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ORIGINAL RESEARCH

Oncogene mutational analysis in Chinese gastrointestinal stromal tumor patients

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Background: Gastrointestinal stromal tumors (GISTs) are the most common mesenchymal tumors and exhibit a high frequency of oncogenic *KIT* or *PDGFRA* mutations. Tyrosine kinase inhibitors (TKIs) have been mainly used in the treatment of GISTs bearing *KIT/PDGFRA* mutations. However, other mutation profiles have been found to affect the sensitivity to and effectiveness of TKIs in the treatment of GISTs.

Purpose: The aim of the present study was to describe the mutational status of multiple genes in GIST samples and to provide information for finding potential predictive markers of therapeutic targets in Chinese GIST patients.

Patients and methods: MassARRAY spectrometry was used to test 40 Chinese GIST patients for 238 mutations affecting 19 oncogenes.

Results: A total of 14 oncogenes with 43 mutations were detected in 38 samples, with a mutation frequency of 95%. Among these mutation samples, 26 GISTs were found for *KIT* or *PDGFRA* mutations, while 12 were *KIT/PDGFRA* wild-type. Approximately half of the GIST samples harbored multiple mutations. The most frequent mutations were found in *KIT* (62.5%), *CDK4* (17.5%), *NRAS* (15%) and *EGFR* (12.5%). Other mutations included *PIK3CA* and *AKT1* (10%), *BRAF* and *ABL1* (7.5%), *PDGFRA*, *ERBB2* and *HRAS* (5%), and *AKT2*, *FLT3* and *KRAS* (2.5%). New mutated genes (*CDK4*, *AKT2*, *FLT3*, *ERBB2*, *ABL1* and *AKT1*), a higher *BRAF* mutation frequency (7.5%) and new BRAF mutation sites (G464E) were found in Chinese GIST patients.

Conclusion: This study demonstrated useful mutations in a small fraction of Chinese GIST, but targeted therapeutics on these potential predictive markers need to be investigated in depth especially in Oriental populations.

Keywords: gastrointestinal stromal tumor (GIST), mutation, tyrosine kinase receptor, oncogene

Introduction

Gastrointestinal stromal tumors (GISTs) are the most common mesenchymal tumors originating in different parts of the digestive tract. GISTs have a characteristic morphology and biological continuum, and they are mostly incidentally discovered.[1] Despite clinicopathological differences, most GISTs share a similar genetic profile, including oncogenic *KIT* or *PDGFRA* mutations.

Previous studies reported that *KIT* mutations are identified in 60%–85% of GISTs, while *PDGFRA* mutations are identified in 5%–10%.[2] These mutations appear to be mutually exclusive, encoding a tyrosine kinase receptor type III.[3,4] Thus, tyrosine kinase inhibitors (TKIs), such as imatinib, sunitinib, or sorafenib, are considered the main treatment for GISTs. However, previous reports suggest that *KIT* and *PDGFRA* mutations in GISTs mainly affect exons that code for functional domains of the KIT and PDGFRA receptors. Therefore, *KIT* and *PDGFRA* genotyping may be of value in predicting sensitivity to TKIs and selecting the optimal clinical treatment. For example, *KIT* exon

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11 mutants respond well to imatinib, while exon 9 mutants (Ala502-Tyr503dup) are less sensitive to this TKI. *PDGFRA* exon 18 mutants (Asp842Val) are resistant to imatinib, and *KIT* exon 13 and 14 mutants are sensitive to sunitinib.[5] However, *KIT*-negative GISTs present a true diagnostic challenge.

In addition, ~10%–15% of GISTs do not have detectable *KIT* or *PDGFRA* mutations (*KIT*/*PDGFRA* wild-type [WT] GISTs), suggesting that other molecular pathways may also be involved in the pathogenesis of these tumors. Mutations in *NF*[6,7] and *BRAF* (V600E),[8,9] or SDH complex genes,[10] were detected in *KIT*/*PDGFRA* WT GISTs. Thus, GISTs are also characterized by five categories of oncogenic abnormalities, including *KIT* mutant, *PGDFRA* mutant, *SDH*-deficient, *RAS*/*BRAF*/*NF1* mutant, or quadruple (*KIT*/*PDGFRA*/*SDH*/*RAS-P*) WT GISTs.[11] The pathogenesis and underlying biology of quadruple WT GISTs is currently unknown. Further molecular and clinicopathological characterization of quadruple WT GISTs may help determine their prognosis as well as assist with the optimization of medical management, including clinical testing of novel therapies.[11] Therefore, additional genetic testing may help identify therapeutic targets and develop novel therapeutic strategies for managing GISTs.

The aim of the present study was to describe the mutational status of multiple genes in GIST samples using the MassARRAY spectrometry platform. The results revealed 14 oncogenes with 43 mutations in 40 Chinese GIST patients, including 68.42% *KIT* or *PDGFRA* mutations and 31.58% *KIT*/*PDGFRA* WT GISTs. New mutation genes (*CDK4*, *AKT2*, *FLT3*, *ERBB2*, *ABL1*, and *AKT1*), a higher *BRAF* mutation frequency (7.5%), and new *BRAF* mutation sites (G464E) were identified in Chinese GIST patients. These mutation genes found in the present study may work as predictive markers for novel therapeutic targets in Chinese GIST patients.

Materials and methods

Patients and samples

Formalin-fixed paraffin-embedded samples from 40 patients with pathologically diagnosed GISTs were retrieved from the NanFang Hospital, Southern Medical University (Guangzhou, People's Republic of China), between June 2006 and September 2011. All the cases were clinically treated with tumor resection. The clinical and follow-up data were updated in September 2011. This study was approved by the NanFang Hospital Ethics Committee, and written informed consent was obtained from all the participants.

Oncomutation detection

The OncoCarta panel (v1.0; Sequenom Inc., San Diego, CA, USA) was used to detect oncomutations in 40 GIST samples. This panel is a set of prevalidated assays for sensitive and efficient mutation screening by parallel analysis of 238 somatic mutations across 19 common oncogenes. The mutation types of each gene are listed in Table S1. DNA was extracted from each GIST sample using a QIAamp DNA formalin-fixed paraffin-embedded tissue kit (Qiagen, Hilden, Germany), according to the manufacturer's instructions. DNA (20 ng) was amplified using 24 sets of OncoCarta PCR primers. An extension reaction based on the OncoCarta extension primers was then performed. After salts were removed by the addition of a cation exchange resin, the reaction analyses were spotted onto a SpectroCHIP (Sequenom Inc.) and were analyzed using a MassARRAY matrix-assisted laser desorption/ionization time-of-flight mass spectrometry platform (Sequenom Inc.).

Analytical and statistical methods

Mutation data were analyzed by MassARRAY Typer Analyzer software 4.0.4.20 (Sequenom Inc.), using a cut-off mutation frequency of 1%. Automated mutation calls identified with the Typer software were generated using computational algorithms by quantifying the heights ratio of raw spectral peaks corresponding to the mutant and WT signals, noise-to-peak-height ratio, and area under the curve. In addition, the mutation report was manually reviewed by 3 investigators.

Results

Patient characteristics

Our study included 40 patients with GISTs who had undergone surgical resection. Mutation detection with the OncoCarta panel (ver.1.0; Sequenom Inc.) was performed in all the samples. The clinical characteristics of the patients are summarized in Table 1. The median age was 49 years (range, 20–84 years). Only 5% of these patients exhibited tumor recurrence or succumbed to the disease. A total of 80% of the patients were treated only with surgical resection and received no imatinib therapy, whereas 95% of the patients were insulin-like growth factor 1 receptor (IGF1R)-positive. All these results indicated that these tumors were low risk, with a low incidence of recurrence.

Mutation status in 40 GIST cases

Of the 40 GIST tumors, 38 (95%) were found to harbor oncogenic mutations. Of the 238 hotspot mutations in 19 common oncogenes, 14 oncogenes with 43 mutations were detected. The most frequent mutations were found in *KIT* (62.5%, 25/40), *CDK4* (17.5%, 7/40), *NRAS* (15%, 6/40), and *EGFR* (12.5%, 5/40). Other mutations included *PIK3CA* and *AKT1* (10%, 4/40), *BRAF* and *ABL1* (7.5%, 3/40),

Table 1 Clinical characteristic of 40 GIST patients

Characteristic	Number of patients
Sex	
Male	17 (42.5%)
Female	23 (57.5%)
Ages (years)	
Median	49
≤49	20 (50%)
>49	20 (50%)
Overall survival	
Survival	38 (95%)
Death	2 (5%)
Imatinib therapy	
No	32 (80%)
Yes	8 (20%)
Risk classification	
High	16 (40%)
Intermediate	10 (25%)
Low	12 (30%)
Very low	2 (5%)
IGF1R	
Positive	2 (5%)
Negative	38 (95%)
Morphology	
Spindle cells	26 (65%)
Epithelioid cells	1 (2.5%)
Mixed cells	13 (32.5%)

Abbreviations: GIST, gastrointestinal stromal tumor; IGF1R, insulin-like growth factor 1 receptor.

PDGFRA, *ERBB2*, and *HRAS* (5%, 2/40), and *AKT2*, *FLT3*, and *KRAS* (2.5%, 1/40). The identified mutations are outlined in Figure 1.

A total of 12 (30%) cases were found to be *KIT*/*PDGFRA* WT GISTs, including 4 cases with 2 or 3 coexisting mutations and 8 cases with a single mutation (Table 2). Sample 805823 harbored multiple mutations in *ABL1* (E255K), *AKT1* (rs11555435), and *PIK3CA* (E545K). Sample 707660 had two mutations in *BRAF* (G464E) and *HRAS* (G13S). Sample 8071414 harbored two mutations in *ABL1* (T315I) and *CDK4* (R24C). Sample 610972 harbored two mutations in *ABL1* (G250E) and *NRAS* (G12D).

The profiles of 26 cases with KIT or PDGFRA mutations are shown in Table 3. Also, most of the cases harbored multiple mutations.

Discussion

Cancer genetic information may provide important reference data for clinical diagnosis and treatment. Our research aimed to provide such information for identifying novel therapeutic targets by analyzing the mutational status of Chinese GIST patients for 238 hotspot mutations in 19 common oncogenes. A total of 43 mutations in 14 oncogenes were detected in 38 samples, with an overall mutation frequency of 95%. This result is consistent with a previous study reporting a single center's experience with 275 GIST cases, among which mutations were identified in 93.8% of the cases.[12] A total of 26 GISTs were detected for *KIT* or *PDGFRA* mutations, while 12 were found to be *KIT*/*PDGFRA* WT GISTs.

KIT is a cytokine receptor that belongs to the type III receptor tyrosine kinase family. It is structurally similar to PDGFRs, colony-stimulating factor-1 receptor, and fms-like tyrosine kinase. It has been reported that GISTs are generally positive for CD117 (c-kit) and are primarily caused by activating mutations in *KIT* or *PDGFRA*. Previous studies have demonstrated that *KIT* mutations are found in 60%–85% of GISTs, while *PDGFRA* mutations are found in 5%–10%.[2]

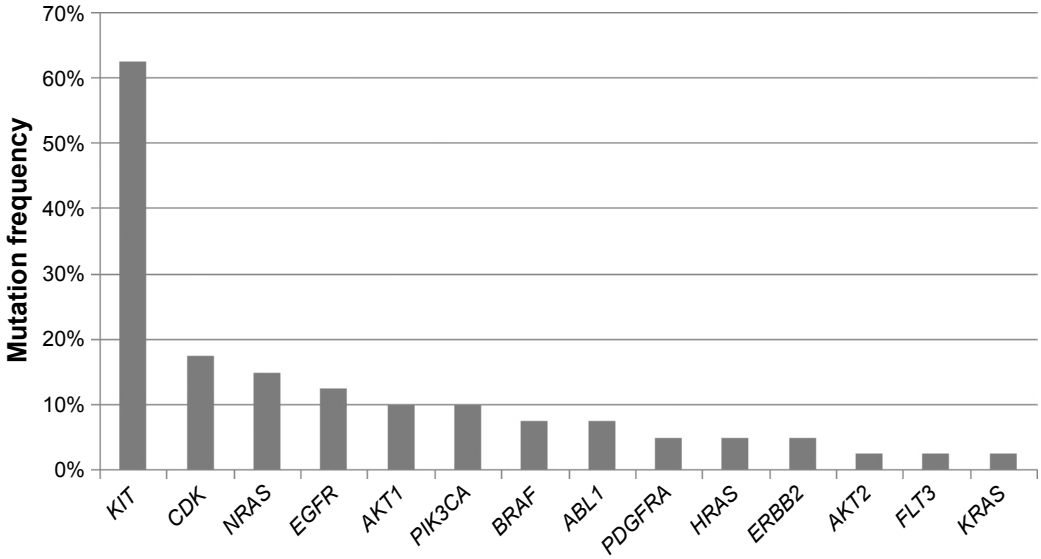

Figure 1 Mutation status in 40 GIST cases.
Abbreviation: GIST, gastrointestinal stromal tumor.

Table 2 Mutation analysis of GIST wild types

Sample ID	Gene	Mutation	Frequency	
			WT	MT
1002103	*CDK4*	R24C	0.93	0.07
900187	*EGFR*	S752I/F	0.74	0.26
8104799	*CDK4*	R24C	0.92	0.08
807165	*PIK3CA*	E542K	0.9	0.1
702442	*NRAS*	G12D	0.88	0.1
700675	*KRAS*	G12C	0.88	0.12
920127	*ERBB2*	G776S	0.87	0.14
100546	*AKT1*	rs11555435	0.83	0.17
805823	*ABL1*	E255K	0.92	0.08
	AKT1	rs11555435	0.85	0.15
	PIK3CA	E545K	0.91	0.09
707660	*BRAF*	G464E	0.94	0.1
	HRAS	G13S	0.84	0.2
8071414	*ABL1*	T315I	0.9	0.1
	CDK4	R24C	0.94	0.06
610972	*ABL1*	G250E	0.9	0.1
	NRAS	G12D	0.89	0.11

Abbreviations: GIST, gastrointestinal stromal tumor; MT, mutation type; WT, wild type.

In the present study, the *KIT* and *PDGFRA* mutation frequencies were 62.5 and 5%, respectively, which is consistent with previous reports. The most common mutation of *PDGFRA* (D842V) was not identified in the present study; on the contrary, T674I (exon 14) and D1071N (exon 22) were identified. *PDGFRA* T674I is an imatinib-resistant type of *PDGFRA*, and this mutation status may provide useful information for the clinical treatment of GISTs.

Table 3 Mutation profiles of GIST in *KIT* or *PDGFRA* mutations

Sample ID	Mutation subtypes
809610	*CDK* (R24C), *KIT* (W557G)
900087	*CDK* (R24C), *KIT* (V560del, V560D)
900308	*AKT1* (rs34409589), *EGFR* (T790M), *KIT* (W557R), *NRAS* (G13S)
901117	*KIT* (L576P)
902827	*EGFR* (T790M), *KIT* (V560del, E561K)
904328	*PDGFRA* (T674I, D1071N)
1002262	*KIT* (V559A)
1004320	*KIT* (V560del, V560D)
1004799	*KIT* (W557G), *PDGFRA* (D1071N), *PIK3CA* (E545K)
100601	*ERBB2* (P780_Y781insGSP), *KIT* (V560del, V560D, K550_K558del)
1008731	*CDK* (R24C), *KIT* (P551_V555del)
1013505	*KIT* (V559_V560del, V559D)
1013727	*KIT* (V550_V558del)
601653	*KIT* (V560del)
609820	*KIT* (Y503_F504insAY)
612020	*CDK* (R24C), *KIT* (K642E)
612113	*NRAS* (G12S), *KIT* (D579del)
701468	*BRAF* (L597S), *FLT3* (I836del), *AKT1* (rs11555436), *AKT2* (R371H), *KIT* (V559A), *NRAS* (A18T)
704876	*KIT* (Y503_F504insAY), *NRAS* (A18T), *PIK3CA* (H1047Y)
708681	*KIT* (W557G), *HRAS_2* (G12D)
803389	*EGFR* (D770_N771insG), *KIT* (K642E)
804077	*BRAF* (G464E), *KIT* (V559D), *BRAF* (L597S)
900879	*EGFR* (S752I/F), *KIT* (V560del, V560D)
905970	*KIT* (V559G)
908812	*KIT* (Y503_F504insAY)
920543	*KIT* (K642E)

Abbreviation: GIST, gastrointestinal stromal tumor.

KIT/*PDGFRA* WT GISTs are another type of GIST without *KIT* and *PDGFRA* mutations (10%–15%), in which the responsible pathogenetic pathways remain unknown. In our study, a high frequency (30%) of *KIT*/*PDGFRA* WT GISTs was detected among the 40 GIST samples, with 3 mutations in *CDK4* and *ABL1*, 2 mutations in *AKT1*, *PIK3CA*, and *NRAS*, and 1 mutation in *EGFR*, *HRAS*, *KRAS*, *ERBB2*, and *BRAF*. Mutational analysis revealed that *KRAS* and *ABL1* mutations were only detected in *KIT*/*PDGFRA* WT GISTs, and all *ABL1* mutations were part of a multiple mutation status (results not shown).

It is well established that the RAS/RAF/ERK pathway plays an important role in tumor development, and *KRAS*, *HRAS*, and *NRAS* are the main components of the RAS/RAF/ERK pathway. Mutations in these genes occur in at least one-third of all human cancers, with *KRAS* mutations being the most common.[13–15] In the present study of Chinese patients with GISTs, mutations of *KRAS*, *NRAS*, and *HRAS* were also detected. Among the 40 GISTs, 1 case (2.5%) of a *KRAS* G12C mutation was identified, which did not occur simultaneously with *KIT*, *PDGFRA*, or *BRAF* mutations. This mutation site differed from that reported by Hechtman et al[16] in 2015, where one case with a *KRAS* G12V mutation was detected among 267 GISTs. Furthermore, 2 cases (5%) of *HRAS* mutations and 6 cases (15%) of *NRAS* mutations were detected among the 40 GISTs, whereas 1 *HRAS* mutation (G13S) and 2 *NRAS* mutations (G12D) were harbored by *KIT*/*PDGFRA* WT GISTs. It was previously reported that *KRAS*, *NRAS*, and *HRAS* mutations are scarce in GISTs.[17] Although our results support that *KRAS* and *HRAS* mutation are scarce in GISTs, *NRAS* mutations were detected at a higher frequency among Chinese GIST patients. This result suggests that the role of *NRAS* mutations may differ among various populations, and it may play a key role in the RAS/RAF/ERK pathway in Chinese GIST patients.

EGFR mutation is one of the most important targets for biological therapy, particularly in non-small-cell lung cancer and colorectal cancer.[18] However, there are very few literature on *EGFR* mutation in GISTs.[19] In the present study, 4 *EGFR* mutations (D770_N771insG, T790M, and S752I/F) were detected among the 40 GISTs. Among these mutations, D770_N771insG and T790M occurred together with *KIT*, *NRAS*, or *AKT1* mutations, whereas only the S752I/F mutation was harbored by *KIT*/*PDGFRA* WT GISTs. This result may overturn the hypothesis of Shi et al[19]

that *EGFR* mutations are mutually exclusive with *KIT*, *PDGFRA*, *KRAS*, or *BRAF* mutations in primary GISTs. In addition, the *EGFR* mutation frequency detected in our study is higher compared with previous reports. Therefore, we hypothesized that GISTs may be candidates for anti-EGFR-targeted therapy.

BRAF mutations are common in cancer and represent the most frequent genetic events in malignant melanoma. Multiple studies reported *BRAF* mutation V600E in *KIT*/*PDGFR* WT GISTs.[8,20,21] In the present study, 4 cases of *BRAF* mutations (L597S and G464E) were detected, and G464E coexisted with the *HRAS* mutation G13S in *KIT*/*PDGFR* WT GISTs. This result infers a higher *BRAF* mutation frequency (7.5%) and indicates the presence of new mutation sites in GISTs.

The P13K 110 α subunit encoded by *PIK3CA*, a downstream effector in the *KIT* signaling pathway, has been identified in different types of cancer. In GISTs, *PIK3CA* mutations were also reported in a recent study.[22] Similarly, in the present study, 4 cases (10%) were found to harbor *PIK3CA* mutations (H1047Y, E542K, and E545K). All the mutation sites identified in the present study have been reported in association with other tumors. A previous study based on immunohistochemistry suggested that activation of the mTOR signaling pathway is characteristic in *PDGFRA* mutant and WT GISTs, rather than *KIT* mutant GISTs.[23] In the present study, 2 cases harbored E542K and E545K hotspot mutations of *PIK3CA* in *KIT*/*PDGFRA* WT GISTs. Thus, *PIK3CA* mutations may play a role in WT GIST pathogenesis.

Cyclin-dependent kinase 4, encoded by the *CDK4* gene, is a member of the cyclin-dependent kinase family, is also referred to as cell division protein kinase 4, and is a catalytic subunit of the protein kinase complex that is important for cell cycle G1 phase progression. *CDK4* mutations are associated with tumor cell growth. However, there has been no report of this gene's mutations in GISTs to date. In the present study, 7 cases (17.5%) harbored *CDK4* mutations at R24C (2 hotspots of R24C and R24H in *CDK4* were detected). Nishida et al[24] reported that genotyping and cell cycle analysis may be crucial for GIST risk stratification. Analyzing the GIST risk classification among these *CDK4* mutation cases, it was observed that all these cases were high- or intermediate risk. This result was consistent with the study by Nishida et al.[24] Taking the function of *CDK4* into consideration, it was hypothesized that cyclin-dependent kinase inhibitors for tumor cell quiescence (associated with *CDK4* mutations) may be a new therapeutic target in GISTs.

In addition, *AKT2*, *FLT3*, and *ERBB2* mutations, concurrently with *KIT* mutations, were separately observed in 3 different cases. A total of 4 cases (10%) harbored *AKT1* mutations and 3 cases were *ABL1* mutation-positive among *KIT*/*PDGFRA* WT GISTs. To the best of our knowledge, there has been no report of these mutations in GISTs to date. Thus, *AKT2*, *FLT3*, and *ERBB2* mutations are rarely present in GISTs. However, *AKT2*, *FLT3*, *ERBB2*, *ABL1*, and *AKT1* were reported to be associated with GIST therapy.[25–27] Therefore, the mutations observed in the present study may provide useful information for the clinical treatment of GISTs.

Conclusion

The present study using MassARRAY spectrometry screened 238 mutations affecting 19 oncogenes in 40 Chinese GIST patients. Fourteen oncogene mutations were detected in the samples, including *KIT*, *CDK4*, *NRAS*, *EGFR*, *PIK3CA*, *AKT1*, *BRAF*, *ABL1*, *PDGFRA*, *ERBB2*, *HRAS*, *AKT2*, *FLT3*, and *KRAS*. Approximately half of the GIST samples harbored multiple mutations. A higher frequency of *KIT*/*PDGFRA* WT GISTs was detected in the present study. In addition, *CDK4*, *EGFR*, *PIK3CA*, *NRAS*, *KRAS*, *ERBB2*, and *AKT1* were single-point mutations detected in *KIT*/*PDGFRA* WT GISTs. New mutation genes (*CDK4*, *AKT2*, *FLT3*, *ERBB2*, *ABL1*, and *AKT1*) were also identified in Chinese GIST patients, along with a higher *BRAF* mutation frequency (7.5%) and new *BRAF* mutation sites (G464E). It is noteworthy that, although new mutations were detected in Chinese GIST patients, the sample size was insufficient to draw definitive conclusions. Therefore, further studies with larger samples that screen for mutations in full-length sequences are required to confirm our results.

Disclosure

The authors report no conflicts of interest in this work.

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Supplementary material

Table S1 Mutations detected with OncoCarta

Gene mutation	Gene mutation	Gene mutation
ABL1-G250E	*EGFR*-L747_E749del, A750P	KIT-P585P
ABL1-Q252H	*EGFR*-E746_A750del	KIT-D579del
ABL1-Y253H	*EGFR*-L747_E749del, A750P	KIT-K642E
ABL1-Y253F	*EGFR*-L747_S752del, P753S	KIT-D816V
ABL1-E255K	*EGFR*-E746_T751del, V ins	KIT-D816H/D816Y
ABL1-E255V	*EGFR*-L747_S752del, Q ins	KIT-V825A
ABL1-D276G	*EGFR*-L747_S752del, Q ins	KIT-E839K
ABL1-F311L	*EGFR*-E746_T751del, S752D/SNP C2255T	KIT-M552L
ABL1-T315I	*EGFR*-D770_N771>AGG/V769_ D770insASV/V769_D770insASV	KIT-Y568D
ABL1-F317L	*EGFR*-D770_N771insG	KIT-F584S
ABL1-M351T	*EGFR*-L747_T750del, P ins	KIT-P551_V555del
ABL1-E355G	*EGFR*-E746_A750del	KIT-P551_V555del
ABL1-F359V	*EGFR*-E746_T751del, I ins	KIT-Y553_Q556del
ABL1-H396R	*EGFR*-L747_T751del	KIT-Y553_Q556del
AKT1-rs11555435	*EGFR*-L747_T751del	*KRAS*-G12V/A/D/C/S/R/F
AKT1-rs11555431	*EGFR*-E746_A750del, V ins	*KRAS*-G13C/S/V/D
AKT1-rs11555432	*EGFR*-E746_A750del, V ins	*KRAS*-A59T
AKT1-rs12881616	*EGFR*-S752_I759del	*KRAS*-Q61E/K/L/R/P/H
AKT1-rs11555433	ERBB2-L755P	*MET*-R970C
AKT1-rs11555436	ERBB2-G776S/G776LC	*MET*-T992I
AKT1-rs34409589	ERBB2-G776VC	*MET*-Y1230C
AKT2-S302G	ERBB2-G776VC/G776VC	*MET*-Y1235D
AKT2-R371H	ERBB2-M774_A775insYVMA	*MET*-M1250T
BRAF-G464R	ERBB2-A775_G776insYVMA	*NRAS*-G12V/G12A/G12D
BRAF-G464V/G464E	ERBB2-P780_Y781insGSP	*NRAS*-G12C/G12R/G12S
BRAF-G466V/G466G/G466E	ERBB2-P780_Y781insGSP	*NRAS*-G13V/G13A/G13D
BRAF-G466R	ERBB2-S779_P780insVGS	*NRAS*-G13C/G13R/G13S
BRAF-F468C	FGFR1-S125L	*NRAS*-A18T
BRAF-G469S/E/A/V/R	FGFR1-P252T	*NRAS*-Q61L/Q61R/Q61P
BRAF-D594V| G	FGFR3-R248C	*NRAS*-Q61H
BRAF-F595L	FGFR3-S249C	*NRAS*-Q61E/Q61K
BRAF-G596R	FGFR3-G370C	PDGFRA-V561D
BRAF-L597S/R/Q/V	FGFR3-Y373C	PDGFRA-T674I
BRAF-T599I	FGFR3-A391E	PDGFRA-F808L
BRAF-V600E/K/R/L	FGFR3-K650Q/E	PDGFRA-D846Y
BRAF-K601N/E	FGFR3-K650T/M	PDGFRA-N870S
CDK-R24C/H	FLT3-I836del	PDGFRA-D1071N
EGFR-R108K	FLT3_2	PDGFRA-D842_H845del
EGFR-T263P	FLT3_3	PDGFRA-I843_D846del
EGFR-A289V	FLT3-D835H/D835Y	PDGFRA-S566_E571>K
EGFR-G598V	*HRAS*-G12V/D	PDGFRA-I843_S847>T
EGFR-E709K/E709H	*HRAS*-G13C/R/S	PDGFRA-D842V
EGFR-E709A/E709G/E709V	*HRAS*-G13V/D	*PIK3CA*-R88Q
EGFR-G719S/G719C	*HRAS*-Q61H	*PIK3CA*-N345K
EGFR-G719A	*HRAS*-Q61H/L/R/P/K	*PIK3CA*-C420R
EGFR-M766_A767insAI	JAK2-V617F	*PIK3CA*-P539R
EGFR-S768I	KIT-D52N	*PIK3CA*-E542K
EGFR-V769_D770insASV	KIT-Y503_F504insAY	*PIK3CA*-E545K
EGFR-V769_D770insCV	KIT-W557R/W557R/W557G	*PIK3CA*-Q546K
EGFR-D770_N771>AGG/V769_ D770insASV/V769_D770insASV	KIT-V559D/V559A/V559G	*PIK3CA*-H701P
EGFR-D770_N771insG	KIT-V559I	*PIK3CA*-H1047R/H1047L
EGFR-N771_P772>SVDNR	KIT-V560D/V560G	*PIK3CA*-H1047Y

(*Continued*)

Table S1 (*Continued*)

Gene mutation	Gene mutation	Gene mutation
EGFR-P772_H773insV	KIT-K550_K558del	*PIK3CA*-G1049R
EGFR-H773>NPY	KIT-K558_V560del	*PIK3CA*-R38H
EGFR-H773_V774insNPH/H773_V774insPH/H773_V774insH	KIT-K558_E562del	*PIK3CA*-C901F
EGFR-V774_C775insHV	KIT-V559del	*PIK3CA*-M1043I/M1043I
EGFR-T790 M	KIT-V559_V560del	RET-C634R
EGFR-L858R	KIT-V560del	RET-C634W/Y
EGFR-L861Q	KIT-Y570_L576del	RET-E632_L633del
EGFR-L747_T750del, P ins/E746_A750del, T751A	KIT-E561K	RET-M918T
EGFR-E746_T751del, I ins/S752_I759del	KIT-L576P	RET-A664D