tan KY: **Current research and treatment for gastrointestinal stromal tumors**. *World journal of gastroenterology* 2017, **23**(27):4856-4866.
103. Demetri GD, van Oosterom AT, Garrett CR, Blackstein ME, Shah MH, Verweij J, McArthur G, Judson IR, Heinrich MC, Morgan JA *et al*: **Efficacy and safety of sunitinib in patients with advanced gastrointestinal stromal tumour after failure of imatinib: a randomised controlled trial**. *Lancet* 2006, **368**(9544):1329-1338.
104. Demetri GD, Reichardt P, Kang YK, Blay JY, Rutkowski P, Gelderblom H, Hohenberger P, Leahy M, von Mehren M, Joensuu H *et al*: **Efficacy and safety of regorafenib for advanced gastrointestinal stromal tumours after failure of imatinib and sunitinib (GRID): an international, multicentre, randomised, placebo-controlled, phase 3 trial**. *Lancet* 2013, **381**(9863):295-302.
105. Mir O, Cropet C, Toulmonde M, Cesne AL, Molimard M, Bompas E, Cassier P, Ray-Coquard I, Rios M, Adenis A *et al*: **Pazopanib plus best supportive care versus best supportive care alone in advanced gastrointestinal stromal tumours resistant to imatinib and sunitinib (PAZOGIST): a randomised, multicentre, open-label phase 2 trial**. *Lancet Oncol* 2016, **17**(5):632-641.
106. Heinrich MC, Jones RL, Mehren Mv, Schoffski P, Bauer S, Mir O, Cassier PA, Eskens F, Shi H, Alvarez-Diez T *et al*: **Clinical activity of BLU-285 in advanced gastrointestinal stromal tumor (GIST)**. *Journal of Clinical Oncology* 2017, **35**(15_suppl):11011-11011.

107. Heinrich MC, Jones RL, von Mehren M, Schöffski P, Serrano C, Kang YK, Cassier PA, Mir O, Eskens F, Tap WD *et al*: **Avapritinib in advanced PDGFRA D842V-mutant gastrointestinal stromal tumour (NAVIGATOR): a multicentre, open-label, phase 1 trial**. *Lancet Oncol* 2020, **21**(7):935-946.
108. Serrano C, Leal A, Phallen J, Marino-Enriquez A, Kuang Y, Triplett O, Morgan JA, Barysauskas C, Wagner AJ, Demetri GD *et al*: **Phase Ib study of rapid alternation of sunitinib (SU) and regorafenib (RE) in patients (pts) with advanced gastrointestinal stromal tumor (GIST)**. *Journal of Clinical Oncology* 2018, **36**(15_suppl):11510-11510.
109. von Mehren M, Serrano C, Bauer S, Gelderblom H, George S, Heinrich M, Schöffski P, Zalcberg J, Chi P, Jones RL *et al*: **LBA87 - INVICTUS: A phase III, interventional, double-blind, placebo-controlled study to assess the safety and efficacy of ripretinib as≥4th-line therapy in patients with advanced gastrointestinal stromal tumors (GIST) who have received treatment with prior anticancer therapies (NCT03353753)**. *Annals of Oncology* 2019, **30**:v925-v926.
110. Montemurro M, Schöffski P, Reichardt P, Gelderblom H, Schütte J, Hartmann JT, von Moos R, Seddon B, Joensuu H, Wendtner CM *et al*: **Nilotinib in the treatment of advanced gastrointestinal stromal tumours resistant to both imatinib and sunitinib**. *European Journal of Cancer* 2009, **45**(13):2293-2297.
111. Sawaki A, Nishida T, Doi T, Yamada Y, Komatsu Y, Kanda T, Kakeji Y, Onozawa Y, Yamasaki M, Ohtsu A: **Phase 2 study of nilotinib as third-line therapy for patients with gastrointestinal stromal tumor**. *Cancer* 2011, **117**(20):4633-4641.
112. Cauchi C, Somaiah N, Engstrom PF, Litwin S, Lopez M, Lee J, Davey M, Bove B, von Mehren M: **Evaluation of nilotinib in advanced GIST previously treated with imatinib and sunitinib**. *Cancer chemotherapy and pharmacology* 2012, **69**(4):977-982.
113. Reichardt P, Blay JY, Gelderblom H, Schlemmer M, Demetri GD, Bui-Nguyen B, McArthur GA, Yazji S, Hsu Y, Galetic I *et al*: **Phase III study of nilotinib versus best supportive care with or without a TKI in patients with gastrointestinal stromal tumors resistant to or intolerant of imatinib and sunitinib**. *Annals of Oncology* 2012, **23**(7):1680-1687.
114. Adenis A, Blay JY, Bui-Nguyen B, Bouché O, Bertucci F, Isambert N, Bompas E, Chaigneau L, Domont J, Ray-Coquard I *et al*: **Masitinib in advanced gastrointestinal stromal tumor (GIST) after failure of imatinib: a randomized controlled open-label trial**. *Annals of oncology : official journal of the European Society for Medical Oncology / ESMO* 2014, **25**(9):1762-1769.
115. Kang YK, Yoo C, Ryoo BY, Lee JJ, Tan E, Park I, Park JH, Choi YJ, Jo J, Ryu JS *et al*: **Phase II study of dovitinib in patients with metastatic and/or unresectable gastrointestinal stromal tumours after failure of imatinib and sunitinib**. *British journal of cancer* 2013, **109**(9):2309-2315.
116. Joensuu H, Blay JY, Comandone A, Martin-Broto J, Fumagalli E, Grignani G, Del Muro XG, Adenis A, Valverde C, Pousa AL *et al*: **Dovitinib in patients with gastrointestinal stromal tumour refractory and/or intolerant to imatinib**. *British journal of cancer* 2017, **117**(9):1278-1285.
117. Kindler HL, Campbell NP, Wroblewski K, Maki RG, D'Adamo DR, Chow WA, Gandara DR, Antonescu C, Stadler WM, Vokes EE: **Sorafenib (SOR) in patients (pts) with imatinib (IM) and sunitinib (SU)-resistant (RES) gastrointestinal stromal tumors (GIST): Final results of a University of Chicago Phase II Consortium trial**. *Journal of Clinical Oncology* 2011, **29**(15_suppl):10009-10009.
118. Park SH, Ryu MH, Ryoo BY, Im SA, Kwon HC, Lee SS, Park SR, Kang BY, Kang YK: **Sorafenib in patients with metastatic gastrointestinal stromal tumors who**

failed two or more prior tyrosine kinase inhibitors: a phase II study of Korean gastrointestinal stromal tumors study group. *Invest New Drugs* 2012, **30**(6):2377-2383.
119. Serrano C, George S, Valverde C, Olivares D, Garcia-Valverde A, Suarez C, Morales-Barrera R, Carles J: **Novel Insights into the Treatment of Imatinib-Resistant Gastrointestinal Stromal Tumors**. *Targeted oncology* 2017, **12**(3):277-288.
120. Sodergren SC, White A, Efficace F, Sprangers M, Fitzsimmons D, Bottomley A, Johnson CD: **Systematic review of the side effects associated with tyrosine kinase inhibitors used in the treatment of gastrointestinal stromal tumours on behalf of the EORTC Quality of Life Group**. *Critical Reviews in Oncology/Hematology* 2014, **91**(1):35-46.
121. van Oosterom AT, Judson I, Verweij J, Stroobants S, Donato di Paola E, Dimitrijevic S, Martens M, Webb A, Sciot R, Van Glabbeke M *et al*: **Safety and efficacy of imatinib (STI571) in metastatic gastrointestinal stromal tumours: a phase I study**. *Lancet* 2001, **358**(9291):1421-1423.
122. Jorg Thomas H, Michael H, Hans-Georg K, Hans-Peter L: **Tyrosine Kinase Inhibitors – A Review on Pharmacology, Metabolism and Side Effects**. *Current Drug Metabolism* 2009, **10**(5):470-481.
123. Lin NU, Sarantopoulos S, Stone JR, Galinsky I, Stone RM, Deangelo DJ, Soiffer RJ: **Fatal hepatic necrosis following imatinib mesylate therapy**. *Blood* 2003, **102**(9):3455-3456.
124. Ridruejo E, Cacchione R, Villamil AG, Marciano S, Gadano AC, Mandó OG: **Imatinib-induced fatal acute liver failure**. *World journal of gastroenterology* 2007, **13**(48):6608-6111.
125. Cross TJ, Bagot C, Portmann B, Wendon J, Gillett D: **Imatinib mesylate as a cause of acute liver failure**. *Am J Hematol* 2006, **81**(3):189-192.
126. Yachoui R: **Early onset imatinib mesylate-induced hepatotoxicity in a patient with gastrointestinal stromal tumors**. *Am J Ther* 2014, **21**(5):e148-150.
127. Nassar I, Pasupati T, Judson JP, Segarra I: **Histopathological study of the hepatic and renal toxicity associated with the co-administration of imatinib and acetaminophen in a preclinical mouse model**. *Malays J Pathol* 2010, **32**(1):1-11.
128. Ahmadieh H, Salti I: **Tyrosine kinase inhibitors induced thyroid dysfunction: a review of its incidence, pathophysiology, clinical relevance, and treatment**. *Biomed Res Int* 2013, **2013**:725410-725410.
129. Fallahi P, Ferrari SM, Vita R, Di Domenicantonio A, Corrado A, Benvenga S, Antonelli A: **Thyroid dysfunctions induced by tyrosine kinase inhibitors**. *Expert Opinion on Drug Safety* 2014, **13**(6):723-733.
130. Yin M, Mackley HB, Drabick JJ, Harvey HA: **Primary female breast sarcoma: clinicopathological features, treatment and prognosis**. *Sci Rep* 2016, **6**:31497.
131. Mitus JW, Blecharz P, Jakubowicz J, Reinfuss M, Walasek T, Wysocki W: **Phyllodes tumors of the breast. The treatment results for 340 patients from a single cancer centre**. *Breast* 2019, **43**:85-90.
132. Pencavel T, Allan CP, Thomas JM, Hayes AJ: **Treatment for breast sarcoma: A large, single-centre series**. *European Journal of Surgical Oncology (EJSO)* 2011, **37**(8):703-708.
133. Seinen JM, Styring E, Verstappen V, Vult von Steyern F, Rydholm A, Suurmeijer AJ, Hoekstra HJ: **Radiation-associated angiosarcoma after breast cancer: high recurrence rate and poor survival despite surgical treatment with R0 resection**. *Ann Surg Oncol* 2012, **19**(8):2700-2706.

134. Hodgson NC, Bowen-Wells C, Moffat F, Franceschi D, Avisar E: **Angiosarcomas of the Breast: A Review of 70 Cases**. *American journal of clinical oncology* 2007, **30**(6).
135. Rubino C, Shamsaldin A, Le MG, Labbe M, Guinebretiere JM, Chavaudra J, de Vathaire F: **Radiation dose and risk of soft tissue and bone sarcoma after breast cancer treatment**. *Breast Cancer Res Treat* 2005, **89**(3):277-288.
136. Arora TK, Terracina KP, Soong J, Idowu MO, Takabe K: **Primary and secondary angiosarcoma of the breast**. *Gland surgery* 2014, **3**(1):28-34.
137. Kadouri L, Sagi M, Goldberg Y, Lerer I, Hamburger T, Peretz T: **Genetic predisposition to radiation induced sarcoma: possible role for BRCA and p53 mutations**. *Breast Cancer Res Treat* 2013, **140**(1):207-211.
138. Cui L, Zhang J, Zhang X, Chang H, Qu C, Zhang J, Zhong D: **Angiosarcoma (Stewart-Treves syndrome) in postmastectomy patients: report of 10 cases and review of literature**. *Int J Clin Exp Pathol* 2015, **8**(9):11108-11115.
139. Stewart FW, Treves N: **Lymphangiosarcoma in postmastectomy lymphedema; a report of six cases in elephantiasis chirurgica**. *Cancer* 1948, **1**(1):64-81.
140. Fury MG, Antonescu CR, Van Zee KJ, Brennan MF, Maki RG: **A 14-year retrospective review of angiosarcoma: clinical characteristics, prognostic factors, and treatment outcomes with surgery and chemotherapy**. *Cancer J* 2005, **11**(3):241-247.
141. Fayette J, Martin E, Piperno-Neumann S, Le Cesne A, Robert C, Bonvalot S, Ranchere D, Pouillart P, Coindre JM, Blay JY: **Angiosarcomas, a heterogeneous group of sarcomas with specific behavior depending on primary site: a retrospective study of 161 cases**. *Annals of oncology : official journal of the European Society for Medical Oncology / ESMO* 2007, **18**(12):2030-2036.
142. Merfeld E, Gabani P, Spraker MB, Zoberi I, Kim H, Van Tine B, Chrisinger J, Michalski JM: **Clinical Outcomes and Prognostic Features of Angiosarcoma: Significance of Prior Radiation Therapy**. *Clin Oncol (R Coll Radiol)* 2019, **31**(4):232-241.
143. Li GZ, Fairweather M, Wang J, Orgill DP, Bertagnolli MM, Raut CP: **Cutaneous Radiation-associated Breast Angiosarcoma: Radicality of Surgery Impacts Survival**. *Annals of Surgery* 2017, **265**(4):814-820.
144. Styring E, Klasson S, Rydholm A, Vult von Steyern F: **Radiation-associated angiosarcoma after breast cancer: Improved survival by excision of all irradiated skin and soft tissue of the thoracic wall? A report of six patients**. *Acta Oncologica* 2015, **54**(7):1078-1080.
145. Parker SJ, Harries SA: **Phyllodes tumours**. *Postgraduate Medical Journal* 2001, **77**(909):428-435.
146. Bernstein L, Deapen D, Ross RK: **The descriptive epidemiology of malignant cystosarcoma phyllodes tumors of the breast**. *Cancer* 1993, **71**(10):3020-3024.
147. Salvadori B, Cusumano F, Del Bo R, Delledonne V, Grassi M, Rovini D, Saccozzi R, Andreola S, Clemente C: **Surgical treatment of phyllodes tumors of the breast**. *Cancer* 1989, **63**(12):2532-2536.
148. Strode M, Khoury T, Mangieri C, Takabe K: **Update on the diagnosis and management of malignant phyllodes tumors of the breast**. *The Breast* 2017, **33**:91-96.
149. Lim SZ, Ng CCY, Rajasegaran V, Guan P, Selvarajan S, Thike AA, Nasir NDBM, Koh VCY, Tan BKT, Ong KW *et al*: **Genomic profile of breast sarcomas: a comparison with malignant phyllodes tumours**. *Breast Cancer Research and Treatment* 2019, **174**(2):365-373.

150. Tan BY, Acs G, Apple SK, Badve S, Bleiweiss IJ, Brogi E, Calvo JP, Dabbs DJ, Ellis IO, Eusebi V *et al*: **Phyllodes tumours of the breast: a consensus review**. *Histopathology* 2016, **68**(1):5-21.
151. Zhou ZR, Wang CC, Yang ZZ, Yu XL, Guo XM: **Phyllodes tumors of the breast: diagnosis, treatment and prognostic factors related to recurrence**. *J Thorac Dis* 2016, **8**(11):3361-3368.
152. Rodrigues MF, Truong PT, McKevitt EC, Weir LM, Knowling MA, Wai ES: **Phyllodes tumors of the breast: The British Columbia Cancer Agency experience**. *Cancer/Radiothérapie* 2018, **22**(2):112-119.
153. Elhamili A, Bergquist J: **A method for quantitative analysis of an anticancer drug in human plasma with CE-ESI-TOF-MS**. *Electrophoresis* 2011, **32**(13):1778-1785.
154. Zhang Q, Li Z, Xu K, Qian Y, Chen M, Sun L, Song S, Huang X, He Z, Li F *et al*: **Intracellular concentration and transporters in imatinib resistance of gastrointestinal stromal tumor**. *Scand J Gastroenterol* 2019, **54**(2):220-226.
155. Hompland I, Bruland OS, Ubhayasekhera K, Bergquist J, Boye K: **Clinical implications of repeated drug monitoring of imatinib in patients with metastatic gastrointestinal stromal tumour**. *Clinical sarcoma research* 2016, **6**:8.
156. Holmebakk T, Bjerkehagen B, Hompland I, Stoldt S, Boye K: **Relationship between R1 resection, tumour rupture and recurrence in resected gastrointestinal stromal tumour**. *Br J Surg* 2019, **106**(4):419-426.
157. Rutkowski P, Nowecki ZI, Michej W, Debiec-Rychter M, Woźniak A, Limon J, Siedlecki J, Grzesiakowska U, Kakol M, Osuch C *et al*: **Risk criteria and prognostic factors for predicting recurrences after resection of primary gastrointestinal stromal tumor**. *Ann Surg Oncol* 2007, **14**(7):2018-2027.
158. Ahmed I, Welch NT, Parsons SL: **Gastrointestinal stromal tumours (GIST) - 17 years experience from Mid Trent Region (United Kingdom)**. *Eur J Surg Oncol* 2008, **34**(4):445-449.
159. Zhi X, Jiang B, Yu J, Røe OD, Qin J, Ni Q, Sun L, Xu M, Zhu J, Ma L: **Prognostic role of microscopically positive margins for primary gastrointestinal stromal tumors: a systematic review and meta-analysis**. *Sci Rep* 2016, **6**:21541.
160. Demetri GD, Wang Y, Wehrle E, Racine A, Nikolova Z, Blanke CD, Joensuu H, von Mehren M: **Imatinib plasma levels are correlated with clinical benefit in patients with unresectable/metastatic gastrointestinal stromal tumors**. *Journal of clinical oncology : official journal of the American Society of Clinical Oncology* 2009, **27**(19):3141-3147.
161. Widmer N, Decosterd LA, Csajka C, Leyvraz S, Duchosal MA, Rosselet A, Rochat B, Eap CB, Henry H, Biollaz J *et al*: **Population pharmacokinetics of imatinib and the role of α1-acid glycoprotein**. *British Journal of Clinical Pharmacology* 2006, **62**(1):97-112.
162. Ito F, Miura M, Fujioka Y, Abumiya M, Kobayashi T, Takahashi S, Yoshioka T, Kameoka Y, Takahashi N: **The BCRP inhibitor febuxostat enhances the effect of nilotinib by regulation of intracellular concentration**. *International Journal of Hematology* 2020.
163. Taffurelli M, Pellegrini A, Meattini I, Orzalesi L, Tinterri C, Roncella M, Terribile D, Caruso F, Tazzioli G, Pollini G *et al*: **Secondary breast angiosarcoma: A multicentre retrospective survey by the national Italian association of Breast Surgeons (ANISC)**. *Breast* 2019, **45**:56-60.
164. Salminen SH, Sampo MM, Bohling TO, Tuomikoski L, Tarkkanen M, Blomqvist CP: **Radiation-associated sarcoma after breast cancer in a nationwide population: Increasing risk of angiosarcoma**. *Cancer medicine* 2018, **7**(9):4825-4835.

165. Monroe AT, Feigenberg SJ, Mendenhall NP: **Angiosarcoma after breast-conserving therapy**. *Cancer* 2003, **97**(8):1832-1840.
166. Strobbe LJA, Peterse HL, van Tinteren H, Wijnmaalen A, Rutgers EJT: **Angiosarcoma of the breast after conservation therapy for invasive cancer, the incidence and outcome. An unforeseen sequela**. *Breast Cancer Research and Treatment* 1998, **47**(2):101-109.
167. Early Breast Cancer Trialists' Collaborative G, Darby S, McGale P, Correa C, Taylor C, Arriagada R, Clarke M, Cutter D, Davies C, Ewertz M *et al*: **Effect of radiotherapy after breast-conserving surgery on 10-year recurrence and 15-year breast cancer death: meta-analysis of individual patient data for 10,801 women in 17 randomised trials**. *Lancet* 2011, **378**(9804):1707-1716.
168. Wickberg Å, Holmberg L, Adami H-O, Magnuson A, Villman K, Liljegren G: **Sector Resection With or Without Postoperative Radiotherapy for Stage I Breast Cancer: 20-Year Results of a Randomized Trial**. *Journal of Clinical Oncology* 2014, **32**(8):791-797.
169. Doke K, Butler S, Mitchell MP: **Current Therapeutic Approaches to DCIS**. *Journal of Mammary Gland Biology and Neoplasia* 2018, **23**(4):279-291.