100,000 trials). *MED12* is also involved in oestrogen signalling and its mutations have been proposed to comprise the driver genetic event of the more benign end of the fibroepithelial tumour spectrum.[7] This suggests that *RARA* mutations might also represent early events in formation of PTs,[3] possibly giving rise to the low variant allele frequencies observed in sections 2, 9, 10, 12 and 13, which displayed more aggressive histology.

As mentioned above, a malignant spindle cell tumour on histology observed in Case 2 gives rise to differential diagnoses of malignant PT, SCMBC or breast sarcoma. Studies that have sequenced SCMBC are few,[8–10] and none of the mutations detected corresponded to our panel except for *PIK3CA*, *TP53*, *PTEN* and *TERT* promoter. Alterations in *PIK3CA*, *TP53* and *PTEN* are common in breast cancer and thus not particularly helpful in distinguishing a PT from SCMBC. Interestingly, in Krings and Chen's study, no mutations in *TP53* were observed for SCMBC compared to other metaplastic breast carcinoma subtypes. Furthermore, to our knowledge, no alterations in *MED12* exon 2 have been identified in SCMBC, with only *MED12* G44D mutation seen in a metaplastic breast carcinoma of squamous subtype by Ross and team.[8] The discovery of only *MED12* exon 2 and *TP53* mutations in Case 2 helped to lean the diagnosis towards PT rather than SCMBC. Although the morphological diagnosis was a malignant spindle cell tumour favouring a sarcoma, the presence of *MED12* exon 2 mutation suggests an underlying PT. Studies have shown that malignant PTs and breast sarcomas appear to be closely related, with similar clinicopathological features and clinical outcomes.[11,12]

In summary, we report two histologically challenging cases: one where grading of the PT was in question, while the second case was a diagnostic dilemma posed by a malignant spindle cell tumour. Application of the 16-gene mutation panel helped support the revised grading of an originally diagnosed malignant PT to borderline, and was also useful in differentiating between malignant PT and SCMBC. Therefore, this mutation panel shows potential use as an adjunctive tool in diagnostic pathology, and validation in larger cohorts is ongoing.

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Primary adenosquamous carcinoma in a patient with Lynch syndrome

Sir,

Adenosquamous colorectal carcinoma (ASCC) is rare and reported to represent between 0.06% and 0.20% of colorectal carcinoma (CRC).[1] Microsatellite instability-high (MSI-H) CRC caused by DNA mismatch repair (MMR) deficiency is detected in 15% of CRC, of which 12% are sporadic. These are caused by hypermethylation of MLH1 gene promoter, whilst the remaining 3% are inherited and associated with Lynch syndrome (LS).[2] Recently, ASCC has been reported in association with MSI-H and LS, raising the possibility of MSI in the molecular pathogenesis and whether it may represent another histological subtype of MSI-H CRC. The molecular pathogenesis of ASCC remains poorly studied to date. Herein, we report the first case of ASCC with detailed molecular analysis by next-generation sequencing (NGS) of the glandular and squamous components, highlighting

potential insights into the underlying molecular pathogenesis and a review of the literature.

A 38-year-old previously fit and well female patient presented with sudden onset fever, nausea, vomiting and abdominal pain on a background of a 2-week history of diarrhoea. Computed tomography (CT) abdomen and pelvis scan revealed a heterogeneous 80×70 mm hepatic flexure lesion and she proceeded to have an extended right hemicolectomy with *en bloc* cholecystectomy, partial duodenectomy and pancreatectomy. Further history revealed that she belonged to a LS family with early-onset CRC in her mother and sister, and confirmed underlying *MSH2* germline mutation. She had previously been offered but had declined genetic testing due to personal reasons. Post-operatively she was managed with adjuvant chemotherapy and during follow up of 9 months was negative for residual disease or recurrence.

Histopathology examination showed a pT4bN0M1a, 95 mm, ulcerated hepatic flexure ASCC demonstrating areas of poorly-differentiated adenocarcinoma (Fig. 1A) and squamous cell carcinoma (Fig. 1B) with intimate admixture and zones of transition. There was direct invasion into the adherent duodenum and pancreas, no metastases in 22 regional lymph nodes, but metastatic tumour in one non-regional lymph node. The tumour was associated with a peritumoural Crohn's-like inflammatory reaction and tumour infiltrating lymphocytes (>4 per high power field). There was extensive lymphovascular invasion, including extramural venous invasion, and perineural invasion.

Histochemical staining with Alcian blue/PASD showed apical, intraluminal and focal intracytoplasmic mucin within the glandular component and intermingled within squamous areas (Fig. 2A). Immunohistochemistry (IHC) showed the tumour to be positive for CK7 and negative for CK20 in both components. Variable CDX2 nuclear staining was observed in both components. The glandular component was positive for MOC31, BerEP4, CEA, EMA and MUC1 (Fig. 2B), whereas the squamous component stained for p40, CK5/6 and p63 (Fig. 2C), with cells in areas of transition demonstrating variable combined expression of both markers. The tumour showed preservation of MLH1 and PMS2 expression and loss of MSH2 and MSH6 DNA mismatch repair (MMR) protein expression. No BRAFV600E mutant protein was detected by IHC.

MSI testing was performed using commercially available pentaplex MSI analysis kit (promega) containing five mononucleotide repeat markers (BAT-25, BAT-26, NR-21, NR-24 and MONO-27) and showed deletions in all five markers for both components, consistent with MSI-H tumour. NGS was performed using TruSight Tumour 26 gene kit (Illumina, USA) with pathogenic sequence variants detected in the *KRAS*, *PIK3CA*, *TP53* and *GNAS* genes in both components (Table 1). Variants of clinical significance were not detected in target regions of other causally associated genes of *PTEN*, *AKTI*, *SMAD4*, *MSH6*, *APC* or *FBXW7*.

ASCC was first reported in 1907 and is a rare subtype of colorectal carcinoma comprising of malignant glandular and squamous elements, either as separate areas within the tumour or admixed,[3] the squamous component consisting of more than just occasional small foci of squamous differentiation. Clinically, ASCC presents in a similar fashion to conventional colonic adenocarcinomas. Occasional patients with ASCC can present with paraneoplastic syndrome related hypercalcaemia.[4] Most reported ASCC occur in the sixth to seventh decade and affect both males and females without ethnic predilection. Any area of the colon can be affected, although some studies report a predominance of proximal involvement.[5] Prior to a diagnosis of ASCC it is important to rule out other possible causes of malignant squamous elements in the large intestine, particularly metastasis or direct invasion, and confirm lack of continuity of the colorectal tumour with squamous lined sites or epithelia, including malignant transformation within squamous lined fistulous tract or invasion by anal SCC in distal sites (especially within 8 cm of the dentate line).[6]

The pathogenesis of colorectal ASCC is poorly understood. Four hypotheses have been previously suggested including malignant transformation of embryological nests of ectodermal cells, squamous metaplasia, presence of pluripotent stem cells of endodermal origin capable of multidirectional differentiation and direct transformation/dedifferentiation of glandular epithelium.[5] It has also been proposed that an abnormal mucosal stimulus from conditions such as ulcerative colitis, schistosomiasis, radiation, or human papillomavirus (HPV) may allow squamous metaplasia and subsequent malignant transformation.[1] The role of HPV in ASCC is controversial with an association with high risk HPV genotype 16 reported by some investigators[7] but not others.[6]

Traditionally, mixed tumours have been considered to potentially represent one of two subgroups: (1) composite tumours in which both components demonstrate intimate admixture or transition and suggestive of clonal relationship with divergent differentiation; or (2) collision tumours with coexistence and juxtaposition of two adjacent but histologically distinct tumours and suggestive of clonally unrelated tumours.[8] The former consideration has gained favour over time, and advances in molecular diagnostics have provided support for this notion, including adenosquamous carcinomas in extra-colonic sites.

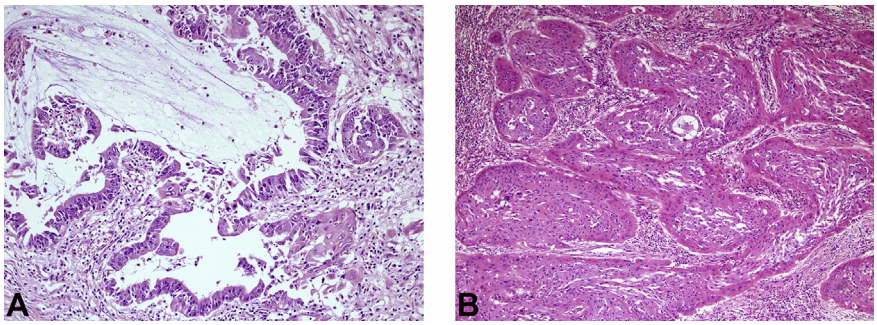

Fig. 1 (A) H&E of glandular component. (B) H&E of squamous component.

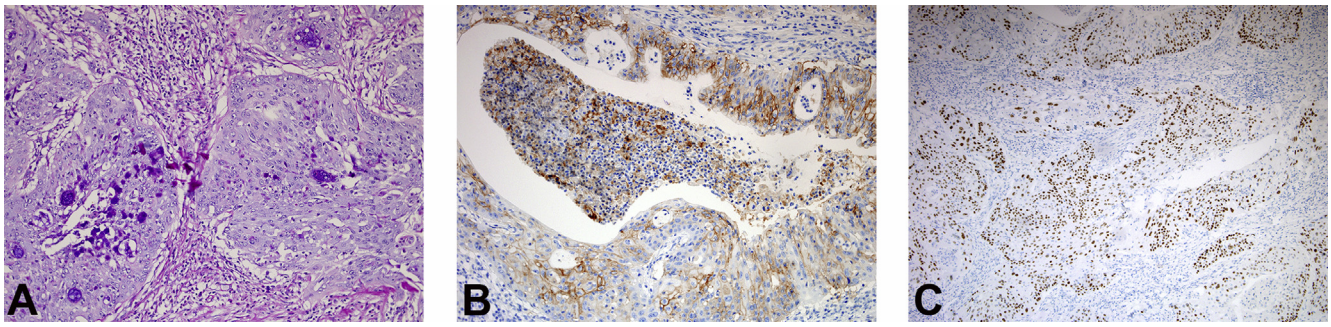

Fig. 2 (A) Histochemical staining with Alcian blue/PASD highlighting mucin. (B) Immunohistochemistry staining glandular component with BerEP4. (C) Immunohistochemistry staining squamous component with p40.

In our study, separate molecular analysis following microdissection on the glandular and squamous components of the tumour identified similar molecular abnormalities, supporting the concept of divergent differentiation from a common precursor rather than a collision tumour. The molecular abnormalities identified overlap with and include variants that have been commonly reported in conventional CRC. These findings raise the possibility of ASCC arising initially as adenocarcinoma prior to undergoing subsequent transdifferentiation, a concept that has been suggested in lung adenosquamous carcinomas.[9] The different histological features detected within the lesion almost certainly have unique molecular profiles and are driven by distinct sets of genetic and epigenetic alterations, as demonstrated for the different precursor colonic lesions such as sessile serrated adenomas and adenomatous polyps. We did not identify any additional underlying molecular genetic abnormalities within the squamous component that were not identified in the glandular component which might suggest possible underlying molecular genetic abnormalities that may be involved in driving phenotypic switch. However, the gene panel used is not sufficiently broad to provide further insight into this. We hope the information provided will serve as a starting point for future studies. Interestingly, a PIK3CA variant was identified, which has also been detected in adenosquamous carcinomas of other sites, including breast, lung and biliary tract, raising the possibility of PIK3CA inhibitors as novel treatment agents.[10] Extensive literature review did not identify any studies reporting on the role of immunotherapy in ASCC.

Recently, two cases of ASCC have been reported in association with MSI-H,[8,11] one of which was confirmed to represent a LS-related tumour. This raises interesting questions regarding the underlying molecular pathogenesis and its potential to represent another histological subtype associated with MSI-H and LS. Coincidentally, recent studies on the distribution of ASCC have noted a predilection for the proximal colon, a feature shared with MSI-H CRC. Furthermore, Petrelli *et al.*[1] reported that ASCC may be associated with increased risk of synchronous adenocarcinoma, a feature shared with LS-associated tumours. These findings add further circumstantial support for this notion. This case represents the third case of ASCC associated with MSI-H and the second confirmed case of LS-associated ASCC in peer reviewed literature. Although it is evident that at least a proportion of ASCC may be associated with MSI-H and LS, analogous to the situation in conventional CRC, the degree and strength of this relationship remains to be determined and the exact role of MSI-H in the molecular pathogenesis of ASCC. The role of MSI-H in the molecular pathogenesis of ASCC may have potential therapeutic implications if confirmed, raising the possibility of the consideration of immunotherapy in the treatment armamentarium.[12] As a corollary, documentation of efficacy of immunotherapy in adenosquamous carcinoma may provide indirect support of the potential of MSI-H in the pathogenesis of this rare tumour; however, to the best of our knowledge, there have been no definitive studies demonstrating efficacy of immunotherapy in adenosquamous carcinoma of the colon to date.

ASCC is an aggressive subtype of colorectal carcinoma and is associated with worse prognosis when compared with usual colorectal carcinoma. This difference in stage-matched prognosis is less pronounced in early stage tumours without lymph node metastases but significantly worse with nodal metastasis.[5] The overall 5-year survival rate of ASCC is reported at 31% compared to 66% in adenocarcinomas, with the 5-year survival rate for node negative disease at 85%, falling to 23% in node positive disease.[5] Apart from well accepted poor prognostic factors such as metastasis, high stage and high grade disease, other suggested poor prognostic predictors include right sided lesions, ulcerated or annular carcinomas and association with ulcerative colitis.[6] Surgical resection is considered definitive treatment of ASCC. The role of adjuvant chemotherapy remains unclear.

In conclusion, we report the first case of ASCC with detailed molecular analysis of the glandular and squamous components, and document only the second case of confirmed LS-related MSI-H ASCC. Our findings support the concept of divergent differentiation from a common precursor rather than a collision tumour, with a mutational profile that overlaps with and includes mutations identified in

Table 1 Breakdown of the same pathogenic sequence variants detected in the glandular and squamous components

Gene	VAF	Read depth	HGVSc	HGVSp
Glandular component				
KRAS	18.17	4343	c.38G>A	p.Gly13Asp
PIK3CA	17.91	4959	c.3140A>G	p.His1047Arg
GNAS	17.87	4549	c.2531G>A	p.Arg844His
TP53	18.07	3801	c.541C>T	p.Arg181Cys
Squamous component				
KRAS	11.4	921	c.38G>A	p.Gly13Asp
PIK3CA	12.87	987	c.3140A>G	p.His1047Arg
GNAS	10.03	1825	c.2531G>A	p.Arg844His
TP53	11.14	2038	c.541C>T	p.Arg181Cys

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