Prevalence of CKIT and PDGFRA Mutation in Gastrointestinal Stromal Tumors among Filipinos

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ABSTRACT

Background. Gastrointestinal stromal tumors (GIST) are defined as specific, typically kit (CD117)-positive and CKIT or platelet-derived growth factor receptor alpha (PDGFRA) mutation-driven mesenchymal tumors that can occur anywhere in the GI tract. GIST diagnosis relies heavily on immunohistomorphology. However, with the advent of molecular testing, the classification, diagnosis, and targeted therapy for gastrointestinal mesenchymal tumors have been improved. In the Philippines, molecular testing is not yet readily available as in other countries. The local molecular profile of gastrointestinal stromal tumors is a point of investigation as treatment may be more tailored to the patients' needs.

Objective. This study aims to determine the prevalence of CKIT and PDGFRA mutations among formalin-fixed and paraffin embedded gastrointestinal stromal tumors and other gastrointestinal mesenchymal tumors in St. Luke's Medical Center – Quezon City.

Methodology. A retrospective cross-sectional study of formalin fixed and paraffin embedded tumor samples diagnosed as Gastrointestinal Stromal Tumor from January 1, 2009 to December 31, 2017 will be analyzed for KIT and PDGFRA mutations.

Results. The epidemiology of GIST remains constant in that mean age group is the 5th to 6th decade, with equal gender distribution, and stomach followed by small bowel are the most common sites. Mutational analysis of the GISTs predominantly showed KIT Exon 11 (47.83%) followed by CKIT Exon 9 (13.04%) and PDGFRA Exon 18 (10.87%). For KIT Exon 11, deletion is the most common mutation followed by point mutations. No mutation is detected in 47.83% of GISTs.

Conclusion. Mutational analysis for CKIT-PDGFRA is warranted among GIST patients, as it may significantly influence the treatment protocol of patients.

Key words: Gastrointestinal Stromal Tumors, GIST, Sequencing, CD117, CKIT, PDGFRA

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INTRODUCTION

Gastrointestinal stromal tumors (GIST) can be defined as specific, typically kit (CD117)-positive and KIT or platelet-derived growth factor receptor alpha (PDGFRA) mutation-driven mesenchymal tumors that can occur anywhere in the gastrointestinal (GI) tract.[1,2] It is a relatively rare soft tissue sarcoma which commonly arises in the stomach (60%), followed by jejunum and ileum (30%), duodenum (5%), colorectal (<5%), and rarely in the esophagus or appendix.[3] GISTs may also occur as primary tumors outside of the GI tract, in the retroperitoneum or abdomen (e.g., omentum, mesentery), and such tumors have been referred to as extra-gastrointestinal stromal tumors.[1] GISTs arise mostly in middle-aged or older individuals, and some arise as congenital tumors in children, with no sex predilection. These may be asymptomatic or manifest with GI bleeding and abdominal pain. Other clinical symptoms include nausea, vomiting, weight loss and the presence of abdominal mass.[4-6] The vast majority of GISTs are

sporadic with no known associated risk factors, however, approximately 5% are associated with a tumor syndrome, including neurofibromatosis type 1 (NF1), Carney's triad (pulmonary chordoma, paraganglioma, GIST), and familial GIST syndrome.[7,8]

GISTs are either derived from or differentiate toward interstitial cells of Cajal, which act as the pacemaker cells of the gut and serve as intermediaries between the GI autonomic nervous system and smooth muscle cells to regulate GI motility and coordinate peristalsis.[3,9] GISTs were originally considered to be of smooth muscle origin, due to their histology. Due to its spindle cell characteristic, in the past, these tumors were classified as other gastrointestinal muscle tumors (GMT) such as leiomyomas, leiomyosarcomas, leiomyoblastomas and spindle cell neoplasms.[10] Hence, their true frequency is unknown. Epidemiologic data provided by the National Cancer Institute's Surveillance, Epidemiology and End Results (SEER) program may be difficult to interpret since the early definition "malignant GIST" was taken from the criteria published in 1990, before the molecular classification of GIST.[6] Current epidemiologic studies done showed an annual incidence of 14.5 per million in Swedish,[2] 11 per million in Icelandic[11] and annual incidence of at least 4,000 to 6,000 new cases per year in the United States.[3,6] In Taiwan, the reported incidence is 1.13 per 100,000 in 1998, with an increase to 1.97 per 100,000 in 2008.[11] Shanghai epidemiologic studies showed average crude incidence of GISTs of 2.11 per 100,000 between 2004 and 2008.[12] However, in the Philippines, no studies have been done. Since CKIT and PDGFRA mutation testing has not been previously performed in the Philippines, we will compare the prevalence of mutations in these genes among our GIST cases with from the literature.

At present, GIST is diagnosed in our institution using the following immunohistochemical stains: CKIT/CD117, DOG1, CD34, smooth muscle actin, S100 and desmin. Among these stains, CKIT/CD117, a very specific and sensitive marker in the differentiating GIST from other mesenchymal tumors in the GI tract is most widely used.[13,14] Despite the significant therapeutic implications of CKIT/CD117 positivity, the intensity, extent and patterns of KIT staining does not correlate with the type of KIT mutation or its response to available medications.[15]

Molecular advancements in pathology have established that KIT mutations, majority of which are somatic which cause the constitutive activation of the kinase, are found in 70-80% of GISTs.[1,16] The oncogenic activation of KIT is the dominant pathogenetic mechanism in GIST.[16] However, about 5% of GISTs lacking KIT gene mutations harbor activating mutations in PDGFRA.[3] Molecular findings have led to the development of tyrosine kinase inhibitors, the prototype of which is imatinib. These inhibit the c-KIT and PDGFRA by competing with the adenosine triphosphate-binding site required for phosphorylation and activation of the receptor, hence, inhibiting tumor proliferation.[1,3,16,17] Imatinib has been considered as the standard treatment for GIST. Partial response is achieved in 65 to 70%, but 15–20% maintain stable disease.[3]

GIST is classified into three molecular categories based on the mutations of the KIT and PDGFRA gene: GIST with KIT mutations, GIST with PDGFRA mutations, and non-KIT or PDGFRA somatic mutations that are designated as wild type.[18] The wild type variation is considered complex due to the existence of different subgroups with distinct molecular hallmarks, such as deletion mutations of succinate dehydrogenase subunit A (SDHA) and mutations of neurofibromatosis type 1 (NF1), RAS, or BRAF.[19] Advancement in molecular pathology, has identified PDGFRA mutations, in 5 to 10% of GIST's.[1,16] Mutations of PDGFRA on exon 12, 14, and 18 are mostly implicated. However, PDGFRA exons 12 and 14 mutations have a low frequency of <1%, with PDGFRA exon 18 having a relatively higher frequency of 6 to 7%.[16] PDGFRA, although a close homologue of CKIT, are more gastric in location, and is associated with epithelioid morphology and indolent course.[16,20] Ultimately, GIST can be characterized as a cancer with comparatively small genetic variation; hence, the precise treatment of the cancer gene map for GISTs has become seemingly evident and apt.

Recent data show that GIST patients respond differently to tyrosine kinase inhibitors (drugs like imatinib and sunitinib), depending on the specific mutations displayed by their tumors.[3] Most deletions and deletions preceded by substitutions result to active conformation of the normal kinase activation loop.[16] KIT mutations in exon 11 is the most common mutation, and is seen in 70% of cases. These are commonly seen in the gastric and small bowel and has a higher risk of relapse after surgical resection.[21] The second most common KIT mutation is seen in the extracellular domain encoded by exon 9.[20] It has a frequency of 10 to 15%, and may reach up to 18.1%.[1,16,21] Exon 9 mutations are usually seen in the small bowel, and with an aggressive clinical behavior. Less than 1% of GIST harbor mutations in the exon 13 and 17.[1,16]

Mutational analysis of the KIT gene (exons 11, 9, 13, and 17) and PDGFRA gene (exons 12, 14, and 18) may aid in confirming GIST if immunohistochemical stains fail to support the diagnosis.[16] At present, GIST mutational analysis is recommended in the NCCN (National Comprehensive Cancer Network) and ESMO (European Society for Medical Oncology) clinical recommendations.[16,22,23] Such recommendations have provided clinical significance in therapeutic aspects for its predictive value for sensitivity to molecular-targeted therapy (including dosage) and prognostic value.[16] A study done by Heinrich and Corless et al., indicate a stronger response to imatinib in patients with KIT exon 11 mutations than patients with exon 9 mutations. Patients with an exon 11 GIST mutation were much more likely to have a partial response with imatinib therapy than those with exon 9 or no mutations.[1,3] In contrast, patients with KIT exon 9 mutations, resistant to imatinib, showed better response to a tyrosine kinase inhibitor (sunitinib).[16] GISTs with PDGFRA exon 18 mutation (D842V) show primary resistance to imatinib both in vivo and in vitro.[1,16] Another utility of mutation testing involves the identification of newly acquired secondary mutations, not initially detected in the primary tumor, that can confer drug resistance to imatinib.

The spectrum of mutations in gastrointestinal stomal tumor is still unknown among Filipino patients. The diagnosis and treatment of GIST currently relies on immunohistochemical staining of GIST tumor with CD117 antibody. This study aims to characterize the CKIT and PDGFRA mutations among Filipino patients diagnosed with GIST in our institution.

METHODOLOGY

Following approval by the institutional review board, a retrospective review of all formalin fixed paraffin embedded (FFPE) tumor samples diagnosed with GIST from the period of January 2009 to December 2017 was performed. All samples were from pre-treatment procedures and were from primary tumor sites. No samples were taken from recurrence or metastatic sites. The age, sex, histopathologic diagnosis, and location of the tumors were recorded.

Sample collection

DNA was isolated from FFPE samples after deparaffinization and extraction of 3–5 mm thick paraffin sections in xylene and by using the QIAamp DNA FFPE Tissue Kit (Qiagen) per the manufacturer's instruction. In samples with DNA concentration of less than 5 ng/ml, a second extraction from another tissue block was performed. Those with DNA concentration less than 5 ng/ml after second extraction were excluded. DNA purity was measured using Nanodrop 1000. A A260/280 ratio of between 1.7-2 was deemed acceptable for subsequent reactions. Suboptimal samples were also excluded.

Primer identification

Using data from Ensembl (www.ensembl.org), forward and reverse primers were designed to identify mutations found in the different exons or different regions in a single exon CKIT (exon: 9, 11, 13 and 17) and PDGFRA (exon: 12, 14 and 18) genes (Tables 1 and 2).

Table 1. NIH consensus classification criteria for defining risk of aggressive clinical course of primary GISTs

Risk Category	Tumor size in largest dimension	Mitotic count (per 50 HPFs)
Very low	<2 cm	<5
Low	2-5 cm	<5
Intermediate	<5 cm	6-10
	5-10 cm	<5
High	>5 cm	>5
	>10 cm	Any mitotic rate
	Any size	>10

Sanger sequencing

Sequential testing of mutations was done to determine the CKIT and PDGFRA mutations. PCR amplifications were performed using specific primer pairs to amplify exons 9, 11, 13 and 17 of CKIT gene as well as exons 12, 14 and 18 of PDGFRA gene. The samples negative for CKIT exon 9 and 11 mutations underwent another round of PCR amplification using specific primer pairs to amplify the remaining mutations CKIT (exon 13 and 17) and PDGFRA (exon 12,14 and 18).

Data analysis

The prevalence of CKIT and PDGFRA mutations for GIST was described. The association of the CKIT and PDGFRA mutations with tumor size, mitotic count, location, and risk stratification[24] (Table 1) was determined using Fisher's exact test. A p-value of <0.05 was considered significant.

RESULTS

For the duration of the study period, a total of 85 FFPE Gastrointestinal lesions suspected of GIST were retrieved and 58 cases were confirmed by immunohistochemical stain. Table 2 summarizes the characteristics of patients diagnosed with GIST. Out of 58 samples, 46 (79.3%) were resection specimens and 12 (20.7%) were biopsy specimens. The mean age at diagnosis was 60.12 years (29-86 years). Gender distribution was equal (1:1). Among 58 cases, 36 (62.1%) cases were found to have mutations (CKIT or PDGRAF or double mutation) while 22 (37.9%) had no mutations. Overall, the most common tumor site was gastric (63.8%). The patients' age did not differ between the two groups (p=0.090). The presence of mutation was not associated with gender (p=0.787) and tumor location (p=0.177).

Tumor profile was available in 46 cases (Table 3). Based on risk classification, 37% were classified as low risk, 6.5% as intermediate, and 56.5% as high risk. Mitotic count, tumor size, and risk classification were not associated with presence of mutation (p=0.371, p=0.660, p=0.625, respectively). Immunoreactivity to CD117, DOG1 and CD34 are high at 93.5%, 92.31% and 67.7%, respectively. Some GISTs did test positive for SMA (12.05%) and S100 (5.26%).

Mutational analysis of the GIST cases showed predominantly KIT mutation (29/36, 80.6%). There were 5 (13.9%) PDGFRA mutations and two (5.6%) cases with double mutation (CKIT and PDGFRA). The mutational profile of the cases is summarized in Table 4. CKIT11

Table 2. Patient characteristics of GIST cases

Variable	Overall (n=58)	No mutation (n=22)	With mutation (n=36)	P-value
Mean age (range) in years	60.12 (29-86)	56.27 (29-78)	62.47 (38-86)	0.090[a]
Gender (M:F)	29:29	10:12	19:17	0.787[b]
Tumor location				
Esophagus	1 (1.7%)	1 (4.5%)	0	0.177[b]
Gastric	37 (63.8%)	15 (68.2%)	22 (61.1%)	
Duodenum	2 (3.4%)	0	2 (5.6%)	
Jejunum	6 (10.3%)	1 (4.5%)	5 (13.9%)	
Ileum	5 (8.6%)	1 (4.5%)	4 (11.1%)	
Colorectal	4 (6.9%)	1 (4.5%)	3 (8.3%)	
Extra-gastrointestinal	3 (5.2%)	3 (13.6%)	0	

[a]Mann-Whitney U Test; [b]Fisher's Exact Test

Table 3. Tumor profile and risk stratification of GIST cases

Variable	Overall (n=46)	No mutation (n=16)	With mutation (n=30)	P-value
Mitosis (per 50 HPFs)				
<5	35 (76.1%)	12 (75%)	23 (76.7%)	0.371
6-10	5 (10.9%)	3 (18.8%)	2 (6.7%)	
>10	6 (13%)	1 (6.3%)	5 (16.7%)	
Tumor size (cm)				
2-5	14 (30.4%)	6 (37.5%)	8 (26.7%)	0.660
5-10	10 (21.7%)	4 (25%)	6 (20%)	
>10	22 (47.8%)	6 (37.5%)	16 (53.3%)	
Risk stratification				
Low	17 (37%)	7 (43.8%)	10 (33.3%)	0.625
Intermediate	3 (6.5%)	0	3 (10%)	
High	26 (56.5%)	9 (56.3%)	17 (56.7%)	

HPF = High Power Field

Table 4. KIT and PDGFRA mutational profile

Gene	Exon	Mutation detected	n (%)
CKIT	9	Internal tandem duplication of AY502-503	6 (16.7)
	11	Deletions between and including K550-G565	14 (38.9)
		Point mutations at Y553, W557, V559, V560	5 (13.9)
		Insertion at D579	3 (8.3)
	9 and 11	N/A	1 (2.8)
PDGFRA	18	V824V silent mutation (GTC to GTT)	2 (5.6)
		Point mutations at D842	3 (8.3)
Double mutation	CKIT11 and PDGFRA18	Deletions between and including K550-G565 Point mutations at D842	2 (5.6)

deletion was the most common mutation (38.9%) followed by CKIT9 tandem duplication (16.7%).

Table 5 summarizes the patient characteristics, tumor profile, and risk stratification between patients with CKIT or PDGFRA mutation. Patient age did not differ between the two groups (p=0.851) and gender was not associated with the type of mutation (p=0.335). The most common tumor site was gastric in both mutations (62.1% in CKIT and 80% in PDGFRA). Most patients were also classified as high risk with 54.2% of the CKIT mutation and 50% of PDGFRA mutation. However, tumor location, mitotic count, tumor size, and risk stratification were not associated with the type of mutation (p-values: 0.360, 0.342, 1.00, and 0.547, respectively). The most common morphology was spindle cell at 69.0% and 60% for samples with CKIT mutation and PDGFRA mutation, respectively. Of those with CKIT mutations and spindle cell morphology, 6 (16.7%) had CKIT 9 mutation and 14 (38.9%) had CKIT 11 mutation. Three samples which showed epithelioid (n=1), and mixed spindle and epithelioid histomorphology (n=2) had CKIT 11 mutations.

Table 5. Patient characteristics, tumor profile and risk stratification by CKIT and PDGFRA mutation

Variable	CKIT mutation (n=29)	PDGFRA mutation (n=5)
Mean age (range) in years	63.14 (43-86)	59.00 (38-78)
Gender (M:F)	13:16	4:1
Tumor location		
Gastric	18 (62.1%)	4 (80%)
Duodenum	2 (6.9%)	0
Jejunum	5 (17.2%)	0
Ileum	3 (10.3%)	0
Colorectal	1 (3.4%)	1 (20%)
Mitosis (per 50 HPF)	n=24	n=4
<5	19 (79.2%)	3 (75%)
6-10	1 (4.2%)	1 (25%)
>10	4 (16.7%)	0
Tumor size (cm)	n=24	n=4
2-5	7 (29.2%)	1 (25%)
5-10	5 (20.8%)	1 (25%)
>10	12 (50%)	2 (50%)
Risk stratification	n=24	n=4
Low	9 (37.5%)	1 (25%)
Intermediate	2 (8.3%)	1 (25%)
High	13 (54.2%)	2 (50%)
Histomorphology		
Spindle	20 (69.0%)	3 (60%)
Epithelioid	1 (3.4%)	1 (20%)
Mixed spindle and epithelioid	2 (6.9%)	0 (0%)
Not specified	6 (20.7%)	1 (20%)

DISCUSSION

Across geographic regions, the epidemiology of GIST remains constant in that mean age group is between the 5th to 6th decade, with no gender preponderance, and gastric being the most common tumor site.[25] The same observations were demonstrated in the present study.

The advent of molecular pathology has brought about paradigm shift in the classification, diagnosis and targeted therapy for gastrointestinal mesenchymal tumors.[26] Prior to the wide use of immunohistochemical stains, GISTs were thought to be smooth muscle tumors and classified as cellular leiomyomas, leiomyoblastomas, and leiomyosarcomas of the GI tract.[10] Histomorphology alone has several limitations as GISTs has a wide morphologic spectrum ranging from spindle cell to epithelioid morphology.[27] The broad histologic differential diagnosis of GIST has brought about the importance of immunohistochemical testing. At present, commonly used immunohistochemical analysis to diagnose GIST includes CD34, CD117 and the much newer DOG1.[28,29] About 95% of GISTs are immunoreactive for CD117, however, more recent studies have shown

that 4 to 15% of these tumors may be negative.[14,30,31] Such occurrence is most commonly observed in gastric GISTs having epithelioid morphology and PDGFRA mutation.[32] In 2004, West R et al., demonstrated that the novel marker, DOG1 is ubiquitously expressed in gastrointestinal tumors. It is more sensitive to CD117 for gastric epithelioid GISTs than those with PDGFRA mutations.[31,33] CD117 and DOG1 have an overall sensitivity of 94.7% and 94.4%, respectively.[32] Nevertheless, in a large-scale study conducted by Miettinen et al., 2.6% of GISTs were noted to be negative to both CD117 and DOG1. In the same study, 11/24 DOG1-negative spindle cell neoplasm was noted to be positive for KIT and PDGFRA mutations, supporting the diagnosis of GIST. Further investigations show that other mesenchymal tumors such as retroperitoneal leiomyomas, peritoneal leiomyomatosis and synovial sarcomas may be immunoreactive to DOG1.[32] Immunohistochemical findings in the present study showed a similar result as majority of the GISTs were positive for CD117, DOG1 and CD34. Several cases also showed positivity to SMA and S100. Most clinical trials on GIST are commonly conducted in Western countries as compared to the limited number in Asia, indicating that Asian GIST patients have limited access to investigational drugs after standard therapy.[34]

Targeted therapy for gastrointestinal stromal tumors was developed with the discovery of KIT mutations.[27] Similar to published literature, the majority of the GIST mutations are that of KIT exon 11. In a review by Szucs et al., 69 to 83% of all GISTs show KIT mutations, specifically exon 11.[35] This is in line with the present data where 81% of mutations were KIT mutations and exon 11 was involved 76% of these cases. Among the mutations of this exon, the most studied is that of deletions. Exon 11 deletions are in 23.2 to 27.7% of all GIST cases.[36] A large-scale study done by Wozniak R et al., showed that tumors with exon 11 deletion, especially those affecting codons 557-558, are usually larger and have high mitosis.[36] Hence, tumors are usually classified as high risk for progressive disease. A similar profile was observed in the current study where 39% of CKIT mutations were exon 11 deletions and 54% with CKIT mutation were classified as high risk. The GISTs mostly have tumors >10 cm, with some accompanied by high mitotic rate. In the same review, contrary to KIT Exon 11 deletions, GISTs with point mutations have an indolent course, with smaller tumors and low mitosis. As seen in the present study, the GIST cases with point mutations have small size, 0 to 1 mitotic rate and are classified as low risk. Although global data suggests an equal distribution of GISTs among genders, CKIT Exon 9 has been reported more in males and may be seen in the lower intestinal tract.[36,37] Clinical behavior of this mutation can be contradicting in some studies. Künstlinger et al., concluded that exon 9 mutations per se do not have a prognostic relevance as they are not associated with high risk and metastasizing tumors.[38] Data in the present study also show that KIT Exon 9 mutations, although located in the lower intestinal tract, have low risk for progression. In spite of this, caution must be taken on Exon 9 mutation. A study done by Zhao et al., indicated the importance of Exon 9 mutation as it may be implicated in the mutations having resistance to Gleevec. A more recent publication showed that Exon 9 mutations have better response to another tyrosine kinase inhibitor, Sutent.[39] PDGFRA D842V GIST mutations, as previously discussed in recent publications is of importance due to its contradicting behavior and therapeutic response.[40] GISTs with PDGFRA D842V usually have an epithelioid morphology, indolent course and remain localized with low risk of recurrence. However, GISTs harboring this mutation are usually resistant to imatinib.[7] Imatinib was the first FDA-approved as the first-line drug for metastatic and recurrent GIST.[41] However, it was observed in several studies that resistance develops in two years.[42] Recent publications have implicated the presence of a secondary mutation, commonly KIT and PDGFRA as the cause of resistance.[43] Our current two cases were noted to have double mutations seen as KIT Exon 11 and PDGFRA 18 on mutational analysis. On investigation, one of the GIST cases is already on recurrence after treatment with Imatinib.

Ultimately, this study supported by other materials highlights the significance of molecular level analysis to efficiently identify mutations associated with GISTs and recommend individualized treatments depending on the specific mutation's sensitivity. Furthermore, treatment resistance may provide a genetic basis for developing new GIST therapeutic drugs.

CONCLUSION

Although gastrointestinal stromal tumor is the most common mesenchymal tumor of the gastrointestinal tract, it remains rare compared to other tumors. Given its varying histomorphology, mutational analysis has aided its diagnosis. Mutational analysis also has a significant impact in the treatment and prognosis of gastrointestinal stromal tumors. The presence of resistant mutation (PDGFRA D842V) would warrant alternative treatment. In the Philippines, diagnosis is based on immunohistomorphology of the cases only, and is not optimal for long term management of the patient. As seen in the findings of this study, mutational analysis, in correlation with immunohistomorphology can greatly aid the diagnosis and management of GISTs. Among the 62% of CKIT and PDGFRA wild type GIST, additional testing for other genes (Neurofibromatosis type 1 and Succinate dehydrogenase deficiency) would be warranted.

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STATEMENT OF AUTHORSHIP

The authors certified fulfillment of ICMJE authorship criteria.

AUTHOR DISCLOSURE

The authors declared no conflict of interest.

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APPENDICES

Appendix 1. KIT and PDGFRA mutations to be analyzed

Gene	Exon	Mutation detected
CKIT	9	Internal tandem duplication of AY502-503
CKIT	11	Point mutations at Y553, W557, V559, V560
		Deletions between and including K550-G565
		P573R (CCA to CGA) à novel
		L576P (CTT to CCT)
		Insertion at D579
CKIT	13	K642E (AAA to GAA)
		V654A (GTG to GCG)
	17	Point mutations at D816, D820
		N822K (AAT to AAA/AAG)
		Y823D (TAT to TGT)
		silent mutation (ACT to ATT)
PDGFRA	12	silent mutation (ACT to ATT), S566-E571, Y582-W586
		V561D (GTC to GAC)
		E571K (GAA to AAA)
		insertions following R560
		L580P (CTT to CCT)
	14	K646E (AAG to GAG)
		N659K, Y (AAC to AAG/TAC)
	18	V824V silent mutation (GTC to GTT)
		Point mutations at D842
		Y849C (TAT to TGT)
		Deletions between and including D842-846

Appendix 2. List of PCR primer sequences used to amplify all the CKIT and PDGFRA exons for the Sanger sequencing analysis

Gene	Exon	Mutation Detected	Primer ID	Forward Primer (5' to 3')	Primer ID	Reverse Primers (5' to 3')
CKIT	9	Internal tandem duplication of AY502-503	CKIT9F	ATGCTCTGCTTCTGTACTGCC	CKIT9R	CAGAGCCTAAACATCCCCTTA
CKIT	11	Point mutations at Y553, W557, V559, V560	CKIT11F	CCAGAGTGCTCTAATGACTG	CKIT11R	ACCCAAAAAGGTGACATGGA
		Deletions between and including K550-G565				
		P573R (CCA to CGA) à novel				
		L576P (CTT to CCT)				
		Insertion at D579				
CKIT	13	K642E (AAA to GAA)	CKIT13F	CATCAGTTTGCCAGTTGTGC	CKIT13R	ACACGGCTTTACCTCCAATG
		V654A (GTG to GCG)				
	17	Point mutations at D816, D820	CKIT17F	TGTATTCACAGAGACTTGGC	CKIT17R	GGATTTACATTATGAAAGTCACAGG
		N822K (AAT to AAA/AAG)				
		Y823D (TAT to TGT)				
		silent mutation (ACT to ATT)				
PDGFRA	12	silent mutation (ACT to ATT), S566-E571, Y582-W586	PDGFRA12F	TCCAGTCACTGTGCTGCTTC	PDGFRA12R	GCAAGGGAAAAGGGAGTCTT
		V561D (GTC to GAC)				
		E571K (GAA to AAA)				
		insertions following R560				
		L580P (CTT to CCT)				
	14	K646E (AAG to GAG)	PDGFRA14F	TGGTAGCTCAGCTGGACTGAT	PDGFRA14R	GGGATGGAGAGTGGAGGATT
		N659K, Y (AAC to AAG/TAC)				
	18	V824V silent mutation (GTC to GTT)	PDGFRA18F	CAGCTACAGATGGCTTGATCC	PDGFRA18R	TGAAGGAGGATGAGCCTGAC
		Point mutations at D842				
		Y849C (TAT to TGT)				
		Deletions between and including D842-846				