

Rapid Relapse after Resection of a Sunitinib-resistant Gastrointestinal Stromal Tumor Harboring a Secondary Mutation in Exon 13 of the *c-KIT* Gene

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Abstract. We describe a case with rapid relapse after resection of a sunitinib-resistant gastrointestinal stromal tumor (GIST). Liver metastases and foci of left retroperitoneal recurrence developed during adjuvant imatinib treatment. The tumors did not shrink after sunitinib treatment, and hepatectomy and retroperitoneal tumorectomy were performed. Histological examination showed a Ki67 labeling index of over 50% in viable tumor cells. Genomic analysis revealed mutations in exons 11 and 13 of the *c-KIT* gene. Computed-tomographic scan revealed retroperitoneal recurrence at the surgical site five weeks post-operatively. In this case, high proliferative activity of the recurrent foci was associated with resistance to sunitinib and rapid recurrence during the perioperative withdrawal of sunitinib. It is important to consider the possibility of an exon 13 mutation with an aggressive phenotype when treating sunitinib-resistant GISTs. Surgical intervention for sunitinib-resistant GISTs should be carefully considered if R0 resection is not possible.

Gastrointestinal stromal tumor (GIST) is the most common mesenchymal tumor of the gastrointestinal tract. The majority of GISTs have a gain-of-function mutation of the *c-KIT* or platelet-derived growth factor receptor (PDGFR) alpha gene in the interstitial cells of Cajal (1-3). Most of the *c-KIT* mutations are located in exon 11, which encodes for the KIT receptor juxtamembrane domain, while others are located in exons 9, 13, or 17 (1, 4-6). Although surgery is the most effective treatment for resectable primary GISTs, post-operative recurrence or metastasis are difficult to cure with surgery alone.

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The orally bioactive tyrosine kinase inhibitor imatinib mesylate (Gleevec, Gleevec; Novartis, Basel, Switzerland) that inhibits KIT and PDGFR has been used to treat patients with unresectable or metastatic GISTs (7-9). Although imatinib is thought to be the most effective agent for treating GISTs, secondary resistance often arises during therapy due to secondary mutations in exons 13 or 17 of the *c-KIT* gene (10-12). Sunitinib malate (Sutent, Pfizer Inc., New York, NY, USA) is a tyrosine kinase inhibitor with efficacy in imatinib-resistant GISTs and has been used as a second-line therapy for recurrent or metastatic GISTs (13). The effectiveness of sunitinib depends on the location of the primary and secondary mutations. Sunitinib is reported to be effective for GIST with primary mutation in exon 9 or with wild-type *c-KIT* genotype. In cases of imatinib-resistant GISTs with primary mutation in exon 11 of the *c-KIT* gene, sunitinib is more effective for tumors with secondary mutations in exon 13 or 14 than in exon 17 of the *c-KIT* gene (14, 15). Since no other agents have been approved for imatinib-resistant GISTs, surgery may be considered if the sunitinib-resistant tumor seems resectable. However, the feasibility of such surgery for imatinib- and sunitinib-resistant GISTs still needs to be evaluated.

We report a case showing rapid relapse during post-operative withdrawal of sunitinib after resection of a sunitinib-resistant recurrent GIST, harboring a secondary mutation in exon 13 of the *c-KIT* gene.

Case Report

A 59-year-old man was referred to our hospital for treatment of recurrent GIST. He had undergone proximal gastrectomy after neoadjuvant imatinib therapy for gastric GIST with perigastric invasion. Nine months after the initial surgery, imatinib had been introduced for the locally-recurrent GIST. The patient had undergone three cytoreductive operations consisting of distal pancreatectomy, left adrenalectomy, local tumorectomy, left nephrectomy, and partial diaphragmectomy. When he was admitted to our hospital,

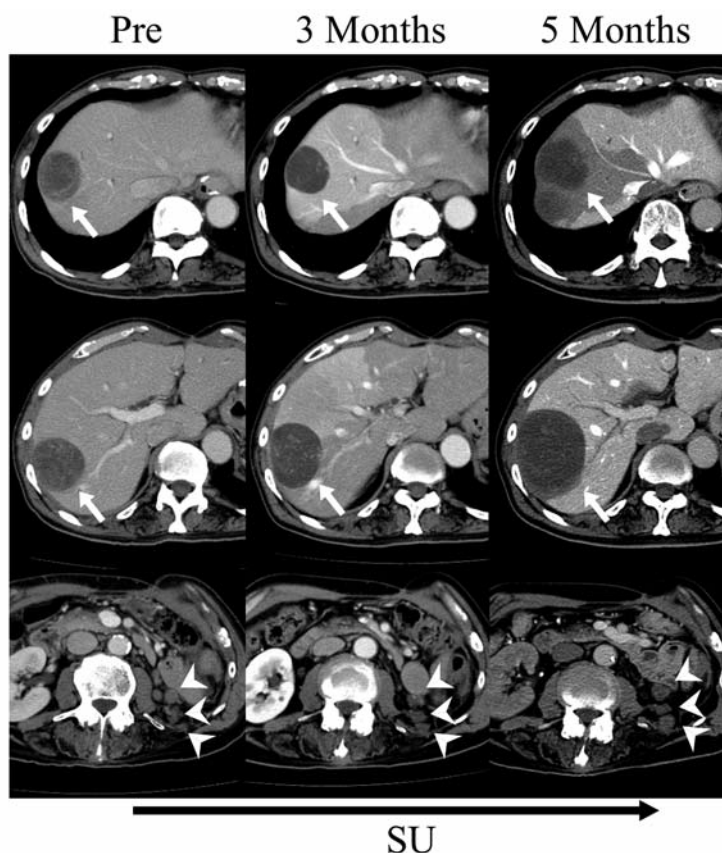


Figure 1. Computed-tomographic (CT) scans of the abdomen before (left panel) and after (middle and right panels) sunitinib (SU) treatment. There are multiple liver metastases in the right lobe (top and middle panels, arrows), one of which was sensitive (top panel) and the other of which was resistant (middle panel) to sunitinib. Recurrent tumors were seen in the retroperitoneal space (bottom panel, arrowheads).

four years after the initial gastrectomy and one month after the last cytoreductive surgery, computed-tomographic (CT) scan did not show any residual tumor in the abdominal cavity. The patient continued imatinib treatment at 400 mg/day. At four months after the last cytoreductive surgery, the CT scan revealed metastatic GISTs in the right lobe of the liver and local recurrence in the left retroperitoneal space (Figure 1, left panels). Imatinib was replaced by sunitinib, initially at 50 mg/day for four weeks with 2-week intervals between treatments, then was reduced to 37.5 mg/day from the second course because of general fatigue. The retroperitoneal tumors and one of the two liver metastases were controlled by sunitinib (Figure 1, top and bottom panels), but the other liver metastasis continued to grow (Figure 1, middle panel). Overall, the sum of the diameters of all tumors increased by 13% after four courses of sunitinib treatment, which was evaluated as disease progression according to the criteria of Choi *et al.* for response assessment (16). As there are no other agents approved for the treatment of GIST in Japan, surgical resection was considered. One month after the temporary

withdrawal of sunitinib, right hepatectomy and retroperitoneal tumorectomy were performed to reduce the volume of sunitinib-resistant tumors (Figure 2A).

Histological examination showed degenerative changes in parts of the tumors, presumably due to sunitinib administration. In both metastatic liver tumors and retroperitoneal tumors, including the tumors controlled by sunitinib, viable tumor cells positive for KIT, discovered on GIST-1 (DOG-1) and cluster of differentiation 34 (CD34), were observed (Figure 2B). The Ki67 labeling index in viable tumor cells was over 50%. Genomic DNA was extracted from each of two metastatic liver tumors and from three retroperitoneal tumors. Mutation analysis of the *c-KIT* genes was performed, as previously described (17). The primary mutation in all tumors consisted of a three-base deletion at codon position 560 (V560del) with a K558N point mutation in exon 11 of the *c-KIT* gene, while no tumors exhibited mutations in exons 9 or 17 of the *c-KIT* gene. Secondary mutations were additionally observed in exon 13 of the *c-KIT* gene (V654A) in all of the liver metastases and retroperitoneal tumors (Figure 2C).

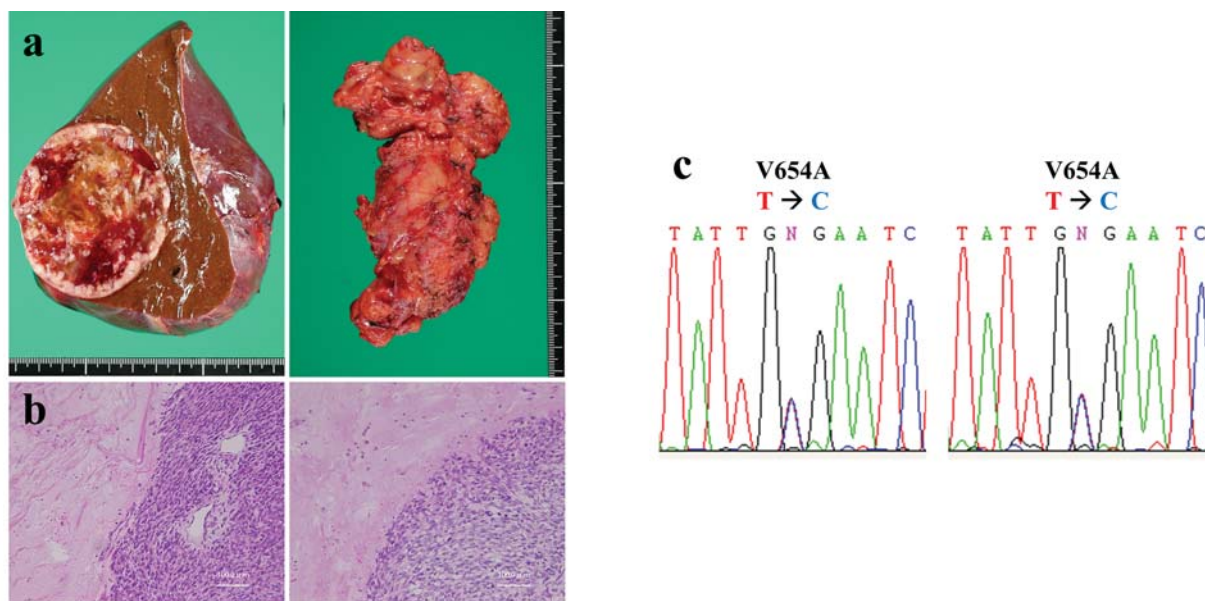


Figure 2. A: Macroscopic findings of the resected liver (left panel) and retroperitoneal gastrointestinal stromal tumors (GISTs) (right panel). B: Histological findings of the resected GISTs. Hematoxylin and eosin staining showed viable GIST cells as well as tumor cells with degenerative changes both in the liver (left panel) and retroperitoneum (right panel). C: DNA sequencing of the *c-KIT* gene at exon 13. Both liver (left panel) and retroperitoneal tumors (right panel) exhibited a T to C point mutation which resulted in a V654A amino acid alteration.

The patient was discharged four weeks after surgery and was planning to restart sunitinib treatment. However, he experienced left upper abdominal pain shortly after discharge and returned to our hospital. A CT scan revealed retroperitoneal recurrence at the previous surgical site (Figure 3, left middle panel). Retrospectively, one of the recurrent tumors was visible on the CT scan performed two weeks after his last surgery (Figure 3, left panel), suggesting that the recurrent tumors had been overlooked intraoperatively. Non-steroidal anti-inflammatory drugs (NSAIDs) were administered, and subsequently sunitinib was reintroduced at 50 mg/day to control the recurrent tumors. His severe pain disappeared soon after starting this treatment. A repeat CT scan three weeks after the reintroduction of sunitinib showed cystic changes in the recurrent tumors (Figure 3, right middle panel). Later, the dose of sunitinib was reduced to 25 mg/day because of severe general fatigue. One of the tumors subsequently started to regrow, probably due to an insufficient dose of sunitinib (Figure 3, right panel). The patient has been alive for six months after the surgery, and is receiving sunitinib and opioid.

Discussion

Post-operative recurrence or metastasis has been observed in 40-90% of patients with primary GISTs (18, 19). Although imatinib is regarded as the most effective treatment for patients with metastatic or unresectable GISTs, acquired *c-KIT* mutations have been reported to cause secondary

resistance to imatinib (10-12). Sunitinib is known to inhibit the KIT receptor, as well as PDGFRs, vascular endothelial growth factor receptors, and the receptor encoded by the *RET* proto-oncogene, and has been used as second-line therapy for imatinib-resistant GIST (13, 20). However, the response rate to sunitinib treatment is low, particularly in patients with GIST harboring a primary *c-KIT* exon 11 mutation (partial response rate 5%). Furthermore, long-term administration of sunitinib is often difficult because of its adverse effects (13). Secondary mutations in the activation loop of *c-KIT* or *PDGFRA* have also been reported to cause resistance to sunitinib (21, 22). Surgical management following second-line treatment has, therefore, recently been discussed.

We previously reported that metastatic liver foci had a higher Ki67 labeling index than primary gastric GISTs (17). Our report also described a case in which a small part of the primary gastric GIST harboring loss of heterozygosity of the *c-KIT* gene metastasized to the liver (17). These findings indicate that malignant clones with highly proliferative activity could arise or be selected in the process of metastasis or recurrence, and perhaps during treatment with imatinib or sunitinib. In the present case, the resected GISTs were highly malignant, with a high Ki67 labeling index of over 50%. As these recurrent foci had a secondary mutation in exon 13 of the *c-KIT* gene, sunitinib was expected to be effective (15). However, the tumors did not shrink after administration of sunitinib. A plausible explanation for the resistance to sunitinib is that the high proliferative activity might have overcome the

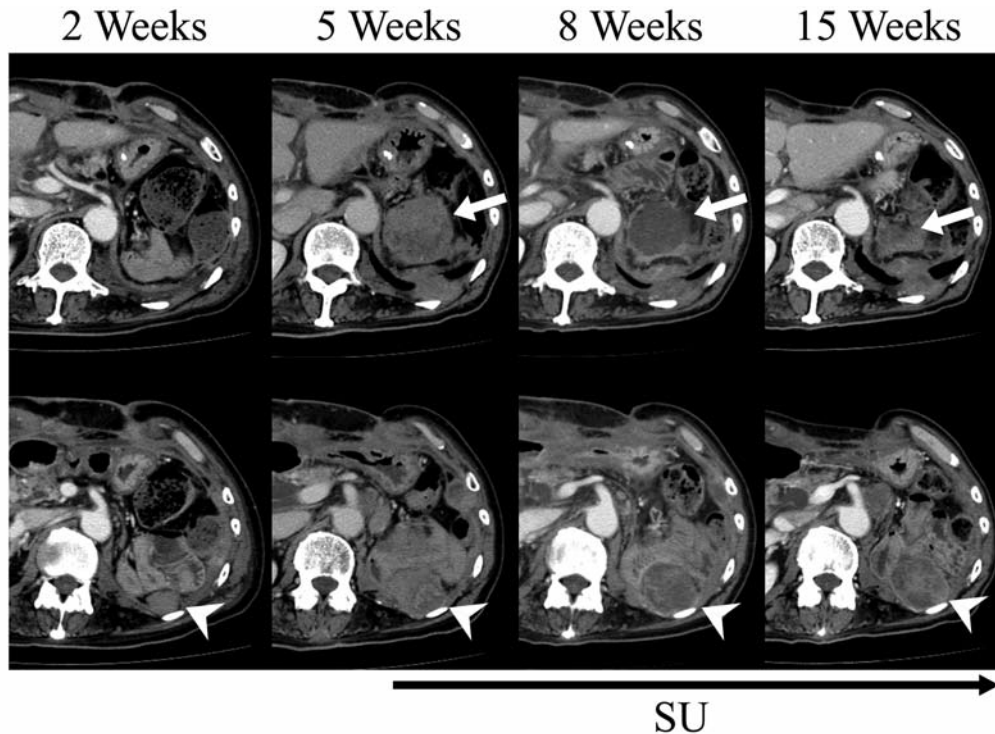


Figure 3. Post-operative computed-tomographic (CT) scans of the abdomen before (left two panels) and after (right two panels) sunitinib (SU) treatment. CT images show recurrent tumors in the retroperitoneal space, one of which was sensitive (upper panel, arrows) and the other of which was resistant (lower panel, arrowheads) to sunitinib.

antitumor effect of sunitinib at 37.5 mg/day. It seems that intraoperatively overlooked tumors relapsed rapidly during the temporary withdrawal of sunitinib. It is therefore necessary to consider the possibility of an exon 13 mutation with an aggressive phenotype when treating sunitinib-resistant GIST. In contrast, the effect of perioperative sunitinib withdrawal may be minimal in the case of a sunitinib-resistant GIST harboring a secondary mutation in exon 17, since such tumors are generally refractory to sunitinib (15).

Cytoreductive surgery has been reported to be feasible in patients with metastatic GIST on imatinib therapy, when their disease is stable or responsive to imatinib (23-25). Recent retrospective studies demonstrated the feasibility of cytoreductive surgery in patients with metastatic GIST treated with sunitinib (26). However, the feasibility of surgical intervention for metastatic or recurrent GISTs may differ between patients treated with sunitinib and with imatinib, even at best response. Considering the high proliferative activity of the sunitinib-resistant tumor with an exon 13 mutation in the present case, special care was needed to avoid incomplete resection. When considering surgery for the treatment of sunitinib-resistant GISTs, immediate pre-operative CT scan should be performed to accurately re-evaluate tumor volume and location before surgery. Randomized controlled studies or prospective cohort

studies are needed to clarify the effects of cytoreductive surgery on the survival of patients receiving imatinib or sunitinib and develop recurrent GISTs.

Surgical intervention may not be feasible in cases of sunitinib-resistant GISTs, and should be carefully considered if R0 resection is not possible.

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