Article is available online at http://www.webio.hu/por/2003/9/1/0013

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Gastrointestinal Stromal Tumors (GIST): C-kit Mutations, CD117 Expression, Differential Diagnosis and Targeted Cancer Therapy with Imatinib

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Gastrointestinal stromal tumors (GISTs) have been recognised as a biologically distinctive tumor type, different from smooth muscle and neural tumors of the gastrointestinal tract. They constitute the majority of gastrointestinal mesenchymal tumors. They are defined and diagnosed by the expression of a protooncogene protein called CD117 detected by immunohistochemistry. It is now believed that GISTs originate from gastrointestinal pacemaker cells known as interstitial cells of Cajal, that control gut motility or from a precursor of these cells. The identification of mutations mostly in exon 11 and to a lesser extent in exons 9 and 13 of the c-kit protooncogene coding for c-kit (CD117) in many GISTs, has resulted in a better understanding of their oncogenic mechanisms. The finding of remarkable antitumor effects of the molecular inhibitor, imatinib (Glivec™) in metastatic and inoperable GISTs, has necessitated accurate diagnosis of GISTs and their distinction from other gastrointestinal mesenchymal tumors. To achieve this, pathologists need to be familiar with the spectrum of histological appearances shown by GISTs and have a high index of suspicion for these tumors. This review summarises recent advances in knowledge regarding the histogenesis, pathology, molecular biology, genetics and differential diagnosis of GISTs and the basis for the novel targeted cancer therapy with imatinib. (Pathology Oncology Research Vol 9, No 1, 13–19, 2003)

Keywords: Gastrointestinal stromal tumors, c-kit, CD117, imatinib

Introduction

During the past four years or so, unprecedented new knowledge regarding the previously rather neglected, confusing and controversial subject of gastrointestinal stromal tumors (GISTs) has resulted in GISTs being defined as a biologically distinctive tumor type, different from smooth muscle (leiomyomas and leiomyosarcomas) and neural (schwannomas) tumors of the gastrointestinal tract. The expression of CD117 (a protooncogene protein) has emerged as the most important defining feature and probably the gold standard for diagnosing GISTs,[15,19,46,48] which are now known to constitute the majority of all gastrointestinal mesenchymal tumors.[29] The identification of mutations in the c-kit gene in many GISTs has resulted in a better understanding of their oncogenic mechanisms.[1,14,15,19,46] The most clinically relevant breakthrough has been the finding of the remarkable antitumor effects of the molecular inhibitor, imatinib (Glivec™ Novartis, Basel, Switzerland) in GIST, a tumor that was previously regarded as being generally resistant to conventional chemotherapy.[7,13] This review summarises recent advances in knowledge regarding the histogenesis, pathology, molecular biology, genetics and differential diagnosis of GISTs and the basis for the novel targeted cancer therapy with imatinib.

Historical aspects

Traditionally, spindle cell tumors of the gastrointestinal tract were classified as smooth muscle tumors (leiomyoma, leiomyosarcoma) because of the site of origin with-

Received: Jan 2, 2003; *accepted:* March 5, 2003

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in the muscularis and the close morphologic resemblance to smooth muscle tumors at other sites. Smooth muscle tumors with epithelioid cells were initially designated as leiomyoblastoma and later as epithelioid leiomyoma or epithelioid leiomyosarcoma. With the advent of electron microscopy and immunohistochemistry came the realisation that only some of these were of smooth muscle origin (smooth muscle actin and/or desmin positive, presence of myofilaments) whereas others appeared to be undifferentiated or have a neurogenic origin (S100 positive with a "neural" appearance).[26] The term gastrointestinal stromal tumor was used to encompass smooth muscle, neural and undifferentiated tumors on the basis that the variable histogenesis was of unknown clinical relevance.[41] The term gastrointestinal autonomic nerve tumor (GANT) was used to describe tumors with ultrastructural evidence of autonomic nervous system differentiation.[21,56]

The demonstration of CD34 expression by many gastrointestinal stromal tumors suggested a specific entity distinct from smooth muscle tumors.[32,34,53] It was the subsequent observation of CD117 expression[46] that ultimately led to a major reappraisal of the classification and the current acceptance that gastrointestinal mesenchymal tumors can be divided into GISTs (CD117 positive), true smooth muscle tumors and, much less often, true schwann cell tumors. A large majority of tumors previously diagnosed as smooth muscle tumors of the gastrointestinal tract are probably GISTs. GANT is now regarded as a variant of GIST.[23,31]

Histogenesis

The immunophenotypic (CD117 positive) and ultrastructural resemblance of GISTs to the interstitial cells of Cajal, gastrointestinal pacemaker cells which control gut motility, suggests a histogenesis from the latter cells.[19] Furthermore one study has shown that an embryonic form of smooth muscle myosin in GIST is similar to that found in Cajal cells.[44] Cajal cells are known to originate from common intestinal mesenchymal precursor cells that also give rise to smooth muscle cells and it has been proposed that CD117 is required for differentiation into Cajal cells.[22,25,59] This would explain the morphological resemblance of GISTs to smooth muscle tumors and also the occurrence of the GISTs outside the bowel wall, in the omentum and mesentery, where Cajal cells are not normally found.[31]

Incidence

Data from population based studies in Finnland suggest that the annual incidence of all GISTs is around 10-20/million and that of malignant GISTs is about 4 cases per million of population.[29,30] The true incidence may be higher because of under-diagnosis in the past, and the reporting of some tumors as smooth muscle tumors or as sarcomas of uncertain histogenesis. Informal estimates of the annual incidence of clinically detected new cases of GIST in the United States have increased from perhaps 300-500 per year to 5000-6000 per year due to renewed interest and better diagnosis[10] and there has been a rapid accrual of patients to clinical trials.

Clinical features

GISTs predominantly affect middle-aged and older patients with a median age of 50-60 years.[31] They are rare before the age of 40-years and very rare in children.[29] About 60-70% of GISTs occur in the stomach, 20-30% in the small intestine and 10% or less in the oesophagus, colon and rectum.[10,28,31,32,51,52] Similar tumors, sometimes known as extragastrointestinal stromal tumors (EGIST), may arise in the omentum, mesentery, or retroperitoneum.[40] The clinical presentation depends on the size and site of the tumor. Small asymptomatic GISTs, usually less than 2cm in diameter, are detected incidentally on the serosal surfaces at laparotomy, in specimens that have been resected for other conditions, and at endoscopy, usually in the gastric submucosa.[29] Symptomatic tumors often present with vague abdominal discomfort.[31] Larger GISTs with central ulceration present with acute or chronic gastrointestinal haemorrhage. Lesions in the oesophagus may present with dysphagia,[32] while intestinal tumors may cause obstruction, perforation, bleeding or altered bowel habit.[29] Very large GISTs presenting as externally palpable intra-abdominal masses are likely to be malignant.[31] Some patients may present with liver metastases.

Macroscopic appearances

GISTs are usually unencapsulated but well circumscribed masses, with a whorled fibroid-like or a softer more fleshy appearance on cut surface. Larger lesions often show cystic degeneration or central necrosis. Ulceration of the overlying mucosa is common. Some tumors protrude both into the lumen and from the serosa of the bowel, resulting in a dumbbell appearance. Seeding of tumor deposits into the serosa or omentum indicates malignancy.[4,6]

Histological spectrum

Familiarity of the spectrum of histological appearances shown by GISTs will enable pathologists to have a high index of suspicion for these tumors. The predominant pattern, seen in 70-80% of GISTs, is of a spindle cell tumor (*Figure 1*) with a fascicular or storiform growth pattern.[10,31] They may be solidly cellular or more myxoid.[10] The cells show less cytoplasmic eosinophilia than smooth

muscle tumors.[31] The nuclei tend to be oval rather than cigar shaped. Some tumors, especially those in the stomach, may show striking perinuclear vacuolation (*Figure 2*).[10] Others may show nuclear palisading simulating nerve sheath tumors.[10] Often there is cystic degeneration. Thin walled vessels and stromal haemorrhage are commonly seen.[10] Rectal and colonic GISTs are usually spindle cell tumors with rectal ones often having a bland appearance.[28,29,51,52]

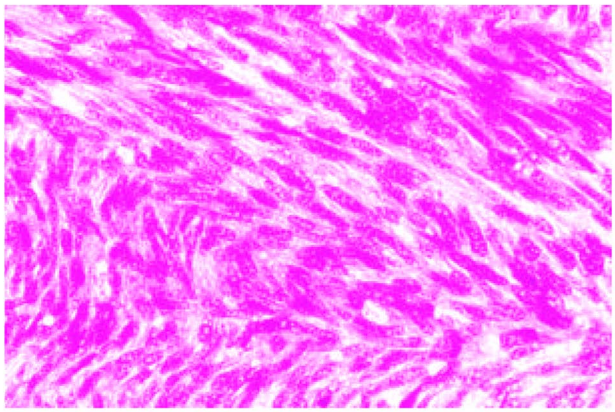

Figure 1. *GIST showing fascicles of spindle cells (HE x200).*

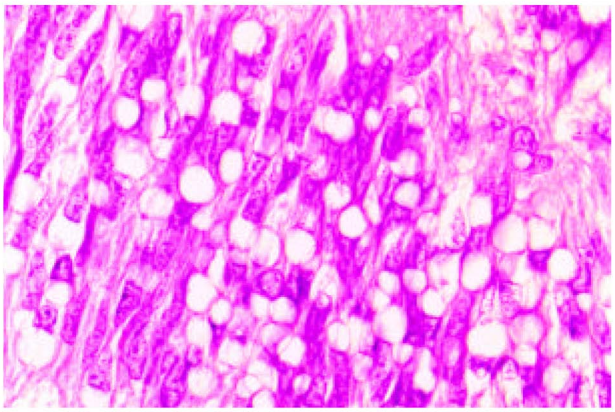

Figure 2. *Spindle celled GIST showing perinuclear vacuolation (HE x400).*

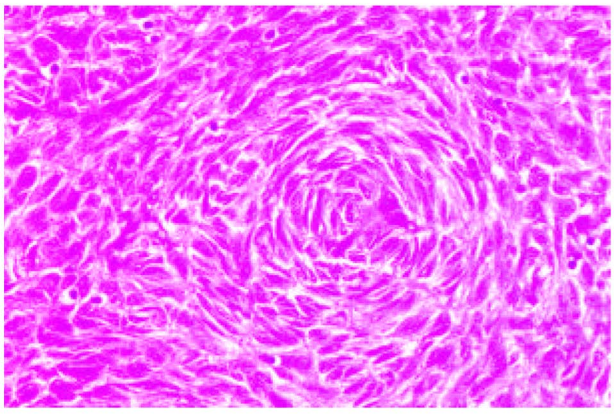

Figure 3. *GIST showing whorled appearance of more epithelioid cells (HE x200).*

About 20-30% of tumors are predominantly composed of large round or polygonal epithelioid cells with abundant often eosinophilic or clear cytoplasm (*Figure 3*).[10,31] They may show a packeted architecture resembling a paraganglioma or carcinoid tumor.[2,10] These epithelioid tumors correspond with those previously designated as leiomyoblastomas and epithelioid smooth muscle tumors and are commoner in the stomach.[2] Mixed spindle and epithelioid tumors are common.

The stroma often contains a prominent inflammatory cell infiltrate. Small intestinal GISTs may contain eosinophilic, hyaline, PAS positive diastase resistant, extracellular globules or more elongated collagen (*Figure 4*) known as skeinoid fibres.[35] GISTs may show focal or prominent stromal hyalinization.[49] Stromal calcification[12] and metaplastic bone formation[51] have been reported. Prominent nuclear pleomorphism is unusual in GISTs.[10,12,29]

CD117 and immunohistochemical features of GISTs

CD117, the c-kit proto-oncogene protein is a transmembrane receptor for a growth factor known as stem cell factor (SCF) or mast cell growth factor. It is encoded by the c-kit protooncogene located on chromosome 4q11-21.[55] It has extracellular, intramembranous and intracellular domains. Binding of SCF ligand to the receptor leads to dimerization of the receptor. This activates tyrosine kinase located in the intracellular domain, leading to activation of further intracellular signalling cascades controlling cell proliferation, adhesion and differentiation. CD117 is functionally important and expressed in haematopoietic stem cells, mast cells, germ cells, some epithelial cells and in the interstitial cells of Cajal.[50]

Expression of CD117 is seen in almost all GISTs regardless of the site of origin, histologic appearance and biologic behaviour, and is therefore considered to be best defining feature of GISTs.[10,15,19,46,48,51] CD117 commonly shows strong diffuse cytoplasmic positivity (Figure 5) but may also show dot positivity (so called "golgi pattern") within the cytoplasm.[10] As yet, it is not known whether these patterns reflect different forms of mutation of the c-kit gene. The proportion of CD117 cells in GISTs is usually at least 90%, but may be as low as 5%-20% in occasional tumors.[10] This may account for the rare CD117 negativity in small biopsies of tumors with the morphology of a GIST. There remains a small problematic group of tumors with morphological features of GISTs without expression of CD117.[29] Some pathologists designate these as GIST-like tumors. Mast cells and cells of Cajal in normal bowel wall are useful internal

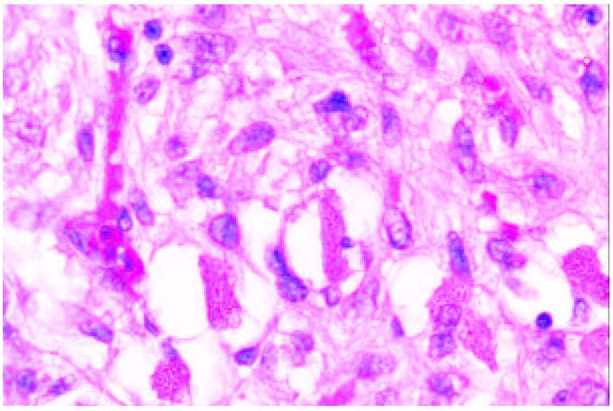

Figure 4. *Skeinoid fibres in a small intestinal GIST (HE x400).*

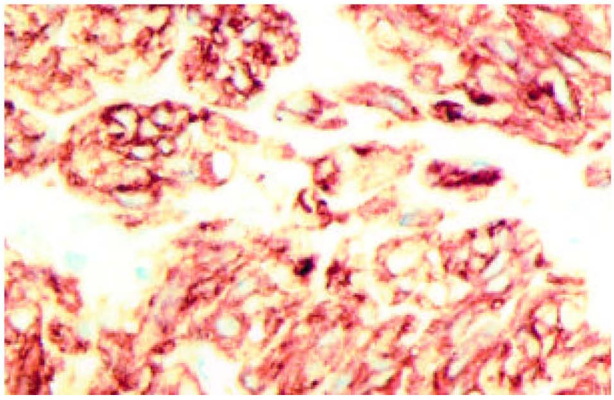

Figure 5. *Diffuse CD 117 positivity in a GIST (x400)*

positive controls to supplement the normal positive and negative controls. CD117 is also expressed by a large number of other tumor types such as dermatofibrosarcoma protuberans, synovial sarcoma, rhabdomyosarcoma, anaplastic large cell lymphoma, glioma, germinoma, melanoma, acute myeloid leukaemia and mastocytosis,[12,16] but few of these occur within the gastrointestinal tract, and unusual CD117 positivity is more often due to technical artifact.[10] As in all diagnostic immunohistochemistry, immunostaining must be interpreted in the light of morphology and clinical context.

GISTs are nearly uniformly positive for high-molecular weight caldesmon, an actin-binding cytoskeleton-associated protein expressed by smooth muscle and myoepithelial cells. [12,31] Approximately 60-80% of GISTs express CD34,[10,31,33,34,53] malignant GISTs showing a slightly lower frequency than benign ones.[46] One study showed that expression of CD34 was lower in small intestinal GISTs.[12] About 20-40% of GISTs show focal reactivity for smooth muscle actin,[10,33,34,53] fewer than 2% express desmin and under 5% stain for S100 protein.[10] Neurone-specific enolase immunoreactivity may be seen in GISTs, but is a non-specific feature because true smooth muscle tumors may also be positive.[31]

Mutations of c-kit

Although the precise proportion is disputed, mutations of the c-kit gene are known to be present in over half of GISTs, most commonly in exon 11 which encodes the juxta-membrane domain. These mutations (including deletions and point mutations) result in gain of function with permanent activation of the receptor, in the absence of binding of the stem cell factor ligand.[14,15,38] Less commonly mutations involve exons 9 and 13, which encode the extracellular and kinase domains respectively.[14,24,41] Some studies have shown that c-kit mutations in exon 11 predominantly occur in GISTs that are clinically and histologically malignant,[20] but others have found them in benign ones as well.[5,42,57] The c-kit mutations which alter the enzymatic portion of the kit protein are called enzymatic mutations, while those that involve the regulatory portion are called regulatory-type mutations.[14] In vitro studies suggest that GISTs with regulatory-region KIT mutations are more likely to respond to ST1-571 than GISTs with enzymatic–region mutations.[14] The c-kit mutations probably occur as early events in the process of development of GISTs, and such mutations have been demonstrated in GISTs measuring one centimetre or less in diameter.[5]

It seems that even some of those GISTs that lack c-kit mutations do have high kit kinase activity. Such GISTs might contain c-kit mutations which are not readily detected by conventional screening methods or activation might be due to nonmutational mechanisms.[14]

Uncommonly, GISTs develop in families, and in these patients germline mutations of c-kit have been identified particularly in exons 11 and 13.[14,39] These patients develop diffuse hyperplasia of the cells of Cajal, which is regarded as a pre-neoplastic lesion. Those patients with mutations of exon 11 develop cutaneous mastocytosis with or without cutaneous hyperpigmentation but those with mutations of exon 13 do not have these associated features.[3,17] C-kit mutations have not been demonstrated in true leiomyomas and leiomyosarcomas,[24,37] thus further confirming that GISTs are different from these tumors.

Cytogenetic findings

A variety of chromosomal abnormalities have been described in GISTs,[1] most typically monosomy of chromosomes 14 and 22 and deletions of 1p.[1,8,45] Malignant GISTs often show additional abnormalities including amplification of 8q and 17q.[43] These cytogenetic aberrations are undoubtedly important in the pathogenesis of GIST but are currently not used as diagnostic adjuncts.

Differential diagnosis

GIST has to be distinguished from true smooth muscle tumors of the gastrointestinal tract. Leiomyomas are more commonly observed in the oesophagus.[31] Morphologically smooth muscle tumors appear less cellular and their cells contain more eosinophilic cytoplasm.[31] Immunohistochemically, they will be consistently positive for desmin and actin and negative for CD117. About 10-15% of smooth muscle tumors are positive for CD34.[10]

Schwannomas are composed of spindle cells showing nuclear palisading and may have a typical lymphoid cuff surrounding the tumor.[31] Immunohistochemically they will be positive for S100 and negative for CD117. Schwannomas (especially Antoni B areas) may sometimes stain with CD34.[10]

Sometimes intra-abdominal fibromatosis may involve the bowel wall and may express CD117, but usually the histological appearances are distinctive with parallel arrangement of spindle cells in long sweeping fascicles and presence of keloid like collagen.[58] The spindle cells of fibromatosis do not stain with CD34.[58] Fibromatosis will show nuclear β-catenin positivity whereas GISTs will not.[36] Inflammatory fibroid polyps often express CD34, but not CD117. Inflammatory myofibroblastic tumor, although usually a tumor of childhood, may occur in adults with involvement of the bowel wall. It can be confused with a GIST containing prominent inflammatory cells but will not express CD34 and CD117.

More epithelioid GISTs can resemble paragangliomas. Awareness of the problem and use of CD117 will readily solve this difficulty. Malignant melanoma has a tendency to metastasise to the gastrointestinal tract and may express CD117, but can be distinguished by expression of S100, HMB-45 and melan-A and identification of the primary tumor.

Prognostic factors

The prediction of malignant potential of GISTs based on clinicopathological features is often difficult. Large, mitotically active tumors, with necrosis, predictably behave aggressively, but even small cytologically bland and poorly mitotic tumors may on occasion give rise to metastases.[4,30] Tumors in the small intestine are known to behave most aggressively than those in the stomach.[9,27,30] Tumors that have metastasized at the time of presentation,[27] and those with peritoneal seeding at the time of the primary operation,[4,6] have a very poor prognosis. Mucosal invasion in the form of a diffuse lymphoma-like pattern of growth between the glandular elements but not the mere presence of an ulcer is associated with malignant GISTs.[27]

The two main methods of spread of malignant GISTs are liver metastases and intraabdominal spread.[11] Bone and lung metastases are rare.[29] Soft tissue metastases may occur in the internal aspect of the abdominal wall or the subcutaneous tissue.[29]

It is now accepted that categorising GISTs into low, intermediate and high-risk tumors based on an estimation of their potential for recurrence and metastases is more appropriate than dividing them into benign and malignant categories. ***Figure 6*** shows an algorithm based on the consensus approach for assessing the risk of malignancy reached at a National Institutes of Health workshop held in April 2001.[10] It should be kept in mind that a small subset of cases will behave in an unpredictable manner and that

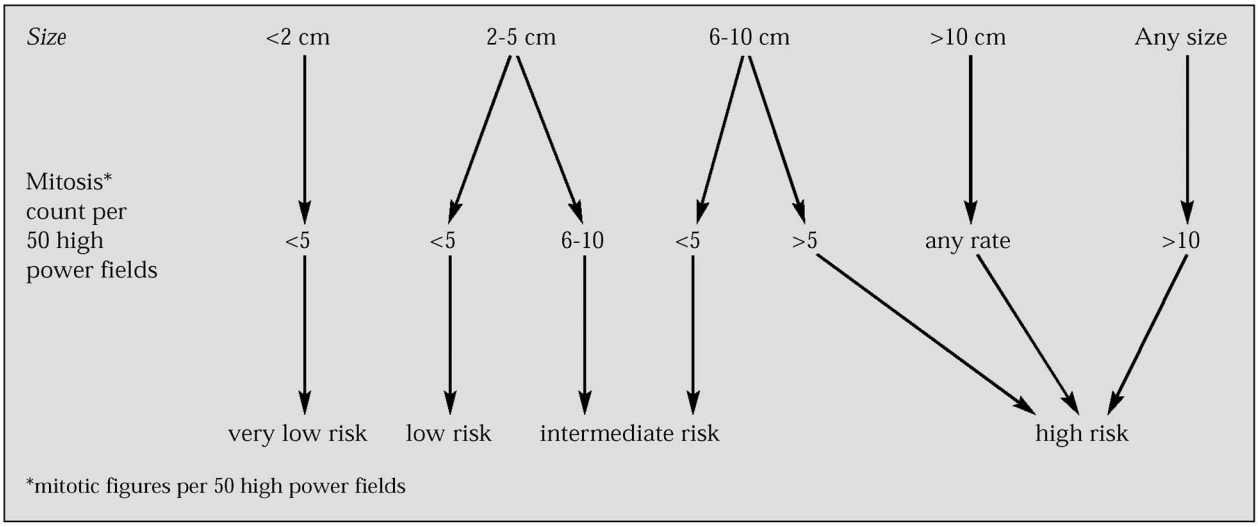

Figure 6. *Algorithm based on the consensus approach for assessing the risk of malignancy of GIST reached at a National Institutes of Health workshop held in April 2001.*[10]

no lesion can be labelled as unequivocally benign.[10] Thus all patients with GIST should be carefully followed up for an indefinite period.

Treatment of GISTs and targeted therapy using Imatinib

When possible, complete surgical excision is the treatment of choice for localised GISTs. The role of radiotherapy is limited by the potential toxicity to surrounding structures, especially the intestines.[7] There has been a 40-69% partial response of inoperable and metastatic GISTs to targeted therapy using imatinib (Glivec, Novartis).[7,13] This is indeed remarkable for a tumor that was previously regarded as being generally resistant to conventional chemotherapy.[7] Imatinib mesylate (Glivec) is a synthetic tyrosine kinase inhibitor, which now has an established role in the management of interferon resistant chronic myeloid leukaemia (CML).[47] CML is characterised by a translocation between chromosomes 9 and 22 which produces a chimaeric protein (BCR-ABL) with tyrosine kinase activity. Imatinib acts by occupying the kinase pocket of the BCR-ABL oncoprotein, preventing phosphorylation of its substrate.[47] Imatinib is also effective against a number of other tyrosine kinases including c-kit and platelet derived growth factor (PDGF).[47] It was initially shown to have striking antitumor effect in a single Finnish patient with metastatic GIST,[18] a finding confirmed by larger trials in America and in Europe.[7,13,54] It is now considered to be the drug of choice for metastatic and inoperable GIST.[7,13]

The use of Imatinib is a classic example of a drug targeted to a specific molecular defect of a tumor and marks a new era of rational and targeted molecular inhibition of cancer. Hopefully the development of such drugs will increase in the near future. The use of such drugs will necessitate more specific diagnosis of mesenchymal tumors, using immunohistochemistry, cytogenetics and molecular biology.

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