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IDH1-mutated Crohn's disease-associated small bowel adenocarcinomas: Distinctive pathological features and association with *MGMT* methylation and serrated-type dysplasia

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IDH1-mutated Crohn's disease-associated small bowel adenocarcinomas: Distinctive pathological features and association with *MGMT* methylation and serrated-type dysplasia

Aims: Patients with Crohn's disease (CrD) have an elevated risk for the development of small bowel adenocarcinomas (SBAs). Actionable isocitrate dehydrogenase 1 (*IDH1*) mutations have been reported to be more frequent in CrD-SBAs than in sporadic SBAs. The present study aimed to investigate the clinicopathological and immunophenotypical features, as well as methylation profiles, of *IDH1*-mutated CrD-SBAs.

Methods and results: An international multicentre series of surgically resected CrD-SBAs was tested for *IDH1* mutation. Clinicopathological features, immunophenotypical marker expression and O6-methylguanine-DNA methyltransferase (*MGMT*) and long interspersed nuclear element-1 (LINE-1) methylation were compared between *IDH1*-mutated and *IDH1* wild-type CrD-SBAs. Ten (20%) of the 49 CrD-SBAs examined harboured an *IDH1* mutation and all the mutated cancers harboured the R132C variant. Compared to *IDH1* wild-type cases, *IDH1*-mutated CrD-SBAs showed significantly lower rates of cytokeratin 7 expression ($P = 0.005$) and higher rates of p53 overexpression ($P = 0.012$) and *MGMT* methylation ($P = 0.012$). All three dysplastic growths associated

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with *IDH1*-mutated SBAs harboured the same *IDH1* variant (R132C) of the corresponding invasive cancer, and all were of non-conventional subtype (two serrated dysplastic lesions and one goblet cell-deficient dysplasia). In particular, non-conventional serrated dysplasia was significantly associated with *IDH1*-mutated CrD-SBAs ($P = 0.029$). No significant cancer-specific survival difference between *IDH1*-mutated CrD-SBA patients and *IDH1* wild-type CrD-SBA patients was found (hazard ratio = 0.55, 95% confidence interval = 0.16–1.89; $P = 0.313$).
Conclusions: *IDH1*-mutated CrD-SBAs, which represent approximately one-fifth of total cases, are characterised by distinctive immunophenotypical features and methylation profiles, with potential therapeutic implications. Moreover, *IDH1*-mutated non-conventional, serrated dysplasia is likely to represent a precursor lesion to such CrD-SBAs.

Keywords: immune-mediated disorder, non-conventional dysplasia, small intestinal carcinoma

Introduction

Although small bowel adenocarcinoma (SBA) is a rare malignancy, patients with Crohn's disease (CrD) have an increased risk for the development of SBAs related to long-standing intestinal inflammation.[1–4] Crohn's disease-associated SBAs (CrD-SBAs), which arise predominantly from inflamed areas of the ileum, show distinctive clinicopathological and immunophenotypical features in comparison with sporadic or coeliac disease-associated SBAs.[1,5–14] In addition, although the pathogenetic mechanisms of CrD-SBA development and progression remain poorly known, accumulating evidence suggests that CrD-SBAs may emerge through a distinct molecular pathway of tumorigenesis.[7–9,15] On one hand, CrD-SBAs have been reported to show a lower frequency of somatic *APC* and *KRAS* mutations in comparison with sporadic SBAs and less frequent mismatch repair (MMR) deficiency and nuclear β-catenin accumulation compared to coeliac disease-associated SBAs. On the other hand, *SMAD4* and isocitrate dehydrogenase 1 (*IDH1*) mutations have been reported to be more frequent in CrD-SBAs than in sporadic SBAs.[9,15] Interestingly, among SBAs, *IDH1* mutations seem to be almost unique to CrD-SBAs.[15]

IDH1 encodes for the cytoplasmic and peroxisomal isoform of a Krebs cycle enzyme, which catalyses the conversion of isocitrate to α-ketoglutarate. Originally identified in gliomas and myeloid neoplasms, *IDH1* mutations have been more recently observed in several solid tumours.[16–20] Such *IDH1* mutations are neomorphic, in that they convert ketoglutarate into the oncometabolite 2-hydroxyglutarate which, in turn, is thought to drive cell transformation by altering a diverse range of cellular processes, including metabolic and epigenetic changes.[21,22] In gliomas, promoter methylation of O6-methylguanine-DNA methyltransferase (*MGMT*), a DNA repair enzyme that removes alkyl groups from the O-6 position of guanine, has been positively correlated with *IDH1* mutation and global DNA methylation surrogate long interspersed nuclear element-1 (LINE-1) methylation.[23,24]

Given the relatively high rate of potentially targetable *IDH1* mutations in CrD-SBAs (28.6% in their cohort), Aparicio *et al.*[15] suggested that *IDH1* status should be screened in all CrD-SBAs. However, very few *IDH1*-mutated (*IDH1*-MUT) CrD-SBAs have been reported to date (11 cases from the literature).[9,14,15,25,26]

Starting from these premises, in the present study, we aimed to test an international multicentre series of surgically resected CrD-SBAs for *IDH1* mutations and to compare the clinicopathological features, as well as *MGMT* and LINE-1 methylation profiles, between *IDH1*-MUT and *IDH1* wild-type (*IDH1*-WT) CrD-SBAs.

Methods

STUDY POPULATION

This retrospective multicentre international study included 49 CrD patients who underwent surgical resection for primary, non-ampullary SBA derived from: (i) a population of 162 SBA patients enrolled from Italian Centres participating in the Small Bowel Cancer Italian Consortium, (ii) data sets of the Department of Gastroenterology and Hepatology at the University Hospitals Leuven (Leuven, Belgium) and (iii) databases of the Division of Anatomic Pathology, Department of Laboratory Medicine and Pathology, Mayo Clinic (Rochester, MN, USA). CrD diagnosis was ascertained according to international criteria.[27] This study was approved by the Ethics Committee of Pavia (protocol number: 20140003980).

HISTOLOGY AND IMMUNOHISTOCHEMISTRY

A central histopathological review of all tumours was performed by two gastrointestinal pathologists (G.A. and A.V.) for the parameters required by the College of American Pathologists' (CAP) protocol.[28] Histologically, SBAs were classified into six histological subtypes: (i) SBAs, not otherwise specified (SBAs-NOS), (ii) poorly cohesive carcinomas (PCCs), (iii) mixed-poorly-cohesive-glandular SBAs (mixed-PCG-SBAs), (iv) medullary-type carcinomas, (v) mucinous adenocarcinomas and (vi) low-grade tubuloglandular adenocarcinomas, as previously reported.[7,8,13,25,28,29] Tumour budding (Tb) was analysed along the SBA invasive front, according to the International Tumour Budding Consensus Conference criteria, as previously reported,[30,31] and divided into two classes (low Tb: 0–9 buds; high Tb: ≥ 10 buds), associated with prognosis in a large SBA study.[32]

Tumour slides were also reviewed to identify small intestinal dysplastic lesions adjacent to SBAs (i.e. detected on the same slides as the cancer and morphologically distinguishable from the invasive cancer). The histological subtype of dysplasia was recorded as either conventional or non-conventional. Non-conventional dysplasias, recently described in the small bowel of CrD patients in association with SBAs,[12] were subcategorised based on the criteria applied to large bowel dysplastic lesions of patients with inflammatory bowel disease.[33,34]

Immunohistochemistry was performed on 4-μm-thick sections using a Dako Omnis platform (Dako Agilent, Glostrup, Denmark) using monoclonal antibodies against cytokeratin (CK) 7 (clone OV-361-TL12/30; Dako), CK20 (Js20.8; Dako), MUC5AC (CLH2; Abcam, Cambridge, UK), MUC6 (CLH5; Leica Biosystems, Wetzlar, Germany), CDX2 (DAK-CDX2; Dako), β-catenin (beta-catenin-1; Dako), p53 (DO-7; Dako), MLH1(ES05; Dako), MSH2 (FE11; Dako), MSH6 (EP49; Dako) and PMS2 (EP51; Dako). Tumours were considered positive if at least 10% of cells showed membranous/cytoplasmic (CK7, CK20, MUC5AC, MUC6) or nuclear (CDX2, β-catenin) immunoreactivity.[8] p53 staining was interpreted as negative (weak uneven positivity) or overexpressed/positive (strong immunoreactivity in ≥ 50% of the nuclei).[5] No case with complete loss of p53 staining in tumour cells was observed. SBAs were regarded as MMR-deficient (dMMR) if complete loss of nuclear expression of at least one MMR protein was observed in the presence of an adequate internal positive control; the remaining cases were considered MMR-proficient (pMMR).

MOLECULAR ANALYSES

Haematoxylin and eosin-stained slides for each surgical specimen were reviewed to separately select the areas of interest, including both the SBA and, if identified, the adjacent dysplastic lesion, for DNA extraction and molecular testing. DNA was isolated and purified using NucleoSpin® Gel and PCR clean-up kit (Macherey-Nagel, Düren, Nordrhein-Westfalen, Germany).

IDH1 status was assessed by DNA Sanger sequencing. Briefly, polymerase chain reaction amplifications were carried out using *IDH1* primer sequences designed by Patel *et al.*[35] (F-CGGTCTTCAGAGAAGCC ATT, R-GTCATGTTGGCAATAATGTG), while Sanger sequencing was performed by capillary gel electrophoresis on 3130 Genetic Analyser (Thermo Fisher Scientific, Waltham, MA, USA).[36] Sequences obtained from base calling were analysed using Chromas application (Technelysium Pty Ltd, South Brisbane, QLD, Australia) and FASTA sequences were aligned with the reference sequence in the Ensembl Genome Browser website (http://www.ensembl.org/index.html) using the BLAST (http://blast.ncbi.nlm.nih.gov/Blast.cgi).

MGMT promoter methylation was performed as previously described.[37] Methylation status of six CpGs of *MGMT* promoter was assessed by pyrosequencing using MGMT Plus kit (Diatech Pharmacogenetics, Jesi, Ancona, Italy). A cut-off of 15%, defined by calculating the limit of blank for each cytosine, was set to score the presence of *MGMT* methylation. Global DNA methylation status was performed by using bisulphite pyrosequencing addressing four consecutive CpG sites in the LINE-1 region (GenBank accession number: X58075).[38,39]

STATISTICAL ANALYSIS

Data were described using the median and 25–75th percentiles if continuous and with counts and percentages if categorical; they were compared between groups with the Mann–Whitney *U*-test and Fisher's exact test, respectively. Median follow-up (25–75th percentile) was computed with the reverse Kaplan–Meier method. Follow-up was computed from diagnosis of cancer to death or last available follow-up for censored patients. Mortality rates were computed per 100 person-years together with their 95% confidence intervals (95% CI). The log-rank test was used to compare survival between groups and Cox regression to derive hazard ratios (HR) and 95% CI. Kaplan–Meier cumulative survival was plotted. Due to the low number of events, only univariable analyses could be performed. A two-sided $P < 0.05$ was considered statistically

significant. Stata software (version 18; StataCorp, College Station, TX, USA) was used for computation.

Results

Comparison of clinicopathological, immunohistochemical and methylation features between IDH1-mut and IDH1-wt CrD-SBAs

Ten (20%) of the 49 CrD-SBAs harboured a R132C *IDH1* mutation, while the remaining cases (80%) were IDH1-WT. Clinicopathological, immunophenotypical and molecular data of *IDH1*-MUT and *IDH1* WT cases and their comparison are summarised in Table 1.

All *IDH1*-MUT CrD-SBAs were located in the ileum. The median patient age at cancer diagnosis was similar between the *IDH1*-MUT (58.5 years) and *IDH1*-WT CrD-SBAs (56 years) and male gender was predominant in both groups. Histologically, *IDH1*-MUT (60%) CrD-SBAs were more frequently classified as SBAs-NOS compared to IDH1-WT CrD-SBAs (51%), although the difference did not reach statistical significance. No case fulfilled the criteria for mucinous adenocarcinoma or low-grade tubuloglandular carcinoma; however, one *IDH1*-MUT SBA (case 1 in Table 2) showed a low-grade tubuloglandular pattern in 40% of the tumour.

Compared to *IDH1*-WT CrD-SBAs, *IDH1*-MUT cases expressed CK7 (Figure 1) significantly less frequently ($P = 0.005$), whereas they showed a significantly higher rate of p53 overexpression ($P = 0.012$).

In 31 cases with available tumour sections, *MGMT* and LINE-1 methylation was tested. *MGMT* promoter methylation was observed in all but two *IDH1*-MUT cases (six of eight cases, 75%), whereas it was found in 22% of *IDH1*-WT cancers ($P = 0.012$). LINE-1 methylation levels were significantly higher in *IDH1*-MUT cancers compared to *IDH1*-WT cases ($P = 0.005$).

Eight cases exhibited a dMMR phenotype, including two (20%) *IDH1*-MUT CrD-SBAs (one with a combined loss of MLH1 and PMS2 and the other with an isolated MSH6 loss) and six (15%) *IDH1*-WT cancers (all showing a combined loss of MLH1 and PMS2). No patient had known Lynch syndrome or family cancer history suspicious for Lynch syndrome. No significant difference in LINE-1 methylation levels between dMMR (median = 63.98%, 25–75th = 61.40–65.20) and pMMR cases (median = 62.03%, 25–75th = 52.00–64.60) was found ($P = 0.395$).

Patients were followed for a median time of 69 months (25–75th = 2–117). One patient died

Table 1. Clinicopathological, immunohistochemical and molecular features of *IDH1* mutated and *IDH1* wild-type Crohn's disease associated SBAs

	IDH1-MUT CrD-SBAs ($n = 10$)	*IDH1*-WT CrD-SBAs ($n = 39$)	*P*-value
Patient age at SBA diagnosis, median (25–75th)	58.5 (55–62)	56 (47–69)	0.814
Patient age at Crohn's disease diagnosis, years, median (25–75th)	46 (33–52)	39 (27–58)	0.896
Crohn's disease duration before SBA development, months, median (25–75th)	132 (52–216)	156 (1–264)	0.871
Female gender, *n* (%)	4/10 (40%)	10/39 (26%)	0.442
Tumour site, *N* (%)			
Duodenum	0/10 (0%)	2/39 (5%)	1.000
Jejunum	0/10 (0%)	2/39 (5%)	
Ileum	10/10 (100%)	35/39 (90%)	
Histological subtype, *n* (%)			
SBAs-NOS	6/10 (60%)	20/39 (51%)	0.581
Medullary SBA	1/10 (10%)	1/39 (3%)	
PCCs	2/10 (20%)	10/39 (26%)	
Mixed-PCG-SBAs	1/10 (10%)	8/39 (20%)	
Lymphovascular invasion, *n* (%)	8/10 (80%)	33/39 (85%)	0.659
Perineural invasion, *n* (%)	3/10 (30%)	19/39 (49%)	0.478
High tumour budding, *n* (%)	5/10 (50%)	25/39 (64%)	0.480
Adjacent dysplastic lesions, *n* (%)	3/10 (30%)	12/39 (31%)	1.000
pT stage, *n* (%)			
pT1	1/10 (10%)	2/39 (5%)	0.767
pT2	0/10 (0%)	2/39 (5%)	
pT3	5/10 (50%)	22/39 (57%)	
pT4	4/10 (40%)	13/39 (33%)	

Table 1. (*Continued*)

	IDH1-MUT CrD-SBAs (n = 10)	*IDH1*-WT CrD-SBAs (n = 39)	*P*-value
Lymph node metastases, *n* (%)	6/10 (60%)	19/39 (49%)	0.725
AJCC stage, *n* (%)			
I	1/10 (10%)	4/39 (10%)	0.604
II	3/10 (30%)	16/39 (41%)	
III	6/10 (60%)	14/39 (36%)	
IV	0/10 (0%)	5/39 (13%)	
CDX2 expression, *n* (%)	5/10 (50%)	21/39 (54%)	1.000
CK20 expression, *n* (%)	7/10 (70%)	21/39 (54%)	0.482
CK7 expression, *n* (%)	1/10 (10%)	24/39 (61%)	**0.005**
MUC5AC expression, *n* (%)	5/10 (50%)	14/37 (38%)*	0.496
MUC6 expression, *n* (%)	0/10 (0%)	7/37 (19%)*	0.318
p53 overexpression, *n* (%)	9/10 (90%)	16/37 (43%)*	**0.012**
β-catenin nuclear expression, *n* (%)	1/9 (11%)*	8/37 (22%)*	0.664
MMR-deficiency, *n* (%)	2/10 (20%)	6/39 (15%)	0.659
LINE1 methylation, median (25–75th)	64.95% (63.95–66.53)*	61.30% (51.50–63.90)*	**0.005**
MGMT methylation (%)	6/8 (75%)*	5/23 (22%)*	**0.012**

Bold type indicates significant *P*-values. AJCC, American Joint Committee on Cancer; CrD-SBA, Crohn's disease associated small bowel adenocarcinoma; CK, cytokeratin; *IDH1*-MUT, *IDH1*-mutated; *IDH1*-WT, *IDH1* wild-type; mixed-PCG-SBA, mixed-poorly-cohesive-glandular small bowel adenocarcinoma; MMR, mismatch repair; PCC, poorly cohesive carcinoma; SBA, small bowel adenocarcinoma; SBA-NOS, small bowel adenocarcinoma, not otherwise specified.

*MUC5AC, MUC6 and p53 expression were assessed in 47 cases with available tumour sections, whereas β-catenin was evaluated in 46 cases; *MGMT* methylation and LINE1 methylation testing was possible in 31 cases with available tumour material.

perioperatively and was excluded from survival analysis. Three patients died in the *IDH1*-MUT group and 18 in the IDH1-WT group, corresponding to mortalities of 7.7 per 100 (95% CI = 2.5–24.0) and 11.7

Table 2. Clinicopathological, immunohistochemical and molecular features of *IDH1*-mutated Crohn's disease-associated SBAs with associated *IDH1*-mutated dysplastic lesions

Case	Patient age at SBA diagnosis	Patient sex	Tumour stage at diagnosis	Patient outcome	Histologic SBA subtype	Histologicalal DYS subtype	*IDH1* mutation variant		*MGMT* methylation		p53 overexpression		MMR deficiency		CK7 expression	
							SBA	DYS	SBA	DYS	SBA	DYS	SBA	DYS	SBA	DYS
1	67	M	II (pT3N0)	AWD (35 mo)	SBA-NOS	Non-C (TSA-like)	R132C	R132C	Yes	Yes	Yes	No	Yes	Yes	No	No
2	58	M	I (pT1N0)	AWD (42 mo)	SBA-NOS	Non-C (GCD)	R132C	R132C	NV	NV	Yes	No	No	No	No	No
3	55	F	III (pT4N2)	DOD (7 mo)	Mixed-PCG-SBA	Non-C (TSA-like)	R132C	R132C	Yes	Yes	Yes	Yes	No	No	No	No

AWD, alive without disease; CK, cytokeratin; DYS, dysplasia; DOD, dead of disease; GCD, goblet cell deficient; mixed-PCG-SBA, mixed-poorly-cohesive-glandular small bowel adenocarcinoma; MMR, mismatch repair; mo, months after surgery; Non-C, non-conventional; NV, not evaluable; SBA, small bowel adenocarcinoma; SBA-NOS, small bowel adenocarcinoma, not otherwise specified; TSA, traditional serrated adenoma.

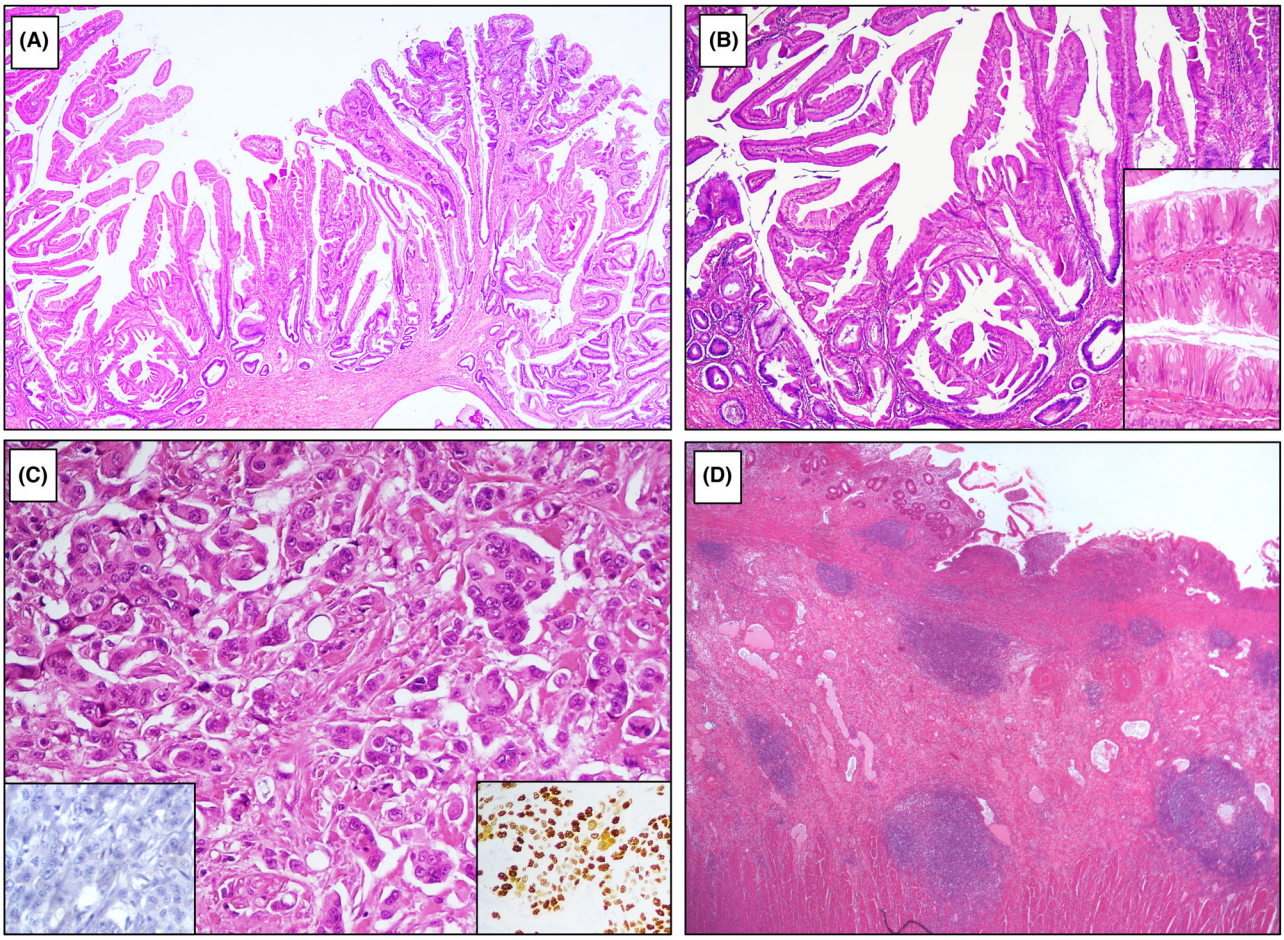

Figure 1. A Crohn's disease-associated *IDH1*-mutated small bowel adenocarcinoma, with an adjacent traditional serrated adenoma (TSA)-like non-conventional dysplastic lesion. (A, B) The TSA-like dysplastic component, featuring slit-like serrations, eosinophilic cytoplasm and ectopic crypt formation (inlet) (haematoxylin and eosin; A, original magnification ×100; B, original magnification ×200 and, in the inlet, ×400). (C) The invasive component (haematoxylin and eosin, original magnification ×200), showing a lack of immunoreactivity for cytokeratin 7 (inlet in the bottom left corner, cytokeratin 7 immunohistochemistry, original magnification ×200), and an immunohistochemical overexpression of p53 (inlet on the bottom right corner, p53 immunohistochemistry, original magnification ×200). (D) Ileum adjacent to the cancer showing histologic features consistent with active Crohn's disease (haematoxylin and eosin, original magnification ×20).

person-years (95% CI = 7.4–18.6), respectively. Survival analysis showed no significant difference between *IDH1*-MUT and *IDH1*-WT CrD-SBA patients (HR = 0.55, 95% CI = 0.16–1.89; $P = 0.313$) (Figure 2). Moreover, the six patients with both *IDH1*-MUT and *MGMT* hypermethylated tumours had a similar survival to the remaining patients (HR = 0.99, 95% CI = 0.21–4.70; $P = 0.992$).

DYSPLASTIC LESIONS ASSOCIATED WITH IDH1-MUT AND IDH1-WT CRD-SBAS

CrD-associated small bowel dysplastic lesions were seen in 15 ileal SBAs, including three (30%) *IDH1*-MUT and 12 (31%) *IDH1*-WT cancers. All dysplasias adjacent to *IDH1*-MUT cancers were classified as non-conventional and encompassed two serrated dysplastic lesions resembling traditional serrated adenomas (TSAs), i.e. TSA-like dysplasias (Figure 1), and one goblet cell-deficient dysplasia, while the dysplastic lesions associated with *IDH1*-WT cancers included nine conventional and three non-conventional dysplasias (one hypermucinous and two goblet cell-deficient dysplastic lesions). Serrated dysplasia was found significantly more commonly in association with *IDH1*-MUT CrD-SBAs compared to *IDH1*-WT cases ($P = 0.029$).

Interestingly, all three dysplastic growths associated with *IDH1*-MUT SBAs harboured the same *IDH1* mutation variant (R132C) of the corresponding invasive cancer. The more relevant clinicopathological

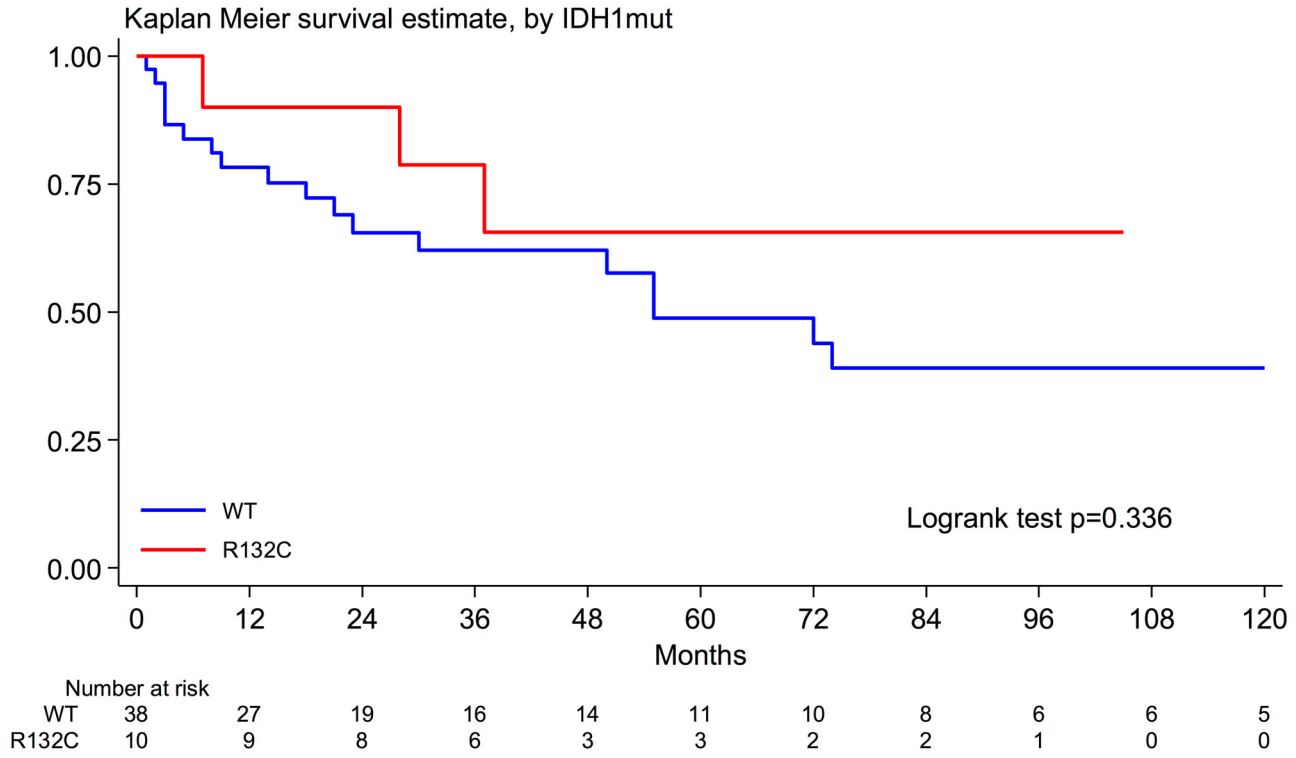

Figure 2. Kaplan–Meier cancer-specific survival estimate by *IDH1* mutation.

and molecular features of the three *IDH1*-MUT CrD-SBA cases with an associated *IDH1*-MUT dysplastic lesion are summarised in Table 2. All these *IDH1*-MUT non-conventional dysplastic lesions showed lack of CK7 expression, like the corresponding SBA. Two (both TSA-like) *IDH1*-MUT dysplasias showed *MGMT* methylation (like the corresponding carcinomas).

Discussion

In the present study, we found that *IDH1* mutations occurred in approximately 20% of CrD-SBAs and that *IDH1*-MUT CrD-SBAs, all of which arising in the ileum, showed distinctive immunophenotypical features and a higher rate of *MGMT* methylation compared to *IDH1*-WT cancers.

The frequency of *IDH1* mutation in CrD-SBAs found in our larger series is similar to those reported in previous studies on SBAs and/or colorectal carcinomas (CRCs) associated with CrD, ranging from 18 to 29%, whereas it appears to be extremely rare in CRCs associated with ulcerative colitis, as well as in sporadic SBAs and CRCs.[9,15,19,20,25] *IDH1* mutations have also been described in many different types of neoplasms, including gliomas, intrahepatic cholangiocarcinoma, chondrosarcoma and acute myeloid leukaemia.[17,40–46] Interestingly, all *IDH1*-mutated SBAs and most *IDH1*-mutated intrahepatic cholangiocarcinomas show the *IDH1* R132C mutation variant, while the most frequently observed *IDH1* variant in gliomas is reported to be R132H.[47]

Our study examines, for the first time to our knowledge, both global DNA hypomethylation and site-specific gene hypermethylation in CrD-SBAs, demonstrating a key role of *IDH1* in driving DNA hypermethylation from the early steps of tumorigenesis in this cancer type. In recent years, several reports demonstrated that few mutated driver genes are associated with genome-wide patterns of aberrant hypomethylation or CpG island hypermethylation in specific cancer types and that somatic mutations could directly or indirectly affect cancer methylomes.[22,48–52] It is well known that mutated *IDH1*/*IDH2* produce abnormal 2-hydroxyglutarate leading to CpG island methylator phenotype (CIMP) by inhibiting the 10–11 translocation (TET)-mediated demethylation pathway and that a link between *IDH1* mutations and *MGMT* promoter methylation is frequently observed in gliomas and intrahepatic cholangiocarcinomas.[53–55]

Hartman *et al.*[25] suggested that *IDH1* mutation may be an early molecular change in a subset of intestinal

cancers associated with CrD. Accordingly, we found the same *IDH1* R132C mutation variant in both the dysplastic and the invasive components of all three *IDH1*-mutated CrD-SBAs associated with a morphologically distinguishable dysplastic lesion in our study. Interestingly, all such preinvasive lesions were classified as non-conventional dysplasias. These findings suggest that a subset of CrD-associated ileal dysplasias with non-conventional patterns, despite their generally low-grade cytological atypia, may be precursor lesions to CrD-SBA harbouring *IDH1* mutation. Two of the three aforementioned *IDH1*-mutated dysplastic lesions were subcategorised as TSA-like dysplasias which, in our study, proved to be significantly associated with *IDH1*-mutated SBAs. TSA-like dysplasia is a rare and poorly characterised form of non-conventional dysplasia, essentially described in the large intestine of patients with inflammatory bowel disease.[34,56–58] To the best of our knowledge, no previous case of TSA-like dysplasia has been reported in the small intestine. Hartman *et al.*,[25] however, described a case of *IDH1*-mutated CrD-SBA in the ileum associated with an *IDH1*-WT dysplasia with serrated features which, according to the authors, did not fulfil the histological criteria of TSA-like dysplasia; they found a significant association between *IDH1*-mutated IBD-associated intestinal carcinomas and precursor lesions exhibiting serrated morphology. Further investigations are needed to explore whether *IDH1* mutation is a peculiar molecular feature of CrD-associated ileal non-conventional dysplastic lesions, especially those with TSA-like features, or whether it may be also be seen in colorectal non-conventional dysplasia and/or in the rare sporadic TSAs of the small bowel.[59]

In our series of CrD-SBAs an association between *IDH1* mutation and CK7 negativity was identified. This finding is interesting, as CK7 has been reported to be expressed in the majority of CrD-SBAs and to strongly correlate with worse patient survival; therefore, its negativity may help to identify a subset of CrD-SBAs enriched for *IDH1* mutations.[8,12] However, no significant association of *IDH1* mutation with CrD-SBA patient prognosis could be identified. In addition, the positive association between *IDH1* mutation and p53 overexpression found in our series is in keeping with findings by Liao *et al.*,[9] who found a concurrent mutation of *TP53* and *IDH1* in all *IDH1*-mutated CrD-SBAs.

IDH mutations may represent an appealing therapeutic target in CrD-SBAs, as several IDH-inhibitors have been developed and are being investigated in various clinical trials. In addition, *IDH* mutated cancers appear to be defective in homologous recombination ('BRCAness phenotype'), which offers sensitivity to poly(ADP-ribose) polymerase inhibitors.[60] Our identification of *MGMT* methylation in a subset of CrD-SBAs may also have therapeutic implications, as temozolomide-based regimens have been reported to be effective in *MGMT*-methylated gliomas and gastrointestinal carcinomas.[61–63]

We acknowledge that this study has limitations, such as its inherently retrospective nature and the relatively low sample size related to the rarity of the disease. Nevertheless, the involvement of international centres with referral experience in the field and the centralised histological review and molecular testing are indicative of data quality. Moreover, Sanger sequencing has a relatively low sensitivity in identifying mutations, and we cannot exclude that minor subclones of *IDH1*-MUT cancer cells were missed. Further studies using next-generation sequencing are needed to compare the genetic landscape of *IDH1*-MUT and *IDH1*-WT CrD-SBAs.

In conclusion, when compared to *IDH1*-WT cases, *IDH1*-MUT CrD-SBAs, which represent approximately one-fifth of cases, are characterised by significantly higher rates of CK7 immunohistochemical negativity, p53 overexpression and *MGMT* hypermethylation, with potential therapeutic implications. Moreover, *IDH1*-MUT serrated dysplasia might represent a precursor lesion to such CrD-SBAs.

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Conflicts of interest

The authors have disclosed that they have no significant relationships with, or financial interest in, any commercial companies pertaining to this article.

Data availability statement

The data sets used and/or analysed during the current study are available from the corresponding author upon reasonable request.

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