Effects of CDX2 on prognosis and chemotherapy responsiveness in mismatch repair-deficient colorectal cancer

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Background: Caudal-related homeobox transcription factor 2 (CDX2) is an intestine-specific transcription factor implicated in tumour differentiation, proliferation, cell adhesion and migration. Negative CDX2 status (CDX2−) is associated with worse prognosis in colorectal cancer and may identify high-risk stage II disease that benefits from adjuvant chemotherapy. This observational study investigated whether CDX2− is associated with prognosis or response to chemotherapy in the mismatch repair-deficient (dMMR) phenotype of colorectal cancer.

Methods: Patients with resectable dMMR colorectal cancer were eligible for inclusion. The prognostic and predictive value of CDX2 expression on the presence of lymph node metastasis (LNM) and survival was investigated. CDX2 status was determined via immunohistochemistry using the Leica Bond™ CDX2 (clone EP25) ready-to-use primary antibody.

Results: Some 235 of 238 consecutive dMMR tumours were assessed for CDX2 status. CDX2− was observed in 15.7 per cent of colorectal cancer. Interobserver agreement was excellent (κ = 0.863; P < 0.001). CDX2− was significantly associated with female sex, increased size, advanced stage, worse conventional and poorly differentiated cluster (PDC) grade, mucinous morphology, perineural and lymphovascular invasion, and pN status (all P ≤ 0.038). CDX2− was not associated with LNM or survival in multivariable analysis. Independent predictors of LNM were PDC grade (odds ratio (OR) 4.12, 95 per cent c.i. 1.76 to 9.63; P = 0.001) and extramural venous invasion (OR 3.79, 1.62 to 8.85; P = 0.002). Budding (hazard ratio (HR) 2.79, 95 per cent c.i. 1.60 to 4.87; P < 0.001), pT status (HR 3.59, 1.29 to 10.01; P = 0.015) and adjuvant chemotherapy (HR 2.07, 1.15 to 3.74; P = 0.016) were independently associated with worse disease-free survival.

Conclusion: CDX2− does not confer a worse prognosis in the dMMR phenotype of colorectal cancer. The MMR status of patients with colorectal cancer should be determined before assessing CDX2 status.

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Introduction

Although molecular profiles of colorectal cancers have been characterized in detail in recent years¹, for practical purposes there are two main molecular subtypes: those with microsatellite instability (MSI) (15–20 per cent) where errors in replication go unchecked due to deficient DNA mismatch repair (dMMR), and mismatch repair (MMR)-proficient (pMMR) tumours (80–85 per cent)².

The majority of dMMR cancer is sporadic and caused by MLH1 promoter hypermethylation, occurring on a background of global gene promoter hypermethylation known as the CpG island methylator phenotype (CIMP)¹. Such tumours are usually diagnosed at advanced age with a female preponderance, are associated with BRAF mutation, and originate from sessile serrated lesions (SSLS)². Between 3 and 5 per cent of all colorectal cancers, however,
are thought to be due to constitutional mutations in the MMR genes (Lynch syndrome)\(^1\). Aside from helping to identify patients with this syndrome\(^1\), interest in determining MMR status has grown as a result of a role in prognostic stratification\(^6,7\), and as a predictor of response to chemotherapy\(^8\) and novel immunotherapies\(^9,10\).

CDX2 is a homeobox gene/intestine-specific transcription factor essential for intestinal development and differentiation\(^11\). It is restricted to the adult small intestine and colon, and is an important biomarker of mature colonic epithelial tissue. It has been proposed to govern diverse processes such as cell proliferation, adhesion and migration, and tumorigenesis, and may have both oncogenic and tumour-suppressing potential\(^12\). Colorectal migration, and tumorigenesis, and may have both onco-diverse processes such as cell proliferation, adhesion and migration, and may have both oncogenic and tumour-suppressing potential\(^12\). Colorectal cancer with negative CDX2 status (CDX2\(^−\)) is associated with an increased likelihood of aggressive features such as lymph node metastasis (LNM), poor differentiation, lymphovascular (LVI), perineural (PNI) and extramural (EMVI) invasion, \(BRAF\) mutation and CIMP\(^12\). CDX2\(^−\) has been shown to be associated with a worse disease-free survival (DFS) in colorectal cancer\(^13\). It has been hypothesized that the prognostic effect of CDX2\(^−\) could be explained by its capacity to function as a single biomarker for many biological risk factors, under the common motif of an immature stem cell phenotype. Moreover, CDX2 status may identify high-risk stage II disease (CDX2\(^−\) disease) that has a high risk of recurrence and in which the hazards of chemotherapy may be offset by a survival benefit\(^13\).

Several studies have shown that dMMR colorectal cancer has a better prognosis than pMMR colorectal cancer\(^6,7\), whereas experimental and clinical evidence suggests a reduced response to 5-fluorouracil (5-FU)-based adjuvant chemotherapy in dMMR tumours\(^8,14\). This has led to the recommendation that MMR testing should be considered for all patients with stage II disease\(^15\). Loss or downregulation of CDX2 expression, however, occurs more frequently in the dMMR phenotype (approximately 15 per cent) than in colorectal tumours overall (less than 5 per cent)\(^12,13\).

The aim of the present study was to investigate the apparently counterintuitive association of dMMR colorectal cancer, with its reported good prognosis and resistance to 5-FU, with CDX2\(^−\) disease, a phenotype conferring an adverse prognosis and a benefit from 5-FU.

**Methods**

This was a single-centre observational study of patients tested for dMMR between 1 January 2005 and 1 January 2015. Patients with primary resectable colorectal cancer were retrieved from an institutional database. All had a confirmed histological diagnosis of colorectal cancer and had undergone surgical resection. Patient selection for colorectal cancer resection was in accordance with the Royal College of Surgeons in Ireland guidelines, and all patients were discussed at a multidisciplinary meeting\(^16\). The study protocol was reviewed and approved by St Vincent’s University Hospital research and ethics committee. A full description of all methods is available in Appendix S1 (supporting information).

**Mismatch repair status**

Mismatch status was assessed using immunohistochemistry (IHC) for MMR proteins, human mutL homologue (hMLH1) 1 (clone G168–728; BD Biosciences\(^®\), Franklin Lakes, New Jersey, USA), human postmeiotic segregation (hPMS) 2 (clone A16–4; BD Biosciences\(^®\)), human mutS homologue (hMSH) 2 (clone FE11; Calbiochem\(^®\), San Diego, California, USA) and hMSH6 (clone 44; BD Biosciences\(^®\)), as described previously\(^17\).

**CDX2 status**

Formalin-fixed, paraffin-embedded tissue sections were stained with Leica Bond\(^TM\) CDX2 (clone EP25; Leica Biosystems, Wetzler, Germany) ready-to-use primary antibody. IHC was performed on the automated Leica BOND\(^TM\) platform. Antigen retrieval with Leica Bond\(^TM\) ER2 solution (30 min) was performed with an antibody incubation time of 15 min. Visualization of the antibody–antigen reaction was via the Leica Bond\(^TM\)
Polymer Refine Detection system (Leica Biosystems). Percentage CDX2 expression and the intensity of the immunoreaction were estimated in each case. To assess CDX2 status, the scoring system developed by Dalerba and colleagues was used. Two consultant histopathologists reviewed all available specimens. When there was disagreement, a third histopathologist decided on the CDX2 status.

### Follow-up and outcomes

The prognostic and predictive value of CDX2 expression on the presence of LNM and survival was investigated. Clinical follow-up was at 6 weeks and 3–6-monthly intervals thereafter, and included endoscopic assessment. For 3 years, all patients had 6-monthly CT of the thorax, abdomen and pelvis. Survival and recurrence were recorded in a prospectively developed institutional colorectal cancer database. Locoregional recurrence was defined as recurrence, either biopsy-proven or with convincing imaging and concurrent increase in tumour markers, located in the abdominal or pelvic nodes, at the anastomotic site or rectal stump, or in the peritoneum, presacral area or retroperitoneum as a soft tissue mass. Mortality status and cause of death were confirmed from data obtained from the National Cancer Registry in Cork and the General Registrar’s Office in Dublin, Ireland. Primary care physicians were contacted as necessary to complete survival data if cause of death was unclear. The last date of follow-up was 31 March 2016.

### Statistical analysis

All results were analysed using IBM SPSS® Statistics version 21.0 (2012) for Mac OS® (IBM, Armonk, New York, USA).}

### Table 2

Comparison of detailed histopathological and molecular features according to CDX2 status colorectal cancer with mismatch repair-deficient phenotype

| Feature                          | CDX2− (n = 37) | CDX2+ (n = 198) | P*  |
|----------------------------------|----------------|-----------------|-----|
| **BRAF**                         |                |                 |     |
| Wild-type                        | 62             | 9 (15)          | 53 (85) |
| Positive                         | 143            | 23 (16-1)       | 120 (83.9) |
| **WHO grade**                    |                |                 |     |
| 1–2                              | 149            | 11 (7-4)        | 138 (92.6) |
| 3–4                              | 86             | 26 (30)         | 60 (70) |
| **Tumour budding**               |                |                 |     |
| No                               | 130            | 10 (7-7)        | 120 (92.3) |
| Yes                              | 37             | 5 (14)          | 32 (86) |
| **PDC grade**                    |                |                 |     |
| Low                              | 126            | 7 (5-6)         | 119 (94-4) |
| High                             | 41             | 8 (20)          | 33 (80) |
| **Mucinous (%)**                 |                |                 |     |
| ≤ 50                             | 197            | 37 (18-8)       | 160 (81-2) |
| > 50                             | 38             | 0 (0)           | 38 (100) |
| **Signet ring**                  |                |                 |     |
| No                               | 216            | 33 (15-3)       | 183 (84-7) |
| Yes                              | 19             | 4 (21)          | 15 (79) |
| **LVI**                          |                |                 |     |
| No                               | 106            | 6 (7-5)         | 98 (92-5) |
| Yes                              | 129            | 29 (22-5)       | 100 (77-5) |
| **PNI**                          |                |                 |     |
| No                               | 201            | 27 (13-4)       | 174 (86-6) |
| Yes                              | 34             | 10 (29)         | 24 (71) |
| **EMVI**                         |                |                 |     |
| No                               | 159            | 22 (13-8)       | 137 (86-2) |
| Yes                              | 75             | 15 (20-0)       | 60 (80) |
| **Type of margin**               |                |                 |     |
| Expansile                        | 115            | 15 (13-0)       | 100 (87-0) |
| Infiltrative                     | 119            | 22 (18-8)       | 97 (81-5) |
| **pT category**                  |                |                 |     |
| pT1–2                            | 40             | 2 (5)           | 38 (95) |
| pT3–4                            | 195            | 35 (17-9)       | 160 (82-1) |
| **pN category**                  |                |                 |     |
| pN0                              | 166            | 20 (12-0)       | 146 (88-0) |
| pN1–2                            | 69             | 17 (25)         | 52 (75) |

Values in parentheses are percentages. CDX, caudal-related homeobox transcription factor; PDC, poorly differentiated cluster; LVI, lymphovascular invasion; PNI, perineural invasion; EMVI, extramural vascular invasion. *Fisher’s exact test.

### Table 3

Binary logistic regression analysis of histopathological predictors of lymph node metastasis

| Predictor                          | Odds ratio | P     | Odds ratio | P     |
|------------------------------------|------------|-------|------------|-------|
| **Univariable analysis**           |            |       |            |       |
| Age (years)                        | 1.00       | 0.8-12| 0.712      |       |
| Male sex (versus female sex)       | 0.56       | 0.3-10| 0.069      |       |
| Left side (versus right side)      | 0.97       | 0.4-2.09| 0.945     |       |
| CDX2− (versus CDX2+)               | 2.39       | 1.16-4.90| 0.018     |       |
| **BRAF+ (versus wild-type)**       | 1.08       | 0.57-2.05| 0.822     |       |
| High WHO grade (versus low grade)  | 2.50       | 1.4-4.43| 0.002     |       |
| High budding (versus low budding)  | 5.18 < 0.001| 2.35-11.43| 0.001     |       |
| High PDC grade (versus low grade)  | 6.52 < 0.001| 2.98-14.28| 4.12 0.001|       |
| LVI present (versus absent)        | 4.44 < 0.001| 2.33-8.47| 1.76-9.63|       |
| PNI present (versus absent)        | 5.11 < 0.001| 2.38-10.96| 0.002     |       |
| EMVI present (versus absent)       | 6.09 < 0.001| 3.31-11.23| 3.79 0.002|       |
| Infiltrative margin present (versus absent) | 2.81 0.001 | 1.56-5.07| 1.62-8.85|       |
| High pT category (versus low category) | 4.50 < 0.001| 1.54-13.18| 0.006     |       |

Values in parentheses are 95 per cent confidence intervals. CDX, caudal-related homeobox transcription factor; PDC, poorly differentiated cluster; LVI, lymphovascular invasion; PNI, perineural invasion; EMVI, extramural vascular invasion.
York, USA) and GraphPad Prism® version 7.0 (2016) for Mac OS® (GraphPad Software, San Diego, California, USA). Cohen’s κ coefficient was used to test interobserver reliability. The statistical association between CDX2 status and the various histological parameters was investigated using Fisher’s exact test for categorical data. Student’s t test and the Mann–Whitney U test were used for continuous variables as appropriate. Variables with \( P < 0.200 \) in univariable analysis were included in multivariable analysis. The Hosmer–Lemeshow test was used to assess the goodness-of-fit of the binary logistic regression model. Kaplan–Meier curves, log rank test and Cox regression were used to associate survival with CDX2 status, and the various molecular and pathological characteristics were expressed as hazard ratios (HRs) with 95 per cent confidence intervals. All tests of significance were two-tailed, with \( P < 0.050 \) indicating statistical significance.

**Results**

Some 238 patients with primary dMMR cancers were identified. No further tissue was available for CDX2 analysis on three dMMR slides that had been referred from external institutions for MMR testing. Therefore, only 235 patients and tumours were available for CDX2 assessment.

The median length of follow-up for all 238 patients with dMMR cancers was 45 (range 3–144) months. The mean(s.d.) age of the cohort was 71.2(13.8) (range 23–97) years. The majority of patients were women (65.5 per cent), and tumours occurred most frequently (84.0 per cent) in the right colon (*Table S1*, supporting information). Of 204 patients with MLH1- and MLH1/PMS2-deficient tumours, 199 (97.5 per cent) underwent reflex *BRAF* mutational testing to screen for Lynch syndrome, of whom 58 (29.1 per cent) were *BRAF* wild-type. Twenty-five patients with dMMR had a confirmed Lynch syndrome constitutional mutation. CDX2− was observed in 15.7 per cent of dMMR colorectal cancers (*Fig. S1*, supporting information). Agreement between observers was excellent (κ = 0.863, \( P < 0.001 \)) with regard to the final assessment of CDX2 status.

**Comparisons with other demographic and histopathological features**

*Table 1* compares basic patient and tumour characteristics between patients with CDX2− and those with CDX2+ tumours. CDX2− was significantly associated with patient sex \( (P = 0.037) \), tumour size \( (P = 0.002) \) and AJCC (seventh edition) stage \( (P = 0.019) \). With regard to histopathological features, CDX2 status was associated with WHO grade \( (P < 0.001) \), poorly differentiated cluster (PDC) grade \( (P = 0.012) \), mucinous type \( (P = 0.001) \), PNI \( (P = 0.038) \), LVI \( (P = 0.002) \) and pN category \( (P = 0.016) \) (*Table 2*).

**Risk factors for lymph node metastasis**

LNM was observed in 29.4 per cent of tumours (70 of 238) in the entire dMMR cohort. Of the 235 tumours that were assessed for CDX2 status, LNM rate was 46 per cent (17 of 37) in CDX2− tumours and 26.3 per cent (52 of 198) in CDX2+ tumours. CDX2 status \( (P = 0.018) \), WHO grade \( (P = 0.002) \), tumour budding \( (P < 0.001) \), PDC grade \( (P < 0.001) \), LVI \( (P < 0.001) \), PNI...
Table 4 Cox regression analysis of disease-free survival in patients with colorectal cancer with mismatch repair-deficient phenotype

|                | Univariable analysis | Multivariable analysis |
|----------------|----------------------|------------------------|
|                | Hazard ratio | P     | Hazard ratio | P     |
| Age (years)    | 0.99       | 0.074 |                |        |
| Male sex (versus female sex) | 1.11   | 0.636 |                |        |
| Left side (versus right side) | 1.40   | 0.215 |                |        |
| CDX2− (versus CDX2+) | 1.42   | 0.198 |                |        |
| BRAF+ (versus wild-type) | 1.06   | 0.829 |                |        |
| High WHO grade | (versus low grade) | 1.57   | 0.036 |                |        |
| High budding (versus low budding) | 2.91   | <0.001 | 2.79 | <0.001 |        |
| High PDC grade | (versus low grade) | 2.42   | 0.033 |                |        |
| LVI present (versus absent) | 1.70   | 0.020 |                |        |
| PNI present (versus absent) | 2.44   | <0.001 |                |        |
| EMVI present (versus absent) | 2.78   | <0.001 |                |        |
| Infiltrative margin present (versus absent) | 2.39   | <0.001 |                |        |
| High pT category (versus low category) | 4.18   | 0.002 | 3.59 | 0.015 |        |
| Positive nodal status (versus negative status) | 2.45   | <0.001 |                |        |
| Adjuvant chemotherapy (versus surgery alone) | 2.06   | 0.004 | 2.07 | 0.016 |        |

Values in parentheses are 95 per cent confidence intervals. CDX, caudal-related homeobox transcription factor; PDC, poorly differentiated cluster; LVI, lymphovascular invasion; PNI, perineural invasion; EMVI, extramural vascular invasion. Schoenfeld residual test: P = 0.427.

Table 5 Cox regression analysis of overall survival in patients with colorectal cancer with mismatch repair-deficient phenotype

|                | Univariable analysis | Multivariable analysis |
|----------------|----------------------|------------------------|
|                | Hazard ratio | P     | Hazard ratio | P     |
| Age (years)    | 0.99       | 0.989 |                |        |
| Male sex (versus female sex) | 1.07   | 0.806 |                |        |
| Left side (versus right side) | 1.24   | 0.490 |                |        |
| CDX2− (versus CDX2+) | 2.03   | 0.010 |                |        |
| BRAF+ (versus wild-type) | 1.40   | 0.303 |                |        |
| High WHO grade | (versus low grade) | 1.57   | 0.036 |                |        |
| High budding (versus low budding) | 2.91   | <0.001 |                |        |
| High PDC grade | (versus low grade) | 2.42   | 0.003 |                |        |
| LVI present (versus absent) | 1.70   | 0.020 |                |        |
| PNI present (versus absent) | 2.44   | <0.001 | 2.74 | 0.010 |        |
| EMVI present (versus absent) | 2.78   | <0.001 | (1-27, 7-20) |        |
| Infiltrative margin present (versus absent) | 2.39   | <0.001 |                |        |
| High pT category (versus low category) | 4.18   | 0.002 |                |        |
| Positive nodal status (versus negative status) | 2.45   | <0.001 | 2.13 | 0.026 |        |
| Adjuvant chemotherapy (versus surgery alone) | 0.80   | 0.487 |                |        |

Values in parentheses are 95 per cent confidence intervals. CDX, caudal-related homeobox transcription factor; PDC, poorly differentiated cluster; LVI, lymphovascular invasion; PNI, perineural invasion; EMVI, extramural vascular invasion.

(P < 0.001), EMVI (P < 0.001), type of margin (P = 0.001) and pT category (P = 0.006) were associated with LNM in univariable analysis (Table 3). The only independent predictors of LNM, however, were PDC grade (OR 4.12, 95 per cent c.i. 1.76 to 9.63; P = 0.011) and EMVI (OR 3.79, 1.62 to 8.85; P = 0.002) (Table 3).

Survival

Some 40 patients (16.8 per cent) had recurrent cancer, most frequently locoregional recurrence (37 patients, 93 per cent); all three distant recurrences were to the lung. There was no significant difference in 5-year disease-free survival according to CDX2 status (Fig. 1a). CDX2− carcinomas were associated with reduced 3-year (60 per cent versus 77.8 per cent for CDX+ tumours; P = 0.018) and 5-year (51.0 versus 70.1 per cent respectively; P = 0.009) overall survival (Fig. 1b). However, the prognostic effect of CDX2 expression for overall survival in dMMR colorectal cancer was not independent of stage (Fig. S2, supporting information).

Chemotherapy was not associated with an improvement in 3-year disease-free survival once adjusted for stage (Fig. S3, supporting information). There was no difference in response based on CDX2 expression. Using Cox regression, treatment with adjuvant chemotherapy was associated with reduced disease-free survival (HR 2.06, 95 per cent c.i. 1.25 to 3.38; P = 0.004) in univariable analysis. This effect remained significant in the multivariable model (HR 2.07, 1.15 to 3.74; P = 0.016) (Table 4). Other variables associated

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with worse disease-free survival in multivariable analysis were pT category (HR 3.59, 1.29 to 10.01; $P=0.015$) and budding (HR 2.79, 1.60 to 4.87; $P<0.001$). Only PNI (HR 2.74, 1.27 to 7.20; $P=0.010$) and the presence of LNM (HR 2.13, 1.10 to 4.15; $P=0.026$) were independently associated with worse overall survival (Table 5).

Discussion

In this study CDX2− status in dMMR colorectal cancer was associated with a number of adverse prognostic variables such as stage, conventional WHO grade, PDC grade, PNI and LVI, but not with survival. This shows that dMMR colorectal cancer is a heterogenous phenotype with different prognostic factors compared with pMMR disease. CDX2 testing does not appear to provide additional prognostic or predictive information in the management of dMMR tumours. In the authors’ opinion, colorectal cancer should be tested for MMR status first, and CDX2 testing should be considered only when the tumour is pMMR.

The recent international consensus molecular subtype (CMS) classification divides colorectal cancer into four CMS groups based on gene expression profiling1, and emerging evidence18 suggests that these have distinct immune orientation. Initial studies19–21 have found an association between lack of CDX2 expression and reduced survival in dMMR colorectal cancer (CMS1/immune subtype). Some studies22–24 have reported discordant results. Olsen and colleagues23 found that low CDX2 protein or mRNA expression was not associated with recurrence risk in a study of 119 patients with colorectal cancer, including 44 dMMR tumours. In a study of 469 colorectal cancers including 81 dMMR tumours, Pilati and co-workers22 demonstrated that although CDX2− identified a group of patients with a particularly poor prognosis both globally and in the CMS4 (stem cell–related/mesenchymal) group, it was not associated with a worse prognosis within the CMS1 subtype. More recently, Neumann et al.24 found, in a cohort of 503 patients with colorectal cancer (FIRE-3) and in a case–control collection of 50 right-sided tumours with and without synchronous distant metastasis, that CDX2 was not an independent prognostic biomarker in colorectal cancer. They found that the prognostic impact of CDX2 depended on the MMR and $BR4F$ mutational status of these tumours. The present study has validated these findings in a large cohort of dMMR colorectal cancer.

Recently, Fessler and colleagues25 showed that transforming growth factor (TGF) $\beta$ stimulation in an SSL model strongly reduced CDX2 expression, downregulated the expression of genes associated with colorectal intraepithelial neoplasia and induced the expression of mesenchymal marker genes. Thus, SSLs may progress to either CMS1 (dMMR/MSI) or CMS4, depending on the level of TGF$\beta$ signalling activity. The assumed better prognosis of dMMR colorectal cancer may reflect the marked immune cell infiltration of its tumour microenvironment26. TGF$\beta$ in the tumour microenvironment also stimulates the recruitment of immune cells, which have a significant role in the regulation of tumour progression, while directly inhibiting their antitumour effector functions27. Therefore, in poorly differentiated tumours there may be two distinct scenarios. In CDX2− CMS4 tumours TGF$\beta$ may polarize infiltrating lymphocytes towards a more regulatory phenotype, with less potent antitumour effector functions27, whereas in CDX2− CMS1 tumours the absence of TGF$\beta$ may result in a more robust cytotoxic T-cell response18. In the present study, tumour budding, associated with both TGF$\beta$ and epithelial mesenchymal transition28, was a stage-independent predictor of worse outcome.

In some studies the supposed survival advantage of dMMR seems to be independent of tumour stage29,30, whereas in others it appears to be confined to stage II31 or stage III32 disease, although these data are likely confounded by the effect of adjuvant therapy33. Preclinical evidence suggests that dMMR might be one mechanism for tumour resistance to 5-FU14 and, although results were conflicting, numerous clinical data suggest a possible reduced response to 5-FU in dMMR colorectal cancer8. In contrast, dMMR tumour cells appear sensitive to oxaliplatin34, and retrospective analyses of a number of large clinical trials suggest that treatment with FOLFOX (folinic acid–5-FU–oxaliplatin) is superior to 5-FU in dMMR colorectal cancer8. In the present study, neither therapy demonstrated a survival advantage above that of surgery alone. Unlike the findings of Dalerba and colleagues33, there was no difference in response to chemotherapy based on CDX2 expression.

Limitations of this study include the absence of a comparator group of patients with stage- and aged-matched pMMR colorectal cancer and the retrospective design, which may have led to selection bias. Evolving knowledge regarding the heterogeneity of colorectal cancer poses questions regarding the appropriateness of controls. Findings arising from colorectal cancer trials of novel therapies, and prognostic and predictive biomarkers, may not be applicable to all tumours.

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### Supporting information

Additional supporting information can be found online in the Supporting Information section at the end of the article.