Mutational spectrum and therapy response of metastasized GIST in Central Switzerland - a population-based study

Rössle, M; Hirschmann, A; Diebold, J
Mutational spectrum and therapy response of metastasized GIST in Central Switzerland - a population-based study

Abstract

Our data support previous observations, that PDGFRα mutations play no important role in metastasized GISTs. The influence of Imatinib and Sunitinib therapy in metastasized GISTs with wild type genotype and c-kit exon 9 mutations needs further investigation.
Original article:

„Mutational Spectrum and Therapy Response of metastasized GIST in Central Switzerland - a population-based study“

Authors: Matthias Rössle¹,², Astrid Hirschmann¹, and Joachim Diebold¹

¹ Institute of Pathology, Luzerner Kantonsspital, Lucerne, Switzerland
² Institute of Clinical Pathology, University Hospital Zurich, Zurich, Switzerland

Corresponding author:

Matthias Rössle, MD
Institute of Clinical Pathology
University Hospital Zurich
Schmelzbergstrasse 12
8091 Zurich
Switzerland

Phone: +41 (0)44 255 96 86
Fax:  +41 (0)44 255 44 16
E-Mail: matthias.roessle@usz.ch
Abstract

Background: Until now, no population-based studies investigated the mutational status of primary GIST (PT) and corresponding metastases and correlated these data with response to Imatinib or Sunitinib therapy.

Patients and methods: In a retrospective observation study, all metastatic GISTs of the last 15 years of our institution were investigated for mutations in c-kit and in PDGFRα gene in each PT and corresponding metastasis. Correlation with clinical outcome and response to Imatinib or Sunitinib therapy was performed.

Results: In 13 PT c-kit mutations in exon 9 (3), exon 11 (7) and exon 13 (1), 2 wild type genotypes, and no PDGFRα mutation were detected. In 3 metastases a switch from heterozygosity to homozygosity and one additional exon 13 mutation were observed. All 10 persons with available follow-up received Imatinib as first-line chemotherapy. 5 of them (3 exon 9 mutations, 1 wild type, 1 additional exon 13 mutation) stopped Imatinib due to tumour progression. In 3 cases, Sunitinib as second-line chemotherapy was ended due to same reasons.

Conclusions: Our data support previous observations, that PDGFRα mutations play no important role in metastasized GISTs. The influence of Imatinib and Sunitinib therapy in metastasized GISTs with wild type genotype and c-kit exon 9 mutations needs further investigations.

Keywords: Gastrointestinal stromal tumour; GIST; mutations; imatinib; sunitinib; c-kit; PDGFRA
Introduction

Gastrointestinal stromal tumours (GIST) are the most common mesenchymal neoplasms of the gastrointestinal tract with an incidence of approximately 0.65 – 1.5 cases per 100’000 inhabitants (1-6). The most frequently affected sites are stomach and small intestine, but GISTs may also develop in any other part of the GI tract, peritoneum and retroperitoneum. The finding, that approximately 95% of these tumours express CD117 (c-kit tyrosine kinase receptor) (7-8), has dramatically increased the diagnostic accuracy of these tumours. On a molecular level most GISTs harbour hyperactivating somatic mutations either in c-kit or PDGFRα genes, occurring mostly in the juxtamembrane domains or in the extracellular region (9-10). This is the rationale for the treatment option with tyrosine kinase inhibitors like Imatinib (Gleevec®, Novartis, Switzerland) or Sunitinib (Sutent®, Pfizer, USA). The localisation of these mutations determines the efficiency of these substances, to inhibit the stimulatory effect of c-kit and PDGFRα proteins on cell growth (11-12).

In the last few years some unselected population-based studies from various European regions have been published, in which the mutational status of primary GISTs was investigated (2-6). In one of these studies, the genomic status of metastases was reported (2), but no correlation to the genomic status of corresponding primary tumour on the one hand and to the response after treatment with Imatinib and Sunitinib on the other hand was given.

The aim of our study was to evaluate the status of c-kit and PDGFRα mutations in metastasized GISTs of an unselected population in Central Switzerland, to describe the mutational spectrum of primary and corresponding metastatic tumours. In addition we correlated these results with the response to treatment with tyrosine kinase inhibitors Imatinib and Sunitinib.
**Materials and methods**

*Ethical considerations*

This was a retrospective study with no study-driven clinical intervention. No material was sent to external institutions. Approval of the local ethics committee was obtained (reference number EK 915).

*Retrieval of cases*

The record files of the Institute of Pathology of the Cantonal Hospital Lucerne were searched for all patients with GIST diagnosed in the period from 1995 – 2009. The search included all sites of the gastrointestinal tract as well as the intra-abdominal, mesenteric, omental, pelvic and retroperitoneal regions. In addition to GIST the search included all benign and malignant mesenchymal neoplasm as well as tumour-like conditions including fibromatosis, desmoids and inflammatory pseudotumour. The pathology reports and histological slides of patients with metastasized disease were re-evaluated (M.R.) and, in questionable cases, GIST diagnosis was confirmed by CD117 immunohistochemistry (see below).

Since only metastasized tumours were investigated no risk stratification of malignancy or pathological staging of the GIST was performed.

Clinical data including the treatment procedures (e.g. chemotherapy with Imatinib or Sunitinib, radiation, other therapies) after diagnosis of the primary and metastatic tumour, the response to therapy, and the state of disease at the end of the observation period were obtained from the treating oncologists.

*Immunohistochemical and molecular analysis*

Tissue probes of primary and metastatic tumours were fixed in 4% buffered formalin, embedded in paraffin and, after histopathological diagnosis, archived at the Institute of Pathology, Cantonal Hospital, Lucerne, Switzerland. All formalin- fixed paraffin-embedded
tumour blocks were reviewed (M.R.) for quality and tumour content, and a single representative tumour block from primary tumour and every available metastases of each case was selected for immunohistochemical and molecular analyses.

Immunohistochemical reactions were performed with a Benchmark automatic immunostaining device (Ventana Medical System, Tucson, AZ, USA) according to the manufacturer’s instructions. Three-µm thick tissue sections were incubated after heat induced antigen retrieval with antibodies against CD117 [clone A4502, DakoCytomation (Glostrup, Denmark), 1:50 dilution]. Positive and negative controls were included in each slide run. For molecular analysis, micro-dissection of selected areas of the representative tumour tissue blocks was performed.

For molecular analysis of \textit{c-kit} and \textit{PDGFRα} gene, DNA was extracted using the QIAamp DNA FFPE Tissue Kit (Qiagen, Hilden, Germany). PCR for \textit{c-kit} exons 8, 9, 11-18 and \textit{PDGFRα} exons 10, 12, 14, 18 was performed in 20 µl volumes using 50 ng of template DNA, 0.2 U HotstarTaq polymerase (Qiagen, Hilden, Germany), 2.5 mM MgCl2, 0.2 mM of dNTP (peqlab, Erlangen, Germany) and 400 nM of each primer (see Table 1). The cycling conditions were as follows: one cycle of 95°C 15 min; 50 cycles of 95°C 30 sec, 60°C 30 sec and 72°C 45 sec; one cycle of 72°C 5 min. The PCR products were purified with ExoSapIT (usb, Cleveland, Ohio). The sequencing reaction was carried out with BigDye Terminator Mix v 1.1 (Applied Biosystems, Foster City, CA) according to the manufacture’s protocol. After cleaning the products with DyeEx (Qiagen) the probes were analysed using the abi 3130 sequencing system (Applied Biosystems) and the Chromas software. Each sequencing reaction was performed at least twice starting from independent PCR reactions. Detected mutations were confirmed in the sequence as sense and antisense strands. Our results were compared with normal sequence available on gene ID 3815 (KIT) and gene ID 5156 (PDGFRα).
Results

Of the 87 GIST (crude incidence rate: 0.97 per 100’000 inhabitants of Central Switzerland per year), diagnosed from 1995 to 2009 in our institution, 13 patients (14.9%) had metastatic disease, which equals a crude incidence rate of 0.14 per 100’000 inhabitants of Central Switzerland per year. All primary and all metastatic tumours showed a strong CD117 expression. In three of the 13 metastatic cases, the metastases were diagnosed at the same time as the primary tumour.

Patients and tumour characteristics are summarized in Table 2. The patients collective included 8 women (61.5%) and 5 men (38.5%), with a median age of 68 years (range 31 – 80) at the first GIST diagnosis. Primary tumours (PT) arose in stomach (6 cases; 46.2%), small intestine (4 cases; 30.8%), and 1 case each in pancreas, gastrocolic ligament, and rectovaginal septum. Metastases were found in the liver (6 cases, 33.3%), peritoneum, not otherwise specified (4 cases, 22.2%), omentum (3 cases, 16.7%), and retroperitoneum, mesenterium, rectovaginal septum, lymph nodes and bone (1 case each, 5.6%). 5 of the 13 patients (38.5%) had metastases in different organs (see Table 2). In 2 other patients with peritoneal metastases (patient A and C), tissue samples of 2 different, not otherwise specified peritoneal tumour sites were examined.

Histologically 10 of 13 PT showed a spindle cell type (76.9%), 2 PT a mixed spindle cell / epitheloid type (15.4%), and one tumour of the small intestine an epitheloid type (7.7%).

The median of the greatest diameter of PT was 9.5 cm (range 4 – 25 cm). Only 1 PT was less than 5 cm (7.7%), 6 PT ranged between 5 and 10 cm (46.2%), and 5 PT were greater than 10 cm (38.5%). For one PT, confirmed by biopsy, no reliable tumour size was available.

The mitotic count (per 50 high power fields (hpf)) ranged from 1 to 100 with a median of 8. The highest counts were observed in PT of the stomach (up to 100 mitoses per 50 hpf) and the small intestine (up to 40 mitoses per 50 hpf), whereas the extragastrointestinal PT showed a low mitotic count (range 1 to 6 mitoses per 50 hpf).
Molecular analyses could be performed in all PT and all above described corresponding metastases (see also Table 2). In patients with more than one metastatic site, the different metastases of the respective patient showed always the same genetic alterations. In 2 cases with PT in the stomach (patient C and H; 15.4%) no genetic alterations in the c-kit or in the PDGFR-α genes could be detected neither in PT nor in metastases. The other 11 cases (84.6%) showed changes in the c-kit gene, but none in the PDGFR-α gene. C-kit exon 9 alterations could be detected in 2 PT of the small intestine and in the one PT of the pancreas. In all 3 cases the same well known duplication of codon 502-503 (p.502_503dup AY) was found in PT as well as in metastatic tumours in heterozygous form. Both PT and metastasis of the GIST of the septum rectovaginale showed a heterozygous exon 13 point mutation (p.K642E). The vast majority of PT (7 patients with PT in stomach, small intestine, and lig. gastrocolicum) showed heterozygous exon 11 alterations: One point mutation (p.W557G; case I), 5 deletions (p.W557_K558del in case D and J, p.K558_E562del in case G, p.E554_N567del in case K, and p.V559_G565del in case M), and 1 duplication (p.P577_R588dup12 in case L). In two of the cases (Patients J and L) metastatic tumours showed homozygosity for the same alteration as in the PT. In the metastatic tumour of patient D, two changes compared to the PT could be seen: Homozygosity of the exon 11 deletion and an additional heterozygous point mutation in exon 13 (p.V654A). Of the above mentioned genetic alterations, all but two have been previously described. The two exceptions are the exon 11 deletion (p.E554_N567del) of patient K and the exon 11 duplication (p.P577_R588dup12) of patient L.

Full clinical data were available in 10 of 13 cases. In 2 patients metastatic GIST was initially diagnosed within the last 3 months of the study period, so no clinical data of the disease progression exist. One patient did not return for a follow-up after diagnosis of metastatic disease (25 months after PT diagnosis). The median observation period was 47 months (range 1 – 180). The progression free survival after diagnosis of PT ranged from 6 to 114 months.
At the end of the follow-up period (January 15<sup>th</sup> 2010) 4 patients had died of disease, 3 patients had a stable disease, and 3 patients showed no evidence of disease.

The range of overall survival after diagnosis of PT was 6 to 180 months (median 47 months). All 10 patients with appropriate clinical data received Imatinib as first-line tyrosine kinase inhibitor therapy during the observation period. Among these, the therapeutic regime varied considerably (see also Table 2): One patient (case C) received Imatinib as preoperative therapy (400mg/day for 1 month) of PT and then again after resection of metastatic tumour (800 mg/day). Another patient (case J) received Imatinib as postoperative therapy (400 mg/day) of PT for 12 months and again after relapse of disease (400mg/day). The other 8 patients received Imatinib after the radiological and/or histological diagnosis of metastases or local recurrency with an initial dose of 400 mg/day. So in 4 patients (C, D, H; J) the tissue probes of metastases used in this study were taken after the start of imatinib therapy and in six patients (A, B, E, F, G, I) before.

In 1 case (patient A) a dose elevation to 600mg/day was performed after 38 months for another 25 months. In 2 other patients (E and F) the imatinib dosage was increased to 800 mg/day after 20 month for 1 month and after 38 months for 3 months, respectively. The therapy was switched to Sunitinib in 5 patients (37.5 mg in 4 cases, 50 mg in one case, see also Table 2) due to tumour progression (3 patients), intolerance (1 patient) or a combination of both (1 patient) during imatinib application. The Sunitinib therapy in 3 of these 5 patients was stopped due to the same reasons (2 patients with intolerance, 1 patient with tumour progression). In patient E, Imatinib therapy was replaced by radiotherapy (30 Gy for 2 weeks). In two patients, Imatinib therapy was terminated without substitution because of tumour progression (patient B) and side effects (patient H), respectively.

In all 3 patients with alterations of the <i>c-kit</i> exon 9 a tumour progression was observed during Imatinib therapy, which was therefore stopped. Whereas 1 of the 2 remaining patients, in which the Imatinib therapy had to be ended due to tumour progression, showed no detectable
genetic alteration, the other one showed more genetic alterations in the metastasis compared
to the PT: The heterozygous deletion in exon 11 of the \textit{c-kit} gene of the PT changed to a
homozygous deletion in metastasis and an additional point mutation in exon 13 of the \textit{c-kit}
gene was detected.
Unfortunately, no clinical follow-up data exists for the two cases (patients K and L) that
harbour the novel complex genetic alterations in exon 11 of the \textit{c-kit} gene.
Discussion

The present study is, to our knowledge, the first, which focuses on metastatic GIST in a population-based collective, analysing both the mutational status and response to tyrosine kinase inhibitor therapy.

Our population data with a GIST crude incidence of 0.97 per 100’000 inhabitants per year and a proportion of 14% metastasized GIST is comparable to data reported by other groups (2-6). Compared to the study of Braconi and colleagues (2), which provides data of primary metastasized tumours of their whole GIST collective, the mutational status of our collective, included primary and secondary metastasized tumours, shows a slightly higher amount of wild type GIST (15.4% vs. 5%) and a similar proportion of c-kit mutations (84.6% vs. 90%). In contrast to their study (5%) we could not detect any PDGFRα mutation.

Also the site distribution of tumours (46.2% stomach, 30.8% small intestine, 24% other sites) is comparable to the data presented by Braconi et al. A difference could be observed in the histologic type of the tumours: While the vast majority of tumours in the present series showed a spindle cell type (76.9%) and only 15.4% and 7.7% a mixed or epitheloid type, respectively, more epitheloid (26%) and mixed type (31%) GISTs were reported in the metastatic group in their study.

The genetic alterations of the investigated metastatic GIST’s are comparable to those, reported in literature for recurrent and metastasized GIST. The most frequently altered exon in the primary tumours of our study group was c-kit exon 11 (54%) with 5 deletions, 1 duplication, and 1 missense point mutation. Other observed genetic alterations occurred in the c-kit exon 9 (23%) and c-kit exon 13 (8%), while 2 PT (15%) showed a c-kit and PDGFRα wild type status. Similar results have also been described in previous publications (2, 9, 13-14).
The fact that we could not detect any PDGFRα mutations in our group of malignant GIST supports the assumption, that GISTs with this genetic alteration are associated with a lower malignant potential and therefore have a better prognosis (10, 14). The exon 11 alterations, which mostly occur in gastric GIST (5, 14), were also mostly detected in our PT of the stomach (4 of 7 cases). In 3 of these 4 cases, for which a clinical follow-up was available, a stable disease or no evidence of disease was observed, which is congruent with published data (12). No correlation between outcome and type of exon 11 alterations could be seen. So the PT of patient D, who died of disease, had the same heterozygous exon 11 deletion (p.W557_K558) as Patient J, who showed no evidence of disease. Interestingly, both showed a switch to homozygosity in their metastatic tumours, which was described as a prognostically unfavourable factor (15). In addition the metastasis of patient D harboured a point mutation in exon 13. Previous studies showed, that exon 13 alterations result in poor response to tyrosine kinase inhibitor therapy (16). This could be responsible for the more aggressive course of the disease of this patient, while receiving Imatinib and Sunitinib without any clinical or radiological response. It remains debatable, whether the genetic changes form PT to metastasis in these 2 cases, in which the here investigated metastatic tissue probes were taken after the start of the drug therapy, were a consequence of the administration of imatinib and/or sunitinib. The other 3 patients with exon 11 alterations, who showed a stable disease or no evidence of disease, initially received Imatinib therapy. In one patient the therapy was stopped after the end of the adjuvant cycle (1 year) with no evidence of residual disease. In another patient a change to Sunitinib was necessary due to liver toxicity.

Exon 9 alterations generally seem to be associated with a worse course of disease. In our study all three observed cases showed the frequent p.502_503AY duplication (12, 17-19) heterozygously in PT and in metastases. The 2 patients with exon 9 mutations and PT in the small intestine showed a tumour progression under tyrosine kinase inhibitor therapy (Imatinib
and Sunitinib) and died of disease. One of these two patients (A) showed a tumour progression despite a dosage elevation of imatinib up to 600 mg/day. The third patient with exon 9 alteration, whose PT was located in the pancreas, shows stable disease under Sunitinib treatment after Imatinib therapy was stopped due to tumour progression and side effects. One of the two patients with wild type GIST was diagnosed only 6 month before the end of our observation period, so no reliable follow-up data is available. The other wild type patient died of disease, showing a rapid tumour progression during tyrosine kinase inhibitor treatment (Imatinib, Sunitinib). This poor response of wild type GIST patients was also repeatedly reported in literature (9, 11-12).

In two patients (K and L) we detected novel exon 11 alterations so far not described: A 14 amino acids deletion (p.E554_N567) in case K with heterozygosity in both PT and metastasis and a 12 amino acids duplication (p.P577_R588) in case L, being heterozygous in PT and homozygous in the metastasis. Unfortunately, no clinical follow-up was available.

Until now only a few cases of GIST in the rectovaginal septum have been described in the English literature (20-29). In all these reports, similarly to our case, this localization seems to be associated with a favourable prognosis. In the few cases with reported genotyping (24-25), no c-kit exon 13 alteration – like in our case – was described.

Taken together, our study, which is based on a population-based collective of metastatic GIST, confirms the observations of other researchers: GISTs with a malignant behaviour are mostly associated with genetic alterations in exons 9 and 11 of the c-kit gene or harbour wild-type c-kit. PDGFRα alterations on the other hand only rarely result in metastatic disease.

In here described setting of a population-based observational study with a small number of cases with different, not standardized treatment modalities, it remains difficult, to draw certain conclusions for a possible relation between the genetic alterations of c-kit and PDGFRα in PT and metastases, respectively, and the response to tyrosine kinase inhibitor therapy. In tendency, our observations support the results of previous studies, in that GISTs with a wild
type genotype or a *c-kit* exon 9 alteration in primary tumour or a secondary mutation in metastasis show a worse response to Imatinib or Sunitinib treatment than other *c-kit* alterations. But, especially in patients with exon 9 mutations and treatment with elevated doses of imatinib according to the results of the MetaGIST group (30), further studies with a larger number of patients with metastasized GIST are needed to confirm these findings.
Funding / Conflict of Interest

This work was supported by an unrestricted research grant of Novartis Pharma Schweiz AG

Acknowledgments

The authors thank the treating oncologists A. Gschwend, R. Sperb, R. Winterhalder and T. Froesch for providing clinical data. We are very grateful to A. Vogetseder for linguistic revision of the manuscript.
References

<table>
<thead>
<tr>
<th>EXON</th>
<th>Primer sequence 5' --&gt; 3'</th>
</tr>
</thead>
<tbody>
<tr>
<td>c-kit exon 8 forward</td>
<td>TTCTGCCCTTTGAACTTGCT</td>
</tr>
<tr>
<td>c-kit exon 8 reverse</td>
<td>AATTGCGACCTTTCCCTCCTC</td>
</tr>
<tr>
<td>c-kit exon 9 forward</td>
<td>TGCTAGATAGAGCCAGGGCT</td>
</tr>
<tr>
<td>c-kit exon 9 reverse</td>
<td>CAGAGCTAAACATCCCCCTTA</td>
</tr>
<tr>
<td>c-kit exon 11 forward</td>
<td>CCAGACGTCTTAATGACCTGA</td>
</tr>
<tr>
<td>c-kit exon 11 reverse</td>
<td>CCTAAAAGTCACTGTATGATTGACC</td>
</tr>
<tr>
<td>c-kit exon 12 forward</td>
<td>GGTTCGCAAGAAACACATCG</td>
</tr>
<tr>
<td>c-kit exon 12 reverse</td>
<td>CAAAAGACACAACTGGGCAA</td>
</tr>
<tr>
<td>c-kit exon 13 forward</td>
<td>TCTGTATGGTACGTGACGTG</td>
</tr>
<tr>
<td>c-kit exon 13 reverse</td>
<td>AAAGGCACCTTGGCACCCAGCAC</td>
</tr>
<tr>
<td>c-kit exon 14 forward</td>
<td>TGGGAGCCAGATTAAATCTC</td>
</tr>
<tr>
<td>c-kit exon 14 reverse</td>
<td>AACCCCTATGACCCCCCATGA</td>
</tr>
<tr>
<td>c-kit exon 15 forward</td>
<td>GACCCAGATGAGCTCCCTCTC</td>
</tr>
<tr>
<td>c-kit exon 15 reverse</td>
<td>TTGACCAATTGGGACTTGCTAC</td>
</tr>
<tr>
<td>c-kit exon 16 forward</td>
<td>GCCACTGTCTTTTCTCTTCTC</td>
</tr>
<tr>
<td>c-kit exon 16 reverse</td>
<td>TGCTCTAAATGCTCTGTATCC</td>
</tr>
<tr>
<td>c-kit exon 17 forward</td>
<td>TGTATTCACAGAGACTTGGC</td>
</tr>
<tr>
<td>c-kit exon 17 reverse</td>
<td>GCAAGACTGTCAAGCAGAGA</td>
</tr>
<tr>
<td>c-kit exon 18 forward</td>
<td>CATTTCAGCAACAGCAGCAT</td>
</tr>
<tr>
<td>c-kit exon 18 reverse</td>
<td>CAAGGAAGCAGGACACCAAT</td>
</tr>
<tr>
<td>pdgfr-α exon 10 forward</td>
<td>GCCACTGTCTTTTCTCTTCC</td>
</tr>
<tr>
<td>pdgfr-α exon 10 reverse</td>
<td>TCCACCAATTTGCACTTGATCA</td>
</tr>
<tr>
<td>pdgfr-α exon 12 forward</td>
<td>GGTTGCACTGGAGCTTGGTAATCCAC</td>
</tr>
<tr>
<td>pdgfr-α exon 12 reverse</td>
<td>AGAATGGGCTGAGCTGATGAG</td>
</tr>
<tr>
<td>pdgfr-α exon 14 forward</td>
<td>CAAGGACAAAGATGGTACAC</td>
</tr>
<tr>
<td>pdgfr-α exon 14 reverse</td>
<td>TTCACAACCAATTTGATCCA</td>
</tr>
<tr>
<td>pdgfr-α exon 18 forward</td>
<td>CCAGTCTTGGACGGGATGATCTAT</td>
</tr>
<tr>
<td>pdgfr-α exon 18 reverse</td>
<td>AACAGCCACGAAATCTCTAGAAGC</td>
</tr>
</tbody>
</table>
### Table 2 Patients' and tumour characteristics

<table>
<thead>
<tr>
<th>Patient ID</th>
<th>Sex</th>
<th>age (years)</th>
<th>Site primary tumor (PT)</th>
<th>PT diameter (cm)</th>
<th>PT histology</th>
<th>Mutation PT</th>
<th>Site of metastasis (M)</th>
<th>Mutation M</th>
<th>Observation period (months)</th>
<th>Progression free survival (months)</th>
<th>Time spread PT - M (months)</th>
<th>State of disease</th>
<th>Duration (months) and dosage</th>
<th>Withdrawal Imatinib therapy (reason)</th>
<th>Duration (months) and dosage Sunitinib therapy (reason)</th>
<th>Withdrawal Sunitinib therapy (reason)</th>
</tr>
</thead>
<tbody>
<tr>
<td>A</td>
<td>m</td>
<td>71</td>
<td>small intestine</td>
<td>9</td>
<td>S</td>
<td>c-kit exon 9 (p.502_503dupAY) heterozygous</td>
<td>peritoneum</td>
<td>c-kit exon 9 (p.502_503dupAY) heterozygous</td>
<td>93</td>
<td>23</td>
<td>23</td>
<td>23 (400mg/day)</td>
<td>DOD</td>
<td>38 (400mg/day) and 23 (400mg/day)</td>
<td>Yes (TP)</td>
<td>1 (50mg/day)</td>
</tr>
<tr>
<td>B</td>
<td>f</td>
<td>68</td>
<td>jejunum</td>
<td>8</td>
<td>S</td>
<td>c-kit exon 9 (p.502_503dupAY) heterozygous</td>
<td>mesenterium</td>
<td>c-kit exon 9 (p.502_503dupAY) heterozygous</td>
<td>47</td>
<td>19</td>
<td>19</td>
<td>19 (300mg/day)</td>
<td>DOD</td>
<td>3 (400mg/day)</td>
<td>Yes (TP, SE)</td>
<td>No</td>
</tr>
<tr>
<td>C</td>
<td>f</td>
<td>31</td>
<td>stomach</td>
<td>21</td>
<td>S</td>
<td>none</td>
<td>peritoneum</td>
<td>none</td>
<td>52</td>
<td>20</td>
<td>20</td>
<td>20 (400mg/day)</td>
<td>DOD</td>
<td>1 (400mg/day before PT surgery) and 4 (800mg/day after metastasis)</td>
<td>Yes (TP)</td>
<td>1 (37.5 mg/day)</td>
</tr>
<tr>
<td>D</td>
<td>m</td>
<td>64</td>
<td>stomach</td>
<td>23</td>
<td>S</td>
<td>c-kit exon 11 (p.W557_K558del) heterozygous</td>
<td>retroperitoneum</td>
<td>c-kit exon 11 (p.W557_K558del) homozygous; c-kit exon 13 (p.V654A) heterozygous</td>
<td>79</td>
<td>17</td>
<td>78</td>
<td>78 (400mg/day)</td>
<td>DOD</td>
<td>28 (400mg/day)</td>
<td>Yes (TP)</td>
<td>30 (37.5 mg/day)</td>
</tr>
<tr>
<td>E</td>
<td>f</td>
<td>72</td>
<td>septum rectovaginale</td>
<td>n/a (biopsy)</td>
<td>S</td>
<td>c-kit exon 13 (p.K642E) heterozygous</td>
<td>septum rectovaginale</td>
<td>c-kit exon 13 (p.K642E) heterozygous</td>
<td>180</td>
<td>114</td>
<td>114</td>
<td>11 (400mg/day)</td>
<td>SD</td>
<td>20 (400mg/day) and 1 (800mg/day)</td>
<td>Yes (SE)</td>
<td>No</td>
</tr>
<tr>
<td>F</td>
<td>f</td>
<td>66</td>
<td>pancreas</td>
<td>4</td>
<td>S</td>
<td>c-kit exon 9 (p.502_503dupAY) heterozygous</td>
<td>liver, omentum</td>
<td>c-kit exon 9 (p.502_503dupAY) heterozygous</td>
<td>168</td>
<td>104</td>
<td>104</td>
<td>104 (400mg/day)</td>
<td>SD</td>
<td>38 (400mg/day) and 3 (800mg/day)</td>
<td>Yes (TP, SE)</td>
<td>12 (37.5 mg/day)</td>
</tr>
<tr>
<td>G</td>
<td>f</td>
<td>65</td>
<td>stomach</td>
<td>8</td>
<td>S</td>
<td>c-kit exon 11 (p.K558_E562del) heterozygous</td>
<td>liver</td>
<td>c-kit exon 11 (p.K558_E562del) heterozygous</td>
<td>41</td>
<td>11</td>
<td>11</td>
<td>11 (400mg/day)</td>
<td>SD</td>
<td>18 (400mg/day)</td>
<td>No</td>
<td>No</td>
</tr>
<tr>
<td>H</td>
<td>f</td>
<td>68</td>
<td>stomach</td>
<td>11</td>
<td>S</td>
<td>none</td>
<td>liver, lymph node</td>
<td>none</td>
<td>6</td>
<td>6</td>
<td>6</td>
<td>6 (400mg/day)</td>
<td>NED</td>
<td>4 (400mg/day)</td>
<td>Yes (SE)</td>
<td>No</td>
</tr>
<tr>
<td>I</td>
<td>f</td>
<td>68</td>
<td>jejunum</td>
<td>8</td>
<td>S</td>
<td>c-kit exon 11 (p.W557G) heterozygous</td>
<td>omentum, peritoneum</td>
<td>c-kit exon 11 (p.W557G) heterozygous</td>
<td>35</td>
<td>22</td>
<td>22</td>
<td>22 (400mg/day)</td>
<td>NED</td>
<td>1 (400mg/day)</td>
<td>Yes (SE)</td>
<td>9 (50 mg/day)</td>
</tr>
<tr>
<td>J</td>
<td>m</td>
<td>65</td>
<td>stomach</td>
<td>10</td>
<td>S</td>
<td>c-kit exon 11 (p.W557_K568del) heterozygous</td>
<td>liver</td>
<td>c-kit exon 11 (p.W557_K568del) homozygous</td>
<td>26</td>
<td>24</td>
<td>24</td>
<td>24 (400mg/day after PT) and 12 (400mg/day after metastasis)</td>
<td>NED</td>
<td>1 (400mg/day after metastasis)</td>
<td>No</td>
<td>No</td>
</tr>
<tr>
<td>K</td>
<td>m</td>
<td>55</td>
<td>stomach</td>
<td>7.5</td>
<td>S/E</td>
<td>c-kit exon 11 (p.K554_N565del) heterozygous</td>
<td>liver</td>
<td>c-kit exon 11 (p.K554_N565del) heterozygous</td>
<td>n/a</td>
<td>n/a</td>
<td>0</td>
<td>n/a (n/a)</td>
<td>n/a</td>
<td>n/a</td>
<td>n/a</td>
<td>n/a</td>
</tr>
<tr>
<td>L</td>
<td>f</td>
<td>74</td>
<td>lig. gastrocolicum</td>
<td>25</td>
<td>S/E</td>
<td>c-kit exon 11 (p.P577_K558del) heterozygous</td>
<td>liver, bone</td>
<td>c-kit exon 11 (p.P577_K558del)2 heterozygous</td>
<td>n/a</td>
<td>n/a</td>
<td>25</td>
<td>n/a (n/a)</td>
<td>n/a</td>
<td>n/a</td>
<td>n/a</td>
<td>n/a</td>
</tr>
<tr>
<td>M</td>
<td>m</td>
<td>89</td>
<td>small intestine</td>
<td>15</td>
<td>S</td>
<td>c-kit exon 11 (p.V559_G565del) heterozygous</td>
<td>omentum, peritoneum</td>
<td>c-kit exon 11 (p.V559_G565del) heterozygous</td>
<td>n/a</td>
<td>n/a</td>
<td>0</td>
<td>n/a (n/a)</td>
<td>n/a</td>
<td>n/a</td>
<td>n/a</td>
<td>n/a</td>
</tr>
</tbody>
</table>

Sex (m=male; f=female); PT histology (S=spindle cellular; E=epitheloid); state of disease (DOD=death of disease; SD=stable disease; NED=no evidence of disease); n/a=not available; TP=tumour progression; MD=side effects.