

Imatinib in the Management of Multiple Gastrointestinal Stromal Tumors Associated With a Germline *KIT* K642E Mutation

Janet Graham, MBChB, MRCP(UK); Maria Debiec-Rychter, MD, PhD; Christopher L. Corless, MD, PhD;
Robin Reid, BSc, MBChB, FRCPath(UK); Rosemarie Davidson, MBChB, MRCP(UK), BSc MedSci;
Jeff D. White, MBChB, DM, MRCP(UK)

• Gastrointestinal stromal tumors (GISTs) are the most common mesenchymal tumors of the gut and are distinguished by expression of CD117 (c-Kit). Oncogenic mutations in the *KIT* or *PDGFRA* gene are detected in approximately 85% of sporadic GISTs. In recent years, examples of familial GIST have been reported in which germline mutations of *KIT* or *PDGFRA* result in multiple GISTs, skin disorders, and other abnormalities. The most common germline mutations are in *KIT* exon 11, mutations in exons 8 and 17 have also been described, and there are 2 families with germline *PDGFRA* mutations. We present a case in which a germline *KIT* exon 13 mutation (K642E) was discovered in a patient with multiple GISTs of rectum, small intestine, and esophagus, as well as diffuse hyperplasia of the interstitial cells of Cajal. To our knowledge, this is only the second germline example of this particular mutation. The patient's esophageal tumors were stabilized with imatinib.

(Arch Pathol Lab Med. 2007;131:1393–1396)

Gastrointestinal stromal tumors (GISTs) are rare tumors of the gastrointestinal (GI) tract that were previously classified as leiomyoma, leiomyosarcoma, or leiomyoblastoma. Now recognized as a distinct clinicopathologic entity, these tumors are distinguished by their common expression of CD117 (c-Kit) and CD34 antigens. Oncogenic *KIT* and *PDGFRA* mutations have been identified as primary events in GIST tumorigenesis.¹ Despite the low general incidence of these tumors, they have received a great deal of attention recently because of the effectiveness of tyrosine kinase in-

hibitors imatinib mesylate and sunitinib in the treatment of patients with advanced disease.^{2,3}

Activating mutations of Kit are common in GISTs. The frequency of detection varies from 20% to more than 80% according to the technique used.¹ The most frequent mutations are seen in the juxtamembrane domain of Kit, which is coded by exon 11.⁴ Mutations in the extracellular domain of Kit, exon 9, are seen in 10% to 18%,¹ and tumors bearing this type of mutation originate almost exclusively from the small intestine.^{5,6} The rarest mutations are reported in the kinase 1 domain of Kit, encoded by exon 13 (0.8 to 4.1% of cases)¹ and in the activating loop of Kit, encoded by exon 17 (<1% of all described mutations).¹

Gastrointestinal stromal tumors usually occur sporadically and as single tumors, as a result of somatic mutations. However, more than 30 cases of multiple GISTs have been reported, and they are usually observed in a familial setting. Here we report a case of multiple GISTs with a rare mutation in *KIT* exon 13 (K642E) present in the germline. Details of the patient's presentation and management with imatinib are discussed.

CASE PRESENTATION

A 57-year-old white man presented to the oncology clinic in May 2004. He was referred by the surgeons with a background history of multiple tumors removed from his GI tract. His first surgery was 15 years before when he was evaluated for bloating and diarrhea and was found to have a perirectal tumor. The lesion was diagnosed as a low-grade leiomyosarcoma. In 2003, the patient presented with melena, and an upper GI endoscopy showed 2 lesions in the stomach, which were removed and were described as GISTs. In January 2004, he presented with melena and anemia, and on this occasion 14 lesions were removed from the fourth part of the duodenum to the ileum.

The lesions from 2003 and 2004 were reviewed by a pathologist with expertise in sarcomas who confirmed that the tumors were GISTs with strong expression of CD117 and CD34. In several of the lesions the adjacent myenteric plexus appeared expanded, which was interpreted as diffuse hyperplasia of interstitial cells of Cajal. This is illustrated using CD117 staining in Figure 1, A and B.

MOLECULAR PATHOLOGY

The patient's history of diffuse interstitial cells of Cajal hyperplasia and multiple GISTs suggested the possibility of a germline mutation in *KIT*. Therefore, the patient and his family were referred to the clinical genetics team. Ge-

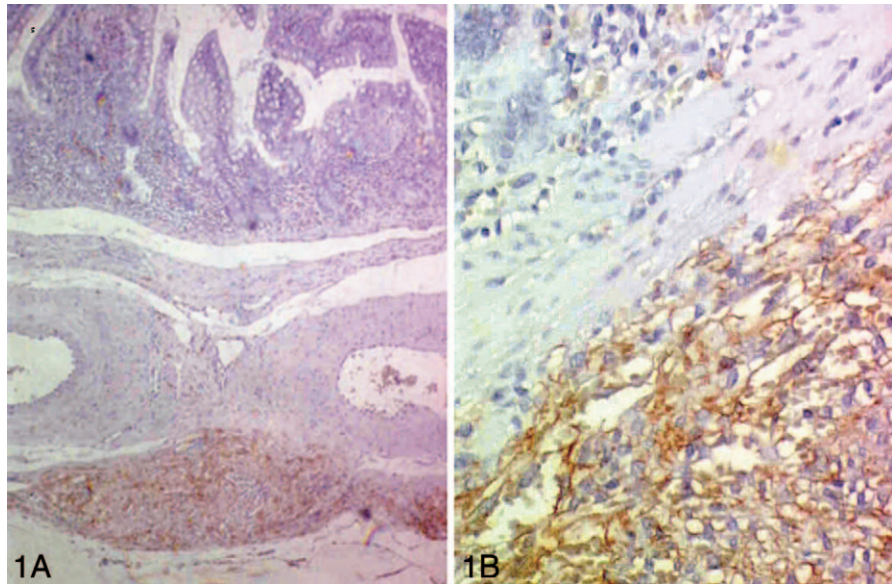
Accepted for publication March 2, 2007.

From the Beatson Oncology Centre, Glasgow, United Kingdom (Drs Graham and White); the Department of Human Genetics, University Hospital Leuven, Leuven, Belgium (Dr Debiec-Rychter); the Cancer Pathology Shared Resource, Oregon Health and Science University, Portland (Dr Corless); Osteoarticular Pathology, Western Infirmary, Glasgow, United Kingdom (Dr Reid); and the West of Scotland Regional Genetics Service, Ferguson-Smith Centre for Clinical Genetics, Yorkhill Division, Glasgow, United Kingdom (Dr Davidson).

Dr Corless has received honoraria and consulting fees from Novartis Pharma (<\$10 000 per year). The other authors have no relevant financial interest in the products or companies described in this article.

Reprints: Jeff D. White, MBChB, DM, MRCP(UK), Beatson Oncology Centre, Dumbarton Road, Glasgow G11 6NT, United Kingdom (e-mail: jeff.white@northglasgow.scot.nhs.uk).

Figure 1. A, CD117 immunostaining. Small intestine: low-power view, showing immunopositivity in the hyperplastic layer of Cajal cells (indirect immunoperoxidase, original magnification $\times 20$). B, CD117 immunostaining. Small intestine: detail of membranous expression of Kit in Cajal cells and not in any other cells (indirect immunoperoxidase, original magnification $\times 100$).



nomic DNA was prepared from representative paraffin blocks of the patient's tumor and from peripheral blood leukocytes. A combination of polymerase chain reaction and denaturing high-performance liquid chromatography was used to screen tumor and blood-derived DNA for mutations in *KIT* gene exons 9, 11, 13, and 17, as previously described. The high-performance liquid chromatography profile for *KIT* exon 13 indicated a sequence anomaly in both the tumor and blood-derived DNAs (Figure 2). Bidirectional DNA sequencing of both samples confirmed the presence of a heterozygous, single base missense mutation leading to the substitution K642E (Lys \rightarrow Ala).

Array-based comparative genomic hybridization using a 1-Mb platform was performed on a sample of the patient's tumor obtained at endoscopy in April 2005.⁷ This analysis indicated loss of chromosomes 14 and 22 (Figure 3, A and B), a feature that is characteristic of GIST but is not associated with aggressive disease.⁵

The patient's father is alive and well, age 80, and his mother died recently, age 79 (not because of malignancy). His maternal uncle died of lung cancer and maternal grandmother had cancer of uncertain etiology (Figure 4). His father and brother had a number of moles removed. A *KIT* gene mutation screening of the proband's family members has been completed. His father and 4 siblings do not have the K642E mutation. His children have chosen not to be tested. It is likely that our patient acquired a de novo mutation, but in the absence of genotypic information from his mother we are unable to prove this.

CLINICAL FOLLOW-UP

At the time of referral the patient was asymptomatic. He was followed up with 6 monthly endoscopies and computed tomography (CT) scans of chest, abdomen, and pelvis. Prophylactic imatinib was discussed, but it was felt there was a lack of evidence to support this approach at that time.

The patient underwent an upper GI endoscopy in January 2005 that showed no evidence of recurrence. However, a CT scan 1 month later showed a 2.8×2.6 -cm soft tissue mass in the wall of the esophagus from the aortic arch to the subcarinal area and a 3.8×1.8 -cm mass in the

distal esophagus, as well as further mucosal thickening in the region of the pylorus. The appearances were highly suggestive of recurrent stromal tumors. He remained asymptomatic from these lesions. Surgery was deemed not appropriate. Four months later the patient developed symptoms that were felt to be disease related; namely, nonproductive cough occurring when he lay on his left side, suggestive of acid reflux leading to bronchial irritation. It was felt this may have been related to the mass in his esophagus demonstrated on earlier CT imaging.

He was commenced on imatinib at 400 mg/day along with a proton pump inhibitor and a prokinetic agent. This resulted in an improvement in his symptoms. A repeat CT scan 3 months later showed stable disease in the esophageal lesions and some regression of the pyloric thickening. He tolerated the first 4 months of imatinib very well but then developed generalized pruritus and a mild grade I erythematous rash over his forearms. This responded to antihistamines and a reduction in his imatinib dose to 300 mg/day. The drug-related rash was associated with eosinophilia, as previously reported in another patient with imatinib-related skin toxicity.⁸ He also manifested grade 1 hypertension.

The patient had a 20-year history of vitiligo and was referred to our dermatology colleagues to assess the possibility of a relationship with his GISTs, as various skin conditions have been described in individuals with germline *KIT* mutations. The dermatologists felt that the vitiligo was unrelated. Although germline *KIT* mutations have been associated with piebaldism, these mutations result in loss of function rather than oncogenic activation of the kinase.⁹ In contrast, generalized lentiginos and skin hyperpigmentation have been described in patients with activating-type germline mutations in *KIT* exon 11.^{10,11} An association with urticaria pigmentosa has also been described.¹²

The patient remains on imatinib treatment at 300 mg and a recent CT scan confirms ongoing stable disease after 19 months.

CONCLUSION

Familial GISTs have been observed in kindreds harboring germline mutations of either *KIT* or *PDGFRA*.¹³ Af-

Imatinib, Multiple GISTs, and kit K642E Mutation—Graham et al

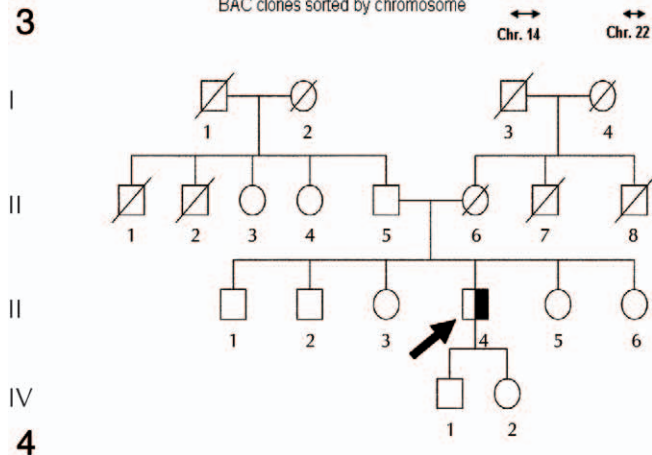
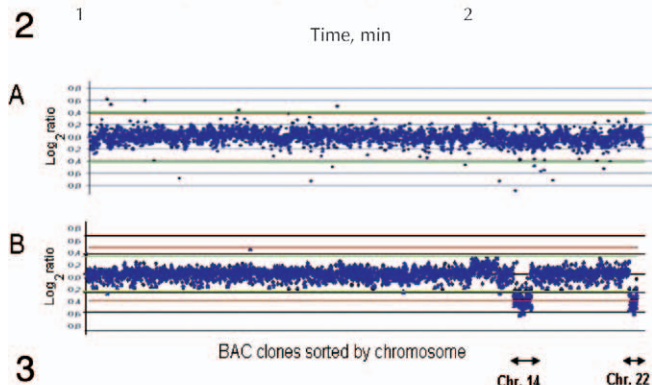
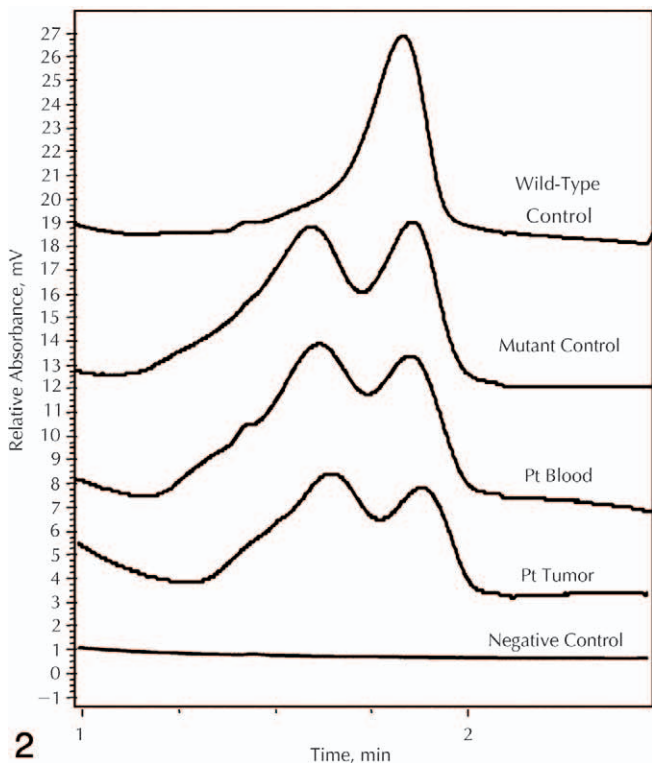


Figure 2. Denaturing high-performance liquid chromatography profiles for KIT exon 13 amplicons. Pt indicates patient.

Figure 3. Array comparative genomic hybridization ratio profiles from the 2 endoscopy specimens from the thickening in the region of the pylorus (A) and the esophagus mass (B) obtained from the patient. The latter reveals losses of chromosomes 14q and 22q (marked with arrows), which are reported as early genomic changes in sporadic gastrointestinal stromal tumors.

Figure 4. Family tree of our patient. Arrow indicates our patient.

affected individuals may develop multiple GISTs, which are generally indolent but may behave aggressively. In addition, patients may have hyperplasia of the interstitial cells of Cajal, which correlates with dysphagia and GI dysmotility, and they may suffer urticaria pigmentosa or other disorders of skin pigmentation.¹⁴ Most reported kindreds have germline mutations in *KIT* exon 11. Families have also been described with heritable mutations in *KIT* exon 8, *KIT* exon 17, *PDGFRA* exon 12, or *PDGFRA* exon 18. To our knowledge, only 1 family with a *KIT* exon 13 mutation has been reported.¹² Two members of this French family shared the same germline mutation of the kinase 1 domain (K642E) that was found in our patient. As in our case, the tumors in the affected mother and son predominated in the small bowel, were uniformly low grade, and were accompanied by interstitial cells of Cajal hyperplasia. These patients did not have vitiligo or other pigmentation abnormalities.

Imatinib has proven effective in controlling the progression of our patient's esophageal GISTs. This is consistent with the observation that the K642E mutant form of Kit is inhibited by imatinib *in vitro*.² It also fits with observations from clinical trials of imatinib for the treatment of advanced, sporadic GISTs, in which a correlation between tumor genotype and imatinib response has been observed. The best responses are seen in tumors with *KIT* exon 11 mutations, whereas those with an exon 9 mutation or no detectable *KIT* or *PDGFRA* mutation are less likely to benefit from therapy.^{2,15} Clinical data are more limited for tumors with exon 13 mutations. Nevertheless, in the initial phase II trial, both patients with a K642E-positive tumor achieved a partial response to imatinib therapy.² In a follow-up phase III trial, 4 of 6 patients with this mutation had a partial response and the remaining 2 had stable disease on imatinib.¹⁵ Thus, sporadic tumors with this genotype are responsive to imatinib treatment. In the case of our patient with a germline mutation, imatinib not only may serve to control his clinically significant esophageal tumors but may prevent the development of additional lesions elsewhere in the GI tract.

We thank Michael Heinrich, MD, and the members of his laboratory for mutational analyses and also Fiona Cowie, MBBS, MSc, MD, MRCP(UK), FRCR; Dawn Currie, RGN, RM, BSc, MSc; Saranya Kakumanu, MBBS, MRCP, FRCR; and Paddy O'Dwyer, MD, FRCS.

References

1. Corless CL, Fletcher JA, Heinrich MC. Biology of gastrointestinal stromal tumors. *J Clin Oncol*. 2004;22:3813–3825.
2. Heinrich MC, Corless CL, Demetri GD, et al. Kinase mutations and imatinib response in patients with metastatic gastrointestinal stromal tumor. *J Clin Oncol*. 2003;21:4342–4349.
3. Verweij J, Casali PG, Zalcberg J, et al. Progression-free survival in gastrointestinal stromal tumours with high-dose imatinib: randomised trial. *Lancet*. 2004;364:1127–1134.
4. Hirota S, Isozaki K, Moriyama Y, et al. Gain-of-function mutations of c-kit in human gastrointestinal stromal tumors. *Science*. 1998;279:577–580.
5. Lasota J, Wozniak A, Sarlomo-Rikala M, et al. Mutations in exons 9 and 13 of *KIT* gene are rare events in gastrointestinal stromal tumors: a study of 200 cases. *Am J Pathol*. 2000;157:1091–1095.
6. Antonescu CR, Sommer G, Saran L, et al. Association of *KIT* exon 9 mutations with nongastric primary site and aggressive behavior: *KIT* mutation analysis and clinical correlates of 120 gastrointestinal stromal tumors. *Clin Cancer Res*. 2003;9:3329–3337.
7. Wozniak A, Sciot R, Guillou L, et al. Array CGH analysis in primary gastrointestinal stromal tumors: cytogenetic profile correlates with anatomic site and tumor aggressiveness, irrespective of mutational status. *Genes Chromosomes Cancer*. 2007;46:261–276.
8. Scott LC, White JD, Reid R, Cowie F. Management of skin toxicity related to the use of imatinib mesylate (STI571, Glivec[®]) for advanced stage gastrointestinal stromal tumours. *Sarcoma*. 2005;9(3–4):157–160.
9. Syrris P, Heathcote K, Carrozzo R, et al. Human piebaldism: six novel mutations of the proto-oncogene *KIT*. *Hum Mutat*. 2002;20:234.

10. Shibusawa Y, Tamura A, Mochiki E, Kamisaka K, Kimura H, Ishikawa O. c-kit mutation in generalized lentiginos associated with gastrointestinal stromal tumor. *Dermatology*. 2004;208:217–220.
11. Maeyama H, Hidaka E, Ota H, et al. Familial gastrointestinal stromal tumor with hyperpigmentation: association with a germline mutation of the c-kit gene. *Gastroenterology*. 2001;120:210–215.
12. Iozaki K, Terris B, Belghiti J, Schiffmann S, Hirota S, Vanderwinden JM. Germline-activating mutation in the kinase domain of KIT gene in familial gastrointestinal stromal tumors. *Am J Pathol*. 2000;157:1581–1585.
13. Li FP, Fletcher JA, Heinrich MC, et al. Familial gastrointestinal stromal tumor syndrome: phenotypic and molecular features in a kindred. *J Clin Oncol*. 2005;23:2735–2743.
14. O’Riain C, Corless CL, Heinrich MC, et al. Gastrointestinal stromal tumors: insights from a new familial GIST kindred with unusual genetic and pathologic features. *Am J Surg Pathol*. 2005;29:1680–1683.
15. Debiec-Rychter M, Sciot R, Le Cesne A, et al. KIT mutations and dose selection for imatinib in patients with advanced gastrointestinal stromal tumours. *Eur J Cancer*. 2006;42:1093–1103.