Metastatic gastrointestinal stromal tumour of the ileum with dual primary c-KIT missence mutations

Sir,

Gastrointestinal stromal tumours (GISTs) are mesenchymal neoplasms, most of which harbour activating mutations in either the KIT or platelet-derived growth factor receptor-α (PDGFRA) receptor tyrosine kinase genes. Most such mutations are gain-of-function mutations which lead to receptor activation, which may be therapeutically targeted by drugs such as imatinib mesylate. Response to imatinib correlates with the presence and type of these mutations, which may arise through codon deletion, duplication or point mutation. Most such mutations arise sporadically but germline mutation does occur and results in familial GIST. Mutation of KIT exon 11 is the most common (around 67% of GISTs, arising at all anatomical sites) and predicts a better response to imatinib as compared to exon 9, 13 and 17 mutations. PDGFRA mutations are less common (around 5–7% of GISTs), occur in exons 12, 14 or 18, and are more common in gastric tumours. Activation loop mutations at D842 V are predictive of imatinib resistance. We report a unique case of ileal GIST with dual primary KIT mutations in exon 13 (lysin substitution to glutamic acid at codon 642, p.Lys642Glu) and in exon 17 (asparagine substitution to lysine at codon 822, p.Asn822Lys).

A 64-year-old man presented in February 2011 with an episode of melaena while recovering from an elective left hip haemiarthroplasty performed a few weeks earlier. He had a history of hypertension and diabetes mellitus with mild renal impairment. There was no history of abdominal pain, weight loss or change in bowel habit. He was also found to have extensive left above knee deep vein thrombosis requiring anticoagulation. He was managed with intravenous fluids and packed red blood cell transfusion. A gastroscopy showed a small non-bleeding benign gastric ulcer but abdominal computed tomography (CT) scan revealed multiple abdominal masses without liver involvement. A positron emission tomography (PET) scan showed that the disease process was only confined to the abdomen.

A fine needle aspiration biopsy of the right iliac fossa mass showed a spindle cell malignancy and subsequently a laparotomy was performed. Intraoperatively he was found to have numerous peritoneal tumour deposits and a primary in the wall of the distal ileum. Most of the macroscopic disease was removed with no tumour rupture or immediate complications. The primary tumour measured >15 cm across and further multiple metastatic deposits resected weighed in excess of 2 kg in aggregate. Histological examination (Fig. 1) revealed GIST with predominantly spindle cell morphology but focal epithelioid areas. Frequent mitoses were present (>50/mf/50 hpf) and there was patchy coagulative tumour necrosis. Immunoperoxidase staining confirmed diffuse strong positivity for DOG-1, diffuse weak positivity for CD117 and patchy weak to moderate positivity for SMA, whereas CD34 and desmin were essentially negative.

DNA sequencing using Sanger method demonstrated two missense mutations in KIT (Fig. 2), one in exon 13 (Lys642Glu) and one in exon 17 (Asn822Lys). These mutations were found in both the primary and two of the metastases. The polymerase chain reaction (PCR) amplification followed by automated sequencing on two separate peripheral blood samples did not detect germline mutation.

Due to high risk morphological features, ileal primary site and intra-abdominal dissemination, imatinib mesylate was initiated at a dose of 400 mg daily in April 2011. A partial response was confirmed on CT scan 3 months later. His disease remained stable for 8 months when imaging suggested increased size of mesenteric and peritoneal nodules, including a large subdiaphragmatic lesion. Hence the dose of imatinib was escalated to 800 mg daily in January 2012 which was tolerated with minimal toxicity. The most recent CT scan after 14 months of high dose imatinib confirmed progressive disease. He is currently being treated with sunitinib malate 50 mg orally once daily on a 4 weeks on, 2 weeks off, dosing schedule.

Sporadic GIST occurs predominantly in older people, with a median age of 63 years. Familial GIST associated with germline KIT mutations occurs at a younger age and is associated with multiple tumours and a familial syndrome in up to 15% of cases. The majority of which arose in the small intestine. Primary mutations in KIT gene exons 13 and 17 are less common as compared to acquired mutations. Usually primary KIT mutations are heterozygous while secondary mutations are acquired following imatinib exposure. This acquired resistance after treatment has been ascribed to loss of the remaining wild-type KIT allele. It has been observed that the secondary mutations occur post-treatment, particularly in exon 17, and are typically absent in the pre-imatinib or non-resistant tumours.

Homozygous KIT exon 11 mutations occur in the setting of disease progression and predict aggressive behaviour. Both the Lys642Glu and Asn822Lys mutations result from single nucleotide substitution at protein level and are the most common KIT exon 13 and 17 primary mutations, respectively. The Lys642Glu mutation has been seen in both sporadic and familial GIST syndromes. There are no data reported on the association of Asn822Lys with familial tumours. Gastric GISTs with Lys642Glu mutation have more aggressive clinical behaviour than other gastric GISTs. This tendency is not seen in small intestinal GISTs harbouring either Lys642Glu or Asn822Lys mutation.

Historically, tumour size, location and mitotic activity are the most important prognostic determinants in GISTs. In general, small intestinal tumours tend to follow a more aggressive course than their gastric counterparts. The type of mutation in KIT and PDGFRA correlates with clinical response to imatinib.
GISTs with exon 11 mutations have a higher response rate, longer time to progression and improved overall survival compared with tumours harbouring KIT exon 9 or wild type. Patients with exon 9 mutation seem to have better disease-free survival but not overall survival when treated with higher imatinib doses (800 versus 400 mg). The higher dose benefit with respect to time to progression has not been seen in exon 11 mutant tumours. Both KIT exon 13 and 17 mutations have

![Diagram](image-url)

**Fig. 1** (A) Typical spindle cell appearance of GIST (H&E). (B) Areas of coagulative necrosis present (H&E). (C) There was diffuse but rather weak staining for CD117, with both membranous and paranuclear dot positivity. (D) Diffuse strong positivity for DOG-1.

**Fig. 2** (A) KIT Exon 13 c.1924A>G: p.Lys642Glu. (B) KIT Exon 17 c.2466T>A: p.Asn822Lys.
demonstrated sensitivity to imatinib in in vitro studies. The benefit from dose escalation in exon 13 and 17 mutant GISTs is unknown because of the rarity of these types. It is quite possible that these GISTs may also need a higher dose of imatinib mesylate to achieve a response quite similar to that observed in KIT exon 9 mutant tumours. In summary, we have described a unique case of GIST harbouring two rare primary mutations. The long term outcome of sporadic GISTs with exon 13 or 17 mutation when treated with imatinib is unknown. Our patient achieved a partial response with standard dose of imatinib, with a duration of response of 8 months. After dose escalation to 800 mg, his disease remained stable for another 14 months. He has recently been commenced on sunitinib due to disease progression. Sunitinib malate has modest activity in exon 13 secondary mutation but there are no data on primary exon 13 and 17 mutations at present.

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Laeeq Malik* Christine Hemmings†§ Victoria Beslany∥ Stephen Fox∥ Desmond Yip‡¶

*Medical Oncology Unit, The Canberra Hospital, Garran. †ANU Medical School, Australian National University, Acton, ACT. ‡School of Surgery, University of Western Australia. §§John of God Pathology, Subiaco, WA, and ¶Department of Pathology, Peter MacCallum Cancer Centre, Melbourne, Vic, Australia

Contact Associate Professor D. Yip.
E-mail: desmond.yip@anu.edu.au


BAP1 expression in cutaneous melanoma: a pilot study

Sir,

BAP1 is a tumour suppressor gene, the protein product of which (BAP1) interacts with the RING finger domain of BRCA1 protein. BAP1 also plays a role in chromatin dynamics as part of multi-protein complexes that regulate transcription by modification of histones.

Recent family studies revealed that germline mutations in BAP1 conferred inherited susceptibility to cutaneous melanocytic tumours (predominantly epithelioid/spitzoid tumours, and less commonly melanoma) and uveal melanoma. and to other tumours, such as mesothelioma and meningioma. BAP1 might also play a role in sporadic melanocytic tumours. Sequencing of BAP1 in 60 sporadic cutaneous melanomas revealed mutations in the gene in three (5%) cases, but these tumours were not described in detail. BAP1 mutations were associated with the development of metastasis and poor prognosis in patients with uveal melanoma, but the prognostic significance of BAP1 expression in cutaneous melanomas has not been studied to date, to the best of our knowledge.

Absence of nuclear BAP1 expression by immunohistochemistry (IHC) has been shown to correlate very well with functional inactivation (by mutation or epigenetic mechanisms) of the BAP1 gene. The aim of this pilot study was to describe the IHC expression of BAP1 in cutaneous melanomas, and to investigate associations of BAP1 expression with clinical and pathological parameters. A secondary aim was to determine whether there were any associations between BAP1 expression and clinical outcomes.

The study was carried out with institutional Ethics Review Committee approval. Tissue microarrays (TMAs) were prepared from paraffin embedded, primary cutaneous melanomas from patients treated at Melanoma Institute Australia (MIA) between 1992 and 2009. The tumours represented thick melanomas of various melanoma subtypes. The tumour specimens had been fixed in 10% buffered formalin, routinely processed, and embedded in paraffin blocks. IHC was performed on 4 μm thick sections of the TMAs using an automated IHC system (Ventana Benchmark XT; Ventana Medical Systems, USA) according to the manufacturer's instructions. Briefly, following deparaffinisation of paraffin sections of the TMAs and heat-induced antigen retrieval, the sections were incubated with BAP1 antibody (clone C-4, 1:50 dilution; Santa Cruz Biotechnology, USA) for 1 h. A subsequent amplification step was followed by incubation with haematoxylin II counterstain for 4 min and then with blueing reagent for 4 min.

Only nuclear staining was considered to be positive. Nuclei of keratinocytes, fibroblasts, and lymphocytes served

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