conventional CRC, raising the possibility of trans-differentiation. The exact role of MSI-H in ASCC and whether this could represent yet another histological subtype of CRC associated with MSI-H and LS requires further studies including molecular analysis, with an aim to identify potential triggers for the phenotypic switching.

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## Gastrointestinal stromal tumour with *CDKN2A* deletions: a report of three cases

Sir,

The proto-oncogene *KIT* encodes c-kit protein, a type 3 transmembrane receptor for MGF (mast cell growth factor, also known as stem cell factor). *KIT* mutations are found in gastrointestinal stromal tumors (GIST), mast cell disease, acute myelogenous leukaemia, and piebaldism. Mutations of *KIT* have been reported in ~85% of GISTs[1] and have been found in exon 11 (~70%), exon 9 (10–15%), exon 13 (1–3%), and exon 17 (1–3%).[1] Imatinib, a tyrosine kinase inhibitor, inhibits cellular proliferation and cell survival by occupying the ATP-binding pocket of *KIT*.[2] Although imatinib is an effective and systemic treatment, some cases have shown imatinib resistance.

Primary or secondary *KIT* mutations and *KIT* amplifications have been identified in imatinib-resistant cases.[3,4] In addition, imatinib is also a representative therapeutic drug for chronic myeloid leukaemia (CML) and Philadelphia chromosome-positive acute lymphoblastic leukaemia (Ph+ ALL). Several recent reports have suggested that a *CDKN2A* deletion is related to tyrosine kinase inhibitor resistance in patients with Ph+ ALL.[5,6] However, a relationship between *CDKN2A* deletion and imatinib resistance has not been reported in GISTs. Here, we report three GIST cases with concurrent *KIT* mutations and *CDKN2A* deletion and the clinical significance of those genetic alterations.

Case 1 was 56-year-old woman who presented after a diagnosis of rectal GIST invading to the uterine wall. After imatinib treatment for 6 months, the size of the mass did not decrease, suggesting it was less sensitive to imatinib. The patient underwent surgery for the rectal mass with a size of 8 cm. The cut surface was an ivory to tan coloured hard lesion with focal haemorrhage and myxoid degeneration. On microscopy, the majority of the tumour showed myxoid and hyaline degeneration, representing imatinib treatment response, but in multifocal areas, pathological responses for imatinib were not observed, with high mitotic activity (17/6 HPFs), high cellularity, and moderate cellular atypia (Fig. 1A,B). In those areas, c-kit and DOG-1 were diffusely positive and Ki-67 index was 45% (Fig. 2A,B).

We investigated the viable tumour using a next generation sequencing (NGS) cancer panel test to determine more appropriate treatment for the patient and found a *KIT* mutation (p.Trp557_Glu561del) and *CDKN2A* deletion (copy number: 0).

Case 2 was a 55-year-old man who visited our institution for abdominal pain and weight loss. Multiple heterogeneous masses were observed along the peritoneum and mesentery, and the sizes were up to 13 cm on abdominal computed tomography (CT). The patient had undergone a core biopsy on the mass. He was diagnosed with GIST and imatinib treatment was started subsequently. The core needle-biopsied tumour was composed of oval to spindle-shaped cells with moderate cellular atypia. The immunohistochemistry tests were focally positive for c-kit and diffusely positive for DOG-1, and the Ki-67 index was 30% (Fig. 2C,D). We tested the biopsy specimen with the NGS cancer panel and found a *KIT* mutation (p.Trp557Arg) and *CDKN2A* deletion (copy number: 0).

Case 3 was a 56-year-old man who presented to our institute in 2008 for suspected pancreas cancer with multiple liver metastases. Metastatic gastrointestinal stromal tumour was diagnosed with biopsied liver, and neoadjuvant imatinib treatment was started. Three years later, bleeding of the primary duodenal GIST and progress of the disease in spite of the dose escalation of imatinib prompted the patient to undergo pylorus preserving pancreatoduodenectomy. The tumour was composed of spindle cells with high cellularity and frequent mitoses (19/50 HPFs) and imatinib treatment effects which were observed in 40% of tumour

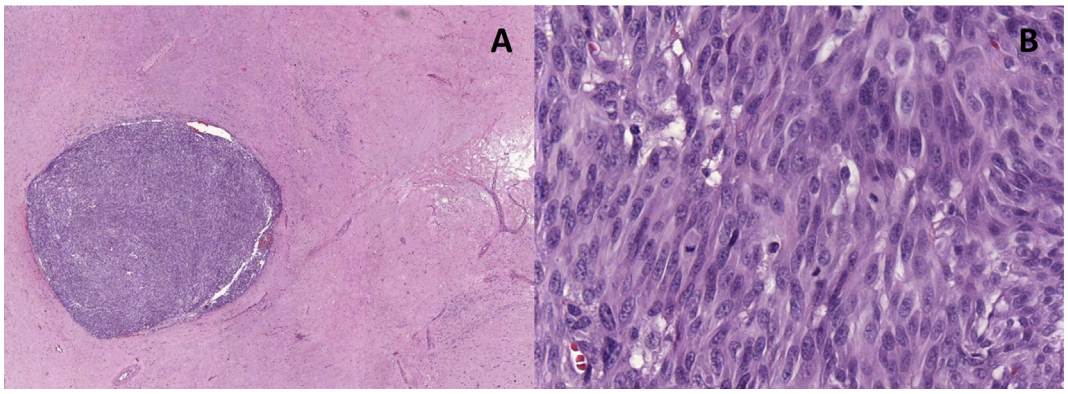


**Fig. 1** Microscopic findings of the first case. Viable gastrointestinal stromal tumour cell clusters in the background of marked hyaline degeneration associated with prior imatinib treatment (A, low power). High cellularity with atypia and frequent mitotic activity were observed (B, high power).

volume. Complex *KIT* mutations (p.Met552_Trp557del in exon 11 and p.Asn822Lys in exon 17) were detected in this duodenal resection specimen by Sanger sequencing. Despite continued high dose imatinib and sunitinib treatment, the hepatic masses progressed. Metastasectomies were performed twice and therapeutic responses were found in less than 5% of the tumour volume. NGS cancer panel test with this hepatic resection specimen showed the same *KIT* exon 11 deletion mutation (p.Met552_Trp557del), additional exon 17 mutation (p.Asp820Val) and heterozygous *CDKN2A* deletion (copy number: 0.4) associated with imatinib resistance.

Most GIST patients respond to therapy with imatinib, but eventually become resistant with a median time to progression of 2 years. The mechanism of acquired resistance to imatinib and oncogenic KIT signal transduction in GISTs have not been well defined.[7] Primary (exon 9 or wild type) or secondary *KIT* mutations (exon 13, 14, 17, or 18) and *KIT* amplifications have been found to activate other pathways or increase drug efflux pumps and cause resistance.[4] Among them, the most frequent genetic alterations of resistance are secondary *KIT* mutations occurring late in tumour progression after a response or a progression-free survival time exceeding 3−6 months,[4] as demonstrated in our first case. The secondary *KIT* mutations, which hinder imatinib binding by changing the c-kit conformation, have been found in 50−70% of late progression cases.[4] Amplification of *KIT* is suggested as another potential mechanism.[4,8] This alteration could increase the number of c-kit molecules for inhibition. In our first and third cases, there was no secondary mutation,

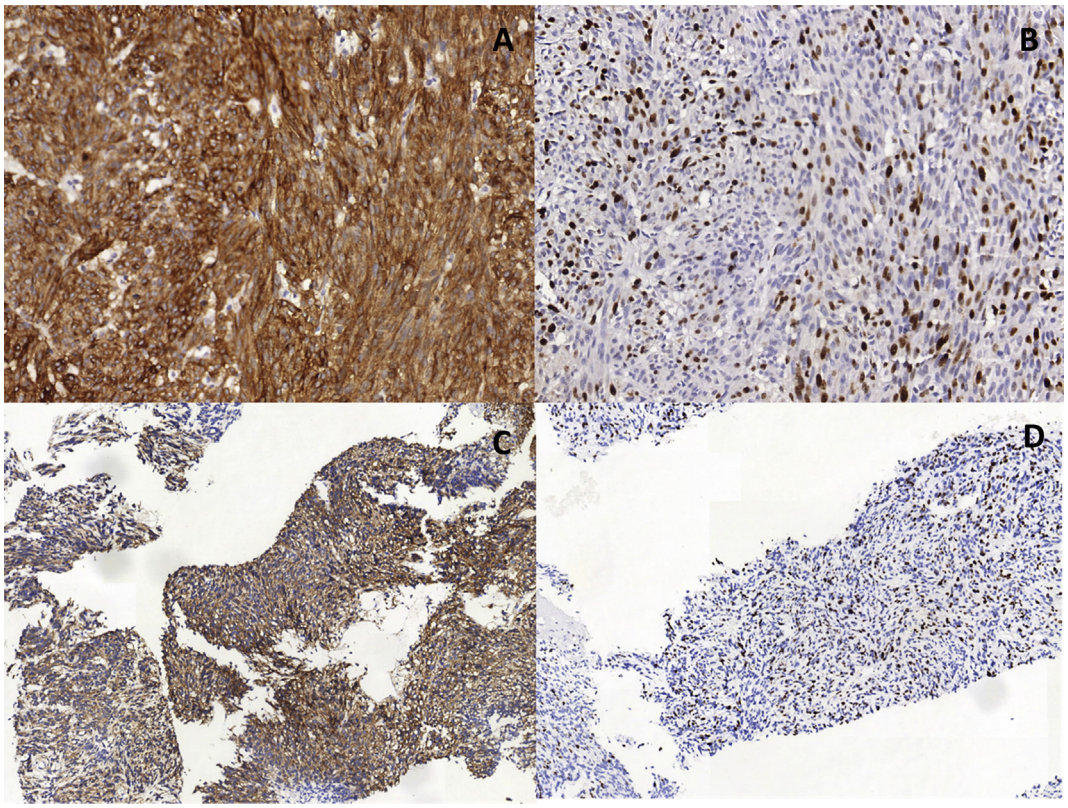


**Fig. 2** Immunohistochemical staining of the first case which showed strong positivity for c-kit (A) and high Ki-67 index (B). A core biopsy of the second case showed strong positivity for DOG-1 immunohistochemistry (C) and high Ki-67 index (D).

rather a *CDKN2A* deletion was found in imatinib-insensitive areas after prior imatinib treatment. The relationship between a *CDKN2A* deletion and imatinib resistance has not been reported in GISTs but has been reported in Ph+ ALL.[5,6] The rapid proliferation of a leukaemic clone was suggested as a mechanism for *CDKN2A* deletion based on the imatinib resistance.[5] In GIST, mutations of *CDKN2A* have not been reported.[9] However, deletion of chromosome 9p arms harbouring *CDKN2A/B* loci have been identified by array comparative genomic hybridisation in 10% of GISTs as a predictive marker of recurrence or metastasis.[10] To find the frequencies of *CDKN2A* deletions in GIST, we searched all the NGS cancer panel test results performed in our institute. For the last 2 years, the NGS test has been performed in 19 patients with metastatic GIST treated with imatinib and showed evidence of progress of disease. Among them, three cases (15.7%) showed *CDKN2A* deletions. In the previous NGS study with 83 primary untreated GISTs, *CDKN2A* deletion was not reported.[9]

Based on these observations and our three cases having both *KIT* mutations and *CDKN2A* deletions, and showing progress of disease after imatinib treatment, *CDKN2A* deletions would lead to proliferation of mutant clones and cause those tumour cells insensitive to imatinib in GISTs.

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## A novel case of linear IgG4-antibody mediated tubulointerstitial nephritis with concomitant HLA-B7, ANCA-MPO

Sir,

IgG4-related disease (IgG4-RD) is a usually systemic disease with raised serum IgG4, and when the kidney is involved there is abundant IgG4 positive plasma cells infiltration with a storiform interstitial fibrosis.[1] The anti-neutrophil cytoplasmic antibodies (ANCAs), most commonly myeloperoxidase (MPO) and proteinase 3 (PR3), are usually associated with pauci-immune systemic vasculitis and crescentic glomerulonephritis (Cr GN). The occurrence of double MPO-ANCA and anti-glomerular basement membrane antibody nephritis (anti-GBM-Ab nephritis) with linear IgG, C3 along the GBM are uncommon, and known to occur in only 0.73% (10) of 1,373 cases of crescentic GN.[2] Only 10 cases have been identified in the literature of IgG4-RD and ANCA-associated vasculitis. A rare case of concomitant IgG4-RD tubulointerstitial nephritis with pauci-immune IgG4-MPO-ANCA with necrotising crescentic GN with C3 and fibrinogen has been reported.[3] We report a novel case of a young adult male with IgG4 tubulointerstitial nephritis (IgG4-TIN) with no necrotising crescentic nephritis, with linear IgG4 along the tubular basement membrane (TBM), and with concomitant HLA-B7 and persistently elevated ANCA-MPO, and with no GBM involvement by immunoglobulins or complements. There was rapidly progressive renal failure treated by plasma exchange (PEX) and immunosuppression.

A 23-year-old Caucasian male was referred in April 2015 with past history of correction of transposition of great arteries at the age of two. Serum creatinine was 146 µmol/L, eGFR 58 mL/min/1.73 $m^2$, positive (MPO) ANCA 15 U/mL (normal <3.5 U/mL), and bland urine sediment with normal urine albumin creatinine ratio (uACR). Ultrasound showed normal renal cortico-medullary differentiation. In January 2016, renal function declined with eGFR 44 mL/min/1.73 $m^2$. Percutaneous renal biopsies were performed for diagnosis and follow up. Renal core biopsies were placed in 10% buffered formalin for light microscopy, in optimal cutting temperature (OCT) compound then snap frozen in liquid nitrogen for immunofluorescence microscopy, or in 2.5% glutaraldehyde for electron microscopy. For light microscopy, fixed tissues were routinely processed, paraffin-embedded and 2 µm sections were stained with haematoxylin and eosin (H&E), periodic acid-Schiff (PAS), periodic acid methenamine silver (PAMS) or modified Mallory's trichrome. Sections were also examined by immunohistochemistry using antibodies against cell markers of T and B cells, macrophages and plasma cells, CD3 (clone2GV6),