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## Response to imatinib mesylate of a gastrointestinal stromal tumor with very low expression of KIT

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**Abstract** More than 90% of gastrointestinal stromal tumors (GISTs) express the receptor tyrosine kinase KIT, and activating mutations of the KIT gene are detectable in the vast majority of these tumors. Imatinib mesylate (formerly STI571) is a potent inhibitor of KIT kinase activity and has been proven to be highly active in patients with unresectable or metastatic GIST expressing immunohistochemically detectable KIT protein. Here we report a patient with metastatic GIST who responded well to imatinib mesylate treatment despite the near absence of KIT expression in two different samples

of his tumor. The tumor was morphologically typical for a GIST, stained positively for CD34, and harbored an in-frame deletion (WK 557–558) in *KIT* exon 11 that is common in GISTs. Our experience with this patient suggests that even GISTs with very low levels of KIT expression may respond to imatinib mesylate therapy.

**Keywords** Gastrointestinal stromal tumor · GIST · Imatinib · KIT immunonegativity

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### Introduction

Gastrointestinal stromal tumors (GISTs) are the most common mesenchymal tumors of the gastrointestinal tract. Expression of the KIT (CD117) receptor tyrosine kinase is immunohistochemically detectable in more than 90% of GISTs and represents one of the key markers in the differential diagnosis of GIST (Fletcher et al. 2002). Gain-of-function mutations of KIT are present in the majority of GISTs, even in very small tumors, and are thought to be involved in the early pathogenesis of these tumors (Corless et al. 2002). The importance of KIT activation in the growth of GISTs is supported by recent clinical trial data, which have demonstrated remarkable activity of the tyrosine kinase inhibitor imatinib mesylate (formerly STI571), a potent inhibitor of KIT, in the treatment of patients with advanced GIST (van Oosterom et al. 2001; Demetri et al. 2002a). In the largest of these trials, the partial response rate was 54%, and an additional 41% of patients experienced disease stabilization (Demetri et al. 2002b). Because these trials of imatinib mesylate were limited to patients with GISTs that were unequivocally KIT-positive (as assessed by immunohistochemistry), the activity of this drug against GISTs that express very little or no KIT has not been explored. Here we detail the response of a patient with such a tumor.

## Materials and methods

### Patient history

In December 1999, a 36-year-old patient was diagnosed with a gastric tumor infiltrating the adrenal gland, the pancreas and the jejunum. Histopathological examination of the resected tumor yielded a diagnosis of GIST; immunohistochemistry for KIT (which was not routine at the time) was not performed. In April 2001, several hepatic metastases with a maximum diameter of 7 cm were detected, and a biopsy confirmed metastatic GIST. When the patient presented to our hospital in June 2001, computed tomography (CT) scans showed progression of the hepatic lesions to a maximum diameter of 16 cm. A PET scan highlighted the intrahepatic metastases as well as peritoneal nodules. To assess the patient's eligibility for a clinical trial with imatinib mesylate, a section of the liver metastasis biopsy was immunohistochemically stained for KIT (CD117), but was found to be negative. Conventional chemotherapy was therefore begun in July 2001 with a gemcitabine-containing regimen and his disease stabilized until January 2002. A paraffin-embedded sample of the primary gastric tumor was subsequently obtained for KIT immunohistochemistry and *KIT* gene mutation screening. Treatment with imatinib (400 mg daily) was initiated in February 2002 in an off-label setting and at the time of writing was being continued.

### Immunohistochemistry

Immunohistochemistry was performed on sections of the primary gastric tumor at Oregon Health & Science University (C.C.) using a DAKO automated immunostainer (DAKO Corporation, Carpinteria, Calif.), DAKO polyclonal rabbit antibody (DAKO A4502) at a 1:300 dilution, goat biotinylated anti-rabbit secondary antibody

(Vector Laboratories, Burlingame, Calif.) and a Vectastain Elite kit (Vector Laboratories), as previously described (Corless et al. 2002). Endogenous mast cells served as internal-positive controls.

Immunohistochemistry of the liver biopsy specimen was performed at the University of Essen Medical School (O.D.) using the DAKO polyclonal rabbit antibody (DAKO A4502) at a dilution of 1:50 in phosphate-buffered saline, pH 7. Incubation was done for 2 h at room temperature. Detection was performed using a DAKO ChemMate kit (DAKO K 5005). Link and label were incubated for 30 min at room temperature. Counterstaining was done using hemalum. Endogenous mast cells served as internal-positive controls.

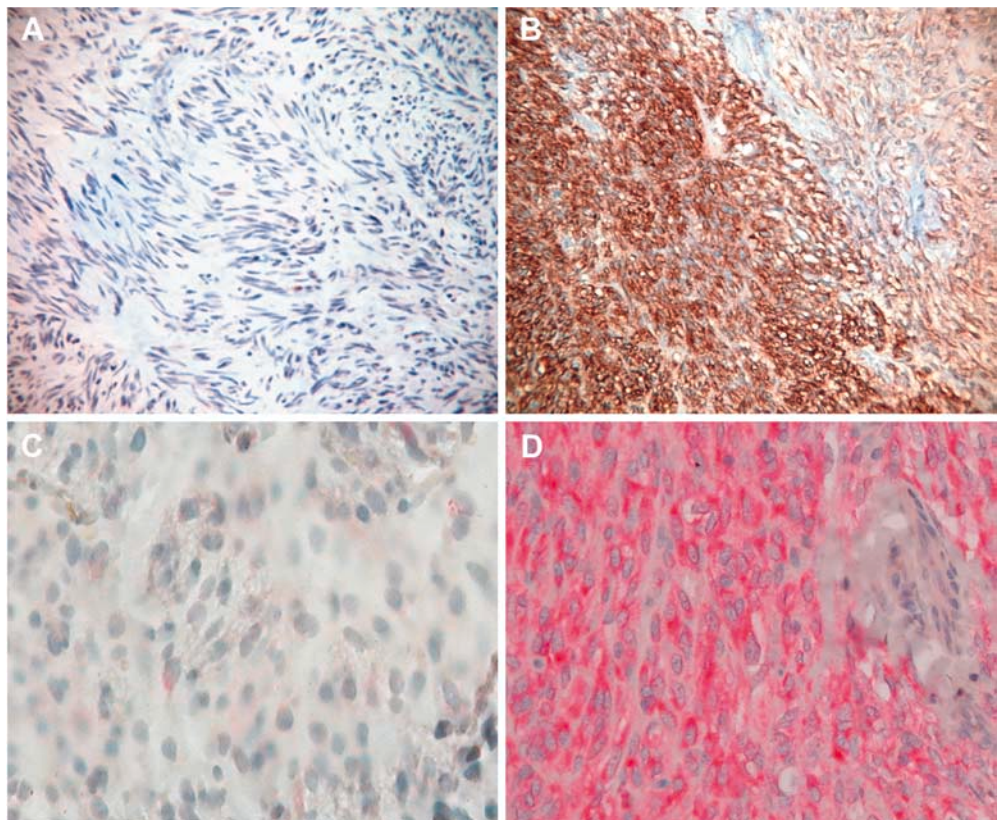
### DNA sequencing

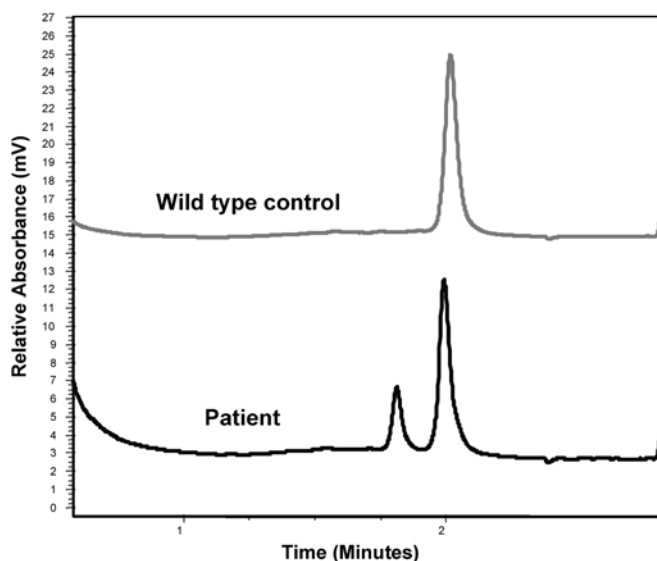
Tumor tissue obtained from the primary gastric tumor specimen was prepared by microdissection and deparaffinization through serial extractions in xylene and ethanol. DNA was purified using a Qiagen minikit (no. 51304; Qiagen, Valencia, Calif.) in accordance with the manufacturer's recommendations. Exons 9 and 11 of the *KIT* gene were amplified by PCR as previously described (Corless et al. 2002), except that the following primer pair was used for exon 11: CTCCTTTGCTGATTGGTTTCGT (forward); TTTCCCAGAAACAGGCTGAG (reverse). PCR amplimers were screened for mutations by denaturing high-pressure liquid chromatography (D-HPLC), and the detected mutation in exon 11 was confirmed by bidirectional DNA sequencing, as previously described (Corless et al. 2002).

### Radiographic imaging

Pretreatment and follow-up evaluations of the patient's tumor lesions were performed with a dual-modality PET/CT tomograph (Biograph, Siemens Medical Solutions, Hoffman Estates, Ill.).

**Fig. 1A–D** Immunohistochemical analysis of the primary gastric stromal tumor (horseradish peroxidase method) showed very weak staining for KIT (CD117) (A) as compared with the staining for CD34 (B). Similarly, a KIT stain of the liver metastasis (alkaline phosphatase method) was nearly negative (C). A section of GIST from another patient shows strong positive staining for KIT by this method (D)





**Fig. 2** HPLC profile of *KIT* exon 11 amplimers from control (wild-type) DNA and DNA extracted from the patient's primary gastric GIST analyzed at a non-denaturing temperature (50°C). The presence of a second peak in the patient's sample is indicative of a length-type (deletion or insertion) mutation, which was confirmed by direct DNA sequence analysis

Standardized  $^{18}\text{F}$ -deoxyglucose (FDG) uptake values (SUV) were calculated for the lesions with the most intense uptake at baseline. Tumor measurements were done using the sum of the products of two perpendicular diameters of each measurable liver metastasis.

## Results

Initial histopathologic assessment of the primary gastric tumor revealed a spindle cell neoplasm consistent with a GIST. By immunohistochemistry, the tumor cells were positive for CD34 and negative for desmin and S-100. The follow-up liver metastasis biopsy showed similar morphology, and the tumor cells were again positive for CD34 and negative for S-100. The liver specimen was subsequently stained for KIT with a CD117 antibody, but failed to show a detectable expression (Fig. 1C). The primary gastric tumor was also stained with the CD117 antibody and showed only very faint, focal staining (Fig. 1A), which was insufficient to permit enrollment of the patient into an ongoing trial of imatinib mesylate.

By D-HPLC screening, DNA obtained from the primary gastric tumor was wild-type for *KIT* exon 9 but showed clear evidence of an exon 11 deletion/insertion-type mutation (Fig. 2). Subsequent DNA sequence analysis confirmed an in-frame deletion of six nucleotides resulting in deletion of amino acids WK 557–558 in exon 11.

The patient began imatinib mesylate therapy in February 2002. Serial PET scans and/or PET-CT scans showed a 34% reduction (minor response) in the cross-sectional area of all measurable tumor after 2 months of imatinib therapy (initial size 383 cm<sup>2</sup>, 2 months 252 cm<sup>2</sup>). The liver metastasis that displayed the most

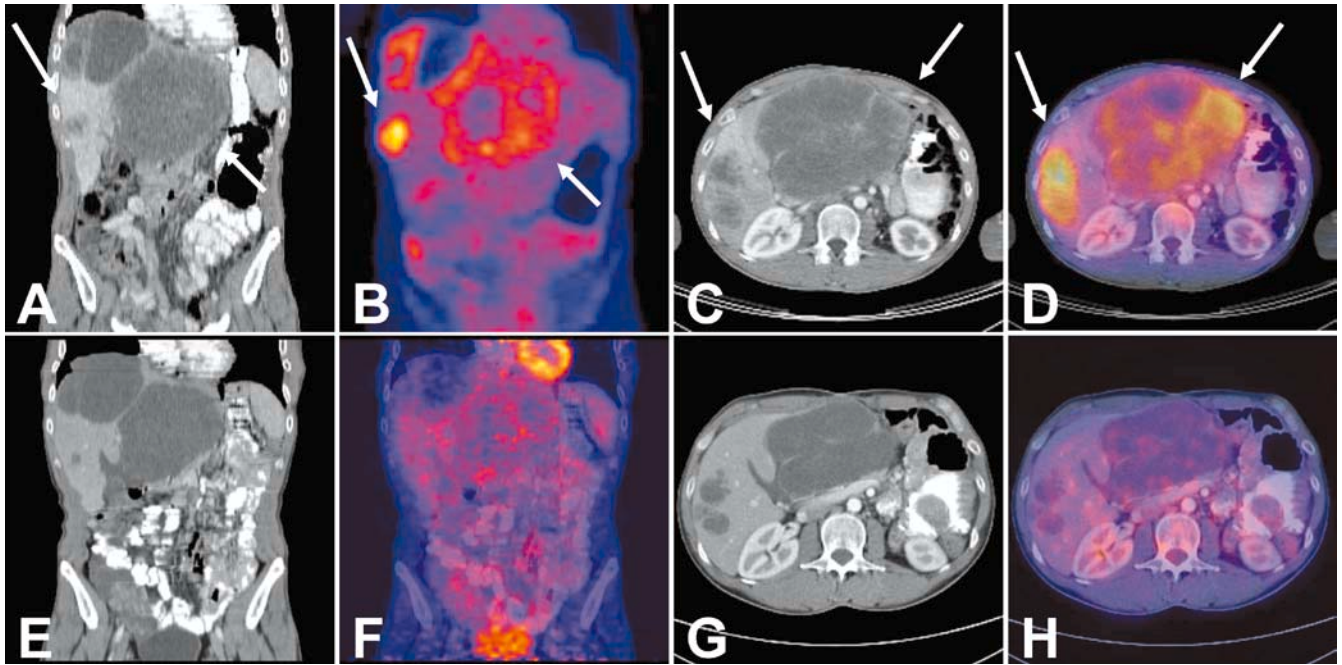
intense  $^{18}\text{F}$ FDG uptake at baseline (SUV of 4.1) showed a significant reduction of SUV within the first 10 days and was negative for  $^{18}\text{F}$ FDG uptake on a scan performed after 2 months of treatment. In that scan most of the liver metastases showed less uptake than the normal liver tissue, indicating either complete metabolic inhibition or necrosis (Fig. 3). At follow-up in July 2002 the patient was clinically doing well under continued treatment with imatinib, without any measurable side effects.

## Discussion

The discovery that oncogenic activation of KIT is common in GISTs has, in less than 4 years, led to a marked improvement in both the diagnosis and treatment of this tumor. Expression of KIT is found in the vast majority of GISTs and is regarded as the single best immunophenotypic marker for these tumors (Fletcher et al. 2002). However, despite the use of anti-CD117 (KIT) antibodies that have been validated by internal and external controls, as well as refined immunohistochemical procedures including antigen retrieval, a small proportion of GISTs (about 5%) either show very faint expression of KIT or none at all. Since inhibition of KIT tyrosine kinase activity is believed to be the major antitumoral effect of imatinib mesylate in GISTs, immunopositivity for KIT has been a precondition for treatment with this drug, both within and (following the recent registration of imatinib mesylate for GIST) outside clinical trials. For this reason, very little is known of the efficacy of imatinib mesylate in patients with GISTs that show little or no expression of KIT.

In the case presented here, the tumor was negative for KIT expression in the liver metastasis and showed only focal, very weak staining for KIT in the gastric primary, yet the patient had a clear radiologic and clinical response to imatinib mesylate therapy. There are several possible explanations for this apparent discrepancy between the KIT immunostaining results and the response of the tumor to treatment. For example, problems with specimen handling and/or fixation may have interfered with the preservation of KIT epitopes in the tumor. This is unlikely, however, because two independent samples of the tumor had little or no staining for KIT; one of these samples was tested in a second laboratory and the result was confirmed (there was insufficient material to re-test the other sample). It is also unlikely that mutation-related alterations in KIT epitopes accounted for the low level of KIT staining, because the tumor retained a wild-type allele. Moreover, the exon 11 deletion that was found in the tumor has previously been reported in GISTs that are strongly KIT-positive (Rubin et al. 2001).

Another potential explanation is that another imatinib mesylate-sensitive kinase accounted for the observed response in our patient. For example, PDGF receptors alpha and beta are both inhibited by imatinib mesylate in vitro (Capdeville et al. 2002). We favor the view that KIT was the target in our patient's tumor, despite its



**Fig. 3A–H** Coronal (A, B, E, F) and axial (C, D, G, H) PET/CT scans of the 36-year-old patient with hepatic metastases from a GIST. Before treatment with imatinib (A–D), multiple metastatic lesions were present in the liver (arrows) with heterogeneous  $^{18}\text{F}$ FDG uptake especially in the larger metastases. After 2 months of treatment with imatinib (E–H), the metastases had decreased in size, and appeared more hypodense on CT.  $^{18}\text{F}$ FDG-uptake diminished in all tumor nodules to a level below that of normal liver tissue

apparently very low level of expression, for the following reasons. First, mutant KIT isoforms with exon 11 deletions similar to that found in our patient's tumor have constitutive kinase activity when expressed in cultured cells (Hirota et al. 1998; Heinrich et al. 2001), but are still sensitive to imatinib mesylate (Heinrich et al. 2001). Second, in the CSTIB2222 phase II treatment trial of imatinib mesylate therapy for advanced GIST, the highest probability of response was observed among patients whose tumor harbored a *KIT* exon 11 mutation (Heinrich et al. 2002). Finally, it is known that the degree of KIT activation (phosphorylation) varies in extracts of typical GISTs and is not necessarily proportional to the total KIT protein present (Rubin et al. 2001). A relatively small amount of activated KIT may be all that is necessary to sustain GIST cells, such that total KIT may be at or below the threshold of immunohistochemical detection in some tumors.

In conclusion, this case demonstrates that the near absence of KIT in a GIST does not preclude a clinical response to imatinib mesylate. It is interesting to note that in non-GIST tumors, the opposite appears to be true. Despite positive immunostaining for KIT there have been no clinical responses to imatinib mesylate among patients with small-cell lung carcinoma (Johnson et al. 2002) or other KIT-positive malignancies (Apperley 2002). For GISTs that are positive for KIT expression, the *KIT* gene mutation status is the single best

predictor of imatinib mesylate response (Heinrich et al. 2002). Further studies of KIT-low and KIT-negative GISTs are needed, but in the meantime molecular screening of such tumors for *KIT* gene mutations can serve to confirm the pathologic diagnosis and may provide preliminary prognostic information on the likelihood of a drug response.

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