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REVIEW AND PERSPECTIVE

Gastrointestinal stromal tumors

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Abstract Gastrointestinal stromal tumors (GISTs) have emerged from being poorly defined, treatment-resistant tumors to a well-recognized, well-understood, and treatable tumor entity within only one decade. The understanding of GIST biology has made this tumor a paradigm for molecularly targeted therapy in solid tumors and provides informative insights into the advantages and limitations of so-called targeted therapeutics. Approximately 85% of GISTs harbor activating mutations in *KIT* or the homologous receptor tyrosine kinase *PDGFRA* gene. These mutations are an early event in GIST development and the oncoproteins serve as a target for the small molecule tyrosine kinase inhibitors imatinib and sunitinib. The existing and emerging treatment options demand exact morphologic classification and risk assessment. Although, KIT (CD117) immunohistochemistry is a reliable diagnostic tool in the diagnosis of GIST, KIT-negative GISTs, GISTs showing unusual morphology as well as GISTs which progress during or after treatment with imatinib/sunitinib can be a challenge for pathologists and clinicians. This review focuses on GIST pathogenesis, morphologic evaluation, promising new immunohistochemical markers, risk assessment, the role of molecular analysis, and the increasing problem of secondary imatinib resistance and its mechanisms.

Keywords Gastrointestinal stromal tumor · GIST · Imatinib · Sunitinib · Tyrosine kinase inhibitors · Resistance · KIT · PDGFRA

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Introduction

Gastrointestinal stromal tumors (GISTs) are the most common mesenchymal tumors in the gastrointestinal (GI) tract. Malignant examples of this tumor type were once viewed as the most treatment-refractory sarcomas, with fewer than 10% of patients showing clinical response to conventional chemo- or radiation therapies [1, 2]. For decades, prior to the late 1990s, these mesenchymal tumors arising in the GI tract were most often classified as smooth muscle tumors or neural tumors [3]. In 1983, Mazur and Clark introduced the term "stromal tumor" [4], but it was not widely accepted until the early 1990s, when CD34 was discovered as a marker for stromal tumors arising in the gastrointestinal tract, at that time being regarded as relatively specific [5]. In the 1990s, investigators noted similarities between GIST cells and the interstitial cells of Cajal, a group of cells located in the muscularis propria and around the myenteric plexus throughout the GI tract, serving as pacemakers for peristaltic contraction [6–10]. Studies revealed that interstitial cells of Cajal express KIT and are developmentally dependent on stem cell factor which is regulated through the KIT kinase [7, 11]. In 1998, a groundbreaking publication by Hirota and colleagues, showed activating mutations in the *KIT* receptor tyrosine kinase (RTK) gene in GIST as well as expression of KIT

protein by immunohistochemistry [6]. These insights, together with the subsequent introduction of highly effective tyrosine kinase inhibitor (TKI) treatments, led to immense research interest in GIST. Subsequent studies confirmed these findings; and in 2003, Heinrich and colleagues [12] additionally identified platelet-derived growth factor receptor alpha (*PDGFRA)* gene mutations as an alternative pathogenetic event in GISTs lacking *KIT* gene mutations. To date, approximately 85% of GISTs are reported to harbor activating mutations in *KIT* or the homologous RTK gene, *PDGFRA* [12–14], and KIT immunohistochemistry has proven to be a reliable and sensitive tool in GIST diagnosis [8, 15, 16]. With the possibility of inhibiting the activated oncoproteins KIT and PDGFRA with TKI therapies (imatinib and sunitinib), inoperable or metastatic GISTs are now treatable, and a number of additional alternative drugs are already in clinical trials. Increased understanding of GIST biology has made this tumor a paradigm of molecularly targeted therapy in solid tumors and provides informative insights into the advantages and limitations of so-called targeted therapeutics.

Oncogenic *KIT* and *PDGFRA* mutations and signaling pathways in GIST

The *KIT* and *PDGFRA* genes map to chromosome 4q12 [17]. Both encode type III receptor tyrosine kinases sharing closely related structural features. These kinases are composed of an extracellular (EC) ligand-binding region containing five immunoglobulin-like repeats, a transmembrane sequence, a juxtamembrane domain (JM), and two cytoplasmic kinase domains (TK[I]: ATP-binding pocket; and TK[II]: kinase activation loop; Fig. 1) [18, 19].

KIT and PDGFRA are activated by binding of their respective ligands, stem cell factor and PDGFA, to the EC region. Ligand binding results in receptor homodimerization and subsequent cross-phosphorylation of cytoplasmic tyrosines which serve as binding sites for various signaling proteins, leading to phosphorylation cascades with activation of signaling substrates regulating cell proliferation, adhesion, motility, and survival [20] (Fig. 2). KIT tyrosine kinase activity is regulated by its JM domain, which inhibits KIT kinase activity in the absence of KIT ligand [21]. Overall, KIT activation has been shown to regulate important cell functions including proliferation, apoptosis, adhesion, and chemotaxis, [22–25] and KIT is critical for the development and maintenance of several cell types, e.g., germ cells, hematopoietic cells, mast cells, melanocytes, and interstitial cells of Cajal [20, 26, 27].

In GISTs, *KIT* or *PDGFRA* mutations cause constitutive oncogenic signaling in the absence of their ligands. The uncontrolled RTK activity results in the activation of the PI3K-AKT and MEK-MAPK pathways accompanied by relatively low level signal transducer and activation of transcription (STAT)1 and STAT3 activation [25, 28, 29], leading to alterations in cell cycle, protein translation, metabolism, and apoptosis (Fig. 2).

Mutations in the *KIT* or *PDGFRA* gene involve two main regions, the receptor regulatory domains (dimerization domain in the EC region and JM domain) and the enzymatic domains (TK[I] and TK[II]). In GISTs most *KIT* mutations (~65%) involve the JM domain (exon 11) followed by mutations involving the EC dimerization

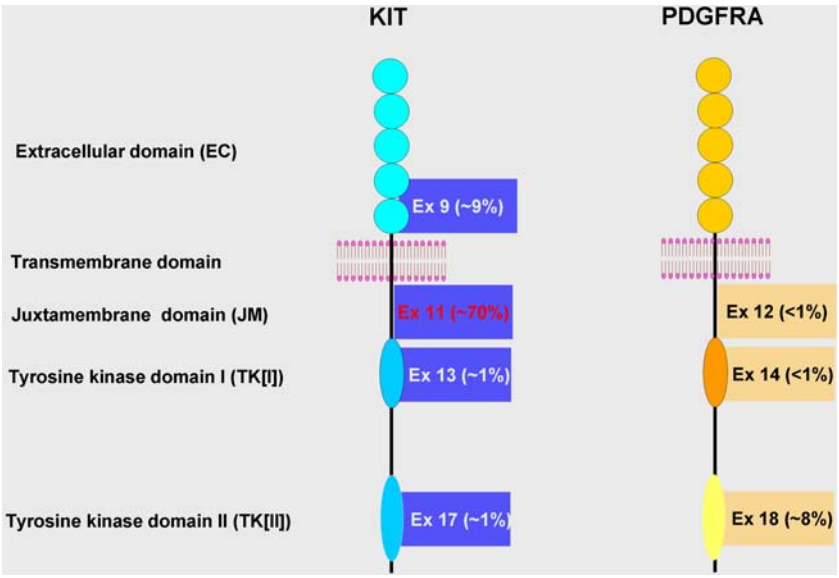

Fig. 1 Schematic structure of KIT and PDGFRA receptor tyrosine kinases and distribution of KIT mutations in GIST

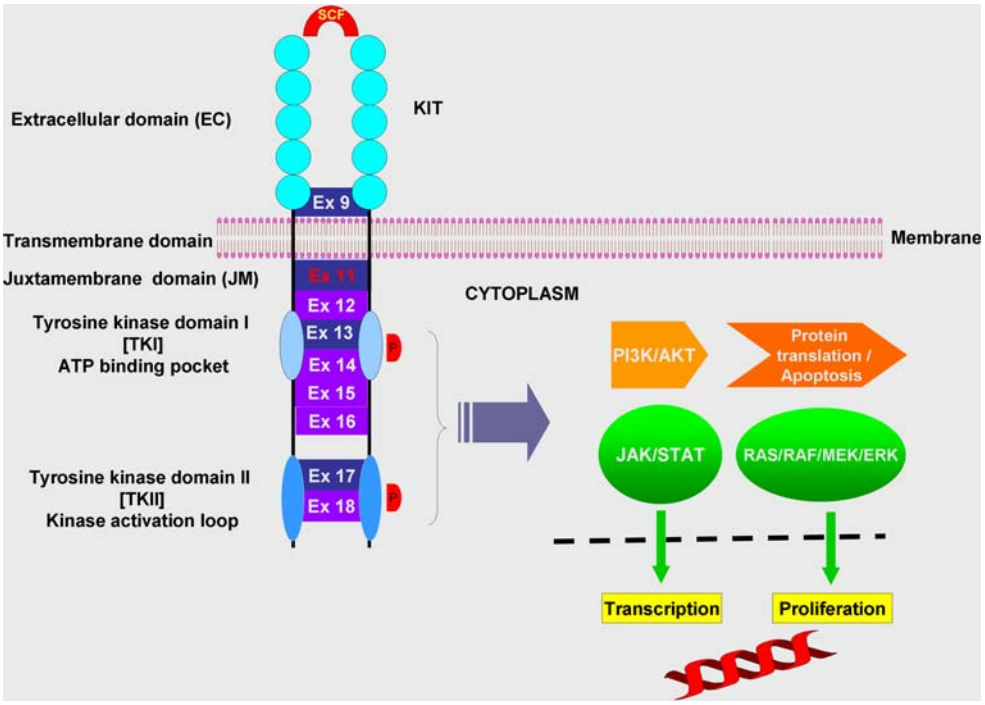

Fig. 2 Summary of the major signaling pathways activated by KIT

domain (exon 9) which are seen in about 9% of cases [13, 14]. Primary *KIT* mutations can also occur in exon 13 (TK [I]: ATP binding pocket) and exon 17 (TK[II]: kinase activation loop), but these mutations are rare (~2%) and data are quite limited.

GISTs harboring an exon 11 mutation can occur throughout the GI tract. Various types of mutation can be found in exon 11 including missense mutations, insertions, and deletions. Tandem repeat mutations are infrequently seen in the distal part of *KIT* exon 11. These changes were predominantly found in female stomach and have been proposed to be associated with a quite indolent clinical course [30–33]. More controversial are GISTs with deletion mutations in and around *KIT* exon 11 codon 557–558. Some studies have shown an aggressive clinical course and poor prognosis, but these findings have not been confirmed by others [30, 32, 33]. Loss of heterozygosity in the *KIT* locus has been associated with high proliferative activity and increased metastatic potential [31, 32].

GISTs with an exon 9 mutation arise most commonly in the small intestine and are frequently high-risk tumors [31, 34, 35]. In the vast majority of cases, exon 9 mutations are characterized by insertion of six base pairs, a duplication of Ala and Tyr and are found in primary as well as relapsed or advanced GISTs [32, 36]. According to a recent study, *KIT* exon 13 and exon 17 mutant GISTs are slightly over-represented among the intestinal group of GISTs, and if tumors with an exon 13 mutation occur in the stomach, they tend to be slightly larger and more aggressive than "average"gastric GISTs. The majority of *KIT* exon 13 and 17 mutations are substitutions and, in small intestinal GISTs, these mutations have no substantial impact on clinicopathologic features when compared to the "average" small intestinal GIST [37].

PDGFRA mutations, identified in approximately 8% of GISTs, involve mainly (~6–7%) either exon 18 (TK[II]: kinase activation loop) or exon 14 (TK[I]: ATP-binding pocket) and rarely (less than 1%) exon 12 (JM) [12, 38–40]. Mutations in *PDGFRA* exon 14 and 18 are mostly missense mutations. The subset of GISTs with a *PDGFRA* mutation that is associated with a commonly benign clinical course is limited to the stomach and omentum, lack KIT expression by immunohistochemistry (IHC), and preferentially shows epithelioid morphology [41, 42]. GISTs with a mutation D842V in exon 18 of *PDGFRA* are resistant to imatinib and sunitinib [13, 43–45].

Mutations in the JM domain impair its auto-inhibitory functions, causing kinase activation [46, 47], whereas mutations in the EC region may lead to ligand-independent receptor dimerization [13]. *KIT* and *PDGFRA* mutations are mutually exclusive.

Most recently, *BRAF* exon 15 V600E mutations were identified in 7–13% of adult wild-type GISTs [48–50]. GISTs with *BRAF* mutation seem to show a predilection for the small bowel [49, 50]; however, the association with high-risk malignancy is controversial as yet [48–50].

Role of cytogenetics in GIST progression

KIT or *PDGFRA* mutations are an early event in GIST development. Mutations are found irrespective of tumor

size. They are observed in "silent" microscopic and multiple incidental GISTs detected in gastrectomies, performed for other causes, and in 10–20% of normal patients over the age of 60 [51, 52]. These findings underscore that mutations per se are involved in the oncogenesis and proliferation of GISTs, but seem to be of little importance in malignant transformation [53]. Therefore, additional genetic hits are important in clinical tumor progression. Approximately two-thirds of *KIT* and *PDGFRA* mutant GISTs show either monosomy 14 or partial loss of 14q [12, 54, 55]. Two 14q regions, 14q11.2–q12 and 14q23–q24, seem to harbor tumor suppressor genes important in early GIST development [54, 56]. Another common event, seen in approximately 50% of GISTs, is loss of the long arm of chromosome 22. This finding is associated with progression to borderline/malignant GIST [54, 57–59]. Less frequently observed are losses on chromosomes 1p, 9q, 11p, 17q, and gains on chromosomes 8q and 17q, which are also associated with malignant behavior [56, 57, 59–61]. GISTs without mutations in *KIT*, *PDGFRA* or *BRAF*, whether pediatric or adult, have been shown to exhibit a much lower level of cytogenetic progression than observed in mutant GISTs [10, 62] underscoring that mechanisms leading to tumor progression are different in mutant and wild-type GISTs.

Epidemiology

The exact incidence of GIST in the USA and Europe is hard to determine, as GISTs have only been properly recognized and uniformly diagnosed as an entity since the late 1990s. Recent population-based studies performed in Sweden [63], Holland [64] and Iceland [65] found incidences of approximately 14.5, 12.7, and 11 cases/million/year, respectively. These findings would translate into an annual incidence in Europe of ~8,000-9,000 cases and in the USA of ~4,000-5,000 cases a year. Nevertheless, the prevalence of GIST is higher, as many patients live with the disease for many years or develop small GISTs only detected at autopsy or if a gastrectomy is performed for another cause. A German study performed on consecutive autopsies revealed small GISTs (1-10 mm) in 22.5% of individuals over the age of 50 years [66]. These minute GISTs are immunoreactive for KIT and often contain an oncogenic mutation in the *KIT* or *PDGFRA* gene [66]. Similar findings have been reported by other groups [51–53, 67]. These findings suggest that small GISTs do not progress often (or rapidly) into large tumors despite the presence of *KIT* or *PDGFRA* mutations.

At the time of diagnosis, the majority of patients with GIST are between 40 and 80 years old, with a median age of approximately 60 years; GISTs have no clear gender predilection [68]. Rarely, GISTs occur in children and young adults. Pediatric GISTs are considered a separate clinicopathologic entity (see below) and occur predominantly in the second decade [10, 69, 70]. Most GISTs are sporadic, but families with germ-line *KIT* mutations and GISTs are well described [71–79]. Furthermore, hereditary syndromes such as neurofibromatosis type I [80–82], Carney's triad (gastric GIST, paraganglioma, and pulmonary chondroma) [83, 84], and Carney's dyad (paraganglioma, gastric GIST) [78] can be associated with the development of GISTs.

Clinical and pathological aspects

Clinical features

GISTs occur throughout the GI tract and are most commonly seen in the stomach (60%), jejunum and ileum (30%), duodenum (5%), colorectum (4%), and rarely the esophagus and appendix [35, 68, 70, 85]. Tumors lacking any association with the bowel wall are known as extra-gastrointestinal stromal sarcomas and more often occur in the omentum, mesentery, or retroperitoneum [86, 87]. Clinical symptoms associated with GIST include abdominal pain, fatigue, dysphagia, satiety, and obstruction. Patients may present with chronic GI bleeding (causing anemia) or acute GI bleeding (caused by erosion through the gastric or bowel mucosa) or rupture into the abdominal cavity causing life-threatening intraperitoneal hemorrhage. Previously, a population-based study revealed that approximately 70% of GISTs were associated with clinical symptoms, 20% were not, and 10% were detected at autopsy [63]. The median tumor size in each of these categories was 8.9, 2.7, and 3.4 cm, respectively [63]. Small GISTs mainly present as incidental findings during endoscopy, surgery, or radiologic studies for other reasons, whereas patients with malignant GIST often present with disseminated disease. Metastases can quite often occur 10-15 years after initial surgery, and therefore long-term follow-up is required. Metastases develop primarily in the abdominal cavity and liver, rarely in the soft tissue and skin, and exceptionally rarely in lymph nodes or in the lung [85]. Clinically, it is essential to differentiate metastatic GIST from multifocal GISTs observed in patients with germline *KIT* or *PDGFRA* mutations, in patients with neurofibromatosis 1 and multiple sporadic GISTs, mainly occurring in the proximal stomach [88]. The pathogenesis of multiple sporadic GISTs is poorly understood; however, these GISTs have been shown to harbor different *KIT* mutations in separate individual lesions from the same patient [88]. Generally speaking, the clinical history, clinical presentation (see below), morphology, mitotic activity and, in rare cases, mutational analysis should allow exact precise classification.

Macroscopic features

GISTs present most often as well-circumscribed, highly vascular tumors associated with the stomach or the intestine. On gross examination, these tumors appear fleshy pink or tan-white and may show hemorrhagic foci, central cystic degenerative changes, or necrosis (Fig. 3).

Microscopic features

Morphologic evaluation reveals three principal subtypes of GIST depending on the cytomorphology. Spindle cell GIST (Fig. 4a), accounting for approximately 70% of cases, is composed of cells with palely eosinophilic fibrillary cytoplasm, ovoid nuclei, and ill-defined cell borders, often with a syncytial appearance, arranged in short fascicles or whorls. GIST with epithelioid cell morphology (Fig. 4b), accounting for approximately 20%, is composed of round cells with eosinophilic to clear cytoplasm arranged in sheets and nests. Finally, approximately 10% of GISTs show mixed morphology, being composed of both spindle and epithelioid cells (Fig. 4c). Variable cellularity as well as sclerotic, collagenous, or myxoid stromal changes can be seen in each subtype. Spindle cell GISTs can show nuclear palisading (Fig. 4d) or a storiform growth pattern as well as prominent paranuclear vacuolation (Fig. 4e), a morphologic feature formerly proposed to be suggestive of smooth muscle origin but far more commonly seen in GISTs. Overall, GISTs are characterized as uniform and monotonous tumors; however, pleomorphic GISTs [89] and dedifferentiated GISTs are seen very occasionally (Fig. 4f) [90].

GISTs after treatment with TKI may show a dramatic decrease in tumor cellularity and marked sclerosis (Fig. 5a) or prominent stromal alterations including myxoid change (Fig. 5b). In the majority of cases, the cytomorphology remains comparable with the primary tumor. However, changes from spindle to purely epithelioid cytomorphology, a pseudopapillary epithelioid growth pattern [91] as well as, in very rare cases, rhabdomyosarcomatous differentiation (Fig. 5c, d), has been reported after TKI treatment [92]. These findings can cause major diagnostic problems especially if the tumor also loses KIT expression. Under such circumstances, mutational analyses facilitate the diagnosis, as these tumors still retain their primary *KIT* or *PDGFRA* mutation after treatment [91, 92]. Although reports about these unusual findings are limited to date, the use of several different TKIs or other drugs over a longer period may predispose to the development of treatment-resistant tumor clones showing unusual morphology. A somewhat surprising observation in this context is that tumors showing these unusual morphologic alterations generally lack secondary *KIT* mutations [91, 92], the most common cause for secondary TKI treatment resistance. Interestingly, in one case, a metastatic peritoneal nodule showed a *BRAF* mutation in addition to the primary *PDGFRA* mutation after treatment with Imatinib [49].

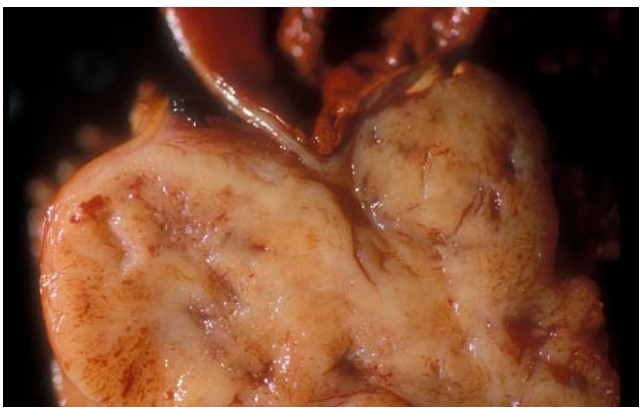

Fig. 3 Typically fleshy tan-white surface of a large gastric serosal GIST

GIST and neurofibromatosis type I

The occurrence of multiple small GISTs in the small bowel, other than in the setting of disseminated sporadic GIST, is significantly associated with neurofibromatosis type I (NF1). These GISTs show spindle cell morphology, are usually mitotically inactive, and express KIT, usually in the absence of *KIT* and *PDGFRA* mutations [80–82]. Clinically, they are usually benign. In rare cases, clinically malignant GISTs in association with multiple benign tumor nodules have been described [35].

Pediatric GISTs

Approximately 1-2% of GISTs occur in the pediatric age group, predominantly in the second decade. Pediatric GISTs are associated with a marked female predominance, are preferentially located in the stomach, and show mainly epithelioid morphology [10, 69, 70]. Although these tumors consistently express KIT protein, the majority lack *KIT* or *PDGFRA* mutations [10, 69, 70]. Unlike adult GISTs, these tumors quite often spread to lymph nodes. Interestingly, pediatric *KIT* wild-type GISTs lack the typical cytogenetic deletions seen in adult *KIT*-mutant GISTs and progress to malignancy without acquiring large-scale chromosomal aberrations [10]. The difference between pediatric *KIT* wild-type and adult GISTs of the stomach is further demonstrated by their separate clustering by gene expression profiling [69], and it is very likely that these tumors are a separate clinicopathologic entity. In the pediatric wild-

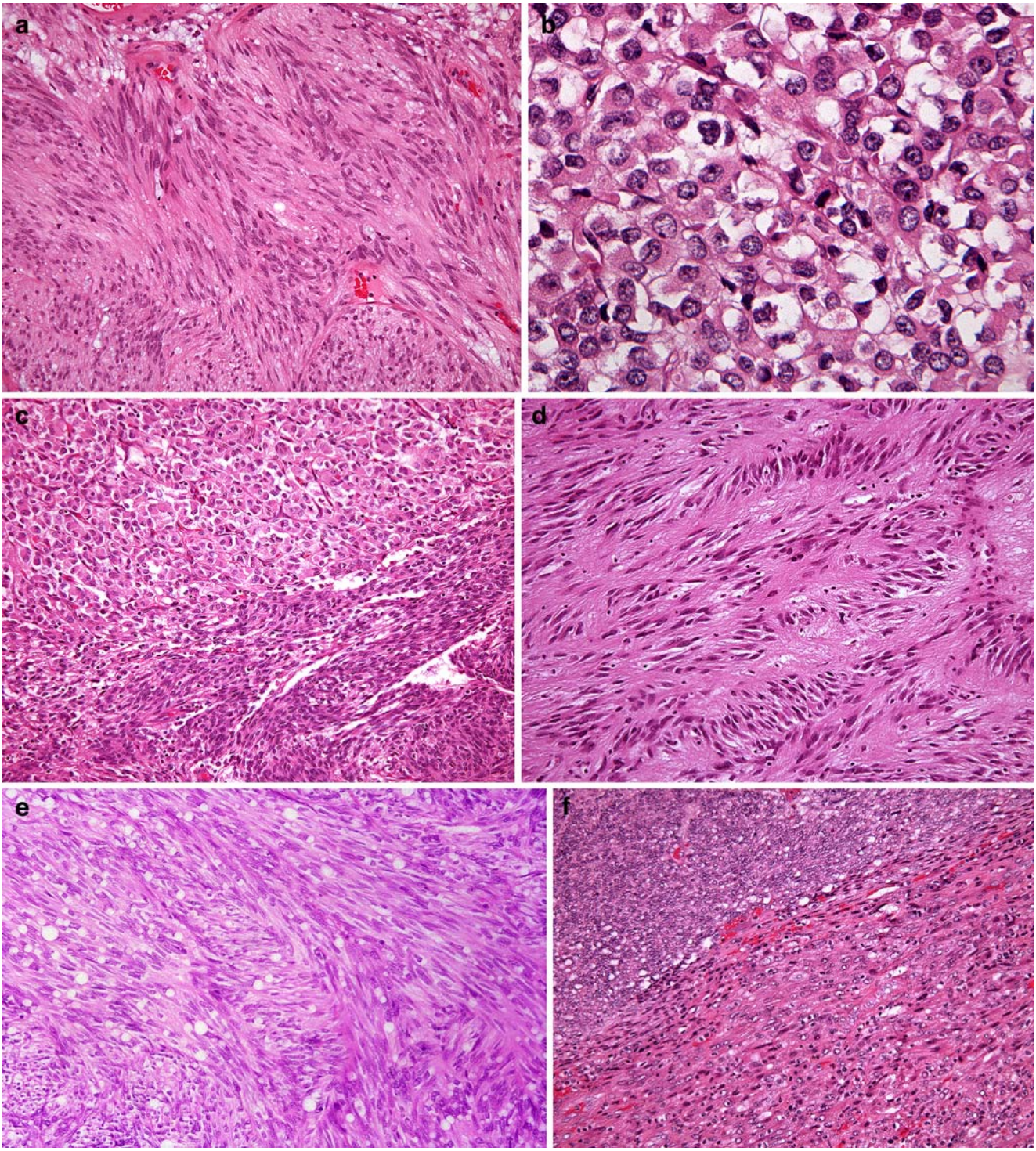

Fig. 4 **a** GIST composed of spindle cells with uniform ovoid or tapering nuclei and palely eosinophilic fibrillary cytoplasm with ill-defined cell borders. **b** GIST composed of epithelioid cells with eosinophilic or clear cytoplasm. **c** GIST with mixed spindle cell and epithelioid cytomorphology. **d** Spindle cell GIST showing prominent nuclear palisading (tumors such as this were often formerly labeled as GANT). **e** Spindle cell GIST showing prominent paranuclear cytoplasmic vacuolation. **f** Dedifferentiated GIST showing abrupt transition from conventional GIST to undifferentiated sarcoma composed of much larger epithelioid and spindle cells, which were KIT-negative

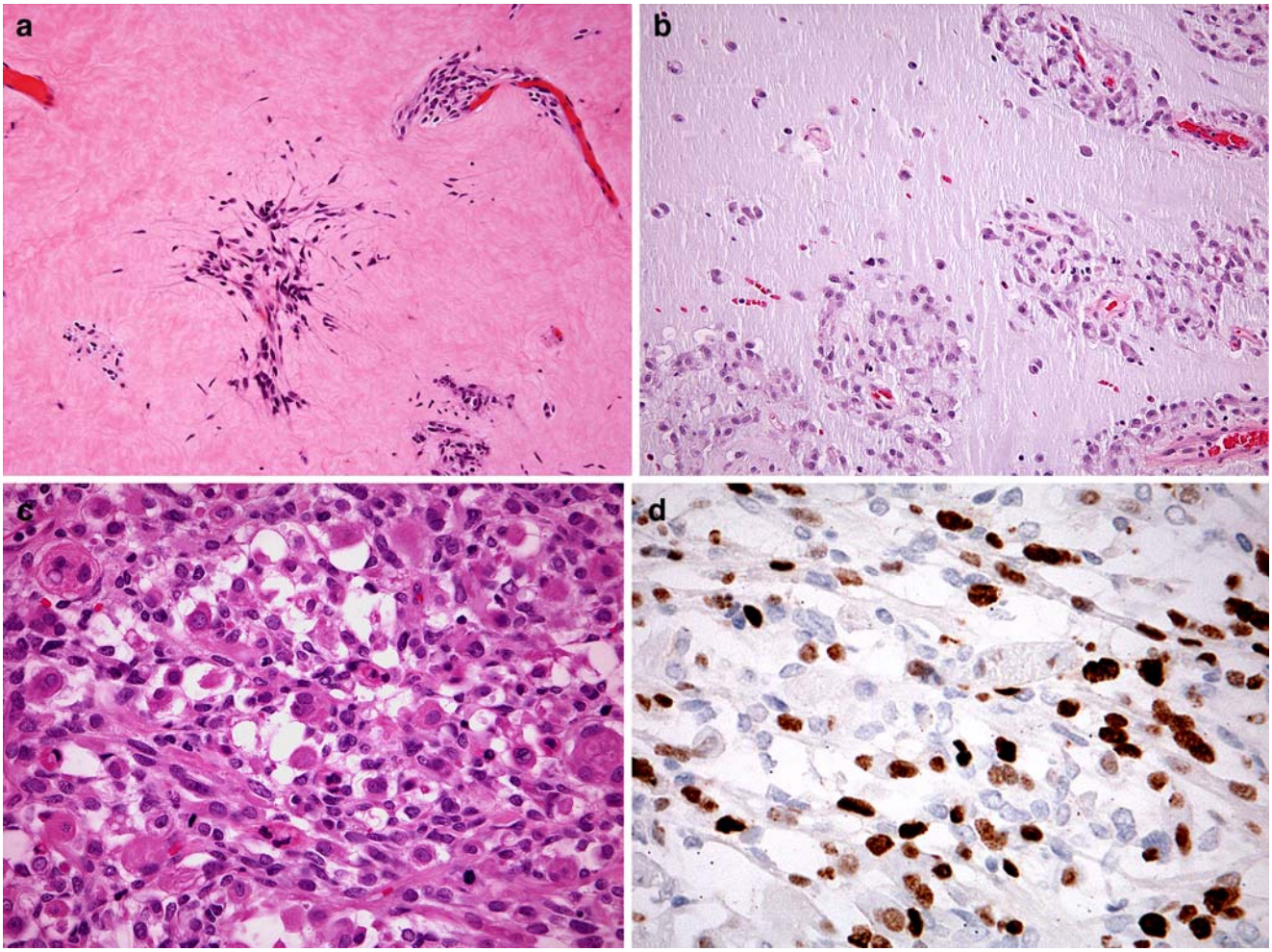

Fig. 5 **a** Treated GIST with marked stromal sclerosis and reduced cellularity. **b** Treated GIST showing myxoid stromal change. **c** Treated GIST showing heterologous rhabdomyosarcomatous differentiation. **d** Strong nuclear expression of myf-4 (myogenin) in a GIST showing heterologous rhabdomyosarcomatous differentiation

type GIST group, time to tumor progression was significantly longer on sunitinib than on prior imatinib treatment, indicating that this patient group could benefit from sunitinib as first-line treatment [93, 94].Sometimes pediatric GISTs are associated with pulmonary chondromas and/or paragangliomas referred to as Carney's triad [83]. In the pediatric age group, Carney's triad should be considered in any patient with GIST, especially if patients also present with lung nodules.

Familial GISTs

Heritable mutations in *KIT* and *PDGFRA* have been reported in some families [71–79]. The penetrance in these kindreds is high, and most affected family members will develop one or more GISTs during their life span. The mean age of onset (44 years) is younger than that of sporadic GISTs (around 60 years) without gender differences. Most of these GISTs follow a benign course, and their morphology does not differ from that of their sporadic counterparts. Interestingly, patients harboring a heritable *KIT* exon 13 or 17 mutation develop multiple GISTs, whereas patients harboring a heritable *KIT* exon 11 mutation can also develop skin hyperpigmentation and mast cell disease [71–75].

GISTs in association with Carney's triad and Carney's dyad (Carney-Stratakis syndrome)

GISTs are part of Carney's triad (gastric GIST, paraganglioma, and pulmonary chondroma) [83, 84] and Carney's dyad (paraganglioma, gastric GIST) [78], and these GISTs are *KIT/PDGFRA* wild-type. The genetic basis for Carney's triad is not known, although it is thought to be sporadic rather than familial. In both conditions, the presence of multiple gastric GISTs is common. Carney's

dyad is transmitted as an autosomal dominant trait, and recently Pasini et al. demonstrated, in a subset of these GISTs, germline mutations of the genes coding for succinate dehydrogenase subunits B, C, or D, implying that other molecular mechanisms, rather than *KIT/PDGFRA* mutations, may play a role in the pathogenesis of GISTs in this setting [78]. Recently, Zhang et al. reported a study of 104 GISTs in Carney triad, emphasizing the differences from sporadic GISTs [95]. The clinical features of the triad include occurrence at a young age, female predilection, tumor multifocality, slow growth, frequent metastasis (often to lymph nodes), lack of response to imatinib treatment, and sometimes fatal outcome. In addition, this subset of GISTs occurs mainly in the gastric antrum, shows predominantly epithelioid morphology, and lacks *KIT*, *PDGFRA*, or *SDH* mutations [95]. Interestingly, there is no correlation between conventional risk assessment and tumor behavior and, even with metastatic disease, clinical behavior is unpredictable [95].

Immunohistochemical markers in the diagnosis of GIST

KIT has been demonstrated to be a very specific and sensitive marker in the differential diagnosis of mesenchymal tumors in the GI tract, and around 95% of GISTs express KIT [6, 8, 16, 96]. Different KIT-staining patterns can be observed [16]. Most GISTs show strong and diffuse cytoplasmic KIT staining (Fig. 6a) often associated with dot-like ("Golgi-pattern") staining. Only in a minority of cases is an exclusively dot-like or even a membranous staining pattern observed. The extent and patterns of KIT staining do not correlate with the type of *KIT* mutation and have no impact on the likelihood of response to imatinib. However, GISTs showing weak or focal KIT expression and those GISTs completely negative for KIT are more likely *KIT* wild-type or *PDGFRA* mutant GISTs [38, 39]. Approximately, 4-5% of GISTs are KIT negative [38, 39]. KIT-negative GISTs preferentially occur in the stomach and usually show pure epithelioid or mixed (spindle and epithelioid) cytomorphology.

Other commonly expressed but less sensitive and specific markers are CD34, h-caldesmon, and SMA. CD34 is expressed in approximately 80% of gastric tumors, 50% of those in the small intestine, and 95% of GISTs in the esophagus and rectum [35, 97], whereas h-caldesmon is expressed in more than two-thirds of GISTs [86, 98] and SMA in 30%. S-100 and cytokeratin are only infrequently expressed in GISTs. Desmin expression has been reported rarely in GISTs, but in our experience approximately 30% of KIT-negative GISTs, especially those located in the stomach and showing epithelioid morphology, are desmin positive (Fig. 6b) [99]. In this context, it should be noted

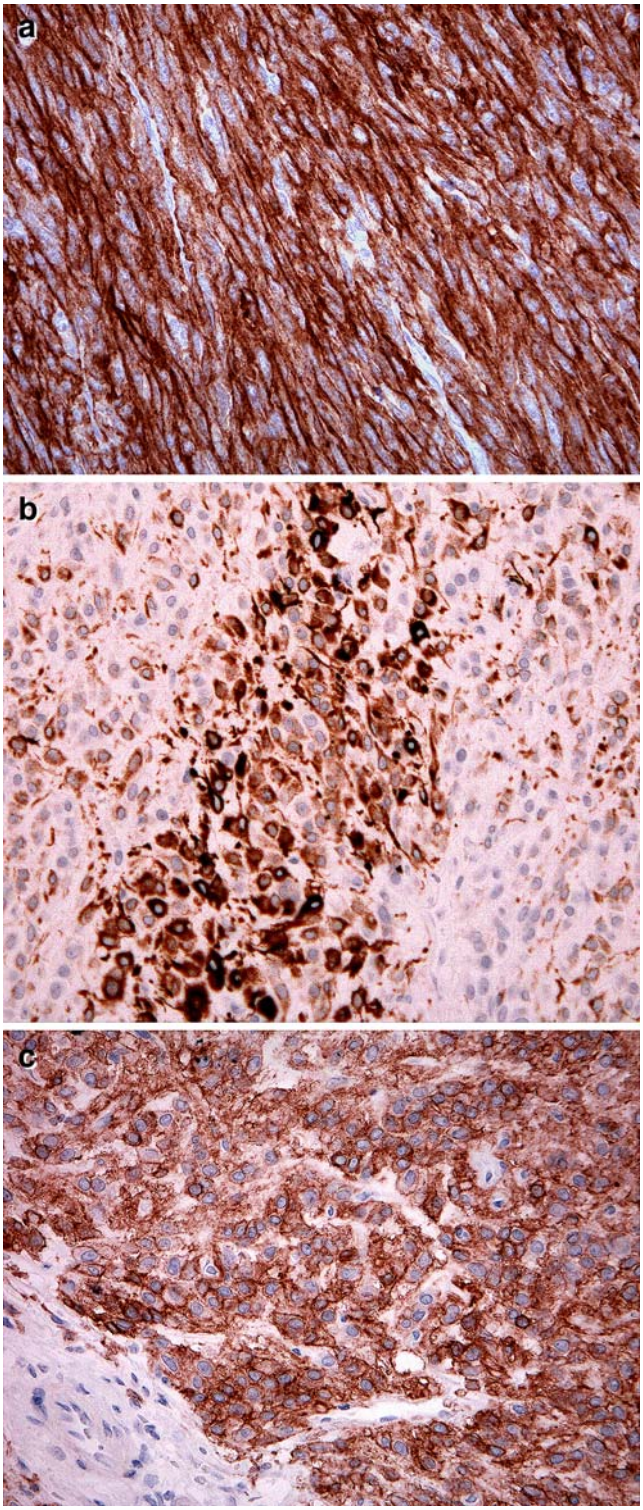

Fig. 6 **a** Spindle-cell GIST showing strong and diffuse cytoplasmic KIT expression. **b** Desmin staining in a KIT-negative epithelioid GIST (with *PDGFRA* mutation). **c** Cytoplasmic and membranous DOG1.1 staining in a KIT-negative GIST

that KIT negativity by no means justifies denying patients therapy with TKI (imatinib or sunitinib), as some wild-type GISTs as well as some tumors with *PDGFRA* mutations respond to treatment with TKI (see below).

In recent years, alternative antibodies for the diagnosis of GIST have emerged. These immunomarkers were mainly identified through molecular studies and are of special interest in the subgroup of KIT-negative GISTs.

One promising marker is discovered on GIST (DOG1), also known as TMEM16A, which is a transmembrane protein recently shown to be up-regulated in GISTs by gene expression profiling [100]. Two recent studies have suggested that antibodies against DOG1 have greater sensitivity and specificity than KIT (CD117) and CD34, and that these antibodies could serve as specific immunohistochemical markers for GIST irrespective of the underlying *KIT/PDGFRA* mutation or KIT expression by IHC [100, 101]. In our own experience, DOG1.1 is a very sensitive marker for GIST, works well on paraffin-embedded tissue, and is highly expressed in *KIT* mutant GISTs as well as in unusual subtypes of GIST lacking *KIT/PDGFRA* mutations, namely pediatric GISTs and GISTs associated with NF1 [99]. Although, DOG1.1 stains about one-third of KIT-negative GISTs (Fig. 6c), in our experience the remaining two-thirds, while often being morphologically typical, are difficult to validate immunohistochemically [99]. Depending on the DOG1 antibody used in different studies, staining in normal gastric epithelium, carcinomas, germ cell tumors, melanomas, and rarely in some mesenchymal tumors has been reported [101, 102].

Protein kinase C (PKC)-theta is a member of the protein kinase C family and is expressed in virtually all GISTs and is very specific, at least by Western blots. Immunohistochemical staining for PKC-theta has been reported in GIST; however in our experience, the commercially available antibodies to this protein show high background staining and limited specificity and are therefore of limited diagnostic utility [28, 103, 104].

PDGFRA alpha is a receptor tyrosine kinase closely related to KIT. Antibodies to this kinase have been proposed to be of use in the identification of KIT-negative GISTs harboring a *PDGFRA* mutation [105–108]. However, in our experience (and that of other major centers), the commercially available antibodies to PDGFRA do not show reproducible immunohistochemical results in paraffin sections.

Nestin, an intermediate filament protein, has been proposed to be expressed in GISTs as well as in a subset of glial, epithelial, melanocytic, and vascular tumors [109] and the intensity of staining has been suggested to correlate with risk stratification [109]. Although, our personal experience is limited with this marker, it seems to work well on paraffin-embedded tissue. Further evaluation of this antibody, especially on KIT-negative GISTs, will be necessary to determine its potential use in routine diagnosis.

Carbonic anhydrase II (CA II) has been proposed as a novel biomarker in GIST [110]. Independent of mutational status, 95% of GISTs express CA II and, interestingly, 50% of KIT negative cases show strong CA II expression [110]. In addition CA II expression also seems to be associated with a better disease-specific survival rate compared to low or no expression [110]. Although, we do not have personal experience with this marker, the results are promising that CA II could be used as an additional diagnostic as well as prognostic marker.

Despite these developments, a subset of KIT-negative tumors remains a diagnostic challenge, at least in terms of immunohistochemical verification; and mutational analysis should be strongly considered under these circumstances.

Recently, a PCR-based assay based on the identification of a single gene set identified through top scoring pair analysis (a method able to identify a gene pair where the relative expression is reversed between two cancers) has been proposed to be highly accurate to differentiate GISTs from leiomyosarcomas as well as to accurately identify KIT-negative GISTs and GISTs with weak or heterogeneous KIT expression [111]. Price et al. proposed an estimated accuracy for this test of around 98% [111]. Although, such testing appears to be promising, it has not yet been evaluated by independent groups.

Differential diagnosis

The main differential diagnoses of spindle-cell GIST that should be considered are smooth muscle tumors, desmoid fibromatosis, schwannoma, inflammatory myofibroblastic tumor, inflammatory fibroid polyp, and solitary fibrous tumor. Smooth muscle tumors show brightly eosinophilic cytoplasm with defined cell borders rather than the syncytial appearance typically seen in GIST. Although SMA and caldesmon are expressed in both tumor types, desmin expression is relatively specific for smooth muscle tumors and rarely positive in spindle-cell GISTs. However, in our experience, desmin expression is mainly seen in epithelioid GISTs located in the stomach and lacking KIT expression [99]. Schwannomas occurring in the gastrointestinal tract typically show a distinctive peripheral cuff of lymphocytes and express S-100 protein and GFAP [112]. Intraabdominal desmoid fibromatosis is morphologically characterized by long sweeping fascicles of fibroblastic/myofibroblastic spindle cells set within a collagenous matrix. Immunohistochemistry reveals nuclear beta-catenin positivity in approximately 75% of cases [113, 114]. In the absence of antigen retrieval, KIT positivity in desmoid fibromatoses is infrequent [115]. Inflammatory myofibroblastic tumors mainly occur in children and young adults. They are cellular, fascicular, fibroblastic/myofibroblastic

tumors with a prominent inflammatory infiltrate composed mainly of plasma cells. ALK expression by immunohistochemistry can facilitate the diagnosis [116] but is seen in at most 50% of cases (mostly in children). Inflammatory fibroid polyp (IFP) has a collagenous or more myxoid granulation tissue-like stroma containing fibroblasts in a pattern-less array and inflammatory cells, including numerous eosinophils. Perivascular fibrosis is commonly seen. The fibroblasts usually express CD34. PDGFRA expression by immunohistochemistry and mutations in *PDGFRA* has been reported in IFPs [117, 118]. However, in contrast to GIST, IFPs do not express KIT and DOG1 [101, 102]. The differential diagnosis for epithelioid GIST includes neuroendocrine carcinoma, glomus tumor, malignant melanoma, epithelioid leiomyosarcoma, epithelioid MPNST, and clear cell sarcoma. Neuroendocrine carcinomas are characterized by cytokeratin, synaptophysin, and chromogranin positivity. Glomus tumors are rarely seen in the GI tract and are morphologically and immunohistochemically identical to glomus tumors in other locations. Melanoma, clear cell sarcoma, and epithelioid malignant peripheral nerve sheath tumor (MPNST) express S-100 protein; the first two may also express second-line melanoma markers that are not expressed in MPNST. Furthermore, clear cell sarcomas are characterized by *EWSR1–CREB1* or *−ATF1* gene fusions [119, 120].

Morphologic risk assessment in GIST

Although the vast majority of GISTs smaller than 2 cm are essentially clinically benign, occasional patients develop metastases sometimes 5 years or more after primary excision. Therefore, older classifications using the terminology "benign" or "malignant" GIST have been replaced by stratification schemata to help predict the risk of aggressive clinical behavior. The first widely accepted scheme to predict the risk of aggressive clinical behavior in GIST was published in 2002 by Fletcher and colleagues after a consensus workshop held at the National Institutes of Health [3, 121]. The proposed risk assessment was based on tumor size and mitotic activity [per 50 high power fields (HPF)], with the most important cut-offs being tumor size of 5 cm and 5 mitoses/50 HPF as indicators of aggressive clinical behavior. According to the 2002 consensus guidelines, all GISTs may have malignant potential. In 2005 and 2006, Miettinen and colleagues from the AFIP presented two very large studies of gastric GISTs and jejunal/ileal GISTs [35, 68], providing strong evidence that GISTs located in the stomach have a much lower rate of aggressive behavior than jejunal and ileal GISTs of similar size and mitotic activity. Based on these publications, anatomic location is now included as an additional parameter in risk assessment for GIST according to the recently updated consensus NCCN guidelines from 2007 [1] (for summary, see Table 1, adapted from Miettinen et al [122]). According to the new guidelines, GISTs smaller than 2 cm can be regarded as essentially benign. In addition to these widely accepted and preferentially used risk assessment schemes, additional schemes have also been proposed by Joensuu and Woodall et al [123, 124]. Of interest is the prognostic nomogram for recurrence-free survival after complete surgical resection of localized primary GISTs using size, mitotic index and site to predict the probability of 2- and 5-year recurrence-free survival [125]. This nomogram may potentially be of help in stratifying patients for adjuvant TKI treatment.

Other proposed prognostic markers in GISTs

The tumor suppressor gene *CDKN2A* (p16) on chromosome 9p is an important inhibitor of the cell cycle and has been demonstrated to be inactivated in a significant proportion of malignant GISTs [126–130]. Evaluation of p16 by immunohistochemistry has been proposed, and downregulation of p16

Table 1 Risk stratification of primary GIST by mitotic index, size and anatomic location

Risk stratification of primary GIST					
Tumor parameter		Risk of progressive disease[a]			
Mitotic index	Size	Gastric	Duodenum	Jejunum/ileum	Rectum
≤5/50 HPF	≤2 cm	None	None	None	None
≤5/50 HPF	>2≤5 cm	Very low (1.9%)	Low (8.3%)	Low (4.3%)	Low (8.5%)
≤5/50 HPF	>5≤10 cm	Low (3.6%)	-[c]	Moderate (24%)	-[c]
≤5/50 HPF	>10 cm	Moderate (10%)	High (34%)	High (52%)	High (57%)
>5/50 HPF	≤2 cm	None[b]	-[c]	High[b]	High (54%)
>5/50 HPF	>2≤5 cm	Moderate (16%)	High (50%)	High (73%)	High (52%)
>5/50 HPF	>5≤10 cm	High (55%)	-[c]	High (85%)	-[c]
>5/50 HPF	>10 cm	High (86%)	High (86%)	High (90%)	High (71%)

[a] Defined as metastasis or tumor-related death

[b] Limited number of cases

[c] Insufficient data

The table is based on Miettinen et al, Semin Diagn Pathol, 2006 [122]. Data based on long-term follow-up of 1,055 gastric, 629 small intestinal, 144 duodenal, and 111 rectal GISTs [35, 68].

has been shown to correlate with aggressive behavior, even in tumors classified as low risk according [40] to the standard morphologic evaluation [127, 128, 130]. However, most recently, Steigen et al. demonstrated contrary results [131].

The p27 cell cycle inhibitor can also be downregulated in malignant GISTs and upregulation of cell cycle regulatory proteins (cyclins B1,D, and E; cdc2, CDK2, CDK4, and CDK6), as well as increased expression of p53, RB, and cyclinA by immunohistochemistry, have been proposed to be detected more commonly in high-risk GISTs [128, 130, 132–136]. In addition to the markers mentioned above, an increasing list of prognostic factors has been reported in GIST for example Ezrin, Raf kinase inhibitor protein, COX-2, bcl-2, CA II [110, 136–139]. However, standardized protocols for staining and interpretation of these markers have not been established as yet.

Role of molecular testing with regard to prognosis and treatment

Objective clinical response to imatinib has been shown to depend on the underlying RTK mutation, and a molecular classification of GISTs has been proposed [14]. Based on four clinical trials (phase I-III) investigating more than 700 genotyped GISTs, the objective response rates for *KIT* exon 11 mutant GISTs, *KIT* exon 9 mutant GISTs, and wild-type GISTs are 72-86%, 38-48%, and up to 28%, respectively [1, 13, 43, 140–142]. Primary resistance to imatinib has been likewise demonstrated in 5% of GISTs showing *KIT* exon 11 mutation and in 16% and 23% of *KIT* exon 9 mutant and *KIT* wild-type GISTs, respectively. *PDGFRA* mutant GISTs have been shown to respond to imatinib, with the exception of the exon 18 D842V mutation [13, 43–45]. Overall, the best response rates to imatinib {the longest median time to tumor progression (~24 months) and longest median survival (~63 months)} [13, 43, 140] are seen in GISTs harboring a *KIT* exon 11 mutation. In these patients, the median time to tumor progression is more than 1 year longer than for *KIT* exon 9 mutant or other common genotypic subsets.

Generally, patients with *KIT* exon 11 mutant GISTs are treated with 400 mg imatinib/day, and dose escalation to 800 mg/day is recommended if patients progress on 400 mg. Clinical data did not reveal a significant benefit for *KIT* exon 11 mutant GISTs whether treated initially with 400 or 800 mg of imatinib [140]. However, patients with *KIT* exon 9 mutations have better progression-free survival if treated with imatinib 800 mg/day than 400 mg [36, 43]. This observation provides the rationale for recent consensus that *KIT* mutation status be evaluated routinely in inoperable GISTs, and with imatinib dose escalated immediately to 800 mg/day if a *KIT* exon 9 mutation is found.

To date, routine mutational testing on all GISTs remains very controversial, as most institutions treat patients with imatinib as a first-line therapy, irrespective of mutational status. Nevertheless, scientific data coming from major academic institutions in Europe and the USA, where molecular analysis of GISTs is routinely performed, suggest that mutational testing should be performed for unresectable and metastatic GISTs, such that patients with *KIT* exon 9 mutants can be dose-escalated to 800 mg/day.

Mutational testing could additionally be considered before adjuvant imatinib treatment for primary "intermediate-high risk" GISTs [1, 143]. Adjuvant treatment with imatinib has been reported to extend progression-free survival in patients with high-risk primary GISTs [144, 145]. Recent study results provide encouraging data on imatinib as an adjuvant therapy. In the ACOSOG Z9001, a phase III randomized trial, 778 patients with localized GIST, who underwent complete surgical resection, were treated for 1 year with imatinib (400 mg/day) versus placebo. The patients were only stratified by tumor size. Adjuvant imatinib treatment significantly improved recurrence-free survival in the imatinib group versus the placebo group as well as in all tumor size stratification groups. The greatest difference between the treatment and the placebo arm was observed in high risk patients (tumor size ≥10 cm). However, the overall survival in the imatinib and placebo group was the same [146]. Based on these results, imatinib was approved as adjuvant therapy for GIST by the US Federal Drug Administration and the European Medicines Agency. Although, an overall consensus as to which patients should receive adjuvant treatment does not exist yet, it is widely accepted that patients with high-risk GISTs should definitely get adjuvant imatinib treatment.

Although state-of-the-art first-line treatment for all patients with metastatic/unresectable GIST is imatinib, it is likely that this treatment regimen will change in the future based on the underlying mutational status [36, 94].

Supportive arguments for routine mutational testing of all GISTs are also based on data that *KIT* exon 9 mutant GISTs in the small intestine and colon are more aggressive than tumors with a *KIT* exon 11 mutation [31, 34], whereas tumors with a *PDGFRA* mutation are less aggressive than those harboring a *KIT* mutation [41, 147].

Resistance mechanisms in GIST

The vast majority of patients with unresectable or metastatic GIST show a response to imatinib. Treatment response can be seen on CT scan as reduction of the tumor mass or as decreased FDG uptake on a PET scan. In a subset of cases, the tumor remains stable under treatment, whereas, in the minority of cases, tumor re-growth is noted within

the first 6 months. Tumor progression within the first 6 months of imatinib treatment is referred to as primary resistance. In this group, *KIT* exon 9 and *KIT* wild-type tumors are over-represented compared with exon 11 mutant tumors [13, 43]. The majority of patients showing initially good response or stable disease will develop tumor progression in one or more lesions usually after 12-36 months, referred to as secondary resistance. Secondary resistance is most commonly caused by secondary (acquired) mutations in the *KIT* kinase domain, and rarely other resistance mechanisms including *KIT/PDGFRA* genomic amplification and activation of alternative oncogenes have been reported [148–150]. Secondary *KIT* kinase mutations are non-randomly distributed single nucleotide substitutions affecting codons in the ATP binding pocket (exons 13 and 14) and the kinase activation loop (exon 17 and 18). Several studies have demonstrated resistance mutations in 44-67% of GISTs progressing after imatinib therapy [142, 151–153]. Sunitinib, the second-line TKI used after imatinib failure, has been shown to be effective against secondary mutations located in the ATP binding pocket (exon 13 and 14) but not against mutations in the kinase activation loop (exon 17 and 18) based on in vitro and in vivo studies [36, 94].

We demonstrated substantial inter- and intralesional heterogeneity in TKI resistance mutations in patients treated with imatinib alone or imatinib and sunitinib: 83% of patients in this study had secondary drug-resistant *KIT* mutations, including 67% with two to five different secondary mutations in separate metastases, and 34% with two secondary *KIT* mutations in the same metastasis [150]. The most frequent secondary resistance mutation in patients whose GISTs have primary *KIT* exon 11 mutations and who eventually progress during imatinib treatment is the V654A point mutation in the ATP-binding pocket [142, 154]. Interestingly, the V654A mutation, which is sunitinib-sensitive based on in vitro studies [94], was found in ~27% of our samples after clinical progression on sunitinib [150]. In addition, these same samples showed minimal-to-low morphologic evidence of treatment response. Such observations suggest that sunitinib is cytostatic rather than cytocidal in GISTs with secondary V654A mutations, in keeping with the clinical evidence from a randomized, placebo-controlled, multicenter trial demonstrating that stable GIST was the best overall tumor response on sunitinib treatment [155]. The presence of low-level TKI resistance mutations on the same *KIT* alleles encoding the V654A may also account for persistence of V654A alleles during sunitinib therapy.

New treatment options in GISTs

Imatinib currently remains the standard first-line treatment option for patients with unresectable and metastatic GISTs, especially those harboring an exon 11 mutation. However, accumulating evidence suggests that sunitinib could be effective as a first-line treatment for GISTs harboring *KIT* exon 9 mutation and for *KIT/PDGFRA* wild-type GISTs (including pediatric GISTs) [36, 94, 152]. Sunitinib is effective against secondary imatinib-resistance mutations in the ATP-binding pocket [94]. However, the substantial heterogeneity of resistance mutations (as discussed above) highlights the therapeutic challenges involved in salvaging patients, especially after clinical progression on TKI monotherapies. With regard to treatment approaches, the role of newer generation KIT and PDGFRA kinase inhibitors (e.g., nilotinib, dasatinib, etc.) remains to be determined in GIST patients who are multiply resistant, i.e., after imatinib and sunitinib treatment, to TKIs. Nilotinib has been shown to be effective in advanced imatinib- and sunitinib-resistant GISTs [156]. Using nilotinib 400 mg twice a day, the median progression-free survival and the median overall survival were 12 and 34 weeks, respectively [156]. In vitro data, using cell lines expressing imatinib-resistant *PDGFRA* (D842V) mutants, suggest that dasatinib, a dual SRC/ABL kinase inhibitor, and IPI-504, a heat shock protein 90 inhibitor, may be a therapeutic option for patients with a GIST harboring the *PDGRA* (D842V9) mutation [157]. Histone deacetylase inhibitors (HDACI) alone or in combination with imatinib show inhibition of cell proliferation, KIT activity and expression as well as activation of downstream pathways in KIT-positive cell lines, providing preclinical evidence that HDACI may expand the treatment options in KIT-positive GISTs [158]. IGFR inhibitors in combination with imatinib have been proposed as a treatment option mainly for wild-type GISTs which tend to be less responsive to imatinib-based therapies. The rational for this treatment is based on detected amplification of IGF1R and protein over-expression predominantly in WT and pediatric GISTs [159, 160].

It seems clear that multi-agent treatment modalities are needed in the future. Combination therapies with various such inhibitors could prolong GIST remissions, in a manner analogous to treatment approaches used in HIV, by suppressing a broader spectrum of tumor clones from the outset of therapy. Similarly, therapeutic options less dependent on specific molecular mechanisms of KIT or PDGFRA activation are needed to overcome the substantial heterogeneity of secondary *KIT* kinase mutations responsible for treatment failure in the vast majority of patients. Such treatment options include inhibition of the KIT chaperone HSP90 [161], which may result in KIT oncoprotein degradation, irrespective of the TKI-resistance mutations present, or the use of flavopiridol, which acts as a *KIT* transcriptional repressor and is not expected to be altered by any nucleotide mutation in the

KIT coding sequence [162]. Compelling data has been presented recently by demonstrating the effects of bortezomib on imatinib-sensitive and imatinib-resistant cell lines. The results are especially promising as this drug has been shown to be effective against GIST cells harboring various resistance mutations and preliminary in vitro data confirm the potency of bortezomib in transgenic mice [163].

Other broadly relevant therapeutic strategies include blockage of crucial KIT-mediated signaling pathways, as might be accomplished via PI3-K [164], PKC-theta [165], MEK, or AKT-inhibitors [142].

Conclusion

This review provides an overview of GIST pathogenesis, morphologic evaluation, new diagnostically promising immunohistochemical markers, risk assessment, the role of molecular analyses as well as the increasing problem of secondary imatinib resistance and its mechanisms. The review demonstrates that within just one decade, GISTs have emerged from being poorly defined, treatment-resistant tumors to a well-recognized, well-understood, and treatable tumor entity. The small molecule tyrosine kinase inhibitors imatinib and sunitinib have fundamentally changed the overall survival for patients with metastatic GIST. However, the increased understanding of GIST biology has also provided informative insights into the limitations of so-called targeted therapeutics and highlights the upcoming problem of secondary resistance. Exact morphologic classification and risk assessment are an essential part of optimal patient care. Although, the precise role of molecular analysis is controversial to date, the potential value of molecular testing in at least a subset of GISTs is clear.

Conflict of interest statement We declare that we have no conflict of interest.

References

1. Demetri GD, Benjamin RS, Blanke CD et al (2007) NCCN Task force report: management of patients with gastrointestinal stromal tumor (GIST)—update of the NCCN clinical practice guidelines. J Natl Compr Canc Netw 5(Suppl 2):S1–S29
2. Edmonson JH, Marks RS, Buckner JC et al (2002) Contrast of response to dacarbazine, mitomycin, doxorubicin, and cisplatin (DMAP) plus GM-CSF between patients with advanced malignant gastrointestinal stromal tumors and patients with other advanced leiomyosarcomas. Cancer Investig 20(5–6):605–612
3. Fletcher CD, Berman JJ, Corless C et al (2002) Diagnosis of gastrointestinal stromal tumors: a consensus approach. Human Pathol 33:459–465
4. Mazur MT, Clark HB (1983) Gastric stromal tumors. Reappraisal of histogenesis. Am J Surg Pathol 7(6):507–519
5. Miettinen M, Virolainen M, Maarit Sarlomo R (1995) Gastrointestinal stromal tumors—value of CD34 antigen in their identification and separation from true leiomyomas and schwannomas. Am J Surg Pathol 19(2):207–216
6. Hirota S, Isozaki K, Moriyama Y et al (1998) Gain-of-function mutations of c-kit in human gastrointestinal stromal tumors. Science 279(5350):577–580
7. Huizinga JD, Thuneberg L, Kluppel M et al (1995) W/kit gene required for interstitial cells of Cajal and for intestinal pacemaker activity. Nature 373(6512):347–349
8. Kindblom L, Ramotti H, Aldenborg F et al (1998) Gastrointestinal pacemaker cell tumor (GIPACT): gastrointestinal stromal tumors show phenotypic characteristics of the interstitial cells of Cajal. Am J Pathol 152:1259–1269
9. Robinson TL, Sircar K, Hewlett BR et al (2000) Gastrointestinal stromal tumors may originate from a subset of CD34-positive interstitial cells of Cajal. Am J Pathol 156(4):1157–1163
10. Janeway KA, Liegl B, Harlow A et al (2007) Pediatric KIT wild-type and platelet-derived growth factor receptor alpha wild-type gastrointestinal stromal tumors share KIT activation but not mechanisms of genetic progression with adult gastrointestinal stromal tumors. Cancer Res 67(19):9084–9088
11. Isozaki K, Hirota S, Nakama A et al (1995) Disturbed intestinal movement, bile reflux to the stomach, and deficiency of c-kit-expressing cells in Ws/Ws mutant rats. Gastroenterology 109 (2):456–464
12. Heinrich MC, Corless CL, Duensing A et al (2003) PDGFRA-activating mutations in gastrointestinal stromal tumors. Science 299(5607):708–710
13. Heinrich MC, Corless CL, Demetri GD et al (2003) Kinase mutations and imatinib response in patients with metastatic gastrointestinal stromal tumor. J Clin Oncol 21(23):4342–4349
14. Corless CL, Fletcher JA, Heinrich MC (2004) Biology of gastrointestinal stromal tumors. J Clin Oncol 22(18):3813–3825
15. Hornick JL, Fletcher CD (2002) Immunohistochemical staining for KIT (CD117) in soft tissue sarcomas is very limited in distribution. Am J Clin Pathol 117(2):188–193
16. Hornick JL, Fletcher CD (2007) The role of KIT in the management of patients with gastrointestinal stromal tumors. Human Pathol 38(5):679–687
17. Stenman G, Eriksson A, Claesson-Welsh L (1989) Human PDGFA receptor gene maps to the same region on chromosome 4 as the KIT oncogene. Genes Chromos Cancer 1(2):155–158
18. Hubbard SR (2004) Juxtamembrane autoinhibition in receptor tyrosine kinases. Nat Rev Mol Cell Biol 5(6):464–471
19. Pawson T (2002) Regulation and targets of receptor tyrosine kinases. Eur J Cancer 38(Suppl 5):S3–S10
20. Blume-Jensen P, Claesson-Welsh L, Siegbahn A et al (1991) Activation of the human c-kit product by ligand-induced dimerization mediates circular actin reorganization and chemotaxis. Embo J 10(13):4121–4128
21. O'Farrell AM, Abrams TJ, Yuen HA et al (2003) SU11248 is a novel FLT3 tyrosine kinase inhibitor with potent activity in vitro and in vivo. Blood 101(9):3597–3605
22. Casteran N, De Sepulveda P, Beslu N et al (2003) Signal transduction by several KIT juxtamembrane domain mutations. Oncogene 22(30):4710–4722
23. Duensing A, Medeiros F, McConarty B et al (2004) Mechanisms of oncogenic KIT signal transduction in primary gastrointestinal stromal tumors (GISTs). Oncogene 23(22):3999–4006
24. Kitayama H, Kanakura Y, Furitsu T et al (1995) Constitutively activating mutations of c-kit receptor tyrosine kinase confer factor-independent growth and tumorigenicity of factor-dependent hematopoietic cell lines. Blood 85(3):790–798

25. Rossi F, Ehlers I, Agosti V et al (2006) Oncogenic Kit signaling and therapeutic intervention in a mouse model of gastrointestinal stromal tumor. Proc Natl Acad Sci U S A 103(34):12843–12848
26. Lev S, Blechman J, Nishikawa S et al (1993) Interspecies molecular chimeras of kit help define the binding site of the stem cell factor. Mol Cell Biol 13(4):2224–2234
27. Maeda H, Yamagata A, Nishikawa S et al (1992) Requirement of c-kit for development of intestinal pacemaker system. Development 116(2):369–375
28. Duensing A, Joseph NE, Medeiros F et al (2004) Protein kinase C theta (PKCtheta) expression and constitutive activation in gastrointestinal stromal tumors (GISTs). Cancer Res 64 (15):5127–5131
29. Rubin BP, Singer S, Tsao C et al (2001) KIT activation is a ubiquitous feature of gastrointestinal stromal tumors. Cancer Res 61(22):8118–21
30. Corless CL, Heinrich MC (2008) Molecular pathobiology of gastrointestinal stromal sarcomas. Annu Rev Pathol 3:557–586
31. Lasota J, Dansonka-Mieszkowska A, Stachura T et al (2003) Gastrointestinal stromal tumors with internal tandem duplications in 3′ end of KIT juxtamembrane domain occur predominantly in stomach and generally seem to have a favorable course. Mod Path 16(12):1257–1264
32. Lasota J, Miettinen M (2008) Clinical significance of oncogenic KIT and PDGFRA mutations in gastrointestinal stromal tumours. Histopathology 53(3):245–266
33. Wardelmann E, Losen I, Hans V et al (2003) Deletion of Trp-557 and Lys-558 in the juxtamembrane domain of the c-kit protooncogene is associated with metastatic behavior of gastrointestinal stromal tumors. Int J Cancer 106(6):887–895
34. Antonescu CR, Sommer G, Sarran L et al (2003) 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 9 (9):3329–3337
35. Miettinen M, Makhlouf H, Sobin LH et al (2006) Gastrointestinal stromal tumors of the jejunum and ileum: a clinicopathologic, immunohistochemical, and molecular genetic study of 906 cases before imatinib with long-term follow-up. Am J Surg Pathol 30(4):477–489
36. Heinrich MC, Owzar K, Corless CL et al (2008) Correlation of kinase genotype and clinical outcome in the North American intergroup phase III trial of imatinib mesylate for treatment of advanced gastrointestinal stromal tumor: CALGB 150105 study by Cancer and Leukemia Group B and Southwest Oncology Group. J Clin Oncol 26(33):5360–5367
37. Lasota J, Corless CL, Heinrich MC et al (2008) Clinicopathologic profile of gastrointestinal stromal tumors (GISTs) with primary KIT exon 13 or exon 17 mutations: a multicenter study on 54 cases. Mod Path 21(4):476–484
38. Debiec-Rychter M, Wasag B, Stul M et al (2004) Gastrointestinal stromal tumours (GISTs) negative for KIT (CD117 antigen) immunoreactivity. J Pathol 202(4):430–438
39. Medeiros F, Corless CL, Duensing A et al (2004) KIT-negative gastrointestinal stromal tumors: proof of concept and therapeutic implications. Am J Surg Pathol 28(7):889–894
40. Wardelmann E, Hrychyk A, Merkelbach-Bruse S et al (2004) Association of platelet-derived growth factor receptor alpha mutations with gastric primary site and epithelioid or mixed cell morphology in gastrointestinal stromal tumors. J Mol Diagn 6 (3):197–204
41. Lasota J, Dansonka-Mieszkowska A, Sobin LH et al (2004) A great majority of GISTs with PDGFRA mutations represent gastric tumors of low or no malignant potential. Lab Invest 84 (7):874–883
42. Lasota J, Stachura J, Miettinen M (2006) GISTs with PDGFRA exon 14 mutations represent subset of clinically favorable gastric tumors with epithelioid morphology. Lab Invest 86(1):94–100
43. Debiec-Rychter M, Sciot R, Le Cesne A et al (2006) KIT mutations and dose selection for imatinib in patients with advanced gastrointestinal stromal tumours. Eur J Cancer 42(8):1093–1103
44. Corless CL, Schroeder A, Griffith D et al (2005) PDGFRA mutations in gastrointestinal stromal tumors: frequency, spectrum and in vitro sensitivity to imatinib. J Clin Oncol 23(23):5357–5364
45. Hirota S, Ohashi A, Nishida T et al (2003) Gain-of-function mutations of platelet-derived growth factor receptor alpha gene in gastrointestinal stromal tumors. Gastroenterology 125(3):660–667
46. Mendel DB, Laird AD, Xin X et al (2003) In vivo antitumor activity of SU11248, a novel tyrosine kinase inhibitor targeting vascular endothelial growth factor and platelet-derived growth factor receptors: determination of a pharmacokinetic/pharmacodynamic relationship. Clin Cancer Res 9(1):327–337
47. Ma Y, Cunningham ME, Wang X et al (1999) Inhibition of spontaneous receptor phosphorylation by residues in a putative alpha-helix in the KIT intracellular juxtamembrane region. J Biol Chem 274(19):13399–13402
48. Agaimy A, Terracciano LM, Dirnhofer S et al (2009) V600E BRAF mutations are alternative early molecular events in a subset of KIT/PDGFRA wild-type gastrointestinal stromal tumours. J Clin Pathol 62(7):613–616
49. Agaram NP, Wong GC, Guo T et al (2008) Novel V600E BRAF mutations in imatinib-naive and imatinib-resistant gastrointestinal stromal tumors. Genes Chromos Cancer 47(10):853–859
50. Hostein I, Faur N, Primois C et al (2010) BRAF mutation status in gastrointestinal stromal tumors. Am J Clin Pathol 133(1):141–148
51. Agaimy A, Wunsch PH, Dirnhofer S et al (2008) Microscopic gastrointestinal stromal tumors in esophageal and intestinal surgical resection specimens: a clinicopathologic, immunohistochemical, and molecular study of 19 lesions. Am J Surg Pathol 32(10):1553–1559
52. Kawanowa K, Sakuma Y, Sakurai S et al (2006) High incidence of microscopic gastrointestinal stromal tumors in the stomach. Human Pathol 37(12):1527–1535
53. Corless CL, McGreevey L, Haley A et al (2002) *KIT* mutations are common in incidental gastrointestinal stromal zumors one centimeter or less in size. Am J Pathol 160(5):1567–1572
54. Debiec-Rychter M, Lasota J, Sarlomo-Rikala M et al (2001) Chromosomal aberrations in malignant gastrointestinal stromal tumors: correlation with c-KIT gene mutation. Cancer Genet Cytogenet 128(1):24–30
55. Fukasawa T, Chong JM, Sakurai S et al (2000) 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 91(12):1241–1249
56. El-Rifai W, Sarlomo-Rikala M, Miettinen M et al (1996) DNA copy number losses in chromosome 14: an early change in gastrointestinal stromal tumors. Cancer Res 56(14):3230–3233
57. Bergmann FGB, Hermanns B et al (1998) 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 82:275–278
58. Kim NG, Kim JJ, Ahn JY et al (2000) Putative chromosomal deletions on 9P, 9Q and 22Q occur preferentially in malignant gastrointestinal stromal tumors. Int J Cancer 85(5):633–638
59. Lasota J, vel Dobosz AJ, Wasag B et al (2007) Presence of homozygous KIT exon 11 mutations is strongly associated with malignant clinical behavior in gastrointestinal stromal tumors. Lab Invest 87(10):1029–1041
60. El-Rifai W, Sarlomo-Rikala M, Andersson LC et al (2000) High-resolution deletion mapping of chromosome 14 in stromal

tumors of the gastrointestinal tract suggests two distinct tumor suppressor loci. Genes Chromos Cancer 27(4):387–391
61. Schurr P, Wolter S, Kaifi J et al (2006) Microsatellite DNA alterations of gastrointestinal stromal tumors are predictive for outcome. Clin Cancer Res 12(17):5151–5157
62. Belinsky MG, Skorobogatko YV, Rink L et al (2009) High density DNA array analysis reveals distinct genomic profiles in a subset of gastrointestinal stromal tumors. Genes Chromos Cancer 48(10):886–896
63. Nilsson B, Bumming P, Meis-Kindblom JM et al (2005) Gastrointestinal stromal tumors: the incidence, prevalence, clinical course, and prognostication in the preimatinib mesylate era-a population-based study in western Sweden. Cancer 103 (4):821–829
64. Goettsch WG, Bos SD, Breekveldt-Postma N et al (2005) Incidence of gastrointestinal stromal tumours is underestimated: results of a nationwide study. Eur J Cancer 41(18):2868–2872
65. Tryggvason G, Gislason HG, Magnusson MK et al (2005) Gastrointestinal stromal tumors in Iceland, 1990-2003: the icelandic GIST study, a population-based incidence and pathologic risk stratification study. Int J Cancer 117(2):289–293
66. Agaimy A, Wunsch PH, Hofstaedter F et al (2007) Minute gastric sclerosing stromal tumors (GIST tumorlets) are common in adults and frequently show c-KIT mutations. Am J Surg Pathol 31(1):113–120
67. Abraham SC, Krasinskas AM, Hofstetter WL et al (2007) "Seedling" mesenchymal tumors (gastrointestinal stromal tumors and leiomyomas) are common incidental tumors of the esophagogastric junction. Am J Surg Pathol 31(11):1629–1635
68. Miettinen M, Sobin LH, Lasota J (2005) Gastrointestinal stromal tumors of the stomach: a clinicopathologic, immunohistochemical, and molecular genetic study of 1,765 cases with long-term follow-up. Am J Surg Pathol 29(1):52–68
69. Prakash S, Sarran L, Socci N et al (2005) Gastrointestinal stromal tumors in children and young adults: a clinicopathologic, molecular, and genomic study of 15 cases and review of the literature. J Pediatr Hematol Oncol 27(4):179–187
70. Miettinen M, Lasota J, Sobin LH (2005) Gastrointestinal stromal tumors of the stomach in children and young adults: a clinicopathologic, immunohistochemical, and molecular genetic study of 44 cases with long-term follow-up and review of the literature. Am J Surg Pathol 29(10):1373–1381
71. Beghini A, Tibiletti MG, Roversi G et al (2001) Germline mutation in the juxtamembrane domain of the kit gene in a family with gastrointestinal stromal tumors and urticaria pigmentosa. Cancer 92(3):657–662
72. Isozaki K, Terris B, Belghiti J et al (2000) Germline-activating mutation in the kinase domain of KIT gene in familial gastrointestinal stromal tumors. Am J Pathol 157(5):1581–1585
73. Kang DY, Park CK, Choi JS et al (2007) Multiple gastrointestinal stromal tumors: clinicopathologic and genetic analysis of 12 patients. Am J Surg Pathol 31(2):224–232
74. Maeyama H, Hidaka E, Ota H et al (2001) Familial gastrointestinal stromal tumor with hyperpigmentation: association with a germline mutation of the c-kit gene. Gastroenterology 120(1):210–215
75. Nishida T, Hirota S, Taniguchi M et al (1998) Familial gastrointestinal stromal tumours with germline mutation of the KIT gene. Nat Genet 19(4):323–324
76. O'Riain C, Corless CL, Heinrich MC et al (2005) Gastrointestinal stromal tumors: insights from a new familial GIST kindred with unusual genetic and pathologic features. Am J Surg Pathol 29(12):1680–1683
77. Kleinbaum EP, Lazar AJ, Tamborini E et al (2008) Clinical, histopathologic, molecular and therapeutic findings in a large kindred with gastrointestinal stromal tumor. Int J Cancer 122 (3):711–718
78. Pasini B, McWhinney SR, Bei T et al (2008) Clinical and molecular genetics of patients with the Carney-Stratakis syndrome and germline mutations of the genes coding for the succinate dehydrogenase subunits SDHB, SDHC, and SDHD. Eur J Hum Genet 16(1):79–88
79. Li FP, Fletcher JA, Heinrich MC et al (2005) Familial gastrointestinal stromal tumor syndrome: phenotypic and molecular features in a kindred. J Clin Oncol 23(12):2735–2743
80. Andersson J, Sihto H, Meis-Kindblom JM et al (2005) NF1-associated gastrointestinal stromal tumors have unique clinical, phenotypic, and genotypic characteristics. Am J Surg Pathol 29 (9):1170–1176
81. Maertens O, Prenen H, Debiec-Rychter M et al (2006) Molecular pathogenesis of multiple gastrointestinal stromal tumors in NF1 patients. Hum Mol Genet 15(6):1015–1023
82. Miettinen M, Fetsch JF, Sobin LH et al (2006) Gastrointestinal stromal tumors in patients with neurofibromatosis 1: a clinicopathologic and molecular genetic study of 45 cases. Am J Surg Pathol 30(1):90–96
83. Carney JA (1999) Gastric stromal sarcoma, pulmonary chondroma, and extra-adrenal paraganglioma (Carney triad): natural history, adrenocortical component, and possible familial occurrence. Mayo Clin Proc 74(6):543–552
84. Carney JA, Stratakis CA (2002) Familial paraganglioma and gastric stromal sarcoma: a new syndrome distinct from the Carney triad. Am J Med Genet 108(2):132–139
85. DeMatteo RP, Lewis JJ, Leung D et al (2000) Two hundred gastrointestinal stromal tumors: recurrence patterns and prognostic factors for survival. Ann Surg 231(1):51–58
86. Miettinen M, Monihan JM, Sarlomo-Rikala M et al (1999) Gastrointestinal stromal tumors/smooth muscle tumors (GISTs) primary in the omentum and mesentery: clinicopathologic and immunohistochemical study of 26 cases. Am J Surg Pathol 23 (9):1109–1118
87. Reith JD, Goldblum JR, Lyles RH et al (2000) Extragastrointestinal (soft tissue) stromal tumors: an analysis of 48 cases with emphasis on histologic predictors of outcome. Mod Path 13 (5):577–585
88. Agaimy A, Markl B, Arnholdt H et al (2009) Multiple sporadic gastrointestinal stromal tumours arising at different gastrointestinal sites: pattern of involvement of the muscularis propria as a clue to independent primary GISTs. Virchows Arch 455(2):101–108
89. Verma P, Corless C, Medeiros F et al (2005) Pleomorphic gastrointestinal stromal tumors: diagnostic and therapeutic implications. Mod Pathol 18(suppl 1):121A, abstract
90. Antonescu CR, Hornick JL, Nielsen GP et al (2007) Dedifferentiation in gastrointestinal stromal tumor (GIST) to an anaplastic KIT-negative phenotype—a diagnostic pitfall. Mod Pathol 20 (suppl 2):11A, abstract
91. Pauwels P, Debiec-Rychter M, Stul M et al (2005) Changing phenotype of gastrointestinal stromal tumours under imatinib mesylate treatment: a potential diagnostic pitfall. Histopathology 47(1):41–47
92. Liegl B, Hornick JL, Antonescu C et al (2009) Rhabdomyosarcomatous differentiation in gastrointestinal stromal tumors after tyrosine kinase inhibitor therapy: a novel form of tumor progression. Am J Surg Pathol 33(2):218–226
93. Janeway KA, Albritton KH, Van Den Abbeele AD et al (2009) Sunitinib treatment in pediatric patients with advanced GIST following failure of imatinib. Pediatr Blood Cancer 52(7):767–771
94. Heinrich MC, Maki RG, Corless CL et al (2008) Primary and secondary kinase genotypes correlate with the biological and clinical activity of sunitinib in imatinib-resistant gastrointestinal stromal tumor. J Clin Oncol 26(33):5352–5359

95. Zhang L, Smyrk TC, Young WF Jr et al (2010) Gastric stromal tumors in Carney triad are different clinically, pathologically, and behaviorally from sporadic gastric gastrointestinal stromal tumors: findings in 104 cases. Am J Surg Pathol 34(1):53–64
96. Sarlomo-Rikala M, Kovatich A, Barusevicius A et al. (1998). CD117: a sensitive marker for gastrointestinal stromal tumors that is more specific than CD34. Mod Path (11):728–734
97. Miettinen M, Lasota J (2005) KIT (CD117): a review on expression in normal and neoplastic tissues, and mutations and their clinicopathologic correlation. Appl Immunohistochem Mol Morphol 13(3):205–220
98. Orosz Z, Tornoczky T, Sapi Z (2005) Gastrointestinal stromal tumors: a clinicopathologic and immunohistochemical study of 136 cases. Pathol Oncol Res 11(1):11–21
99. Liegl B, Hornick JL, Corless C et al (2009) Monoclonal antibody DOG 1.1 shows higher sensitivity than KIT in the diagnosis of Gastrointestinal stromal tumors, including unusual subtypes. Am J Surg Pathol 33(3):437–446
100. West RB, Corless CL, Chen X et al (2004) The novel marker, DOG1, is expressed ubiquitously in gastrointestinal stromal tumors irrespective of KIT or PDGFRA mutation status. Am J Pathol 165(1):107–113
101. Espinosa I, Lee CH, Kim MK et al (2008) A novel monoclonal antibody against DOG1 is a sensitive and specific marker for gastrointestinal stromal tumors. Am J Surg Pathol 32(2):210–218
102. Miettinen M, Wang ZF, Lasota J (2009) DOG1 antibody in the differential diagnosis of gastrointestinal stromal tumors: a study of 1,840 cases. Am J Surg Pathol 33(9):1401–1408
103. Blay P, Astudillo A, Buesa JM et al (2004) Protein kinase C theta is highly expressed in gastrointestinal stromal tumors but not in other mesenchymal neoplasias. Clin Cancer Res 10(12 Pt 1):4089–4095
104. Lee HE, Kim MA, Lee HS et al (2008) Characteristics of KIT-negative gastrointestinal stromal tumours and diagnostic utility of protein kinase C theta immunostaining. J Clin Pathol 61(6):722–729
105. Rossi G, Valli R, Bertolini F et al (2005) PDGFR expression in differential diagnosis between KIT-negative gastrointestinal stromal tumours and other primary soft-tissue tumours of the gastrointestinal tract. Histopathology 46(5):522–531
106. Peterson MR, Piao Z, Weidner N et al (2006) Strong PDGFRA positivity is seen in GISTs but not in other intra-abdominal mesenchymal tumors: immunohistochemical and mutational analyses. Appl Immunohistochem Mol Morphol 14(4):390–396
107. Zheng S, Chen LR, Wang HJ et al (2007) Analysis of mutation and expression of c-kit and PDGFR-alpha gene in gastrointestinal stromal tumor. Hepatogastroenterology 54(80):2285–2290
108. Miselli F, Millefanti C, Conca E et al (2008) PDGFRA immunostaining can help in the diagnosis of gastrointestinal stromal tumors. Am J Surg Pathol 32(5):738–743
109. Yang XH, Wu QL, Yu XB et al (2008) Nestin expression in different tumours and its relevance to malignant grade. J Clin Pathol 61(4):467–473
110. Parkkila S, Lasota J, Fletcher J A et al. (2010) Carbonic anhydrase II. A novel biomarker for gastrointestinal stromal tumors. Mod Pathol (in press)
111. Price ND, Trent J, El-Naggar AK et al (2007) Highly accurate two-gene classifier for differentiating gastrointestinal stromal tumors and leiomyosarcomas. Proc Natl Acad Sci U S A 104 (9):3414–9
112. Sarlomo-Rikala M, Miettinen M (1995) Gastric schwannoma—a clinicopathological analysis of six cases. Histopathology 27 (4):355–360
113. Carlson JW, Fletcher CD (2007) Immunohistochemistry for beta-catenin in the differential diagnosis of spindle cell lesions: analysis of a series and review of the literature. Histopathology 51(4):509–514
114. Montgomery E, Torbenson MS, Kaushal M et al (2002) Beta-catenin immunohistochemistry separates mesenteric fibromatosis from gastrointestinal stromal tumor and sclerosing mesenteritis. Am J Surg Pathol 26(10):1296–1301
115. Lucas DR, al-Abbadi M, Tabaczka P et al (2003) C-Kit expression in desmoid fibromatosis. Comparative immunohistochemical evaluation of two commercial antibodies. Am J Clin Pathol 119(3):339–345
116. Cessna MH, Zhou H, Sanger WG et al (2002) Expression of ALK1 and p80 in inflammatory myofibroblastic tumor and its mesenchymal mimics: a study of 135 cases. Mod Path 15(9):931–938
117. Lasota J, Wang ZF, Sobin LH et al (2009) Gain-of-function PDGFRA mutations, earlier reported in gastrointestinal stromal tumors, are common in small intestinal inflammatory fibroid polyps. A study of 60 cases. Mod Path 22(8):1049–56
118. Schildhaus HU, Cavlar T, Binot E et al (2008) Inflammatory fibroid polyps harbour mutations in the platelet-derived growth factor receptor alpha (PDGFRA) gene. J Pathol 216(2):176–182
119. Antonescu CR, Nafa K, Segal NH et al (2006) EWS-CREB1: a recurrent variant fusion in clear cell sarcoma—association with gastrointestinal location and absence of melanocytic differentiation. Clin Cancer Res 12(18):5356–5362
120. Lyle PL, Amato CM, Fitzpatrick JE et al (2008) Gastrointestinal melanoma or clear cell sarcoma? Molecular evaluation of 7 cases previously diagnosed as malignant melanoma. Am J Surg Pathol 32(6):858–866
121. Fletcher CD, Berman JJ, Corless C et al (2002) Diagnosis of gastrointestinal stromal tumors: a consensus approach. Int J Surg Pathol 10(2):81–89
122. Miettinen M, Lasota J (2006) Gastrointestinal stromal tumors: pathology and prognosis at different sites. Semin Diagn Pathol 23(2):70–83
123. Joensuu H (2008) Risk stratification of patients diagnosed with gastrointestinal stromal tumor. Human Pathol 39(10):1411–1419
124. Woodall CE 3rd, Brock GN, Fan J et al (2009) An evaluation of 2,537 gastrointestinal stromal tumors for a proposed clinical staging system. Arch Surg 144(7):670–678
125. Gold JS, Gonen M, Gutierrez A et al (2009) Development and validation of a prognostic nomogram for recurrence-free survival after complete surgical resection of localised primary gastrointestinal stromal tumour: a retrospective analysis. Lancet Oncol 10(11):1045–1052
126. Perrone F, Tamborini E, Dagrada GP et al (2005) 9p21 locus analysis in high-risk gastrointestinal stromal tumors characterized for c-kit and platelet-derived growth factor receptor alpha gene alterations. Cancer 104(1):159–169
127. Ricci R, Arena V, Castri F et al (2004) Role of p16/INK4a in gastrointestinal stromal tumor progression. Am J Clin Pathol 122 (1):35–43
128. Sabah M, Cummins R, Leader M et al (2004) Loss of heterozygosity of chromosome 9p and loss of p16INK4A expression are associated with malignant gastrointestinal stromal tumors. Mod Pathol 17(11):1364–1371
129. Schneider-Stock R, Boltze C, Lasota J et al (2003) High prognostic value of p16INK4 alterations in gastrointestinal stromal tumors. J Clin Oncol 21(9):1688–1697
130. Schneider-Stock R, Boltze C, Lasota J et al (2005) Loss of p16 protein defines high-risk patients with gastrointestinal stromal tumors: a tissue microarray study. Clin Cancer Res 11(2 Pt 1):638–645
131. Steigen SE, Bjerkehagen B, Haugland HK et al (2008) Diagnostic and prognostic markers for gastrointestinal stromal tumors in Norway. Mod Path 21(1):46–53
132. Feakins RM (2005) The expression of p53 and bcl-2 in gastrointestinal stromal tumours is associated with anatomical site, and p53 expression is associated with grade and clinical outcome. Histopathology 46(3):270–279

133. Nemoto Y, Mikami T, Hana K et al (2006) Correlation of enhanced cell turnover with prognosis of gastrointestinal stromal tumors of the stomach: relevance of cellularity and p27kip1. Pathol Int 56(12):724–731
134. Pruneri G, Mazzarol G, Fabris S et al (2003) Cyclin D3 immunoreactivity in gastrointestinal stromal tumors is independent of cyclin D3 gene amplification and is associated with nuclear p27 accumulation. Mod Path 16(9):886–892
135. Tornillo L, Duchini G, Carafa V et al (2005) Patterns of gene amplification in gastrointestinal stromal tumors (GIST). Lab Invest 85(7):921–931
136. Romeo S, Debiec-Rychter M, Van Glabbeke M et al (2009) Cell cycle/apoptosis molecule expression correlates with imatinib response in patients with advanced gastrointestinal stromal tumors. Clin Cancer Res 15(12):4191–4198
137. Martinho O, Gouveia A, Silva P et al (2009) Loss of RKIP expression is associated with poor survival in GISTs. Virchows Arch 455(3):277–284
138. Turkoz HK, Alkan I, Sisman S et al (2009) Cyclooxygenase-2 expression and connection with tumor recurrence and histopathologic parameters in gastrointestinal stromal tumors. APMIS 117 (11):825–830
139. Wei YC, Li CF, Yu SC et al (2009) Ezrin overexpression in gastrointestinal stromal tumors: an independent adverse prognosticator associated with the non-gastric location. Mod Path 22 (10):1351–1360
140. Blanke CD, Demetri GD, von Mehren M et al (2008) Long-term results from a randomized phase II trial of standard- versus higher-dose imatinib mesylate for patients with unresectable or metastatic gastrointestinal stromal tumors expressing KIT. J Clin Oncol 26(4):620–625
141. Blanke CD, Rankin C, Demetri GD et al (2008) Phase III randomized, intergroup trial assessing imatinib mesylate at two dose levels in patients with unresectable or metastatic gastrointestinal stromal tumors expressing the kit receptor tyrosine kinase: S0033. J Clin Oncol 26(4):626–632
142. Heinrich MC, Corless CL, Blanke CD et al (2006) Molecular correlates of imatinib resistance in gastrointestinal stromal tumors. J Clin Oncol 24(29):4764–4774
143. Casali PG, Jost L, Reichardt P et al (2008) Gastrointestinal stromal tumors: ESMO clinical recommendations for diagnosis, treatment and follow-up. Ann Oncol 19(Suppl 2):ii35–ii38
144. DeMatteo R, Owzar K, Antonescu C R, et al. (2008). Efficacy of adjuvant imatinib mesylate following complete resection of localized, primary gastrointestinal stromal tumor (GIST) at high risk of recurrence: the US intergroup phase II trial ACOSOG Z9000. Gastrointestinal Cancer Symposium, Orlando, Florida. Proceedings No. 8, p73
145. DeMatteo RPACR, Chadaram V, et al. (2005). Adjuvant imatinib mesylate in patients with primary high risk gastrointestinal stromal tumors (GIST) following complete resection: Safety results from the U.S. Intergroup Phase II trial ACOSOG Z9000. J Clin Oncol 23(ASCO Annual Meeting Proceedings. No. 16S, Part I of II (June 1 Supplement))
146. DeMatteo RP, Ballman KV, Antonescu CR et al (2009) Adjuvant imatinib mesylate after resection of localised, primary gastrointestinal stromal tumour: a randomised, double-blind, placebo-controlled trial. Lancet 373(9669):1097–1104
147. Lasota J, Miettinen M (2006) KIT and PDGFRA mutations in gastrointestinal stromal tumors (GISTs). Semin Diagn Pathol 23 (2):91–102
148. Fletcher J, Corless C, Dimitrijevic S et al. (2003) Mechanisms of resistance to imatinib mesylate (IM) in advanced gastrointestinal stromal tumors (GIST). Proc Am Soc Clin Oncol (22):3275–3277
149. Debiec-Rychter M, Cools J, Dumez H et al (2005) Mechanisms of resistance to imatinib mesylate in gastrointestinal stromal tumors and activity of the PKC412 inhibitor against imatinib-resistant mutants. Gastroenterology 128(2):270–279
150. Liegl B, Kepten I, Lee C et al (2008) Heterogeneity of kinase inhibitor resistance mechanisms in GIST. J Pathol 216(1):64–74
151. Agaram NP, Besmer P, Wong GC et al (2007) Pathologic and molecular heterogeneity in imatinib-stable or imatinib-responsive gastrointestinal stromal tumors. Clin Cancer Res 13(1):170–181
152. Prenen H, Cools J, Mentens N et al (2006) Efficacy of the kinase inhibitor SU11248 against gastrointestinal stromal tumor mutants refractory to imatinib mesylate. Clin Cancer Res 12(8):2622–2627
153. Wardelmann E, Merkelbach-Bruse S, Pauls K et al (2006) Polyclonal evolution of multiple secondary KIT mutations in gastrointestinal stromal tumors under treatment with imatinib mesylate. Clin Cancer Res 12(6):1743–1749
154. Antonescu CR, Besmer P, Guo T et al (2005) Acquired resistance to imatinib in gastrointestinal stromal tumor occurs through secondary gene mutation. Clin Cancer Res 11(11):4182–4190
155. Demetri GD, van Oosterom AT, Garrett CR et al (2006) Efficacy and safety of sunitinib in patients with advanced gastrointestinal stromal tumour after failure of imatinib: a randomised controlled trial. Lancet 368(9544):1329–1338
156. Montemurro M, Schoffski P, Reichardt P et al (2009) Nilotinib in the treatment of advanced gastrointestinal stromal tumours resistant to both imatinib and sunitinib. Eur J Cancer 45 (13):2293–2297
157. Dewaele B, Wasag B, Cools J et al (2008) Activity of dasatinib, a dual SRC/ABL kinase inhibitor, and IPI-504, a heat shock protein 90 inhibitor, against gastrointestinal stromal tumor-associated PDGFRAD842V mutation. Clin Cancer Res 14 (18):5749–5758
158. Muhlenberg T, Zhang Y, Wagner AJ et al (2009) Inhibitors of deacetylases suppress oncogenic KIT signaling, acetylate HSP90, and induce apoptosis in gastrointestinal stromal tumors. Cancer Res 69(17):6941–6950
159. Pantaleo MA, Astolfi A, Di Battista M et al (2009) Insulin-like growth factor 1 receptor expression in wild-type GISTs: a potential novel therapeutic target. Int J Cancer 125(12):2991–2994
160. Tarn C, Rink L, Merkel E et al (2008) Insulin-like growth factor 1 receptor is a potential therapeutic target for gastrointestinal stromal tumors. Proc Natl Acad Sci U S A 105(24):8387–92
161. Bauer S, Yu LK, Demetri GD et al (2006) Heat shock protein 90 inhibition in imatinib-resistant gastrointestinal stromal tumor. Cancer Res 66(18):9153–9161
162. Sambol EB, Ambrosini G, Geha RC et al (2006) Flavopiridol targets c-KIT transcription and induces apoptosis in gastrointestinal stromal tumor cells. Cancer Res 66(11):5858–5866
163. Bauer S, Parry JA, Muhlenberg T et al (2010) Proapoptotic activity of bortezomib in gastrointestinal stromal tumor cells. Cancer Res 70(1):150–159
164. Bauer S, Duensing A, Demetri GD et al (2007) KIT oncogenic signaling mechanisms in imatinib-resistant gastrointestinal stromal tumor: PI3-kinase/AKT is a crucial survival pathway. Oncogene 26(54):7560–7568
165. Ou WB, Zhu MJ, Demetri GD et al (2008) Protein kinase C-theta regulates KIT expression and proliferation in gastrointestinal stromal tumors. Oncogene 18:5624–5634