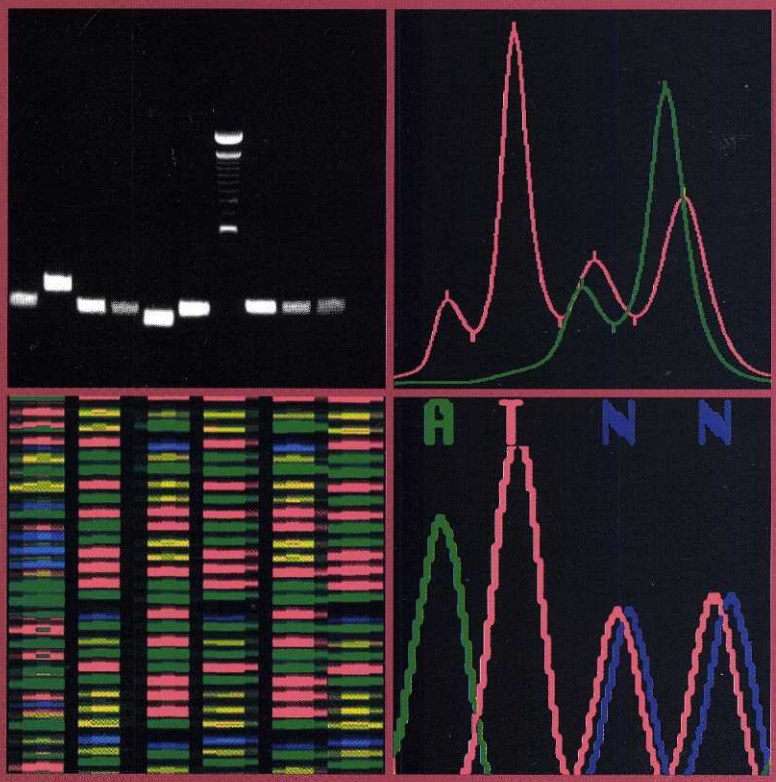
GASTROINTESTINAL STROMAL TUMORS - PATHOGENETIC MECHANISMS, PHENOTYPIC CHARACTERIZATION AND PROGNOSIS

Johanna Andersson



Göteborg 2005

GASTROINTESTINAL STROMAL TUMORS – PATHOGENETIC MECHANISMS, PHENOTYPIC CHARACTERIZATION AND PROGNOSIS

Akademisk avhandling

som för avläggande av medicine doktorsexamen vid Göteborgs Universitet kommer att offentligen försvaras i föreläsningssalen "Arvid Carlsson", Academicum, medicinaregatan 3, Göteborg, fredagen den 29 april 2005, kl. 13:00.

av

Johanna Andersson

Fakultetsopponent: Professor Olle Larsson,
Institutionen för onkologi-patologi, Enheten för patologi,
Karolinska Institutet, Stockholm

Avhandlingen baseras på följande delarbeten:

I. **Andersson J**, Sjögren H, Meis-Kindblom JM, Stenman G, Åman P, Kindblom LG. The complexity of KIT gene mutations and chromosome rearrangements and their clinical correlation in gastrointestinal stromal (pacemaker cell) tumors.
Am J Pathol 160(1):15-22, 2002

II. Bümming P, **Andersson J**, Meis-Kindblom JM, Klingenstierna H, Engström K, Stierner U, Wängberg B, Jansson S, Ahlman H, Kindblom LG, Nilsson B. Neoadjuvant, adjuvant and palliative treatment of gastrointestinal stromal tumors (GIST) with imatinib: a centre-based study of 17 patients.
British J Cancer 89(3):460-464, 2003

III. **Andersson J**, Bümming P, Meis-Kindblom JM, Sihto H, Nupponen N, Joensuu H, Gustavsson B, Kindblom LG, Nilsson B. Gastrointestinal stromal tumors with *KIT* exon 11 deletions are associated with poor prognosis.
Submitted

IV. **Andersson J**, Sihto H, Meis-Kindblom JM, Joensuu H, Nupponen N, Kindblom LG. NF1 associated gastrointestinal stromal tumors have unique clinical, phenotypic, and genotypic characteristics.
Am J Surg Pathol 29(5): In press, 2005

Avhandlingen kommer att försvaras på svenska.

ABSTRACT

GASTROINTESTINAL STROMAL TUMORS – PATHOGENETIC MECHANISMS, PHENOTYPIC CHARACTERIZATION AND PROGNOSIS

Johanna Andersson

Lundberg Laboratory for Cancer Research, Department of Pathology, Sahlgrenska Academy at Göteborg University, Sahlgrenska University Hospital, SE-413 45 Göteborg, Sweden

Gastrointestinal stromal tumor (GIST), the most common non-epithelial neoplasm of the gastrointestinal tract, has historically been problematic both conceptually and clinically. Recently, GIST has been shown to share phenotypic features with the interstitial cells of Cajal (ICC), including the almost uniform expression of the tyrosine kinase receptor KIT. GIST have frequently been found to have activating mutations of the *KIT* gene or more rarely the platelet derived growth factor receptor alpha (*PDGFRA*) gene; these apparently play a key role in GIST's pathogenesis. The occurrence of GIST lacking *KIT* and *PDGFRA* mutations indicates, however, that alternative pathogenetic mechanisms exist.

Clinical studies regarding the correlation of mutations with disease outcome in GIST have been contradictory. Mutation analysis of the *KIT* gene in 14 GIST revealed exon 11 mutations in 9 tumors. Mutations were detected in benign as well as malignant GIST. Cytogenetic and SKY analyses revealed several recurrent structural and numerical abnormalities; losses of 1p, 14 and 22 being the most common. No correlation was found between biologic behavior, *KIT* mutation status and chromosome aberrations. All tumors preferentially expressed the shorter, tumorigenic, splice variant of exon 9 of the *KIT* gene.

Imatinib mesylate, a new designer drug, selectively inhibits type III receptor tyrosine kinases and has a dramatic, anti-tumor effect on GIST. In a center-based study of 17 patients with GIST, we studied the relationship between *KIT* exon 11 mutation status and treatment response. Imatinib was especially effective in tumors with exon 11 mutations - *KIT* exon 11 mutations were detected in 8 of 9 patients with partial response, whereas no mutations were detected in 3 patients with stable or progressive disease.

Earlier studies of receptor tyrosine kinase mutations indicate that different types of mutations are associated with distinctive phenotypes and potentially clinical behavior in GIST. In a large, retrospective, population-based series of GIST from the pre-imatinib mesylate era, we examined whether mutation type correlated with phenotype and clinical course. *KIT* exon 11 mutations were detected in GIST from 117 of 233 patients (69 deletions, 27 missense mutations and 21 duplications). The deletion subgroup had a significantly decreased disease-free survival. *KIT* exon 11 deletions were found to be an independent adverse prognostic factor with respect to disease-free survival.

GIST have been reported to occasionally occur in patients with neurofibromatosis type I (NF1). NF1 is the most common autosomal dominant inherited disorder, with an incidence of 1/3-4000 individuals. The clinical spectrum of NF1 is extremely wide and includes organic as well as mental manifestations. We analyzed GIST arising in 15 NF1 patients and found that this subset of GIST has a unique phenotype, including an almost uniformly benign appearance histologically, a propensity for multicentricity, association with ICC hyperplasia, predominant location in the small intestine, and a spindle cell morphology as well as benign or very low grade malignant behavior. The NF1-associated GIST also have a unique genotype in that they lack *KIT* and *PDGFRA* mutations, indicating that different pathogenetic mechanisms are involved in their evolution.

In summary, we have shown that the role of *KIT* mutations in the biological behavior of GIST is more complex than previously recognized and that the type of mutation influences clinical behavior as well as response to imatinib treatment. NF1-associated GIST are a pheno-and genotypically unique subset of GIST that lack activating mutations in the *KIT* and *PDGFRA* genes.

Key words: Gastrointestinal stromal tumor (GIST), neurofibromatosis type I (NF1), mutation, prognosis, denaturating high performance liquid chromatography (dHPLC), sequencing, *KIT*, *PDGFRA*, *NF1*
ISBN 91-628-6431-9, Göteborg 2005

GASTROINTESTINAL STROMAL TUMORS – PATHOGENETIC MECHANISMS, PHENOTYPIC CHARACTERIZATION AND PROGNOSIS

Johanna Andersson

Lundberg Laboratory for Cancer Research
Department of Pathology
Sahlgrenska Academy at Göteborg University
Sahlgrenska University Hospital
2005

ISBN 91-628-6431-9
Göteborg 2005

fortsätt längta
hitta mod
våga hoppas
våga tro
tro på drömmen
ta ett kliv
vält den värld du lever i
och var en galning
var naiv
det är det som håller oss vid liv

/Peter LeMarc, "Det som håller oss vid liv"

ABSTRACT

GASTROINTESTINAL STROMAL TUMORS – PATHOGENETIC MECHANISMS, PHENOTYPIC CHARACTERIZATION AND PROGNOSIS

Johanna Andersson

Lundberg Laboratory for Cancer Research, Department of Pathology, Sahlgrenska Academy at Göteborg University, Sahlgrenska University Hospital, SE-413 45 Göteborg, Sweden

Gastrointestinal stromal tumor (GIST), the most common non-epithelial neoplasm of the gastrointestinal tract, has historically been problematic both conceptually and clinically. Recently, GIST has been shown to share phenotypic features with the interstitial cells of Cajal (ICC), including the almost uniform expression of the tyrosine kinase receptor KIT. GIST have frequently been found to have activating mutations of the *KIT* gene or more rarely the platelet derived growth factor receptor alpha (*PDGFRA*) gene; these apparently play a key role in GIST's pathogenesis. The occurrence of GIST lacking *KIT* and *PDGFRA* mutations indicates, however, that alternative pathogenetic mechanisms exist.

Clinical studies regarding the correlation of mutations with disease outcome in GIST have been contradictory. Mutation analysis of the *KIT* gene in 14 GIST revealed exon 11 mutations in 9 tumors. Mutations were detected in benign as well as malignant GIST. Cytogenetic and SKY analyses revealed several recurrent structural and numerical abnormalities; losses of 1p, 14 and 22 being the most common. No correlation was found between biologic behavior, *KIT* mutation status and chromosome aberrations. All tumors preferentially expressed the shorter, tumorigenic, splice variant of exon 9 of the *KIT* gene.

Imatinib mesylate, a new designer drug, selectively inhibits type III receptor tyrosine kinases and has a dramatic, anti-tumor effect on GIST. In a center-based study of 17 patients with GIST, we studied the relationship between *KIT* exon 11 mutation status and treatment response. Imatinib was especially effective in tumors with exon 11 mutations - *KIT* exon 11 mutations were detected in 8 of 9 patients with partial response, whereas no mutations were detected in 3 patients with stable or progressive disease.

Earlier studies of receptor tyrosine kinase mutations indicate that different types of mutations are associated with distinctive phenotypes and potentially clinical behavior in GIST. In a large, retrospective, population-based series of GIST from the pre-imatinib mesylate era, we examined whether mutation type correlated with phenotype and clinical course. *KIT* exon 11 mutations were detected in GIST from 117 of 233 patients (69 deletions, 27 missense mutations and 21 duplications). The deletion subgroup had a significantly decreased disease-free survival. *KIT* exon 11 deletions were found to be an independent adverse prognostic factor with respect to disease-free survival.

GIST have been reported to occasionally occur in patients with neurofibromatosis type I (NF1). NF1 is the most common autosomal dominant inherited disorder, with an incidence of 1/3-4000 individuals. The clinical spectrum of NF1 is extremely wide and includes organic as well as mental manifestations. We analyzed GIST arising in 15 NF1 patients and found that this subset of GIST has a unique phenotype, including an almost uniformly benign appearance histologically, a propensity for multicentricity, association with ICC hyperplasia, predominant location in the small intestine, and a spindle cell morphology as well as benign or very low grade malignant behavior. The NF1-associated GIST also have a unique genotype in that they lack *KIT* and *PDGFRA* mutations, indicating that different pathogenetic mechanisms are involved in their evolution.

In summary, we have shown that the role of *KIT* mutations in the biological behavior of GIST is more complex than previously recognized and that the type of mutation influences clinical behavior as well as response to imatinib treatment. NF1-associated GIST are a pheno- and genotypically unique subset of GIST that lack activating mutations in the *KIT* and *PDGFRA* genes.

Key words: Gastrointestinal stromal tumor (GIST), neurofibromatosis type I (NF1), mutation, prognosis, denaturating high performance liquid chromatography (dHPLC), sequencing, *KIT*, *PDGFRA*, *NF1*
ISBN 91-628-6431-9

LIST OF PUBLICATIONS

This thesis is based on the following papers, which will be referred to in the text by their Roman numerals (I-IV):

I. **Andersson J**, Sjögren H, Meis-Kindblom JM, Stenman G, Åman P, Kindblom LG. The Complexity of *KIT* Gene Mutations and Chromosome Rearrangements and Their Clinical Correlation in Gastrointestinal Stromal (pacemaker cell) Tumors.
Am J Pathol, 2002; 160(1):15-22.

II. Bümming P, **Andersson J**, Meis-Kindblom JM, Klingenstierna H, Engström K, Stierner U, Wängberg B, Jansson S, Ahlman H, Kindblom LG, Nilsson B. Neoadjuvant, adjuvant and palliative treatment of gastrointestinal stromal tumours (GIST) with imatinib: a centre-based study of 17 patients.
British J Cancer, 2003; 89(3):460-464.

III. **Andersson J**, Bümming P, Meis-Kindblom JM, Sihto H, Nupponen N, Joensuu H, Gustavsson B, Kindblom LG, Nilsson B. Gastrointestinal stromal tumors with *KIT* exon 11 deletions are associated with poor prognosis.
Submitted

IV. **Andersson J**, Sihto H, Meis-Kindblom JM, Joensuu H, Nupponen N, Kindblom LG. NF1 associated gastrointestinal stromal tumors have unique clinical, phenotypic, and genotypic characteristics.
Am J Surg Pathol, 2005; 29(5):In Press

CONTENTS

ABSTRACT 5

LIST OF PUBLICATIONS 6

CONTENTS 7

ABBREVIATIONS 8

INTRODUCTION 11

GENETIC BASIS OF CANCER 11
Oncogenes 11
Tumor suppressor genes 11
DNA repair genes 12
GASTROINTESTINAL CANCER 12
GASTROINTESTINAL STROMAL TUMORS (GIST) 12
CLINICAL PRESENTATION AND PROGNOSIS OF GIST 12
THE EVOLVING CONCEPT OF GIST 13
RECENT BREAKTHROUGHS IN THE UNDERSTANDING OF GIST 15
ICC 15
KIT and GIST 15
Platelet derived growth factor alpha (PDGFRA) and GIST 17
Treatment of malignant GIST 18
CYTOGENETIC ALTERATIONS IN GIST 18
NF1 AND GIST 18

OBJECTIVES 20

MATERIALS AND METHODS 21

TUMOR MATERIAL 21
METHODS 21
Denaturating high performance liquid chromatography (dHPLC) 21
Immunohistochemical, molecular genetic, cytogenetic, and statistical methods 22

RESULTS AND DISCUSSION 23

THE COMPLEXITY OF *KIT* GENE MUTATIONS AND CHROMOSOMAL REARRANGEMENTS AND THEIR CLINICAL CORRELATION IN GASTROINTESTINAL STROMAL TUMORS (PAPER I) 23
KIT exon 11 mutations and biologic behavior 23
Cytogenetic and SKY analyses 23
GIST preferentially express the shorter, tumorigenic, isoform of exon 9 of the KIT transcript 24
NEOADJUVANT, ADJUVANT AND PALLIATIVE TREATMENT OF GASTROINTESTINAL STROMAL TUMORS (GIST) WITH IMATINIB: A CENTRE-BASED STUDY OF 17 PATIENTS (PAPER II) 24
GASTROINTESTINAL STROMAL TUMORS WITH *KIT* EXON 11 DELETIONS ARE ASSOCIATED WITH POOR PROGNOSIS (PAPER III) 25
NF1 ASSOCIATED GASTROINTESTINAL STROMAL TUMORS HAVE UNIQUE CLINICAL, PHENOTYPIC, AND GENOTYPIC CHARACTERISTICS (PAPER IV) 27

CONCLUSIONS 29

FUTURE PERSPECTIVES 30

GENE EXPRESSION ANALYSES 30
NF1 GENE ALTERATIONS 30
SPLICE ISOFORM EXPRESSION 30
CHROMOSOMAL REARRANGEMENTS 31
MUTATION TYPE SPECIFIC TREATMENT RESPONSE TO IMATINIB MESYLATE IN GIST 31

POPULÄRVETENSKAPLIG SAMMANFATTNING 32

ACKNOWLEDGEMENTS 34
REFERENCES 36
PAPERS I-IV 45

ABBREVIATIONS

ABL	Abelson murine leukemia viral oncogene
ACN	Acetonitrile
ATP	Adenosine triphosphate
BCR	Breakpoint cluster region
bp	base pair
CD117	KIT protein product
cDNA	Complementary DNA
CML	Chronic myelogenous leukemia
dHPLC	Denaturating high performance liquid chromatography
DNA	Deoxyribonucleic acid
DVB	Divinyl benzene
GAP	Guanosine triphosphate activating protein
GDP	Guanosine diphosphate
GTP	Guanosine triphosphate
gDNA	genomic DNA
GANT	Gastrointestinal autonomal nerve tumor
GIPACT	Gastrointestinal pacemaker cell tumor
GIST	Gastrointestinal stromal tumor
hpf	high power field
ICC	Interstitial cells of Cajal
kb	kilo base pair
kDa	kilo Dalton
Ki67	nuclear antigen expressed in proliferating cells
KL	Kit ligand
LOH	Loss of heterozygosity
MAPK	Mitogen activated protein kinase
MGF	Mast cell Growth factor
mRNA	messenger RNA
NF1	Neurofibromatosis type 1
PCR	Polymerase chain reaction
PDGFRA	Platelet derived growth factor receptor alpha gene
PDGFRα	Platelet derived growth factor receptor alpha protein

PI3K	Phosphatidylinositol 3-kinase
RNA	Ribonucleic acid
RTK	Receptor tyrosine kinase
SCF	Stem cell factor
SF	Steel factor
SKY	Spectral karyotyping
STAT	Signal transducer and activator of transcription
TEAA	Triethylammonium amine
TP53	Tumor protein 53
WT	Wild type

INTRODUCTION

Genetic basis of cancer

Over the past few decades, cancer has been shown to be a genetic disease. Its development is a multi-step process characterized by the stepwise transformation of a normal cell into a tumor cell.[1,2] During cancer development, somatic cells accumulate a number of genetic changes that lead to uncontrolled cell growth, loss of differentiation, and frequently increased motility.[3] Through these changes, which may be subtle, as in point mutations, deletions and insertions, or which may involve chromosomal rearrangements, such as gains and losses, amplifications and translocations, the cell acquires the necessary functional capabilities to become a fully transformed malignant tumor cell. In a landmark publication, Hanahan & Weinberg[4] described six such functional capabilities believed to be essential for malignant neoplasms, namely: self sufficiency in growth signals; insensitivity to anti-growth signals; limitless replicative potential; evasion of apoptosis; sustained angiogenesis; and tissue invasion and metastasis. The majority of genes that are responsible for or control these capabilities can be divided into the three major classes of cancer genes, *i.e.* oncogenes, tumor suppressor genes and DNA repair genes.[5]

Oncogenes

Proto-oncogenes are normal genes that are often involved in the control of cell growth and proliferation, processes that are normally strictly regulated in the cell. Conversion of normal proto-oncogenes into activated oncogenes is an important step in the tumorigenesis of many tumors. Oncogenes are activated by dominant, "gain-of-function" mutations and consequently only one allele is normally affected by the mutation. They may be divided into different classes or families depending on the function of the proteins they encode, such as growth factors, growth factor receptors and signal transduction molecules. Several receptor tyrosine kinases act as oncogenes, including KIT and PDGFRα.

Tumor suppressor genes

Tumor suppressor genes are also known as the gatekeepers of the cell and they function as negative regulators of cell proliferation, encoding cell cycle inhibitors, pro-apoptotic factors and transcriptional repressors. The mutations that affect tumor suppressor genes are recessive, "loss-of-function" mutations; both the maternal and the paternal alleles must be inactivated in order for these genes to contribute to tumorigenesis as postulated by Knudson in the "two-hit" hypothesis published in 1971.[6] Patients with hereditary forms of cancer often inherit a germline mutation in one of the alleles of a tumor suppressor gene.

Mutations of *TP53* are among the most common aberrations known to occur in human cancers, sporadic as well as hereditary. For example, patients suffering from the Li Fraumeni syndrome have germline mutations in this tumor suppressor gene and are

predisposed to develop various malignancies including soft tissue sarcomas. *TP53*, perhaps the most well-known tumor suppressor gene in the human genome, encodes a protein that mediates cell cycle arrest and promotes apoptosis in response to DNA damage.[7-9] Inactivation of TP53 prevents the cell from undergoing DNA-damage induced cell cycle arrest or apoptosis and leads to genetic instability due to the accumulation of mutations.[10]

DNA repair genes

The third class of cancer associated genes includes the DNA repair genes ("caretakers"). These genes encode proteins that maintain the integrity of the genome. Loss of this function, due to mutations, results in genomic instability.[11,12] There are several mechanisms for DNA repair, including direct repair, base excision repair, and mismatch repair (reviewed in Lehmann, 1995).[13,14] Defects in the DNA repair mechanisms indirectly increase the risk of cancer development by increasing the mutation rate.

Gastrointestinal cancer

By far the most common type of gastrointestinal cancer is adenocarcinoma, particularly involving the colon, rectum and stomach. After breast, lung, and prostate cancer, colorectal cancer is the most common malignant tumor occurring in the western world. Malignant tumors of the small intestine are rare and account for less than 2% of all gastrointestinal cancers.[15,16]

Malignant mesenchymal tumors (sarcomas) of the gastrointestinal tract are relatively uncommon. The National Cancer Institute Surveillance, Epidemiology, and End Results (SEER) data from 1995 report that sarcomas account for 2.2% of gastric malignancies, 13.9% of small bowel malignancies and only 0.1% of all colorectal malignancies.[17]

Gastrointestinal stromal tumors (GIST)

Clinical presentation and prognosis of GIST

Gastrointestinal stromal tumors (GIST), the focus of this thesis, are the most common mesenchymal (non-epithelial) neoplasms of the gastrointestinal tract. A recent population-based study of GIST in western Sweden has indicated a yearly incidence of $14.5/10^6$ inhabitants and a prevalence of $129/10^6$ inhabitants.[18] GIST typically occur in middle aged and older individuals; in the population-based study of 288 GIST in western Sweden, the median age was 69 years (range 10-92 years).[18] Most studies show an equal gender distribution.[18-20] Tumor sizes range from 2 – 30 cm at clinical presentation. The majority of tumors are detected due to symptoms but approximately 30% are incidental findings at surgery or autopsy. In the population-based study of 288 GIST in western Sweden, 69% of the tumors were symptomatic, causing abdominal pain, gastrointestinal bleeding, gastrointestinal obstruction or a palpable mass.[18] The stomach is the most common site (50-70%) for GIST, followed by the small intestine (20-30%), and colon-

rectum (<10%). More rarely, GIST occur in the esophagus or in other sites such as the omentum or the mesentery (less than 5%).[18-20]

There has been much confusion and controversy regarding the prognostication of GIST. Previously, a substantial proportion of GIST have been regarded as benign. Recently, however, most GIST, including very small, incidentally detected tumors, have been shown to be capable of metastasis.[21] In 2001, a consensus risk stratification system for GIST was formulated based on expert opinions. This system classifies GIST according to their malignant potential; based on size and mitotic activity, the tumors are divided into four different risk groups, as shown below:[22]

Risk group	Size (cm)	Mitotic count
Very low risk	<2	<5/50hpf
Low risk	2-5	<5/50hpf
Intermediate risk	<5	6-10/50hpf
	5-10	<5/50hpf
High risk	>5	>5/50hpf
	>10	Any mitotic rate
	Any size	>10/50hpf
Overtly malignant*	Metastasis at presentation	

*: Subgroup added in the analysis of Nilsson et. al. 2005.

In the population-based study of 288 GIST in western Sweden, Nilsson and co-workers found that 63% and 83% of high risk and overtly malignant GIST were associated with tumor related deaths (median follow-up intervals, 40 months and 16 months, respectively) compared to only 2 of 170 (1.2%) patients with very low, low or intermediate risk GIST. The authors proposed a refined prognostication system for GIST based on tumor size and proliferative index.[18] The maximum tumor size (in centimeters) and the maximum Ki67 proliferative index (in percentage) are added to give a risk score. Risk scores ≥ 7 are associated with a decreased 5 year survival; the likelihood of dying increases rapidly with higher risk scores. This risk score system is simpler and therefore probably more reproducible than other existing prognostication systems, further stratifies risk groups, and is potentially more useful in the evaluation of treatment protocols.

The evolving concept of GIST

GIST have a wide morphological spectrum, ranging from small clinically benign lesions with a bland histologic appearance to large, highly malignant tumors with spindled and/or epithelioid cellular features.[23-25] Problems regarding criteria for diagnosis, appropriate nomenclature, identification of reliable clinical and morphologic prognostic factors, and

type of differentiation have existed for decades. Conflicting views regarding their differentiation, classification and prognosis have been reflected by a variety of names used for these tumors. In the past, they were usually believed to be of nerve or nerve sheath origin. Later, most mesenchymal tumors of the gastrointestinal tract were thought to be of smooth muscle origin, since they histologically had some smooth muscle-like features, thus terms such as leiomyoma, leiomyoblastoma, and leiomyosarcoma, sometimes with the prefix "epithelioid", were commonly used. In the 1980s and early 1990s, however, immunohistochemical and ultrastructural studies showed that true smooth muscle tumors are very rare in the gastrointestinal tract. The majority of gastrointestinal mesenchymal tumors differ from true smooth muscle tumors of other sites in that they rarely express desmin and smooth muscle actin and ultrastructurally lack well-developed myofilaments. In 1983, Mazur and Clark published a paper documenting the lack of smooth muscle differentiation, which led to a significant nomenclature change.[26] They proposed the term "stromal tumors", later modified to "gastrointestinal stromal tumor" (GIST) to reflect that these tumors display different lines of differentiation rather than true smooth muscle. The use of immunohistochemical and ultrastructural techniques to classify GIST also led to the recognition that a subset of these tumors express some features of nerve cell differentiation, and in 1984, Herrera and co-workers introduced the term "plexosarcoma".[27] This subset of GIST was later labeled as gastrointestinal autonomic nerve tumors (GANT).[28,29]

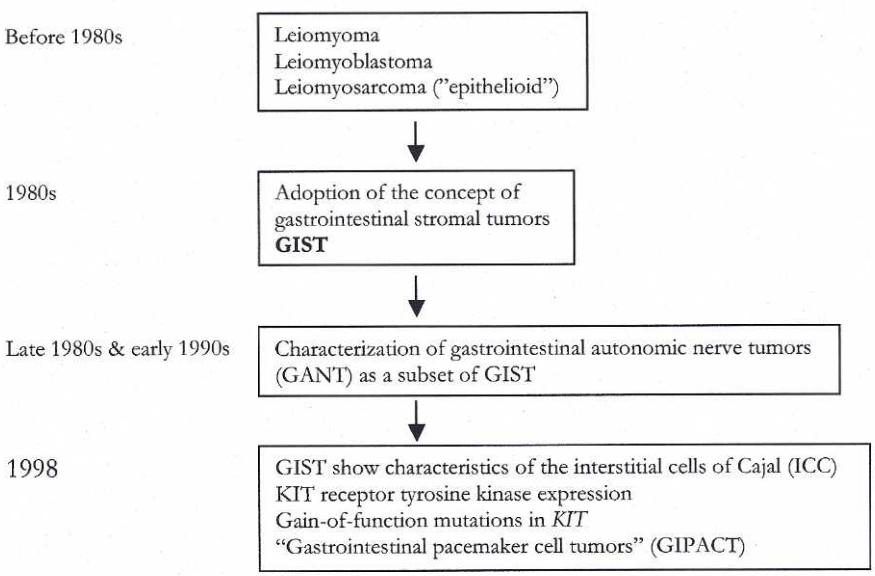


Figure 1. Schematic illustration of the evolving concept and terminology of GIST. (Adapted from Chan, 1999)

Recent breakthroughs in the understanding of GIST

Recent studies have demonstrated a close phenotypic resemblance between GIST and the interstitial cells of Cajal (ICC).[30,31] In 1998, Kindblom and co-workers demonstrated striking ultrastructural similarities between the ICC and GIST (78 cases); they also found that GIST, similar to the ICC, strongly expressed the KIT receptor tyrosine kinase.[30] These observations were an important breakthrough in the understanding of the origin of GIST, indicating that they originate from the ICC or from a progenitor cell that differentiates toward a gastrointestinal pacemaker cell phenotype. The term gastrointestinal pacemaker cell tumor (GIPACT) was proposed, since it more accurately reflects current knowledge regarding differentiation than the non-committal and purely descriptive term GIST.[30] The simultaneous discovery of gain-of-function mutations in the *KIT* gene in 5 of 6 GIST by Hirota and co-workers provided fundamental insights into the pathogenesis of GIST. Hirota et al. also showed that the mutant but not the wild type form of KIT induced malignant transformation of lymphoid cells.[32]

ICC

The interstitial cells of Cajal (ICC), so named after the neuroanatomist Santiago Ramon y Cajal from Valencia, form a complex network of cells within the gastrointestinal tract wall. They appear to have two major functions: (1) initiating spontaneous smooth muscle contraction, i.e., pacemaker function by generating slow waves, and (2) acting as intermediaries in the control of gastrointestinal motility by the enteric nervous system as shown by their heavy innervation and junction formation with smooth muscle cells.[33] Expression of the *KIT* proto-oncogene is essential for the development of the ICC. Disruption of KIT during development results in the absence of a functional ICC network, manifested by lack of peristalsis of the gut.[34,35]

KIT and GIST

The *KIT* proto-oncogene, located on chromosome 4q11-21, is the cellular homologue of the transforming element of the Hardy-Zuckerman IV feline sarcoma virus.[36-39] KIT (CD117) is a type III receptor tyrosine kinase, which belongs to the immunoglobulin supergene family.[40] The molecule consists of 976 amino acids (135kDa) and is divided into an extracellular domain and an intracellular domain. The extracellular part comprises five immunoglobulin-like domains, with the second and third loops being involved in ligand binding. The intracellular tyrosine kinase domain contains a consensus ATP-binding site and a split tyrosine kinase domain (Fig. 2).[40,41]

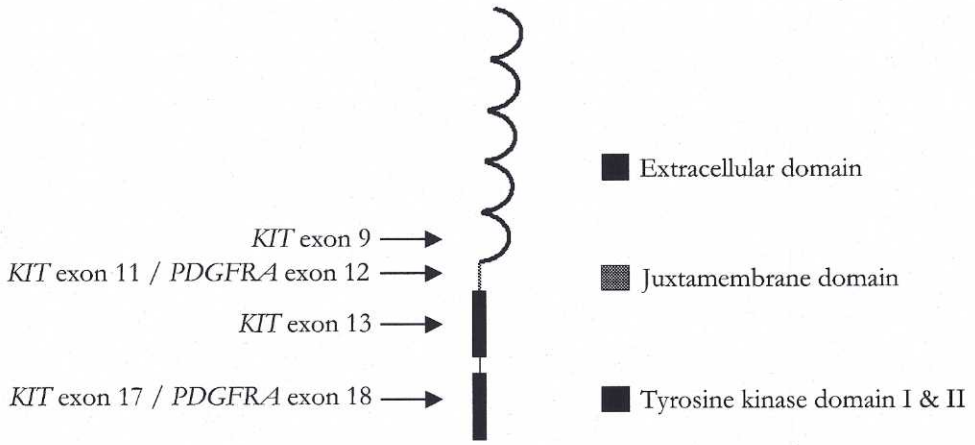


Figure 2. Schematic illustration of the structure of type III receptor tyrosine kinases. Arrows indicate the location of the gain-of-function mutations frequently found in the *KIT* or, more rarely, *PDGFRA* genes in GIST.

The ligand for KIT is stem cell factor (SCF), also known as KIT ligand (KL), Steel Factor (SF), or mast cell growth factor (MGF).[42] Ligand binding initiates dimerization and autophosphorylation that activates downstream signaling pathways. These include the phosphatidylinositol 3-kinase (PI3K) pathway, the Ras-Raf-MAPK pathway*, and the STAT pathway, leading to proliferation, differentiation, and/or apoptosis (Fig. 3a).

A functional KIT is essential for normal development of hematopoetic cells, germ cells, melanocytes, and mast cells, as well as for the development of the interstitial cells of Cajal (ICC).[43] Mutations in the *KIT* gene can result in a variety of diseases related to these cell types.[44-46]

Gain-of-function mutations result in ligand-independent dimerization, autophosphorylation and activation of downstream signaling pathways (Fig. 3b). As previously mentioned, Hirota and co-workers reported gain-of-function mutations in exon 11 of the *KIT* gene in GIST,[32] a finding that led to a number of studies investigating the correlation between such mutations and biological behavior. The results were, however, conflicting. While some investigators reported a strong correlation between the presence of mutations and malignant behavior,[47-50] others were not able to confirm such a correlation.[51,52] Most of these early studies were limited to the juxtamembrane domain, encoded by exon 11 in the *KIT* gene. However, in a few studies, the entire coding region was analyzed, leading to the detection of mutations in exon 9[53-55] and rarely exon 13[53,54,56] of the *KIT* gene in a small subset of GIST lacking exon 11 mutations. Rare mutations were also subsequently reported to occur in exon 17 of the *KIT* gene.[57]

*: An interactive animation of the MAP Kinase signal transduction pathway is shown at:

http://www.bio.davidson.edu/courses/Immunology/Flash/MAPK.html

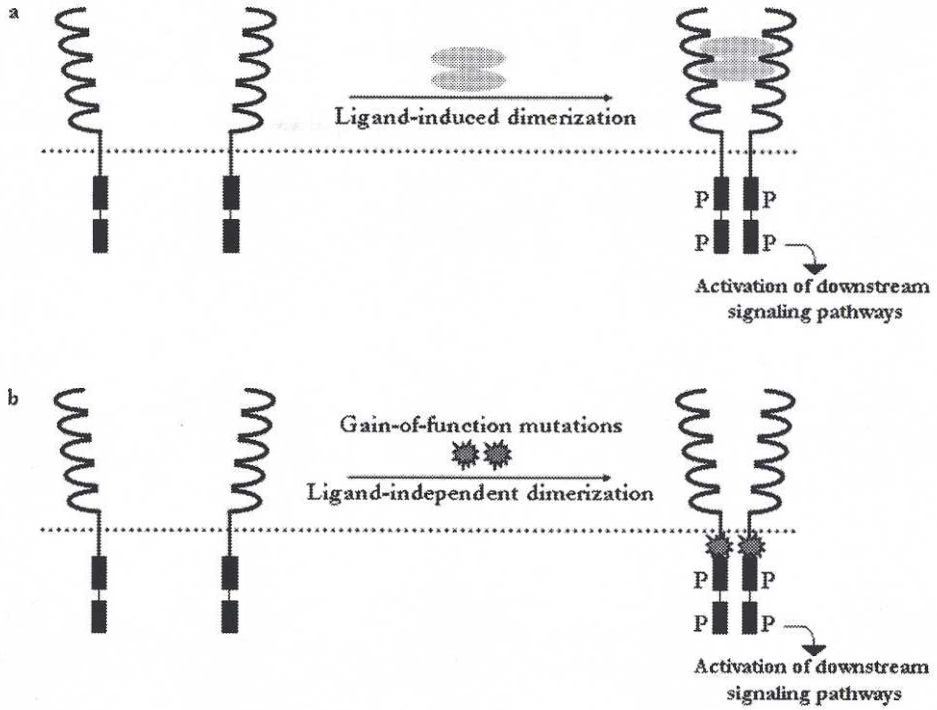


Figure 3. Activation of type III receptor tyrosine kinases. **a**: Normally, binding of the ligand induces dimerization and autophosphorylation, activating downstream signaling pathways. **b**: In GIST, gain-of-function mutations in the *KIT* or *PDGFRA* genes result in ligand-independent dimerization, autophosphorylation and activation.

Platelet derived growth factor alpha (PDGFRA) and GIST

The *PDGFRA* gene is located next to the *KIT* gene on chromosome 4q11-21. PDGFRα and KIT belong to the same family of receptor tyrosine kinases and consequently show extensive structural homology.[36,58] The ligands for PDGFRα are the PDGF dimers AA, AB, BB, CC, and DD.[59-62] Ligand binding initiates receptor dimerization that autophosphorylates cytoplasmic tyrosine residues (Fig. 3a) and triggers the signal transduction pathway through phosphatidylinositol 3-kinase, phospholipase Cγ, and GTPase activating proteins (reviewed in Heldin, 1992).[41,63]

PDGFRα is believed to play a key role in the development of glia.[64] Expression of PDGFRα lacking a part of the fourth and fifth Ig-like extracellular domains have been reported in primary brain tumors of glial origin.[65]

Recently, gain-of-function mutations have been reported to occur in exons 12 and 18 of the *PDGFRA* gene in a subset of GIST that lack *KIT* mutations.[66,67] *KIT* and *PDGFRA* mutations are mutually exclusive events in GIST. Tumors with *PDGFRA* mutations often lack immunoreactivity for KIT, have a predominantly gastric location and reportedly have a relatively benign clinical course.[66-69]

Treatment of malignant GIST

Malignant GIST are virtually resistant to conventional chemotherapy and radiation therapy. Until recently, surgical resection has been the only available treatment. For advanced GIST, surgery alone has been inadequate and the outcome for most patients with malignant GIST has been poor.[70]

A major breakthrough in the treatment of malignant GIST came in 2001 when imatinib mesylate (Glivec®, STI-571, Novartis Pharma, Basel Switzerland) was introduced as a treatment for patients with unresectable and / or metastatic GIST. Imatinib was originally designed as a treatment for chronic myelogenous leukemia (CML). In CML, imatinib blocks the binding of adenosine triphosphate to the ABL kinase in the BCR-ABL fusion gene that drives tumor proliferation. Subsequently, imatinib was found to not only inhibit ABL but also the closely related type III receptor tyrosine kinases, KIT and PDGFRα.[71] The first successful treatment of a patient with metastatic GIST with imatinib, a model of the new, rapidly growing group of molecularly targeted cancer therapies, was reported by Joensuu and co-workers in 2001.[72] Imatinib thus represents the first safe and effective treatment for patients with malignant GIST, as proven by several clinical trials and ongoing clinical studies.[73-76]

Cytogenetic alterations in GIST

Both losses and gains of chromosomes have been reported in GIST. Monosomy of chromosome 14 or partial loss of 14q are seen in up to two-thirds of tumors.[52,66,77-81] At least two regions of this chromosome, 14q11.1-q12 and 14q22-24, seem to be hot spots for deletions and most likely represent sites for putative tumor suppressor genes that may play an important role in GIST development.[80,82] Loss of the long arm of chromosome 22 is the second most common chromosomal aberration in GIST, detected in approximately 50% of the tumors.[77,78,80,83] Losses on chromosomes 1p and 9p are less frequent, but may be more clearly associated with malignancy.[66,77,83-85]

Gene amplifications have also been reported in GIST and gains on chromosome 8q and 17q have been associated with metastatic behavior.[80,82,84]

NF1 and GIST

Neurofibromatosis type 1 (NF1), also known as von Recklinghausen's disease, is the most common autosomally dominant inherited disorder in humans, occurring in approximately 1 in 3,500 individuals.[86] It is caused by mutations in *NF1*, a tumor suppressor gene which has been mapped to the long arm of chromosome 17 (17q11.2).[87-89] The *NF1* gene is one of the largest genes in the human genome. It spans 290 kb of genomic DNA and contains 60 exons, encoding a 12-kb mRNA transcript.

Neurofibromin, the *NF1* gene product, displays partial homology to GTPase activating protein (GAP), a family of *RAS* regulatory proteins.[90] The GAP-related domain, encoded by exons 20-27a, is the only region of neurofibromin to which a biologic function has been ascribed. Neurofibromin stimulates GTPase activity and negatively regulates *RAS* by catalyzing the conversion of the active GTP-bound RAS to its inactive GDP-bound form. *NF1* mutations disrupt the normal function of neurofibromin and result in constitutive

RAS activation.[91] This activation increases downstream signaling through the MAP kinase pathway.[92]

The clinical spectrum of NF1 is extremely wide and includes a plethora of organic and mental manifestations of varying degrees of severity.[93,94] Most affected individuals exhibit café-au-lait spots, neurofibromas, Lisch nodules, and axillary freckling. A wide variety of neuroectodermal, neuroendocrine and mesenchymal tumors have also been associated with NF1 as well as an increased risk of developing malignancies in general.[95]

A number of case reports and a few small series[96-98] have indicated an association between NF1 and GIST. In an autopsy series of NF1 patients from Göteborg, Sweden, GIST were incidentally detected in up to one-third of the patients.[95]

OBJECTIVES

The main objectives of this thesis were to further elucidate the phenotypic characteristics and pathogenetic mechanisms involved in sporadic as well as NF1-associated gastrointestinal stromal tumors.

The specific aims of the different studies in this thesis were:

I. to investigate the spectrum of *KIT* gene mutations as well as *KIT* isoform expression and chromosomal rearrangements in GIST in relationship to morphologic features and clinical behavior. (Paper I)

II. to study the effects of imatinib mesylate treatment in neoadjuvant, adjuvant and palliative clinical settings and to evaluate treatment response with respect to *KIT* mutational status. (Paper II)

III. to detect any possible correlation between different types of receptor tyrosine kinase mutations and phenotypic characteristics, risk group assessment and clinical course in a large population-based series of patients with GIST. (Paper III)

IV. to investigate the clinical and phenotypic characteristics of NF1-associated GIST and to determine mutational patterns with respect to *KIT* and *PDGFRA* in an effort to elucidate on their pathogenesis. (Paper IV)

MATERIALS AND METHODS

Tumor material

In this thesis, we have examined 259 GIST from 246 patients and 50 NF1-associated GIST from 15 NF1 patients.

Paper I: cDNA was prepared from fresh frozen tissue from 14 tumors (12 patients). Chromosome preparations were made from exponentially growing primary cultures from 10 tumors (8 patients).

Paper II: cDNA was prepared from fresh frozen tissue from 9 tumors (9 patients). Genomic DNA was extracted from formalin-fixed, paraffin-embedded tissue from 8 tumors (8 patients).

Paper III: Genomic DNA was extracted from formalin-fixed, paraffin-embedded tissue from 245 tumors (233 patients).

Paper IV: Molecular analyses were performed on genomic DNA extracted from paraffin-embedded tissue of 24 tumors (12 patients). In three patients, no material was available for molecular analyses. Immunohistochemical analyses were performed on 50 tumors (15 patients).

Methods

Denaturating high performance liquid chromatography (dHPLC)

Denaturating high performance liquid chromatography (dHPLC) is based on ion paired reverse phase chromatography. A stationary phase contained in the column is used to retain samples and a mobile phase is used to release the sample from the column. In dHPLC, the stationary phase usually consists of divinyl benzene (DVB). The mobile phase is an aqueous buffer with a mixture of acetonitrile and triethylammonium amine (TEAA). By increasing the concentration of acetonitrile, the DNA is released from the column. In contrast to regular HPLC, dHPLC is carried out at temperatures where the DNA is partially denaturated.

In wild type DNA, the alleles form two homoduplexes. In samples with mutations, on the other hand, there is a mismatch and both homoduplexes and heteroduplexes are formed. Heteroduplexes have a weaker affinity for the stationary phase than homoduplexes and are therefore eluted from the column at lower concentrations of the mobile phase. Figure 4 shows a dHPLC chromatogram illustrating heteroduplexes giving rise to two extra peaks.

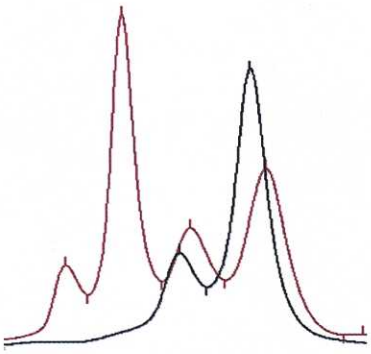


Figure 4. dHPLC chromatogram showing one wild type and one mutated sample. Two homoduplexes are present in the wild type sample, shown in green. In the mutated sample, shown in red, two heteroduplexes, containing a sequence mismatch, are also present, giving rise to the two additional peaks in the chromatogram.

The major advantages of using dHPLC to screen for mutations are that: (1) it is the most sensitive method for detecting mutations,[99] since samples with <10% mutant DNA can be detected; (2) it is a fast, cost effective, technique; and (3) it predicts the type of mutation. The major disadvantage is that it is a qualitative technique and sequencing must be performed in order to identify the mutated sequence.

Immunohistochemical, molecular genetic, cytogenetic, and statistical methods

The other immunohistochemical, molecular genetic and cytogenetic techniques, as well as statistical methods used in this thesis, including immunohistochemistry, PCR, cloning, nucleotide sequencing, G-banding and spectral karyotyping, are described in more detail in papers I-IV or in references therein and will not be commented on further here.

RESULTS AND DISCUSSION

The complexity of *KIT* gene mutations and chromosomal rearrangements and their clinical correlation in gastrointestinal stromal tumors (paper I)

KIT exon 11 mutations and biologic behavior

There have been conflicting reports regarding the effect of gain-of-function mutations in *KIT* on the biologic behavior of GIST. While some investigators have reported a strong correlation between mutations and a more malignant phenotype,[47-50] others have not been able to confirm such a correlation.[51,52]

In paper I, we analyzed the mutational status of the *KIT* gene in 14 GIST from 12 patients. Typical gain-of-function mutations were detected in one benign tumor, in one borderline tumor, and in eight malignant tumors. The reported S715del is now known to be an alternative splice form of *KIT*, not a true mutation. In the four malignant GIST that lacked exon 11 mutations, no further mutations were found after analysis of the entire coding sequence of *KIT*. Our results indicate that the correlation between *KIT* mutations and biological behavior is more complex than previously reported and that alternative mechanisms driving tumor development exist.

Subsequent studies have shown that mutations also occur in exons 9, 13, and 17 of the *KIT* gene as well as in exons 12 and 18 of the closely related *PDGFRA* gene in GIST lacking *KIT* exon 11 mutations.[66,67] The exon 9 mutations have been reported to occur particularly in small intestinal GIST and to have an aggressive behavior.[100,101] Mutations in *PDGFRA* seem to be associated with gastric GIST of low malignant potential that frequently lack KIT immunoreactivity.[66-69] These findings led us to further investigate the correlation between specific mutations types and clinical behaviors (paper III).

Cytogenetic and SKY analyses

A limited number of cytogenetic and molecular cytogenetic studies have been reported describing chromosomal rearrangements in GIST. Complete or partial loss of chromosomes 14, 22 and 1p seems to be the most common abnormalities. Using traditional G-banding as well as spectral karyotyping (SKY analysis) for the first time in selected cases, we could confirm complete or partial losses of chromosomes 1, 14 and 22 as being the most common rearrangements in GIST. Loss of chromosome 1p was detected in 2 tumors, loss of chromosome 14 in 2 tumors, and loss of chromosome 22 in 5 tumors. We were not able to detect any correlation between chromosomal aberrations and *KIT* mutational status.

Later studies have indicated that loss of chromosomes 14 and 22 are very common in both benign and malignant GIST.[52,66,77-81,83] Deletions of 1p and 9p are rarer and associated with malignant behavior,[66,77,83-85] indicating that putative tumor suppressor genes are encoded in these regions.

GIST preferentially express the shorter, tumorigenic, isoform of exon 9 of the KIT transcript

Alternative splicing in the 3´-end of exon 9 of the *KIT* transcript gives rise to two isoforms of the KIT protein, GNNK+ and GNNK-. The shorter isoform, GNNK-, has been shown to induce malignant transformation when transfected into NIH3T3 cells.[102] Preferential expression of the shorter isoform has been observed in a number of neoplasms including acute myelogenous leukemia[103] and human germ cell tumors.[45]

In paper I, cDNA was extracted from fresh frozen tumor tissue, enabling us to analyze the expression profile of the two isoforms in GIST. All 13 analyzed tumors showed a preferential expression of the shorter isoform. The expression pattern of normal ICC has not been analyzed; hence the biologic significance of the overexpression is unknown. Similar overexpression in other malignancies suggests, however, that it may play a role in the tumorigenesis of GIST, particularly in those tumors that lack activating *KIT* mutations; overexpression of the shorter isoform could serve as an alternative mechanism for increased KIT signaling. Further studies of larger series will be needed to confirm possible clinical correlations.

Neoadjuvant, adjuvant and palliative treatment of gastrointestinal stromal tumors (GIST) with imatinib: a centre-based study of 17 patients (paper II)

Gastrointestinal stromal tumors (GIST) are virtually resistant to conventional chemotherapy and radiation. Surgery has been the mainstay of treatment for malignant GIST, but surgery alone is often inadequate.[70] Imatinib mesylate, an inhibitor of type III receptor tyrosine kinases, was originally developed to inhibit the tyrosine kinase ABL in the BCR-ABL fusion protein, driving tumorigenesis in chronic myelogenous leukemia. Lately, imatinib has been shown to have a dramatic antitumor effect in GIST, since it inhibits not only ABL, but also the closely related KIT and PDGFRα tyrosine kinase receptors.[71] Earlier reports have indicated that specific mutation sites influence response to imatinib treatment. Preliminary studies had indicated that GIST patients with exon 11 mutations may respond better to imatinib treatment than patients whose tumors have other types of mutations or wild type *KIT*.

In a center-based study of 17 consecutive patients with high risk or overtly malignant GIST, we analyzed the effectiveness of imatinib in correlation to *KIT* exon 11 mutations. Imatinib was used in three different clinical settings – neoadjuvantly, adjuvantly, and palliatively. Clincal response to imatinib treatment correlated morphologically with tumor necrosis, fibrosis and reduced proliferative activity within the GIST. Typical gain-of-function mutations in exon 11 of the *KIT* gene were found in 12 of 17 patients. Missense mutations alone were found in 2 patients and deletions in 10 patients (in three patients in combination with missense mutations). Eight of 9 patients that had a partial response to imatinib had mutations. In contrast, partial response was only seen in one of the patients whose GIST lacked mutations. A *KIT* exon 9 mutation was later detected in this patient, a finding that may explain the partial treatment response since GIST with this mutation have been shown to respond to imatinib mesylate. None of the patients with stable or progressive disease had GIST carrying *KIT* exon 11 mutations.

Subsequently, treatment response has been shown to clearly correlate with the location of the mutation in *KIT* or *PDGFRA*.[75] GIST with *KIT* exon 11 mutations have the best treatment response, while certain tyrosine kinase domain mutations (*KIT* exon 17 or *PDGFRA* exon 18) render the tumors resistant to imatinib. Thus, mutation screening of GIST has become an essential part of the clinical work up of patients.

Gastrointestinal stromal tumors with *KIT* exon 11 deletions are associated with poor prognosis (paper III)

Gain-of-function mutations in the *KIT* and more rarely the *PDGFRA* genes have been shown to play a critical oncogenic role in the majority of GIST. Mutations are most common in the juxtamembrane domain of KIT, encoded by exon 11. Clinical studies regarding the correlation of exon 11 mutations with disease outcome have been contradictory. While some investigators[47,48,50,104,105] have indicated that GIST with exon 11 *KIT* mutations have a more aggressive course then those with wild type *KIT*, others have not.[51,52,106] Most previous studies have lumped together the different mutation types and compared them with tumors having wild type *KIT*. Some earlier studies have, however, indicated that different types of mutations may be associated with distinctive phenotypic features and possibly also clinical behavior.[100,107]

In a large population-based series of 246 GIST (from 233 patients) with long term follow-up, we determined if the mutation type among GIST with *KIT* and *PDGFRA* mutations correlated with tumor phenotype, risk group assessment and clinical course. Wild type (WT) *KIT* and *PDGFRA* were detected in 102/233 (44%) patients. *KIT* exon 11 mutations were detected in 117/233 (50%) patients; deletions were detected in 69 (30%) patients (4 of these also had a missense mutation); missense mutations were detected in 27 (12%), and duplications in 21 (9%). *KIT* exon 9 mutations were detected in 7 (7/233, 3%) patients, *PDGFRA* exons 12 and 18 mutations in 3 patients each (6/233, 2.6%), and *KIT* exon 17 mutation in one patient (1/233, 0.4%). No mutations were detected in *KIT* exon 13.

GIST with *KIT* exon 11 deletions were significantly larger in size and showed significantly more prominent histological atypia than WT tumors. Those GIST with exon 11 deletions also had a significantly higher proportion of tumors belonging to the high risk or overtly malignant risk groups and, correspondingly, a significantly greater proportion of tumors with a risk score >7 (tumor size (cm) + Ki67 max (%)) compared to the WT tumors. The deletion subgroup also had a significantly decreased disease-free survival (Fig. 5).

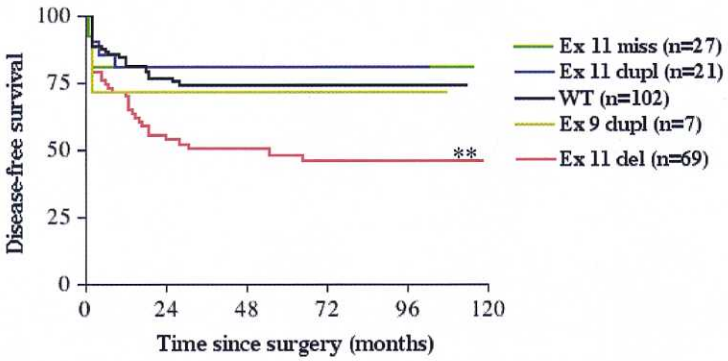


Figure 5. Kaplan-Meier estimates of disease-free survival among 226 GIST patients with different types of *KIT* exon 11 mutations, *KIT* exon 9 duplications and WT *KIT* and *PDGFRA*. ** p=0.005 between patients with exon 11 deletions and patients with WT tumors. P values determined by log-rank test.

Using a hazard function analysis, the independent influence of *KIT* exon 11 deletions on disease-free survival was determined. The simultaneous influence of tumor size and proliferative index were corrected for by setting identical sizes and proliferative index values. Disease-free survival was significantly worse in GIST patients with exon 11 deletions compared to GIST patients with WT and all other types of mutations combined (p=0.0358). Disease-free survival in GIST patients with exon 11 deletions compared to those with WT tumors alone was nearly significant (p=0.0788).

Internal tandem duplications in exon 11 of the *KIT* gene have previously been observed in a small subgroup of GIST. These mutations cluster in the 3´-end of the exon and have been reported to be associated with gastric location, a female predominance, and usually follow a benign clinical course. In this series, we found a higher proportion of tumors with duplications (21/233, 9%) than previous reports. We did not observe any gender preference. In contrast to other reports, almost one-third of the tumors belonged to the high risk or overtly malignant risk groups and 19% of the patients died of GIST. In conclusion, duplications may be more frequent and the clinical outcome poorer than previously recognized.

Similar to previous reports,[100,101] all 7 GIST in our series with *KIT* exon 9 mutations occurred in the small intestine. In contrast, however, we did not find the clinical course in this small group of patients to be clearly worse than GIST with WT *KIT* and *PDGFRA*. Five of the 6 patients with GIST having *PDGFRA* mutations belonged to the very low, low or intermediate risk groups and had benign clinical courses. Four of these were gastric and 3 were entirely or partially of epithelioid cell type. These findings correlate well with previous observations of this subset of GIST.[66,68,108,109] GIST with *PDGFRA* mutations frequently lack immunoreactivity for KIT and the relatively low number of mutations detected in this series, particularly with regard to exon 18, may be due to the fact that immunoreactivity for KIT was used as a diagnostic criteria in the selection of cases for this population-based study.

In conclusion, this study demonstrates that in addition to phenotypic and prognostic differences between GIST involving different receptor tyrosine kinase genes and different exon mutations, there are different subgroups of mutations within the same exon. The underlying reasons for the biological differences between GIST with different types of *KIT* exon 11 mutations remains unclear. The juxtamembrane domain in *KIT* (exon 11) normally functions to inhibit receptor dimerization in the absence of ligand. Thus, a possible explanation could be that deletions, which are clustered in the 5´-end of the exon, particularly disrupt this function.

NF1 associated gastrointestinal stromal tumors have unique clinical, phenotypic, and genotypic characteristics (paper IV)

There are several reports in the literature indicating that the association between NF1 and GIST is more than mere chance.[96-98,110-112] A high incidence of GIST among NF1 patients has been shown in a prospective 12-year follow-up study of 70 NF1 patients; 12 of these patients were autopsied and incidental GIST were detected in 4 (33%). We studied the association between GIST and NF1 from another perspective by reviewing a consecutive series of 100 GIST patients (scrutinized for NF1) and found 5 NF1 patients. This suggests that patients with GIST carry a 150-fold increased risk of having NF1 (normal incidence approximately 1:3000). The frequent occurrence of GIST among NF1 patients suggests that NF1 patients, particularly those presenting with gastrointestinal bleeding, anemia, abdominal pain or a palpable abdominal mass, should be clinically investigated for GIST.

A total of 50 macroscopically detected NF1-associated GIST from 15 NF1 patients were histologically reviewed and immunohistochemically analyzed. We found that NF1-associated GIST have a unique phenotype. In addition to a propensity for multicentricity within the gastrointestinal tract, detected in 9/15 patients (3 to >100 tumors), their distribution deviates from that of sporadic GIST. The 15 NF1 patients in this series had GIST involving the jejunum and/or ileum and 4 had duodenal tumors; only 4 had gastric tumors and all of these were also associated with small intestinal tumors. The NF1-associated GIST had a predominantly spindle cell morphology. Only 3 of 50 histologically examined tumors in this series were partially of the epithelioid cell type; the remainders were entirely spindled. This is in contrast to sporadic GIST in which one-third of tumors are partly or entirely of the epithelioid type.[18] Also striking is the almost uniformly histological low-grade appearance of the NF-associated GIST. A unique finding in this series was the occurrence of focal or diffuse ICC hyperplasia in the vicinity of the primary tumor as well as within distant separate foci of the gastrointestinal wall. The striking ICC hyperplasia seen in our cases of NF1-associated GIST is also highly suggestive of a precursor lesion for GIST.

Activating, oncogenic *KIT* mutations have been reported in 50-80% of sporadic GIST in most series.[21,32,113] Recently, one-third of GIST with wild type *KIT* have been found to have activating *PDGFRA* mutations. *KIT* and *PDGFRA* mutations seem to be mutually exclusive.[66,67] In contrast, no such mutations were detected in *KIT* or *PDGFRA* in our series of 24 NF1-associated GIST. Thus, this study shows that NF1-associated GIST are genotypically distinct from sporadic GIST. This is the first large series unequivocally demonstrating the absence of *PDGFRA* mutations in NF1-associated GIST. Two

recently reported NF1-associated GIST with wild type *KIT* were also found to lack *PDGFRA* mutations.[98] Our findings further support the contention that the underlying pathogenesis of NF1-associated GIST differs from most sporadic GIST.

In summary, the incidence of GIST is markedly increased in NF1 patients. NF1-associated GIST are unique with regard to multicentricity, predominant location in the small bowel, spindle cell morphology and lack of *KIT* and *PDGFRA* mutations. In contrast to sporadic GIST, the vast majority of NF1-associated GIST have a favorable clinical course.

CONCLUSIONS

The main observations and conclusions of this thesis can be summarized as follows:

I. The role of *KIT* mutations in the biological behavior of GIST is more complex than previously recognized. A malignant phenotype can occur without activating mutations and mutations may occur in clinically benign tumors.

Losses of whole or parts of chromosomes are the most common cytogenetic abnormalities in GIST, suggesting that loss of multiple chromosomal regions, presumably containing putative tumor suppressor genes, is an important genetic event in GIST.

GIST preferentially express the shorter, tumorigenic isoform (GNNK-) of *KIT* exon 9. Preferential overexpression of this isoform could be significant in tumors lacking *KIT* and *PDGFRA* mutations and serve as an alternative mechanism for increased KIT signaling.

II. Imatinib mesylate is an effective treatment of malignant GIST, particularly for patients whose tumors harbor *KIT* exon 11 mutations.

III. The type of *KIT* exon 11 mutation influences phenotype, biological behavior and clinical outcome in GIST.

Patients with tumors having *KIT* exon 11 deletions have a significantly poorer prognosis than patients with tumors having other mutation types or wild type *KIT* and *PDGFRA*.

In addition to tumor size and proliferative index, *KIT* exon 11 deletion is an independent adverse prognostic factor in GIST.

IV. There is a strong association between GIST and NF1.

NF1 associated GIST represent a unique subset of GIST that typically are multicentic, of low malignant potential and appears to originate from hyperplastic foci of interstitial cells of Cajal (ICC). The lack of activating mutations in the *KIT* and *PDGFRA* genes in NF1 associated GIST indicates that different pathogenetic mechanisms are involved.

FUTURE PERSPECTIVES

Gene expression analyses

The completion of the human genome (HUGO) project and recent important technical breakthroughs have made large scale gene expression profiling feasible. Recently, distinct gene expression profiles have been detected in GIST with *KIT* and *PDGFRA* mutations.[114] These findings may be of clinical importance and may lead to the development of highly selective therapeutic targets. Similarly, studies of gene expression profiles in GIST with different subtypes of *KIT* exon 11 mutations could potentially help to explain the prognostic differences among GIST subgroups and be of future therapeutic importance.

Using oligo microarray, we will analyze the expression profiles of different subgroups of GIST. These subgroups include tumors of different risk groups; tumors with or without mutations in the *KIT* and *PDGFRA* genes; tumors with mutations in different exons of *KIT* or *PDGFRA*; and tumors with different types of mutations in exon 11 of the *KIT* gene. Our aim is to gain further understanding of the pathogenetic mechanisms involved in the development and progression of GIST.

NF1 gene alterations

NF1 associated GIST are genotypically and phenotypically distinct from sporadic GIST, suggesting that the pathogenetic mechanisms underlying tumor development differ. There is a connection between *KIT/PDGFRA* and *NF1*. Neurofibromin, encoded by the *NF1* gene, is a negative regulator of RAS. RAS is involved in the MAPK signaling pathway that can be triggered by activation of KIT or PDGFRα. High levels of KIT expression have also been detected in neurofibromin-deficient Schwann cells derived from a neurofibroma of an NF1-patient.[115] This is in contrast to non-NF1 associated Schwann cells, which do not express KIT.

In paper IV, we examined the mutation status of the *KIT* and *PDGFRA* genes in a rather large series of NF1 associated GIST. Using the same NF1-associated as well as sporadic GIST, we will expand the study and investigate the *NF1* gene for mutations or other aberrations such as loss of heterozygosity (LOH) with the aim to identify pathogenetic mechanisms involved in tumor development and progression in this subset of GIST. The possibility of somatic NF1 mutations in sporadic GIST will also be explored.

Splice isoform expression

The shorter isoform of *KIT* exon 9 has been shown to be tumorigenic.[102] Preferential overexpression of this isoform is seen in human malignancies such as myelogenous leukemia[103] and human germ cell tumors.[45] In paper I, we reported the same expression profile in GIST. The biological significance is unknown, but it may serve as an alternative mechanism for KIT activation in GIST that lack activating mutations. We would like to extend the analysis of the expression pattern of the two isoforms using a large series of GIST, including tumors of all risk groups and tumors with different mutational status, in

order to further elucidate the biological and clinical relevance of the preferential isoform expression.

Chromosomal rearrangements

Losses of parts of or whole chromosomes frequently occur in GIST. Loss of 14 and 22 are the most common and are believed to be early events in the tumor development. Loss of 1p and 9, in contrast, occur later and have been associated with malignant behavior, indicating that the deleted regions may contain putative tumor suppressor genes.

The INK4a locus, encoding the $p16^{INKK4a}$ and the $p14^{ARF}$ genes on chromosome 9p21, is frequently inactivated in malignant tumors. Using our unique population-based material, we will study losses of chromosomes or parts of chromosomes in GIST. In particular, the study will focus on loss of chromosome 9p, encoding the INK4a locus, since deletions of this region have been reported to be associated with malignant behavior. Our aim is to further elucidate receptor tyrosine kinase mutations and pathogenetic mechanisms involved in the progression of GIST.

Mutation type specific treatment response to imatinib mesylate in GIST

Imatinib mesylate has been shown to be an effective drug treatment for patients with malignant GIST. The response differs, however, with regard to the mutation status of the *KIT* or *PDGFRA* genes. Tumors with mutations in *KIT* exon 11 are more responsive to imatinib mesylate than tumors with other mutations or with WT *KIT* and *PDGFRA*. Some mutations (in *KIT* exon 17 and *PDGFRA* exon 18) render GIST resistant to drug treatment with imatinib mesylate.

In paper III, we showed that not only the location of the mutation, but also the type of mutation within the exon influence the biological behaviour of GIST. Tumors with *KIT* exon 11 deletions are associated with a decreased disease-free survival. Whether the effectiveness of imatinib mesylate treatment varies within subgroups of GIST with *KIT* exon 11 mutations – deletions, missense mutations and duplications - is unclear. Information regarding the possible correlation between mutation type status and treatment response could be of value in identifying which patients will benefit most from treatment with imatinib mesylate.

POPULÄRVETENSKAPLIG SAMMANFATTNING

Under de senaste decennierna har det blivit klarlagt att cancer är en genetisk sjukdom. Ca 90 % av alla tumörer orsakas av att fel uppstått i en eller, i de flesta fall, flera gener. Människans arvsmassa (DNA) består av ca 25000 gener. Generna kodar för proteiner som bygger upp och utför cellens processer. Cellers tillväxt (proliferation) är normalt strikt kontrollerad, men ibland brister denna kontroll och en tumör (cancer) kan börja utvecklas. En cancercell är en från början normal cell, som genom en ansamling av fel i arvsmassan fått förmågan att växa okontrollerat och invadera omgivande vävnad (metastasera). Dessa fel, s.k. mutationer, drabbar ofta gener som har som funktion att kontrollera proliferation, apoptos (celldöd) eller att reparera skador i arvsmassan. Det finns tre huvudtyper av mutationer; deletioner - som innebär att en del av genen försvunnit, substitutioner - som innebär att en byggsten i DNA bytts ut mot en annan och duplikationer - som innebär att en del av genen dubblerats.

Tumörer som uppstår i ben och mjukdelar kallas sarkom. Till gruppen sarkom räknas gastrointestinala stromacellstumörer (GIST), som denna avhandling fokuserar på. GIST är den vanligaste formen av gastrointestinala sarkom och uppkommer oftast i magsäcken, men förekommer i hela mag-tarmkanalen. Ungefär 15 individer per en miljon innevånare och år drabbas av GIST. Den är en relativt heterogen tumörtyp och det kliniska förloppet varierar kraftigt mellan olika patienter. Ungefär en tredjedel av tumörerna är små och långsamväxande, de ger sällan symptom utan upptäcks ofta av en slump i samband med andra undersökningar. Prognosen för patienter med denna typ av GIST är relativt god. Resterande två tredjedelar är stora och snabbväxande tumörer som oftast har en dålig eller mycket dålig prognos. Vad som orsakar denna variation är inte helt klarlagt.

Det huvudsakliga syftet med denna avhandling har varit att studera dels hur vanliga mutationer är i två olika gener i GIST, dels hur dessa mutationer påverkar det biologiska beteendet hos tumörerna. Generna, som heter *KIT* och *PDGFRA* (platelet derived growth factor receptor alpha), fungerar som en slags gaspedal för cellen, som när de aktiveras får signaler att börja dela sig. Gener av denna typ kallas proto-onkogener. I många olika typer av tumörer är dessa gener muterade, vilket leder till att de blir överaktiva och övergår till att bli onkogener.

Tidiga studier av den biologiska betydelsen av mutationer i GIST har varit motsägelsefulla. Vissa grupper har visat ett starkt samband mellan mutationer och dålig prognos, men andra har inte kunnat verifiera detta. I vårt första arbete studerade vi *KIT* genen i 14 GIST och fann att 9 av dessa hade mutationer. Vi fann mutationer i benigna såväl som i flera av de maligna tumörerna. Det fanns även maligna tumörer som helt saknade mutationer. Våra resultat tyder på att sambandet mellan mutationer och malignt beteende är mer komplex än man tidigare trott.

I det tredje arbetet fortsatte vi studera betydelsen av mutationer i *KIT* och *PDGFRA* i GIST. Materialet bestod av 233 GIST patienter och det är en av de största serier som undersökts samt den första populations-baserade studien av GIST. Vi fann att drygt hälften av patienterna hade mutationer; vanligast var deletioner i en specifik del av *KIT* genen (exon 11). För första gången kunde vi visa att tumörer med deletioner har en signifikant sämre prognos än tumörer med andra mutationstyper eller tumörer som saknar

mutationer. Tumörer med deletioner hade högre tillväxt och var signifikant större. Tumörstorlek och proliferationshastighet har tidigare visats vara oberoende prognostiska faktorer för GIST. I denna studie kunde vi visa att även deletioner i *KIT* exon 11 är en oberoende faktor för dålig prognos, oavsett tumörstorlek och proliferationshastighet.

GIST är i stort sett helt resistenta mot strålning och kemoterapi (cellgifter) och den enda behandlingsformen har länge varit kirurgi. Tyvärr kan man inte operera alla patienter, beroende på tumörens utbredning, och prognosen för dessa var tidigare mycket dålig. De senaste åren har detta kommit att ändrats sedan ett nytt läkemedel, imatinib mesylat (Glivec), introducerats. Imatinib mestylat inaktiverar onkogenerna KIT och PFGFR_ och resultatet blir att tumörens tillväxt avstannar. I vårt andra arbete studerade vi om det fanns ett samband mellan behandlingssvar och förekomst av mutationer i *KIT* i en serie av 17 GIST patienter. Vi kunde visa att så är fallet; det är de patienter som har mutationer i exon 11 som svarar bäst på behandling. Åtta av de 9 patienter som svarade bra hade mutationer, men det hade ingen av de patienter som inte svarade på behandlingen.

Neurofibromatos typ I (NF1) är en av de vanligaste ärftliga sjukdomarna. Patienter med NF1 drabbas oftare än normalbefolkningen av olika tumörer, bl.a. GIST. I det sista arbetet studerade vi GIST som uppstår hos NF1 patienter och jämförde dem med sporadiska GIST, d.v.s. från patienter som inte har NF1. Materialet bestod av 50 GIST tumörer från 15 NF1 patienter, och är en av de största serier som publicerats. Våra resultat visar att NF1 associerade GIST är en unik undergrupp av GIST. Patienterna har ofta många tumörer som framför allt uppkommer i tunntarm och det kliniska förloppet är godartat. Även genetiskt skiljer sig NF1 associerade GIST från sporadiska GIST i det att de saknar mutationer i *KIT* och *PDGFRA*. Detta tyder på att de mekanismer som driver tumörutvecklingen är annorlunda.

Vi har visat att den biologiska betydelsen av *KIT* mutationer i GIST är mer komplex än vad man tidigare trott och att mutationstypen påverkar kliniskt beteende och behandlingssvar med imatinib mesylat. NF1 associerade GIST är en unik undergrupp av GIST som saknar *KIT* och *PDGFRA* mutationer och har ett benignt kliniskt förlopp.

ACKNOWLEDGEMENTS

For me it is a dream come true to be involved in cancer research! These years have been the most stimulating and rewarding time of my life and I would like to express my deepest gratitude to all of you who, in one way or another, have helped me fulfill this dream:

My supervisors *Lars-Gunnar Kindblom* and *Jeanne Meis-Kindblom*, for giving me the opportunity to explore the fascinating world of science and tumor biology in particular. Thank you also for your warmth, support and guidance and above all for your never ending enthusiasm - it's contagious! I truly enjoy working with you!

My assistant supervisor *Bengt Nilsson* and also *Per Bümming*, for stimulating discussions and for the clinical perspective. Thank you for all the nice times, whenever, wherever… You make me smile!

My initial supervisor *Pierre Åman*, for generously sharing your knowledge about molecular biology in practice. Thank you also for amusing stories and all the delicious (and some not so delicious?) cookies.

Håkan Ahlman, *Katarina Engström*, *Bengt Gustafsson*, *Svante Jansson*, *Hans Klingenstierna*, *Göran Stenman*, *Ulrika Stierner*, and *Bo Wängberg*, for co-authorship.

Co-workers and friends in the Kindblom-lab: *Gabriella Arne*, *Anna-Carin Ericson*, *Carina Karlsson*, and *Sibylle Widéhn*, for your willingness to always lend a hand, for your support and engagement (in my research and beyond ☺) and most importantly for a million laughs.

Former co-workers in the Åman-lab: *Carola Andersson* (for never letting go of the rope), *Anita Olofsson*, *Annika Krona* (☺), *Melker Göransson* (I miss the jokes), *Agnetha Josefsson*, *Mattias Andersson*, *Christina Kåbjörn* (for all the great partys), *Erik Elias*, and *Lisbeth Gustafsson*, for help whenever needed and for your encouragement and support.

My co-workers at Biomedicum Helsinki for fruitful collaboration and co-authorship; *Heikki Joensuu*, for generously welcoming me in your lab; *Nina Nupponen*, for sharing your knowledge about dHPLC and Sequencher and for all the nice e-mails and chats; *Harri Sihto*, for invaluable help on various projects and for all the fun, special moments. Kiitos!

Past and present PhD-students and Postdocs at the Lundberg Laboratory for Cancer Research, especially; *Anna-Karin Ström*, *Fredrik Enlund* (for clarifying things), *Fredrik Persson*, *Helene Sjögren* (for nice chats – most often about anything but cancer research), and *Marta Winnes*, for your help and support and for all the fun times.

All colleagues at the Lundberg Laboratory for Cancer Research, in particular *Barbro Wedell*, *Ywonne Andrén*, and *Ellinor Andersson*, for sharing knowledge about methods and apparatus, and for making LLCR such a nice place to work at.

All colleauges and staff at the Department of Pathology, especially *Ali-Reza Mozlemi* and *Gittan Kollberg* for help with sequencing; *Kerstin Grahn*, *Gunilla Möllmark*, and *Birgitta Börjesson* for secretarial assistance; *Ulla-Britt Strandlund*; and the computer wizards *Ulric Pedersen* and *Ivan Novak*.

The *Levan-lab*, especially *Fredrik Trossö* (always inspiring me to reach for the top) and *Tatjana Adamovic*, for being such nice PhD-room companions and for good times during coffeebreaks.

The KIF-crew; *Enn Päärt*, *Per Karlsson*, and *Owe Fredholm*, for interesting courses and seminars and for all you help and support.

Bo Jämterud - you are one of the best teachers I've ever had.

Peter Abrahamsson (NOT an annoying sales representative after all ☺), for nice lunches with lots of crème brulle.

My best friends *Eva Eriksson* and *Karin Sundholm*, for ...everything. You are simply the best!

All my other *wonderful friends*, no one mentioned and no one forgotten, for support and for making my life brighter and a lot more fun. You mean the world to me!

My precious family; *Bellsa*, *Terry*, *Rikard*, *Jewelia*, and *Skye*, for love and support and for giving me many "highlights of the day"; my mum *Agneta* and dad *Sören*, for unconditional love, encouragement, and for always believing in me. I love you!

This work was supported by grants from the Swedish Cancer Society, the IngaBritt and Arne Lundberg Research Foundation, the Johan Jansson Foundation for Cancer Research, the Assar Gabrielsson Research Foundation, the King Gustav V Jubilée Clinic Cancer Research Foundation, the Sahlgrenska University Hospital Foundations and the Willhelm and Martina Lundgren Research Foundation.

Wherever you are it is your friends who make your world.
/William Jones

REFERENCES

1. Bishop JM. The molecular genetics of cancer. *Science 1987;235:305-311.*
2. Weinberg RA. Oncogenes, antioncogenes, and the molecular bases of multistep carcinogenesis. *Cancer Res 1989;49:3713-3721.*
3. Klein G, Klein E. Evolution of tumours and the impact of molecular oncology. *Nature 1985;315:190-195.*
4. Hanahan D, Weinberg RA. The hallmarks of cancer. *Cell 2000;100:57-70.*
5. Bishop JM. Molecular themes in oncogenesis. *Cell 1991;64:235-248.*
6. Knudson AG, Jr. Mutation and cancer: statistical study of retinoblastoma. *Proc Natl Acad Sci U S A 1971;68:820-823.*
7. Lane DP. Cancer. p53, guardian of the genome. *Nature 1992;358:15-16.*
8. Levine AJ. The tumor suppressor genes. *Annu Rev Biochem 1993;62:623-651.*
9. Haffner R, Oren M. Biochemical properties and biological effects of p53. *Curr Opin Genet Dev 1995;5:84-90.*
10. Cooper GM. The cell: A molecular approach. Washington, D.C.: ASM Press, 1997:612-628.
11. Kinzler KW, Vogelstein B. Cancer-susceptibility genes. Gatekeepers and caretakers. *Nature 1997;386:761, 763.*
12. Roth DB, Gellert M. New guardians of the genome. *Nature 2000;404:823-825.*
13. Lehmann AR. Nucleotide excision repair and the link with transcription. *Trends Biochem Sci 1995;20:402-405.*
14. Martin NM. DNA repair inhibition and cancer therapy. *J Photochem Photobiol B 2001;63:162-170.*
15. Barclay TH, Schapira DV. Malignant tumors of the small intestine. *Cancer 1983;51:878-881.*
16. Neugut AI, Jacobson JS, Suh S, Mukherjee R, Arber N. The epidemiology of cancer of the small bowel. *Cancer Epidemiol Biomarkers Prev 1998;7:243-251.*
17. Thomas RM, Sobin LH. Gastrointestinal cancer. *Cancer 1995;75:154-170.*
18. Nilsson B, Bumming P, Meis-Kindblom JM, Oden A, Dortok A, Gustavsson B, Sablinska K, Kindblom LG. Gastrointestinal stromal tumors: the incidence, prevalence, clinical course, and prognostication in the preimatinib mesylate era--a population-based study in western Sweden. *Cancer 2005;103:821-829.*
19. Pidhorecky I, Cheney RT, Kraybill WG, Gibbs JF. Gastrointestinal stromal tumors: current diagnosis, biologic behavior, and management. *Ann Surg Oncol 2000;7:705-712.*
20. Miettinen M, Lasota J. Gastrointestinal stromal tumors--definition, clinical, histological, immunohistochemical, and molecular genetic features and differential diagnosis. *Virchows Arch 2001;438:1-12.*
21. Corless CL, McGreevey L, Haley A, Town A, Heinrich MC. KIT mutations are common in incidental gastrointestinal stromal tumors one centimeter or less in size. *Am J Pathol 2002;160:1567-1572.*
22. Fletcher CD, Berman JJ, Corless C, Gorstein F, Lasota J, Longley BJ, Miettinen M, O'Leary TJ, Remotti H, Rubin BP, Shmookler B, Sobin LH, Weiss SW. Diagnosis of gastrointestinal stromal tumors: A consensus approach. *Hum Pathol 2002;33:459-465.*

23. Appelman HD. Smooth muscle tumors of the gastrointestinal tract. What we know now that Stout didn't know. *Am J Surg Pathol 1986;10:83-99.*
24. Appelman HD. Mesenchymal tumors of the gastrointestinal tract. In: Ming S-C, Goldman H, eds. Pathology of the Gastrointestinal Tract. Philadelphia: WB Saunders, 1992:310-350.
25. Evans HL. Smooth muscle tumors of the gastrointestinal tract. A study of 56 cases followed for a minimum of 10 years. *Cancer 1985;56:2242-2250.*
26. Mazur MT, Clark HB. Gastric stromal tumors. Reappraisal of histogenesis. *Am J Surg Pathol 1983;7:507-519.*
27. Herrera GA, Pinto de Moraes H, Grizzle WE, Han SG. Malignant small bowel neoplasm of enteric plexus derivation (plexosarcoma). Light and electron microscopic study confirming the origin of the neoplasm. *Dig Dis Sci 1984;29:275-284.*
28. Walker P, Dvorak AM. Gastrointestinal autonomic nerve (GAN) tumor. Ultrastructural evidence for a newly recognized entity. *Arch Pathol Lab Med 1986;110:309-316.*
29. Herrera GA, Cerezo L, Jones JE, Sack J, Grizzle WE, Pollack WJ, Lott RL. Gastrointestinal autonomic nerve tumors. 'Plexosarcomas'. *Arch Pathol Lab Med 1989;113:846-853.*
30. Kindblom LG, Remotti HE, Aldenborg F, Meis-Kindblom JM. Gastrointestinal pacemaker cell tumor (GIPACT): gastrointestinal stromal tumors show phenotypic characteristics of the interstitial cells of Cajal. *Am J Pathol 1998;152:1259-1269.*
31. Sircar K, Hewlett BR, Huizinga JD, Chorneyko K, Berezin I, Riddell RH. Interstitial cells of Cajal as precursors of gastrointestinal stromal tumors. *Am J Surg Pathol 1999;23:377-389.*
32. Hirota S, Isozaki K, Moriyama Y, Hashimoto K, Nishida T, Ishiguro S, Kawano K, Hanada M, Kurata A, Takeda M, Muhammad Tunio G, Matsuzawa Y, Kanakura Y, Shinomura Y, Kitamura Y. Gain-of-function mutations of c-kit in human gastrointestinal stromal tumors. *Science 1998;279:577-580.*
33. Faussone-Pellegrini MS. Histogenesis, structure and relationships of interstitial cells of Cajal (ICC): from morphology to functional interpretation. *Eur J Morphol 1992;30:137-148.*
34. Huizinga JD, Thuneberg L, Kluppel M, Malysz J, Mikkelsen HB, Bernstein A. W/kit gene required for interstitial cells of Cajal and for intestinal pacemaker activity. *Nature 1995;373:347-349.*
35. Kluppel M, Huizinga JD, Malysz J, Bernstein A. Developmental origin and Kit-dependent development of the interstitial cells of cajal in the mammalian small intestine. *Dev Dyn 1998;211:60-71.*
36. Yarden Y, Kuang WJ, Yang-Feng T, Coussens L, Munemitsu S, Dull TJ, Chen E, Schlessinger J, Francke U, Ullrich A. Human proto-oncogene c-kit: a new cell surface receptor tyrosine kinase for an unidentified ligand. *Embo J 1987;6:3341-3351.*
37. Chabot B, Stephenson DA, Chapman VM, Besmer P, Bernstein A. The proto-oncogene c-kit encoding a transmembrane tyrosine kinase receptor maps to the mouse W locus. *Nature 1988;335:88-89.*
38. Geissler EN, Ryan MA, Housman DE. The dominant-white spotting (W) locus of the mouse encodes the c-kit proto-oncogene. *Cell 1988;55:185-192.*

39. Spritz RA, Strunk KM, Lee ST, Lu-Kuo JM, Ward DC, Le Paslier D, Altherr MR, Dorman TE, Moir DT. A YAC contig spanning a cluster of human type III receptor protein tyrosine kinase genes (PDGFRA-KIT-KDR) in chromosome segment 4q12. *Genomics 1994;22:431-436.*
40. Andre C, Martin E, Cornu F, Hu WX, Wang XP, Galibert F. Genomic organization of the human c-kit gene: evolution of the receptor tyrosine kinase subclass III. *Oncogene 1992;7:685-691.*
41. Ullrich A, Schlessinger J. Signal transduction by receptors with tyrosine kinase activity. *Cell 1990;61:203-212.*
42. Williams DE, Eisenman J, Baird A, Rauch C, Van Ness K, March CJ, Park LS, Martin U, Mochizuki DY, Boswell HS, et al. Identification of a ligand for the c-kit proto-oncogene. *Cell 1990;63:167-174.*
43. Fleischman RA. From white spots to stem cells: the role of the Kit receptor in mammalian development. *Trends Genet 1993;9:285-290.*
44. Nakata Y, Kimura A, Katoh O, Kawaishi K, Hyodo H, Abe K, Kuramoto A, Satow Y. c-kit point mutation of extracellular domain in patients with myeloproliferative disorders. *Br J Haematol 1995;91:661-663.*
45. Tian Q, Frierson HF, Jr., Krystal GW, Moskaluk CA. Activating c-kit gene mutations in human germ cell tumors. *Am J Pathol 1999;154:1643-1647.*
46. Longley BJ, Jr., Metcalfe DD, Tharp M, Wang X, Tyrrell L, Lu SZ, Heitjan D, Ma Y. Activating and dominant inactivating c-KIT catalytic domain mutations in distinct clinical forms of human mastocytosis. *Proc Natl Acad Sci U S A 1999;96:1609-1614.*
47. Ernst SI, Hubbs, A.E., Przygodzki, R.M., Emory, T.S., Sobin, L.H., O´Leary, T.J. KIT Mutation Portends Poor Prognosis in Gastrointestinal Stromal/Smooth Muscle Tumors. *Lab Invest 1998;78:1633-1636.*
48. Lasota J, Jasinski M, Sarlomo-Rikala M, Miettinen M. Mutations in exon 11 of c-Kit occur preferentially in malignant versus benign gastrointestinal stromal tumors and do not occur in leiomyomas or leiomyosarcomas. *Am J Pathol 1999;154:53-60.*
49. Moskaluk CA, Tian Q, Marshall CR, Rumpel CA, Franquemont DW, Frierson HF, Jr. Mutations of c-kit JM domain are found in a minority of human gastrointestinal stromal tumors. *Oncogene 1999;18:1897-1902.*
50. Taniguchi M, Nishida T, Hirota S, Isozaki K, Ito T, Nomura T, Matsuda H, Kitamura Y. Effect of c-kit mutation on prognosis of gastrointestinal stromal tumors. *Cancer Res 1999;59:4297-4300.*
51. Sakurai S, Fukasawa T, Chong JM, Tanaka A, Fukayama M. C-kit gene abnormalities in gastrointestinal stromal tumors (tumors of interstitial cells of Cajal. *Jpn J Cancer Res 1999;90:1321-1328.*
52. Andersson J, Sjogren H, Meis-Kindblom JM, Stenman G, Aman P, Kindblom LG. The Complexity of KIT Gene Mutations and Chromosome Rearrangements and Their Clinical Correlation in Gastrointestinal Stromal (Pacemaker Cell) Tumors. *Am J Pathol 2002;160:15-22.*
53. Lux ML, Rubin BP, Biase TL, Chen CJ, Maclure T, Demetri G, Xiao S, Singer S, Fletcher CD, Fletcher JA. KIT extracellular and kinase domain mutations in gastrointestinal stromal tumors. *Am J Pathol 2000;156:791-795.*
54. Lasota J, Wozniak A, Sarlomo-Rikala M, Rys J, Kordek R, Nassar A, Sobin LH, Miettinen M. Mutations in exons 9 and 13 of KIT gene are rare events in

gastrointestinal stromal tumors : A study of 200 cases. *Am J Pathol 2000;157:1091-1095.*

55. Hirota S, Nishida T, Isozaki K, Taniguchi M, Nakamura J, Okazaki T, Kitamura Y. Gain-of-function mutation at the extracellular domain of KIT in gastrointestinal stromal tumours. *J Pathol 2001;193:505-510.*
56. Isozaki K, Terris B, Belghiti J, Schiffmann S, Hirota S, Vanderwinden JM. Germline-activating mutation in the kinase domain of KIT gene in familial gastrointestinal stromal tumors. *Am J Pathol 2000;157:1581-1585.*
57. Rubin BP, Singer S, Tsao C, Duensing A, Lux ML, Ruiz R, Hibbard MK, Chen CJ, Xiao S, Tuveson DA, Demetri GD, Fletcher CD, Fletcher JA. KIT activation is a ubiquitous feature of gastrointestinal stromal tumors. *Cancer Res 2001;61:8118-8121.*
58. Matsui T, Heidaran M, Miki T, Popescu N, La Rochelle W, Kraus M, Pierce J, Aaronson S. Isolation of a novel receptor cDNA establishes the existence of two PDGF receptor genes. *Science 1989;243:800-804.*
59. Claesson-Welsh L, Eriksson A, Westermark B, Heldin CH. cDNA cloning and expression of the human A-type platelet-derived growth factor (PDGF) receptor establishes structural similarity to the B-type PDGF receptor. *Proc Natl Acad Sci U S A 1989;86:4917-4921.*
60. Claesson-Welsh L, Hammacher A, Westermark B, Heldin CH, Nister M. Identification and structural analysis of the A type receptor for platelet-derived growth factor. Similarities with the B type receptor. *J Biol Chem 1989;264:1742-1747.*
61. Gilbertson DG, Duff ME, West JW, Kelly JD, Sheppard PO, Hofstrand PD, Gao Z, Shoemaker K, Bukowski TR, Moore M, Feldhaus AL, Humes JM, Palmer TE, Hart CE. Platelet-derived growth factor C (PDGF-C), a novel growth factor that binds to PDGF alpha and beta receptor. *J Biol Chem 2001;276:27406-27414.*
62. Bergsten E, Uutela M, Li X, Pietras K, Ostman A, Heldin CH, Alitalo K, Eriksson U. PDGF-D is a specific, protease-activated ligand for the PDGF beta-receptor. *Nat Cell Biol 2001;3:512-516.*
63. Heldin CH. Structural and functional studies on platelet-derived growth factor. *Embo J 1992;11:4251-4259.*
64. Yeh HJ, Silos-Santiago I, Wang YX, George RJ, Snider WD, Deuel TF. Developmental expression of the platelet-derived growth factor alpha-receptor gene in mammalian central nervous system. *Proc Natl Acad Sci U S A 1993;90:1952-1956.*
65. Kumabe T, Sohma Y, Kayama T, Yoshimoto T, Yamamoto T. Amplification of alpha-platelet-derived growth factor receptor gene lacking an exon coding for a portion of the extracellular region in a primary brain tumor of glial origin. *Oncogene 1992;7:627-633.*
66. Heinrich MC, Corless CL, Duensing A, McGreevey L, Chen CJ, Joseph N, Singer S, Griffith DJ, Haley A, Town A, Demetri GD, Fletcher CD, Fletcher JA. PDGFRA activating mutations in gastrointestinal stromal tumors. *Science 2003;299:708-710.*
67. Hirota S, Ohashi A, Nishida T, Isozaki K, Kinoshita K, Shinomura Y, Kitamura Y. Gain-of-function mutations of platelet-derived growth factor receptor alpha gene in gastrointestinal stromal tumors. *Gastroenterology 2003;125:660-667.*

68. Lasota J, Dansonka-Mieszkowska A, Sobin LH, Miettinen M. A great majority of GISTs with PDGFRA mutations represent gastric tumors of low or no malignant potential. *Lab Invest 2004;84:874-883.*
69. Miettinen M, Sobin LH, Lasota J. Gastrointestinal Stromal Tumors of the Stomach: A Clinicopathologic, Immunohistochemical, and Molecular Genetic Study of 1765 Cases With Long-term Follow-up. *Am J Surg Pathol 2005;29:52-68.*
70. DeMatteo RP, Lewis JJ, Leung D, Mudan SS, Woodruff JM, Brennan MF. Two hundred gastrointestinal stromal tumors: recurrence patterns and prognostic factors for survival. *Ann Surg 2000;231:51-58.*
71. Buchdunger E, Cioffi CL, Law N, Stover D, Ohno-Jones S, Druker BJ, Lydon NB. Abl protein-tyrosine kinase inhibitor STI571 inhibits In vitro signal transduction mediated by c-Kit and platelet-derived growth factor receptors. *J Pharmacol Exp Ther 2000;295:139-145.*
72. Joensuu H, Roberts PJ, Sarlomo-Rikala M, Andersson LC, Tervahartiala P, Tuveson D, Silberman SL, Capdeville R, Dimitrijevic S, Druker B, Demetri GD. Effect of the Tyrosine Kinase Inhibitor STI571 in a Patient with a Metastatic Gastrointestinal Stromal Tumor. *N Engl J Med 2001;344:1052-1056.*
73. van Oosterom AT, Judson I, Verweij J, Stroobants S, Donato di Paola E, Dimitrijevic S, Martens M, Webb A, Sciot R, Van Glabbeke M, Silberman S, Nielsen OS. Safety and efficacy of imatinib (STI571) in metastatic gastrointestinal stromal tumours: a phase I study. *Lancet 2001;358:1421-1423.*
74. Demetri GD, von Mehren M, Blanke CD, Van den Abbeele AD, Eisenberg B, Roberts PJ, Heinrich MC, Tuveson DA, Singer S, Janicek M, Fletcher JA, Silverman SG, Silberman SL, Capdeville R, Kiese B, Peng B, Dimitrijevic S, Druker BJ, Corless C, Fletcher CD, Joensuu H. Efficacy and safety of imatinib mesylate in advanced gastrointestinal stromal tumors. *N Engl J Med 2002;347:472-480.*
75. Heinrich MC, Corless CL, Demetri GD, Blanke CD, von Mehren M, Joensuu H, McGreevey LS, Chen CJ, Van den Abbeele AD, Druker BJ, Kiese B, Eisenberg B, Roberts PJ, Singer S, Fletcher CD, Silberman S, Dimitrijevic S, Fletcher JA. Kinase mutations and imatinib response in patients with metastatic gastrointestinal stromal tumor. *J Clin Oncol 2003;21:4342-4349.*
76. Verweij J, Casali PG, Zalcberg J, LeCesne A, Reichardt P, Blay JY, Issels R, van Oosterom A, Hogendoorn PC, Van Glabbeke M, Bertulli R, Judson I. Progression-free survival in gastrointestinal stromal tumours with high-dose imatinib: randomised trial. *Lancet 2004;364:1127-1134.*
77. Bergmann F, Gunawan B, Hermanns B, Hoer J, Schumpelick V, Fuzesi L. Cytogenetic and morphologic characteristics of gastrointestinal stromal tumors. Recurrent rearrangement of chromosome 1 and losses of chromosomes 14 and 22 as common anomalies. *Verh Dtsch Ges Pathol 1998;82:275-278.*
78. Breiner JA, Meis-Kindblom J, Kindblom L, McComb E, Liu J, Nelson M, Bridge JA. Loss of 14q and 22q in gastrointestinal stromal tumors (Pacemaker cell tumors). *Cancer Genet Cytogenet 2000;120:111-116.*
79. Fukasawa T, Chong JM, Sakurai S, Koshiishi N, Ikeno R, Tanaka A, Matsumoto Y, Hayashi Y, Koike M, Fukayama M. Allelic loss of 14q and 22q, NF2 mutation, and genetic instability occur independently of c-kit mutation in gastrointestinal stromal tumor. *Jpn J Cancer Res 2000;91:1241-1249.*

80. Debiec-Rychter M, Lasota J, Sarlomo-Rikala M, Kordek R, Miettinen M. Chromosomal aberrations in malignant gastrointestinal stromal tumors. correlation with c-KIT gene mutation. *Cancer Genet Cytogenet 2001;128:24-30.*
81. Heinrich MC, Rubin BP, Longley BJ, Fletcher JA. Biology and genetic aspects of gastrointestinal stromal tumors: KIT activation and cytogenetic alterations. *Hum Pathol 2002;33:484-495.*
82. El-Rifai W, Sarlomo-Rikala M, Andersson LC, Miettinen M, Knuutila S. High-resolution deletion mapping of chromosome 14 in stromal tumors of the gastrointestinal tract suggests two distinct tumor suppressor loci. *Genes Chromosomes Cancer 2000;27:387-391.*
83. Kim NG, Kim JJ, Ahn JY, Seong CM, Noh SH, Kim CB, Min JS, Kim H. Putative chromosomal deletions on 9P, 9Q and 22Q occur preferentially in malignant gastrointestinal stromal tumors. *Int J Cancer 2000;85:633-638.*
84. El-Rifai W, Sarlomo-Rikala M, Andersson LC, Knuutila S, Miettinen M. DNA sequence copy number changes in gastrointestinal stromal tumors: tumor progression and prognostic significance. *Cancer Res 2000;60:3899-3903.*
85. O'Leary T, Ernst S, Przygodzki R, Emory T, Sobin L. Loss of heterozygosity at 1p36 predicts poor prognosis in gastrointestinal stromal/smooth muscle tumors. *Lab Invest 1999;79:1461-1467.*
86. Neurofibromatosis. Conference statement. National Institutes of Health Consensus Development Conference. *Arch Neurol 1988;45:575-578.*
87. Cawthon RM, Weiss R, Xu GF, Viskochil D, Culver M, Stevens J, Robertson M, Dunn D, Gesteland R, O'Connell P, et al. A major segment of the neurofibromatosis type 1 gene: cDNA sequence, genomic structure, and point mutations. *Cell 1990;62:193-201.*
88. Viskochil D, Buchberg AM, Xu G, Cawthon RM, Stevens J, Wolff RK, Culver M, Carey JC, Copeland NG, Jenkins NA, et al. Deletions and a translocation interrupt a cloned gene at the neurofibromatosis type 1 locus. *Cell 1990;62:187-192.*
89. Wallace MR, Marchuk DA, Andersen LB, Letcher R, Odeh HM, Saulino AM, Fountain JW, Brereton A, Nicholson J, Mitchell AL, et al. Type 1 neurofibromatosis gene: identification of a large transcript disrupted in three NF1 patients. *Science 1990;249:181-186.*
90. Xu GF, O'Connell P, Viskochil D, Cawthon R, Robertson M, Culver M, Dunn D, Stevens J, Gesteland R, White R, et al. The neurofibromatosis type 1 gene encodes a protein related to GAP. *Cell 1990;62:599-608.*
91. Maruta H, Burgess AW. Regulation of the Ras signalling network. *Bioessays 1994;16:489-496.*
92. Chian R, Young S, Danilkovitch-Miagkova A, Ronnstrand L, Leonard E, Ferrao P, Ashman L, Linnekin D. Phosphatidylinositol 3 kinase contributes to the transformation of hematopoietic cells by the D816V c-Kit mutant. *Blood 2001;98:1365-1373.*
93. Riccardi VM. Von Recklinghausen neurofibromatosis. *N Engl J Med 1981;305:1617-1627.*
94. Huson S, Hughes R. The Neurofibromatoses: A Clinical and Pathogenetic Overview. Chapman & Hall, 1994.

95. Zoller ME, Rembeck B, Oden A, Samuelsson M, Angervall L. Malignant and benign tumors in patients with neurofibromatosis type 1 in a defined Swedish population. *Cancer 1997;79:2125-2131.*
96. Kinoshita K, Hirota S, Isozaki K, Ohashi A, Nishida T, Kitamura Y, Shinomura Y, Matsuzawa Y. Absence of c-kit gene mutations in gastrointestinal stromal tumours from neurofibromatosis type 1 patients. *J Pathol 2004;202:80-85.*
97. Cheng SP, Huang MJ, Yang TL, Tzen CY, Liu CL, Liu TP, Hsiao SC. Neurofibromatosis with gastrointestinal stromal tumors: insights into the association. *Dig Dis Sci 2004;49:1165-1169.*
98. Yantiss RK, Rosenberg AE, Sarran L, Besmer P, Antonescu CR. Multiple gastrointestinal stromal tumors in type I neurofibromatosis: a pathologic and molecular study. *Mod Pathol 2005;18:475-484.*
99. Xiao W, Oefner PJ. Denaturing high-performance liquid chromatography: A review. *Hum Mutat 2001;17:439-474.*
100. Antonescu CR, Sommer G, Sarran L, Tschernyavsky SJ, Riedel E, Woodruff JM, Robson M, Maki R, Brennan MF, Ladanyi M, DeMatteo RP, Besmer P. Association of KIT exon 9 mutations with nongastric primary site and aggressive behavior: KIT mutation analysis and clinical correlates of 120 gastrointestinal stromal tumors. *Clin Cancer Res 2003;9:3329-3337.*
101. Lasota J, Kopczynski J, Sarlomo-Rikala M, Schneider-Stock R, Stachura T, Kordek R, Michal M, Boltze C, Roessner A, Stachura J, Miettinen M. KIT 1530ins6 mutation defines a subset of predominantly malignant gastrointestinal stromal tumors of intestinal origin. *Hum Pathol 2003;34:1306-1312.*
102. Caruana C, Cambareri A.C., Ashman L.K. Isoforms of c-KIT differ in activation of signalling pathways and transformation of NIH3T3 fibroblasts. *Oncogene 1999;18:5573-5581.*
103. Piao X, Curtis JE, Minkin S, Minden MD, Bernstein A. Expression of the Kit and KitA receptor isoforms in human acute myelogenous leukemia. *Blood 1994;83:476-481.*
104. Nishida T, Hirota S. Biological and clinical review of stromal tumors in the gastrointestinal tract. *Histol Histopathol 2000;15:1293-1301.*
105. Kim TW, Lee H, Kang YK, Choe MS, Ryu MH, Chang HM, Kim JS, Yook JH, Kim BS, Lee JS. Prognostic significance of c-kit mutation in localized gastrointestinal stromal tumors. *Clin Cancer Res 2004;10:3076-3081.*
106. Wardelmann E, Neidt I, Bierhoff E, Speidel N, Manegold C, Fischer HP, Pfeifer U, Pietsch T. c-kit mutations in gastrointestinal stromal tumors occur preferentially in the spindle rather than in the epithelioid cell variant. *Mod Pathol 2002;15:125-136.*
107. Singer S, Rubin BP, Lux ML, Chen CJ, Demetri GD, Fletcher CD, Fletcher JA. Prognostic value of KIT mutation type, mitotic activity, and histologic subtype in gastrointestinal stromal tumors. *J Clin Oncol 2002;20:3898-3905.*
108. Medeiros F, Corless CL, Duensing A, Hornick JL, Oliveira AM, Heinrich MC, Fletcher JA, Fletcher CD. KIT-negative gastrointestinal stromal tumors: proof of concept and therapeutic implications. *Am J Surg Pathol 2004;28:889-894.*
109. Sakurai S, Hasegawa T, Sakuma Y, Takazawa Y, Motegi A, Nakajima T, Saito K, Fukayama M, Shimoda T. Myxoid epithelioid gastrointestinal stromal tumor

(GIST) with mast cell infiltrations: a subtype of GIST with mutations of platelet-derived growth factor receptor alpha gene. *Hum Pathol 2004;35:1223-1230.*

110. Fuller CE, Williams GT. Gastrointestinal manifestations of type 1 neurofibromatosis (von Recklinghausen's disease). *Histopathology 1991;19:1-11.*
111. Min KW, Balaton AJ. Small intestinal stromal tumors with skeinoid fibers in neurofibromatosis: report of four cases with ultrastructural study of skeinoid fibers from paraffin blocks. *Ultrastruct Pathol 1993;17:307-314.*
112. Ishida T, Wada I, Horiuchi H, Oka T, Machinami R. Multiple small intestinal stromal tumors with skeinoid fibers in association with neurofibromatosis 1 (von Recklinghausen's disease). *Pathol Int 1996;46:689-695.*
113. Hou YY, Tan YS, Sun MH, Wei YK, Xu JF, Lu SH, SJ AK-S, Zhou YN, Gao F, Zheng AH, Zhang TM, Hou WZ, Wang J, Du X, Zhu XZ. C-kit gene mutation in human gastrointestinal stromal tumors. *World J Gastroenterol 2004;10:1310-1314.*
114. Kang HJ, Nam SW, Kim H, Rhee H, Kim NG, Hyung WJ, Noh SH, Kim JH, Yun CO, Liu ET. Correlation of KIT and platelet-derived growth factor receptor alpha mutations with gene activation and expression profiles in gastrointestinal stromal tumors. *Oncogene 2005;24:1066-1074.*
115. Badache A, Muja N, De Vries GH. Expression of Kit in neurofibromin-deficient human Schwann cells: role in Schwann cell hyperplasia associated with type 1 neurofibromatosis. *Oncogene 1998;17:795-800.*

GÖTEBORGS UNIVERSITET
GÖTEBORGS UNIVERSITETSBIBLIOTEK

Vasastadens
Bokbinderi AB

Bokbinderi & Tryckeri

Tel: 031 - 29 20 45 • Mail: info@vasastadensbokbinderi.se

Lundberg Laboratory for Cancer Research
Department of Pathology
Sahlgrenska Academy at Göteborg University
Sahlgrenska University Hospital
2005