Toward a more individualised treatment of patients with gastrointestinal stromal tumour

PhD thesis by
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1. **Scientific environment**

This PhD project at the Faculty of Medicine at the University of Oslo comprises a collaboration of all specialists involved in the treatment of gastrointestinal stromal tumours in the Sarcoma group, Norwegian Radium Hospital, Oslo University Hospital. The thesis was completed in parallel with holding a fifty per cent position as a consulting oncologist in the same group. As a member of the Sarcoma Research Group I could benefit from collaboration with other fellow clinicians and researchers specialised in sarcoma treatment and research. The work was carried out at the Department of Oncology at the Norwegian Radium Hospital, which is part of Oslo University Hospital.

The project was financially supported by unrestricted research grants from Rakel and Otto Kr Bruun’s Legacy, the Oslo University Hospital Foundation, Unifor Frimed, Inven2, the Radium Hospital Legacy and the National Advisory Unit for Sarcomas in Norway. Senior consultant and career development research fellow, Kjetil Boye, MD, PhD, was the main supervisor, and Professor Øyvind Sverre Bruland was the co-supervisor.
2. Acknowledgement

First, I gratefully acknowledge the financial support provided by unrestricted grants from the different sources mentioned above. I would also like to thank the head of the Department of Oncology at Oslo University Hospital, Professor Stein Kaasa, for his belief in my research and important financial support for the last period of my work.

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I am grateful to all the co-authors, and I want to thank them for their contributions to this work. An extra thanks to Toto Hølmebakk, who has been a vital cooperation partner conducting studies on primary GISTs. His enthusiasm for GIST research has indeed been contagious.

Thanks to the ‘sarcoma family’ at Oslo University Hospital. The passion shown in all aspects of sarcoma evaluation, treatment, follow up and research has been inspiring. I am especially grateful to Kirsten Sundby Hall, who introduced me to sarcoma treatment. Her unlimited support during this project has been very important to me. In addition, thanks to the ‘sarcoma nurses’ Lotta and Stine for putting up with all my visits to their office when writer’s block kicked in.

The unconditional support from my family and friends has truly been important to me. An extra thanks to my brother, Tord, who I shared office with during the first period of this
project. His investigational knowledge surely kickstarted my research. Finally, and most importantly, I would like to thank my wife, Anne-Mette. I am highly grateful for her patience and support throughout this period. She put up with my late evening work and my long talks about GIST and sarcoma. Her quality of ignoring me precisely as required and forcing me to do things other than research has been vital.

Oslo, February 2018

[Signature]
### 3. Abbreviations

<table>
<thead>
<tr>
<th>Abbreviation</th>
<th>Description</th>
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<tbody>
<tr>
<td>AFIP</td>
<td>Armed Forces Institute of Pathology</td>
</tr>
<tr>
<td>AIO</td>
<td>Arbeitsgemeinschaft Internistische Onkologie</td>
</tr>
<tr>
<td>CDR</td>
<td>Cause of Death Registry</td>
</tr>
<tr>
<td>CI</td>
<td>Confidence interval</td>
</tr>
<tr>
<td>CML</td>
<td>Chronic myeloid leukaemia</td>
</tr>
<tr>
<td>CRN</td>
<td>Cancer Registry of Norway</td>
</tr>
<tr>
<td>CT</td>
<td>Computer tomography</td>
</tr>
<tr>
<td>CYP</td>
<td>Cytochrome P</td>
</tr>
<tr>
<td>DSS</td>
<td>Disease-specific survival</td>
</tr>
<tr>
<td>E-GIST</td>
<td>Extra-gastrointestinal GIST</td>
</tr>
<tr>
<td>ECOG</td>
<td>Eastern Cooperative Oncology Group</td>
</tr>
<tr>
<td>ESMO</td>
<td>European Society for Medical Oncology</td>
</tr>
<tr>
<td>EUS</td>
<td>Endoscopic ultrasound</td>
</tr>
<tr>
<td>GI</td>
<td>Gastrointestinal</td>
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<tr>
<td>GIST</td>
<td>Gastrointestinal stromal tumour</td>
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<tr>
<td>HPF</td>
<td>High power field</td>
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<tr>
<td>HR</td>
<td>Hazard ratio</td>
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<tr>
<td>IFFS</td>
<td>Imatinib failure free survival</td>
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<tr>
<td>C&lt;sub&gt;min&lt;/sub&gt;</td>
<td>Plasma trough level</td>
</tr>
<tr>
<td>MCV</td>
<td>Mean corpuscular volume</td>
</tr>
<tr>
<td>MRI</td>
<td>Magnetic resonance imaging</td>
</tr>
<tr>
<td>NCCN</td>
<td>National Comprehensive Cancer Network</td>
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<tr>
<td>NIH</td>
<td>National Institute of Health</td>
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<tr>
<td>OMD</td>
<td>Oligometastatic disease</td>
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<tr>
<td>OS</td>
<td>Overall survival</td>
</tr>
<tr>
<td>OUH</td>
<td>Oslo University Hospital</td>
</tr>
<tr>
<td>PDGFRA</td>
<td>Platelet-derived growth factor alpha</td>
</tr>
<tr>
<td>PET</td>
<td>Positron emission tomography</td>
</tr>
<tr>
<td>PFS</td>
<td>Progression free survival</td>
</tr>
<tr>
<td>PMD</td>
<td>Polymetastatic disease</td>
</tr>
<tr>
<td>RCT</td>
<td>Randomised clinical trial</td>
</tr>
<tr>
<td>REC</td>
<td>Regional ethics committee</td>
</tr>
<tr>
<td>RFS</td>
<td>Recurrence free survival</td>
</tr>
<tr>
<td>SDH</td>
<td>Succinate dehydrogenase</td>
</tr>
<tr>
<td>SSG</td>
<td>Scandinavian Sarcoma Group</td>
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<tr>
<td>TKI</td>
<td>Tyrosine kinase inhibitor</td>
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4. Synopsis

4.1 Background

Although gastrointestinal stromal tumour (GIST) is the most common primary sarcoma\(^1\), it is an uncommon cancer that presents substantial heterogeneity and appears as various clinical entities, showing considerable differences in tumour biology and prognosis\(^2\). Most knowledge concerning GIST outcomes comes from clinical trials and experience from cooperative sarcoma societies. In contrast, this doctoral thesis involved real-life data from the complete cohort of patients with GIST treated at Oslo University Hospital (OUH) over two decades.

4.2 Purpose

The main goal of this thesis was to contribute to a more individualised treatment and follow-up of patients with GIST.

4.3 Key objectives

The key objectives were as follow:

- To study the effect of imatinib plasma concentration measurements in routine clinical practice on metastatic GIST patients;
- To explore factors associated with long-term survival in metastatic GIST patients after the introduction of imatinib, aiming to improve prediction of survival, and subsequently, clinical decision making;
- To improve the risk stratification in localised gastric GISTs by investigating the clinical significance of a strict definition of tumour rupture proposed by our group; and
- To investigate whether tumour genotype is associated with tumour rupture in localised stomach or small bowel GISTs using the definition of rupture proposed by our group.
4.4 Main findings

The main findings of the research were as follows:

- We confirmed the relatively large intra- and interpatient variability in the plasma concentrations in patients with metastatic GIST;
- Low imatinib plasma concentrations were significantly correlated with age, gastric resection and liver metastasis;
- The mean imatinib levels were significantly lower at the time of progression compared to the previous measurement for the same patients;
- Good performance status, small tumour diameter and oligometastatic disease were the factors significantly associated with long-term survival in metastatic GIST;
- The 5- and 10-year overall survival (OS) rates for patients with oligometastatic disease were significantly better compared with patients with polymetastatic disease;
- Tumour rupture according to our strict definition and mitotic index were independently associated with recurrence in gastric GIST patients;
- The 5-year recurrence-free survival (RFS) for gastric GISTs was significantly inferior in patients with tumour rupture compared with patients without rupture;
- Almost all patients with a gastric GIST and tumour rupture relapsed after having completed adjuvant imatinib treatment;
- Increased risk of rupture was observed in patients with KIT exon 11 mutations with deletions or insertions/deletions compared with substitutions or duplications/insertions;
- Patients with gastric GIST with deletions involving codons 557 and 558 (del557/558) had a significantly increased risk of rupture compared with patients with other mutations; and
In multivariable analysis, *KIT* exon 11 del557/558 and tumour size were associated with an increased likelihood of tumour rupture, while the mitotic count was not.

4.5 New knowledge

- The mean imatinib levels were significantly lower at the time of progression compared with the previous measurement for the same patients, suggesting that this could be the reason for loss of disease control in selected patients.

- Oligometastatic disease was as a strong and favourable prognostic factor in patients with metastatic GIST. Patients with oligometastatic GIST has an outcome similar to patients with high-risk localized disease, and should be regarded as a separate category among patients with metastatic GIST.

- Tumour rupture according to our strict definition was independently associated with recurrence in gastric GIST patients. With tumour rupture, patients relapsed despite adjuvant treatment. Without rupture, prognosis was good even in the high-risk group.

- Gastric GISTs with *KIT* exon 11 deletions involving codon 557 and 558 are at increased risk of tumour rupture.
5. List of publications


Review article:

6. **Aims of the present study**

The overall aim of this project was to contribute to a more improved treatment and follow up of patients with gastrointestinal stromal tumour (GIST) by reviewing the experience from treatment and follow up of patients with localised and metastatic GIST at Oslo University Hospital (OUH). The specific aims of each paper were as follows:

- **Paper I**: To assess imatinib plasma concentration repeatedly over several years in a group of patients with metastatic GIST, thereby revealing scenarios where such measurements may have clinical implications;

- **Paper II**: To study the treatment of patients with metastatic GIST at OUH over an 18-year period and identify factors associated with long-term survival after the introduction of imatinib, aiming to improve the prediction of survival, and subsequently, clinical decision making;

- **Paper III**: To improve the risk stratification in localised gastric GISTs by investigating the clinical significance of a strict definition of tumour rupture that was previously defined by the Sarcoma Group at OUH for small intestinal GISTs;

- **Paper IV**: To investigate whether tumour genotype is associated with tumour rupture using the definition of rupture proposed by the Sarcoma Group at OUH; and

- **Paper V**: To present three clinical GIST cases and review the available literature concerning a hypothesis that a long-term response to imatinib and complete surgery of metastatic lesions may lead to a cure and the justification to stop imatinib in selected patients.
7. Introduction/background

7.1 Sarcomas

The term sarcoma is derived from the Greek word sarx, which means ‘flesh’. Sarcomas are rare malignancies that arise from transformed cells of mesenchymal origin, such as bone, fat, muscles and vascular tissue\(^3\). Sarcomas may occur anywhere in the body, and they are divided into dozens of histological categories. Hence, there is an enormous number of possible mixtures of histology and primary site. Sarcomas are generally divided into two main groups – soft-tissue sarcoma and bone sarcoma. Bone sarcomas are rare, accounting for less than 0.2% of registered tumours in Norway\(^4\). Soft-tissue sarcomas occur in tissues like the muscles, nerves, fat and blood vessels\(^3\). Like bone sarcomas, soft-tissue sarcomas are also rare cancers (4–5/100 000/year)\(^5\). Soft-tissue sarcoma comprises a large group of entities, and the recommended treatment options will vary depending upon the stage, grade, type and location of the tumour. Gastrointestinal stromal tumour (GIST) which is studied in this thesis, is considered a soft-tissue sarcoma originating in the gastrointestinal (GI) tract\(^3\).

7.2 GIST – General considerations

GIST is the most frequent primary mesenchymal tumour of the gastrointestinal tract; it is both the most common soft-tissue sarcoma and individual type of sarcoma\(^1\). GISTs were traditionally classified as smooth muscle tumours in the family of leiomyomas, leiomyoblastomas or leiomyosarcomas. The notion of GIST was introduced in the 1980s\(^6\); however, it was not until before the late 1990s that GIST was acknowledge as a well-defined diagnosis and tumour entity due to the identification of the KIT oncogene mutations\(^7\). Some years later, in 2001, the tyrosine kinase inhibitor (TKI) imatinib was introduced and proven exceedingly effective in the treatment of metastatic GIST\(^8,9\). Since this exciting discovery, GIST, as the first solid tumour effectively treated using TKI, has become an important model...
for understanding and a paradigm for the development of molecularly targeted therapies in oncology.

GISTs span from small tumours with benign clinical behaviour to large and highly malignant tumours. GISTs of less than 1 cm are referred to as micro-GISTs\textsuperscript{10}. In previous studies, micro-GISTs were found in almost one-fourth of resected stomachs from elderly individuals\textsuperscript{11-13}. Small GISTs with benign behaviour are more numerous than large, malignant ones\textsuperscript{12,14,15}. However, approximately one-fourth of gastric GISTs (excluding minimal incidental tumours and micro-GISTs) are clinically malignant\textsuperscript{12}. Moreover, around 40\% of localised GISTs develop metastases, and 10–20\% of patients already have metastatic disease at the time of primary diagnosis\textsuperscript{2}. Metastatic disease is most commonly located inside the abdomen, for example, in the liver and peritoneum, whereas extra-abdominal metastases (e.g. lungs and bone) are infrequent, but they do occur\textsuperscript{16}.

GISTs are found throughout the GI tract. The most frequent site of origin is in the stomach (approximately 60\%), followed by the small intestine (about 35\%); they are more rarely seen in the colon and rectum (roughly 5\%) and very seldom in the oesophagus (less than 1\%)\textsuperscript{2}. Approximately 5\% of GISTs are thought to be located in the omentum, mesentery or retroperitoneum, and these are commonly referred to as extra-gastrointestinal GISTs (E-GISTs)\textsuperscript{2}. Whether E-GISTs truly arise from the extra-gastrointestinal sites is debatable\textsuperscript{17}. New evidence indicates that a significant portion, perhaps all, of the E-GISTs originate in the GI tract and belong to the clinicopathological spectrum of advanced gastric or intestinal GISTs rather than being a separate entity\textsuperscript{18}.

GISTs have an equal gender distribution; they are typically found in older patients (median 60–65 years), but they can also develop in children and young adults\textsuperscript{1,2}. Paediatric GISTs are rare and represent a distinct entity showing a female predominance, gastric
multicentric location, frequent lymph node metastases and absence of KIT and platelet-derived growth factor alpha (PDGFRA) mutations\textsuperscript{19}.

The global prevalence of GIST is estimated to be about 130 cases per million population\textsuperscript{20,21}; but the true global incidence is not known. The annual incidence of diagnosed GISTs is considered to be approximately 10 cases per million in Europe\textsuperscript{1,20} and about 7 cases per million in both Europe and the United States when adjusted for age\textsuperscript{1,22,23}. There are some large geographical differences reported, but whether this is due to methodology or true population differences is unknown\textsuperscript{24}. In Norway, two population studies have been performed, one from northern Norway and the other from the south-western Norway\textsuperscript{25,26}. These studies report conflicting results; with an annual incidence of 19 (northern Norway) and 7 (south-Western Norway) per million\textsuperscript{25,26}. The reason for the large difference in the incidence between these two studies is unknown.

7.3 Aetiology and pathogenesis

Interstitial cells of Cajal or their stem cell-like precursors are considered to give rise to GIST\textsuperscript{7,27}. These cells regulate GI motility and autonomic nerve function; they are KIT and KIT-ligand (stem cell factor) positive and located around the myenteric plexus and in the muscularis propria throughout the GI tract\textsuperscript{28,29}. Constitutional activation of KIT by mutations causes Cajal cell proliferation, and ultimately GIST, in mouse models\textsuperscript{30,31}. Normally, binding of the matching ligand activates the KIT receptor. In contrast, a gain-of-function mutation in the gene encoding for the KIT receptor may result in a constitutively active receptor without binding to the corresponding ligand, causing numerous downstream signal transduction pathways, which finally result in malignancy and tumour progression (discussed in detail in Section 7.5.3).
There are no known risk factors for GISTs, but they can arise in the setting of different genetic syndromes. For example, the Carney triad is a syndrome involving the loss of succinate dehydrogenase (SDH) subunite B expression that causes paraganglioma, pulmonary chondromas and gastric GISTs\textsuperscript{32}. A similar rare syndrome (Carney-Stratakis syndrome) with germline mutations in \textit{SDH} subunits often causes GISTs and paragangliomas\textsuperscript{33}. Some patients with neurofibromatosis type I will develop multicentric small bowel GISTs without \textit{KIT}/\textit{PDGFRA} mutations\textsuperscript{34}. Autosomal dominant germline \textit{KIT} mutations are rare, and they often present with multiple GISTs in young individuals\textsuperscript{35}.

\textbf{7.4 Clinical presentation and evaluation}

GISTs are associated with a variety of clinical presentations, which depend on tumour size and anatomical location. Anaemia caused by bleeding of the tumour into the intestine or the peritoneal cavity and abdominal pain are the two most frequent symptoms; more vague symptoms, such as abdominal discomfort, nausea and vomiting, obstipation or diarrhoea and fatigue, can also occur\textsuperscript{22,36,37}. GISTs can sometimes present as severe bleeding from the tumour, bowel perforation due to tumour rupture and intestinal obstruction by the tumour, with the need for emergency surgery. It is not uncommon for GISTs to be discovered as an incidental finding during radiological examination or surgery for other diseases, and some are detected at autopsy\textsuperscript{20,22,37}.

Biopsy is often obtained by endoscopy or as a transcutaneous procedure through the abdominal wall. Transcutaneous biopsy comes with the theoretical risk of tumour spillage; however, a recent study has indicated that this is safe\textsuperscript{38}. Endoscopic ultrasound (EUS) is valuable in the assessment and diagnosis of oesophagus, stomach, duodenal and rectal GISTs\textsuperscript{39}. Contrast-enhanced computed tomography (CT) or magnetic resonance imaging
(MRI) of the abdomen and pelvis is usually adequate, since metastases are uncommon outside the abdominal cavity. Positron emission tomography/CT (PET)/CT is usually not indicated, but this is an option when CT or MRI results are difficult to interpret.

7.5 Pathology

7.5.1 Morphology

As mentioned Section 7.2, GISTs have a wide clinicopathological spectrum, ranging from incidental nodules to large tumours. Tumour size and mitotic activity are key parameters in the evaluation of malignancy; these are discussed in Section 7.6.3. Although their morphological appearances are quite variable, most GISTs fall into three histological categories, as follows: spindle cell type (70%) (Figure 1A), epithelioid type (20%) or mixed type3,40.

7.5.2 Immunohistochemistry

Since GISTs have numerous histological differential diagnoses, immunohistochemical testing is required. The two most important markers are KIT (CD117; Figure 1B) and anoctamin 1 (DOG-1; Figure 1C)41-43. KIT expression is found in most GISTs, and it is quite specific for GIST among the GI mesenchymal tumours44. Some GISTs (less than 5%) are negative for KIT, typically encompassing epithelioid GISTs in the stomach with PDGFRA mutations, and some in this group respond to imatinib45. DOG-1 is also expressed in the overwhelming majority of GISTs, and is positive in many GISTs negative for KIT; thus, it is important in the diagnosis42,43. CD34, a hematopoietic progenitor cell antigen, is often expressed in GISTs, but to a lesser degree than KIT and DOG-1 are46. Alpha smooth muscle actin, heavy caldesmon, and desmin (smooth muscle markers) can be present in GISTs; however, other smooth muscle
tumours of the GI tract often express these markers to a greater degree\textsuperscript{47}.

7.5.3 Molecular pathology

Testing for KIT and PDGFRA mutations should be performed when TKIs are planned as treatment. Different mutations show different clinical behaviours and responses to imatinib treatment. These are summarised in Table 1. About 75–80\% of GISTs have KIT mutations\textsuperscript{7,48}, and the most frequent mutations affect exon 11. Mutations in KIT exon 9 (5–10\%) and exons 13 and 17 (about 2\%) are less frequent\textsuperscript{49}. Approximately 10\% have PDGFRA mutations in domains homologous to those in KIT\textsuperscript{50,51}. Alterations are in-frame deletions, insertions and substitutions or combinations of these. The GIST-associated kinase mutations and their relationship with structural features of the proteins are shown in Figure 2.
**Table 1. Mutations and clinicopathological features**

<table>
<thead>
<tr>
<th>Genetic type</th>
<th>Frequent mutations</th>
<th>Frequency (%)</th>
<th>Characteristics and site</th>
<th>Imatinib sensitivity</th>
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<tr>
<td><strong>KIT mutation</strong></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Exon 8</td>
<td>KIT mutation</td>
<td>80</td>
<td>All sites</td>
<td>Yes</td>
</tr>
<tr>
<td></td>
<td>Exon 9</td>
<td>Insertion of AY 502-503</td>
<td>Rare</td>
<td>Small bowel, colon, spindle, aggressive</td>
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<tr>
<td></td>
<td>Exon 11</td>
<td>Deletions, missense mutations, insertions</td>
<td>Deletion of codon 557 or 558</td>
<td>All sites</td>
</tr>
<tr>
<td></td>
<td></td>
<td>Internal tandem duplication</td>
<td></td>
<td>Aggressive, poor prognosis</td>
</tr>
<tr>
<td></td>
<td>Exon 13</td>
<td>K642E</td>
<td>1</td>
<td>All sites</td>
</tr>
<tr>
<td></td>
<td>Exon 17</td>
<td>D820Y, N822K, Y823D</td>
<td>1</td>
<td>All sites</td>
</tr>
<tr>
<td><strong>PDGFRA mutations</strong></td>
<td></td>
<td>10</td>
<td>Epitheloid, clinically indolent</td>
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<tr>
<td>Exon 12</td>
<td>Missense mutations</td>
<td>1-2</td>
<td>All sites</td>
<td>Yes</td>
</tr>
<tr>
<td>Exon 14</td>
<td>N659K</td>
<td>&lt;1</td>
<td>Stomach, epitheloid</td>
<td>Yes</td>
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<td>Exon 18</td>
<td>D842V</td>
<td>5-10</td>
<td>Stomach, mesentery, omentum, epitheloid</td>
<td>Yes, but no for D842V</td>
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<td><strong>KIT and PDGFRA wild-type</strong></td>
<td></td>
<td>10-15</td>
<td>All sites</td>
<td>Probably no</td>
</tr>
<tr>
<td></td>
<td>SDHA, SDHB, SDHC, and SDHD mutations</td>
<td>~2</td>
<td>Carney–Stratakis syndrome; stomach, multiple, immunohistochemically SDHB negative</td>
<td></td>
</tr>
<tr>
<td></td>
<td>Loss of SDH expression</td>
<td></td>
<td>Carney triad; stomach, clinically indolent, juvenile onset, immunohistochemically SDHB negative</td>
<td></td>
</tr>
<tr>
<td></td>
<td>HRAS and NRAS mutations</td>
<td>&lt;1</td>
<td></td>
<td></td>
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<tr>
<td></td>
<td><strong>NF1 mutation</strong></td>
<td>1-2</td>
<td>Small bowel, clinically indolent, multiple, spindle</td>
<td></td>
</tr>
<tr>
<td></td>
<td>Sporadic paediatric GISTs</td>
<td>~1</td>
<td>Stomach</td>
<td></td>
</tr>
</tbody>
</table>

NF1, neurofibromatosis type I
PDGFRA, platelet-derived growth factor receptor-α
SDH, succinate dehydrogenase.
This table is modified from Nishida et al.63
As mentioned in Section 7.3, binding of their respective ligands activate the dimerisation of both KIT and PDGFRα kinase, and mutations in these kinases disrupt normal function. Alterations in the juxtamembrane regions (KIT exon 11 and PDGFRα exon 12) and the kinase I (KIT exon 13 and PDGFRα exon 14) and II (KIT exon 17 and PDGFRα exon 18) domains of KIT and PDGFRα transmit oncogenic signals via the mitogen-activated protein kinase and phosphoinositide-3-kinase pathways, ultimately leading to tumour growth and progression.
Approximately 10–15% of GISTs lack a mutation in KIT or PDGFRA\textsuperscript{2,54}. These are commonly termed ‘wild-type’ GISTs, and they can occur anywhere in the GI tract. They have an identical morphology to KIT/PDGFRA mutated GISTs, and they also often express high levels of KIT on immunohistochemistry. Phosphorylated KIT is often found in these tumours, and this suggests that KIT is activated\textsuperscript{55}; however, the mechanism of this activation is unknown. Wild-type GISTs include the tumour syndromes mentioned in Section 7.3 (neurofibromatosis type I, Carney-Stratakis syndrome and Carney’s triad) and BRAF mutations, SDH mutations and RAS-family mutations, which all are rare\textsuperscript{32-34,56-59}.

Although KIT and PDGFRA mutations are important in the evolution of GISTs, other genetic events are significant in their development. Chromosomal changes, such as deletions on 14q, 22q, 1p and 9p, are associated with the progression of GISTs\textsuperscript{48}. A gene (chromosome 9p) that encodes an essential cell-cycle regulator (CDKN2A) is often inactivated in GISTs\textsuperscript{60}. Furthermore, gains on chromosomes 8q and 17q have been related to aggressive conduct in GIST\textsuperscript{48}.

### 7.6 Treatment for localised GIST

#### 7.6.1 Surgery

While imatinib has markedly improved the treatment for GIST, surgery is the only modality that safely offers curative treatment\textsuperscript{61-63}. Radical surgical resection (R0) avoiding injuries to the pseudocapsule and tumour rupture\textsuperscript{64} is the treatment of choice for localised GISTs. Small oesophageal or gastric GISTs (less than 2 cm) can be followed by periodic (6–12 months) EUS/CT of the abdomen until the tumours increase in size or become symptomatic\textsuperscript{63}. Small GISTs may be laparoscopically resected with the same oncological principles as for open surgery. In a large series of 540 gastric GIST patients treated with laparoscopic surgery, there
were few complications; R0 resection was achieved in almost all patients (99%), and only 14 (3%) tumours recurred\(^6^5\). Furthermore, a meta-analysis comparing laparoscopy and open surgery in gastric GISTs showed that laparoscopic surgery resulted in less complications and quicker recovery, and most importantly, did not increase the risks of tumour recurrence and metastasis\(^6^6\). However, most of the tumours in this analysis were small, with a very low or low risk of recurrence according to the risk stratification methods (discussed in Section 7.6.4). A prospective randomised trial comparing open and laparoscopic surgery is lacking. Both the European Society for Medical Oncology (ESMO) and the National Comprehensive Cancer Network (NCCN) guidelines state that laparoscopic surgery may be performed for small GISTs, but it is not recommended for large tumours, due to the risk of tumour rupture, causing spillage of tumour content into the abdomen\(^6^1,6^2\). Pretreatment with imatinib before surgery is now the standard of care if there is a risk of multiple organ resection or tumour rupture during surgery (discussed in detail Section 7.6.2).

Large GISTs are often fragile and prone to tumour rupture; thus, they should be handled with the utmost care. Tumour rupture, whether spontaneous or iatrogenic, increases the probability of recurrence after surgery\(^6^4,6^7,6^8\). However, rupture has been inconsistently defined in many studies, and our research group has established a definition that distinguishes between major and minor defects related to tumour integrity in patients with small intestinal GISTs\(^6^9\). Major defects encompass tumour spillage, tumour fracture or piecemeal resection, surgical biopsy, bowel perforation to the abdominal cavity, blood-tinged ascites and microscopic transperitoneal infiltration into an adjacent structure; minor defects include transabdominal core needle biopsy, peritoneal tumour penetration, iatrogenic peritoneal laceration and microscopically involved resection margins\(^6^9\). No differences in recurrence-free survival (RFS) were detected among patients with a minor defect of tumour integrity and patients with no defect. However, patients with a major defect had a significantly increased
risk of recurrence. Hence, our group has suggested that the term ‘tumour rupture’ should be reserved for a major defect\(^6^9\). In Paper III, the definition of tumour rupture proposed by our group was applied to a large cohort of gastric GIST patients, and in Paper IV, we investigated whether tumour genotype is associated with rupture using the same definition.

The aim of surgery is complete resection of the tumour, macroscopic and microscopic negative margins and preserving as much function as possible via wedge resection. A macroscopic margin of 1–2 cm is regarded adequate to achieve microscopically negative margins. How to manage a positive microscopic margin (R1 resection) is not well determined, and the options include re-operation, watchful waiting and postoperative imatinib therapy\(^6^1-^6^3\). A retrospective analysis from two clinical studies suggested that the margin status does not have a significant prognostic effect in the era of imatinib\(^7^0\). In contrast, a larger meta-analysis showed that a R1 resection was an unfavourable prognostic factor\(^7^1\). Further studies are needed to resolve this question. Lymph node removal is not indicated for GISTs due to the rarity of lymphatic metastasis. However, dissection of the lymph nodes may be indicated for paediatric GISTs, where lymph-node metastases are much more frequent\(^3^2,^7^2,^7^3\).

7.6.2 Neoadjuvant imatinib

Upfront surgery is the treatment of choice in localised GISTs. However, if complete resection (R0) is not achievable or cannot be done without mutilating surgery, preoperative treatment with imatinib is now the standard of care\(^6^1,^6^2\). Neoadjuvant imatinib should also be considered if the risk of tumour rupture or bleeding during surgery is high. Tumour shrinkage by imatinib treatment may facilitate R0 resection and organ-sparing surgery\(^7^4\) (Figure 3). There are no randomised studies comparing neoadjuvant imatinib versus primary surgery in locally advanced GISTs. However, prospective nonrandomised studies of preoperative imatinib have
shown that most tumours decrease in size and become less vascular after treatment\textsuperscript{75,76}. A study on patients from 10 sarcoma centres in Europe showed that neoadjuvant imatinib facilitated R0 resection in more than four out of five patients with locally advanced GISTs and the long-term results were excellent\textsuperscript{77}. The current guidelines recommend surgery following maximal tumour response (usually after 6–12 months)\textsuperscript{61,62}. Mutational analysis is mandatory before starting neoadjuvant imatinib to single out patients that do not profit from neoadjuvant imatinib (e.g. PDGFRA D842V). Functional imaging (PET/CT/diffusion weighted MRI) can assess the tumour response rapidly\textsuperscript{78}, and this could especially be considered if mutational analysis is lacking.

Figure 3. Patient with a large gastric GIST. CT of the abdomen at diagnosis (A) with excellent response after approximately 12 months of neoadjuvant imatinib treatment (B).
7.6.3 Survival and prognostic factors in localised GIST

Most patients with operable non-metastatic GISTs are cured by surgery. For primary GISTs without metastases treated by surgery alone, the estimated 5-year RFS is about 70%, while 15-year RFS is almost 60%\(^2\). Many patient and tumour characteristics have prognostic relevance, but the most important prognostic factors are mitotic count, size, origin of the tumour and whether tumour rupture has occurred prior to or during surgery\(^2,64,67,79-81\). Mitoses are counted in the 50 high-power fields (HPFs) of the microscope or 5 mm\(^2\). Non-gastric GISTs have inferior RFS rates compared to gastric GISTs\(^2,67,81,82\). Of the non-gastric GISTs, E-GISTs has a higher risk of recurrence than the other localisations do\(^2\). Tumours with mutations in KIT exon 9 or KIT exon 11 (especially deletions) have an unfavourable outcome, whereas patients with a PDGFR\(A\) D842V mutation usually do better\(^81,83-85\). Furthermore, genomic complexity is strongly predictive of GIST recurrence, and an algorithm to classify primary GISTs according to genomic alterations has been proposed\(^86\).

7.6.4 Risk stratification

There are three commonly used risk-stratification methods to calculate the risk of recurrence after surgery of non-metastatic GISTs, namely the National Institute of Health (NIH) consensus criteria\(^40\), modified NIH criteria\(^87\) and Armed Forces Institute of Pathology (AFIP) criteria\(^47\). These three methods all include the risk factors of tumour size and mitosis, but they differ in that the AFIP criteria also comprise tumour origin, while the modified NIH criteria include tumour site and rupture (Table 2). These schemes have been validated, showing more or less similar prognostic accuracy\(^2,87,88\). Two risk estimation nomograms have also been developed, both including tumour size, mitotic count and tumour site\(^89,90\). Furthermore, prognostic heat maps and contour maps have been developed by Joensuu et al.\(^2\), and these are
moderately more accurate for the calculation of recurrence risk than the three methods mentioned above.

<table>
<thead>
<tr>
<th>Table 2: Criteria for risk-stratification of GIST recurrence after surgery</th>
</tr>
</thead>
<tbody>
<tr>
<td><strong>Tumour parameter</strong></td>
</tr>
<tr>
<td>Diameter (cm)</td>
</tr>
<tr>
<td>-----------------</td>
</tr>
<tr>
<td><strong>Modified NIH criteria</strong></td>
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<tr>
<td>Very low risk</td>
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<td>Intermediate risk</td>
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<tr>
<td>High risk</td>
</tr>
<tr>
<td>High risk</td>
</tr>
<tr>
<td><strong>AFIP criteria</strong></td>
</tr>
<tr>
<td>Group 1</td>
</tr>
<tr>
<td>Group 2</td>
</tr>
<tr>
<td>Group 3a</td>
</tr>
<tr>
<td>Group 3b</td>
</tr>
<tr>
<td>Group 4</td>
</tr>
<tr>
<td>Group 5</td>
</tr>
<tr>
<td>Group 6a</td>
</tr>
<tr>
<td>Group 6b</td>
</tr>
</tbody>
</table>

AFIP=Armed Forces Institute of Pathology.
GIST=gastrointestinal stromal tumour.
HPF=high power field.
NIH=National Institute of Health.
RFS=Recurrence-free survival.
*Data from ten pooled population-based series by Joensuu et al.2
**If rupture is present, any size, count or location is considered high-risk.
***Criteria available for gastric, duodenal, jejunal and ileal, and rectal GIST.
This table is modified from Joensuu et al.109.

7.6.5 Adjuvant treatment

Due to the success of imatinib treatment in metastatic GIST (discussed in Section 7.7.1), studies seeking to test its activity also as an adjuvant therapy were initiated. Three important trials have addressed adjuvant treatment in surgically resected GISTs91-93. The first study enrolled 713 patients with operated non-metastatic GISTs; patients were randomly assigned to receive either imatinib (400 mg) daily or a placebo for 1 year91. This trial showed improved
RFS rates for patients treated with postoperative imatinib compared with the placebo, but there was no overall survival (OS) benefit. Based on these findings, adjuvant imatinib was approved in the United States and Europe. Notably, this first study only required the primary GIST lesion to be at least 3 cm in size, irrespective of the mitotic index\textsuperscript{91}; thus, many enrolled patients had resected GISTs regarded as having a low risk of recurrence.

A cooperative trial between the Scandinavian Sarcoma Group and German Sarcoma Group (SSG XVIII/AIO trial) randomised between imatinib (400 mg daily) for 1 year and 3 years\textsuperscript{92}. In contrast to the previous study, only patients with high-risk GISTs according to the NIH criteria were enrolled. The patients treated with 3 years of imatinib had significantly better RFS rates than did those treated for 1 year (66\% vs. 48\% at 5 years; hazard ratio [HR] 0.46; \( p < 0.001 \)). Most importantly, the OS was superior in the patients treated with imatinib for 3 years (92\% vs. 82\% at 5 years; HR 0.45; \( p = 0.019 \))\textsuperscript{92}. The 5-year follow up continued to show significantly improved OS, with 91.9\% in patients assigned to the 3-year group versus 85.3\% in the 1-year group\textsuperscript{94}.

In a large European randomised study, patients were treated with either 2 years of imatinib or no further therapy after surgery\textsuperscript{93}. This study included GIST patients with intermediate and high risk according to the NIH consensus criteria. Due to the improvement in survival in the context of metastatic GIST, the endpoint of the study was changed from OS to imatinib failure–free survival (IFFS), which was calculated from the date of randomisation to the date of the start of a new systemic treatment for metastatic GIST. In this study, adjuvant imatinib had a clear effect on RFS, but no significant impact on IFFS was detected\textsuperscript{93}.

Mutational analysis is mandatory before deciding whether to administer adjuvant treatment, since some gene mutations are insensitive to imatinib. The mutation \textit{PDGFRA} D842V, seen in 5–10\% of patients with high-risk GIST\textsuperscript{95}, is the most common mutation
insensitive to imatinib\textsuperscript{96-98}. Furthermore, there is no convincing evidence that patients with $KIT$- and $PDGFRA$-negative GIST will profit from adjuvant imatinib\textsuperscript{99,100}. Evidence from metastatic GIST has shown that patients with $KIT$ exon 9 mutations benefit from a higher dose than 400 mg daily\textsuperscript{101}. This may also be the case when selecting the imatinib dose in an adjuvant setting. In the latest update from the SSG XVIII/AIO study, a comprehensive analysis of GIST genotypes was performed\textsuperscript{100}. This showed that patients with a $KIT$ exon 11 deletion mutation in the tumour had the most benefit from 3 years of adjuvant imatinib. In addition, GISTs with $KIT$ exon 11 harbouring deletions in codons 557 and 558 had the best outcomes with adjuvant imatinib therapy. Furthermore, patients with $KIT$ exon 9 mutations, $PDGFRA$ D842V mutations or tumours lacking mutations in either $KIT$ or $PDGFRA$ exhibited no benefit from adjuvant imatinib therapy\textsuperscript{100}.

Based on the aforementioned studies, the recommended duration of adjuvant imatinib is now 3 years\textsuperscript{61,62}. The criteria for patient selection and duration of treatment are still under debate\textsuperscript{102}. The ESMO guidelines state that patients with a significant risk of relapse should receive adjuvant imatinib\textsuperscript{61}, and the NCCN guidelines purport that patients with intermediate or high-risk GISTs should be considered for at least 3 years of imatinib\textsuperscript{62}. Adjuvant imatinib for longer than 3 years has not been studied; however, there are ongoing trials exploring whether treatment durations of 5 years may benefit the patients with high-risk GISTs. Furthermore, it also remains unclear what lower limit of ‘recurrence risk’ should be used to justify adjuvant therapy. Finally, it remains unknown how to treat GISTs without $KIT$/PDGFRA mutations.

7.6.6 Follow up

Very-low-risk GISTs rarely recur, and a routine follow up is probably not necessary\textsuperscript{2}. The
clinical benefit of a routine follow-up for low-risk tumours is not known; however, it is usually carried out with an abdominal CT scan every 6–12 months for 5 years. The ESMO guidelines recommend that patients with intermediate or high-risk tumours should undergo an abdominal CT scan or MRI every 3–6 months during adjuvant therapy; every 3 months for 2 years when adjuvant imatinib is discontinued (due to the increased risk of relapse in the 2 first years); every 6 months until 5 years from stopping adjuvant therapy; and annually until 10 years from the primary surgery\textsuperscript{61}. A study from the SSG XVIII/AIO trial, exploring a mathematical model to adjust timing of CT scans with the hazard of recurrence, supported an imaging schedule where abdominal CT is performed relatively sparsely during administration of adjuvant imatinib (6-month intervals), followed by more frequent imaging (3- to 4-month intervals) during the first 2 years following the discontinuation of adjuvant imatinib treatment when the risk of tumour recurrence is the greatest, and then at 6- to 12-month intervals\textsuperscript{103}. Since GIST recurrence may occur up to 10 years after surgery, follow up extending for 10 years after the operation appears reasonable. Tumour recurrence outside of the abdomen is infrequent\textsuperscript{16}, and thus, imaging of the chest may be omitted. Detection of recurrence at an early stage could be important, since most recurrent GISTs respond to imatinib, and some retrospective studies on advanced GIST have suggested that the time to progression on imatinib is longer the smaller the tumour mass is at the time of imatinib initiation\textsuperscript{104-107} (discussed in detail in Section 7.7.1).

7.7 Treatment of metastatic GIST

7.7.1 Imatinib mesylate

Historically, patients with metastatic GIST had a poor prognosis, with a median OS of 1–2 years\textsuperscript{16,108}. Treatment was mostly limited to surgery, as the response to chemotherapy and
radiotherapy was generally poor\textsuperscript{109}. As mentioned in Section 7.2, in 2001, imatinib was introduced as a beneficial drug in the treatment of metastatic GIST\textsuperscript{8,9}. Imatinib improved the treatment of patients with advanced GIST greatly\textsuperscript{9}, and it is now the standard first-line medical treatment of metastatic GIST\textsuperscript{61,62}.

Imatinib is a selective inhibitor of a few tyrosine kinases, including KIT and PDGFRA\textsuperscript{110}. It is administered orally and taken with food to avoid upper GI tract irritation. The most commonly used daily dosage is 400 mg. However, there is evidence that patients with KIT exon 9 mutation benefit from a higher dose (800 mg/day) than the standard 400 mg daily dose\textsuperscript{101,111}. The median duration of response to imatinib in advanced GIST is 2–3 years, and 10–20\% of the responses last for 10 or more years\textsuperscript{112,113}.

Different mutation genotypes respond differently to imatinib (Table 1). Patients with the KIT exon 11 mutation respond more frequently and often have superior progression-free survival (PFS) and OS on imatinib compared with patients with KIT exon 9 mutated tumours or tumours without KIT or PDGFRA mutations\textsuperscript{101,106,114,115}. Among the KIT exon 11 mutations, deletions at codons 557–558 show better tumour response but shorter PFS than the other KIT exon 11 mutations do\textsuperscript{106}. Furthermore, although a recent study showed a few responses\textsuperscript{116}, the PDGFRA D842V mutation has been regarded as imatinib insensitive\textsuperscript{96,97}.

Imatinib should be taken until GIST progression if it is well tolerated. In the BFR14 trial, patients with advanced GIST who were responding to imatinib were assigned either to continue imatinib or stop at the time of randomisation\textsuperscript{117-119}. Nearly all patients who stopped had GIST progression within 2 years from the date of randomisation, regardless of whether they had been on imatinib for 1, 3, or 5 years prior to imatinib discontinuation. This led to a significantly shorter PFS in the stopping group. However, stopping imatinib did not significantly influence OS, likely because most patients in the group that stopped responded
to imatinib reinstitution\textsuperscript{117-119}. Whether stopping imatinib in highly selected patients with metastatic GIST could be a treatment option is discussed in Paper V.

Evaluating the response to imatinib may be challenging. Responding metastases usually decrease in size and become hypodense on CT. Measurement of lesion density according to the Choi criteria is recommended for response assessment\textsuperscript{120}. PET/CT is usually not recommended, but can it be used to assess the early treatment response\textsuperscript{78,121} (Figure 4). The detection of new metastases, progression of known metastases or a growing lesion (‘node in mass’) in a responding liver lesion are all considered disease progression\textsuperscript{122}.

![Figure 4. Patient with a large gastric GIST with multiple large metastases in the liver on PET CT at diagnosis (A) showing early response after only two weeks of imatinib treatment (B).](image)

Imatinib is usually well tolerated, and if adverse effects occurs, several can be
improved with supportive actions or dose reduction\textsuperscript{123}. While serious adverse events are rare, almost all patients have side effects on imatinib; the most frequent of these are anaemia, periorbital oedema and watery eyes, diarrhoea, muscle cramps (typically in the hands and legs), fatigue and nausea\textsuperscript{9,124} (Table 3). Compliance, that is, adherence to self-administered imatinib, may be demanding for patients on chronic medical therapy. For example, in the SSG XVIII/AIO trial, 26% discontinued imatinib during the allocated treatment period in the absence of disease recurrence in the 36-month group, compared with 13% in the 12-month group\textsuperscript{92}.

### Table 3. Frequently recorded adverse events in patients receiving 400 mg daily from the early pivotal trials B2222\textsuperscript{9} and EORTC/ISG/AGITG\textsuperscript{124}

<table>
<thead>
<tr>
<th>Adverse event $^a$</th>
<th>Any Grade</th>
<th>Grade 3 or 4</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>B2222 (%)</td>
<td>EORTC (%)</td>
</tr>
<tr>
<td>Any</td>
<td>97</td>
<td>99</td>
</tr>
<tr>
<td>Anaemia</td>
<td>5</td>
<td>89</td>
</tr>
<tr>
<td>Leukopenia</td>
<td>6</td>
<td>43</td>
</tr>
<tr>
<td>Neutropenia</td>
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<td>41</td>
</tr>
<tr>
<td>Fatigue</td>
<td>30</td>
<td>68</td>
</tr>
<tr>
<td>Oedema</td>
<td>71</td>
<td>71</td>
</tr>
<tr>
<td>Nausea</td>
<td>51</td>
<td>49</td>
</tr>
<tr>
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<td>48</td>
</tr>
<tr>
<td>Myalgia</td>
<td>37</td>
<td>24</td>
</tr>
</tbody>
</table>

$^a$ Graded according to the National Cancer Institute Common Terminology Criteria for Adverse Events version 2.0.

#### 7.7.2 Treatment failure of imatinib

Less than 10% of patients with metastatic GIST have primary resistance, defined as progression within six months to imatinib\textsuperscript{9,124}. Typically, these patients have tumour genotypes, such as the PDGFRA D842V and KIT/PDGFR\textsubscript{A} wild types, that do not respond to imatinib or causes early progression with its administration\textsuperscript{58,114}. Most patients responding to imatinib develop secondary resistance after an initial response (median 2–3 years)\textsuperscript{104,124}. About 50–80% of patients with progression develop secondary resistance mutations (Figure 5) that escape imatinib binding (KIT exons 13 and 14) or amplify KIT kinase activation.
The metastatic lesions with secondary resistance are distinct among different patients or even different among metastases in the same individual\textsuperscript{128,129}. Whether secondary mutations are present as de novo subclones or develop with imatinib treatment is not known. Although other TKIs are effective against some secondary mutations (e.g. sunitinib and regorafenib as discussed in detail in Section 7.7.4 and 7.7.5, respectively), none is effective against all of them, making it difficult to have a response in all the lesions with salvage TKI monotherapy. Unfortunately, when GIST patients develop imatinib resistance, it seems clear that, in many patients, a general disease resistance to TKIs develops, and they eventually surrender to their disease.

![Figure 5. Tyrosine kinase inhibitor induced secondary mutations in KIT kinase. The location of primary mutations is shown on the left. The protein domains are shown to the right of this panel. To the right, a magnified view of the kinase domain (exons 13-18) is indicated. The relevant KIT exon locations are shown on the figure. The codon location of drug resistance mutations is depicted to the right of the magnified view of the kinase domain. The wild-type amino acid for the relevant codons is shown and reported amino acid substitution mutations are listed.](image-url)
Even though substantial variance in imatinib plasma concentrations between patients (40–60%) has been demonstrated\textsuperscript{130,131}, a standard dose of 400 mg is recommended in patients with metastatic GIST\textsuperscript{61,62}. Whether low amounts of imatinib in the tumour tissue could cause progression with imatinib is not known. However, one study demonstrated a significantly shorter PFS in patients with imatinib plasma trough levels (\(C_{\text{min}}\)) below 1110 ng/mL at day 29\textsuperscript{131}. Another study analysed trough levels after 3 months of treatment and found that concentrations above 760 ng/mL were associated with longer time to progression\textsuperscript{132}. Furthermore, a pharmacokinetic study demonstrated that after 90 days of treatment, a significant decrease of around 30% in plasma imatinib concentration occurred, and stabilisation of the concentration was observed thereafter\textsuperscript{133}. However, the optimal threshold value of imatinib plasma level has yet to be determined. Interestingly, some patients that progress on 400 mg/day may benefit from increasing the dose to 800 mg/day\textsuperscript{134,135}. Hence, in patients with suspected low concentrations, measurement of trough concentrations could be reasonable. If a low concentration is detected, an increase in dose may be justified. In \textbf{Paper III}, we explored repeated monitoring of plasma imatinib in patients with metastatic GIST.

Levels of compliance with imatinib therapy should be ascertained when progression occurs. Poor compliance (in up to 25% of the patients) has been reported for patients with metastatic GIST\textsuperscript{136}. Although, oral oncological medications offer patients the convenience of self-administration at home, it has been demonstrated that adherence to these medications is not optimal\textsuperscript{137,138}. Maintaining proper adherence is important, since evidence has indicated that interruption of imatinib leads to rapid progression (as discussed in Section 7.7.1)\textsuperscript{117-119}. Moreover, drug monitoring may improve compliance with therapy.
7.7.3 Managing imatinib failure

Although there is no benefit of receiving 800 mg daily in metastatic GIST cases (except for tumours with KIT exon 9 mutations)\textsuperscript{111}, dose escalation to 800 mg may be favourable in about one-third of patients who have progressive disease on 400 mg\textsuperscript{134,135,139}. However, a retrospective nonrandomised study comparing sunitinib with dose-escalated imatinib as the second-line in metastatic/advanced KIT exon 11–mutated GIST patients showed favourable PFS rates in patients treated with sunitinib, but no difference in OS\textsuperscript{140}. Interestingly, imatinib was less effective in patients with GISTs having a KIT exon 11 deletion than in those with another type of mutation in exon 11\textsuperscript{140}. Although prospective studies on larger series of patients are needed to confirm the best choice of second-line treatment, dose escalation of imatinib could be considered in individual patients to delay switching to sunitinib.

Patients with focally progressive GISTs can be considered for surgery of the progressive metastases to delay switching to the second-line therapy (Figure 6). Retrospective studies have shown the median PFS after resection is between 8–12 months, but some patients may have longer time to progression\textsuperscript{141,142}. A small retrospective study of patients with focally progressive disease comparing surgery of the progressive metastases to delaying a switch to second-line TKI versus a direct switch to second-line TKI showed a clear benefit in PFS (12 months vs. 6 months; $p < 0.001$) and OS (52 months vs. 26 months; $p = 0.003$) among the patients that underwent surgery\textsuperscript{143}. Radiofrequency ablation is a feasible and a safe alternative to surgery for liver metastases\textsuperscript{144-146}. Evidence has shown that patients progressing at several sites, should not undergo surgery, since this could lead to considerable morbidity, and the time to a second progression is usually short\textsuperscript{141,147}. Radiotherapy is a well-tolerated way to treat patients progressing in one or a few metastases, and it could be considered as an alternative to surgery in selected cases\textsuperscript{148}. 
Figure 6. Patients with multiple liver metastases responding to imatinib at two years of treatment (A). Six months later a ‘node in mass’ was detected (B). The patient had surgery of the progressing liver metastasis in March 2016. He is still on imatinib without further progression (last update December 2017).

7.7.4 Sunitinib

GIST patients that progress during imatinib treatment or are unable to tolerate it are switched to sunitinib\textsuperscript{[61,62]}; a multitarget inhibitor that inhibits KIT, PDGFRA and vascular endothelial growth factor receptors, among others\textsuperscript{[149]}. Sunitinib is given orally at 50 mg/day, with a 4-week-on/2-week-off schedule, or continuous therapy of 37.5 mg/day. In a randomised study of metastatic GIST comparing sunitinib to a placebo in 312 patients with progression on imatinib, the median PFS was superior with sunitinib compared to the placebo (6.3 months vs. 1.5 months; HR 0.33; \( p < 0.001 \))\textsuperscript{[149]}. The side effects of sunitinib (most frequently hypertension, fatigue, diarrhoea, skin rash and nausea) differ from those of imatinib, and they are generally more severe\textsuperscript{[149]}. Primary \textit{KIT} exon 9–mutated GISTs and \textit{KIT/PDGFRA}-negative GISTs have beneficial PFS and OS rates on sunitinib compared with those with \textit{KIT} exon 11 mutations\textsuperscript{[150-152]}. Patients with secondary ATP-binding domain mutations (\textit{KIT} exon 13 or 14) have superior PFS and OS rates on sunitinib compared to patients with secondary activation loop domain mutations (\textit{KIT} exon 17 or 18)\textsuperscript{[151]}. 
7.7.5 Regorafenib

The recommended third-line therapy for metastatic GIST is regorafenib. In one study, patients who had progressed on imatinib and sunitinib were randomly assigned to either regorafenib or a placebo, and regorafenib exhibited a superior median PFS compared with the placebo (4.8 months vs. 0.9 months; HR 0.27; \(p < 0.001\)). Regorafenib is taken orally 160 mg/day, with a 3-week-on/1-week-off regimen. The most common adverse events related to regorafenib are hypertension, hand-foot syndrome and diarrhoea. Long-term follow up from the trial described above showed that patients with primary KIT exon 11–mutated GISTs and those with SDH-deficient GISTs benefited more than those with other mutations.

7.7.6 Other TKIs

Several other TKIs have activity in the treatment of metastatic GIST patients. The only TKI that has been compared with imatinib as the first-line therapy for metastatic GIST in a randomised phase III study is nilotinib. This drug was regarded as inferior to imatinib, as the 2-year PFS was 59.2% in the imatinib group, compared with 51.6% in the nilotinib group (HR 1.47; 95% confidence interval [CI] 1.10–1.95). Furthermore, another randomised phase III trial failed to demonstrate significant activity of nilotinib in the third-line setting.

Pazopanib has shown activity in patients with progressive GIST on both imatinib and sunitinib. Pazopanib was compared to best supportive care alone in a small randomised phase II trial (PAZOGIST) conducted in 81 patients who were refractory to imatinib and sunitinib. The 4-month PFS was significantly higher with pazopanib (45% vs. 18%), and the median PFS was 3.4 versus 2.3 months (HR 0.59, 95% CI 0.37–0.96).

Sorafenib, a multikinase inhibitor comparable to regorafenib, has shown activity in the
third- and fourth-line therapies for metastatic GIST in nonrandomised trials. The largest series of patients (retrospective) showed a partial response in 10% of cases and stable disease in 57%; the median PFS was 6.4 months, and the median OS was 13.5 months\textsuperscript{158}.

Masitinib is a TKI showing more activity and selectivity than imatinib against mutations in the juxtamembrane region (\textit{KIT} exon 11) and wild-type KIT\textsuperscript{159}. Masitinib showed promising activity as a first-line treatment in a small phase II study on 30 patients with metastatic GIST\textsuperscript{160} and in a small randomised phase II trial comparing masitinib to sunitinib in patients with imatinib failure (second-line)\textsuperscript{161}.

Dovotinib, a multikinase inhibitor, has recently been shown to have activity in patients with metastatic GIST refractory to imatinib, with a median PFS of 4.6 months\textsuperscript{162}. In addition, Crenolanib has been shown to be significantly more potent than imatinib in inhibiting the kinase activity of imatinib-resistant PDGFR\textalpha\textit{A} kinases (including D842V-mutated kinase)\textsuperscript{163}, and it is now being explored in a randomised trial (crenolanib vs. placebo) in subjects with \textit{PDGFR}\textalpha\textit{A} D842V mutated GIST (Clin. Gov: NCT02847429). Lastly, combinations of TKIs are now being explored, such as in the ALTGIST trial (Clin. Gov: NCT02365441). This first-line treatment trial on advanced GIST randomises between imatinib alternating with regorafenib and imatinib alone.

\subsection*{7.7.7 Other systemic treatments}

As mentioned in Section 7.7.1, conventional chemotherapy has minimal activity against GISTs\textsuperscript{16,108}. No further studies on chemotherapy have been conducted since the introduction of TKIs. Immunotherapy may be promising, due to evidence that GISTs contains tumour-infiltrating immune cells, and currently, there are several ongoing preclinical and clinical trials exploring this treatment strategy\textsuperscript{164}. Inhibitors of heat-shock protein, such as
AUY922\textsuperscript{165}, are under investigation in patients with metastatic GIST. In addition to these, many other agents are currently being investigated in clinical trials on metastatic GIST\textsuperscript{166}.

7.7.8 Surgery of residual disease

Metastasis surgery combined with TKI therapy may facilitate beneficial outcomes. A small tumour burden has been associated with long PFS on imatinib treatment, and theoretically, surgery to reduce the tumour volume may prolong the time to drug resistance\textsuperscript{104-106}. Some retrospective studies have reported beneficial outcomes with surgery in metastatic GIST patients responding to imatinib\textsuperscript{105,147,167,168}. A small prospective study on 41 patients with liver metastases from GIST randomised patients to imatinib alone versus imatinib followed by metastasis surgery and continuation of imatinib after surgery. The 1-and 3-year survival rates were higher in the surgery group compared with the imatinib-only group (100% and 89\% vs. 85\% and 60\%, respectively)\textsuperscript{169}. A similar small randomised trial demonstrated that patients treated with imatinib and resection of residual disease had longer PFS and OS periods than patients treated with imatinib alone\textsuperscript{170}. Completely powered randomised trials are lacking; however, the removal of the macroscopic tumours might prolong the duration to secondary resistance and lengthen survival in metastatic GIST patients.

7.7.9 Radiotherapy

GIST has generally been considered radiation resistant, and typically, radiotherapy is given only for painful bone metastases. However, case reports have suggested that GIST may respond to radiotherapy\textsuperscript{171}. Only one prospective study has explored radiotherapy in GISTs. This study included 25 patients affected by liver, soft-tissue, intra-abdominal and bone metastases that were treated with external beam radiotherapy, and most patients in the study
had benefitted from radiotherapy. A cumulative dose of approximately 40 Gy was given, while maintaining systemic therapy during irradiation (mostly imatinib). Only two patients achieved partial remission; however, only three progressed, and the rest had a long-term stabilisation of the lesions that received radiotherapy, with a median stabilisation of 16 months. This suggests that GIST metastases are moderately radiosensitive. High-dose radiotherapy, such as stereotactic body radiotherapy, has shown encouraging results in patients with small liver metastases.

7.7.10 Survival and prognostic factors in metastatic disease

Prior to the introduction of imatinib, patients with metastatic GIST had a poor prognosis, with a median OS of 1–2 years. Now, a median OS of 6 years may be expected. However, there is considerable variation in outcomes among patients with metastatic GIST and the time to development of imatinib-resistant disease is probably a key determinant of survival, since second-generation TKIs are not as efficient. Some patients present rapid tumour progression on imatinib, whereas others may stay on the drug for more than 10 years. In clinical trials, resistance to imatinib has occurred at a median time of 18–26 months. Studies in real-life practice have shown a median PFS of 30–40 months.

Factors associated with long-term survival in clinical trials include KIT exon 11 mutation, female sex, normal albumin levels, neutrophil and lymphocyte counts, good performance status and small tumour diameter. There are more limited data regarding treatment and long-term outcomes in routine practice. In a single-institution study from Taiwan, good performance status, absence of primary imatinib resistance and small tumour volume were associated with a longer OS. In addition, a nationwide study from Poland reported that long-term survivors were characterised by small maximum tumour
diameter at imatinib initiation, better haematological and clinical chemistry parameters, good performance status and surgical removal of residual disease\textsuperscript{105}. In \textbf{Paper II}, we expand on this knowledge. Nomograms for PFS and OS have been developed in metastatic GIST, and risk factors included are the size of the largest metastasis, tumour genotype, primary tumour mitotic count, haemoglobin and neutrophil count at commencement of imatinib\textsuperscript{174}. 
8. Material and methods

8.1 Study population

This thesis is based on patients with GIST treated by the Sarcoma Group at Oslo University Hospital (OUH), which serves as a referral centre for soft-tissue and bone sarcoma in the south-eastern region of Norway. This is the largest region in Norway, comprising 10 counties and a population of about 2.8 million people (Figure 7). Since 1980, the Sarcoma Group at OUH has prospectively registered data on all sarcomas in a database. All cases in this thesis were extracted from this database.

Figure 7. Map of Norway. South-eastern region of Norway is marked by dark blue.
8.1.1 Paper I

Patients with GIST treated with imatinib and with regular follow up at our oncological outpatient clinic were included for plasma concentration measurements. We included patients from January 2011 to April 2015. The inclusion criteria were as follows: (1) histologically confirmed GIST; (2) treatment with imatinib initiated more than 90 days prior to study entry; and (3) high-risk tumour requiring adjuvant imatinib, metastatic disease or inoperable primary tumour. In Paper I, we focussed on patients in an advanced or metastatic setting, and patients under (neo-) adjuvant imatinib were excluded. Furthermore, patients who had less than three available plasma samples and where drug intake was not registered were excluded. Thus, 24 patients were included in the final cohort (Figure 8).

Figure 8. Consort diagram Paper I
8.1.2 Paper II

Patients diagnosed with metastatic GIST from January 1st, 1995 to January 1st, 2013 were identified from the sarcoma database. Metastatic GIST was defined as metastases to the liver, intraperitoneal cavity or other organs. Eighteen patients were excluded, and the final cohort consisted of 115 patients with metastatic GIST (Figure 9).

Figure 9. Consort diagram Paper II.

8.1.3 Paper III

Patients with gastric GIST diagnosed between 2000 and 2015 were identified in the sarcoma database. Those who did not undergo surgery for their primary tumour or who had synchronous metastases were excluded (Figure 10). Thus, 242 patients who underwent resection for primary, non-metastatic gastric GISTs were enrolled in the study.
8.1.4 Paper IV

Patients undergoing surgery for GISTs of the stomach or the small intestine from January 2000 to April 2017 were identified in the clinical sarcoma database. Patients with metastatic disease at the time of the surgery were excluded. In all, 375 patients underwent surgery for localised gastric or small intestinal GISTs, of whom 269 (72%) had gastric and 106 (28%) had small intestinal tumours. Mutation analysis was not performed in 153 patients, and in 11 patients, the analysis was technically unsuccessful. Classification of tumour rupture was not possible in two patients. Thus, 209 patients were included in the final study cohort (Figure 11).
8.1.5 Paper V

Three patients from the cohort in Paper II were presented as case-reports.

8.2 Data source and management

As mentioned in Section 8.1, all patients with sarcoma referred to OUH are registered in a prospective database. This database includes clinical information concerning treatment and follow up. Data on all cases in this project were extracted from this database. Information included demographic, clinicopathological and treatment variables. This information was validated and expanded by a retrospective review of the hospital medical records.

8.3 Histopathology

The diagnosis was confirmed by a sarcoma pathologist according to the current recommendations of the World Health Organization. We did not conduct a formal review of
the pathology specimens, but all the cases were re-evaluated based on the histological reports. However, in Paper III and Paper IV, cases where tumour rupture was not explicitly described in the pathology report or where there were missing mitotic counts, the slides were re-examined. Patients with morphological and immunohistochemical documentation of GIST (immunostaining for KIT/CD117 and/or DOG-1 [anoctamin-1]) were included in the four study cohorts. Patients that demonstrated mutation in KIT or PDGFRA could enter the study despite negative immunostaining for KIT and DOG-1 if the tumour histology was compatible with GIST.

8.4 Radiology

We did not review radiographic images as a part of this work. However, radiological reports (usually CT, MRI or PET/CT) were available and reviewed for disease recurrence (Paper III) or progression (Papers I and II). Disease progression was documented by an experienced radiologist. During follow up, radiological evaluation with CT of the abdomen and pelvis was performed every 3–6 months, depending on the clinical scenario.

8.5 Demographic and clinicopathological variables

Information relevant for this study included the following:

I) Demographics: Gender, date of diagnosis, age at primary diagnosis, age at surgery, date of metastatic diagnosis, age at metastatic disease, date of death and cause of death;

II) Baseline parameters at primary diagnosis: Preoperative biopsy, metastasis at the time of diagnosis and anatomic localisation of the primary tumour;

III) Primary tumour data: Histological subtype, tumour size, mitosis, mutations, tumour rupture and risk stratification according to the modified NIH criteria; and
IV) Baseline parameters at metastatic diagnosis: Metastatic site, number of metastases (oligo- vs. polymetastatic disease), maximum size of the largest tumour, baseline blood tests and baseline performance status according to the Eastern Cooperative Oncology Group (ECOG).

Our definitions of the most important parameters are given below.

*Date of diagnosis:* For patients who underwent surgery for a primary GISTs without pre-treatment of imatinib, the time of surgery was the date of diagnosis. For other patients, the date of diagnosis was set when histology revealed GIST.

*Death and cause of death:* The date of death was retrieved from the National Registry of Norway. The cause of death was retrieved from the hospital records and registered as GIST, unknown or others.

*Metastasis at diagnosis:* We defined metastasis within 6 weeks after primary diagnosis as synchronous (metastasis at the time of diagnosis) and metastasis more than 6 weeks after the primary diagnosis as metachronous.

*Tumour size:* Tumour size was measured using surgical specimens or radiological images when the patients did not undergo surgery; it was measured as the maximum length of the tumour in centimetres.

*Mitotic count:* Mitoses were counted on the primary tumour in 50 HPFs of the microscope or 5 mm² (for the more recent years). Mitoses were not counted in patients pretreated with imatinib.

*Mutations:* Mutational analysis was routinely performed on all intermediate and high-risk tumours and discriminately on low- and very-low-risk tumours. In patients who developed metastatic disease, genotyping of the primary tumour or a recurrent tumour was performed in most cases. Mutation analysis was performed as described previously. Genomic DNA was extracted from formalin-fixed paraffin-embedded or fresh frozen tumour
tissue. Screening was performed for the presence of KIT (exons 9, 11, 13 and 17) or PDGFRA (exons 12, 14 and 18) mutations.

Risk stratification: The primary tumours were stratified according to the modified NIH criteria (Table 2; Section 7.6.4) into very low, low, intermediate, and high risk.

Tumour rupture: Patients were stratified according to the definition of tumour rupture proposed by the Oslo Sarcoma Group. Tumour rupture included spillage, piecemeal resection, surgical biopsy, blood-tinged ascites, gastric perforation, and infiltration into an adjacent organ. The following minor defects of tumour integrity were not considered rupture: core needle biopsy, peritoneal tumour penetration, iatrogenic peritoneal laceration and R1 resection.

Metastatic disease: Metastatic GIST was defined as metastases to the liver, intraperitoneal cavity or other organs.

Date of metastatic disease: Date of metastatic disease (recurrence) was recorded if verified on biopsy or indisputable on CT.

Blood tests: In Paper I, biochemical tests, such as for creatinine, calcium, magnesium and phosphate; haematological values, such as the haemoglobin level, white blood cell count, mean corpuscular volume (MCV) and platelet count; and liver function tests, such as ALAT, ASAT, GT, ALP, and albumin, were measured at the same time as plasma samples for imatinib plasma concentration measurements were drawn. The results from these tests were compared with the imatinib plasma level. In Paper II, the baseline haemoglobin level, albumin level, neutrophil count, and lactate dehydrogenase level were analysed and compared according to the OS.

Number of metastases: Patients were stratified according to oligometastatic disease
OMD), defined as three or fewer metastases detectable on CT at the commencement of systemic treatment of metastatic disease, or polymetastatic disease (PMD; more than three metastases).

*Maximal diameter of the largest tumour in metastatic disease:* This was defined as the largest diameter in cm of the largest tumour (primary tumour or metastasis) on CT at the start of systemic treatment.

### 8.6 Treatment variables

The treatment variables were as follows:

I) Primary surgical treatment data: Surgery at or outside sarcoma centre, type of surgery (elective, emergency, incidental finding); type of resection, multivisceral resection, completeness of resection and neoadjuvant or adjuvant treatment; and


The most important definitions are given below.

*Neoadjuvant treatment:* Neoadjuvant treatment with imatinib 400 mg daily was given at the discretion of the multidisciplinary team to patients with marginally resectable tumours, tumours at high risk of rupture or cases where anticipated tumour shrinkage would reduce the extent of the surgery or secure margins.

*Adjuvant treatment:* Most patients with high-risk GISTs according to the NIH or modified NIH criteria were included in the SSG XVIII/AIO trial from May 2004 to August 2008 (1 vs. 3 years of adjuvant imatinib)\(^2\). From September 2008 to May 2011, high-risk patients received adjuvant treatment for 1 year, and from June 2011, they received it for 3 years. From May 2015, gastric GIST-patients with more than 10 mitoses per 50 HPFs, non-
gastric GIST with mitotic counts of more than 5 mitoses per 50 HPFs or patients with tumour rupture have been enrolled in the SSG XXII trial (Clin.gov: NCT02413736; 3 vs. 5 years of adjuvant imatinib).

Systemic treatment in metastatic disease: The date when the patient started systemic treatment was registered. In addition, the number of lines of TKIs and duration of each line were registered.

Surgery in metastatic disease: The number and types of surgical procedures were registered, as well as whether surgery was performed before or during systemic treatment and the reason for the procedures, such as surgery for residual disease, surgery for progressive metastases or surgery for symptomatic (nonprogressive) metastases.

8.7 Blood sampling for pharmacokinetic assessment

In Paper I, plasma samples for imatinib plasma concentrations were collected. Three millilitres of heparin plasma were taken at each follow-up visit, and within 1 hour, the blood samples were centrifuged at room temperature for 15 minutes at 2500 × gravity. The samples were stored at –20°C until analysis. The time of drug intake was registered, and a validated Bayesian method177 was used to extrapolate the measured concentrations to the trough level. Other important variables registered during follow up were body weight, height and biochemical parameters (as described in Section 8.5).

8.8 Measurement of imatinib concentrations

Imatinib plasma concentration measurement was performed via the expert analysis of Professor Jonas Bergquist and his laboratory in Uppsala, and it followed the protocol described by Ubhayasekhera et al.178. Briefly, from the imatinib standard supplied by Novartis, final concentrations of 1 mg/µL were obtained from the stock solutions of imatinib
and internal standard by dissolution in methanol. Imatinib was extracted, and 25 µl of methanol containing 1 µg/mL internal standard and 0.5 mL of methanol was added to 100 µl of plasma, shaken for 10 minutes and centrifuged for another 10 minutes at 4°C at 14,000 × gravity. Aliquots of 10 µl were injected into the liquid chromatography–mass spectrometry system. The chromatography and mass spectrometry approach is not described in detail here, but it was performed as previously described by Professor Bergquist’s research group.

8.9 Statistical analyses

All the statistical analyses were conducted using the Statistical Package for the Social Sciences (SPSS) version 21 (SPSS, Inc., Chicago, IL, USA).

8.9.1 Descriptive statistics

For the descriptive analyses of the demographic and clinicopathological data, the chi-squared test or Fischer’s exact test was used where appropriate for dichotomous data. The Student’s t-test was used for continuous parametric data and for nonparametric data that were log-transformed (Paper I). The Mann–Whitney U test (two subgroups) and Kruskal–Wallis test (more than two subgroups) were used for nonparametric continuous data that were not log-transformed. In Paper I, correlations were analysed to identify associations using univariate linear regression (Pearson) and the independent Student’s t-test. Variables that showed significant correlations (p < 0.05) in univariate analysis were included in a multivariate analysis using a multiple linear regression model with a stepwise, backward elimination of variables. Correlations were also tested using a more stringent linear mixed-models effect analysis to consider account intra-patient correlations.

In Paper IV, multivariable logistic regression analysis was performed to ascertain the effects of tumour genotype, tumour size and mitotic count on the likelihood of tumour
rupture. All tests were two-sided, and p-values less than 0.05 were considered statistically significant.

### 8.9.2 Survival analyses

Estimates for RFS and OS were performed using the Kaplan–Meier method with the log-rank test (categorical variables) and Cox regression model for continuous data. The OS was calculated from the date of diagnosis of metastatic disease in Paper II and the date of primary surgery in Paper III to the date of death from any cause. The RFS was calculated from surgery for the primary tumour until recurrence in Paper III.

Multivariate analysis was conducted using the Cox proportional hazards regression model with the enter method in Paper II and backward, stepwise elimination of variables in Paper III. Gender, age and all covariates significant in univariate analysis were included in the models. All tests were two-sided, and the significance level was set to 5%.

### 8.10 Ethical approval

In Paper I, the study was approved by the Regional Ethics Committee (REC; #S-06133a), and written informed consent was obtained from all patients. The studies in Papers II, III and IV did not require a formal ethical approval from the REC; however, the studies were approved by the local data protection officer, and informed consent was obtained from all patients (Paper II, #2015/749; Paper III, #2016/16853; and Paper IV, #2015/15603 and #2016/16853). In Paper V, signed informed-consent were obtained from all the three patients presented as case-reports.
9. Summary of the results

9.1 Paper I

All 24 patients included in the study received imatinib for metastatic disease, except one patient who was medically inoperable and received imatinib for a large GIST in the small bowel. The median age was 69 years (range: 33–88 years). Ninety-six plasma samples were analysed, and the median duration of imatinib treatment prior to the first sample was 25 months (range: 3–77 months). The median time from the first sample to the last sample was 32 months (range: 4–48 months).

The mean imatinib plasma concentrations were 782 ng/mL for patients taking daily dose of <400 mg \((n = 21)\), 1132 ng/mL for those taking 400 mg \((n = 69)\) and 1665 ng/mL for those taking >400 mg \((n = 6; p = 0.010)\). In patients taking 400 mg, the mean intra-patient variability (coefficient of variation) was 36%, and the mean inter-patient variability was 68%.

Six patients had a dose reduction to 200 mg due to side effects; two of these had relatively high mean plasma levels of 1418 and 2242 ng/mL, whereas the other four had mean plasma concentrations of 387, 437, 565 and 521 ng/mL. Two patients started on 200 mg and had mean plasma concentrations of 1704 and 540 ng/mL.

In the 400 mg group, high imatinib plasma levels were correlated with age \((p = 0.012)\), low body surface area \((p = 0.010)\), low haemoglobin concentration \((p = 0.016)\), low creatinine clearance \((p = 0.050)\), absence of liver metastasis \((p = 0.025)\) and no prior gastric resection \((p = 0.015)\) in univariate analysis. In multivariate analysis, age \((p = 0.049)\), gastric resection \((p = 0.021)\) and liver metastasis \((p = 0.010)\) were included in the final model.

Eight patients had progressive disease during the study. The mean plasma imatinib levels were significantly lower at progression (770 ng/mL) compared with the previous measurement under stable disease for the same patients (1223 ng/mL; \(p = 0.021)\).
There were 69 males and 46 females among the 115 patients with metastatic GIST included in the final cohort. The median age at diagnosis with metastatic disease was 64 years (range: 31-88 years). Imatinib was the first-line treatment for 110 patients and nilotinib was used for 2 patients, while 3 patients never started systemic treatment. The second-line treatment was given to 35 patients, and 19 received third-line treatment. Subsequently, patients were included in studies (nilotinib/pazopanib), treated with off-label use of sorafenib, reinstitution of imatinib or best supportive care. The median length of the first-line treatment was 49 months (range: 1–161 months), for the second-line treatment 7 months (range: 0.2–74 months) and 5 months (range: 1–32 months) for the third-line treatment.

The median OS was 6.9 years (CI: 5.6–8.3), and after a median follow up of 9.0 years, 52 patients (43%) were still alive. Of the 63 patients that died during the study, 57 died of metastatic GIST, whereas six died of other or unknown causes. The following factors were significantly associated with a longer OS in univariate analysis: good baseline performance status (ECOG status ≤1; \( p < 0.001 \)), age below median (64 years; \( p = 0.022 \)), OMD (≤3 metastases; \( p < 0.001 \)), small diameter of the largest metastasis (<5 cm; \( p < 0.001 \)), surgery for metastatic disease (\( p = 0.005 \)), surgery of the primary tumour (\( p = 0.001 \)), normal baseline haemoglobin level (≥11.0 g/100 mL; \( p = 0.050 \)), normal baseline albumin level (≥35 g/L; \( p = 0.001 \)) and normal baseline neutrophil count (≤5.0 10⁹/l; \( p = 0.030 \)). The variables significantly associated with a longer OS in multivariate analysis were OMD (\( p < 0.001 \)), small diameter of the largest tumour (\( p = 0.040 \)), and good baseline performance status (\( p < 0.001 \)). Patients with OMD had a 5-year and 10-year OS of 89% and 71%, respectively, compared to 38% and 20% for patients with PMD (\( p < 0.001 \)).
9.3 Paper III

Among the 242 patients who underwent resection for primary, non-metastatic gastric GISTs included in the study, there were 119 (49%) males, and the median age was 67 years (range: 14–93 years). The median follow up was 49 months (range: 0–175 months). All resections were macroscopically complete, with 213 (88%) R0 and 28 (12%) R1 resections. The median tumour size was 4.8 cm (range: 0.5–34.0 cm), and the median mitotic index was two per 50 HPFs (range: 0–178).

Using our definition, tumour rupture was documented in 22 (9%) patients, minor defects of tumour integrity in 81 (34%) patients and no defect of tumour integrity in 138 (57%) patients. Patients with rupture had a higher mitotic index ($p < 0.001$) and larger tumours ($p < 0.001$) compared with patients without rupture.

There were 27 patients who relapsed at a median time of 26 months (range: 3–126 months) after surgery, 16 with tumour rupture, 6 with minor defects of tumour integrity and 5 with no defect. Overall, the estimated 5-year RFS was 88%, while it was 96% for patients with no defect of tumour integrity, 91% for patients with minor defects ($p = 0.130$) and 37% for patients with tumour rupture ($p < 0.001$).

No patients in the very-low- and low-risk group, 1 in the intermediate group and 25 in the high-risk group exhibited a recurrence. In the high-risk group, 9 (20%) of the 44 patients without rupture had a recurrence, and 16 (73%) of the 22 patients with rupture had a recurrence. For patients with a tumour size larger than 10 cm, the 5-year RFS with rupture was 38%, compared with 86% without rupture ($p = 0.002$). The corresponding number for patients with a mitotic index >5 was 30% versus 86% ($p < 0.001$), while for high-risk patients, it was 37% versus 77% ($p = 0.001$). On multivariate analysis, tumour rupture ($p = 0.004$) and mitotic index ($p < 0.001$) were the only factors independently associated with RFS.
Forty-two (17%) patients, all in the high-risk group, received adjuvant imatinib for a median of 13 months (range: 1–56 months), among whom, 13 had tumour rupture. Eight patients were still under treatment at the latest follow up. Among the 24 patients who completed adjuvant treatment, 12 relapsed, of whom 8 had tumour rupture.

9.4 Paper IV

This study included 209 patients with gastric or small bowel GISTs with available data on tumour rupture and mutational status. *KIT* mutations were found in 167 (80%) tumours and *PDGFRA* mutations in 29 (14%) tumours; in 13 (6%) tumours, no mutation was discovered.

Overall, tumour rupture occurred in 37 (18%) patients and was present in 33 of 167 (20%) of the *KIT*-mutated tumours, 2 of 27 (7%) with *PDGFRA* mutation and 3 of 13 (15%) with no mutation. All patients with rupture and *KIT* mutations had exon 11 mutations, except for 1 patient, who had an exon 13 mutation. Among 86 patients with a deletion or indel, 25 (29%) had rupture, compared to 5 of 50 (10%) for substitutions and 2 of 19 (11%) for duplications/insertions (*p* = 0.014). Rupture occurred in 17 of 46 (37%) of tumours with del557/558 and 15 of 109 (13%) with other exon 11 mutations (*p* = 0.002). This association was restricted to gastric tumours, as 12 of 34 (35%) gastric GISTs with del557/558 had rupture compared to 6 of 77 (8%) with other exon 11 mutations (*p* = 0.001). In small bowel GISTs with del557/558 mutations, rupture was recorded in 5 of 12 (42%) patients, while it was observed in 9 of 32 (28%) cases with other exon 11 mutations (*p* = 0.475). The frequency of del557/558 mutations was similar in both tumour localisations and 34 of 111 (30%) of gastric tumours and 12 of 44 (27%) of the tumours in the small intestine had a del557/558 mutation (*p* = 0.846).

Gastric tumours with del557/558 had a median size of 10.9 cm (range: 2.8–30.0 cm) versus 5.1 cm (range: 0.5–21.0 cm; *p*<0.001) for gastric tumours with other genotypes.
Moreover, they had a higher mitotic count, with median 18 mitoses per 50 HPFs (range: 2–178), versus 3 (range 0–35) for other mutations ($p < 0.001$). In the multivariate logistic regression analysis, del557/558 mutations ($p = 0.042$) and tumour diameter ($p < 0.001$) were associated with an increased likelihood of tumour rupture, while the mitotic count was not ($p = 0.688$).

Fifteen of 37 (41%) patients had potentially avoidable iatrogenic rupture. In 19 (51%) patients, rupture occurred spontaneously before the surgery. Finally, 3 (8%) patients had both iatrogenic and spontaneous rupture.

Ten patients received neoadjuvant imatinib with a median duration of 8 months (range: 1–10 months). Among the 6 patients with gastric tumours and del557/558 mutation who received neoadjuvant treatment, rupture occurred in one.

9.5 Paper V

In this paper, we hypothesise that a long-term response to imatinib and complete resection of the metastatic lesions harbouring foci responsible for drug resistance and subsequent clinical relapse may lead to a cure and the justification to stop imatinib in selected patients. We suggest that this novel strategy, a priori, warrants further investigation. Thus, we reviewed the available literature, presented three clinical cases and put forward for discussion a treatment algorithm that needs confirmation in the context of a prospective clinical study.
10. Methodological considerations

10.1 General considerations

The patient material is based on a single tertiary institution’s treatment and follow up of GIST within a time frame of about two decades (Section 8.1). The main strength of the present study is that all patients had follow up in one institution and were treated by a limited number of sarcoma specialists who all belong to the same multidisciplinary team, making the treatment relatively consistent.

Norway follows the international consensus that sarcoma treatment should take place at highly specialised centres, and diagnostics and treatment are formally centralized at the regional hospital level. The Sarcoma Group at OUH serves as a referral centre in the south-eastern region of Norway, with a catchment area of about 2.8 million (Section 8.1). Our main data source was information retrieved from the sarcoma database at OUH. As mentioned in Section 8.2, this database contains prospective data on all sarcomas treated and followed up at the hospital. Theoretically, this database should include all patients with GISTs from the south-eastern region of Norway, and one could argue that this thesis is a population-based material. However, we have not crosslinked and validated this with the Cancer Registry in Norway (CRN), which has collected data on cancer incidence, mortality and survival in Norway since 1953, based on the mandatory reporting of all cancers180. Hence, we cannot exclude the possibility that some patients have been treated and followed up at their local hospitals. Furthermore, OUH receives some difficult cases from other regions in Norway, further complicating the numbers compared with the CRN statistics. Interestingly, when comparing the numbers from the CRN with the numbers from OUH, we can observe that they are relatively similar. Comparing numbers from 2000–2015, 255 patients with operable, localised gastric GISTs and 111 with operable, localised small intestinal GISTs were reported to the CRN from the catchment area of OUH. For the same period, the sarcoma database at
OUH contains 242 patients (Paper III and IV) with gastric GISTs (95%) and 100 patients (Paper IV) with small intestinal GISTs (91%).

All the data entered into the sarcoma database at OUH have been verified, and the quality of the data has been ensured by senior clinicians belonging to the OUH Sarcoma Group. As expected, the database did not include all the clinical information needed in this thesis. Thus, beyond validating the parameters obtained from the database, a full retrospective review of the patients’ medical records was performed. Indeed, the retrospective review of most of clinical parameters in all four papers is indisputably a limitation of the research. The main obstacles of a retrospective design include a lack of information and uncertainties concerning the validity of the reported information, changes in the treatment over time, follow up and reporting patterns and selection bias of the patients and the material included. In general, the results from retrospective studies should primarily be viewed as hypothesis generating rather than providing definitive answers.

Randomised clinical trials (RCTs) are considered the gold standard for establishing efficacy. However, RCTs use a standardised therapy in a selected group of patients and are typically restricted to evaluating specific interventions one at a time; they fail to assess complex interactions within a study arm or fail to establish continuous relationships between treatments. Thus, RCTs focus more on evaluating the efficacy of simple therapies and less on the delivery of care, and many study arms will be required for assessing the effectiveness of combinations and evaluating all these issues. Furthermore, it is sometimes unethical to randomise between two therapies, as was the case when imatinib was introduced in the treatment of metastatic GIST. A randomised trial comparing imatinib to a placebo was never done due to its excellent survival benefit.

In real-world studies, including this thesis, the care that patients receive in the clinical routine is recorded. Rather than having strict inclusion and exclusion criteria, all the patients
must be treated, including those with co-morbidities. Such studies generate long-term efficacy and safety data, and it is possible to compare multiple interventions and their effects on the disease. Indeed, there are several challenging issues related to collection of real-world data, such as the lack of good quality and sufficiently representative databases, incomplete databases, presence of many asymptomatic cases in the real world (an issue with retrospective observation of data) and more chances of bias and confounding in prospective real-world studies (as they do not involve randomization). Nevertheless, real-world data have an important role in the evaluation of epidemiology, compliance, persistence and health outcomes of different treatments. Furthermore, GIST is recognised as a rare cancer, and Rare Cancer Europe encourages observational clinical studies on rare cancers, as they can secure essential information on the natural history and clinical characteristics of rare entities and serve as external controls for clinical trials.

10.2 Validity

Internal validity is defined as the degree to which a study is representative of the specific group of individuals being studied. It may be weakened by sample selection bias, information bias and statistical confounding (as discussed in Section 10.6). In Paper II, all patients with metastatic GIST, and in Paper III, all that underwent surgery for stomach GIST were included in the study. Hence, the results describe the development of treatment and outcomes for all patients with such diagnoses at our hospital, not only selected groups included by strict inclusion and exclusion criteria. However, the studies may be biased according to the referral pattern and treatment profile of our hospital. Even so, the similar corresponding number from our database and CRN, mentioned in Section 10.1, implies that almost all patients with GIST in the largest region of Norway are referred to our hospital, and in our view, the sample selection bias was minimal. The study in Paper I included
considerably fewer patients, and the inclusion criteria introduced a possible selection bias by excluding patients with fewer than three plasma samples. Although they were not excluded due to progressive disease, a bias towards including patients without progression could have occurred. The study in Paper IV included patients who underwent surgery for either gastric or small intestine GISTs and who had available mutational analysis. Here, we introduced a possible bias toward large tumours since very few low- and very-low-risk tumours had an available mutational status. However, tumour rupture seems to be a general problem in large GISTs and not small GISTs. Finally, we attempted to reduce information bias by validating and reviewing patients’ medical records.

External validity refers to the degree to which a study can be generalised to other settings and individuals. Support for the validity of our results could come from replication of our research design with another sample drawn from the same population, or from other researchers using the same design in other samples. External validity is in general a complex challenge because generalisation depends on the study design, population and statistical model used, and there are many factors limiting generalisation. Theoretically, the same design and method could be applied to other Norwegian or international sarcoma centres. Nevertheless, our results show many of the same tendencies as other real-life studies and clinical studies. Furthermore, treatment guidelines have been established and the treatment principles are largely the same in Europe, in the United States and Asia. Hence, we think that our results will apply to other large sarcoma centres.

10.3 Reliability

Reliability is referred to as the consistency of the recorded data on the measurements. The reliability of data is dependent on the variables that have been analysed (Section 8).
based on general guidelines, such as histopathology, mutational analysis or blood tests, certainly have better reproducibility than, for example, patient-reported compliance (as noted in Paper I). Nevertheless, histological examination is still subjective, and the biochemical references may also depend on each laboratory’s procedures. Furthermore, data collected in clinical trials is usually considered more reliable than corresponding variables from other studies, such as ours, as in clinical trials, data are collected prospectively in a uniform manner. As far as possible, we have tried to improve the data included in this thesis using several principles. First, we aimed to achieve high internal consistency of the data that were analysed through collection and review of the data by the same person(s) using the same guidelines for all patients in the study. For instance, one surgeon (the first author) and one pathologist (the third author) re-evaluated all surgical and pathological reports using the same definition of tumour rupture in Paper III and Paper IV. Second, we included well-defined variables in the cohorts, such as OMD in Paper II (Section 8.5) and tumour rupture in Paper III and Paper IV (Section 8.5). Hence, we assume that the reproducibility of this clinical information is high. Third, we omitted variables with possible low reliability, even though they could have been of interest. For instance, the correlations between imatinib plasma concentrations and side effects and concomitant medications were excluded from Paper I, as we did not register the two variables in a formal and prospective manner. Finally, the fact that all patients in this thesis were treated at the same hospital with only a few persons involved in every aspect of the evaluation, treatment and follow up of GIST, in our view, makes the data more robust and reliable.

10.4 Endpoints

In Paper I, the endpoints were correlations between plasma imatinib and other variables. Although the dates of progression on imatinib and survival dates were registered, we refrained
from survival analysis due to the small sample size. The endpoint in Paper II was OS. Disease-specific survival (DSS) could have been an alternative endpoint. However, of the 63 patients registered as dead, 57 died of metastatic GIST, whereas 4 died of other causes and 2 of unknown causes. Hence, we think that the reported OS data reflect disease mortality and OS is a valid endpoint in this cohort. Indeed, PFS on different lines of systemic treatment could have been of interest; however, this was not investigated, since a strict radiology review would have been mandatory. In Paper III, the RFS was investigated and recurrence was recorded if verified on biopsy or indisputable on CT. Death of other causes than GIST was not registered as recurrence but censored at the time of death. Recurrence dates were re-evaluated by retrospective review of radiological reports. OS was not reported in Paper III, since this was not one of the aims of the paper; however, the Kaplan–Meier curves for OS and DSS are presented in the appendix (Figure 12; Section 13). In Paper IV, we investigated factors associated with tumour rupture. We refrained from analysing the RFS and OS, as this was reported in another study by our group69 and in Paper III.

10.5 Cause of death

In Norway, all deaths are reported to the cause of death registry (CDR) by doctors, who are required to complete a death certificate. However, the exact underlying cause of death may be difficult to assess without an autopsy. Furthermore, the doctors often do not know the patients, and when a patient has a malignant diagnosis in his or her history, this may be reported as the cause of death, although occasionally incorrect. Thus, instead of retrieving data from the CDR, we attempted to verify the cause of death by reviewing the medical records. Patients were divided into three groups based on the cause of death, as follows: (i) GIST: patients that had end-stage GIST and died at the hospital or shortly after submission from the hospital; (ii) other: patients that clearly died of another cause (i.e. other
malignancies, stroke, heart disease); and (iii) unknown: patients in a stable GIST setting who died, and the reason was not found in the medical records.

10.6 Statistical analyses

In Paper I, correlations between imatinib plasma concentrations and other variables were analysed by univariate linear regression (Pearson) and the independent-samples Student’s $t$-test, and variables that showed significant correlations were included in a multivariate analysis using a multiple linear regression model. However, although this per-sample analysis allowed consideration of the variations along the time of dosing, a more stringent linear mixed-models effect analysis to take into account intra-patient correlation was performed, showing the same trends.

The sample sizes in all four main studies were rather small, which implies a risk of type II statistical errors, signifying a failure to detect a difference that is present. For example, in Paper II, contrary to other studies, the tumour mutation status ($KIT$ exon 11) was not statistically significant for better survival. Patients with $KIT$ exon 11 mutations showed a tendency to have better survival, and perhaps with a larger sample size, this could have been statistically significant. Furthermore, the CIs for several survival estimates presented in all papers were rather wide, showing the uncertainty due to the relatively small number of cases.

Multivariate analysis was attempted in all four papers. In Paper II, some important covariates were omitted from the multivariate analysis due to missing values (mutation status and some blood tests). However, including these in the model did not alter the results.
11. Discussion

Most knowledge concerning GIST outcomes stems from clinical trials and experience from cooperative sarcoma societies. In contrast, this PhD-thesis was based on real-life data from patients with GIST treated at OUH. The main goal was to contribute to an improved treatment and follow up of patients with GIST by taking steps toward a more individualised view on each patient. The main findings from Papers I–V, presented in Section 9, are discussed below. Paper I (Section 11.1) is presented separately, while Paper II and Paper V (Section 11.2) and the two papers on tumour rupture (Paper III and Paper IV; Section 11.3) are presented together.

11.1 Imatinib plasma concentrations

A large variability in plasma imatinib concentrations is observed during treatment in patients with GIST, and this may be affected by factors like age, body weight, body surface area, previous major gastric resection, white blood cell count, haemoglobin, creatinine clearance, albumin and alpha glycoprotein levels\textsuperscript{130,184-189}. Several studies have demonstrated that higher plasma concentrations are associated with better outcomes\textsuperscript{131,132,190,191}. Moreover, a recent study in patients in routine care showed that approximately one-third of GIST patients are systematically underexposed with a fixed dose of imatinib\textsuperscript{192}. However, few studies have explored repeated drug monitoring of imatinib during treatment. Hence, in Paper I, we assessed 96 imatinib plasma trough concentrations (C_{\text{min}}) from 24 patients with metastatic GIST over several years trying to determine whether there are clinical scenarios where measurements of imatinib C_{\text{min}} could be beneficial.
11.1.1 Patient characteristics and imatinib plasma concentrations

Patients who underwent gastric surgery prior to the study had low imatinib C\textsubscript{min} compared with patients without gastrectomy, and this is consistent with a previous finding\textsuperscript{189}. Only eight patients underwent gastric surgery in our study, of which only three patients had progression. Hence, we were not able to analyse the prognostic impact of gastrectomy. However, when exploring this in a larger study population from Paper II, we found no difference in outcomes among metastatic GIST patients who underwent major gastrectomy compared with patients who did not (Figure 13; Section 13). Still, this does not exclude the possibility that patients with prior gastric surgery may be underexposed when using the recommended dose, and that a more individualised drug dosage based on imatinib plasma concentrations could be beneficial in some of these patients. A randomised controlled trial (Clin. Gov: NCT01031628), randomising between dose escalations of imatinib to keep the plasma concentration above 1100 ng/ml and no dose escalation, was unfortunately terminated due to poor accrual. Furthermore, the first target concentration intervention trial in chronic myeloid leukaemia (CML) could not formally demonstrate a benefit of routine monitoring of imatinib due to the small patient number (N = 58) and surprisingly limited prescriber adherence to dosage recommendations\textsuperscript{193}.

Elderly patients in our study had significantly higher plasma imatinib C\textsubscript{min}. Although a study showed that a permanent dose reduction was mandatory in almost half the GIST patients above 75 years of age, the OS in elderly patients is similar to that of younger patients, with a median OS of 50.3 months\textsuperscript{194}. Indeed, the decline of organ functions and higher prevalence of polypharmacy among elderly patients may alter the pharmacokinetics and pharmacodynamics of imatinib. Unfortunately, in Paper I, concomitant medication was not registered, and thus, we are unable to report on this. However, individual dosing of imatinib based on plasma concentrations could balance adverse events and anti-tumour benefit more
accurately and be even more useful in elderly than young patients.

The reason for the significantly lower plasma concentrations in patients with liver metastases in our study is uncertain. Imatinib undergoes metabolism in the liver via the cytochrome P (CYP) 450 enzyme system, with CYP3A4 being the primary isoenzyme involved\textsuperscript{195}. However, CYP3A4 activity did not significantly influence the plasma concentrations of imatinib in CML\textsuperscript{179}. The extent of liver involvement in our cohort was relatively modest, with several patients with a low number and small volume of liver metastases. None of the patients had pathological liver function tests. One previous study has shown that imatinib clearance is not affected by low volume liver disease\textsuperscript{133}, and it thus seems unlikely that the liver metastases per se affected the imatinib metabolism in our patients. We are not aware of studies that have reported differences in imatinib plasma concentrations in patients with or without liver metastases, and this issue could be of interest for further studies.

11.1.2 Disease progression and imatinib plasma concentrations

An interesting finding in \textit{Paper I} was that patients encountering progressive disease had significantly lower imatinib levels at the time of progression compared with the previous measurement of imatinib concentration. One may speculate on whether this decrease could explain the loss of tumour control in some patients. Three separate studies have demonstrated that increasing the imatinib dose to 800 mg can be favourable in up to one-third of patients with tumour progression on 400 mg\textsuperscript{101,134,135}, and one recent retrospective study showed superior OS rates in patients who had dose escalation of imatinib compared with patients who received second-line therapy\textsuperscript{139}. However, none of these studies performed imatinib plasma concentration measurements. Perhaps only patients with subtherapeutic imatinib levels will benefit from dose escalation, implying that patients with therapeutic imatinib levels should be
offered second-line therapy directly. As discussed in Section 7.7.3, measures could be put in place to overcome imatinib failure. A more individualised approach when patients encounter progressive disease could be decided in the future based on the situation. For example, if a patient has a focal progression of one or a few metastases, surgery followed by the same dose of imatinib could be chosen. In patients with more widespread progression and subtherapeutic imatinib plasma concentrations, dose escalation could be preferred. Finally, in patients with therapeutic imatinib levels, second-line TKIs based on what the secondary mutations found in the plasma could be reasonable. Hence, measurements of imatinib $C_{\text{min}}$ in patients with tumour progression could be recommended if a lack of patient compliance has been excluded. A study exploring the dose escalation of imatinib in patients with sub-therapeutic imatinib levels could be of interest.

Although an association exists between the plasma levels of imatinib and PFS, it is not known whether the plasma concentration correlates with the intracellular drug concentration, which is the most relevant compartment for the effect of imatinib. A possible explanation for some GISTs developing resistance to TKIs is the interpatient variability in drug transporter activity and expression, leading to decreased intracellular concentrations, as suggested in a study on CML patients\textsuperscript{196}. Furthermore, a small study on three GIST patients showed large variations in imatinib concentrations among plasma, adipose tissue and different sites in the tumours\textsuperscript{197}. Hence, measurements of imatinib concentration in the cytoplasm of the tumour cells could even more precisely predict target inhibition and clinical efficacy. Further clinical investigations on measurements of intracellular imatinib levels in GIST tissues to understand their possible effects on patient outcome are anticipated.
11.1.3 Side effects and imatinib plasma concentration

One previous study on GIST patients have demonstrated that the severity of side effects is associated with high imatinib plasma concentrations\(^{198}\). Unfortunately, we could not discern information on this issue, since side effects were not registered prospectively. However, of the seven patients with dose reductions in our study, two had relatively high plasma concentrations on 200 mg, suggesting that, for some patients, a low dose is enough to achieve a therapeutic plasma level of imatinib. In contrast, the five other patients had low levels, suggesting that patient-reported adverse events are not necessarily associated with high plasma concentrations. Hence, measuring the imatinib plasma levels in patients with self-reported side effects may help in determining whether it is safe to reduce the dose of imatinib. Further studies on the relations between concentration and toxicity are warranted.

11.1.4 Benefits of imatinib plasma concentration

We found relatively large intra- and interpatient variability compared with other cohorts from real-life practice\(^{132,189}\), and this could be explained by reduced compliance. One patient in our study had one plasma sample where imatinib was not detected at all. Monitoring of imatinib plasma levels could enhance patient compliance with imatinib therapy. Furthermore, the large intra- and interpatient variability could suggest that individualised dosing of imatinib based on plasma concentrations could be beneficial. In our opinion, there are still unresolved issues that need to be addressed before imatinib plasma concentration measurements become mandatory. First, the optimal threshold has not yet been determined, as the first study\(^{131}\) showed a threshold of 1100 mmol/L and the second study\(^{132}\) showed a threshold level of 760 mmol/L. This difference could be explained in that the latter study performed imatinib plasma concentration measurements after 3 months of treatment and the former after 29 days of
treatment, since evidence shows a 30% decline in the plasma concentration of imatinib by 3 months of treatment\textsuperscript{133}. Second, it is not known whether increasing the dose of imatinib in patients that are underexposed is truly beneficial. However, although our results from Paper I do not support the monitoring of imatinib plasma levels throughout the treatment in all patients, individual dosing of imatinib based on drug measurement could be beneficial in patients who have had gastrectomy or subjectively reported adverse events, in older patients and at the time of tumour progression.

Chromatography and mass spectrometry, used for imatinib plasma concentration measurements, are generally expensive and not available everywhere. Hence, another option could be to find biomarkers for low imatinib plasma concentrations. Unpublished results from the study on patients in Paper I show that magnesium, phosphate and mean corpuscular volume (MCV) are correlated with plasma imatinib concentrations. Hypophosphatemia and altered bone metabolism have been shown in patients receiving imatinib\textsuperscript{199}, and hypophosphatemia in patients with CML receiving imatinib has been shown to be associated with a good response to imatinib\textsuperscript{200}. The MCV has been shown to be increased in both GIST and CML during imatinib treatment\textsuperscript{201,202}. We are not aware of any studies reporting hypomagnesemia during imatinib treatment. Combining these three parameters into an algorithm, perhaps together with other known factors correlated with imatinib concentrations, could allow us to foresee which patients are at risk of low a concentration. We are currently conducting a study where this is explored. Indeed, this algorithm needs to have a high sensitivity to ensure that all patients with low concentration are recognised.

\textbf{11.2 Long-term survival in metastatic GIST}

Some patients with metastatic GIST have rapid tumour progression on imatinib, and thus,
they have a dismal OS, whereas others may stay on the drug for more than 10 years\textsuperscript{106,112,113}. In clinical trials, long-term survival has been associated with \textit{KIT} exon 11 mutation, female sex, normal albumin levels, neutrophil and lymphocyte counts, good performance status and small tumour diameter\textsuperscript{104,106,112-114}. There are fewer data from routine practice; however, the absence of primary imatinib resistance, small maximum tumour diameter at the start of imatinib, better haematological and clinical chemistry parameters, good performance status and surgical removal of residual disease have been associated with a longer OS\textsuperscript{105,107}. Nomograms for both PFS and OS include the following factors: size of the largest metastasis, tumour genotype, primary tumour mitotic count and haemoglobin and neutrophil count at commencement of imatinib\textsuperscript{174}. In \textbf{Paper II}, the treatment of patients with metastatic GIST at OUH over an 18-year period was reported with the primary goal of identifying factors associated with long-term survival after the introduction of imatinib.

\textit{11.2.1 Oligometastatic GIST}

In \textbf{paper II}, we demonstrated that OMD, defined as three or fewer metastases at the start of systemic treatment, was significantly associated with long-term survival in patients with metastatic GIST. Patients with OMD had an estimated 5-year OS of 89\% and a 10-year OS of 71\% compared with 38\% and 20\% for patients with PMD. In some cancers types, such as some sarcomas and colorectal cancer, patients with OMD are considered for curative treatment by metastasectomy alone or in combination with chemotherapy, with 5-year survival rates of above 30\%\textsuperscript{203,204}. Some regard OMD as an intermediate disease state between locoregional and widespread metastatic disease\textsuperscript{205,206}. Our data support this view in metastatic GIST, as the 5-year OS in our cohort was 89\%, and the 5-year OS in the SSG XVIII/AIO trial with adjuvant treatment for patients with high-risk, localised tumours was 91\%\textsuperscript{92,94}. Thus, we propose that oligometastatic GIST should be considered as a distinct
disease category more comparable to high-risk primary tumours than disseminated metastatic disease. Whether a curative treatment strategy could be justified in GIST patients with OMD is not known. Indeed, patients with OMD may not be cured and only live longer due to the long response to imatinib; hence, they have a similar 5-year OS to high-risk localised disease, where more than one-third of the patients are cured.

Discontinuation of imatinib in responding patients with advanced GIST has been explored in the BRF14 trial\textsuperscript{117-119}. Patients were randomly assigned to continue or stop imatinib, and regardless of whether they had taken imatinib for 1, 3 or 5 years, nearly all the patients who discontinued had tumour progression within 2 years\textsuperscript{117-119}. Interruption of imatinib, however, did not significantly influence the OS, since most patients in the stopping group restarted imatinib and had a new response. In the BRF14 trial, surgical resection of metastatic lesions was not mandatory, and most patients had visible metastatic disease\textsuperscript{117-119}. There is evidence to support a beneficial outcome in patients undergoing resection of residual disease after a response to imatinib, both in retrospective studies\textsuperscript{147,167,168} and two small prospective studies\textsuperscript{169,170}. Furthermore, although not entirely comparable to GIST, a proportion of CML patients, having maintained durable complete molecular remission on imatinib, did not have rapid CML recurrence despite imatinib discontinuation, suggesting that some patients with CML might be cured with imatinib\textsuperscript{207,208}. In Paper V, based on three cases and a review of the literature, we hypothesised that the surgical removal of residual metastatic disease is essential before the discontinuation of imatinib, and that such an individual strategy may be curative for highly selected patients with OMD. This is now being explored in a prospective phase II trial (“SSG XXV: The STOP-GIST trial”; Clin. Gov: NTC02924714; this is discussed in detail in Section 12).
11.2.2 Tumour diameter

In Paper II, we found that patients with a large diameter of the largest tumour at the commencement of imatinib had inferior survival, and this is in agreement with other studies. The time to imatinib resistance is closely related to the PFS and OS, and probably the most important imatinib resistance mechanism is the development of secondary KIT mutations. The incidence of secondary mutations may be reliant on the level of genomic instability (i.e. the frequency with which new mutations take place) and tumour load (i.e. the number of dividing cells). Our findings support this hypothesis, as both patients with small tumour diameter and patients with OMD have a low tumour volume. This may suggest that surgical tumour debulking could be beneficial in some individuals. Interestingly, in our study, patients who did not undergo surgery of their primary tumour (often large) had significantly worse outcomes than patients who had their primary tumour surgically removed. Indeed, this could be explained by a selection bias toward performing surgery in patients with a better performance status, and our study is not in the position to determine whether removing a large tumour load is beneficial. Furthermore, patients with large tumours may have a more biologically aggressive disease, and thus, an inferior survival compared with patients with smaller tumours. This could also be the case in patients with OMD versus PMD. Hence, both a small diameter of the largest tumour and OMD may be measures of a low degree of biological aggressiveness.

11.2.3 Genotype

In contrast to other studies, we did not detect any statistically significant associations between mutations and long-term survival. However, mutation analysis was not
done in all cases, and the number of mutations other than KIT exon 11 (KIT exon 9, n = 11; mutations not detected, n = 10; and other mutations, n = 5) was quite small. Hence, a robust statistical analysis involving these mutations was not possible. When stratifying patients according to KIT exon 11, KIT exon 9, mutations not detected and other mutations, there was a trend toward better survival in patients with KIT exon 11.

11.2.4 PFS and duration of imatinib treatment

PFS was not explored in our trial, as discussed in Section 10.4. The median treatment duration of imatinib was calculated to be 49 months. Although time on imatinib and PFS cannot be directly compared, the duration of imatinib treatment is substantially longer than the PFS of 18–37 months described in other studies\(^9,105,107,124\). Two factors may explain the relatively long duration of imatinib treatment in our study. First, to delay a switching of TKIs, surgical resection or local ablation of focal tumour progression\(^168,170\) was frequently recommended. Second, when considered as the best option for the patient, imatinib was continued beyond progression, either with the same dose or dose escalation. Whether continuation of imatinib beyond progression in selected cases could be beneficial is not adequately investigated in Paper II, but the long median OS suggests that extending the time on imatinib and postponing a switch in TKIs should be further studied.

11.3 Tumour rupture

Tumour rupture was first identified as a risk factor by Rutkowski and co-workers\(^210\), and it was subsequently found to have an independent negative prognostic value in a large meta-analysis of primary GISTs\(^2\). A strong association between rupture and recurrence was also shown in the SSG XVIII/AIO trial\(^67\). However, other large studies have failed to confirm
Inconsistent definitions and deficient reporting could explain the contradictory results on tumour rupture and recurrence. Hence, in Paper III, we assessed the RFS after complete resection of non-metastatic gastric GISTs in 242 patients, classified according to a strict definition of tumour rupture proposed by the Sarcoma Group at OUH.69

11.3.1 Tumour rupture and recurrence in gastric GISTs

According to the definition proposed by our group (Section 8.5), tumour rupture was independently associated with recurrence. The 5-year RFS with rupture was 37%, compared with 91% in patients with minor defects of the tumour integrity and 96% in patients without any defects. Patients with rupture also had an inferior RFS also when the high-risk features, high mitotic index and large tumour diameter were taken into consideration. As mentioned above, our research group has previously demonstrated, by using the same definition, that tumour rupture has the same negative prognostic significance in patients with small intestinal GISTs.69 Since no established definition of tumour rupture has been used in former studies, tumour rupture could have been underreported, and therefore its negative clinical significance for the RFS could have been underrated. This would also underestimate the prognosis of nonruptured high-risk GISTs. In Paper III, we found a significant difference among patients with tumour rupture, who had an estimated 5-year RFS of 37%, and patients with non-ruptured tumours with other high-risk features (high mitotic count and large tumour size), who had a relatively good estimated 5-year RFS of 77%.

A high mitotic index was independently associated with inferior outcomes, and the 5-year RFS for patients with mitotic index above 5 was 30% with rupture and 86% without rupture. Tumour size was not associated with recurrence in multivariable analysis, and patients with nonruptured tumours larger than 10 cm did well; among 21 patients, there were only 3 who relapsed, of whom 2 had a high mitotic index of 35. For patients with a tumour
size larger than 10 cm, 5-year RFS with rupture was 38% versus 86% without rupture. These numbers, showing impressive survival rates in patients with high-risk nonruptured tumours, suggest that patients in the high-risk group have been underestimated in other studies not using a consistent definition of tumour rupture. Our study shows that patients with tumour rupture are at ultra-high risk of recurrence. When excluding patients with tumour rupture from the high-risk group, we demonstrated that the rest of the high-risk patients in fact had an intermediate risk of recurrence.

11.3.2 Adjuvant imatinib treatment

Adjuvant imatinib was given to only 42 patients of the 66 high-risk tumours, and it was not given consistently throughout the inclusion period due to guideline changes over the years. Until May 2004, no routine adjuvant treatment was given; from May 2004, only eligible patients with high-risk GISTs according to the NIH or modified NIH criteria were included in the SSG XVIII/AIO trial (1 vs. 3 years of adjuvant imatinib)\(^92\). From September 2008 to May 2011, high-risk patients received adjuvant for 1 year, and from June 2011, they received it for 3 years. Hence, patients received different durations of imatinib (1 or 3 years). However, of the 29 patients without tumour rupture that received adjuvant imatinib for 1–3 years, 24 were without a recurrence. Conversely, only 2 of 13 patients with rupture were disease-free, including 1 still on treatment. The recommended 1–3 years of adjuvant treatment\(^61,62\) received by the patients was clearly insufficient for patients with tumour rupture. Currently, we include high-risk patients in the SSG XXII trial to explore whether 5 years of adjuvant therapy is beneficial compared with 3 years; however, the definition of tumour rupture is not as well defined as in the present study. Recurrence during adjuvant imatinib treatment is rare, and in Paper III, only one patient relapsed during adjuvant treatment; this patient also progressed during preoperative imatinib administration. Given the poor prognosis after tumour rupture,
we suggest prolonged and perhaps lifelong treatment for patients with rupture, while the relatively good prognosis in patients with high-risk tumours without rupture may point in the direction of no adjuvant treatment in these patients (this is discussed in more detail in Section 13).

11.3.3 Metastatic propensity of ruptured tumours

All six parameters in our definition of tumour rupture (Section 8.5) come with a high risk of tumour spillage into the abdomen. Hence, one would expect a recurrence pattern dominated by peritoneal metastases. The material in Paper III, however, revealed no such pattern; visceral metastases were almost as frequent as peritoneal metastases in patients with rupture. Similarly, in the previous study by our group on small intestinal GISTs, there was no statistically significant difference in the distribution of metastases between patients with and without rupture\textsuperscript{69}. Furthermore, stomach GISTs in Paper III had significantly higher mitotic counts and were significantly larger than tumours without rupture, and this was also true for the tumours in Paper IV. Whether it is the tumour spillage into the abdomen per se or the aggressive behaviour shown by ruptured tumours that causes the high risk of recurrence is not known. The metastatic propensity of ruptured tumours could reflect biological rather than locoregional intraperitoneal dissemination.

11.3.4 Risk of tumour rupture

If the dismal prognosis in patients with ruptured tumours is due to locoregional intraperitoneal dissemination, it would be beneficial to avoid rupture. Hence, a better prediction of patients at risk of rupture could influence therapeutic decisions on whether to give neoadjuvant treatment prior to surgery versus upfront surgery. The current guidelines recommend preoperative imatinib if the surgical management is expected to be safer after preoperative therapy\textsuperscript{61};
however, there are no established criteria to select patients for such treatment. Large tumour size and small bowel GISTs, both known before surgery, and high mitotic count, not known prior to surgery, are all associated with tumour rupture. It was not known whether different genotypes are associated with tumour rupture. However, certain KIT and PDGFRA mutations have a more aggressive phenotype, such as patients with deletions involving codon 557 and 558 in \( KIT \) exon 11, and these generally have worse outcomes. Hence, in Paper IV, we investigated whether tumour genotype was associated with tumour rupture in 209 patients with available mutational statuses and primary GISTs in the small intestine or stomach. We used the same definition of rupture as in Paper III.

11.3.5 Neoadjuvant imatinib treatment

As mentioned above, it seems important to avoid tumour rupture, and neoadjuvant imatinib could potentially decrease this risk of rupture. Unfortunately, the studies on preoperative imatinib treatment in locally advanced GISTs have not reported rupture data, and whether neoadjuvant imatinib reduces the risk of rupture is not known. However, it is assumed that tumours become less fragile after a response to neoadjuvant treatment, and that tumour regression will make most tumours less prone to rupture. In Paper IV, the rupture was iatrogenic and potentially avoidable in 15 of the 37 patients with tumour rupture (41%). Indeed, the risk of progression could be a concern during neoadjuvant imatinib treatment. Of the 10 patients who were treated with preoperative imatinib in our cohort, one had radiological progression prior to surgery. However, the overall risk of progression during imatinib treatment in previous neoadjuvant series has been below 5%. As mentioned in Section 7.7.1, different genotypes respond differently to imatinib. For example, GISTs with the del557/558 mutation usually respond well to imatinib treatment, with higher response rates compared to other mutations. However, unfortunately, they more rapidly develop
secondary mutations, and hence, have an inferior PFS\textsuperscript{106}. Furthermore, in metastatic GIST, very few patients, including those with del557/558 mutations, had progression during the first year of treatment\textsuperscript{106}. Thus, a duration of preoperative imatinib of less than 1 year should be safe. Still, given the increased risk of developing secondary mutations in tumours with a del557/558 mutation, patients with this genotype should be carefully followed. Some tumours with certain genotypes, such as PDGFRA D842V mutations, do not respond to imatinib, and thus, they should not be considered for neoadjuvant therapy. Hence, mutational analysis before starting neoadjuvant imatinib is mandatory.

11.3.6 Tumour rupture and genotype

In Paper IV, we found that gastric tumours with deletions involving codons 557 and 558 of KIT exon 11 bear a high risk of rupture. As far as we know, the association between tumour genotype and rupture has been reported by only one former study, where tumours with deletions involving codon 557 or 558 had a statistically non-significantly increased risk of rupture\textsuperscript{217}. However, rupture data were missing in almost 20\% of the patients, and a consistent definition of rupture was not applied. In another small study of 23 ruptured GISTs, 14 patients had KIT exon 11 mutations, and of these, 7 had del557/558\textsuperscript{64}. Contrary to gastric GISTs, a statistically significant difference was not detected in small intestinal GISTs in our study, as rupture was recorded in 42\% with del557/558 compared to 28\% with other exon 11 mutations ($p = 0.475$). However, in intestinal GISTs, only 12 patients had del557/558 mutations and 32 had other exon 11 mutations, and with a larger sample size, a statistically significant difference could have been found.

Tumours with del557/558 are generally large, and they have a high mitotic count and poor prognosis\textsuperscript{54,83,85}. It has been demonstrated that specific downstream signalling events are activated by del557/558\textsuperscript{218}, suggesting that certain uncharacterised phenotypic changes
induced by distinct *KIT* and *PDGFRA* mutations may influence the risk of rupture. Hence, del557/558 mutated GISTs are likely to grow faster and are usually more necrotic than tumours with other mutations are, resulting in a more brittle structure. Our study supports this hypothesis, as del557/558 was also associated with rupture when the size and mitotic count were included in a logistic regression analysis, suggesting an independent risk associated with this mutation. However, tumour size is still the main risk factor for rupture, as only one gastric tumour smaller than 10 cm ruptured in our study.

11.3.7 Present indication for neoadjuvant treatment

As mentioned above, there are currently no established criteria for selecting patients for neoadjuvant imatinib treatment. In a recent prospective nonrandomised phase II study that included 56 patients with gastric GISTs above 10 cm, the R0 resection rate was 91%, and preservation of at least half of the stomach was achieved in 42 of 48 patients with R0 resection^{219}. Awaiting future clinical trials involving locally advanced GISTs, we suggest that all gastric GISTs larger than 10 cm should be considered for neoadjuvant imatinib based on the increased risk of rupture, and preoperative treatment should be standard in the presence of a del557/558 mutation in large tumours.
11. Conclusions

The ambitious goal of this thesis was to improve the treatment and follow up of patients with GIST by taking steps toward a more individualised view on each patient. The patient material is based on a single tertiary institution’s treatment and follow up of GISTs within a time frame of almost two decades. With all the obstacles of a retrospective study considered, we have generated hypotheses that treatment and follow up may be more individualised in several respects, as follows:

I) In metastatic GIST patients, we have revealed clinical scenarios where drug measurement of imatinib could be beneficial, such as in the cases of patients who have undergone gastric resection, suspicion of noncompliance, subjectively reported side effects, in elderly patients and at the time of disease progression. Individual dosing of imatinib based on plasma concentrations may be a good option in these patients;

II) In a single-institution cohort of patients, OMD was a strong prognostic factor in patients with metastatic GIST. Patients with OMD had similar outcomes to patients with high-risk localised disease, and they should be regarded as belonging to a separate category among patients with metastatic GIST;

III) The definition of tumour rupture proposed by the Oslo Sarcoma Group identified a subset of gastric GIST patients at particularly high risk of recurrence. Without tumour rupture, patients had an excellent prognosis, and even in the high-risk group, prospects were good for patients without rupture. With tumour rupture, relapse was the rule, and relapses occurred despite adjuvant treatment, suggesting that extended, possibly lifelong imatinib treatment could be beneficial;

IV) This population-based cohort showed that KIT exon 11 deletions involving codons 557 and 558 are independently associated with tumour rupture in gastric GISTs. This high-risk feature can be identified in the diagnostic workup and should be included in
the assessment when neoadjuvant imatinib treatment is considered. An individualised
decision concerning treatment based on genotype could be beneficial.

Given the retrospective design and relatively small patient cohorts, our results and
conclusions should be confirmed in larger cohorts, and ideally in prospective clinical trials, if
applicable (as discussed further in Section 12).
12. Future perspectives and studies in progress

An interesting finding in Paper I was that, in patients with progressive disease, the mean imatinib levels were significantly lower at the time of progression compared to the previous measurement for the same patients. A study measuring imatinib plasma concentrations in patients with progressive disease and increasing the dose in patients with subtherapeutic imatinib levels could be of interest. However, even more important could be finding the patients underexposed on a fixed dose of imatinib before progression. As mentioned in Section 11.1.4, the optimal threshold has not yet been determined, and whether increasing the dose of imatinib in patients that are underexposed is beneficial is not known. Both these issues could be addressed by a randomised study comparing individualised dosing dependent on plasma concentrations versus a fixed dose of 400 mg. Unfortunately, such a clinical trial (Clin. Gov: NCT01031628) was terminated due to poor accrual and it seems unlikely that such a study will be done. Awaiting further knowledge, imatinib concentration measurements could indeed be favourable in some clinical settings, as discussed in Section 11.1.4. Furthermore, as discussed in Section 11.1.4, finding biomarkers for low imatinib plasma concentrations could be of interest, and we are currently conducting a study where we aim to establish an algorithm that will single out patients at risk of having low imatinib concentrations.

In Paper II we found that patients with oligometastatic GIST have excellent outcomes. Based on these results and the literature review in Paper V, we have started an open-label, one-group, prospective, international multicentre phase II trial (Clin. Gov: NCT02924714). Here, patients treated with imatinib for longer than 5 years for oligometastatic GIST (fewer than four metastases) and who no longer have detectable GIST lesions on CT/MRI imaging following R0/R1 resection or radiofrequency ablation of the metastases are assigned to discontinue imatinib. The primary endpoint is 3-year PFS, and the secondary endpoints are the OS and quality of life. This study began in January 2017 with a
planned enrolment of 31 patients. Eight patients are already included from OUH. In this study, we hypothesise that in such highly selected patients, stopping imatinib may lead to durable maintained complete remissions without the imatinib-associated side effects. Perhaps some GISTs may not recur during the entire lifetime of such patients. Ideally, this should be a randomised study, where patients are drawn to either continue or discontinue imatinib. However, due to the rarity of such highly selected patients, a randomised trial was not considered feasibly, as also the case in the imatinib discontinuation trials carried out in the context of CML207,208.

In Paper III, we found that patients with tumour rupture had a dismal 5-year RFS (37%). It is not known whether tumour rupture should be treated as a primary high-risk GISTs with adjuvant imatinib or regarded as metastatic GIST and be treated with longer duration or even lifelong treatment. Our data support the latter, since almost all patients recurred following adjuvant imatinib. In contrast, when excluding the patients with tumour rupture from the high-risk group, the patients actually had an impressive RFS (77%). The evidence shows that more than one-third of patients with high-risk GISTs are cured with surgery alone2, and thus, they do not need imatinib treatment. The SSG XVIII/AIO trial has demonstrated a small, but significant, OS benefit in patients treated with 3 years of imatinib as the first update of the trial showed a significant increase in the 5-year survival (92% vs. 82%)92. In the latest update, although still significant, the difference had tapered to 92% versus 85%94. Whether this difference will persist over time is not known. Furthermore, if relapse occurs after surgery, the risk of relapse is highest in the first 2 years. Hence, based on the relatively good RFS in patients with high-risk tumours without rupture from Paper III and the excellent survival in patients with a low tumour burden (OMD and small tumour diameter) from Paper II, one may speculate as to whether adjuvant imatinib is truly beneficial in high-risk patients without tumour rupture. Could more frequent monitoring
during the first years and early commencement of imatinib for patients with gastric GISTs without rupture be a sound strategy that results in equally good results to adjuvant treatment for all high-risk patients? Our studies are indeed not in the position to answer this question. In an ideal setting, a new randomised trial comparing no adjuvant imatinib versus adjuvant imatinib in high-risk GIST patients without tumour rupture and a randomised trial comparing 3–5 years of adjuvant imatinib versus lifelong therapy in patients with tumour rupture, would be necessary to draw a definitive conclusion on these matters. However, these randomised trials would be challenging due to the extremely long follow up needed to explore clinical differences in OS and the amounts of patients needed in each arm to find a statistically significant difference between the two arms. For example, when calculating the numbers of patients needed to find a statistically significant difference of 5% in the 10-year OS in a non-inferiority designed trial with a power of 80% comparing no adjuvant imatinib versus adjuvant imatinib in high-risk GIST patients without tumour rupture, 1039 patients are needed in each treatment arm.

Although risk stratification schemes have been developed and risk factors for recurrence have been widely studied, more precise information on which patients are truly high-risk is anticipated. We are planning to investigate more variables in the same dataset as in Paper III. We have registered certain biological characteristics and detailed anatomical location within the stomach, and we are currently analysing the relationships between these factors and clinical variables, histopathological characteristics, KIT and PDGFRA mutation status and outcomes.

In Paper IV, we showed that gastric tumours with deletions involving codons 557 and 558 of KIT exon 11 carry a high risk of rupture, an finding that we proved predicts a poor outcome in a previous study on small bowel GISTs. This has now also been shown in stomach GISTs in Paper III. An important, but unsettled, issue is whether neoadjuvant
imatinib attenuates the deleterious consequences of tumour rupture. Our study is in no position to answer this. A randomised trial comparing pretreatment with imatinib versus upfront surgery in large GISTs with tumour rupture as the main endpoint could settle this issue. Moreover, a well-designed trial could also settle the question of whether neoadjuvant treatment truly is beneficial for patients with large primary tumours. However, in our view, randomising patients with large tumours clearly in the need of imatinib pretreatment to have upfront surgery would not be ethically justified, and hence, such a study is not likely to be executed.

In conclusion, this thesis was performed using patient data from a single institution and has indeed added knowledge toward a more individualised treatment of GIST. The more knowledge gained on GIST, the clearer it becomes that individualised treatment is beneficial. However, the more individualised the treatment becomes, the more difficult it will be to organise clinical trials. When the treatment for GIST becomes even more individualised based on factors like genotypes, plasma concentrations on different TKIs and the number of metastases (oligometastatic vs. polymetastatic), the numbers of patients eligible for specific trials becomes a challenge. Hence, more collaboration across borders is encouraged. Indeed, most knowledge concerning GIST outcomes comes from international, multicentre clinical trials and experiences from cooperative sarcoma societies, such as the Scandinavian Sarcoma Group.
13. Appendix

Figure 12. Kaplan-Meier survival curves for overall survival (A) and disease-specific survival (B) of the patients who underwent surgery for a primary gastrointestinal stromal tumour of the stomach (Paper III).

Figure 13. Kaplan-Meier curve for overall survival in patients with metastatic gastrointestinal stromal tumour (Paper II) stratified according to whether or not the patients underwent major gastrectomy. HR=Hazard ratio. CI=Confidence interval.
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15. Papers I-V
Clinical implications of repeated drug monitoring of imatinib in patients with metastatic gastrointestinal stromal tumour

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Abstract

Background: Imatinib mesylate (IM) is the preferred treatment for the majority of patients with metastatic gastrointestinal stromal tumour (GIST). Low trough IM concentration (C\text{min}) values have been associated with poor clinical outcomes in GIST patients. However, there are few studies of repeated measurements of IM levels, and therapeutic drug monitoring is not yet a part of routine clinical practice. This study was conducted to reveal clinical scenarios where plasma concentration measurement of IM trough level (C\text{min}) is advantageous.

Methods: Patients with advanced GIST receiving IM were included from January 2011 to April 2015. Heparin plasma was collected at each follow-up visit. Ninety-six samples from 24 patients were selected for IM concentration measurement. Associations between IM plasma concentration and clinical variables were analyzed by Students’ t test, univariate and multivariate linear regression analyses.

Results: The mean IM C\text{min} plasma concentrations for patients taking <400, 400 and >400 mg daily were 782, 1132 and 1665 ng/mL, respectively (p = 0.010). High IM C\text{min} levels were correlated with age, low body surface area, low haemoglobin concentration, low creatinine clearance, absence of liver metastasis and no prior gastric resection in univariate analysis. In multivariate analysis age, gastric resection and liver metastasis were included in the final model. Eight patients had disease progression during the study, and mean IM levels were significantly lower at time of progression compared to the previous measurement for the same patients (770 and 1223 ng/mL, respectively; p = 0.020).

Conclusions: Our results do not support repeated monitoring of IM levels on a routine basis in all patients. However, we have revealed clinical scenarios where drug measurement could be beneficial, such as for patients who have undergone gastric resection, suspicion of non-compliance, subjectively reported side effects, in elderly patients and at the time of disease progression.

Keywords: Gastrointestinal stromal tumour, Drug monitoring, Imatinib, Plasma concentration

Background

Since the introduction of imatinib mesylate (IM) [1], the outcome of metastatic gastrointestinal stromal tumour (GIST) has improved considerably [2]. IM is an inhibitor of receptor tyrosine kinases, including the stem cell factor receptor KIT and the platelet-derived growth factor receptor alpha (PDGFRA), the main drivers of tumour development in GIST [3]. Several clinical trials have demonstrated the efficacy and safety of IM, and it has become the treatment of choice for the majority of patients with metastatic GIST [2, 4, 5]. The median duration of response to IM in metastatic GIST is 29 months [2], with approximately 20% of the responses lasting 10 years or more [6]. Still, most patients eventually progress on IM, requiring second- and third-line therapy with other tyrosine kinase inhibitors such as sunitinib and regorafenib [7].
In patients with chronic myeloid leukaemia (CML) and GIST, pharmacokinetic (PK) studies have shown that IM has >90% bioavailability following oral administration [8]. IM plasma concentration is influenced by various factors such as age, body weight, body surface area (BSA), previous major gastric resection, white blood cell (WBC) count, haemoglobin, creatinine clearance, albumin, and alpha glycoprotein (AGP) levels [9–15]. A retrospective sub-study from the B2222 trial [4], the first trial showing safety and efficacy of IM in metastatic GIST patients, presented a significantly shorter time to progression in patients with IM trough levels ($C_{\text{min}}$) below 1110 ng/mL at day 29 [16]. Additionally, a retrospective study in patients with CML in chronic phase reported that $C_{\text{min}}$ of IM could predict clinical outcome [13]. However, the optimal threshold value of IM $C_{\text{min}}$ has yet to be determined; both in patients with GIST and CML. A prospective PK study showed a significant decrease of approximately 30% in plasma IM concentration after 90 days of treatment [17], indicating that drug monitoring should preferentially be done after 3 months. This finding was recently supported by a study in real-life practice, where $C_{\text{min}}$ was analysed after more than 3 months of treatment, and concentrations above 760 ng/mL were associated with longer progression-free survival (PFS) [18].

Although considerable inter-patient variability in IM plasma concentrations (40–60%) has been observed in several studies [15, 16], a fixed dose of 400 mg IM is the standard of care in patients with metastatic GIST [7]. Patients that progress on 400 mg/day and patients with KIT exon 9 mutations may benefit from increasing the dose to 800 mg/day [2, 19, 20]. Treatment with 400 mg IM is generally well tolerated, but patients still experience side effects such as anaemia, periorbital oedema, muscle cramps, and diarrhoea [2, 4, 5]. Several of these can be ameliorated with supportive measures, but some patients need dose modifications [21]. Compliance, i.e. adherence to self-administered drugs, is a general challenge for patients on any long-term treatment, as also reported for patients with GIST [22]. However, the extent of non-compliance is often not known and might be a larger problem than expected. Altogether, there are several situations where IM plasma concentration measurements might have a considerable clinical impact in patients with metastatic GIST. However, at present, therapeutic drug monitoring (TDM) is not yet a part of routine clinical practice.

The aim of this study was to assess IM plasma concentration repeatedly over several years in a group of patients with metastatic GIST and thereby revealing scenarios where such measurements might have clinical implications.

Patients and methods

Patients

Patients with GIST treated with IM were included from January 2011 to April 2015. Inclusion criteria were as follows: (1) histologically confirmed GIST; (2) treatment with IM initiated >90 days prior to study entry; (3) high-risk tumour in the need of adjuvant IM, metastatic disease or inoperable primary tumour. Fifty-three patients were enrolled, of whom 19 received IM in a neoadjuvant/adjuvant setting and 34 received IM for metastatic disease or inoperable primary tumour. For the present investigation we focused on patients in advanced or metastatic setting. We further excluded eight patients who had less than three available plasma samples and two patients where drug intake was not registered. Twenty-four patients were included in the final cohort. All patients attended regular 3- to 6-month follow-up visits and were seen by the same physician (OSB). Radiological evaluation with computed tomography of the abdomen and pelvis was performed every 3–6 months depending on the clinical scenario. Disease progression was objectively documented by an experienced radiologist. Secondary review using RECIST or CHOI criteria was not performed. Clinicopathological data were collected retrospectively by reviewing medical records. Body weight, height and biochemical parameters were measured at the time of blood sampling for PK assessment. Creatinine clearance was estimated using the Cockcroft-Gault formula: estimated creatinine clearance = (140 – age in years) × (weight in kilograms) × (0.85 if female)/(72 – serum creatinine) [23]. The study was approved by the Regional Ethics Committee (#S-06133a), and written informed consent was obtained from all patients. Patients were asked if they took the drug as prescribed, and divided into three groups based on drug compliance: Excellent compliance: Never forget to take IM; Intermediate compliance: Forget to take IM on occasions, less than once a week; Poor compliance: Not taking IM regularly with gaps for several days.

Sample collection

Three millilitre heparin plasma was collected at each follow-up visit. Within 1 h of the collection, the blood samples were centrifuged in room temperature for 15 min at 2500×g, and were stored at −20 °C until analysis. Samples were drawn in a routine clinical setting and not at the time of trough level. The time of drug intake was registered, and the validated Bayesian method developed by Gotta and colleagues [24] was used to extrapolate the measured concentrations to $C_{\text{min}}$. 

Measurements of IM concentrations
The determination of the IM plasma concentrations followed the protocol as described in Ubhayasekhera et al. [25]. IM standard was kindly provided by Novartis (Basel, Switzerland). All chemicals including internal standard (Trazodone) and ultrapure solvents were purchased from Sigma Aldrich (Stockholm, Sweden), unless otherwise stated. The stock solutions of IM and internal standard were prepared by dissolving methanol to obtain a final concentration of 1 mg/mL. Protein precipitation was applied as a sample pretreatment. Twenty-five microliter of methanol containing 1 μg/mL internal standard and 0.5 mL of methanol were added to 100 μL of plasma, shaken in 10 min and centrifuged for 10 min at 4 °C at 14,000g. The supernatant was dried by vacuum centrifugation and the residue was reconstituted in 100 μL of 5% acetonitrile containing 0.1% formic acid. Aliquots of 10 μL were injected into the LC–MS system. Chromatography and mass spectrometry was performed as previously described [25, 26].

Statistical analysis
All statistical analyses were performed by using SPSS 21.0 (SPSS, Chicago, IL, USA). Differences in plasma concentrations between dose groups were assessed by Kruskal–Wallis test. The IM C_{min} values were log-transformed for the subsequent analyses. To assess the characteristics of the plasma samples in a homogenous cohort, we focused on the samples being drawn in patients taking 400 mg daily (n = 69). Correlations between IM C_{min} and other variables were analysed by univariate linear regression (Pearson) and independent samples Student’s t test. Variables that showed significant correlations (p < 0.05) with IM C_{min} in univariate analysis were included in a multivariate analysis using a multiple linear regression model with stepwise, backward elimination of variables. Correlations were also tested using a more stringent linear mixed models effect analysis to take into account intra-patient correlation. All tests were two-sided, and p values less than 0.05 were considered statistically significant.

Results
Patient characteristics
Ninety-six samples from 24 patients included in the study were analysed. There were 4 patients with three samples, 16 patients with four samples and 4 patients with five samples. The median duration of IM treatment prior to the first sample was 25 months (range 3–77 months). The median time from the first sample to the last sample was 32 months (range 4–48 months). All patients received IM for metastatic disease, except one patient who was medically inoperable and received IM for a large GIST in the small bowel. The median age was 69 years (range 33–88). The clinical and pathological features of all patients are listed in Table 1. Sixteen patients reported excellent compliance, seven had intermediate compliance and one patient poor compliance. No patients experienced serious life-threatening adverse events. Seven patients had dose reductions: Six patients from 400 to 200 mg due to self-reported side effects and one patient from 800 to 400 mg due to severe fluid retention and haematological toxicity.

C_{min} plasma concentrations
Plasma samples were grouped according to the IM dose at time of sampling: <400 mg group (100 mg: n = 2, 200 mg: n = 19), 400 mg (n = 69) and >400 mg (600 mg: n = 1, 700 mg: n = 1 and 800 mg: n = 4). Mean ± standard deviation values of IM C_{min} plasma concentrations were 782 ± 589, 1132 ± 712 and 1665 ± 924 ng/mL, respectively (Fig. 1a). The difference between the groups was statistically significant (p = 0.010). Intra-patient and inter-patient variability was relatively large. The mean intra-patient variability was relatively large.

Table 1 Baseline clinical and pathological characteristics of the 24 patients enrolled in the study

<table>
<thead>
<tr>
<th>Characteristic</th>
<th>Number (%)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Gender</td>
<td></td>
</tr>
<tr>
<td>Female</td>
<td>8 (33)</td>
</tr>
<tr>
<td>Male</td>
<td>16 (67)</td>
</tr>
<tr>
<td>Primary tumour site</td>
<td></td>
</tr>
<tr>
<td>Stomach</td>
<td>8 (33)</td>
</tr>
<tr>
<td>Small bowel</td>
<td>13 (54)</td>
</tr>
<tr>
<td>Rectum</td>
<td>2 (8)</td>
</tr>
<tr>
<td>Unknown</td>
<td>1 (4)</td>
</tr>
<tr>
<td>Histological subtype</td>
<td></td>
</tr>
<tr>
<td>Spindle cell</td>
<td>17 (71)</td>
</tr>
<tr>
<td>Epithelioid</td>
<td>1 (4)</td>
</tr>
<tr>
<td>Mixed</td>
<td>3 (13)</td>
</tr>
<tr>
<td>ND</td>
<td>3</td>
</tr>
<tr>
<td>Mutation analysis</td>
<td></td>
</tr>
<tr>
<td>KIT exon 11</td>
<td>18 (75)</td>
</tr>
<tr>
<td>KIT exon 9</td>
<td>2 (8)</td>
</tr>
<tr>
<td>PDGFRA exon 12</td>
<td>1 (4)</td>
</tr>
<tr>
<td>Mutations not detected</td>
<td>2 (8)</td>
</tr>
<tr>
<td>ND</td>
<td>1</td>
</tr>
<tr>
<td>Metastatic site</td>
<td></td>
</tr>
<tr>
<td>Liver</td>
<td>13 (54)</td>
</tr>
<tr>
<td>Intrapertoneal cavity</td>
<td>7 (29)</td>
</tr>
<tr>
<td>Liver + intrapertoneal cavity</td>
<td>3 (13)</td>
</tr>
<tr>
<td>No metastasis (inoperable primary tumour)</td>
<td>1 (4)</td>
</tr>
</tbody>
</table>

ND not determined

(coefficient of variation) in patients taking 400 mg was 36% and the highest intra-patient variability 69%, with maximum plasma concentration 1188 ng/mL and minimum of 195 ng/mL. The mean inter-patient variability in patients taking 400 mg was 68%, with the highest measured concentration of 4491 ng/mL and the lowest concentration 195 ng/mL. Among the six patients with a dose reduction to 200 mg, two had relatively high mean plasma levels of 1418 and 2242 ng/mL, whereas the other four had mean plasma concentrations of 387, 437, 565 and 521 ng/mL. Two patients started on 200 mg and had mean plasma concentrations of 1704 and 540 ng/mL.

Patient characteristics and $C_{\text{min}}$ plasma concentrations

Correlations between IM $C_{\text{min}}$ and clinical characteristics were analysed in patients receiving the standard dose 400 mg. The results presented below refer to the per-sample analysis. Linear mixed model effects analyses gave similar trends, although without reaching statistical significance. In univariate analysis, high IM $C_{\text{min}}$ was significantly correlated with age ($\beta = 0.303, p = 0.012$), BSA ($\beta = -0.300, p = 0.010$), low haemoglobin concentration ($\beta = -0.290, p = 0.016$), low creatinine clearance ($\beta = -0.234, p = 0.050$), but not with albumin ($p = 0.061$) or calcium level ($p = 0.999$), tumour diameter ($p = 0.368$), gender ($p = 0.915$), WBC ($p = 0.832$) or platelet count ($p = 0.816$).
Nine patients (38%) had undergone subtotal or total gastrectomy, and IM $C_{\text{min}}$ was significantly lower in these patients ($865 \pm 476$ ng/mL; $n = 28$) than in plasma samples from patients without gastric surgery ($1315 \pm 790$ ng/mL; $n = 41$; $p = 0.015$) (Fig. 1b). Furthermore, IM $C_{\text{min}}$ was significantly lower in the plasma samples from patients with liver metastases ($1003 \pm 710$ ng/mL, $n = 46$) compared to patients without liver metastases ($1390 \pm 657$ ng/mL, $n = 23$; $p = 0.025$) (Fig. 1c).

Multivariate analysis was performed including variables that were associated with IM $C_{\text{min}}$ in univariate analysis in the 400 mg group. Gastric resection ($p = 0.021$), age ($p = 0.049$) and liver metastases ($p = 0.010$) were the covariates significantly associated with IM $C_{\text{min}}$.

**Disease progression and $C_{\text{min}}$ plasma concentrations**

Eight patients had disease progression during the study. In seven of these IM $C_{\text{min}}$ concentrations decreased at the time of progression compared to the previous measurement. The mean IM $C_{\text{min}}$ concentration at the time of progression was $770 \pm 487$ ng/mL, and in the last sample from the time of stable disease from the same patients $1223 \pm 796$ ng/mL ($p = 0.021$; Student’s test). In comparison, there was no statistically significant difference in IM $C_{\text{min}}$ concentration between the two last plasma samples collected in patients with stable disease throughout the study ($1161 \pm 658$ versus $1115 \pm 511$ ng/mL) (Fig. 1d).

**Discussion**

The role of IM $C_{\text{min}}$ measurements in optimizing therapeutic efficacy in GIST is still investigational, despite preliminary estimates of IM blood levels that are associated with improved clinical outcomes ($C_{\text{min}} > 1110$ ng/mL) [16]. In this study, we assessed $C_{\text{min}}$ in a group of patients over several years trying to determine whether there are clinical scenarios where measurements of IM $C_{\text{min}}$ could be advantageous. To the best of our knowledge this is the first study in metastatic GIST with repeated measurements of $C_{\text{min}}$ plasma concentrations including samples at the time of documented progression.

Low IM $C_{\text{min}}$ was associated with major gastrectomy in both univariate and multivariate analysis, which is consistent with previous findings [14]. IM tablets dissolve more rapidly at pH 5.5 or less [8], and lack of gastric acid secretion may explain the lower concentration in such patients. Many patients with metastatic GIST have previously undergone surgery for a primary gastric GIST and one might speculate that such patients could possess an increased risk of sub-therapeutic IM plasma levels and subsequently a less favourable disease outcome. In our cohort, only eight patients had disease progression, of which three had undergone gastric resection. Thus, analysing the prognostic impact of gastric surgery is impossible due to small patient numbers. Still, a more individualized drug dosage based on IM plasma concentrations may be beneficial in patients with prior gastric surgery.

Interestingly, in the current study patients with liver metastases had low IM $C_{\text{min}}$ compared to patients without liver metastases. A previous study has shown that IM clearance is not affected by low volume liver disease [17], and it thus seems unlikely that the liver metastases per se affect IM metabolism in our patients. We are not aware of studies that have reported differences in IM plasma concentration in patients with or without liver metastases, and this issue could be of interest for further studies.

Older patients had higher plasma concentrations in our cohort. The well-known decline of organ functions and increased prevalence of comorbidity and concomitant medication among elderly patients may influence the pharmacokinetics and pharmacodynamics of IM. We did not prospectively register concomitant medications or co-occurring medical conditions, and are thus not able to discern the role of these factors separately. Our results could suggest that individual dosing supported by IM plasma concentration measurement could be even more useful in elderly patients, to balance side effects and antitumour efficacy more precisely.

Although there were no serious adverse events in our study, dose reduction due to subjective side effects were mandatory in seven patients. Two of these patients had relatively high $C_{\text{min}}$ on 200 mg, suggesting that for some patients this dose is enough to ensure optimal therapeutic plasma levels of IM. The four other patients had low levels suggesting that patient-reported side effects are not necessarily associated with high plasma concentrations.

Few studies have explored the relationship between IM plasma concentration and side effects. One study showed that the occurrence and number of side effects correlated with IM total and free plasma concentrations in GIST patients [27], but further studies on relations between concentration and toxicity are warranted. Unfortunately, we did not register side effects in a formal and prospective manner. However, measuring $C_{\text{min}}$ concentrations in patients experiencing subjective side effects (e.g. muscle cramps, dizziness, fatigue etc.) that limit daily activity may help to determine whether it is safe to modify the dose of IM.

The relatively large inter- and interpatient variability in our study compared to other real-life cohorts [14, 18] could be explained by lack of compliance. Although oral cancer therapies offer patients the convenience of self-administration at home, evidence show that adherence to these therapies is far from optimal [28, 29]. The BFR14 Study evaluated the effect of IM interruption in responding patients (complete response, partial response, or
stable disease) after different periods of treatment (1, 3 and 5 years), and results from the study indicated that discontinuation of IM is associated with rapid progression [30–32]. Therefore, maintaining proper adherence may be of great significance and drug monitoring could potentially improve compliance to therapy.

Interestingly, we found a decrease in C_{min} plasma concentration at the time of disease progression, which might explain loss of disease control in certain patients. Measurements of IM C_{min} in case of progressive disease could therefore be indicated if lack of patient compliance has been ascertained. A sub-therapeutic drug level at the prescribed dose could suggest that increasing the dose would be of clinical benefit, in particular in the absence of secondary KIT or PDGFR\(\alpha\) mutations. Studies comparing 400 with 800 mg IM daily in advanced disease showed no clinical benefit of IM 800 mg daily, except for tumours with KIT exon 9 mutations [33]. Despite this, dose escalation to 800 mg can be beneficial in up to 30% of patients upon disease progression on 400 mg [2, 19, 20]. IM plasma concentration measurements have not been performed in dose escalation studies, and perhaps only patients with sub-therapeutic IM levels will benefit from this strategy, whereas the remainder should be offered second-line therapy.

Total IM plasma concentration was measured in our study. Another option is to measure free drug concentrations; i.e. the pharmacologically active fraction not bound by albumin or AGP. The area under the PK curve (AUC) for IM, which can either be measured directly or as the correction of the total drug concentration for binding to AGP, may provide a better surrogate for cellular drug exposure than total IM concentration [15, 26]. Furthermore, IM concentration measurement in the cytoplasm of the tumour cells could even more precisely predict target inhibition and clinical efficacy. A new approach to measurement of intracellular levels of IM in an in vivo setting has been developed, and there were large variation in IM concentrations between plasma, adipose tissue, and different sites within a given tumour [34]. Although only three patients were included in the latter study, this highlights the importance of further clinical investigations on measurements of intracellular IM levels in GIST tissues to understand their possible impact on patient outcome.

Among the limitations of this study are the retrospective registration of the majority of the clinical data and side effects. We neither did review of the radiology nor the pathology, but experienced sarcoma radiologists and pathologists at a major reference centre had already confirmed the diagnostic work-up at start of IM, including analyses of KIT and PDGFR\(\alpha\) mutations that were found in all patients except three. Furthermore, patients with <3 plasma samples were excluded, and median treatment duration of IM before inclusion was 25 months. Even though patients were not excluded due to progressive disease, a bias towards patients without progression could have occurred. Moreover, the plasma samples were not drawn at trough time, but a validated method to extrapolate the samples to trough was used [25]. Even though these issues in general would be considered as shortcomings, they reflect well a routine oncology practice, and our findings could therefore easily be transferred to a routine clinical setting.

In conclusion, our results do not support repeated monitoring of IM levels on a routine basis in all patients. However, we have revealed clinical scenarios where drug measurement could be beneficial, such as for patients who have undergone gastric resection, suspicion of non-compliance, subjectively reported side effects, in elderly patients and at the time of disease progression. Whether dose escalation could be beneficial at disease progression for patients with low IM plasma concentration should be further studied.

**Abbreviations**

GIST: gastrointestinal tumour; IM: imatinib mesylate; C_{min}, trough level; PDGFR\(\alpha\): platelet-derived growth factor receptor \(\alpha\); CML: chronic myeloid leukaemia; PK: pharmacokinetic; BSA: body surface area; WBC: white blood cell; AGP: alpha glycoprotein; PFS: progression-free survival; TDM: therapeutic drug monitoring.

**Authors’ contributions**

IH, OSB and KB were responsible for the concept and design of the study. IH, OSB and KB collected and assembled the clinical data and the plasma samples. KU and JB analysed the plasma IM concentrations. IH and KB analysed the final data. All authors read and approved the final manuscript.

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**Competing interests**

The authors declare that they have no competing interests.

**Availability of data and materials**

The datasets analysed during the current study are available from the corresponding author on reasonable request.

**Ethics approval**

The study was approved by the Regional Ethics Committee (REK) South East Norway (REK-2015-4870).

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Abstract. Patients with advanced gastrointestinal stromal tumors (GIST) are currently recommended for treatment with tyrosine kinase inhibitors (TKI) in a life-long sequence. The standard first-line treatment is imatinib mesylate (IM), which is switched to other drugs at progression or if the patient does not tolerate IM. This strategy has served many patients well as patients with advanced GIST now live for a median of approximately 5 years, compared to 18 months prior the TKI era. The prevailing hypothesis is that IM and other TKIs fail to completely eradicate metastatic GIST and that progression is inevitable if IM treatment is discontinued. Following a response to IM and surgery of metastatic lesions harbouring foci responsible for drug resistance and subsequent clinical relapse, we hypothesize that this may lead to a cure and the justification to stop IM in selected patients. We suggest that this novel strategy, a priori, warrants further investigation. We reviewed the available literature, present three clinical cases and put forward for discussion a treatment algorithm that needs confirmation within the context of a prospective clinical study.

Historically, patients with advanced gastrointestinal stromal tumors (GIST) had a very poor prognosis (1, 2). Treatment was mostly limited to surgery due to the poor response of GIST to conventional chemotherapy and radiotherapy (3). The detection of mutations in the KIT oncogene (4) led to the treatment paradigm of imatinib mesylate (IM) in GIST (5). This small molecule, an inhibitor of tyrosine kinase receptors, including stem cell factor receptor KIT and the platelet-derived growth factor receptor-alpha (PDGFRA), blocks downstream signaling cascades in GIST cells, the main driver of tumor progression (4, 5). Imatinib revolutionized the treatment of patients with advanced GIST (6) and became the model of targeted-therapy in solid tumors; it is now considered to be the standard first-line medical treatment of GIST (7, 8). The median duration of response to IM in advanced GIST is 2-3 years with approximately 20% of the responses lasting for 10 or more years (3, 9). Most GISTs eventually progress on IM, which may then be replaced by other tyrosine kinase inhibitors (TKIs), such as sunitinib (second-line therapy) and regorafenib (third-line therapy) (7, 8). The frequently cited treatment guidelines recommend continuous and ‘life-long’ TKI administration for patients with advanced GIST (7, 8). This has served many patients well, as patients now live for a median of approximately 5 years after detection of metastatic disease, compared to 18 months prior the TKI era (9-11). Currently, a number of new TKIs, alone or in combination (e.g. NCT02365441), are being evaluated alongside treatment options alternative to inhibiting the KIT pathway. Although there has been a dramatic improvement in GIST outcomes, the prevailing hypothesis is that IM and other TKIs fail to completely eradicate the disease and that progression of metastatic GIST is inevitable if IM treatment is discontinued.

Case 1

A 52-year-old man had a complete resection of a 20-cm small bowel GIST with few mitoses in August 2002. In June 2005, the patient had IM initiated due to presence of one large and one small liver metastasis (Figure 1). The metastases were surgically excised in April 2008; they revealed pygnotic KIT-positive cells in hyaline stroma compatible with IM response. Tumor mutation analysis showed KIT exon 11 mutation del556-557. Imatinib was restarted after metastasectomy. The patient has had no
Case 2

A 67-year-old woman had a complete resection of a 9-cm small bowel GIST and five peritoneal lesions in July 2004. Histopathological examination revealed KIT-positive GIST with 13 mitoses per 50 high-power field (HPF). The peritoneal lesions were confirmed as GIST metastases. She was entered into the SSGXVIII/AIO trial (20) and was randomly assigned to the 1-year group. The IM dose of 400 mg/day was reduced to 300 mg/day due to neutropenia. Central pathology review confirmed the diagnosis of GIST. No mutation was found in sequencing of KIT and PDGFRA (KIT exon 17 and PDGFRA exon 12 yielded uninformative results), suggesting an uncommon tumor genetic make-up. Two peritoneal metastases were then detected by computerized tomography of the abdomen (Figure 2) 2 years after discontinuation of adjuvant IM. They were excised in April 2007, but IM was not restarted due to her history of poor tolerance. She has now been followed-up with repeated imaging for 8 years after metastasectomy with no signs of GIST recurrence.

Case 3

A 53-year-old man had a complete resection of a 15-cm stomach GIST in January 2010. Histopathological examination revealed KIT-positive GIST with nine mitoses per 50 HPF. Tumor mutation analysis showed KIT exon 11 mutation ins1754-1789. He received adjuvant IM for 1 year. A solitary liver metastasis was detected on a CT scan of the abdomen 6 months (February 2012) after the discontinuation of adjuvant IM (Figure 3), and IM was re-instituted. PET CT and dynamic MRI showed a remarkable response 1 month after the start of IM (Figure 3), and IM was re-instituted. PET CT and dynamic MRI showed a remarkable response 1 month after the start of IM and IM was restarted due to her history of poor tolerance. She has now been followed-up with repeated imaging for 8 years after metastasectomy with no signs of GIST recurrence.

Discontinuation of Imatinib

A significant body of evidence supports the current practice of administering IM until progression or indefinitely in the absence of progression. Only a small minority (0% to 5%) of patients with advanced GIST achieve complete response (CR) with IM, most responses being partial response (PR) or stable disease (SD) (12-14). In the BFR14 trial, patients with advanced GIST who were responding to first-line IM were randomly assigned either to continue IM or to stop it at the time of randomization (15-17). Almost all patients who stopped had GIST progression within 2 years from the date of randomization, regardless of whether the patient had been on IM 1, 3, or 5 years prior to IM discontinuation. This led to a substantially shorter time to GIST progression in the stop group. Stopping IM did not, however, significantly influence overall survival (OS), likely since most patients in the group that stopped responded to IM reinstitution. Yet, the quality of the responses achieved after restarting IM were generally judged inferior compared to those achieved prior to IM discontinuation (16, 17).

Adjuvant Imatinib in Primary High-risk GIST

Individuals at intermediate or high risk of recurrence are now given adjuvant IM after surgery of a primary GIST (7, 8). Modified National Institutes of Health (NIH) criteria encompass four factors; size, mitotic count, site, and rupture, that are used to select patients for adjuvant treatment (18). A large adjuvant trial that compared 1 year of adjuvant IM to a placebo found that the recurrence-free survival (RFS) benefit achieved with adjuvant IM gradually faded over a long follow-up with no difference in OS between the two groups (19). This indicated that IM controls most GISTs but does not cure patients. The SSGXVIII/AIO trial, however, found that 3 years of adjuvant IM yielded both superior RFS and OS rates compared to 1 year of adjuvant IM (20). Three years of adjuvant IM is now recommended in the treatment guidelines (7, 8). Mature follow-up from the SSGXVIII/AIO trial showed that survival benefits persist (21), lending support to a hypothesis that sufficiently long administration of IM may sometimes eradicate sub-clinical GIST. The role of adjuvant IM has not yet been fully studied, and treatment duration of longer than 3 years may be needed to further reduce or even prevent recurrence. Currently, two trials (NCT02413736 and NCT00867113) are evaluating a duration longer than 3 years of adjuvant IM in GIST.

Imatinib: Toxicity and Side-effects

Imatinib is moderately- to well-tolerated, and several of the adverse effects can be ameliorated with supportive measures or dosing modifications (22). While severe adverse effects are infrequent, almost all treated patients have side-effects, the most frequent being anaemia, periorbital oedema, muscle cramps, and diarrhea (3, 6, 20). Compliance, i.e. adherence to self-administered IM, can be a challenge for patients on chronic therapy, as reported for patients with GIST who were taking IM long term (23). In the SSGXVIII/AIO trial, the proportion of patients who discontinued IM during the assigned treatment period not due to disease recurrence was 25.8% in the 36-month group compared to 12.6% in the 12-month group (20). This suggests that IM for some patients limits quality of life. At present, IM is costly both to individual patients and the health system.
Metastasectomy: Removing a Reservoir of Dormant GIST cells

Metastasectomy may lead to long remission or even to cure in the treatment of some cancer types, including sarcoma (24-27). Approximately 30% of patients with soft tissue sarcoma or osteosarcoma who relapse with lung metastases and who undergo metastasectomy become long-term survivors (24, 28, 29). Similarly, about 30% of selected colorectal cancer patients who have liver metastases resected survive for 5 years or longer after metastasectomy (30, 31). Historically, surgery for metastatic disease in the pre-IM era was universally associated with recurrence, and median survival was 15-20 months (1). In a study of 94 patients who presented with metastatic GIST, complete gross resection was possible in only 30% and the median survival of those treated with surgery alone was 19 months (2). As already mentioned, IM is not curative despite up to 80% of patients with metastatic GIST exhibiting some response or SD on the drug (12-14). This creates the opportunity for surgery to be combined with TKI therapy in order to improve outcomes. Several retrospective studies have reported favourable outcomes of surgery in metastatic GIST patients responding to IM (32-35); however, selection bias cannot be excluded. A large randomized trial that attempted to evaluate metastasis surgery was terminated due to poor accrual (NCT00956072). Hence, it remains unknown whether metastasectomy is beneficial or even harmful in the treatment of advanced GIST. A small prospective study randomized 41 patients with liver metastases from GIST to IM-alone versus IM followed by surgery and then additional IM (36). Patients were followed-up for 36 months and the 1- and 3-year survival rates were higher in the surgery group when compared to the IM-only group (100% and 89% vs. 85% and 60%, respectively). However, whether resection combined with TKI therapy truly confers a survival advantage still awaits a larger randomized controlled trial. Theoretically, surgery of residual disease can prolong durable remission because the excision of the tumor is performed before the development of IM resistance and thus the risk of subsequent disease progression. In the retrospective studies mentioned above, the patients who had metastasis surgery at the time of

Figure 1. The larger liver metastasis detected on a CT scan before the start of IM in case 1.

Figure 2. One of the two intraperitoneal metastases detected on a CT scan following adjuvant IM in case 2.

Figure 3. A small solitary liver metastasis detected on a CT scan in case 3.
GIST progression did poorly compared to those who were operated on while still responding to TKI therapy (32, 33).

**Imatinib: Both Cytotoxic and Cytostatic**

It seems evident that IM does not eradicate overt macroscopic GIST metastases in the great majority of patients. Interestingly, IM is considered both a cytotoxic and a cytostatic drug. The cytotoxic effect is a well-described phenomenon, and evidence shows that tumor cells are replaced by myxoid degeneration after GIST patients have been treated with IM for as little as 4 weeks (5). Myxoid degeneration refers to the proteinaceous material left behind after cell death. This decrease in cellularity suggests that the GIST cells have undergone cell death. Moreover, there have been laboratory studies showing that IM induces apoptosis in GIST cells (37, 38). However, Liu and colleagues demonstrated that IM induces GIST tumour cell quiescence (withdrawal from the cell cycle) in cells that do not enter apoptosis (39). Also, dormant GIST cells are virtually always found in sizable metastases excised from responding patients (19, 33), supporting a hypothesis that there is, despite a cytotoxic effect, also a cytostatic effect. This experience is shared with other TKIs (40). Interestingly, some patients with chronic myeloid leukaemia (CML) who have maintained durable complete molecular remission did not have rapid CML recurrence despite IM discontinuation, suggesting that some patients with CML might be cured with TKIs (41, 42).

Dormant GIST cells may eventually develop IM resistance mutations over time, and since cells within the smallest GIST deposits might be eradicated with IM treatment, complete surgery of larger metastatic foci might, for some patients, potentially lead to cure. A possibility that complete metastasis surgery following durable IM treatment might sometimes lead to cure in GIST patients cannot be refuted. The patient in case 1 had his liver metastases resected, and has in total been on IM for almost 10 years without any signs of recurrence. In that case we have considered stopping IM as an option. The patient described in case 2 had no GIST progression during the 8 years that followed her second metastasectomy. Hence, the decision to refrain from IM served this patient well.
Safe discontinuation: Is it Feasible in Selected Patients Stable on Imatinib?

Can we safely stop IM in patients stable on IM? How should we select these patients? As mentioned above, following primary IM treatment in metastatic GIST patients, more than 80% of patients either respond to or achieve durable SD whereas less than 20% of patients progress (6, 9, 10). If patients relapse after adjuvant IM (1 to 3 years), the first choice of treatment is still IM, seemingly with the same response rate as for primary treatment with IM. In the SSGXVIII/AIO trial, 84% of patients who completed adjuvant IM and then received IM for recurrent GIST responded to IM re-introduction (43). This was independent of the length of prior IM therapy in the adjuvant setting (1 versus 3 years) (43). In the French BFR 14 trial mentioned above, stopping IM did not influence OS, and 96% of the patients responded to the re-institution of IM (15-17). However, patients with PD following IM interruption were not always able to achieve the same degree of tumor control as they had before interrupting therapy (16). This might be of concern because tumor volume (the diameter of the largest metastases) seems to be a negative prognostic factor, both for PFS and OS (10, 44, 45). A concern is that a few patients in the BRF14 trial progressing rapidly after IM interruption had a poorer prognosis (46). Another issue regarding IM interruption is whether it may affect (positively or negatively) the incidence of secondary resistance to IM, which is most often caused by the acquisition of secondary KIT mutations (usually KIT Exon 13, 14, and 17) (47, 48). An analysis in the BFR14 trial compared the time to first progression in patients on continuous IM versus the time to second progression in patients after IM rechallenge. IM-resistant PFS was not significantly different between the continuation and the interruption groups for patients randomized to interruption at 1, 3, or 5 years (49). This might suggest a lack of effect on the IM-resistance selection process by treatment discontinuation. Taking all this into consideration, it seems compelling that it is possible to safely discontinue IM in patients with radically resected metastatic GIST without sacrificing too much for the individual patients. This presupposes that IM is restarted as soon as possible if a recurrence occurs. Strict monitoring with regular radiological imaging is then mandatory to detect any recurrence.

In Figure 4, we present a novel treatment algorithm for GIST put forward for discussion. This should, however, be considered with caution since a rapid progression after discontinuation, a poorer quality of volumetric response at IM re-challenge, and the growth of remaining persistent/resistant sub-clinical disease may impact the long-term outcome of patients. For all these reasons, treatment interruption should not be recommended outside clinical trials unless patients experience substantial toxic effects. In a clinical scenario, such as case 3 (patient experiencing adverse effects limiting his daily activity), we have now decided to explore discontinuation of IM after surgical resection of a solitary liver metastasis. The patient is being carefully followed at the Norwegian Radium Hospital.

Conclusion

While continuous ‘life-long’ IM administration is and should be the standard-of-care for patients with advanced GIST, we propose an experimental treatment strategy in selected patients comprising of IM administration for a few years, metastasectomy, and close surveillance with repeat abdominal imaging after stopping IM. An optimal target group may be patients who, by imaging, have only one or few metastases that can likely be resected without major morbidity and with a documented response to IM. Frequent surveillance by proper imaging modalities to detect recurrence early is then critical. This treatment strategy should, however, not be performed in routine practice but preferentially in a prospective clinical trial or a multinational registry. Even a single-group observational study would likely be informative.

Conflicts of Interest

The Authors have no conflicts of interest.

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