Diplomarbeit

Sequential treatment of a patient with metastatic gastrointestinal stromal tumor (GIST): A case report

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ABBREVIATIONS

ABL	Abelson Protooncogene
AKT	Describes a serine/threonine protein kinase that plays a key role in multiple cellular processes
APES1	Describes a gene encoding a protein that is involved in DNA repair, tumour suppression and apoptosis regulation
ARG	Abelson Related Gene
ATP	Adenosine Triphosphate
BCR-ABL	Describes an oncogene fusion protein consisting of BCR and ABL
c-Myc	Myelocytomatosis Oncogene; Encodes a transcription factor that is located on chromosome 8 and is believed to regulate expression of 15% of all genes
CD34	Cluster of Differentiation molecule; describes a cell surface glycoprotein that is important for cell-cell adhesion
CDKs	Cyclin Dependent Kinases; describes a family of protein kinases that are involved in regulating the cell cycle, transcription, mRNA processing, and the differentiation of nerve cells
CML	Chronic myelogenous leukemia
CNAs	(Genetic) Copy Number Aberrations
CNS	Central Nervous System
CSF1R	Colony stimulating factor 1 receptor; describes a cell-surface protein in humans which is is a receptor for a cytokine called colony stimulating factor 1

CT	X-ray Computed Tomography
DAAM1	Describes a gene encoding a protein that is involved in DNA repair, tumour suppression and apoptosis regulation
DACT1	Describes a gene encoding a protein that is involved in DNA repair, tumour suppression and apoptosis regulation
DNA	Deoxyribonucleic Acid
DOG1	Protein Describes a cell surface protein of yet unknown function selectively expressed in GISTs
E2F1	Transcription Factor E2F1; describes a protein of the E2F family of transcription factors
EC Domain	Extracellular Domain; describes a region of tyrosine kinase receptors like c-KIT
EGIST	Extragastrointestinal Stromal Tumour
ENO1	Enolase 1; encodes the myc-binding protein-1 which downregulates the activity of c-myc protooncogene
EORTC	European Organisation for Research and Treatment of Cancer
ESMO	European Society for Medical Oncology
FDA	Food and Drug Administration
FDG-PET	Fludeoxyglucose Positron Emission Tomography
FGFR	Fibroblast Growth Factor Receptor
FLT3	Receptor Fms-Like Tyrosine Kinase-3 Receptor

FRAP1/mTOR	FK506 binding protein 12 Rapamycin Associated Protein 1/ mammalian Target of Rapamycin; describes a serine/threonine protein kinase that is involved in the regulation of cell growth and proliferation; a member of the phosphatidylinositol 3- kinase-related kinase protein family
GANT	Gastrointestinal Autonomic Nerve Tumour; describes a term used for GISTs with predominant neuronal differentiation before the detection of c-Kit
GI-Tract	Gastrointestinal Tract
GIS	Genomic Instability Stage; a term introduced by Ylipää et al. that describes the tumour cells' stage of key changes in genome levels that might promote oncogenic transformation
GIST	Gastrointestinal Stromal Tumour
GRB2	Growth Factor Receptor-Bound Protein 2; describes an adaptor protein involved in signal transduction/cell communication
HPF	High Power Fields; in relation to microscopy references the area visible under the maximum magnification power of the objective being used
HSP	Heat-Shock Protein
HU	Hounsfield Unit
ICC	Interstitial Cells of Cajal
JM Domain	Juxtamembrane Domain; describes a region of tyrosine kinase receptors like c-KIT
JNK	c-Jun N-terminal Kinase; belongs to the mitogen-activated protein kinase family

KIT-WT	KIT Wild Type; describes the KIT phenotype as it occurs in its typical, physiological form
KIT, C-Kit	CD117, Stem Cell Factor Receptor
MAPKs	Mitogen Activated Protein Kinases
MBP1	Myc-Binding Protein1; downregulates the activity of c-myc protooncogene
MRI	Magnetic resonance imaging
NCCN	National Comprehensive Cancer Network
NCI	National Cancer Institute
NDRG2	Describes a gene encoding a protein that is involved in DNA repair, tumour suppression and apoptosis regulation
NF-1	Neurofibromatosis Type 1
NF2	Neurofibromin 2
OS	Overall Survival; indicates the percentage of people in a study or treatment group who are alive for a given period of time after diagnosis
PARP2	Poly ADP-Ribose Polymerase 2
PCNA	Proliferating Cell Nuclear Antigen
PD	Progressive disease
PDGFRA	Platelet Derived Growth Factor Receptor Alpha Polypeptide
PFS	Progression Free Survival; describes the length of time during and after treatment during which the cancer being treated does not get worse

PI3K	Phosphatidylinositol 3-Kinase; describes an intracellular signal transducer enzyme
PKC-Theta	Protein Kinase C Theta
PR	Partial Response; describes a tumour's response to treatment
Raf Kinases	Describes a family of three serine/threonine-specific protein kinases
Ras Proteins	RAt Sarcoma; describes a protein subfamily of small GTPases that are involved in cellular signal transduction
RB, RB1	Retinoblastoma Protein; describes an important tumor suppressor protein
RECIST	Response Evaluation Criteria In Solid Tumors
RPS6KB1	Ribosomal Protein S6 Kinase Beta-1; describes a serine/threonine kinase that is involved in the regulation of protein synthesis and cell proliferation
RTK	Receptor Tyrosine Kinase
RTN1	Describes a gene encoding a protein that is involved in DNA repair, tumour suppression and apoptosis regulation
S-100 Protein	Describes a low molecular weight protein; its name is derived from the fact that the protein is *100*% **S**oluble in ammonium sulfate at neutral pH
SCF	Stem Cell Factor
SD	Stable Disease; a term that describes a tumour's stable condition
SHC Protein	Src Homology 2 Domain Containing Protein
SMA	Smooth-Muscle Actin

SRC	Describes a proto-oncogenic tyrosine kinase
STAT Proteins	Signal Transducer and Activator of Transcription Proteins; describes a protein family involved in the cellular regulation of growth, survival and differentiation
TK Domain	Tyrosine Kinase Domain; describes a region of tyrosine kinase receptors like c-KIT
TKI	Tyrosine Kinase Inhibitors; e.g. Imatinib
TTP	Time To Progression; describes the period of time until a tumour progresses
UCH-L1	Ubiquitin Carboxy-terminal Hydrolase L1
VEGFR	Vascular Endothelial Growth Factor Receptor
WNT Pathway	Wingless Int 1 Pathway; describes a network of proteins best known for their roles in embryogenesis and cancer

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ABSTRACT

Until recently the prognosis for patients diagnosed with an unresectable GIST was very poor, as conventional chemotherapy and radiotherapy were inefficient in the treatment of this disease. Oncogenic mutations in genes encoding the two closely related tyrosine kinases c-KIT and PDGFRA have been recognized as the initiating features in 85-90% of GIST tumourigenesis. By targeting these auto-activated oncoproteins, imatinib and sunitinib are effective agents for the GIST treatment. Both compounds have been approved for the first- and second-line treatment, respectively. However, at some point of sequential GIST treatment, secondary mutations and consequent resistance to imatinib and sunitinib are likely to occur and present a major therapeutic challenge. Thus, novel multi-TKIs that target alternative spots in oncogenic KIT/PDGFRA become more and more important to sustain subsequent disease control in patients with advanced GIST.

In the first segment of this diploma thesis, the pathology, a historical overview, relevant cyto-pathogenetic aspects, diagnosis, prognostic parameters of GIST as well as established and novel treatment options are presented in detail, according to the latest literature.

The second segment of this diploma thesis demonstrates the case of a female patient first diagnosed with a c-KIT exon 9 mutated GIST in 2005. This patient received TKI treatment for three and a half years. Imatinib and sunitinib were effectively used as first- and second-line therapy. After disease progression, sequential treatment with nilotinib, sorafenib and, to our knowledge for the first time, with pazopanib has been initiated subsequently. While sorafenib and pazopanib resulted in disease stabilisation, nilotinib failed to do so. Observed adverse events were mild to moderate and the patient continued to work until 1 month before decease.

In the following, epidemiologic and general information about GIST is presented as opening part of the first section of this thesis.

ZUSAMMENFASSUNG

Bis vor kurzem war die Prognose für Patienten/innen mit der Diagnose eines Gastrointestinalen Stromatumors (GIST) sehr schlecht, denn traditionelle Chemotherapie und Strahlentherapie zeigten bei der Behandlung dieser Erkrankung keine Wirksamkeit. Onkogene Mutationen in den Genen der beiden eng verwandten Tyrosinkinasen c-KIT und PDGFRA wurden als ursächliche Merkmale zur Tumorentstehung in 85-90% der GIST Fälle erkannt. Imatinib und Sunitinib blockieren gezielt diese beiden Onkoproteine. Beide Substanzen wurden als wirksame First- beziehungsweise Second-line Therapien für Patienten mit GIST eingeführt. Dennoch entstehen in den meisten Fällen im Laufe der sequenziellen Behandlung sekundäre Mutationen. Der dadurch eintretende Wirkungsverlust von Imatinib oder Sunitinib stellt eine große Herausforderung in der weiteren Tumorbehandlung dar. Um auch darauffolgend eine Krankheitskontrolle für Patienten mit fortgeschrittenem GIST zu gewährleisten, gewinnen neu entwickelte Multi-TKIs immer mehr an Bedeutung.

In Berücksichtigung der aktuellen Literatur folgen im ersten Teil dieser Diplomarbeit Ausführungen über die Pathologie, die medizingeschichtlicher Entwicklung dieser Erkrankung, sowie vor allem über relevante zytopathogenetische, diagnostische, prognostische und therapeutische Aspekte.

Im zweiten Teil dieser Diplomarbeit wird der Erkrankungsverlauf einer weiblichen Patientin beschrieben, welche im Jahr 2005 erstmals an GIST erkrankte. Der in c-KIT Exon 9 mutierte Tumor wurde dreieinhalb Jahre mit TKIs behandelt. Die Patientin konnte anfangs erfolgreich mit Imatinib, und vor allem mit Sunitinib behandelt werden. Nach Krankheitsfortschritt unter den beiden letztgenannten Medikamenten zeigten auch die sequenziellen Therapien mit Sorafenib und Pazopanib, welches unserem Wissen nach zum ersten Mal für eine GIST-Behandlung verwendet wurde, klinische Wirkung. Nilotinib konnte bei dieser Patientin keine Wirksamkeit entfalten. Die Nebenwirkungen der Therapien waren gering und die Patientin konnte bis einen Monat vor ihrem Ableben ihrem Beruf nachgehen.

Im Folgenden werden zu Beginn des ersten Teils der Diplomarbeit allgemeine und epidemiologische Aspekte über GIST erläutert.

1 INTRODUCTION

Gastrointestinal stromal tumors (GISTs) are rare neoplasms arising from the wall of the gastrointestinal (GI) tract. Although this type of tumour solely constitutes between 1-3% of all gastrointestinal tumours, with about 80% of mesenchymal neoplasms in the GI tract being GISTs, it represents the majority of lesions in this organ system [1,2].

Within the group of soft tissue sarcomas GISTs have not been identified as a distinct new entity until a few years ago; yet, they have been the object of intensive research since then. Interest has preeminently been drawn towards the role of two specific cellular receptor tyrosine kinases (RTK), in particular the transmembrane receptor proteins c-KIT and, to a lesser degree, the Platelet Derived Growth Factor Receptor Alpha (PDGFRA). Oncogenic mutation of the genes encoding these two kinases results in permanent ligand-independent activation and signal transduction, which is physiologically mediated by these receptors. This mechanism has shown to be of outstanding pathogenetic relevance in GIST tumourigenesis.

Advances in the understanding of the molecular pathophysiology in the evolution of GIST have resulted in the identification of new targets for cancer therapy. Improving the therapeutic options helped transforming the threat of a malignant disease with very poor prognosis to a manageable chronic condition for many patients.

1.1. Epidemiology

Valuable epidemiological studies scrutinizing the occurrence of GIST have been implemented in several European countries [3-5] as well as in the USA [6] and Taiwan [7].

According to these publications the incidence of GIST ranged from 6.8 (USA) to 14.5 (Sweden) new cases per 100.000 individuals per year. The markedly lower number noticed in the US study may be explained by varying case ascertainment. An actual higher incidence close to the number mentioned in the non-US studies seems probable considering the latest observations [8].

Taking all available data into account, nearly equal numbers of GIST incidence by country are observed.

The annual number of persons with newly acquired GIST is estimated to be around 4500-6000 in the USA [9] and 1500 in Germany [10].

Epidemiologic studies evaluating large patient cohorts revealed a median age of 55 to 65 at the time of tumor diagnosis, with less than 10% arising before the age of 40 [1,11]. Although sporadic cases in infancy and adolescence were observed, 80% of patients were above the age of 50 when diagnosed with GIST [6]. Furthermore, certain studies point towards a rising incidence of GIST with increased age [8].

Data overall suggest a slightly higher incidence in men than in women with a distribution of about 55% to 45 % and a higher predisposition for people of black race [6,9].

Of note, however, is the assumption that numerous asymptomatic cases of GIST remain undetected. This hypothesis is supported by the detection of two GISTs per 1000 autopsies performed with the actual number estimated to be much higher, as shown in a recent publication [3].

1.2. Site of occurrence

In general GISTs can potentially develop anywhere in the intestinal tubular tract, from the esophagus to the rectum. In addition to that, the infrequent occurrence of identical lesions in other sites, such as the omentum, the mesentery adjacent as well as the retroperitoneum, has been described.

- **Anatomic distribution**

The majority of primary GISTs originate in the stomach followed by the small bowel, less commonly they arise in the big bowel, the rectum and the esophagus.

In rare occasions lesions are found outside of the gastrointestinal tract elsewhere in the abdominal cavity. The so-called extragastrointestinal GISTs (EGIST) most

frequently emerge in the mesentery, the omentum and the peritoneum. Of note, however, is that due to a lack of symptoms EGISTs may develop to an extensive size at the time of diagnosis, as reported [12,13].

Detailed anatomical distribution of primary GIST is presented in table 1 below:

Table 1: Anatomical allocation of primary GISTs [1]

GIST – Primary Localizations	
Stomach	50-60 %
Small Bowel	20-30 %
Large Bowel	5-15 %
Esophagus	5 %
Omentum, Mesentery	up to 9 %
Retroperitoneum	up to 3 %

- **Rarities**

Highly uncommon sites of primary GISTs were reported to arise in the gallbladder and inside of an inguinal hernial sac [14-16]. There is only one solitary report describing a GIST of the abdominal wall [17].

- **Syndromes**

GISTs may occasionally occur as part of syndromes such as the extremely rare Carney-Syndrome. It has been described for the first time in 1977 and is characterized by the triad of mostly multiple GISTs of the stomach, extra-adrenal paragangliomas and pulmonary chondromas. The majority of patients with this disease are female and younger than 30 years of age [18].

The presentation of solitary or multiple GISTs in patients with Neurofibromatosis 1 (NF1) seems to be of greater significance. As this inactivation of the tumor-suppressor gene NF1, encoding a member of the RAS-regulatory protein family, appears in approximately 1 of 3000 births, it is a relatively common disease. According to studies 5-25% of patients bearing this mutation are diagnosed with GIST during their lifetime. It seems that pathogenetic events leading to tumour genesis differ from those in sporadic GISTs, as KIT- or PDGFRA mutations only occur in very few cases in GISTs of neurofibromatosis patients [19,20].

- **Familial GIST**

Although nearly all GISTs develop sporadically, there are occasional descriptions of familial GISTs, which are almost exclusively associated with germline mutations of the gene encoding the KIT receptor.

The development of multiple GISTs in the entire GI-tract is one of the leading characteristics, and various others like cutaneous hyperpigmentation, nevi and urticaria pigmentosa have been associated with patients carrying a germ line c-KIT mutation. Furthermore, there is an increased risk for carriers of a c-KIT germline mutation to develop GISTs at a much lower age compared to the median age at diagnosis in sporadic GISTs [19,21].

- **Pedriatric GIST**

Despite the rarity of occurrence, gastrointestinal stromal tumours arising in childhood are worth mentioning as they appear to show significant differences to those of adults. Dissimilarities include a much higher incidence in females than in males, the substantially lower mutation frequency of KIT or PDGFRA oncogenes [22], the frequent formation of multiple GISTs with the vast majority of lesions arising in the stomach, and the higher occurrence of regional lymph nodes metastases [23,24].

1.3. Progression of disease

The majority of GISTs are detected after symptoms have developed. For the most part that means the tumour stage is already advanced by the time of first diagnosis. Typically these symptoms are either related to a functional impairment of the GI-tract related to tumour size or to mucosal ulceration and consecutive gastrointestinal bleeding. The mean time between the occurrence of first symptoms and conclusive diagnosis has been reported to be 4-6 months [25].

"*Not infrequently, however, GISTs are discovered incidentally during radiologic imaging for an unrelated condition or as a secondary finding in a surgical resection or autopsy specimen*."[26]

If detected during endoscopy or laparoscopy, tumour stage most of the time is low and locally confined [25].

Surgical resection of the primary tumour is the therapeutic gold standard whenever feasible. The median time of survival after a successful complete resection of a locally confined primary GIST lesion without evidence of metastasis has been reported to be 66 months and the 5-year survival rate is in the range of 50%-65% [27,28].

Unfortunately, tumour recurrence is not infrequently observed even after complete surgical resection. Relapse is commonly detected at the site of resection, but may also present as metastatic dissemination, or both. Up to 50% of GIST patients develop tumour recurrence within the first 5 years after resection [27,28]. As this incident has even been demonstrated among tumours classified with avery low potential of malignancy, not any GIST lesion can be considered truly benign [29]. For this reason, histological features to stratify this tumour for its risk of malignant behaviour have been suggested [29].

It is important to notice that "*...up to 30% of newly diagnosed GISTs are overtly malignant or have features that connote a high malignant potential.*"[26] Furthermore, 20-50% of patients already present with metastatic disease at the time of diagnosis. Not until recently, therapeutic options were very limited for patients with metastatic,

locally advanced and/or recurrent disease, when surgical resection simply was not feasible from a curative standpoint. Both conventional systemic chemotherapy as well as radiotherapy proved to be almost ineffective in the treatment of this tumour, resulting in a median overall survival time of 9-12 months and a 5-year survival rate close to zero for patients with metastatic or recurrent GISTs [30].

Metastases seem to arise in a very limited subset of anatomic sites in recurrent GISTs. The majority of GISTs metastasizes to the liver or to the peritoneal surfaces [1,26]. In very rare cases, metastases are found in lymph nodes, which is important to note, since lymph node resection in clinically and radiologically uninvolved nodes needs not to be performed in localized disease. Metastases to the lung, bones, or CNS, are exceptions. However, since the therapeutic use of imatinib the occurrence of metastases in previously uncommon sites like the CNS has increased [1].

- **The era of imatinib and beyond**

Notably, the molecular definition of important GIST biomarkers and the advent of the development of small-molecule tyrosine kinase inhibitors (TKIs) have considerably improved prognosis for patients with GIST in recent years. Two of these TKIs, imatinib and sunitinib, are currently approved for the treatment of GIST. Especially imatinib, constituting the first of these agents to prove clinical benefit in GIST treatment, displayed a new era of targeted therapy for this disease.

Although there is no reason for limitless optimism as primary or secondary tumour resistances to imatinib treatment may arise quite frequently, the 2-year survival rate increased to 70-80% and the 3-year survival rate to around 60% for patients with metastatic or recurrent disease in the post imatinib era [1].

Potential benefits regarding the adjuvant or neoadjuvant treatment with TKIs, as well as detailed characterization of relevant tyrosine kinases, their therapeutic targets and the evolution of novel targeted compounds for GIST therapy are going to be presented and discussed in the further course of this diploma thesis (see sections 3 and 6).

2 PATHOLOGICAL CHARACTERISTICS

2.1. Macroscopic aspects

Sporadic GISTs predominantly present as a single intramural or submucosal lesion and on palpation they usually appear to be either soft or moderately coarse. Some tumours exhibit distinct extramural patterns of growth and can solely be attached to gastrointestinal walls by a thin serosal or subserosal isthmus.

Ulceration of adjoining mucosa is not an uncommon event, especially in big GISTs. However, most ulcerations are developing in big tumours and thus are rather pressure-related than genuinely caused by tumour infiltration of mucosal structures. The rare proof of true mucosal infiltration, overgrown pre-existing mucosal texture in a lymphoma-like pattern, is one of the few reliable histomorphological criteria of aggressive biological behaviour. Hence, plain pathological distinction of the actual reason for ulceration is of clinical significance.

A typical GIST lesion is bounded plainly or capsule-like. The cut surface of a GIST characteristically appears granular and grey in colour; areas of haemorrhage, necrosis and pseudo cystic regression are encountered in a varying frequency. Features like haemorrhage, necrosis, increased degeneration or a brighter, whiter colour of the cut surface are due to increased cellularity and may occasionally be found in GISTs with higher malignant features [1,31].

2.2. Histology and immunohistochemical features

- **Aetiology**

Gastrointestinal stromal tumours are assumed to derive from interstitial cells of Cajal or their stem-cell precursors, with the latter hypothesis being the favored one today. This histogenetic conclusion has been drawn due to various well-documented, unequivocal ultrastructural and immunophenotypic similarities between GIST and its most probable progenitor [32].

The interstitial cells of Cajal (ICC), who are also known as GI pacemaker cells, physiologically exert an important mediator role between the autonomic innervation of the bowel wall and the function of smooth muscles in the GI tract. It has been described that ICCs regulate peristalsis by producing so-called “slow-waves” and are located embedded between smooth muscle and nervous tissue throughout the gastrointestinal wall.

Although these fibroblast-like cells varyingly display immunophenotypic and ultrastructural features of both smooth muscle and neuronal cells, they also show distinct and important differences to them which are significant for the understanding of GIST biology. Most notably, it was the immunohistochemical detection of KIT (CD117) expressed on ICCs, as well as on the vast majority of GIST cells that helped to differentiate GISTs from true gastrointestinal myogenic or neurogenic tumours in the 1990’s [32]. The characterisation of this important diagnostic feature led the way to a standardized histological GIST diagnosis. Accordingly, at an American GIST-Workshop with the goal of developing consensus diagnostic guidelines for GIST diagnosis in the year 2001 it had been noted that: “...*indeed the term GIST should apply only to neoplasms displaying KIT immunopositivity with very rare exceptions*.”, but, as quoted later on “...*KIT immunopositivity is not in isolation grounds for diagnosing GIST and must always be interpreted in light of the morphologic findings*.”[33]

- **Histological subtypes**

In the majority of GIST-suspect cases plain histologic assessment is already leading towards diagnosis all by itself due to the highly uniform microscopic presentation of GISTs. Most of the time GISTs emerge as 1 of the following 3 categories:

- Spindle cell type (70%)
- Epitheloid type (20%)
- Mixed type

Only a low number of lesions either show prominent myxoid stroma, paraganglioma-like growth patterns or notable cellular pleomorphism as outstanding histological characteristics.

GISTs of spindle cell type are usually composed of quite uniform eosinophilic cells, positioned in storiform or fascicle-like growth patterns. The cytoplasm of these cells is notably less eosinophilic than of smooth muscle neoplasms and frequently shows a fibrillary, syncytial aspect. Nuclear palisading, stromal lymphocytes and cytoplasmic vacuoles are other characteristics of this subtype, encountered in a varying frequency [1,33].

The rounded or rather polygonal cells encountered in epitheloid type GISTs are characteristically arranged in an either nested, trabecular or chondroid architecture with clear cut cell boarders, at times resembling epithelial or melanocytic neoplasm. Cytoplasm in epitheloid GISTs appears variably eosinophilic or clear [1,33].

GISTs composed of mixed cell type may exhibit both, plainly defined areas of spindle or epitheloid cells, as well as areas with commingled segments of these cell types [1,33].

Histological characteristics that may generally be pointed out in GIST lesions include a rather uniform appearance of the tumour cells, microcystic stromal degeneration and focal stromal dispersing with most commonly myxoid, scarcely collagenous sections. Thin-walled vessels are a frequent observation and stromal haemorrhage presents to be a common feature of these tumours. Predominantly GISTs exhibit

smooth tumour margins and infiltration of adjoining mucosal structures is rarely found [1,33].

- **Immunophenotypes displayed in GISTs**

The main characteristic of GIST is the nearly ubiquitous expression of CD117 corresponding to the c-KIT receptor in about 95 % of all lesions. Furthermore, GISTs also show positivity for CD34 in 60-70% and for smooth-muscle actin (SMA) in about 30-40% of cases. Examinations proved significant immunohistochemical differences concerning the frequency of CD34 and SMA expression in GISTs with regard to their

site of manifestation. While positive staining for CD34 was found pre-eminently in esophageal- and colorectal primaries, SMA was detected with a higher frequency in small intestinal GISTs [34].

Immunoreactivity for S-100 protein (about 5% of cases) or desmin (1-2%) are rather uncommon in GISTs [33]. Nevertheless, they play an important role for the exclusion of other mesenchymal tumour types from GISTs.

In a recent paper Miettinen et al [35] discussed the immunohistochemical significance of the DOG1 protein in connection with GIST. DOG1, a receptor-activated chloride channel protein, was found in a preponderant number of Cajal cells and GIST lesions. In the study mentioned above, out of all 1040 GISTs examined, 986 (94.8%) stained positive for DOG1. Furthermore, it could be shown that DOG1 was detected very infrequently in other tissues, attributing DOG1 the most sensitive and specific immunohistochemical role alongside to KIT (CD117). It was concluded that consequently "*DOG1 should be added into the immunohistochemical panel evaluating GI, abdominal, and selected other KIT-negative and positive tumors suspected of GISTs*" [35].

In contrast to DOG1, protein kinase C theta (Θ), another potential antibody target which constitutes a downstream effector in the KIT-signaling pathway, has failed to prove as an objective and distinct marker [35].

Worth mentioning in this regard is the pattern of CD-117 staining. Strong, diffuse cytoplasmatic immunopositivity for CD117 can be demonstrated in the majority of GIST cases. Furthermore, a perinuclear, dot-like accentuation of CD117 staining (so-called “golgi pattern”) is commonly found and in about the half of all cases coexists with the diffuse cytoplasmatic pattern. No obvious findings could be determined regarding potential differences of staining design between spindle- and epitheloid GISTs.

According to some reports, CD117 negativity in highly GIST-suspect lesions most certainly is the result of an already advanced mutational status in the relevant gene locus. In other cases, quite often negative staining either seems to be a result of a sampling error or seems to occur because the examined material presents to be immunohistochemically inert (meaning the process of sample staining simply can not

be performed, for example due to excessive heat during section drying). Hence, due to the fact of strong expression of CD117 in most tumour cells, GIST diagnosis should severely be doubted in case of merely focal or absent expression of the c-KIT protein [1,33,34].

2.3. Differential Diagnosis

GISTs require precise diagnosis in order to provide a possible benefit from efficacious molecular targeted therapy for the patient. Differential diagnosis includes a number of mesenchymal, neural and neuroendocrine neoplasms with a potential manifestation in the abdomen.

Table 2 gives an overview of the most common differential diagnosis regarding GIST:

Table 2: Most common differential diagnosis of GISTs [1,26]

GIST – Histomorphological Differential Diagnosis	
Leiomyoma, leiomyosarcoma	Malignant mesothelioma
Schwannoma, malignant peripheral-nerve sheet tumor	Liposarcoma
Fibromatosis	Metastatic melanoma
Solitary fibrous tumour	Poorly differentiated carcinoma
Inflammatory myofibroplastic tumour	Gastric glomus tumour
Neuroendocrine tumours (carcinoid and islet cell)	Desmoid tumor
Angiosarcoma	Synovial sarcoma

Fibromatosis, leiomyoma and leiomyosarcoma seem to be the tumours most commonly confounded with GIST. Compared to GISTs, primary smooth muscle tumours are quite rare in the GI-tract as well as every other tumour listed in table 2. Moreover, leiomyosarcomas arising in the intestinal tract constitute a veritable exception [1,26].

CD117 as well as DOG1 have proven to be the most specific and sensitive antibodies to skew the diagnosis of suspect lesions towards GIST. These two antibodies should, especially in cases of uncertainty, be combined with staining of other cellular markers, such as CD34, PKC-theta, nestin, smooth muscle actin (SMA), desmin and S100-protein to exclude other tumour entities [36].

The awareness of indicative histologic features and immunohistological staining patterns of the mentioned markers, paired with consideration of the incidences of various different tumours at their respective anatomic sites, should help to exactly

identify GISTs and avoid the diagnostic pitfalls. Additionally, mutational analysis of KIT or PDGFRA genes at an expert centre can be performed either to confirm or to help diagnose GIST in doubtful cases.

3 MOLECULAR PATHOLOGY AND CYTOGENETIC ASPECTS

3.1. History of GIST and focal points of pathogenesis

Until the early 1960s, stromal tumours of the gastrointestinal tract were merely considered to be of smooth muscle genesis, based on light microscopic observations [37]. With the addition of electron microscopic research and the advent of immunohistochemistry in the early 1970s doubt began to spread whether gastrointestinal stromal neoplasms are indeed of myogenic origin [26].

Inconstant expression of muscle markers, such as actin and desmin and, the additional verification of neural markers (S-100, neuron-specific enolase, UCH-L1) supported the growing evidence that the majority of these gastrointestinal tumours were a distinct clinicopathologic entity. Consequently, in the mid-1980s, the term gastrointestinal stromal tumour was introduced, although the cellular origin of these neoplasms was not exactly known by then [38]. At about the same time an alternate diagnosis, gastrointestinal autonomic nerve tumour (GANT), was introduced for those mesenchymal tumours predominantly displaying neural characteristics [39].

Despite being intensely discussed in the pathological community, this terminology did not change until the 1990s. At the beginning of the 1990s, a first attempt on an immunohistochemical definition of gastrointestinal stromal tumours was made when CD34 expression was discovered on most tumour cells [40]. However, even though this observation contributed to a more differentiated perception of GISTs, with an effective number of 60-70% of tumour cells expressing CD34, it quickly turned out not to be the defining feature pathologists were seeking for.

Disappointment did not last for a long time, as the year 1998 marked the beginning of a new GIST-definition which is valid until the current date. Primarily, it were the investigations of Kindblom et al. [32] and Hirota et al. [41] that immunohistochemically identified strong expression of the c-kit protein (CD117) not only on the interstitial

cells of Cajal (ICCs), but also very intense staining on most GIST cells. It could be demonstrated that 95% of GISTs stain positive for KIT [34,42,43]. The detection of the c-KIT receptor on GIST cells confirmed that the origin of these cells could be attributed to ICCs or their stem-cell precursor cells.

- **A prominent role of oncogenic kinase mutations**

At the beginning of the 1990s several groups proved that a mutation of the c-KIT gene was a considerable pathogenetic event in human mast cell neoplasms [44]. Expecting similar mechanisms in ICC cells for GIST development, Hirota et al. substantiated those considerations in what turned out to be a landmark publication [41]. Numerous investigations followed, and it quickly became evident that most GISTs harbour mutations in critical positions of the KIT proto-oncogene, resulting in constitutive phosphorylation and activation of the encoded KIT tyrosine kinase [26].

Physiologically, binding of the KIT ligand SCF leads to receptor dimerization with another receptor protein, ATP-mediated phosphorylation of the tyrosine kinase and further induction of downstream signaling cascades, which are known to promote cellular differentiation, migration and growth [45]. KIT function and activation is decisive not only for the maturational process of ICCs, but also for hematopoietic progenitor cells, mast cells and germ cells. However, in GISTs with oncogenic KIT-gene mutation, cells show permanent and uncontrolled proliferation and growth.

Important pathogenetic insight could also be achieved for a part of the so called KIT-wild type (KIT-WT) GISTs, a term referring to those tumours that are negative for KIT gene mutation. About a third to a half of KIT-WT tumours showed mutations in the Platelet derived growth factor receptor alpha polypeptide (PDGFRA) gene, as well as detectable phosphorylated PDGF receptor A immunoprecipitates [46,47], PDGFRA, denotes a tyrosine kinase receptor with similar structural features as the KIT receptor. The genetic sites of mutations in PDGFRA-mutated GISTs resemble those encountered in KIT-aberrant GISTs [26].

Moreover, the downstream signalling pathways activated in PDGFRA-mutant GISTs seem to be identical to KIT-mutant tumours [47].

KIT and PDGFRA mutations seem to be mutually exclusive, as simultaneous presence of alterations in both genes is yet to be detected. In general, between 85%-90% of gastrointestinal stromal tumours reportedly harbour mutations in either one of these two kinase genes [48]. The overall frequency of primary KIT and PDGFRA mutations in GISTs have been a subject of debate ever since first well executed studies scrutinizing this topic have been implemented. Numbers vary greatly in various studies. Due to some reports the most possible reasons for underlying discrepancies may be found in differently composed cohorts and technical problems in the process of mutation analysis [49].

Numerous different studies have verified the central pathogenetic importance of kinase alterations in GIST development. Corless et al. discovered that the frequency of KIT gene mutations in small, incidentally found gastrointestinal stromal tumours (1 cm or smaller) was highly comparable to that in larger GISTs, suggesting a very early occurrence of the mentioned mutations in GIST genesis [50]. Another study confirmed that the expression of a mutated KIT gene code in transgenic knock-in mice induced the formation of KIT-positive, spindle cellular tumours exhibiting morphologic aspects similar to those in GISTs [51]. On the other hand, a blockade of KIT kinase activation has repeatedly shown to inhibit proliferation and growth of GIST cells in vitro [52] as well as in vivo with patients under imatinib treatment [53]. Even the unfortunate, but commonly seen development of secondary resistance to imatinib at least indicates a persistent tumour dependence on signalling from KIT or PDGFRA kinases [52].

Most KIT/PDGFRA gene alterations occur on the basis of heterozygous mutations, meaning one allele is still showing WT conformation. Thus, KIT or PDGFRA is mentioned as a dominant oncogene, due to the effect of this monoallelic mutation [1].

In conclusion, oncogenic induction of KIT or PDGFRA downstream signal pathways is of central relevance in this disease. Constitutive auto-activation of these kinases caused by differential gene alterations can serve as the initiating event in GIST tumourigenesis.

- **Activated signalling pathways**

A detailed knowledge of intracellular signalling pathways involved in GIST development is believed to have clinical implications by potentially providing further diagnostic and prognostic tools as well as new targeted therapies for the treatment of patients with GISTs.

Oncogenic KIT signalling in GIST not only differs from physiologic KIT signal transduction in ICCs but also from that in haematological neoplasms with activating KIT mutations, like for example in many cases of mast cell cancer [54]. It is suggested that certain types of intrinsic KIT gene mutations result in the activation of specific signaling pathways [54].

Although analyses of tumour extracts constantly demonstrated KIT phosphorylation, the levels of phosphorylated KIT varied significantly from tumour to tumour, even between those harbouring equal mutations [54]. In most primary GISTs KIT is phosphorylated substantially at its GRB2 and PI3K binding sites. In various studies activation and expression of subsequent downstream pathways including mitogen-activated protein kinase MAPK3/1, AKT, RPS6KB1 (p70 S6 kinase) as well as STAT1 and STAT3 were detectable [54,55]. Conversely, no evidence was found for the activation of JNK and STAT5 pathways, oncogenic signalling pathways which are otherwise commonly demonstrable in tumorous diseases [54,55]. Beyond that, it could be verified that constitutive activation of the PI3-kinase/FRAP1 (mTOR) cascade, not the MAPK pathway, is essential in KIT-mediated oncogenic signalling in GISTs [56].

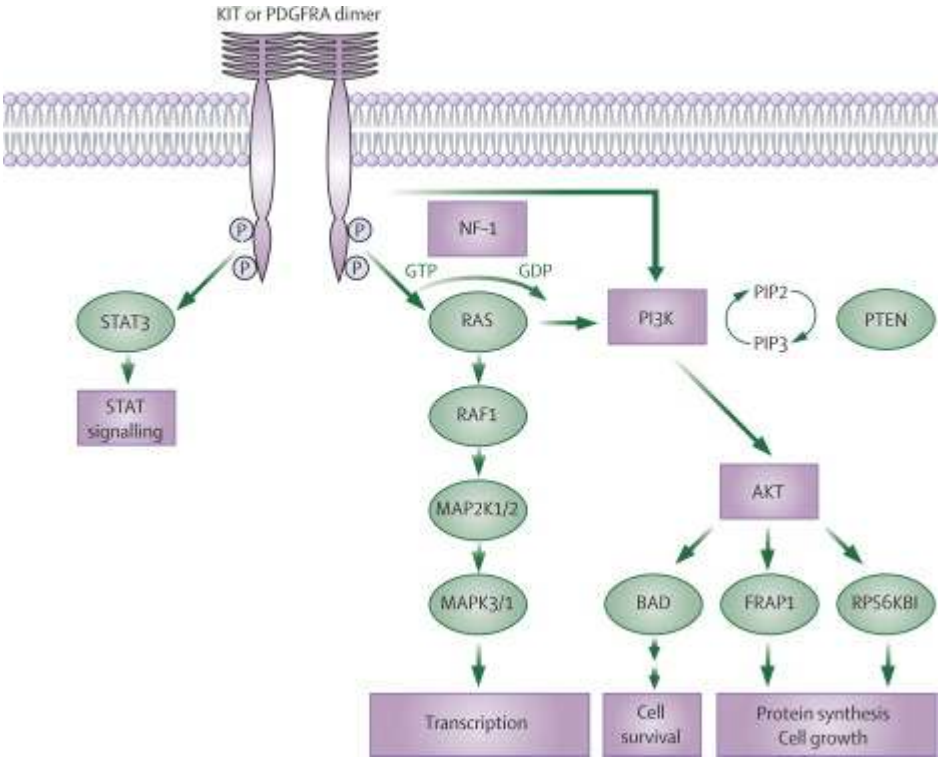
KIT mutated GISTs that showed strong AKT phosphorylation were overall accompanied by higher levels of indicative cell-proliferation markers such as Cyclin A and PCNA (Proliferating Cell Nuclear Antigen). Furthermore, the selective inhibition of the PI3-kinase/mTOR pathway showed to lessen proliferation rates while increasing apoptosis [57].

Generally, signal transduction seems to follow similar patterns in GISTs with constitutively activated PDGFRA [47].

However, despite substantial insights in KIT mediated oncogenic pathways, comparison of gene expression profiles and supplementary analysis of tumour cells broadly suggested "*that additional mechanisms besides the intrinsic genomic KIT mutations influence signalling pathway activation in GISTs*" [54]. A number of observations proved that variable activation of AKT, MAPK and STAT1/3 pathway intermediates was detectable among GISTs with identical KIT mutations. Additional mechanisms may include interactions between the SHC adaptor protein and the KIT receptor, or the process of alternative splicing, which results in the expression of different KIT isoforms [54]. Both of these events result in a differential activation of downstream pathways. Furthermore, the activation of STAT1 and STAT3 proved to be independent from the oncogenic KIT signal [54].

Figure 1 provides a simplified overview of important signalling pathways which are commonly activated in GISTs:

Figure 1: KIT and PDGFRA signalling in gastrointestinal stromal tumours [48]



- **Molecular progression of GISTs**

As mentioned in the first part of this thesis, patients with germline mutations in critical KIT gene spots are very likely to display multiple GISTs. However, their neoplasms do not become clinically significant until early adulthood. This observation clearly suggests the occurrence of additional genetic and/or epigenetic events besides KIT and PDGFRA mutations for GISTs to develop on the basis of an ICC hyperplasia [26].

Cytogenetic studies have shown a wide range of chromosomal alterations in patients with mutant KIT or PDGFRA which are associated with malignant progression. In summary, a common model for chromosomal aberrations in the evolution of GISTs is as follows: from KIT or PDGFRA mutation to 14q deletion, 22q deletion, 1p deletion, 8p gain, 11p deletion, 9p deletion and 17q gain [55].

Most frequently observed in an early tumour stage in about two thirds of GIST cases is 14q complete or partial deletion. Not long ago, two recurrently deleted regions at 14q have been identified, both harbouring important genes involved in DNA repair, tumour suppression and apoptosis regulation, such as PARP2, APES1, NDRG2 [47,59]. Another paper recently reported further 14q gene deletions and subsequently repressed expression of RTN1, DAAM1 and DACT1 which are suggested to play critical roles as tumour suppressors in early tumourigenesis of KIT or PDGFRA mutated GISTs [60]. DACT1 suppresses the basal activity of the WNT/β-catenin pathway, which is described to be decisive for the modulation of cell proliferation and survival [61].

Karyotypes of approximately 50% of GISTs demonstrate loss of the long arm of chromosome 22 [47,55]. NF2 (Neurofibromin 2) is a targeted “hot-spot” gene at this location. As a result of 22q deletion, downregulation of the encoded tumor suppressor protein NF2 also seems to frequently abet GIST progression [59,60].

By similar mechanisms losses on chromosomes 1p, 9p, and 11p favour malignant growth of ICCs. Despite being less common than 14q and 22q losses, the impact of aberrations in 1p, 9p, and 11p seems to be even more severe [59,60]. ENO1 is the target candidate at 1p. It encodes the Myc-binding protein1 (MBP1), which down regulates the activity of c-myc protooncogene [59,60]. 9p deletion is strongly associated with inactivation of CDKN-2A ($p16^{INK4A)}$ and 2B genes under the presence of homozygous deletion. The equally named protein products CDKN2A/2B are important inhibitors of cell-cycle kinases (CDK4/6). When activated in early G1 phase, CDK4/6 phosphorylates RB and thereby frees the transcription factor E2F1 from RB. This subsequently enables mRNA transcription of genes which are essential for G1/S phase transition. In the evolution of GIST, down regulation of CDKN2A/2B seems to be a key contributor to malignant progression, as these two genes were demonstrably inactivated in a significant portion of advanced gastrointestinal stromal tumours [59-63].

Gene amplifications have been detected on chromosomes 8q as well as on 17q and have been associated with metastatic behaviour of GISTs [47].

3.2. KIT, PDGFRA and their common mutations in GISTs

- **KIT and PDGFA receptors**

Obviously, knowledge of the molecular composition of KIT/PDGFRA receptors in GISTs is critical, not only for the understanding of underlying pathogenetic events, but also, and moreover, for consequent implications in therapeutic fields. Responsiveness to treatment with tyrosine kinase inhibitors (TKIs) varies significantly depending on the exonic location of the KIT/PDGFRA mutation.

KIT and PDGFRA genes are located on chromosome 4q and presumably share a common ancestral gene, which is materialized in their highly homologous phenotype. Both genes encode transmembrane glycoproteins which belong to the family of type 3 receptor tyrosine kinases. Type 3 RTKs are characterized by a specific and similar molecular structure, consisting of an extracellular (EC) domain with five immunoglobulin-like loops and a cytoplasmic domain with a juxtamembrane (JM) and a tyrosine kinase (TK) region. A kinase insert further separates the tyrosine kinase domain into an ATP-binding region, TK1, and a phosphotransferase region, TK2. Extracellular and cytoplasmatic domains are connected by a transmembrane region [1,47,49] (Figure 2).

RTKs serve as intermediates of signal transduction, physiologically activated by binding of their respective ligands to the receptor EC domain. This leads to conformational changes of the receptors, subsequent phosphorylation of tyrosine residues in their cytoplasmatic TK domains and induction of multiple downstream signalling pathways. Stem cell factor (SCF) and PDGFs constitute the physiologic ligands to KIT and PDGFRA [1,47,49].

Overview of KIT and PDGFRA mutations in GISTs

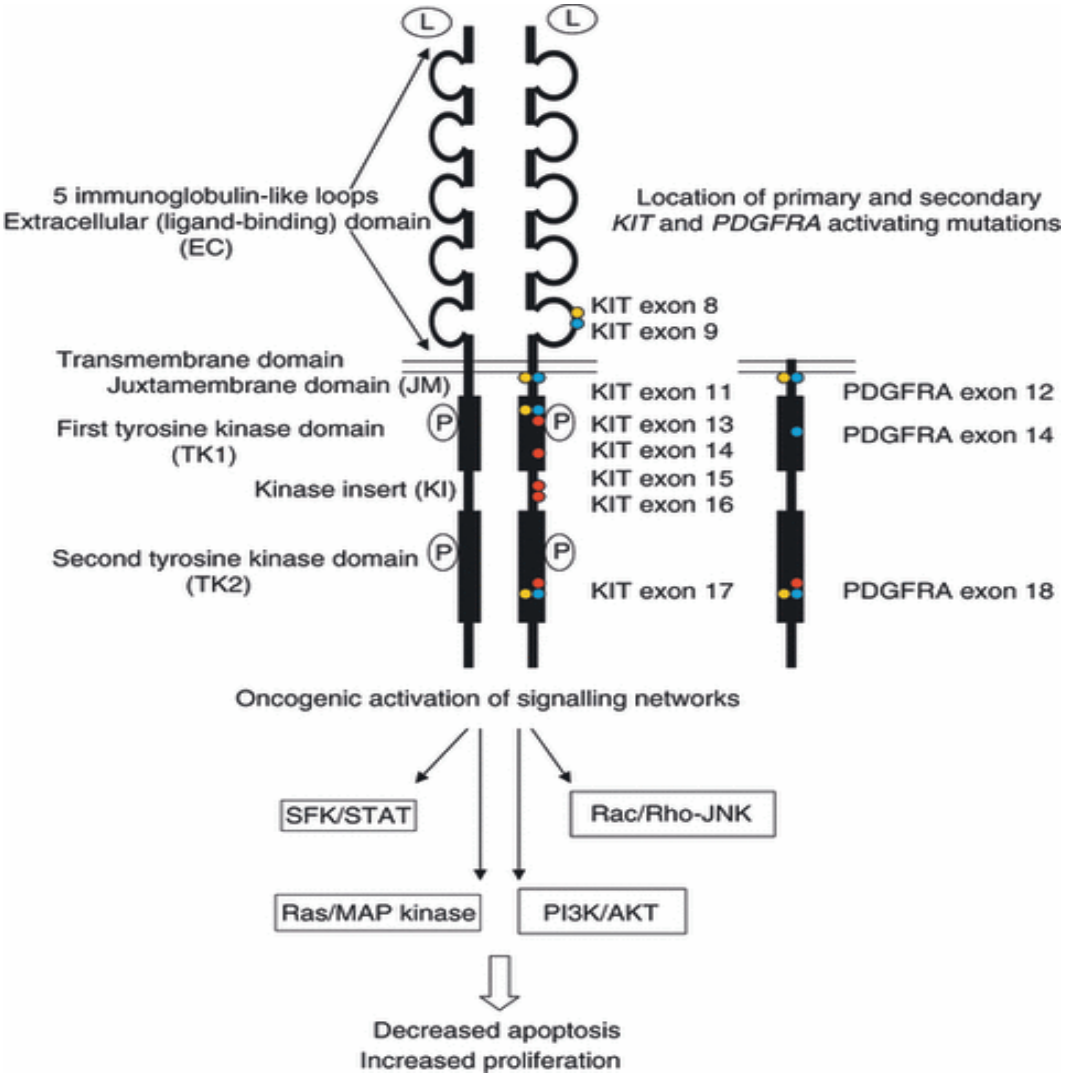
Overall, auto-activating KIT or PDGFRA mutations are found in 85-90% of GISTs [48]. Two categories of KIT and PDGFRA mutations in GISTs have to be separated:

- *Primary KIT and PDGFRA mutations*: detected in primary tumours, evaluated before treatment with a Tyrosine kinase inhibitor (TKI) and related to oncogenic GIST pathogenesis.
- *Secondary KIT and PDGFRA mutations*: evaluated during or after treatment with a TKI, causing resistance to the TKI.

Based on the spot of their appearance, KIT/PDGFRA mutations are separated in two categories. They either occur in the receptor regulatory domain (EC and JM region) or in the enzymatic domain (TK1 and TK2).

Figure 2 presents a plain overview of the structure and common mutational patterns of KIT and PDGFRA receptors:

Figure 2: Structure and mutations of KIT/PDGFRA [49]



Primary KIT mutations are detected in 75-85% of sporadic GIST cases [1,26,48]. The majority of primary KIT mutations affect the JM domain, encoded by exon 11. Mutations in exon 11 with subsequent changes in the JM region result in a lost integrity of its α-helical conformation, which has proven to be essential for receptor autoinhibiton [26,64,65]. Second most commonly, KIT mutations have been described in exon 9, encoding the fifth immunoglobulin-like loop in the receptor EC region [26,47]. Conformational alterations in the EC domain seem to result in spontaneous receptor homodimerization.

Moreover, in exon 9 mutant cells it seems that more different downstream signalling pathways are involved than in KIT-JM mutants [57,65]. Activating KIT mutations in exon 13 (encoding the TK1 region) and exon 17 (encoding the TK2 region) only occur in a low number of GIST cases, and are especially rare in exon 8 (EC, JM domain) [26,65,66].

Activating primary PDGFRA mutations occur in about 5-10% of sporadic GISTs. Most PDGFRA mutations are located in the tyrosine kinase 2 (TK2) domain, encoded by exon 18. About 90% of PDGFRA mutant GISTs seem to harbour mutations at this genomic spot. PDGFRA gene exon 18 mutations result in conformational changes of the activation loop and ligand independent receptor phosphorylation. Mutations in PDGFRA gene exon 12, which is analogically to exon 11 in the KIT gene, are infrequent. Finally, in very rare cases PDFRA mutations may be found at Exon 14. Mutations in this genomic region correlate with altered amino acid structures in the TK2 domain [1,26,47,49].

Table 3 provides an overview of the frequency of primary KIT/PDGFRA mutations in GISTs:

Table 3: Frequency of KIT and PDGFRA mutations in GISTs [1,26,49]

	Frequency
KIT mutation	**80%**
Exon 11	67%
Exon 9	10-13%
Exon 13	1-2%
Exon 17	1%
Exon 8	<1%
PDGFRA mutation	**5-8%**
Exon 18	5%
Exon 12	1%
Exon 14	<1%
Wild-type	**12-15%**

- **Mutation types in KIT and PDGFRA**

Among the most frequent types of primary KIT mutations in GISTs are, in descending order, *deletions*, *substitutions* (single nucleotide substitutions; often called point mutations), *duplications* (the term insertions should not to be used to describe duplications) and *insertions. Complex mutations*, varyingly combining the four mentioned mutation types above, are rarely seen. KIT exon 11 mutations present as a very heterogeneous group when considering the codons and nucleotides affected by the mutations. Deletions constitute the most common entity in KIT exon 11 mutant GISTs. On the other hand, exon 9 mutated GISTs in almost all cases seem to harbour the identical codon 502 and 503 duplication, as detected in our patient, which leads to alanin and tyrosin duplication at the protein level [1,49].

When regarding PDGFRA mutated GIST cells, *single nucleotide substitutions* and *deletions* make up for the most frequent types of mutation. Exon 18 mutations are in 60-80% characterized by the same single nucleotide substitution (point mutation) in codon 842. In this case aspartic acid becomes substituted by the amino acid valine (D842V) [1,49,67].

- **Secondary mutations**

Secondary mutations, per definition only occurring during or after TKI treatment, according to Lasota et al. "*have been found exclusively in the KIT-TK1 and –TK2 (exon 13, 14, 17) domains and KI (exons 15 and 16) and PDGFRA-TK2 (exon 18) domain*."[49] In the same patient different mutations can potentially occur in multiple tumour spots. Some of these mutations seem to alter the imatinib binding spot, and thereby again enable constitutive oncogenic signalling through the respective kinase. Newly acquired KIT mutations seem to be the focal feature underlying the development of secondary imatinib resistance [1,68,69].

4 CLINICAL SYMPTOMS AND DIAGNOSIS

4.1. Clinical Presentation

Overall, about two thirds of primary GISTs are diagnosed in clinically symptomatic patients. Those patients mostly present with non-specific symptoms that are due to general neoplasmatic pathophysiology, a big abdominal mass or tumour disruption [3]. Such symptoms commonly include fatigue, weight loss, nausea, early satiety, bloating and vague abdominal pain or discomfort. Moreover, tumour-induced gastrointestinal bleeding is a very common symptom displayed in more than 50% of symptomatic patients. Depending on the tumour location, GI-bleeding may clinically present as varyingly severe hematemesis, hematochezia, or melena and is typically accompanied by ensuing anaemia and its sequelae. Tumorous expansion may cause perceptibly enlarged abdominal size and a palpable mass. Beyond that, tumour expansion might lead to diagnosis because of internal displacement and functional impairment of other organ systems, such as the urinary tract. This may result in dysuria, hematuria or pollakisuria. Recently there has been a report about a gastric GIST presenting with acute pancreatitis, which was due to a tumorous obstruction of the pancreatic duct. In some cases, especially when arising from the bowel wall, GISTs may provoke diarrhea or intestinal obstruction symptoms. GISTs evolving from the upper GI tract, like the esophagus, cardia or fundus, often cause dysphagia. Big, ruptured GISTs with consequent intraabdominal bleeding sometimes are responsible for cases of acute abdomen [1,11,70,71].

About a fifth of patients, especially those with small primary lesions, do not manifest any symptoms at all and their tumours are detected incidentally at endoscopy, radiological imaging or surgery for other reasons. About one tenth of GISTs are incidentally discovered at autopsy [3].

4.2. Diagnosis, gastroenterological and radiological assessment

- **Gastroenterological and radiological assessment**

Primary gastrointestinal stromal tumours are often discovered incidentally by CT or gastroenterological endoscopy. Disease assessment after the occurrence of suspicious symptoms conventionally includes utilization of:

- Abdominal sonography
- Gastroenterological endoscopy
- Endoscopic ultrasound
- 18F-fluoro-deoxglucose PET (FDG-PET)
- CT-scanning
- MRI-scanning

First examinations are usually made with abdominal ultrasound or conventional endoscopy of the upper or lower gastrointestinal tract [1]. Endoscopic ultrasound proved to be very accurate in locating lesions in the GI wall and generate further information to help distinguishing GIST from other lesions, most notably by detecting intact GI-wall layers and a local widening of the muscularis propria layer [1,72]. CT, MRI or FDG-PET each possesses a prominent role in tumour detection as well as in appropriate tumour staging and preoperative planning [1]. Overall, CT shows the greatest anatomic detail, except for the anorectal region, where MRI offers the better detail [73,74]. FDG-PET generates significant information about baseline metabolic activity of the tumour and thus is not only useful in preoperative staging, but its usage also allows detection of metastasis and, furthermore it considerably contributes to assess therapeutic effectiveness [75]. Ideally, a combination of CT and PET should be approached, because it has shown to provide more information than each procedure alone [76].

- **Biopsy**

The extraction of tumour tissue is essential to obtain conclusive pathologic and mutational analysis of the tumour and to subsequently customize treatment strategies. Due to the fact that GISTs are fragile and strongly vascularised, they must be handled with great care by the time biopsy is taken to avoid tumour rupture and potential intraabdominal tumour dissemination, which is reported to severely lower overall survival rates [28,30].

Generally, preoperative percutaneous biopsy is not recommended for resectable lesions that are highly suspected to be GISTs. Thus, in many cases tumour tissue is examined in postoperative procedures. In cases of unclear diagnosis tumours should be probed with endoscopy techniques, such as fine needle aspiration or biopsy, if they are accessible for that procedure. For lesions that are not accessible for endoscopic biopsy, excision or open biopsy are the standard options [77,78,79]. If metastasis is evident at the time of presentation, biopsy of a metastatic spot may suffice for diagnostic aims [79].

- **Monitoring of clinical response and implementation of CHOI criteria**

Guidelines recommend follow-up CT imaging every 3 to 6 months at least for the first 5 years both for surveillance of recurrent/metastatic disease after complete tumour resection as well as for monitoring of systemic TKI therapy. Since its images are proven to be reliable indicators of TKI response, FDG-PET presents a valuable complement to CT imaging, and combined usage is recommended to examine therapy effects. Also, FDG-PET should be considered as a baseline measure before initiation of TKI treatment. A decrease or absence of uptake on PET screening is predictive of a good response to systemic GIST therapy [77,79].

Contrary to other neoplasms, assessment of clinical response with standard RECIST criteria, which defines PR (partial response) as 30% decrease in tumour size on CT evaluation, is not appropriate for GISTs. In gastrointestinal stromal tumours a decrease in size does not implicitly correspond with therapeutic response. On the other hand, stable or increased size does not necessarily indicate progression,

because after therapy tumours likely become replaced by fibrous tissue and intratumoural oedema or haemorrhage may occur. Also, RECIST criteria misjudges overall clinical benefit by ignoring stable disease as response. These shortcomings have been addressed and specific TKI-response rates for GISTs have been proposed. PR in the modified “CHOI criteria” is now based on either minimum decrease of 10% in tumour size or ≥15% decrease in density (HU) on CT. These new “CHOI criteria” prove to be accurate and sensitive for predicting TTP (time to progression) in advanced GISTs when correlated with clinical response in FDG-PET findings [80,81].

5 INDIVIDUAL RISK ASSESSMENT AND PROGNOSIS

Estimating the individual risk of GIST recurrence is crucial, not only since adjuvant imatinib treatment has proven to prolong recurrence-free survival for patients with significant risk of tumour progression. Tumour size and mitotic activity, measured in the number of mitoses per 50 high power fields (HPF), are long established factors which proved to significantly influence overall survival and risk for recurrence in patients after resection of a primary GIST [82]. Accordingly, in 2001, Fletcher et al stratified the individual risk assessment for patients with GISTs corresponding to the two latter parameters [33].

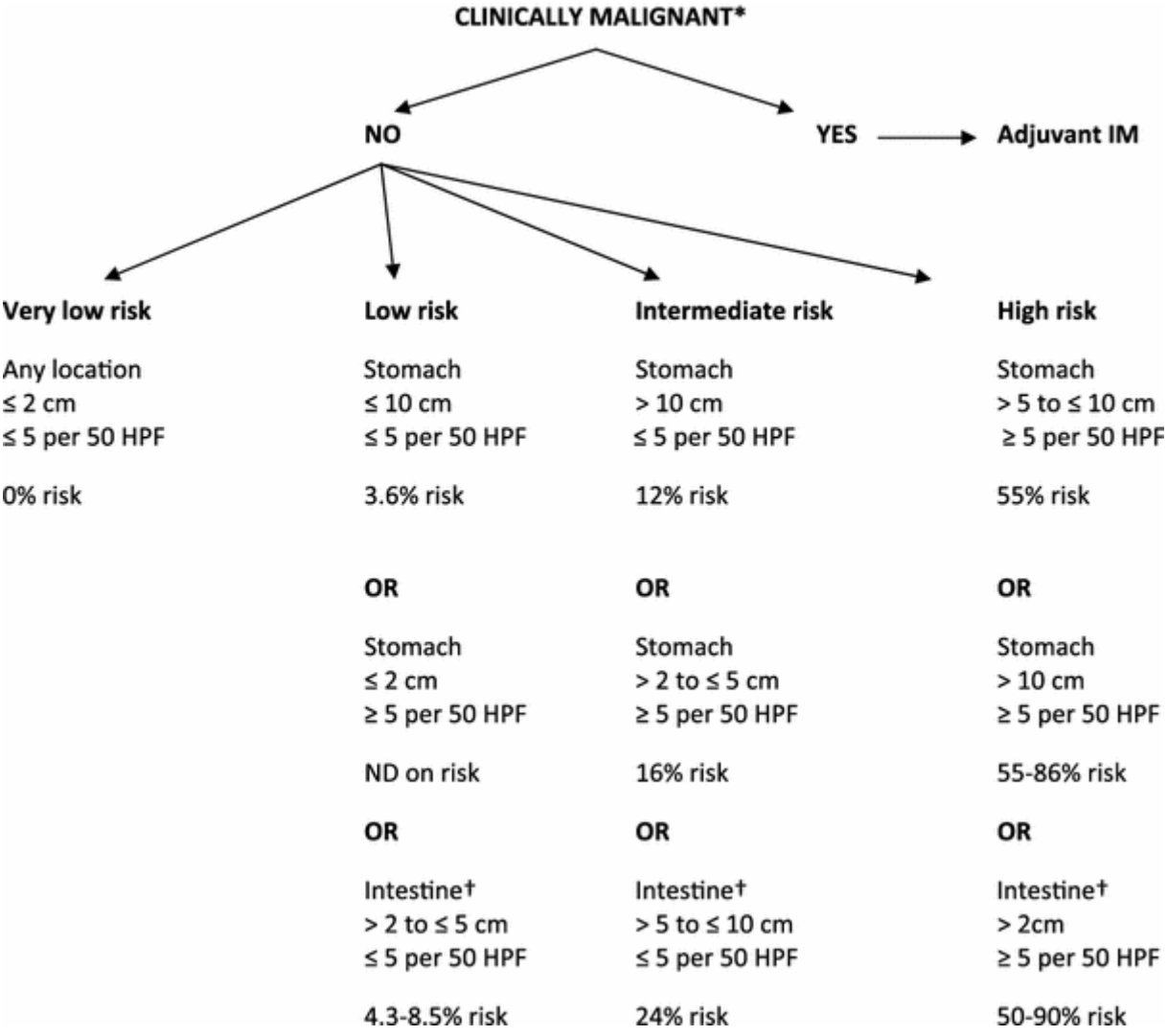
However, intense examination on long-term follow-up data of a great number of GIST cases, performed by Miettinen and Lasota of the Armed Forces Institute of Pathology, indicated a valuable role of the tumour's location for individual prognosis. In the course of these examinations, gastric GISTs constantly demonstrated a favourable course when compared to primary GISTs of intestinal location [82,83]. As a consequence, in 2006, Miettinen and Lasota classified risk categories for GIST patients according to tumour size, mitotic count and tumour location [83].

Lately, Takahashi et al furthermore modified the system for risk stratification by promoting the inclusion of 3 other factors [84]. According to that paper, intraoperative findings of tumour rupture, tumour invasion or peritoneal dissemination are independently related to tumour progression and an unfavourable clinical course [84]. Thus, by adding the latter elements it is possible to provide a more detailed individual prognosis of both, overall risk and tumour recurrence after resection of a primary GIST.

Another interesting approach towards a more comprehensive and individual risk assessment has recently been made by Ylipää et al [85]. The group advocates the integration of a genomic instability stage (GIS) as an additional prognostic factor. 72 GIST cancer samples were analyzed in the study and an obvious correlation between patients' clinical deterioration, i.e. tumour progression, and an increasing count of certain genetic copy number aberrations (CNAs) could be observed. According to the fact that particular key changes in genome levels promote different tumour-steps and interfere with biologic pathways which are crucial for cellular integrity, 4 stages of genomic instability (GIS 1-4) have been established. Chromosomal losses of distal 1p, 19 and 22q define GIS1 lesions, an additional deletion of 14q clearly constitutes GIS2 disease, and further loss of proximal 1p and 15q is characteristic for a tumour at GIS3. The loss of chromosome 10 marks the final group, GIS4. For more detail on this certain topic, see chapter 3.1.

A risk stratification that recognizes and includes all of the parameters mentioned above, besides the GIS-system, is presented in figure 3 [89]:

Figure 3: Recurrence risk stratification after surgical resection based on intraoperative findings, tumour location, size and mitotic rate. *Clinically malignant refers to intra operative findings of peritoneal disease, tumour rupture, tumour invasion. †Intestine denotes duodenum, jejunum/ileum or rectum [89].



6 TREATMENT OF GIST

The treatment paradigm for GIST requires an integration of surgery and molecular therapy. Complete surgical resection is the therapy of choice for newly detected, primary GISTs. In cases of advanced GISTs with locally extensive, metastatic or recurrent disease presentation, tyrosine kinase inhibitors (TKIs) like imatinib and sunitinib present a valuable treatment option since about 10 years. Potential benefits of implementing neoadjuvant TKI regimen, either to facilitate resection of locally advanced, inoperable tumours, or to generally enhance the long-term outcome for patients with resectable, high risk GISTs, have been investigated in the last couple of years. Similar investigations have been made regarding the usage of adjuvant TKI treatment to increase the overall outcome for patients diagnosed with high risk tumours. Both, radiation therapy and conventional systemic chemotherapy have proven to be inadequate for the treatment of GIST.

6.1. Management of resectable disease

- **Surgery**

Surgical resection remains the cornerstone of treatment for primary localised, resectable gastrointestinal stromal tumours when there is no evidence of metastasis [73,77]. Complete gross resection with preservation of an intact tumour pseudocapsule should be the aim of surgical intervention. Similarly it should be the goal to obtain tumour cell negative microscopic margins at every resection of a primary GIST. Usually the procedure can be accomplished with segmental tumour resection of the small intestine or wedge resection of the stomach, but certain cases of tumour expansion and location may indicate extensive surgical intervention such as partial or total gastrectomy. Although every effort should be made to gain negative R0 margins, greatly extended margins have not proven to be beneficial [86].

When performed by skilled surgeons, the resection can be executed laparoscopically for small or intermediate sized tumours (<5 cm). Recurrence rates after laparoscopic surgery are reported to be similar to those after performed open surgery with the benefit of a lower morbidity alongside a shorter hospital stay [87].

As mentioned at another point, GISTs tend to be fragile; hence, intraoperative tumour rupture and consequent intraabdominal tumour spread, which significantly worsens patient's prognosis, must implicitly be avoided [28,30].

ESMO and NCCN guidelines recommend all nodules ≥2 cm to be excised [77,79]. The benefit to resect GISTs smaller than 2 cm is still subject of debate. Thus, ESMO and NCCN guidelines merely suggest excision for GISTs ≤2 cm, if the nodule appears to be symptomatic (e.g. haemorrhage from mucosal erosion) or increases in size. If this is not the case, the lesions are recommended to be closely monitored [77,79]. However, management of small nodules remains controversial, as Canadian guidelines even propose GISTs ≤1 cm to be excised due to the risk of metastasis [88].

GISTs metastasize rarely to lymph nodes and thus locally radical resection with lymphadenectomy is only warranted for proven nodal involvement [89].

Approximately 85% of patients with localised primary GIST receive complete gross resection, and in those cases R0 margins are obtained in 70-95%. Nonetheless, despite complete (R0) resection, many patients have a substantial risk of tumour recurrence and five-year recurrence-free survival rates are reported to be 49% [89].

- **Neoadjuvant imatinib therapy**

In a variety of patients with primary GISTs, surgeons' estimations to achieve R0 margins may be very low and only possible with mutilating resection. For some of those cases, neoadjuvant therapy with imatinib may be considered to gain tumour cytoreduction and size reduction according to European and US guidelines [79,90]. As a consequence, resection may be rendered and morbidity and mortality of a large resection is expected to be lowered significantly. Presumably, preoperative treatment with imatinib is also beneficial in decreasing the risk of intraoperative tumour rupture and peritoneal seeding in large, fragile and hypervascular GISTs.

Moreover, the combined effect of preoperative imatinib cytoreduction and complete resection in large GISTs potentially obviates the genesis of resistant GIST clones [91]. So far, there has only been implemented one prospective multi-institutional neoadjuvant GIST trial, in which the safety and efficacy to down-stage lesions has been tested and confirmed [92].

However, if performed, it is of particular concern to carefully evaluate the response to neoadjuvant imatinib treatment early and constantly. By that potential non-responders to preoperative TKI regimen that should quickly be treated with surgery can be identified. Utilisation of fusion PET/CT has proven to be a valuable option for this monitoring process. After successfully performed resection, adjuvant imatinib should be continued in high risk GISTs [70].

- **Adjuvant imatinib therapy**

Irrespectively of surgical success, recurrence of disease affects a considerable number of patients. Five-year recurrence-free rates and five-year disease-free survival rates of 49% and 65%, respectively, have recently been reported after R0 resections [89].The results from several studies which were investigating the potential benefits of adjuvant 400 mg imatinib treatment indicate a significantly improved outcome for patients under postoperative TKI regimen (one year PFS: 97% vs. 83% in the placebo group) [93,94]. According to these trials, especially patients with high risk tumours seem to benefit from adjuvant imatinib therapy, as the greatest impact could be demonstrated in patients with larger tumours. In the same settings, safety analysis proved that imatinib was well tolerated.

Nevertheless, ESMO guidelines mention that there is no global consensus on adjuvant imatinib treatment for localized GISTs [79]. This discrepancy is mostly due to the lack of overall survival data for postoperative imatinib use. Similarly to NCCN guidelines, ESMO recommends the optional use of at least one year 400 mg adjuvant imatinib daily after complete resection of KIT-positive GISTs for patients with estimated high risk of tumour relapse [79,90].

6.2. Management of advanced disease

Advanced disease includes GISTs that are not curatively resectable and in this regard tumour recurrence should be interpreted like metastatic disease [77]. In the past options for patients with metastatic GISTs ranged from very limited to not existing, a situation that was due to the tumour's poor response rates to conventional chemotherapy or radiotherapy. As mentioned above, before the advent of imatinib the median survival after diagnosis of recurrent/metastatic GIST was limited to 18-24 months [28]. By then, mutilating surgery with R1/R2 resections and tumour debulking was at times performed in patients with advanced GISTs, but is no longer recommended. Instead, systemic therapy with imatinib should be the standard treatment for patients with metastatic GIST [79,90].

For this scenario, partial tumour resection merely presents a considerable option for patients with complicated, acute tumour related symptoms (e.g. gastrointestinal bleeding) and for symptomatic tumour disease (e.g. pain) to improve the patients overall situation in a palliative framework [1].

- **First-line treatment: Imatinib mesylate (Gleevec®)**

Imatinib is a small molecule tyrosine kinase inhibitor that shows potent activity against KIT, PDGFRA, PDGFRB, BCR-ABL, ABL, ARG and eventually CSF1R [94]. Originally tested and confirmed to inhibit constitutive activation of the BCR-ABL kinase in patients with CML, imatinib efficacy against GISTs was first investigated in 2000 in a single patient with advanced, KIT-positive GIST with an exon 11 mutation [95]. At that time, imatinib tolerability has already been well documented, based on its thoroughly scrutinized toxicity profiles in the context of CML treatment. In this patient complete metabolic response under FDG-PET and MRI observation was reported after one month and disease control could be established. On the basis of this encouraging case report and because of the lack of effective treatment strategies for patients with unresectable GIST, numerous clinical phase I, II and III studies followed soon afterwards [96-100]. The first of these trials quickly led the way to imatinib's approval for treating locally advanced, unresectable and metastatic GIST in 2002.

All of the latter trials demonstrated efficacy and tolerability of imatinib 400 and 800 mg/day in the first-line setting. Initial results from the pivotal B2222 trial [97] showed a partial response rate of 53.7% with an additional stable disease rate of 27.9%, combining for a disease control rate of 82% in patients with advanced GISTs. Dosage of 600 mg/day did not display statistically significant advantage to 400 mg/day. Long-term follow up indicated an extended median survival time of 57 months under imatinib regimen. The median time to tumour progression (TTP) was reported to be 24 months and the median duration of response was 29 months [98].

The optimal dosage for treating advanced GIST has been subject of debate. With similar response rates between 400 and 600 mg/day and a reported occurrence of severe adverse events under 1000 mg/day regimen [96], the question still lingered whether to prefer standard, 400 or high dose, 800 mg/day. The S0033 and EORTC 62005 studies initially demonstrated longer progression-free survival (PFS) for patients receiving 400 mg twice per day, with a more significant difference in the EORTC study. Nonetheless, on longer follow-up (median 40 months) PFS for both doses occurred to be similar and no differences in overall survival could be rendered in either trial. The latter findings were congruent with the results of the MetaGIST [101], a large, prospectively planned meta-analysis that scrutinized the imatinib-dose influence on outcome certain patient subgroups. Overall, on a median follow up of 45 months 800 mg/day imatinib did show a small, but meaningful benefit in PFS when compared to 400 mg/day, but overall survival rates were similar in both treatment arms.

Many reports document the affect that the primary mutational status of the KIT or PDGFRA has on response to imatinib treatment [65,101]. Patients with mutations in KIT exon 11 have reportedly higher partial response rates (85%-90%) compared to patients with a mutation in exon 9 (approximately 50%). Furthermore, median overall survival in this context occurs to be longer for patients with exon 11 mutations than for those with exon 9 (63 months vs. 44 months, respectively) [98]. About a third of patients with acquired PDGFRA mutations seem to benefit from imatinib treatment in advanced GIST circumstances. However, the majority of PDGFRA mutations (the PDGFRA exon 18 D842V mutation) and tumours with no detectable KIT mutation seem to exhibit poor or no response to imatinib [65].

NCCN and ESMO guidelines recommend to start treatment of advanced GISTs with an initial dose of 400 mg daily [79,90]. The latter regimen is not indicated for patients with exon 9 mutations. A starting dose of 800 mg/day is instead suggested for this subgroup. Studies revealed that starting with high dose imatinib in patients harbouring primary exon 9 mutations resulted in substantial benefits in terms of PFS compared to 400 mg/day [101]. Dose escalation to imatinib 800 mg/day is recommended for patients with evidence of tumour progression on 400 mg/day. This is one aspect that highlights the clinical value of determining the primary mutational status in GISTs.

A trial implemented in France pointed out a significantly higher risk of rapid tumour progression for patients who interrupted imatinib regimen averagely 3 months after discontinuation [102]. Therefore, it is recommended that patients who benefit from imatinib and sustain disease control, either by experiencing partial response or stable disease, should remain on treatment unless drug resistance or intolerable toxicity occurs [79,102].

The correlation between constant plasma imatinib levels and a favourable clinical outcome is reported to be significant [103]. In this paper it could be demonstrated that patients of the B2222 study that showed imatinib levels above 1100 ng/ml benefited from substantially longer time to progression when compared to patients with lower plasma imatinib levels (median TTP 30.6 months in contrast to 11.3 months). Thus, in case of disease progression during imatinib, quantification of plasma imatinib levels may indicate dose escalation and can thereby result in improved clinical response.

Adverse events

Generally, imatinib shows to have a superior toxicity profile when compared to traditional chemotherapy. Adverse events were mild to moderate in all major clinical studies. According to a study that reviewed common toxicities in a big population of patients under imatinib regimen [104], anaemia (94%) oedema (80%), fatigue (75%) nausea (56%), diarrhoea (54%), neutropenia (42%) and skin rash (37%) were among the most common adverse events, regardless of the severity of the occurring symptoms (consistent with NCI-CTC grades 1-4).

Grade 3 and 4 anaemia was noticed in 13.4% of patients and 7.1% of patients experienced neutropenia of the same severity. Oedema, which in the majority of cases develops periorbital, was grade 3 or 4 in only 6.4% of patients. According to another study, myalgias grade 1 or 2 was noticed as an additional symptom that occurred in 40% of patients with no reported case of myalgia grade 3 or 4 [97]. The occurrence of toxic effects typically intensified with higher imatinib dosage. Furthermore noticeable, the severity of adverse events in many patients seemed to decrease after the first months of imatinib treatment [97]. Ascertaining a patient's adherence to treatment is crucial to maintain constant imatinib plasma levels. In this context, a comprehensive patient education from the start of treatment will contribute to increase a patient's compliance and may help to lower the number of up to 30% of patients who stop taking their pills, as it has been reported in one paper [105].

Tumour progression under imatinib treatment

The effect of imatinib on GIST cells seems to be cytostatic rather than cytocidal [49,65,99]. With that in mind, it must be noted that although imatinib has considerably improved life quality and survival of patients with unresectable GISTs, the majority of patients is not healed and will experience tumour progression at some point [65,99]. Two patterns of progression during imatinib treatment have to be distinguished. Early tumour progression under imatinib occurs during the first 3 to 6 months of treatment, while late progression becomes apparent after the first 6 months of treatment in patients who showed initial response to imatinib [106]. Under the selective pressure of continuous imatinib therapy, tumour cells seem to be additionally altered in critical molecular structures and acquire late progression mainly by this way [106].

According to certain reports, 12-20% of the participating patients experience disease progression on imatinib during the first 3 to 6 months of treatment [97,106]. Primary imatinib resistance, conditioned by the initial mutational status of the respective oncogenic kinase, is the cause for most cases of early progression [29,49]. Secondary KIT/PDGFRA mutations are only detected in 10% of patients with early progression [52]. In patients with late progression on the other hand, secondary KIT or PDGFRA mutations, that generate hampered drug binding at the ATP-binding pocket, seem to be the cause for imatinib resistance in 50-70% [68].

Other mechanisms of acquired, secondary imatinib resistance and late progression include genomic amplification of KIT and consecutive kinase overexpression, activation of alternate RTKs, or functional resistance after novel oncogenic KIT/PDGFRA activation outside the imatinib-sensitive spectrum [29]. A further, KIT/PDGFRA-independent mechanism leading to late progression is altered drug availability, which can be due to increased imatinib clearance, elevated imatinib binding to α_1-acid glycoprotein or disadvantageously altered expression of drug influx/efflux transporters [107-109].

- **Second-line treatment of advanced GIST: Sunitinib (Sutent®)**

Sunitinib is another oral multitargeted kinase inhibitor that prevents signal transduction functions of receptor tyrosine kinases KIT, PDGFRA/B, FLT3 (Fms-like tyrosine kinase-3 receptors) and VEGFR1/2/3 [110]. Due to the additional inhibition of all major VEGFRs, sunitinib successfully inhibits tumour-related angiogenesis, an interesting and relevant feature that imatinib does not demonstrate. Since a few years, sunitinib has become the standard treatment for patients with metastatic renal cell cancer [111]. After disease progression during high dose imatinib therapy or because patients are intolerant to imatinib, switching to sunitinib as a second-line therapy may achieve clinical benefit [112]. For the latter context, sunitinib has been approved by the FDA for GIST treatment in 2006 [113]. 312 patients with imatinib-resistant or imatinib intolerant GIST enrolled in an early phase III trial and therein it could be demonstrated that the median TTP was 27.3 weeks for patients receiving sunitinib 50 mg/day, compared to a TTP of 6.4 weeks for patients receiving placebo [112]. The median PFS was reported to be 24.1 weeks for patients under sunitinib treatment compared to 6.0 weeks for patients getting placebo. Another study examined the long-term survival among the population of the latter study and demonstrated an overall survival of 73.9 weeks vs. 35.7 weeks for sunitinib and placebo, respectively [114].

Sunitinib is recommended for the second-line treatment of advanced GISTs by ESMO and NCCN guidelines if the tumour progresses on imatinib or if intolerance to imatinib occurs [79,90]. In the phase III trial sunitinib was administered at an approved schedule of 50 mg/day for four weeks followed by a two-week rest. An alternative pattern of sunitinib administration is a continuous dosing of 37.5 mg/day. The continuous schedule has proven to have some advantages over the intermittent one, as it may avoid resurgence of the tumour's metabolic activity and progression in the off-treatment interval [115]. Furthermore, the 37.5 mg/day administration schedule was reported to be better tolerated while being equally effective.

Sunitinib was in general terms reported to be well tolerated in the phase III study [112], as the most common drug-induced adverse events overall turned out to be fatigue, diarrhea, skin discoloration and nausea. In the same setting, noteworthy grade 3-4 adverse events were fatigue (10%), hypertension (7%), hand-foot syndrome (6%), diarrhea (5%) and asthenia (5%).

Just as with response to imatinib, the effectiveness of sunitinib seems to be correlated to the mutational status of the tumour. However, contrary to imatinib, patients with KIT exon 9 and KIT/PDGFRA wild-type tumours display significantly better clinical responses, than those patients harbouring exon 11 mutations. The clinical benefit manifested in higher PR rates, longer PFS and longer median overall survival when comparing KIT exon 9 and KIT/PDGFRA wild-type with KIT exon 11 mutations when scrutinized in a phase I/II trial [116]. Moreover, secondary KIT mutations in exon 13 and exon 14 showed to be sensitive to sunitinib, while secondary mutations in KIT exons 17 and 18 did not respond to sunitinib treatment [116].

6.3. Novel TKIs and further options for the treatment of advanced GIST

It is a likely scenario for patients under both imatinib and sunitinib treatment to develop resistance and experience tumour progression to either compound at some stage. Like under imatinib treatment, the selective pressure of sunitinib regimen represents a probable pathway for the genesis of new and resistant kinase genotypes. After failure of sunitinib treatment NCCN and ESMO guidelines recommend to consider an enrolment in a clinical trial, either of a new therapy or combination therapy [79,90]. Several of those novel therapeutic options currently under investigation are going to be introduced in the following section.

- **Nilotinib (AMN107; Tasigna®)**

Nilotinib is a second-generation TKI that inhibits BCR-ABL, KIT, and PDGFRA [117]. This agent was developed to provide a therapeutic option for patients with imatinib resistant CML and in the meantime it has also been examined for the use in patients with GIST and has proved to gain higher intracellular availability than imatinib [118]. Nilotinib was tested in a phase I study as a single treatment option or combined with imatinib and showed encouraging clinical results in patients with advanced GISTs who had progressed on imatinib [117]. Nilotinib was generally well tolerated. Schlemmer et al. recently described an impressive case of a patient with advanced GIST, who highly benefited from nilotinib treatment after progression on imatinib [119].

Other clinical trials evaluating nilotinib in GIST are currently under way. One trial is examining the efficacy and safety of nilotinib in a phase II study in patients with GIST, who experienced progression on or are intolerant to both imatinib and sunitinib [120]. Results are awaited in 2012. The second trial, which is still recruiting participants, is a phase III, open-label study that compares the efficacy and safety of nilotinib 800 mg/day with imatinib 800 mg/day for the first-line treatment of patients with advanced GISTs [121]. Results are not awaited before the year 2021.

- **Sorafenib (Nexavar®)**

Sorafenib is another oral multikinase inhibitor. Apart from targeting KIT, FLT3, PDGFRs and VEGFRs it additionally inhibits Raf kinase, which activates the downstream Ras-Raf-MEK-ERK signaling pathway [122]. Sorafenib is approved for the treatment of advanced renal cell carcinoma and advanced hepatocellular carcinoma. Safety and Pharmacokinetic parameters of sorafenib were investigated in a phase I study [123]. Therein, 800 mg/day was reported to be the recommended dosage and the most notable severe (grade 3) adverse events were fatigue, diarrhea and hand-foot syndrome.

A phase II trial of patients with advanced GIST who progressed on imatinib and sunitinib examined sorafenib efficacy in 23 patients. 18 patients achieved disease control (PR or SD) and PFS turned out to be 22.2 weeks [124].

- **Dasatinib (Sprycel®)**

Dasatinib is an oral ATP-competitive inhibitor with potent activity against ABL, SRC, KIT, PDGFRs and several other tyrosine kinases [125]. In relation to GIST, it could be proven that dasatinib inhibits the kinase activity of both wild-type and mutant KIT isoforms especially of juxtamembrane domain (exon 11) mutant KIT. Impressive clinical results could be rendered in Philadelphia chromosome-positive leukemia patients who were resistant to or who could not tolerate imatinib [126]. A phase II trial that examines the efficacy and safety of dasatinib as a first-line treatment for patients with advanced GIST is currently in progress [127]. Patients are administered dasatinib in a continuous dosing twice a day and the trial is suggested to be completed in 2013.

- **Masitinib (AB1010)**

Masitinib is an orally active TKI with high selectivity against KIT, PDGFRA/B and fibroblast growth factor receptor 3 (FGFR3). Masitinib showed to have stronger in vitro activity and selectivity than imatinib against KIT wild-type and KIT exon 11 mutants and has been tested in a phase I dose-escalation study in patients with advanced GISTs [128]. Masitinib administered at 12 mg/kg daily turned out to be favourable and safe for long-term treatment and the tumour control rate was reported to be encouraging in this trial. A phase II trial followed soon afterwards and examined the efficacy of masitinib at 7.5 mg/kg/d as a first-line treatment of advanced GISTs in imatinib-naive patients [129]. Results showed a disease control rate of 97%, a 2-year PFS rate of 60%, a median PFS of 41 months and a stable OS rate at 2 and 3 years of 90%. Side effects were well tolerated with most frequent grade 3-4 adverse events being rash (10%) and neutropenia (7%). Overall, it could be demonstrated that Masitinib shows comparable results to imatinib in terms of safety and response. One further randomized phase III trial is currently in progress. It compares the efficacy and safety of a first-line treatment of masitinib 7.5 mg/kg/day with imatinib at 400 or 600 mg daily in patients with advanced GISTs [130].

- **Vatalanib (PTK787)**

Vatalanib is a new class III kinase inhibitor that has combined activity against all VEGFRs, KIT, PDGFRB, and c-fms [131]. Vatalanib can be administered orally and due to the agent's potency to inhibit all VEGFRs, it exhibits significant inhibition of tumour angiogenesis. A phase I study indicated an advisable dosage scale between once and twice 750 mg/day [132]. Nausea, vomiting, fatigue and dizziness were the most frequent side effects in that trial. A phase II trial that was implemented in Finland in patients with imatinib-resistant metastatic GISTs revealed clinical benefit in 67% of patients, as 13 % had PR and 53% experienced stable disease [133]. 15 patients participated in this study and vatalanib was given at a dosage of 1250 mg orally once daily. Median time to progression (TTP) was 8.5 months and vatalanib was generally well tolerated by the patients.

- **Motesanib (AMG706)**

Motesanib is a selective oral inhibitor of VEGFR1/2/3, PDGFRs, KIT wild-type and a number of clinically relevant mutated KIT isoforms [134]. Efficacy and safety of motesanib have been tested in a phase II trial in patients with advanced GISTs who were resistant to imatinib [135]. Therein motesanib was administered at a continuous dosage of 125 mg once daily. 102 patients took part at the trial; 59% of them achieved stable disease, TTP was 17 weeks and the median PFS turned out to be 16 weeks. Despite the fact that in this study motesanib was acceptably tolerated and displayed discreet tumor control, it was concluded that further development of motesanib in GIST is not supported due to its insufficient overall efficacy [135].

- **Everolimus (RAD001)**

Everolimus is an inhibitor of the protein kinase mTOR that shows good oral bioavailability. The protein kinase mTOR is an important downstream mediator of the PI3K-AKT pathway. Pharmacologic restriction of this intracellular component is suggested to be effective since the significance of constitutively activated PI3 kinase/FRAP1 (mTOR) cascades in KIT-mediated GIST signalling has been detected [56]. A phase I-II study investigated the therapeutic potency of everolimus in a combined therapy with imatinib [136]. After determining the optimal dose for this combination therapy in phase I, the participating patients were stratified into two groups: patients with progression on imatinib only (stratum 1); and patients with progression after imatinib and sunitinib or another TKI (stratum 2). Everolimus and imatinib were administered at a daily regimen of 2.5 mg and 600 mg, respectively. 17.4% of stratum 1 patients and 37.1% of stratum 2 patients were progression free at a primary end point of 4-months. 36% achieved SD and 54% had PD in group 1, while 2% had PR, 43% SD, and 32% PD in group 2. The treatment was generally well tolerated and it was concluded that the combined use of imatinib and everolimus needs further investigation. Another multicenter, two-stage, phase II trial currently examines the efficacy and safety of everolimus in combination with imatinib mesylate in Germany [137].

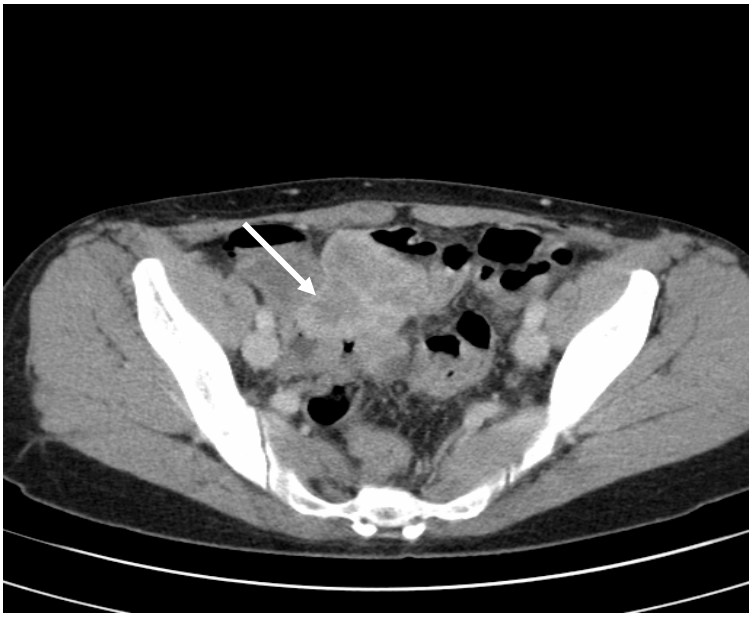
- **Retaspimycin (IPI-504)**

Retaspimycin is an oral inhibitor of the heat-shock protein 90 (HSP-90). HSP-90 is a molecular chaperone that protects proteins, i.a. KIT from protease-mediated degradation [138]. Early research on this new agent was promising and proved evidence of activity in GIST cells [139]. A phase I study with 36 patients participating revealed 1 case of PR, while 24 patients achieved SD. Median TTP was 12 weeks in the same trial [140].

7 CASE REPORT

A 47-year old woman presented with signs of a post-surgical haematoma, one day after a laparoscopically-assisted vaginal hysterectomy (LAVH) in 09/2005. CT imaging of the abdominal and pelvic region revealed a solid-cystic mass 6.5 centimetres in diameter, which was associated to the small bowel. Based on CT scan findings, the expansion was suspected to be a GIST and presumptions were confirmed by diagnostic laparoscopy, which was performed on the same day. There was no evidence of hepatic or pulmonal tumour metastasis at that point. Subsequently and also on the same day, a median laparotomy was conducted with tumour excision and partial resection of the jejunum.

Figure 4: CT scan showing the primary tumour of the jejunum in 09/2010 before surgical intervention (with arrow).



Biopsy specimens were taken and GIST diagnosis was verified after histopathological examination. Immunohistochemically, tumour cells stained highly positive for KIT (CD117) throughout the whole tumour area. Additionally, focal parts of the investigated tumour tissue stained positive for CD34. Histological inspection demonstrated a GIST composed of mixed-cell type with 20 mitoses/10 high power fields (HPF) in epitheloid tumour portions.

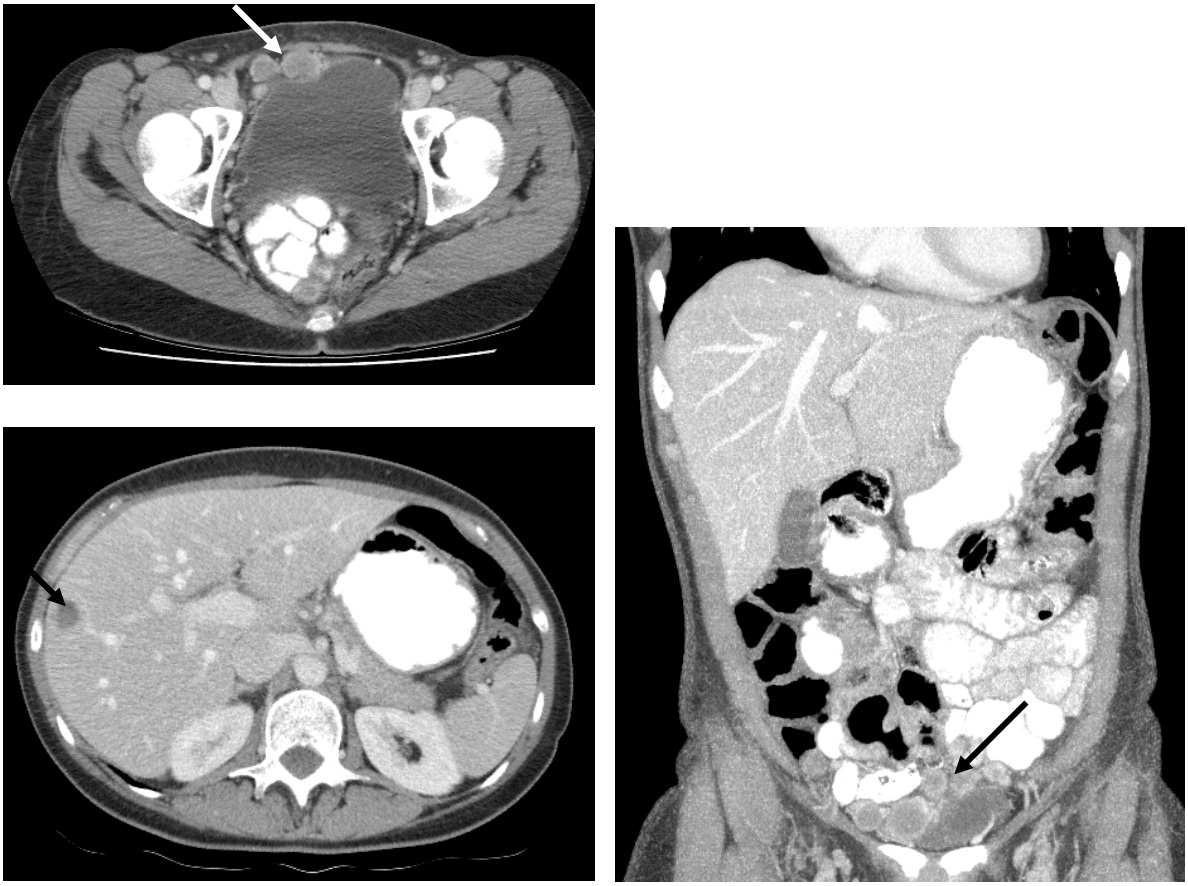
The mucosal layer showed regions of tumour infiltration and tumour-associated ulceration. Pathologists further confirmed R0 resection-margins, as well as tumour-cell free regional lymph nodes. Due to the accomplished tumour evaluation and based on established GIST prognostic criteria, a mainly malignant course was assumed.

At the first outpatient follow up presentation at the Division of Oncology one month later, the patient was free of symptoms, besides postoperative scar pain. All clinically relevant test results were within the normal range. Consequently, follow-up examinations with abdominal and pelvic CT scans, accompanied by examinations at the Division of Oncology, were conducted every three months. Additionally, a PET-FDG scan and lung X-rays were performed one year after tumour resection. No evidence of disease could be demonstrated until 20 months after tumour excision in 05/2007, when hepatic and peritoneal metastasis became evident in a CT scan. At the same time, the patient reported intermittent intraabdominal pain, recurring diarrhea, weight loss and fatigue being present for a few weeks.

Due to the tumour's progression imatinib treatment was initiated at 400 mg/day in 05/2007. Mutational analysis, which was conducted by then, proved that the tumour harboured a KIT exon 9 mutation with in-frame GCCTAT insertion/duplication (Ala502 and Tyr503). After 3 months of imatinib treatment (400 mg/day), stable disease was achieved, but only one month later in 09/2007 progression of hepatic and peritoneal lesions was demonstrated by CT scans.

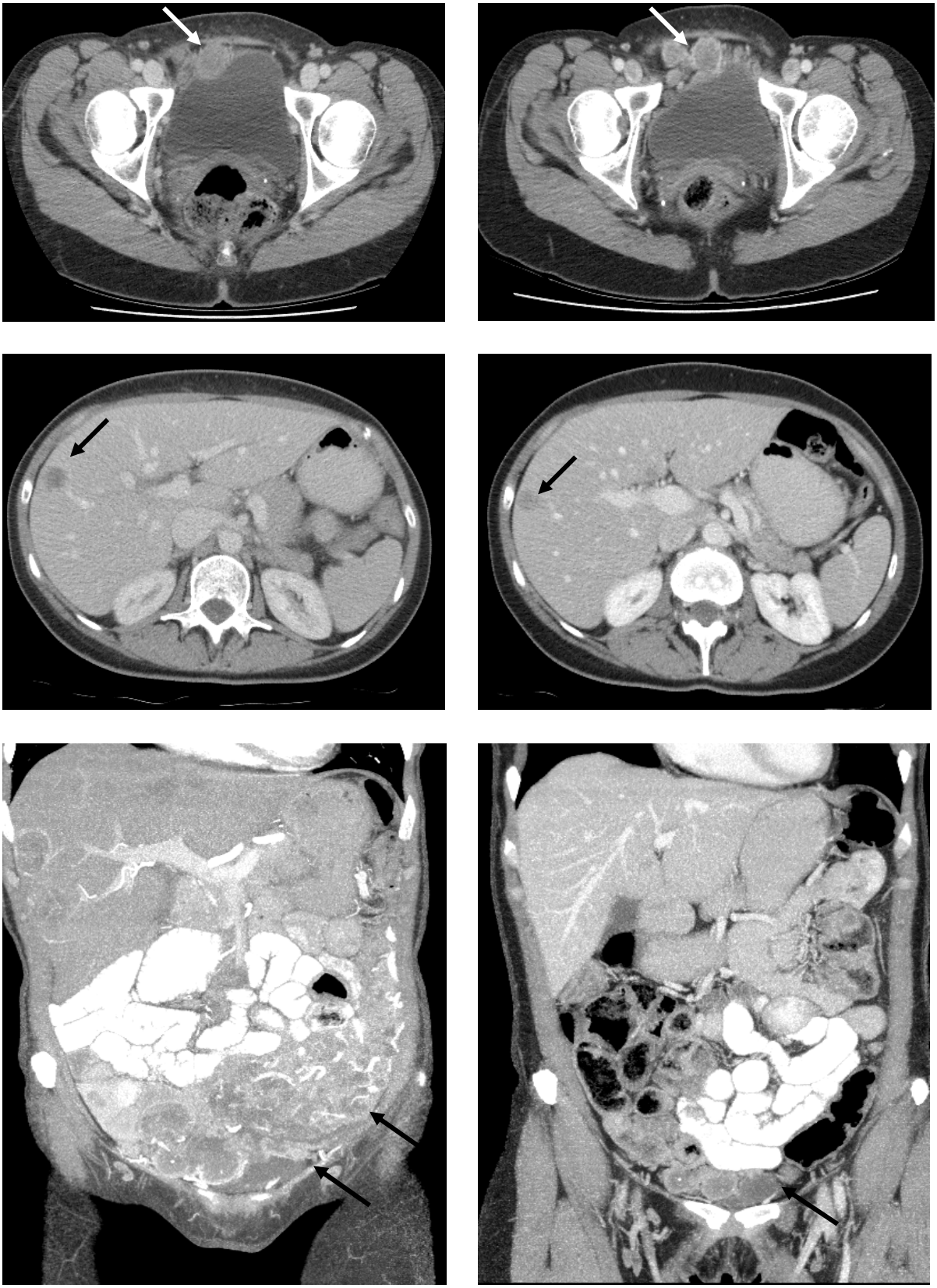
Consequently, imatinib 800 mg/day was initiated by a stepwise dose escalation in 09/2007, but after one month of high-dose imatinib treatment, the patient experienced adverse events. Grade 2 lid-oedema and grade 1 vertigo could each be treated symptomatically, but Grade 2 recurrent abdominal pain and Grade 2 diarrhoea were only manageable by dose reduction to imatinib 700 mg/day. However, after a short period of stable disease in response to imatinib 700 mg/day regimen until 11/2007, CT scans indicated disease progression again in 03/2008.

<u>Figure 5</u>: CT scans showing hepatic and peritoneal metastases in 11/2007 after 6 months of imatinib treatment (with arrows).



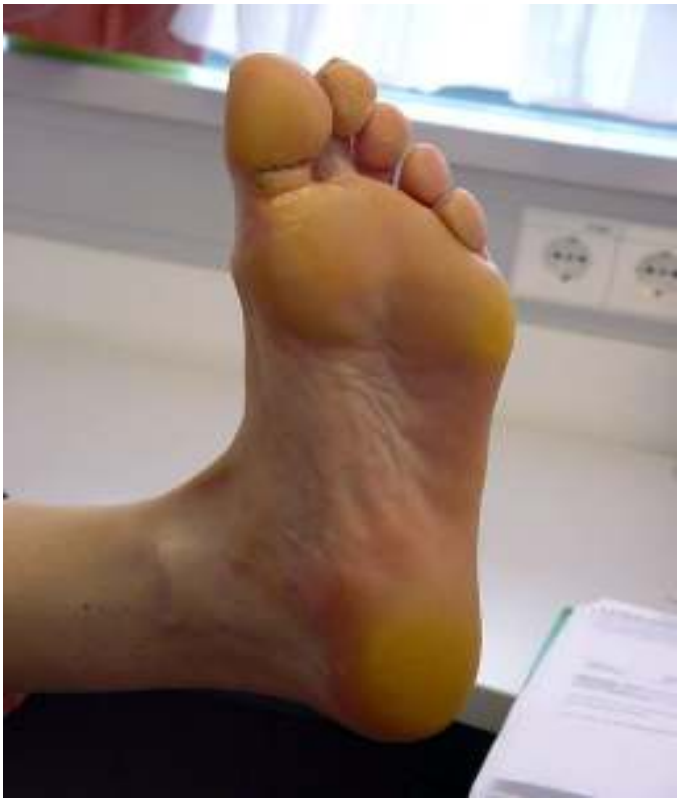
As a consequence, second-line treatment with sunitinib 37.5 mg/day at a continuous administration schedule was started in 03/2008. After 3 months of sunitinib regimen minor response, detected by CT imaging, was achieved. Thereafter, stable disease could be observed for a period of 13 months during sunitinib 37.5 mg/day treatment until disease progression in 08/2009.

Figure 6: CT scans of hepatic and peritoneal metastases at the end of imatinib regimen (left side; 03/2008) and after 3 months of sunitinib treatment (right side; 07/2008). Please note the significant regression of peritoneal carcinomatosis and reduction of size of liver metastasis (arrows).



Observed adverse events due to sunitinib medication were mild to moderate. Hyperkeratosis of the plantar side of both feet and of the palmar side of both hands had developed during sunitinib treatment (See figure 7).

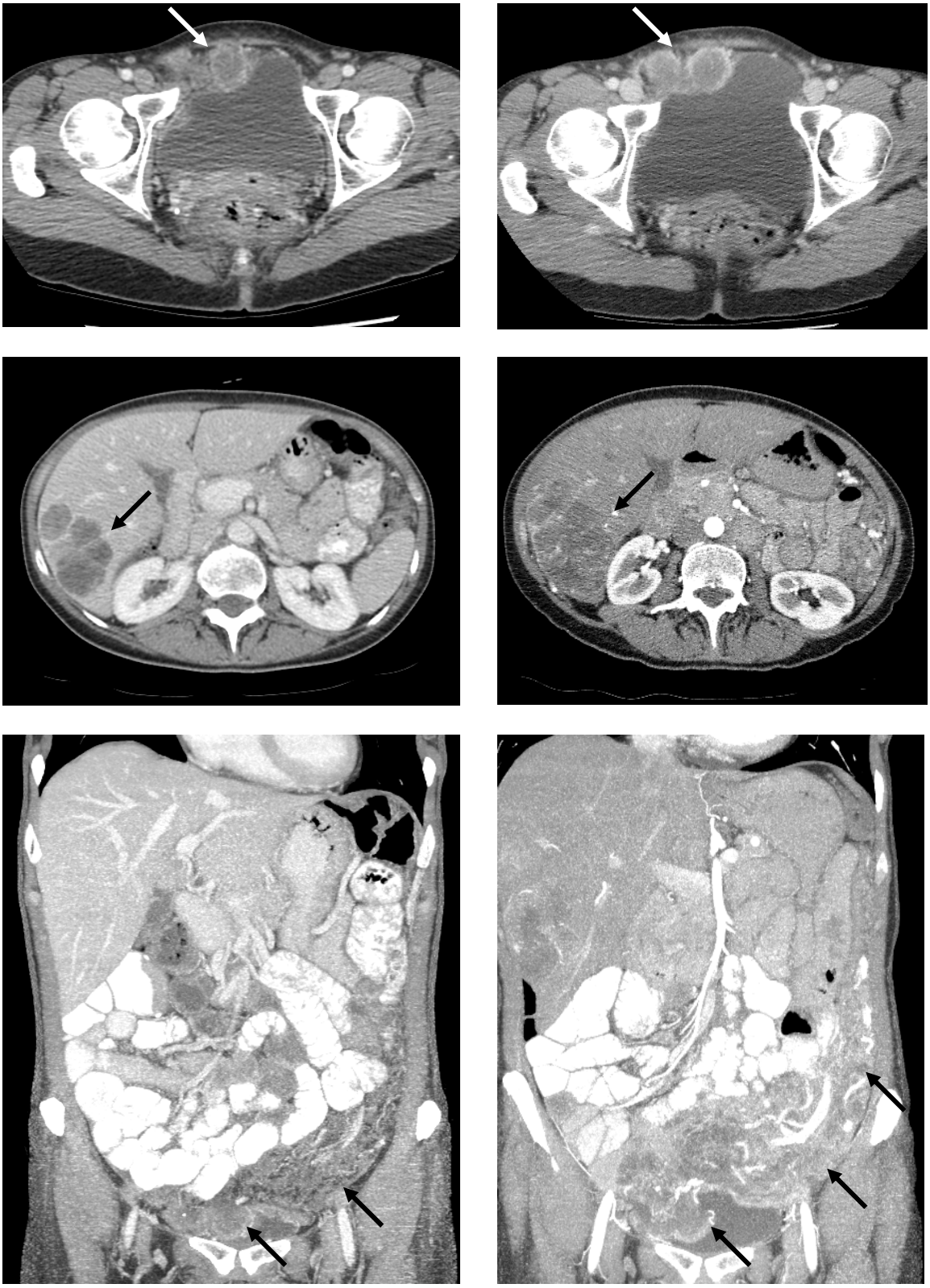
Figure 7: Illustration of hyperkeratosis of the plantar side of the left foot due to sunitinib treatment (10/2008).



Furthermore, the patient experienced grade 1 fatigue, which did not require any symptomatic treatment, and grade 2 depigmentation of the head hair and eyelashes. Beyond that, grade 1 diarrhoea, which was further verified to be due to lactose- and fructose-intolerance, became apparent. Grade 1 weight loss was managed with a high caloric food diet.

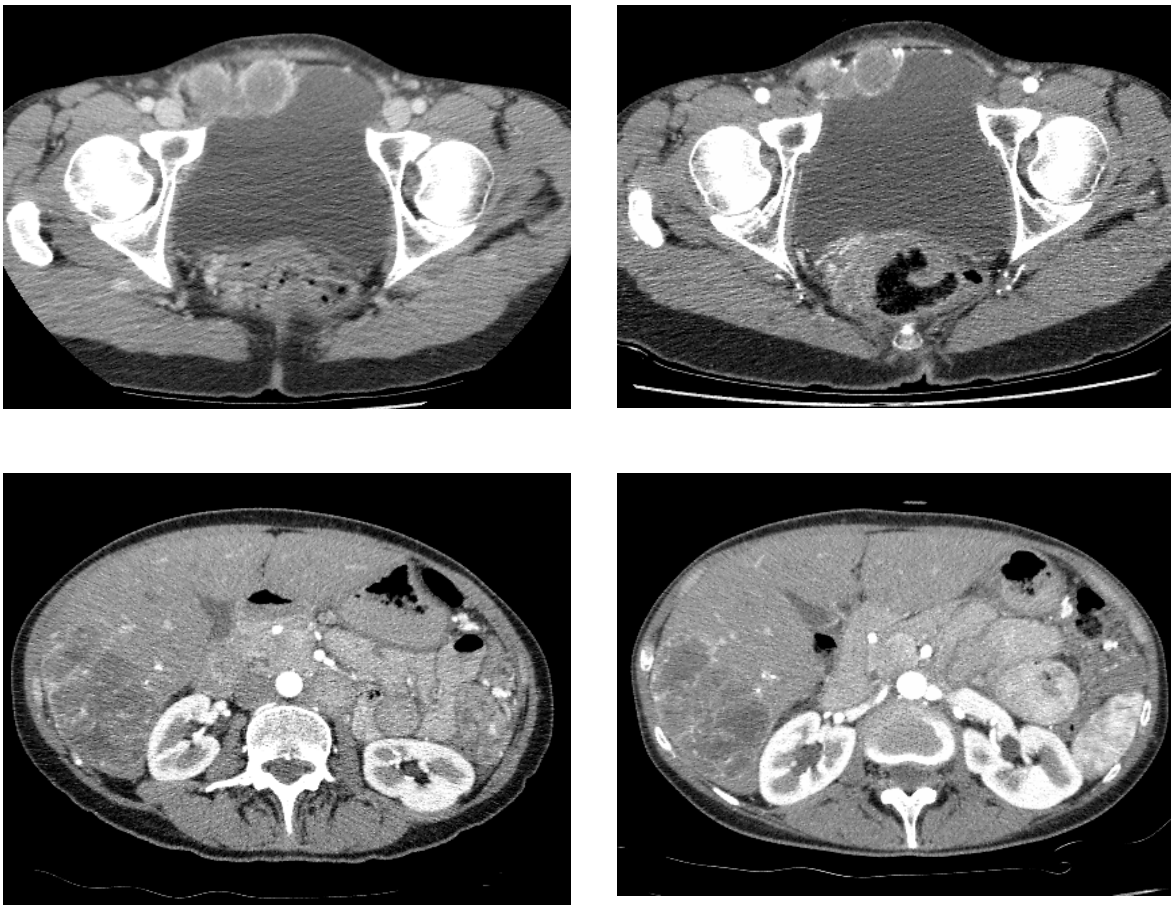
After disease progression on sunitinib, third-line treatment with nilotinib 800 mg/day was initiated in 08/2009. Unfortunately, no clinical response could be observed while nilotinib was administered. Within 2 months rapid tumour progression was detected by abdominal and pelvic CT scans in 10/2009. The only notable adverse event occurring under nilotinib 800 mg/day regimen was grade 1 headache, symptomatically treated with intermittent non-steroidal anti-inflammatory drugs (NSAIDs).

Figure 8: CT scans of hepatic and peritoneal metastases at the end of sunitinib treatment (left side; 08/2009) and after 2 months under nilotinib regimen (right side; 10/2009). Please note the progression of the lesion within the lesion in hepatic metastases and the progression of peritoneal spread (arrows).



As a result, nilotinib administration was discontinued and immediately followed by the start of sequential TKI-treatment with sorafenib 800 mg/day in 10/2009. After 2 months of sorafenib medication, radiologic investigation in accordance with CHOI criteria (decrease of HU in tumorous lesions) proved a partial response of the hepatic and peritoneal metastatic tumour lesions. In addition, the patient improved clinically, documented by improvement of abdominal pain and weight gain of 2 kg of body weight.

Figure 9: CT scans of hepatic and peritoneal metastases at the end of nilotinib treatment (left side; 10/2009) and 2 months after sorafenib initiation (right side; 12/2009). Please note the regression and change in density of the hepatic and peritoneal metastasis during sorafenib treatment suggesting good response (arrows).



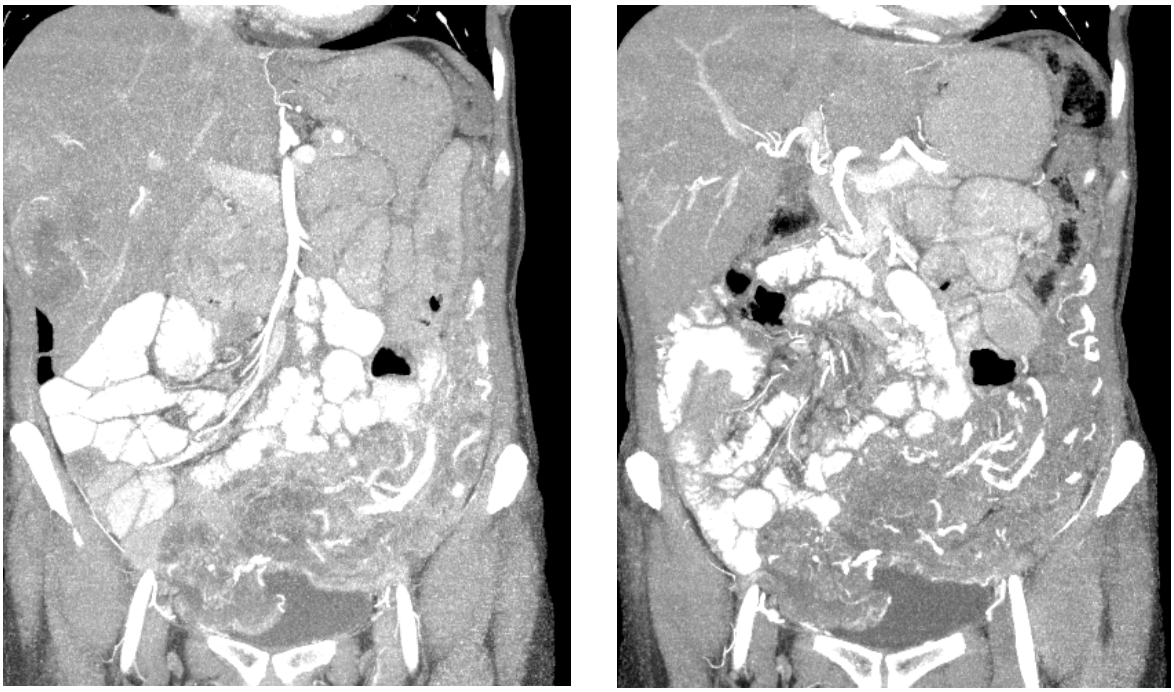


Figure 9: CT scans of hepatic and peritoneal metastases at the end of nilotinib treatment (left side; 10/2009) and 2 months after sorafenib initiation (right side; 12/2009). Please note the regression and change in density of the hepatic and peritoneal metastasis during sorafenib treatment suggesting good response (arrows).

During sorafenib treatment, the patient reported burning pain of hands and feet (hand–foot syndrome grade 2) and the daily dosage was reduced to 600mg. The initially documented partial response due to sorafenib regimen (2 months) was followed by a period of stable disease, which lasted for another 4 months, resulting in a clinical benefit of 6 months until 04/2010.

Sorafenib treatment was continued, but slow progression of hepatic metastasis and peritoneal lesions was observed after three more months of therapy in 07/2010. Radiologic restaging revealed metastatic lesions that had increased in size and indicated tumour progression. In Addition, newly developed pulmonary lesions were reported in CT scans of the thorax. No radiologic signs of intestinal obstruction or perforation were detected. Clinically, the patient appeared cachectic and experienced intermittent abdominal pain.

Because progressive disease became evident again, fifth-line treatment with pazopanib 800 mg/day was started in 07/2010 since this new drug has become available due to the approval as first and second-line treatment of renal cell cancer. Pazopanib is a multi-tyrosine kinase inhibitor with considerable activity in blocking c-KIT receptor. Initial improvement of pain and stop of further weight loss in response to pazopanib and stabilisation of disease could be observed.

Unfortunately, the patient again experienced nausea Grade 2, which required the administration of metoclopramide. Furthermore, the patient repeatedly suffered from hypoglycaemia since the start of pazopanib regimen. The patient was admitted to the emergency department due to severe hypoglycaemic episodes as low as 29 mg/dL serum glucose levels accompanied with syncopes during this period. Laboratory analysis conducted at that time showed maximally suppressed levels of insulin and C-peptide, together with significantly elevated levels of glucagon. Levels of cortisone and ACTH were inside the normal range.

In order to maintain physiological serum-glucose levels the patient was recommended to consistently ensure glucose-rich alimentation at 4-houerly intervals even during the night to prevent severe hypoglycaemia.

Table 4: Exploration of parameters associated with hypoglycaemia during pazopanib treatment

Basal cortisol	87.0	*ng/ml*	(43.0 – 220.0)
Insulin	0.0	*µU/ml*	(2.0 – 25.0)
C-peptide	0.00	*ng/ml*	(0.78 – 1.89)
Basal ACTH	17.2	*pg/ml*	(10.0 – 51.0)
Glucagon	372.1	*pg/ml*	(25.0 – 250.0)

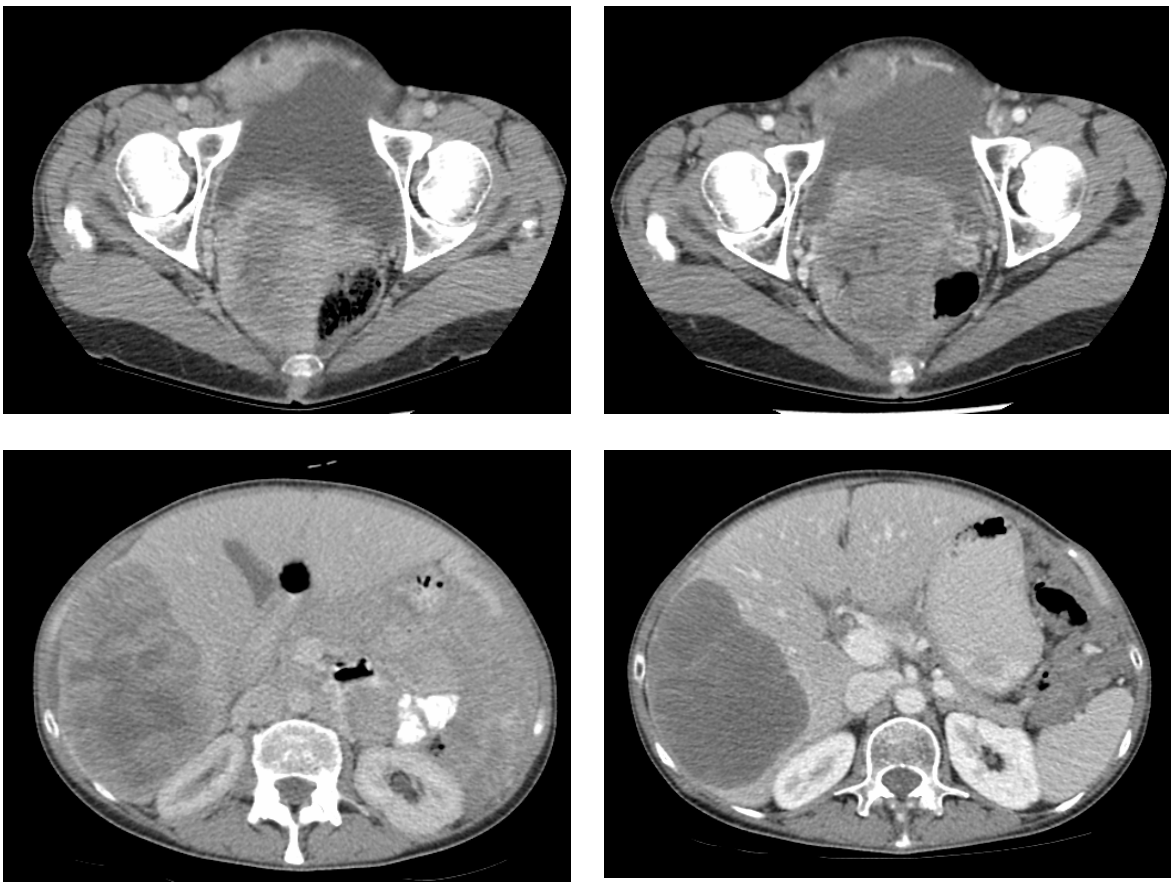
Table 5: Tumour related serum-glucose levels during pazopanib treatment. / *Patient was admitted to emergency room due to severe hypoglycaemia.

dd/mm/yy	02/08/10	11/08/10	15/10/10	08/11/10
Serum-glucose level (*mg/dl*)	41	67	24 *	29 *

To test whether hypoglycaemia was associated with pazopanib the treatment was stopped for 14 days with no significant improvement of hypoglycaemia ruling out the hypothesis that hypoglycaemia might have been a side effect of pazopanib. Therefore, pazopanib was reinitiated in a reduced dose of 600 mg/day since the patient experienced deterioration of performance status without treatment.

Despite experiencing the mentioned side effects, radiologic assessment according to CHOI criteria demonstrated stable disease after 2 and 5 months of treatment in 09/2010 and 12/2010.

Figure 10: CT scans of hepatic and peritoneal metastases at the end of sorafenib regimen (left side; 06/2010) and 2 months after pazopanib initiation (right side; 08/2010). Please note the change in density of the hepatic metastasis during pazopanib treatment suggesting good response according to CHOI criteria.



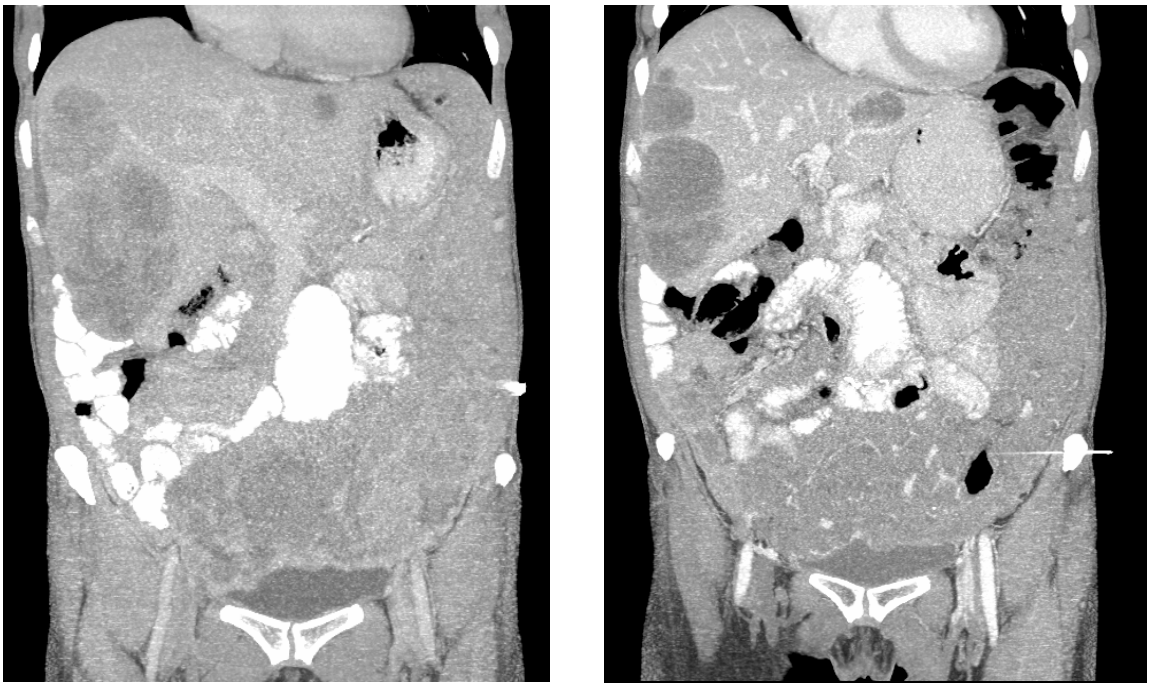


Figure 10: CT scans of hepatic and peritoneal metastases at the end of sorafenib regimen (left side; 06/2010) and 2 months after pazopanib initiation (right side; 08/2010). Please note the change in density of the hepatic metastasis during pazopanib treatment suggesting good response according to CHOI criteria.

Nevertheless, periods of severe hypoglycaemia with syncopic events reoccurred and the patient had to be admitted to hospital since blood glucose levels dropped below 30mg/dL and could not be improved by the stop of pazopanib treatment or continuous high-dose parenteral glucose administration suggesting massive energy depletion by the tumour. Ultimately, the patient died due to tumour-related intestinal obstruction and treatment-refractory hypoglycaemia in a peripheral hospital one month after the last outpatient visit at our clinic.

With the exception of the last two months of her life the patient continued to work as a cleaning lady throughout the whole treatment period.

8 DISCUSSION

Characteristic for this tumour entity, the detection and subsequent diagnosis of the GIST lesion in this case happened incidentally during a radiologic examination for an unrelated condition, performed to exclude haematoma after an accomplished hysterectomy [3,50].

After a neoplastic expansion was verified by laparoscopic examination, surgery was performed. With an observed maximum diameter of 6.5 cm the tumorous lesion markedly exceeded the established resection criteria, which recommends all GIST-suspect nodules ≥2 cm to be excised [77,79]. Beyond that, the lesion's demonstrable intestinal origin indicated an higher malignant potential when compared to a gastric GIST [82,83].

The use of minimally-invasive surgery was not indicated in this case. Studies merely advocate laparoscopic GIST resection for small or intermediate sized tumours which don't exceed a 5 cm diameter [87,141]. Thus, the resection was performed via median laparotomy.

As already mentioned in various parts of this paper, GISTs show a high rate of recurrence, even after excision has successfully been performed by avoiding intraoperative tumour rupture and by obtaining R0 resection margins [27,84,89,]. When considering size (6.5 cm), mitotic count (20 mitoses per 10 HPF) and location (jejunum) of this particular tumorous expansion, all existing risk stratification clearly pointed towards a GIST with a high risk of recurrence and an explicit probability of an unfavourable course of disease [33,83,89]. During the course of sequential therapy later on, mutational analysis of the tumour was conducted and a KIT exon 9 mutation (in-frame Ala502_Tyr503 duplication) could be demonstrated. Well worth mentioning, at the early age of targeted therapy of GISTs, Exon 9 mutations were commonly associated with a rather detrimental outcome when compared to GISTs harbouring the most frequent mutation in exon 11 [49,142]. Interestingly, this correlation could not be verified in subsequent studies. The higher rate of adverse clinical outcomes for KIT exon 9 mutated GISTs proved to be rather due to their overrepresentation among intestinal versus gastric tumours [11,49,143].

Unfortunately, recurrence indeed occurred 16 months after surgery was performed. At that point, the patient herein had developed various hepatic and peritoneal metastatic lesions. When considering the proven significant clinical benefit and safety of imatinib-treatment, the given circumstances obviously provided a strong rationale for its initiation as targeted first-line therapy. To be more precise, a big, multicenter clinical study demonstrated a disease control rate of 82% (partial response rate of 53.7% with an additional stable disease rate of 27.9%) under imatinib medication in patients with advanced/recurrent GISTs [97,98], and further studies underlined these numbers by achieving similar results [99-101]. These accomplishments stand alongside a generally well tolerated toxicity profile of this compound [94,104]. Consequently, in this case imatinib regimen was started at a 400 mg/day basis. It is important to note that a patient's adherence to treatment and thereby sustained constant imatinib plasma levels are substantial to achieve a favourable clinical outcome [103]. Thus, patient information about both, the latter aspect, as well as about the potentially occurring adverse events on the whole, is essential.

On the other hand the argument can be made that despite good clinical response rates, imatinib does not, or only in exceptional cases, induce complete responses and cause cure. Based on imatinib's rather cytostatic than cytocidal impact on GIST cells, the majority of lesions will progress again at some point [65,99]. Furthermore, the genesis of secondary mutations and consequent tumour progression in certain tumour areas seems to be additionally increased under the selective pressure of imatinib administration [106].

After disease progression on imatinib 400 mg/day soon became evident, dose escalation to 800 mg/day was performed. This therapeutical approach was recommended by ESMO and NCCN guidelines in order to treat advanced GISTs [73]. Since 2009, both guidelines rather suggest a starting dose of 800 mg/day for patients with molecular biological verification of a KIT exon 9 mutation [79,90]. This strategy has shown to compare significantly favourable with the 400 mg/day starting regimen in patients who harbour KIT exon 9 mutations [101]. Due to the occurrence of grade 2 diarrhoea, dose reduction to 700 mg/day had to be performed early. Noteworthy, dose-related toxicity is commonly observed in imatinib-treatment at the higher dose level of 800mg/day [104]. The patient in this study achieved stable disease under imatinib 800/700 mg/day for 4 months.

After this period, disease progression necessitated the initiation of a second-line treatment, and sunitinib was the medication of choice for that purpose. In 2006 sunitinib was approved by the FDA [113], for the second-line treatment of patients with advanced GISTs that progressed on imatinib. Since then, ESMO and NCCN guidelines suggest sunitinib treatment for patients in this situation [79,90]. In clinical trials, sunitinib showed to exhibit more potent activity against KIT exon 9-mutated tumours than against exon 11-mutant GISTs [116], which might suggest a minor activity in patients harbouring an exon 11-caused disease. However, sunitinib shows potent efficacy against secondary mutations that alter molecular structures of the ATP binding pocket, which constitutes a common mechanism of imatinib resistance [49,52,116].

When considering sunitinib administration, the question remains which dosage schedule should be chosen: 50 mg/day for 4 weeks followed by a 2-week rest or a continuous 37.5 mg/day regimen. This patient received sunitinib on a continuous 37.5 mg daily basis. This administration schedule has lately proven to be superior to the 4/2 cycle, as it turned out to be generally better tolerated alongside an equal efficacy [115]. Beyond that, tumour regrowth in the 2 weeks off-treatment may be avoided in the continuous schedule [115].

Sixteen months of disease control (PR and SD) could be achieved under sunitinib medication, until, disease progression was evident again. There is no third-line treatment established by ESMO and NCCN guidelines for the treatment of patients who experienced progressive disease after imatinib and sunitinib. Therefore, the implementation of a new therapy or combination therapies is recommended [79,90].Third-line treatment with nilotinib was initiated, based on the drug's verified inhibitory activity against the KIT receptor and its proven efficacy in patients with GISTs [117]. Moreover, certain reports even consider nilotinib for the first-line treatment of GIST due to its effectiveness [119] and according to that, a clinical trial that compares nilotinib and imatinib in a first-line-treatment setting is ongoing [121].

Regrettably, the tumour did not show any signs of disease control under nilotinib regimen. After 3 months, obvious and accelerated tumour growth on maximum dose of nilotinib required a quick transition to another therapy.

Available options for the patient's fourth-line medication were limited. Sorafenib is another TKI that demonstrated to potently inhibit KIT in advanced gastrointestinal stromal tumours [122,124]. A study revealed a recommended dosage of 800 mg/day [123]. Beyond that, sorafenib shows to be an effective therapy after a failure of sunitinib administration in advanced renal cell carcinoma [144]. Thus, fourth-line treatment with sorafenib 800 mg/day was started in the patient of this study, and stable disease could be observed for 9 months.

After that period, the tumour again started to progress more rapidly and pazopanib (Votrient®) regimen was being considered for further treatment. Pazopanib is an orally active and selective multi-targeted receptor-TKI of KIT, PDGFRA/B and all VEGF receptors, which proved to prevent tumour growth [145]. The compound additionally inhibits tumour angiogenesis and has been approved for the treatment of patients with renal cell carcinoma by the U.S. FDA in 2009. Nevertheless, there were no reports about pazopanib efficacy in patients with advanced GISTs.

Mainly due to the marked tumour progression alongside the lack of other therapeutic options and after extensive review of relevant papers, pazopanib 800 mg/day was initiated as a palliative TKI-treatment. The patient was informed about the experimental character of this treatment. As mentioned in detail in the previous chapter, various events of severe hypoglycaemia occurred during pazopanib administration and necessitated dose reduction to 600 mg/day. Due to the fact that stop of drug treatment for 14 days did not ameliorate the frequency of hypoglycaemic events, despite consequent nutritional intake every 4 hours even during the night, we concluded that hypoglycaemia was rather associated to massive energy depletion by the tumour.

At the last clinical examination approximately one month before the patient died, CT-scans demonstrated stable disease of-peritoneal and hepatic lesions, the latter showing response according to CHOI Criteria under pazopanib treatment. This finding suggests anti-tumour activity of pazopanib in patients with advanced GIST and multiple lines of pre-treatment with four other TKIs. To best of our knowledge this is the first report of a patient with GIST responding to pazopanib treatment.

9 CONCLUSION

Treatment options for patients with advanced GIST have multiplied in the past 11 years and thereby the individual patient outcome has significantly improved.

The implementation of imatinib as standard treatment for patients with inoperable or metastatic GIST was a milestone in the progress of medical treatment options for this disease.

Before the initiation of imatinib or any other TKI, mutational analysis of c-Kit and PDGFR receptor alpha is strongly recommended. This case, in accordance with the current literature, has demonstrated that patients with tumours carrying an exon 9 mutation need to be treated with an increased dose of imatinib and may have a more aggressive course of disease.

Sunitinib is the approved second-line treatment for patients failing imatinib treatment. This case report underlines the significant anti-tumour activity of this agent, especially in patients with tumours harbouring the exon 9 mutation. Initial sunitinib treatment for patients with exon 9 mutations is not recommended by NCCN and ESMO guidelines; yet, potential benefits of this procedure should be considered and reassessed.

No standard third-line treatment for patients with GIST has been approved up to now. This case demonstrates that sorafenib and pazopanib are active drugs in this setting.

Further research is warranted to establish the role of sorafenib and pazopanib as new treatment options in imatinib and sunitinib refractory patients with GIST. Also, additional studies are needed to identify treatment options that effectively target intracellular pathways activated in GIST tumourigenesis in order to inhibit oncogenic signalling beyond the c-Kit receptor.

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APPENDIX

- **Publication List David Rühlinger**

Abstracts as a first author:

Rühlinger, D; Szkandera, J; Pichler, M; Eisner, F; Stöger, H; Bauernhofer, T; 2010 Sorafenib as forth-line treatment in a patient with metastatic gastrointestinal stromal tumour: a case report.
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