Review article

Gastrointestinal stromal tumor: a bridge between bench and bedside

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Abstract

Gastrointestinal stromal tumor (GIST) is considered to be driven by a gain-of-function mutation in the *KIT* or *PDGFRA* gene. Cure can be obtained only by complete surgical removal of the GIST; however, imatinib, an inhibitor of KIT and PDGFRA, is indicated for advanced, recurrent, and/or metastatic GISTs. Imatinib exhibited remarkable clinical effects on advanced GISTs, with substantial tolerability. Its efficacy greatly depends on the genotype of GIST. The drug, however, met intrinsic or acquired resistance during the treatment, of which the molecular mechanisms were mostly dependent on the genotype of GIST, including primary mutations or secondary mutations in the kinase domains of the corresponding target genes, respectively. Although sunitinib had substantial effects on imatinib-resistant GIST, this drug also encountered primary or secondary resistance depending on the genotype. Thus, advanced GIST may require multidisciplinary treatment. Because resistance mechanisms show some regularity, it is hoped that, in the near future, we may be able to develop a new drug to which resistance does not occur easily, based on scientific evidence.

Key words *KIT* · *PDGFRA* · Imatinib · Sunitinib · Resistance

Introduction

Overview of GIST: roles of surgery and target agents

Although gastrointestinal stromal tumors (GISTs) are relatively infrequent cancers in the gastrointestinal (GI) tract, GISTs are the most common mesenchymal tumors in the GI tract [1, 2]. Clinically, GIST has an incidence of 10–20/million per year and shows no ethnic or gender differences. Primary GISTs are located mainly in the stomach (60%–70%) and small intestine (20%–30%),

and a few (less than 5%) are found in the colon and the esophagus, and even in peritoneal tissues, including the mesentery, retroperitoneum, and omentum. On immunohistochemical examination, most GISTs (95%) express KIT protein and only a few (5%) do not. The latter, called KIT-negative GISTs, may have mutations either in the *PDGFRA* gene or the *KIT* gene, with the expression of KIT protein at a very low level that cannot be detected by immunohistochemistry. Until recently, there had been no effective treatment for advanced and recurrent GISTs, which have been subjected to repeated but hopeless struggles with surgery, because GISTs are resistant to conventional chemotherapy and radiotherapy. A decade ago, gain-of-function mutations in the *KIT* gene, a monumental milestone, were found in most sporadic GISTs [3, 4], followed by the discovery of similar gain-of-function mutations in the *PDGFRA* gene, a sibling gene of *KIT* [5, 6]. Since then, target agents, including imatinib mesylate (Glivec; Novartis, Basel, Switzerland) and sunitinib malate (Sutent; Pfizer, New York, NY, USA) have been clinically developed. Now, GIST has become a model of targeted therapy for solid tumors, as imatinib has become the first-line treatment for advanced GISTs [2, 7–9].

However, even in an era of molecular-targeted therapy, the primary treatment for GIST is still complete resection by surgery, if it is resectable. Nonetheless, most tumors are clinically diagnosed as abdominal tumors or submucosal tumors (SMTs) before operation, and the diagnosis of GIST is frequently made after surgery by pathological examinations with KIT immunostaining or, less frequently, by genotyping. GIST grows expansively and has infrequent symptoms and signs until it is at an advanced stage, which may result in its being metastatic or at an advanced stage when it is found. GIST mainly metastasizes to the liver hematogenously and/or disseminates in the peritoneal cavity, but rarely shows lymphatic involvement. In Japan, the cancer screening systems may detect relatively small

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SMTs incidentally. These tumors are frequently found as asymptomatic SMTs. When asymptomatic and small SMTs are detected by the screening, the guidelines suggest surgery when SMTs show growth during follow up and clinically malignant features such as irregular margins, ulcer formation, and inhomogeneous parenchyma by echo or computed tomography (CT) scan [9]. Small and asymptomatic SMTs without these features may be followed by periodic endoscopic examinations (Fig. 1). These tumors may be good candidates for laparoscopic surgery as long as the principles of GIST surgery are kept. The roles of laparoscopic surgery in GIST and other cancers are expanding, but this should be discussed separately. The principles of GIST surgery include macroscopically complete resection with safety margins of at least 1 to 2 cm, partial resection with careful consideration of preservation of organ function, no prophylactic lymph node dissection, and avoiding injuries to the tumor pseudocapsule, as well as avoiding tumor rupture by surgical manipulation [7, 9].

When GISTs are inoperable or when achieving curative surgery is difficult, or when there are evident metastases or relapses, imatinib, a selective inhibitor of KIT and PDGFRs, is recommended as the first-line treatment of KIT-positive GIST [7–9]. Although imatinib has shown substantial improvements in the prognoses of patients with advanced GIST, nearly 10% of GISTs show primary resistance to imatinib, 5% of patients exhibit intolerance to imatinib, and half of the patients experience disease progression in the 2–3 years after

commencing imatinib [2, 10–15]. Sunitinib, an oral, multitargeted receptor tyrosine kinase inhibitor of KIT, platelet-derived growth factor receptor- α and - β , vascular endothelial growth factor receptors (VEGFR-1, -2, and -3), FMS-like tyrosine kinase-3 receptor (FLT3), colony-stimulating factor 1 receptor (CSF-1R), and glial cell line-derived neurotrophic factor receptor (RET), is approved for the treatment of advanced GIST after the failure of imatinib [16, 17].

This article will focus on recent advances in GIST therapy, focusing especially on molecular-targeted therapy and its fundamental mechanism of efficacy and resistance.

Pathogenesis of GIST and mechanism of its proliferation

A quick review of GIST oncogenesis and proliferation

Approximately 90%–95% of GISTs are associated with and thus are driven by oncogenic mutations in either the *KIT* or the *PDGFRA* gene; these genes encode similar type III receptor tyrosine kinases: KIT or PDGFR- α , respectively [18, 19]. Both genes are located on chromosome 4 (4q11–q12) nearby. Mutations in the *KIT* or *PDGFRA* gene induce dimerization and autophosphorylation of the corresponding tyrosine kinase without the binding of their ligands,

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Fig. 1. Approaches to submucosal tumor (SMT) or abdominal mass including gastrointestinal stromal tumor (GIST). According to the Japanese GIST guidelines [9], strategies for incidentally found SMTs and GISTs are shown. Asterisk, clinically malignant features include irregular margins, inhomogeneous parenchyma, and ulcer formation. EUS, endoscopic ultrasonography; FNA, fine-needle aspiration; CT, computed tomography. Adapted from reference 9, with permission

stem cell factor and platelet-derived growth factors, respectively.

In the remaining small subgroups of GISTs (wild-type GISTs; 5%–10%) it has been suggested that mutations in the neurofibromatosis type 1 (*NFI*) gene in neurofibromatosis-associated GISTs, amplification of the insulin-like growth factor 1 receptor (IGF1R) in juvenile GISTs with typical clinical features, and b-Raf (BRAF) mutations may contribute to neoplastic proliferation; of note, the expression of KIT and its phosphorylation have been observed even in wild-type GISTs [15, 18, 19].

KIT and PDGFRA mutations in sporadic GISTs

Mutations in the *KIT* or *PDGFRA* gene are mutually exclusive, and cause ligand-independent constitutive activation of the corresponding receptor tyrosine kinase, KIT or PDGFR- α , respectively, and subsequently activate common downstream signaling pathways, including ERK kinases, PI3kinase-mTOR pathways, and STATs pathways. Primary and untreated GIST carries only a single mutation of either the *KIT* (75%–80%) or the *PDGFRA* (~10%) gene. In *KIT*, four hot spots have been identified, i.e., exon 9, exon 11, exon 13, and exon 17, and three hot spots, exons 12, 14, and 18, have been identified for *PDGFRA*. *KIT* mutations in primary GISTs were most frequently found in the juxtamembrane domain (exon 11, 60%–70%), followed by the extracellular domain (exon 9; 6%–10%), but were rarely found in two kinase domains (exons 13 and 17) [18–22]. Similarly, 10%–15% of GISTs have *PDGFRA* mutations either in the juxtamembrane domain (exon 12, 2%–3%) or in the kinase domain (exon 14, ~1%; exon 18, 10%) [19, 21–24]. GISTs with mutations in the *PDGFRA* gene are mostly found in the stomach. Pathological examinations have revealed predominant epithelioid tumor cells in a myxoid stromal background, which sometimes express KIT protein weakly. *PDGFRA* mutations are found in more than 10% of primary GISTs and 5%–10% of recurrent GISTs. GISTs with *PDGFRA* mutations show relatively indolent clinicopathological features. Approximately 5%–10% of GISTs have no mutation in either the *KIT* or the *PDGFRA* gene (Fig. 2).

KIT exon 9 mutation is found exclusively in intestinal GISTs with spindle-cell shape tumor cells and these GISTs show aggressive clinicopathological features [18]. Exon 9 (and exon 8) encode the extracellular domain just beneath five immunoglobulin-like loops (Fig. 2) and mutations in these exons may facilitate receptor dimerization. Exon 9 mutations are almost always the insertion of six base pairs, a duplication of Ala and Tyr, and are detected in 5%–10% of primary GISTs [24] and in 10% to 15% of relapsed or advanced

GISTs [18, 20–22]. Mutations in this region may, thus, render KIT protein prone to dimerization. Mutations in the extracellular domain appear to be still stabilized in an autoinhibited form.

KIT exon 11 and *PDGFRA* exon 12 form the juxtamembrane domain. This domain in both genes not only interacts with the activation loop to stabilize the kinase in an autoinhibited form but interacts with receptor dimerization [25]. Any type of mutation in this domain resulting in loss of its functions leads to conformational changes, to instability of the autoinhibited form, and to the loss of the inhibitory function for dimerization. Thus, various types of mutations, such as missense mutations and insertion and deletion mutations, are found in this region. GIST with *KIT* exon 11 or *PDGFRA* exon 12 mutation is found throughout the GI tract. Interestingly, internal tandem repeat mutations in the distal part of *KIT* exon 11 are infrequent, but are predominantly found in female stomach, and GISTs with these internal tandem repeat mutations show relatively indolent clinical features [18, 26]. Mutations in the juxtamembrane domain tend to be stabilized in an autoinhibited form under unactivated conditions.

Mutations in the kinase domains are uncommonly found in *KIT* exon 13 or 17 as well as in *PDGFRA* exon 14 or 18. Most mutations are a missense mutation. Most of the mutations in these domains, especially in the ATP-binding domain, found in primary GISTs seem to be in an autoinhibited form under unactivated conditions, as seen in exon 9 or 11 mutations, and only the two types of mutations, D816H/V of *KIT* and D842V of *PDGFRA*, show strong conformational equilibrium to the activated form. In general, the kinases preferentially stabilized in the autoinhibited form under unactivated conditions are usually sensitive to both imatinib and sunitinib, while the kinases stabilized in the activated form are extremely resistant to both drugs [27].

Some reports have indicated that GISTs with deletion mutations in and around *KIT* exon 11 codon 557–558, where the juxtamembrane domain mainly interacts with kinase domains and has inhibitory effects on receptor phosphorylation and activation, showed aggressive clinicopathological features and poor prognosis; however, other studies did not [1, 18, 19, 28]. In addition, loss of heterozygosity in the *KIT* locus, that is, a shift from heterozygosity to homozygosity, which was caused by the deletion of wild-type *KIT* and was sometimes associated with an increase in the copy number of mutated *KIT*, was reported to be associated with high proliferative activity and metastatic potential [29, 30]. All these reports were small studies, so further investigations are required to reach a consensus on genotyping and clinicopathological aggressiveness.

Fig. 2. Mutations in the *KIT* and *PDGFRA* genes. Several types of mutations found in primary GISTs and their frequency are shown. *NF-1*, neurofibromatosis type 1

Germline mutations in the KIT and PDGFRA genes cause familial GISTs

Concerning familial GIST, germline mutation in the *KIT* or *PDGFRA* gene was first reported in 1998 [3], the same year that *KIT* mutations in sporadic GIST were reported [4]. Since then, at least 22 pedigrees have been reported with germline mutations in *KIT* exon 8 (1 pedigree), exon 11 (13 pedigrees), exon 13 (2 pedigrees), exon 17 (3 pedigrees), *PDGFRA* exon 12 (2 pedigrees), and exon 18 (1 pedigree; Table 1) [31–51]. Inheritance is autosomal dominant with high penetrance (more than 90%) [52]. The median age of onset (age 44 years) of familial GISTs is younger than that of sporadic GIST (60 years) without gender difference. In these patients, multiple and low-to-intermediate-risk GISTs have been seen in the small intestine, the stomach, and rarely in the colon. Members of four families with germline mutations in *KIT* exon 8 or 17 showed motility disorders of the GI tract including dysphagia and constipation. Importantly, histopathological examinations revealed that most family members had microscopically diffuse proliferation of strongly KIT-expressing cells in the myenteric plexus of normal-appearing GI tract. These results are very consistent with findings in knock-

in mice [53, 54], suggesting that GIST originates from immature mesenchymal cells of the same lineage as the interstitial cells of Cajal, a pacemaker cell of the GI tract. Other distinctive features of familial GISTs are skin pigmentation and skin disorders. Some family members had cutaneous mastocytosis, urticaria pigmentosa, and, rarely, melanoma. Somatic mutations in the *KIT* gene were reported to have a role in the oncogenesis and progression of sporadic mastocytosis and melanoma [55–57].

In spite of the early onset, fewer than 20% of patients with familial GIST die of the disease [52] suggesting that most family members have low-grade risk GISTs. Furthermore, 10%–20% of normal patients over the age of 60, who underwent gastrectomy due to gastric cancer, are reported to have microscopic and multiple GISTs in the upper stomach [58–60], which also harbor mutations in the *KIT* gene [58, 59]. Activating mutations in the *KIT* gene are suggested to be acquired very early in the development of most sporadic GISTs, and *KIT* mutations per se are thought to be of little importance in malignant transformation [61]. These results suggest that mutations in the *KIT* or *PDGFRA* gene are involved in the oncogenesis and proliferation of GIST, but not in malignant changes.

Table 1. Mutations and characteristic features of familial GIST

Pedigree no.	Exons	Mutation type	Locus	Symptoms and signs	Hyperplasia of ICC	Aggressiveness	Reference no.
1	KIT Exon 8	del 419	SB	Diffuse cutaneous mastocytosis, dysphagia	Yes	Benign	31
2	KIT Exon 11	del 559 or 560	G SB	Skin pigmentation	Yes	Benign	4
3	KIT Exon 11	W557R	SB		Yes	Benign	32
4	KIT Exon 11	V559A	G SB	Skin pigmentation, urticaria pigmentosa	Yes	Benign-low malignant	33
5	KIT Exon 11	V559A	G SB	Skin pigmentation	NE	Malignant in benign	34
6	KIT Exon 11	W557R	G SB	Skin pigmentation	Yes	Benign to malignant	35
7	KIT Exon 11	L576_P577insQL	G SB	Skin pigmentation	Yes	Benign	36
8	KIT Exon 11	V559A	G SB	Vitiligo, skin pigmentation, urticaria pigmentosa, and lentigines melanoma	Yes	Benign	37
9	KIT Exon 11	V559A	SB		Yes	Benign	38
10	KIT Exon 11	V560G	SB	Skin pigmentation	Yes	Malignant in benign	39
11	KIT Exon 11	del 579	SB C		Yes	Benign	40
12	KIT Exon 11	del D579	G SB		NE	Benign	42
13	KIT Exon 11	del 579	G SB	Junctional melanocytic nevus	Yes	Benign to malignant	42
14	KIT Exon 11	575_P577del-insH	C		NE	Benign	43
15	KIT Exon 13	K642E	G SB		Yes	Benign-low malignant	44
16	KIT Exon 13	K642E	G SB	Vitiligo	Yes	Benign	45
17	KIT Exon 17	D820Y	G SB	Dysphagia	Yes	Benign	46
18	KIT Exon 17	D820Y	G SB	Dysphagia	Yes	Benign to malignant	47
19	KIT Exon 17	N822Y	G SB		Yes	Benign	48
20	PDGFRA Exon 12	Y555C	SB	Large hand constipation	NE	Benign	49
21	PDGFRA Exon 12	V561D	G SB		NE	Benign	50
22	PDGFRA Exon 18	D846Y	G	Congenital malformation of the hands	Yes	Benign	51

G, stomach; SB, small intestine; C, colon; GIST, gastrointestinal stromal tumor; ICC, interstitial cells of Cajal; NE, not examined

Mechanism of action of imatinib and clinical results

Imatinib, a derivative of 2-phenylaminopyrimidine, has been developed as an agent targeted for the BCR-ABL protein, which is responsible for oncogenesis and proliferation of chronic myelogenous leukemia. Imatinib also inhibits ABL, KIT, and PDGFRs, and the latter two are targets of GIST. Imatinib is very water-soluble, is rapidly absorbed from the GI tract, and its bioavailability is high (98%). Imatinib mainly binds to serum proteins such as albumin and α 1-acid glycoprotein, in the blood, and is metabolized by the cytochrome P450 system, including CYP3A4 and other cytochromes, of the liver (Table 2). The half-lives of imatinib and its main metabolite are 16–18 h and 40 h, respectively. The main metabolite, *N*-demethylated piperazine derivative, is also active.

Sunitinib, a derivative of oxindole, is an oral, small-molecule, multitargeted receptor tyrosine kinase inhibitor. Sunitinib inhibits VEGFR-1, -2, and -3 in the order of K_i : 1, 3 > 2), PDGFRA, PDGFRB > KIT > RET and FLT-3 [17]. Sunitinib appears to be less bioavailable than imatinib, and its binding to serum proteins and metabolic pathways is very similar to that of imatinib. The half-lives of sunitinib and its main metabolite are 40–50 h and 80–110 h, respectively. Although sunitinib has both direct inhibitory effects on KIT and PDGFRA expressing on GIST tumor cells and indirect inhibitory effects via the inhibition of tumor angiogenesis and reduction of blood flow through the inhibition of VEGFR and PDGFR signals, recent data have indicated that direct KIT or PDGFRA inhibition is a principal action for GIST [62, 63]. This will be discussed later.

Both imatinib and sunitinib, which are competitive inhibitors of ATP, enter the ATP-binding pocket of KIT and PDGFRA kinases. These drugs bind to the autoinhibited form, but not the activated form, of these receptor tyrosine kinases, then reversibly inhibit auto-

phosphorylation as well as activation of the kinases, resulting in the silencing of downstream intracellular signaling pathways. The two drugs, however, have different binding loci and modes. Imatinib interacts with several amino acid residues both in the ATP-binding pocket and in the activation loop, and sunitinib may interact with different residues in the ATP-binding pocket [64], which may result in different inhibitory activities of the two drugs.

Clinical trials of imatinib

A number of clinical studies have demonstrated the efficacy and tolerability of imatinib at the recommended dose of 400 mg/day for advanced, metastatic, or recurrent GIST [10–14, 65]. In the clinical trials, complete responses (CRs) were rarely observed (1%), and most patients showed a partial response (PR, 4%–70%) or stable disease (SD, 20%). The progression-free survival (PFS) of patients with long SD (SD of more than 6 months) was very similar to that of patients with a PR [12]. After 6 months of imatinib therapy, if a patient did not experience progressive disease (PD), the patterns of radiological responses (CR, PR, and long SD), determined by tumor size criteria such as RECIST (response evaluation criteria in solid tumors) had no prognostic value for further outcome, and it was recommended that imatinib needed to be continued as long as there was no progression according to RECIST [66]. The disease control rate was nearly 85%. Thus, only 10%–15% of patients with advanced GIST suffered from PD, and up to 5% did not tolerate imatinib. A comparison of two doses of imatinib (400 mg/day vs 800 mg/day) by the European Organization for Research and Treatment of Cancer (EORTC)/intergroup and US S0033 trials indicated that both doses resulted in similar response rates and overall survival (OS) [11, 14], suggesting that the recommended dose be 400 mg/day. However, the higher dose (800 mg/day)

Table 2. Pharmacokinetic parameters of imatinib and sunitinib

	Imatinib mesylate	Sunitinib malate
Drug	Tablet	Capsule
Targets	BCR-ABL, KIT, PDGFR, ABL	VEGFR, KIT, PDGFR, CSF-1, FLT3, RET
Tmax (h)	~3	~8
t1/2 (h)	~8	40–50
Minimal effective dose	300 mg $\leq$	25 mg<
Maximal safe dose	$\leq$ 800 mg	$\leq$ 50 mg
Medication	Oral continuous	Oral interrupted (or continuous)
Bioavailability	>98%	55%–80% (in animals)
Blood distribution	Binding >95% (albumin, α 1-acid glycoprotein)	Binding 95%–90%
Metabolism	Hepatic (CYP3A4)	Hepatic (CYP3A4)
First metabolites	Antitumor effects (+)	Antitumor effects (+)

PDGFR, platelet-derived growth factor receptor; VEGFR, vascular endothelial growth factor receptor; RET, glial cell line-derived neurotrophic factor receptor; Tmax, time that a drug is present at the maximum concentration in serum; t1/2, half-life

showed better PFS than the 400 mg/day dose in the EORTC/intergroup phase III trial, whereas the two doses showed similar PFS in the US S0033 phase III trial. Finally, a meta-analysis of these two trials suggested that there were no significant differences between the two doses in OS, and that treatment with 800 mg/day imatinib showed significantly longer median PFS when a mutation was found in *KIT* exon 9 [15]. In fact, in in vitro studies, a higher concentration was required to inhibit the kinase activity of KIT with exon 9 mutations compared to the concentration required to inhibit the kinase activity of KIT with exon 11 mutations [20].

The BFR14 study randomized advanced GIST patients to receive or to stop ongoing imatinib until progression following 1 or 3 years of tumor control with imatinib [67]. The study showed that imatinib interruption was associated with a high risk of disease progression and that median time to progression (TTP) after stopping either 1-year or 3-year treatment with imatinib was similar (6 months), suggesting that imatinib discontinuation could not be recommended in routine practice even if an apparent CR was obtained. When treatment was restarted, fortunately, disease control was re-established in more than 90% of such cases and OS as well as time to resistance for imatinib appeared to be similar in the stopping and continuous groups. Recently, a pharmacokinetic analysis of the B2222 study [10] has shown that the lowest quartile of imatinib trough levels showed poor clinical outcomes with a lower clinical benefit rate and shorter TTP than the other three quartiles [68]. Lower doses are reported to be risk factors for early progression of the disease [69]. These data suggest that maintenance of the optimal blood level of imatinib is ideal and that blood level testing may be required for optimizing the clinical outcomes of patients with GIST.

From the subanalyses of several clinical trials, factors affecting effectiveness and outcomes of imatinib therapy were indicated [12, 14, 68, 69]. Among them, performance status (PS), tumor mass volume, gender (better prognosis for females), serum level of albumin, imatinib dose, location of the primary tumor (gastric GIST had better prognosis than intestinal), and genotype were commonly indicated to be prognostic.

Genotyping and clinical efficacy

The expression of KIT protein did not always correlate with response to imatinib and PFS, although a significant difference was observed in median OS when KIT-positive tumors were compared with KIT-negative tumors (53 vs 31 months) [14]. Rather, genotypes of *KIT* and *PDGFRA* were correlated well with response to imatinib, PFS, and OS [13, 20–22]. GIST with *KIT*

exon 11 mutations showed a more desirable response to imatinib, followed by GIST with *KIT* exon 9 mutations and wild type. Of note, the beneficial effect of the higher dose (800 mg/day) was confined to GISTs with *KIT* exon 9 mutations, as described above. The number of patients observed is small, but patients with the *PDGFRA* mutation of D842V are highly resistant both to imatinib and sunitinib [62, 63]. These results are consistent with in vitro experiments [6, 62, 64].

With imatinib treatment, the median PFS of patients with advanced GIST appears to be 2 years, and the median OS is approaching 55–57 months, whereas before the imatinib era such patients had an OS of only 9–20 months [12].

Therapy for imatinib-resistant GIST

When imatinib-resistant GIST is diagnosed, we now have a few treatment strategies. When resistant lesions are limited and resectable by operation with substantial safety, one of the therapeutic choices is complete resection of the resistant lesions and continued imatinib therapy. This may add another 8 months of PFS. When the lesions are unresectable, when surgical treatments have substantial risks, or when resistance is systemic, sunitinib is indicated for patients with a good PS (0 or 1). Increase in the imatinib dose to 800 mg/day is another option to imatinib-resistant GIST. This may add another 3–4 months of PFS. In this review, we will focus on sunitinib.

Clinical trials of sunitinib

Sunitinib has been approved multinationally for the treatment of patients with imatinib-resistant GIST or those with imatinib intolerance. Sunitinib was administered at 50 mg/day on a 4-weeks-on and 2-weeks-off schedule in clinical trials, although recently continuous daily doses of 37.5 mg/day have been reported to have similar efficacy and tolerability [16, 70]. From several clinical studies, sunitinib showed response rates of 5%–10% and long SD (SD of more than 6 months) rates of 2%–30%. Thus, the clinical benefit rate (CR+PR+ long SD) was 30%–40%, with a median PFS of 8 months and median OS of 1.5–2 years [71]. Clinical factors affecting outcomes of sunitinib therapy indicated by subgroup analysis of a phase 4 trial were PS, age, and prior imatinib dose (previous higher dose was correlated with a worse prognosis) [15]. More importantly, the *KIT* or *PDGFRA* genotype of GISTs appeared to be correlated with the response to sunitinib and the PFS [62, 63]. The molecular mechanisms of sunitinib resistance are still being investigated. Preliminary results have indicated that genotype and newly developed mutations in the activation loop of the *KIT* or *PDGFRA* gene

appeared to be critical [62, 63]. This will be discussed below.

Molecular mechanisms of resistance to imatinib and sunitinib

Diagnosis of resistance

Half of GISTs under imatinib therapy will show resistance within 2 years. Resistance to imatinib is divided into two categories, primary resistance and secondary resistance. Primary resistance is defined as progression of the disease before any significant effects occur, including CR, PR, and long SD (SD for more than 6 months) and secondary resistance is defined as disease progression after CR, PR, and long SD.

Primary resistance usually appears as enlargement of preexisting tumors or as the appearance of new lesions as defined by RECIST. Primary resistance was shown to be correlated with the genotype [6, 13, 20–22]. Resistance to imatinib was frequently seen in GISTs without mutations in the *KIT* and *PDGFRA* genes, as well as being seen in GISTs with resistant types of mutations in kinase domains (i.e., D816H/V of *KIT* and D842V of *PDGFRA*), and GISTs with *KIT* exon 9 mutations. Secondary resistance is diagnosed by enhanced CT, as enlargement of tumors, a new nodule in a mass, and/or a new lesion. Lesions with “a nodule-in-a-mass” appearance sometimes show reuptake of fluorodeoxyglucose (FDG) on positron emission tomography (PET). Resistance with “a nodule-in-a-mass” appearance has similar prognostic effects and molecular and pathological features to those of the other two resistance patterns [72].

Secondary resistance is mainly due to secondary mutations of the *KIT* or *PDGFRA* gene (70%–80%), and is partly due to the overexpression of *KIT* and/or an increase in the copy number of mutated *KIT* (10%), as well as being partly due to a gain of new but unknown proliferation mechanisms with a concomitant loss of *KIT* control (10%). Secondary mutations and overexpression of *KIT* are generally termed “target resistance” and the last resistant mechanism with other newly activated systems is generally termed “biological resistance”, where *KIT* expression has disappeared and tumors show the morphological appearance of rhabdomyoblastic differentiation including the expression of desmin [73].

Molecular mechanisms of secondary target resistance

The histological response of GISTs to imatinib is heterogeneous. Some tumors may show gross tumor necrosis with cystic degeneration and hyalinization, whereas

the remaining solid areas contain viable tumor cells. Complete loss of tumor cells with hyalinization is rarely seen. The last fact is very consistent with the results of BFR14, where stopping imatinib even after CR induced disease progression [67]. *KIT* expression also varies depending on viable tumor cells. In contrast, most imatinib-resistant tumors exhibited solid tumor masses with strong re-expression of *KIT* [63]. *KIT* and *PDGFRA* are type III tyrosine kinases in which the kinase domain splits into domain I (the ATP-binding domain) and domain II (activation loop). The former domain is mainly composed of exons 13 and 14, and the latter consists mainly of exons 16, 17, and 18. In genetic analyses, almost 80% of resistant lesions were shown to have second mutations either in the ATP-binding domain or in the activation loop [74–86]. Nearly 10% of resistant GISTs having no second mutations were shown to have an increased copy number of mutated *KIT* by fluorescence in situ hybridization (FISH). When secondary mutations were also examined in imatinib-sensitive lesions where various degrees of viable tumors with wide ranges of *KIT* expression were seen, only a few lesions had secondary mutations [82]. Mathematical models have indicated that, in chronic myelogenous leukemia (CML), most secondary mutations might occur after therapy [87]. Recently, however, others have indicated that most mutations found in resistant clones were found before treatment, although the copy number was extremely small [88]. The latter finding indicates that target agents may suppress or kill only tumor cells with sensitive mutations, and may select resistant clones. Whether ready-made clones with resistant mutations are present before the treatment and are selected by the drugs or whether secondary mutations appear during treatment is still to be settled.

Secondary mutations

Secondary mutation in the kinase domains is accompanied by concomitant re-activation of the corresponding tyrosine kinase even in the presence of imatinib [74, 89]. Secondary mutations also have hot spots, including *KIT* exon 13 (codon 654); exon 14 (codon 670); exon 17 of codons 809, 816, 820, 823, and 829; and *PDGFRA* exon 14 and exon 18 (Fig. 3A) [20–22, 74–86]. Secondary mutations in exon 13 of the *KIT* gene are exclusively missense mutation of V654A. Mutation of V654A decreases the binding capacity of imatinib, although the V654A mutation itself is not suggested to be a gain-of-function mutation [90]. This mutation accounts for 40% of secondary mutations found. Secondary mutations found in exon 14 are mostly T670I, which is called a gatekeeper mutation, as reported in other diseases and other genes [91–93]. This type of mutation causes steric hindrance for imatinib binding to *KIT* and also induces

autophosphorylation of the kinase by itself, suggesting a gain-of-function mutation [94]. In GISTs, this gate-keeper mutation was observed in 10% of secondary mutations in the *KIT* gene. Thus, secondary mutations in the ATP-binding domain are mostly confined to the missense mutations in two codons, V654A and T670I, which account for half of the secondary mutations in the *KIT* gene. *KIT* or *PDGFRA* kinase with these mutations in the ATP-binding domain is thought to be stabilized in an autoinhibited form and these forms are sensitive to sunitinib even after they become imatinib-resistant [64].

In the activation loop, missense mutations were frequently detected in codons 816, 820, 822, and 823, and a few deletion mutations were reported. Some mutations found in codons D816, D820, and N822 of secondary resistant GISTs had amino acid replacements similar to those found in the primary GIST, while other mutations were novel and specific for resistant GISTs. The substituted amino acids are relatively constant, as shown in Fig. 3, and most of these mutations are thought to cause autophosphorylation and activation of the kinase [22, 62, 74]. Some mutations found in the activation loop may be considered to destabilize the autoinhibited form by negatively influencing the inhibitory conformation of the juxtamembrane domain, resulting in a shift of conformational equilibrium toward the activated form [64]. So, the mutations found in the activation loop sometimes show resistance to both sunitinib and imatinib in vitro and in vivo.

As previously stated, the incidence of secondary mutations was greater in GISTs with initially imatinib-sensitive mutations, i.e., GISTs with *KIT* exon 11 and *PDGFRA* exon 12 mutations, than in GISTs with the less-sensitive mutation, *KIT* exon 9 [18, 74, 81]. In fact, our summary of the mutational status of secondary imatinib-resistant GISTs reported in several papers indicated that 60% of GISTs with primary *KIT* exon 11 mutations had secondary mutations either in the ATP-binding domain or the activation loop, whereas only one-third of those with a primary *KIT* exon 9 mutation had second mutations. More than 80% of primary wild-type GISTs, which are relatively resistant to imatinib, showed no secondary mutation even after secondary resistance was acquired. These results indicate that the inhibitory effects of imatinib may influence the mechanisms of secondary resistance. More interestingly, almost all of the secondary mutations examined were located in the same gene; that is, when primary GISTs have *KIT* or *PDGFRA* mutations, secondary mutations were found in the *KIT* or *PDGFRA* gene, respectively (Fig. 3B). When no mutation was found in the primary tumor, the GIST still had no mutation even after showing secondary resistance. Furthermore, when the allelic distribution of mutations was examined, it was

found that most secondary mutations occurred in the same allele as the primary mutations, suggesting that secondary mutations are *cis*-mutations (Fig. 3B) [74, 78, 79, 81]. Only a few cases had a second mutation in a different gene and/or a different allele (*trans*) [74, 86]. Similar *cis*-mutations in a resistant lesion were also reported in epidermal growth factor receptor (EGFR)-mutated non-small cell lung cancer after treatment with gefitinib or erlotinib [95]. These results indicate that some specific mechanisms may be involved in the generation of second mutations.

Sunitinib and genotype

Sunitinib was used for imatinib-resistant GISTs and was shown to be effective for GISTs with primary wild-type or primary *KIT* exon 9 mutation [15]. Recently, the correlation between genotype and the efficacy of sunitinib was examined. The reports suggested that GISTs with secondary *KIT* mutations in the ATP-binding domain (*KIT* exons 13 and 14) were sensitive to sunitinib, while GISTs with mutations in the activation loop (*KIT* exons 16, 17, and 18 and *PDGFRA* exon 18) were resistant to sunitinib (Fig. 3B) [62, 63]. These clinical data are very consistent with in vitro data of kinase assays [62, 89]. The correlation of genotype with sunitinib activity appeared to be true for each metastatic lesion of GIST. However, for an individual person treated with sunitinib, the genotype did not always reflect the clinical outcome of the patient, because each patient may have multiple resistant lesions which have different resistance mechanisms and different secondary mutations, resulting in differing sunitinib-sensitivities. Thus, using genotyping, we could not always make correct prognostications for sunitinib, as seen in our findings for imatinib.

Future directions and conclusions

Multidisciplinary treatment for advanced GISTs

As described above, we have noted the roles of targeted therapy and its limitations. Cure could not be obtained for advanced GISTs either by surgery alone or by imatinib or sunitinib alone. One ambitious strategy is surgery plus adjuvant therapy for GISTs at high risk of recurrence. Several trials have investigated adjuvant imatinib therapy. From the published data, adjuvant imatinib therapy for 1 year prolonged recurrence-free survival (RFS), especially for high-risk GIST [96]. The RFS was reported to be influenced by genotype, and patients with *KIT* exon 9 mutations appeared to have a higher chance of recurrence [15]. In the Japanese guidelines [9], the dose of imatinib is 400 mg/day, and target patients may be those with high-risk GISTs and those

Fig. 3A,B. Secondary mutations in the *KIT* and *PDGFRA* genes. **A** Representative mutations (>5%) found in secondarily resistant GISTs are shown. *Asterisk*, the frequency of each mutation type/total secondary mutations was calculated mainly from data obtained from references 75 to 85. **B** Schematic illustration of hot spots where secondary mutations preferentially occurred

with clinically malignant GISTs. Adjuvant therapy for GISTs, however, still has several problems to be overcome, including the optimal duration of the therapy (more than 2 years may be required), effects on OS, and possible induction of resistance to imatinib [97]. These problems will, we hope, be solved by ongoing trials.

Other multidisciplinary treatments for advanced GIST include neoadjuvant treatment and surgery for imatinib-responding GIST, so-called adjuvant surgery. At present, these treatments should be performed only within clinical trials by a team very familiar with GIST therapy.

Personalized medicine

Recurrence risk after complete removal of tumors is dependent on the location of the tumors, tumor size, the biological aggressiveness of the tumors, and genotype. Response to imatinib or sunitinib also depends on the location, genotype, biological aggressiveness, and tumor burden. Using best-fitted risk classification with these factors, we could select the patients who required aggressive therapy [98]. We may be able to choose the optimal dose of imatinib and the best-fitted drug depending on genotype. For example, GISTs with *KIT* exon 9 mutations may be treated with a higher dose of imatinib if tolerable, and GISTs without mutations in the *KIT* and *PDGFRA* genes may be treated with sunitinib. This personalized medicine should be based on scientific evidence.

What can we learn from molecular-targeted therapy in GIST?

Imatinib therapy is the first successful molecular-targeted therapy for solid tumors. What can we learn from this?

First, we could be successful in the development of targeted agents if we use the best-fitted molecules for correct targets of cancer. In this regard, qualitative and quantitative differences between cancer targets and normal tissues seem to be important for achieving high efficacy and low rates of adverse effects. One of the qualitative differences may be mutations, as seen in the *KIT* and *PDGFRA* genes, and one of the quantitative differences may be the overexpression of VEGF and PDGF in renal cell carcinoma.

Second, we would have better results when we treat cancer by its molecular nature but not by its location. For example, cetuximab, a chimeric monoclonal antibody to EGFR, had beneficial effects on EGFR-overexpressing colon cancer without *ras* mutations, but did not have beneficial effects on colon cancer without EGFR overexpression or with *ras* mutations [99].

Third, even for molecular-targeted agents that have shown high efficacy and safety, cancer inevitably acquires resistance to the drugs during treatment. Right now, overcoming drug resistance is critical for patients receiving chemotherapy. As shown in this review, molecular-targeted agents may become a research model of cancer resistance; because the target is simple, the molecular nature of targeted diseases, including downstream pathways, is sometimes elucidated. Cancers targeted by these agents tend to have a nature addicted to their original driving force, as seen with GIST, CML, and EGFR-mutated non-small cell lung cancer.

In summary, here we have provided an overview of GIST as a molecularly characterized cancer. GIST has gain-of-function mutations in the *KIT* or *PDGFRA* gene, which are targets of imatinib as well as sunitinib. Cure can be obtained only by complete removal of a GIST; however, after recurrence or for advanced and/or metastatic GIST, imatinib is indicated. Imatinib showed high efficacy, depending on genotype, and sufficient safety. The drug, however, has met with acquired resistance during treatment, of which the molecular mechanisms have been elucidated to be mostly secondary mutations in the kinase domains of the corresponding targeted genes. Although sunitinib has had substantial effects on imatinib-resistant GIST, this drug has also encountered primary and secondary resistance, depending on the genotype of the imatinib-resistant GIST. Molecular-targeted agents should be developed based on molecular mechanisms.

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