Med Oncol (2008) 25:207–213
DOI 10.1007/s12032-007-9014-2

ORIGINAL PAPER

# Molecular analysis of secondary kinase mutations in imatinib-resistant gastrointestinal stromal tumors

**Ken-Hong Lim · Ming-Jer Huang · Li-Tzong Chen · Tsang-En Wang · Chien-Liang Liu · Cheng-Shyong Chang · Mei-Chin Liu · Reuy-Kuen Hsieh · Chin-Yuan Tzen**

Received: 28 August 2007 / Accepted: 11 September 2007 / Published online: 6 October 2007


**Abstract** Most gastrointestinal stromal tumors (GISTs) are associated with activating kinase mutation in *KIT* or platelet-derived growth factor receptor alpha (PDGFRA) gene, and imatinib has revolutionized the care of advanced GISTs. However, most patients gradually developed resistance to imatinib. We intend to identify the secondary kinase mutations in imatinib-resistant GISTs and to study the relationship between secondary kinase mutations and the clinical response to imatinib. Twelve advanced GIST patients, who have developed resistance to imatinib were included in this study. Paraffin-embedded pretreatment GIST specimens and progression lesions of the tumors after resistance to imatinib were analyzed for kinase mutations in exons 9, 11, 13, and 17 of *KIT* gene and exons of 10, 12,

K.-H. Lim · M.-J. Huang · R.-K. Hsieh
Division of Hematology and Oncology, Mackay Memorial Hospital, 92, Section 2, Chungshan North Road, Taipei 10449, Taiwan

K.-H. Lim · T.-E. Wang · C.-L. Liu · C.-Y. Tzen
Mackay Medicine, Nursing and Management College, 92, Shengjing Road, Taipei 112, Taiwan

M.-J. Huang
School of Medicine, College of Medicine, China Medical University, 91 Hsueh-Shih Road, Taichung 40402, Taiwan

L.-T. Chen
Department of Internal Medicine, Kaohsiung Medical University Hospital, 100, Tzyou 1st Road, Kaohsiung 807, Taiwan

L.-T. Chen
Division of Cancer Research, National Health Research Institutes, Ward 191 Veterans General Hospital, 201, Section 2, Shih-Pai Road, Taipei 112, Taiwan

T.-E. Wang
Division of Gastroenterology, Department of Internal Medicine, Mackay Memorial Hospital, 92, Section 2, Chungshan North Road, Taipei 10449, Taiwan

C.-L. Liu
Department of Surgery, Mackay Memorial Hospital, 92, Section 2, Chungshan North Road, Taipei 10449, Taiwan

C.-S. Chang
Division of Hematology–Oncology, Department of Internal Medicine, Changhua Christian Hospital, 135, Nanhsiao Street, Changhua 500, Taiwan

C.-S. Chang
Department of Medical Education and Research, Changhua Christian Hospital, 135, Nanhsiao Street, Changhua 500, Taiwan

M.-C. Liu
Department of Medical Oncology, Koo Foundation Sun Yat-Sen Cancer Center, 125, Li-De Road, Taipei 112, Taiwan

C.-Y. Tzen (✉)
Department of Pathology, Mackay Memorial Hospital, 45, Minsheng Road, Tamshui, Taipei 251, Taiwan
e-mail: jeffrey@ms2.mmh.org.tw

C.-Y. Tzen
Department of Medical Research, Mackay Memorial Hospital, 92, Section 2, Chungshan North Road, Taipei 10449, Taiwan

C.-Y. Tzen
National Taipei College of Nursing, 365, Min-Te Road, Taipei 11257, Taiwan

C.-Y. Tzen
School of Medical Technology, Taipei Medical University, 250, Wu-Xin Street, Taipei 110, Taiwan

14, and 18 of *PDGFRA* gene. Primary *KIT* mutations have been found in all but one of the primary tumors including one case harboring de novo double *KIT* exon 11 mutations. Secondary kinase mutations in *KIT* and *PDGFRA* were found in seven and 1 of 12 patients, respectively. Two patients harbored more than one secondary *KIT* mutations in different progression sites, and there are four types of clonal or polyclonal evolution being observed. The secondary *PDGFRA* exon 14 mutation H687Y is a novel mutation that has never been reported before. Acquired secondary kinase mutations are the most important cause of secondary imatinib resistance in advanced GISTs. The identification of secondary kinase mutations is important in the development of new therapeutic strategies.

**Keywords** Gastrointestinal stromal tumors · KIT · Platelet-derived growth factor receptor alpha · Kinase mutation · Imatinib · Secondary resistance

## Introduction

Gastrointestinal stromal tumors (GISTs) are characterized by the expression of the KIT receptor. Activating *KIT* mutation or platelet-derived growth factor receptor alpha (PDGFRA) mutation has been found in the majority of GISTs, and has been linked to their molecular pathogenesis [1, 2]. It is the most frequent mesenchymal gastrointestinal tumor with the annual incidence of approximately 10–20 cases per million population [3–5]. The annual incidence of GISTs in Taiwan is estimated to be 300 new cases [5]. Completed surgical resection remains the first line therapy for primary localized GIST. However, the median disease free survival following completed surgical resection for tumors larger than 5 cm in size is only 5 years [6].

Imatinib mesylate (Gleevec, Glivec; Novartis Pharma AG, Basel, Switzerland) is a rationally designed, orally available 2-phenylaminopyrimidine analogue. Imatinib blocks the activity of several tyrosine kinases including KIT and PDGFRA. Recurrent or metastatic GIST patients who are not responsive to conventional chemotherapy or radiotherapy show an excellent response to imatinib. Results from clinical trials have shown an overall response rate around 60% and progression arrest in 80–90% of patients [7]. However, most patients developed resistance to imatinib in a period of 2 years. Current evidences suggest that one of the mechanisms behind secondary imatinib resistance is related to additional mutations in the kinase domain of tumor genotype [8–18]. Therefore, we intend to identify the secondary kinase mutation in imatinib-resistant GISTs in a series of Taiwanese patients, and to study the relationship between secondary kinase mutations and the clinical response to imatinib.

## Materials and methods

### Patients and specimens

From April 2001 to March 2006, 12 advanced GIST patients who have been treated with imatinib and have developed disease progression after initial response and emerged resistance to imatinib were included in this study. All patients received surgical excision of tumors after initial diagnosis and received excision or biopsy of their progression lesions. Paraffin-embedded primary GIST tissues before imatinib therapy and progression lesions of the tumors after resistance to imatinib from all 12 patients were used in the genomic DNA analyses. The study was approved by the local institutional review board. All the tumor specimens were obtained with written informed consent.

### DNA extraction

Briefly, representative paraffin blocks were cut in 8-μm slices using a clean disposable microtome blade for each block. To ensure representative sampling, excess tissue was trimmed before sectioning, and the first and the last sections from each ribbon were examined by light microscope after routine hematoxylin and eosin staining. The paraffin sections were transferred directly to reaction tubes and incubated in 300 μl xylene at 25°C for 5 min, pelleted at 12,000*g* for 5 min, resuspended in 300 μl of absolute alcohol at room temperature, spun down, and then lyophilized. The pellets were then processed using a Puregene DNA isolation kit (Gentra, Minneapolis, MN, USA) according to the manufacturer's instruction, which included proteinase K (300 mg/ml) digestion overnight at 55°C. The final extracts were dissolved in Tris $Na_2$ EDTA buffer and kept at 4°C for later use.

### Polymerase chain reaction (PCR) and DNA sequencing

Four pairs of oligonucleotide primers were used to amplify exons 9, 11, 13, and 17 of *KIT* gene and exons of 10, 12, 14, and 18 of *PDGFRA* gene. The primer pairs to amplify *KIT* were 9R (5′-TGA CAT GGT CAA TGT TGG AA-3′) and 9L (5′-AGC CAG GGC TTT TGT TTT CT-3′) for exon 9, 11R (5′-TGG AAA GCC CCT GTT TCA TA-3′) and 11L (5′-CGT AAT CGT AGC TGG CAT GA-3′) for exon 11, 13R (5′-GCA AGA GAG AAC AAC AGT CTG G-3′) and 13L (5′-CAT GCG CTT GAC ATC AGT TT-3′) for exon 13, and 17R (5'-TGA ACA TCA TTC AAG GGT ACT TTT G-3′) and 17L (5′-TTG AAA CTA AAA ATC CTT TGC AGG AC-3′) for exon 17. The primer pairs to

amplify *PDGFRA* were 10R (5′-AGA TGG TTT GAG AGA TGG TAC TGC-3′) and 10L (5′-GGA CAC AGT AGA GTC CAA CAA CGT-3′) for exon 10, 12F (5′-TCC AGT CAC TGT CGCT GCT TC-3′) and 12R (5′-GCA AGG GAA AAG GGA GTC TT-3′) for exon 12, 14R (5′-CTC ACT CTC ATT CAA ACC TAT CAG C-3′) and 14L (5′-TC ATA CCC ATC TCC TAA CGG C-3′) for exon 14, and 18F (5′-ACC ATG GAT CAG CCA GTC TT-3′) and 18R (5′-TGA AGG AGG ATG AGC CTG ACC-3′) for exon 18.

PCR was carried out according to previously described procedures [2, 19]. The PCR products were sequenced using the ABI PRISM BigDye terminator cycle sequencing ready reaction kit and ABI Prism 377 genetic analyzer (PE Applied Biosystems, Foster City, CA). All PCR products and independent duplicates were sequenced on both strands.

### Statistical analysis

For statistical analysis, the $\chi^2$ test was conducted to analyze associations between kinase mutations and progression free survival (PFS). Statistical analysis was carried out using a commercially available computer program (SPSS; Chicago, IL). Significance was defined at $P < 0.05$; all analyses were two sided.

## Results

### Patients

The demographics of the 12 patients are summarized in Table 1. There were eight male and four female patients, at an average age of 62 years (range, 48–88 years). The primary tumor site was stomach in one patient, small intestine in nine patients, and rectum in two patients. The dosage of imatinib ranged from 300 to 600 mg per day (case 5 took 300 mg due to grade IV eye lid edema, and cases 1, 6, 8, and 12 increased to 600 mg after disease progression). The overall average PFS after imatinib therapy is 17.5 months (range, 2–44 months). There are eight patients still alive with disease and four patients died of their diseases. Interestingly, although imatinib-resistant GIST tumors usually progressed after discontinuation of imatinib therapy, we have observed in case 3 that spontaneous partial regression of the metastatic hepatic tumor 9 weeks after the withdrawal of imatinib therapy (Fig. 1).

### Primary KIT mutations

Activating primary *KIT* mutations have been found in all but one of the primary tumors (Table 2). Exon 11 mutations were found in eight cases (66.6%), including one missense mutation and eight deletions. Interestingly, one case harbored de novo double *KIT* exon 11 mutations including one missense mutation and one deletion (case 12). Exon 9 mutations were found in three cases (25%) and were all duplication of alanine-502 and tyrosine-503. One tumor was found to have wild-type sequences in all the exons examined (case 7). No primary mutation was found in exons 13 and 17 of *KIT* and in exons 10, 12, 14, and 18 of *PDGFRA* in this study.

### Secondary KIT and PDGFRA mutations in imatinib-resistant GISTs

Secondary kinase mutations in *KIT* and *PDGFRA* were found in seven and 1 of 12 patients, respectively (Table 2). Five patients developed only one type of secondary *KIT* mutation in the first or second tyrosine kinase domain in 13

**Table 1** Demographics of 12 patients with advanced GISTs

| No. | Age/Sex | Primary location | Metastasis site | PFS | Outcome |
|---|---|---|---|---|---|
| 1 | 56/M | Duodenal | Liver, Peritoneum | 44 | AWD |
| 2 | 61/F | Jejunum | Liver, Peritoneum | 16 | DOD |
| 3 | 56/F | Small intestine | Liver, Peritoneum | 22 | AWD |
| 4 | 75/M | Stomach | Liver | 24 | DOD |
| 5 | 59/M | Rectum | Liver, Peritoneum | 18 | AWD |
| 6 | 51/F | Rectum | Liver, Lung | 7 | AWD |
| 7 | 88/M | Small intestine | Liver | 23 | AWD |
| 8 | 65/F | Small intestine | Liver, Peritoneum | 25 | AWD |
| 9 | 48/M | Small intestine | Liver, Peritoneum | 12 | DOD |
| 10 | 70/M | Small intestine | Liver, Peritoneum | 2 | DOD |
| 11 | 65/M | Small intestine | Liver | 8 | AWD |
| 12 | 51/M | Small intestine | Liver | 36 | AWD |

PFS, progression free survival; AWD, alive with disease; DOD, died of disease

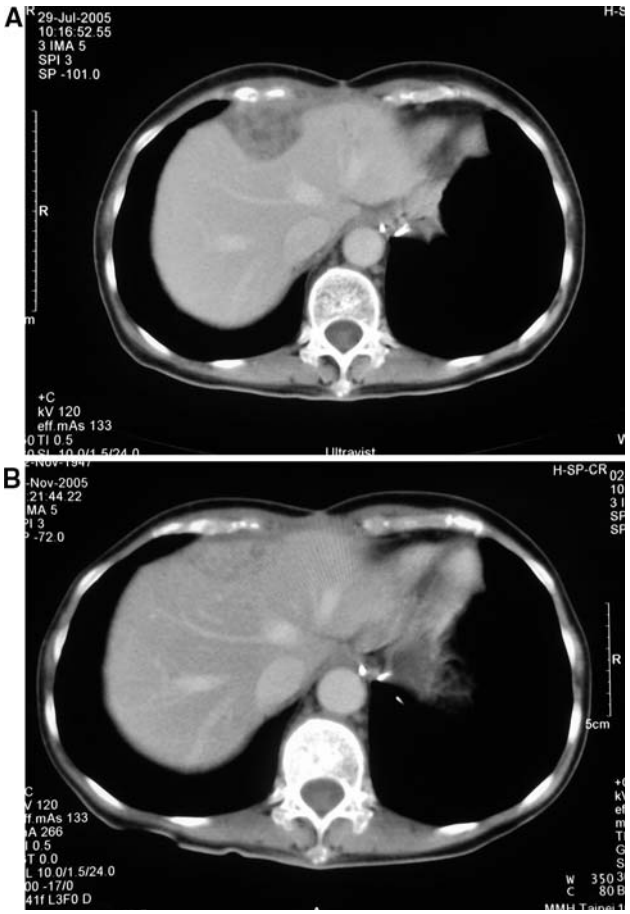


**Fig. 1** Computed tomography of the abdomen of case 3. (**a**) Before discontinuation of imatinib showing a new hepatic metastatic tumor at the anterior aspect of hepatic dome (4.4 × 3.1 cm). (**b**) 9 weeks after the withdrawal of imatinib showing spontaneous partial regression of the metastatic hepatic tumor (4.3 × 1.8 cm)

($n$ = 2) or 17 ($n$ = 3), respectively. One patient (case 1) had two different secondary *KIT* missense mutations in exon 17 at the same codon, each from a different tumor sample (duodenum and liver). Another patient (case 8) had two different secondary *KIT* missense mutations in exons 13 and 17, each from a different tumor sample (jejunum and liver), and one wild-type tumor from the liver. Interestingly, one patients with primary *KIT* mutation developed secondary *PDGFRA* mutations in exons 14 (case 10). Notably, the secondary *PDGFRA* exon 14 mutation H687Y is a novel mutation that has never been reported before, either in untreated or imatinib-treated GIST, while the secondary *PDGFRA* exon 18 mutation V824V in case 11 represents a single nucleotide polymorphism. Wild-type *KIT* and *PDGFRA* sequences were found in two patients with primary *KIT* mutations in exons 9 (case 6) and 11 (case 9), respectively. In only one patient (case 7) with no detectable primary kinase mutation, a *KIT* missense mutation in exon 11 (V559A) was found. All the primary mutation was always detectable along with the secondary mutation in each tumor. No more than two secondary mutations were found in the same tumor sample in this study. In summary, 8 (66.6%) of 12 patients with imatinib-resistant GISTs harbored one or more secondary kinase mutations in our series.

### Correlation of KIT mutation and PFS

Although the mean PFS of GIST patients with primary *KIT* exon 11 mutation (23.63 months ± 11.86) was much longer than that of the patients with primary *KIT* exon 9 mutation (8.33 months), the PFS was not a significant difference between these two groups of patients in this study ($P$ = 0.07, 95% confident interval –1.52, 32.1). Due to only one patient with wild-type *KIT* in our series, the difference of PFS between wild-type *KIT* and primary *KIT* exon 9 or exon 11 mutations could not be compared.

## Discussion

In this study, acquired secondary mutations in *KIT* and *PDGFRA* in imatinib-resistant GISTs were found in 8 out of 12 patients (66.6%). Since *KIT* exon 11 V559A missense mutation should be sensitive to imatinib therapy, it should not be the secondary mutation responsible for imatinib resistance in case 7, and other mechanism of resistance should account for imatinib resistance in this case. It seems likely that this *KIT* exon 11 V559A mutation might have been missed in the primary tumor due to low-level mutations that were not detected by direct sequence analysis. Since mutational level that is less than 25% may not be detected by direct sequence analysis, the use of more sensitive techniques such as denaturing high performance liquid chromatography or cloning may improve the sensitivity of mutation detection in GISTs. All of our patients had initial response to imatinib therapy and received excision or biopsy of their progression lesions for study. The frequency of secondary kinase mutations after secondary resistance to imatinib is high in our study, and is comparable to a recent study by Heinrich et al. [20]. Overall, the frequency of acquired secondary kinase mutations in GISTs with secondary resistance to imatinib ranges from 43.8% to 67% [11, 12, 17, 18]. However, GISTs with primary resistance to imatinib were found to have only 10% (1/10 patients) secondary kinase mutations [18]. This difference in frequency is significant, and may be due to the differences in the underlying biology of these two groups of GISTs.

Most acquired secondary *KIT* mutations are preferentially and nonrandomly located in the first or second

**Table 2** *KIT* and *PDGFRA* sequences of pre-imatinib and imatinib-resistant GISTs

| No. | Primary *KIT* mutation site | Primary *KIT* mutation type | Secondary mutation site | Secondary mutation type | Sample site |
|---|---|---|---|---|---|
| 1 | Exon 11 | Del VQWKVVEEINGNNYVYIDPTQL 555–576V | *KIT* Exon 17 | D820G | Duodenum |
| | | | | D820Y | Liver |
| 2 | Exon 9 | Ins AY502–503 | *KIT* Exon 17 | N822K | Liver |
| 3 | Exon 11 | Del KVV558–560S | *KIT* Exon 13 | V654A | Liver |
| 4 | Exon 11 | Del WKVV557–560 | *KIT* Exon 13 | Insertion 643 nucleotide A frameshift | Liver |
| 5 | Exon 11 | Del WK557–558CE | *KIT* Exon 17 | N822K | Liver |
| 6 | Exon 9 | Ins AY502–503 | Wild type | None detected | Liver |
| 7 | Wild type | None detected | *KIT* Exon 11 | V559A | Liver |
| 8 | Exon 11 | Del VYIDPTQL569–576 | Wild type | None detected | Liver |
| | | | *KIT* Exon 13 | V654A | Jejunum |
| | | | *KIT* Exon 17 | N822K | Liver |
| 9 | Exon 11 | Del MYE552–554K | Wild type | None detected | Liver |
| 10 | Exon 9 | Ins AY502–503 | *PDGFRA* Exon 14 | H687Y | Pelvic |
| 11 | Exon 11 | Del VEEINGNNYVYIDPTQL560–576 | *PDGFRA* Exon 18 | V824V[a] | Jejunum |
| 12 | Exon 11 | Del QWKVVEEINGNNYVYIDPT 556–574 | *KIT* Exon 17 | D820Y | Liver |
| | Exon 11 | V553S | | | |

[a] Single nucleotide polymorphism

tyrosine kinase domains. Our results represent the first secondary kinase mutational report in GISTs from Asian and showed that the occurrence of secondary kinase mutations has no ethnical difference between Taiwanese and Caucasian populations. Additionally, there are no significant correlations between secondary mutation and primary mutation in GISTs. To date, all the reported acquired secondary *KIT* and *PDGFRA* mutations are single amino acid missense mutations. The substitution of single amino acid in mutated tyrosine kinase domain probably leads to a change in the three-dimensional receptor conformation and modification of the ATP-binding pocket, therefore inhibits the binding of imatinib to the receptor [16]. Debiec-Rychter et al. [12] have demonstrated that most imatinib-resistant GISTs still have various levels of constitutive KIT autophosphorylation. In addition, Heinrich et al. [18] have shown that imatinib-resistant GIST cells remain dependent on KIT kinase activity for activation of critical downstream signaling pathways. Hence, KIT-dependent resistant mechanism is the most important mechanism for imatinib resistance in GISTs. However, KIT-independent resistant mechanism is still noted in some GISTs [12], such as case 6 in this study, where total loss of KIT expression and wild-type *KIT* was found in the imatinib-resistant tumor.

Recent studies of imatinib-resistant GISTs have demonstrated that clonal or polyclonal evolution has led to GISTs progression [17, 18]. In patients with GIST harboring primary *KIT* kinase mutation, resistant subclones containing an additional *KIT* or *PDGFRA* mutation emerge in the presence of imatinib. Interestingly, each tumor nodule under progression apparently developed an individual clonal evolution and most patients developed secondary mutation in only one kinase exon. However, Wardelmann et al. [17] reported that three patients developed secondary mutations in two or three kinase exons in their cohort. The cases with more than one secondary *KIT* mutations had a comparable prognosis as those with only one or no secondary mutation. In our cohort, two patients harbored more than one secondary *KIT* mutations in different progression sites. The correlation between occurrences of multiple *KIT* kinase mutations and primary *KIT* kinase mutation was unclear. In our series, there are four types of clonal or polyclonal evolution being observed. First, primary *KIT* mutation turns to secondary *KIT* mutation; second, primary *KIT* mutation turns to multiple *KIT* mutations; third, primary *KIT* mutation turns to wild type; and fourth, primary *KIT* mutation turns to secondary *PDGFRA* mutation. The underlying molecular mechanisms and the relationship of these clonal or polyclonal evolutions between different kinase mutations may warrant further investigations.

The correlation of secondary *KIT* mutations with clinical behavior in imatinib-resistant GISTs is not very clear at present time. However, tumors with secondary *KIT* exon 13 V654A mutations were characterized by a more rapid progression phenotype. In case 3 of our series, tumor with secondary *KIT* exon 13 V654A mutation showed a more

rapid growth after escalated imatinib dose, and spontaneous partial regression of the progression hepatic tumor after withdrawal of imatinib in this case is very impressive. We speculate that secondary *KIT* exon 13 mutations may have some advantage on preferential clonal proliferation under selection pressure of imatinib treatment. Further molecular studies are warranted to clarify the underlying mechanisms in this withdrawal phenomenon.

In contrast to secondary *KIT* kinase mutation, secondary *PDGFRA* kinase mutation is much less common in imatinib-resistant GISTs. To our knowledge, only four cases have been reported including one case with *PDGFRA* exon 18 V824V single nucleotide polymorphism (case 11). It has been clearly shown that primary kinase mutations in *KIT* and *PDGFRA* are mutually exclusive in untreated GISTs [2, 21]. However, secondary *PDGFRA* exon 14 H687Y mutation was found in a GIST harboring a primary *KIT* exon 9 mutation in this study (case 10). This novel *PDGFRA* exon 14 kinase mutation has never been reported and we did not detect this mutation in more than 200 GIST patients in our own series. We believe that this *PDGFRA* exon 14 H687Y mutation is not a polymorphism and is associated with secondary imatinib resistance. Another case of imatinib-resistant GIST with primary *KIT* exon 11 G565R mutation has also been found to have secondary *PDGFRA* exon 18 D842V mutation [12]. The *PDGFRA* exon 18 D842V mutation was also found to associate with secondary imatinib resistance in a GIST harboring a primary *PDGFRA* exon 12 V561D mutation [18]. These cases demonstrated that secondary resistance to imatinib could occur through mutation of a different kinase. It is noteworthy that the activation of downstream signaling intermediates was similar in *KIT*-mutant and *PDGFRA*-mutant GISTs [2, 22]. This observation suggested that mutant PDGFRA provides oncogenic signals that parallel those of mutant KIT [21]. Hence, imatinib-resistant GISTs with primary *KIT* mutation and secondary *PDGFRA* mutation are still dependent on the KIT signaling pathway.

In conclusion, our finding of an association between new emerged *KIT* and *PDGFRA* kinase mutations and disease progression is very impressive in Taiwanese patients. The occurrence of secondary kinase mutations in GISTs has ethnically no difference between Taiwanese and Caucasian populations. Our report on secondary kinase mutations in imatinib-resistant GIST patients indicated that acquisition of secondary kinase mutations might be the most important cause of developing late resistance to imatinib treatment. The identification of secondary kinase mutations in progressive lesions under imatinib treatment can be used to demonstrate imatinib resistance and disease progression in GISTs. These secondary kinase mutations are particularly important in view of new therapeutic strategies including either surgical resection of individual progressive lesion, treatment with other small molecules such as sunitinib malate (Sutent [previously known as SU11248], Pfizer, Inc., New York, New York) [23], or withdrawal of imatinib therapy. The full understanding of the molecular mechanism of oncogenesis and resistance in GISTs may also help gain new insight into and establish further comparable strategies in other cancer types.

**Acknowledgment** This study is supported in part by grant from Mackay Good Neighborhood Foundation.

## References

1. Hirota S, et al. Gain-of-function mutations of c-kit in human gastrointestinal stromal tumors. Science 1998;279:577–80.
2. Heinrich MC, et al. PDGFRA activating mutations in gastrointestinal stromal tumors. Science 2003;299:708–10.
3. Nilsson B, et al. Gastrointestinal stromal tumors: the incidence, prevalence, clinical course, and prognostication in the preimatinib mesylate era—a population-based study in western Sweden. Cancer 2005;103:821–9.
4. Tryggvason G, Gislason HG, Magnusson MK, Jonasson JG. Gastrointestinal stromal tumors in Iceland, 1990–2003: the icelandic GIST study, a population-based incidence and pathologic risk stratification study. Int J Cancer 2005;117:289–93.
5. Tzen CY, et al. Incidence of gastrointestinal stromal tumor: a retrospective study based on immunohistochemical and mutational analyses. Dig Dis Sci 2007;52:792–7.
6. Lin SC, et al. Clinical manifestations and prognostic factors in patients with gastrointestinal stromal tumors. World J Gastroenterol 2003;9:2809–12.
7. Demetri GD, et al. Efficacy and safety of imatinib mesylate in advanced gastrointestinal stromal tumors. N Engl J Med 2002;347:472–80.
8. Chen LL, et al. A missense mutation in KIT kinase domain 1 correlates with imatinib resistance in gastrointestinal stromal tumors. Cancer Res 2004;64:5913–9.
9. Tamborini E, et al. A new mutation in the KIT ATP pocket causes acquired resistance to imatinib in a gastrointestinal stromal tumor patient. Gastroenterology 2004;127:294–9.
10. Wakai T, et al. Late resistance to imatinib therapy in a metastatic gastrointestinal stromal tumour is associated with a second KIT mutation. Br J Cancer 2004;90:2059–61.
11. Antonescu CR, et al. Acquired resistance to imatinib in gastrointestinal stromal tumor occurs through secondary gene mutation. Clin Cancer Res 2005;11:4182–90.
12. Debiec-Rychter M, et al. Mechanisms of resistance to imatinib mesylate in gastrointestinal stromal tumors and activity of the PKC412 inhibitor against imatinib-resistant mutants. Gastroenterology 2005;128:270–9.
13. Tamborini E, et al. KIT/Val654 Ala receptor detected in one imatinib-resistant GIST patient. Cancer Res 2005;65:1115 (author reply 1115).
14. Wardelmann E, et al. Acquired resistance to imatinib in gastrointestinal stromal tumours caused by multiple KIT mutations. Lancet Oncol 2005;6:249–51.
15. Grimpen F, et al. Resistance to imatinib, low-grade FDG-avidity on PET, and acquired KIT exon 17 mutation in gastrointestinal stromal tumour. Lancet Oncol 2005;6:724–7.
16. McLean SR, et al. Imatinib binding and cKIT inhibition is abrogated by the cKIT kinase domain I missense mutation Val654Ala. Mol Cancer Ther 2005;4:2008–15.

17. Wardelmann E, et al. Polyclonal evolution of multiple secondary KIT mutations in gastrointestinal stromal tumors under treatment with imatinib mesylate. Clin Cancer Res 2006;12: 1743–9.
18. Heinrich MC, et al. Molecular correlates of imatinib resistance in gastrointestinal stromal tumors. J Clin Oncol 2006;24: 4764–74.
19. Miettinen M, et al. Gastrointestinal stromal tumors, intramural leiomyomas, and leiomyosarcomas in the duodenum: a clinicopathologic, immunohistochemical, and molecular genetic study of 167 cases. Am J Surg Pathol 2003;27:625–41.
20. Heinrich MC, et al. Sunitinib (SU) response in imatinib-resistant (IM-R) GIST correlates with KIT and PDGFRA mutation status. J Clin Oncol 2006;24(Suppl 18):9502.
21. Corless CL, et al. PDGFRA mutations in gastrointestinal stromal tumors: frequency, spectrum and in vitro sensitivity to imatinib. J Clin Oncol 2005;23:5357–64.
22. Corless CL, Fletcher JA, Heinrich MC. Biology of gastrointestinal stromal tumors. J Clin Oncol 2004;22:3813–25.
23. Demetri GD, et al. Efficacy and safety of sunitinib in patients with advanced gastrointestinal stromal tumour after failure of imatinib: a randomised controlled trial. Lancet 2006;368:1329–38.