

Implications of mismatch repair-deficient status on management of early stage colorectal cancer

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Abstract: For primary colorectal cancers (CRCs), tumor stage has been the best predictor of survival after resection and the key determinant of patient management. However, considerable stage-independent variability in clinical outcome is observed that is likely due to molecular heterogeneity. This is particularly important in early stage CRCs where patients can be cured by surgery alone and only a proportion derives benefit from adjuvant chemotherapy. Thus, the identification of molecular prognostic markers to supplement conventional pathologic staging systems has the potential to guide patient management and influence outcomes. CRC is a heterogeneous disease with molecular phenotypes reflecting distinct forms of genetic instability. The chromosomal instability pathway (CIN) is the most common phenotype, accounting for 85% of all sporadic CRCs. Alternatively, the microsatellite instability (MSI) phenotype represents ~15% of all CRCs and is caused by deficient DNA mismatch repair (MMR) as a consequence of germline mutations in MMR genes or, more commonly, epigenetic silencing of the *MLH1* gene with frequent mutations in the *BRAF* oncogene. MSI tumors have distinct phenotypic features and are consistently associated with a better stage-adjusted prognosis compared with microsatellite stable (MSS) tumors. Among non-metastatic CRCs, the difference in prognosis between MSI and MSS tumors is larger for stage II than stage III patients. On the other hand, the predictive impact of MMR status for adjuvant chemotherapy remains a contentious issue in that most studies demonstrate a lack of benefit for 5-fluorouracil (5-FU)-based adjuvant chemotherapy in stage II MSI-H CRCs, whereas it remains unclear in MSI-H stage III tumors. Here, we describe the molecular aspects of the MMR system and discuss the implications of MMR-deficient/MSI-H status in the clinical management of patients with early stage CRC.

Keywords: Colorectal cancer (CRC); mismatch repair (MMR); microsatellite instability (MSI); adjuvant chemotherapy

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Introduction

Colorectal cancer (CRC) is a common malignancy with more than one million new cases occurring each year (1) and is the second leading cause of cancer deaths in Western countries. Disease stage remains the strongest prognostic variable and is the key determinant of patient management. Although most cases of CRC develop through a CIN pathway, approximately 15% of cases are characterized

by microsatellite instability (MSI), a molecular marker of defective DNA mismatch repair (dMMR). The frequency of MSI varies according to the tumor stage with highest rates in early stage cancers that decreases with progression to locoregional and distant metastases (2). In this review we describe the molecular aspects of the MMR system and discuss the implications of MMR-deficient status in the clinical management of patients with early stage CRC.

Table 1 Characteristics of CRC based on MMR-deficiency status

Status	PCR based assay	IHC	Genetic background	Clinical features	Phenotype
MMR deficient (dMMR; approx. 12-15% of CRC)	MSI-H (>30% of the markers are mutated)	Loss of MMR proteins (esp. other than MLH1)	Germline mutations in MMR genes (<i>MLH1</i> , <i>MSH2</i> , <i>MSH6</i> , <i>PMS2</i>); can have <i>KRAS</i> mutation, but never <i>BRAF</i> mutation	Better prognosis than pMMR tumors; in the proximal colon; lymphocytic infiltrate; poorly differentiated;	Lynch syndrome (aka HNPCC; 2-3% of CRC); younger patients
		Only loss of hMLH1 protein (increasing with aging)	Sporadic epigenetic inactivation of the <i>MLH1</i> gene promoter by DNA hypermethylation (CIMP-related silencing); ~50% of sporadic CRC with MSI are associated with <i>BRAF</i> ^{V600E} mutation	mucinous or signet ring appearance	“Sporadic CRC with MSI”; older patients
MMR proficient (pMMR)	MSI-L (at least 1 but <30% of the markers are mutated) MSS (nothing is mutated)	Intact MMR proteins			

PCR, polymerase chain reaction; IHC, immunohistochemistry; MMR, mismatch repair; CRC, colorectal cancer; MSI, microsatellite instability; MSS, microsatellite stable; CIMP, CpG island methylation phenotype; HNPCC, hereditary non-polyposis colorectal cancer.

Phenotypic features and molecular origin of deficient MMR CRC

CRC patients with dMMR tumor have distinct clinical and pathologic features, such as proximal colon predominance, poor differentiation and mucinous histology, with increased numbers of tumor-infiltrating lymphocytes (*Table 1*) (3). Tumors with dMMR are more common among stage II, and are relatively uncommon among metastatic CRCs (4). MMR-deficiency can arise from two distinct molecular alterations. Lynch syndrome (LS) accounts for approximately 3-4% of all CRCs and one third of all dMMR/MSI-associated CRC. It is inherited autosomal dominant and is caused by inactivating germline mutations in MMR genes (5), including *MLH1*, *MSH2*, and more rarely *MSH6* and *PMS2* (6). Germline mutations in an MMR gene followed by a second hit to the wild-type copy is needed to produce LS, and can occur due to point mutation, loss of heterozygosity or methylation. Patients with LS develop tumors at early ages, often between 20 and 30 years old (compared to median age of 69 years in sporadic CRC) and have increased rates of synchronous CRCs. While cancers of the colon and rectum are most common among LS patients, these patients can also develop cancers of the

uterine endometrium, stomach, ovary, urinary tract, small intestine and other sites (7). The estimated cumulative risks of CRC by age 70 years for LS patients is approximately 50% in case of *MLH1* or *MSH2* mutations, with endometrial cancer as the second most common malignancy in these patients (8). CRCs from LS patients are significantly less likely to carry *BRAF*^{V600E} mutation (*Table 1*). Among dMMR/MSI CRCs, *BRAF*^{V600E} mutation testing thus can be performed to distinguish LS cases from sporadic tumors (9). Patients with suspected hereditary CRC should be referred for genetic counseling, where the identification of germline mutations and evaluation/screening of family members can be appropriately addressed.

Among the 12-15% of all CRC tumors with dMMR/MSI, about two-thirds are sporadics. The majority of these cancers develop in a background of dense promoter hypermethylation of cancer-specific genes known as the CpG island methylator phenotype (CIMP) (10,11). CIMP-related silencing of the *MLH1* gene is known to be responsible for about 80% of cases in which *MLH1*/*PMS2* expression are lost (12). Approximately half of sporadic dMMR cases are associated with *BRAF*^{V600E} mutations (13,14) that serve to distinguish them from LS cases (9). Patients with sporadic CRCs with MSI share

clinicopathological features with LS cases with the exception that sporadics are substantially older at CRC diagnosis compared to LS and there is a female predominance (15).

Measuring MSI and dMMR

The DNA MMR system is composed of proteins (MLH1, MSH2, MSH3, MSH6, PMS2) whose function is to repair base-base mispairs introduced into short and tandemly-repeated sequences (microsatellites), during DNA synthesis to maintain genomic stability (3). Deficient MMR, due to genetic or epigenetic events, results in production of a truncated, nonfunctional protein or loss of a protein which causes MSI phenotype. Tumors with dMMR thus demonstrate a high frequency of MSI (MSI-H), and, in turn, MSI is used as the molecular fingerprint of dMMR.

MSI testing can be performed on paraffin-embedded tumor tissue using a PCR-based assay for detection of instability in selected microsatellite loci (16,17). A panel of microsatellite markers have been validated and recommended as a reference panel (18). On the basis of the MSI status, CRCs can be categorized into three groups: MSI-H, if 2 or more of the 5 microsatellite markers show instability (that is, have insertion/deletion mutations); MSI-L (low-frequency MSI), if only one of the five markers shows instability; and microsatellite stable (MSS) if none of the markers show instability (18). MSI-H corresponds to dMMR, whereas MSI-L and MSS indicate pMMR.

Analysis of MMR protein expression by immunohistochemistry (IHC) is an alternative test that is widely available with the advantages of not requiring a molecular laboratory and the ability to identify the affected gene by detecting loss of its protein product. Since the loss of MMR protein expression by IHC has been shown to be highly concordant with DNA-based MSI testing (17), these two tests are considered to be complimentary. Using IHC, tumors that demonstrate loss of an MMR protein can be collectively referred to as dMMR and expected to be MSI-H. Importantly, only loss of hMLH1 protein expression has been described in sporadic CRCs (12). Tumors with intact MMR proteins can be classified as proficient MMR that are MSS or MSI-low (MSI-L) (Table 1).

Mismatch repair (MMR)-deficient status and clinical outcome

Although the pathologic tumor stage remains the key determinant of CRC prognosis and treatment, there is

considerable stage-independent variability in clinical outcome. Thus, new prognostic and predictive biomarkers are needed to inform prognosis and to guide the use and choice of systemic chemotherapy. Accumulating evidence indicates that dMMR status is one such candidate. Multiple studies have shown that patients with dMMR tumors have a more favorable stage-adjusted prognosis than those with pMMR tumors. These data are largely from retrospective studies that include clinical trials of adjuvant therapy (19-22), and a population-based study (23). A meta-analysis including 32 studies comprising 1,277 MSI cases, among a total of 7,642 patients with stage I-IV CRC, also showed a better prognosis for patients with dMMR compared with pMMR tumors (2). This analysis included untreated patients, as well as patients treated with 5-fluorouracil (5-FU)-based adjuvant chemotherapy. The hazard ratio (HR) for overall survival (OS) associated with dMMR was 0.65 [95% confidence interval (CI), 0.59-0.71]; the benefit was maintained when analyses were restricted to patients with stage II or stage III cancers participating in clinical studies (2). In general, the prognostic impact of dMMR appears to be stronger in earlier stage tumors, i.e., stage II versus node-positive or stage III cancers (24,25).

MMR status and 5-FU based adjuvant chemotherapy

The fluoropyrimidine 5-FU remains the most commonly used chemotherapy drug for the treatment of CRC. Where adjuvant chemotherapy remains optional in stage II CRC patients, capecitabine or 5-FU combined with leucovorin (LV), or combinations of these drugs with oxaliplatin, are considered to be standard treatment options for stage III. Preclinical models have suggested that dMMR tumors were associated with 5-FU resistance (26-31). The preponderance of evidence also suggests that 5-FU-based adjuvant chemotherapy is ineffective in patients with dMMR tumors (32), although some earlier studies suggested that patients with dMMR *vs.* pMMR tumors derive a similar or even a greater benefit from 5-FU-based adjuvant treatment (33-35). Conflicting results were based on studies where patients were not randomly assigned to 5-FU-based treatment versus observation after resection, a relatively small numbers of patients with dMMR colon cancers, and the bimodal age distribution among these patients. Accordingly, the impact of dMMR status as prognostic/predictive classifiers is ideally studied to a clinical trial cohort of same stage patients receiving uniform treatment.

Sargent *et al.* investigated 457 stage II and stage III colon cancer patients who were included in five randomized trials evaluating 5-FU + levamisole or LV as adjuvant chemotherapy *vs.* no post surgical treatment (36). In this analysis, patients with dMMR cancers had significantly better survival than did pMMR patients, although dMMR tumors of either stage did not benefit from 5-FU-based adjuvant therapy. These findings were validated by combining these data with those from a prior study by Ribic *et al.* from the same group (37), yielding a total of 1,027 stage II and stage III colon cancer patients. In the combined dataset, dMMR was associated with more favorable outcome compared to pMMR cancers (DFS: HR =0.51; 95% CI, 0.29-0.89; P=0.009; OS: HR =0.47; 95% CI, 0.26-0.83; P=0.004), and 5-FU adjuvant chemotherapy may attenuate the prognostic advantage of dMMR (DFS: HR =0.79; 95% CI, 0.4-1.25; P=0.30; OS: HR =0.78; 95% CI, 0.49-1.24; P=0.28). Of note, a suggestion of a detrimental effect of 5-FU was seen in patients with stage II dMMR tumors. These data were interpreted to indicate that patients with dMMR stage II CRC should not receive adjuvant 5-FU.

A lack of efficacy for 5-FU as adjuvant chemotherapy in patients with dMMR stage II CRC was observed in the Quick and Simple and Reliable (QUASAR) adjuvant therapy trial where patients with stage II CRCs were assigned to receive 5-FU (n=1,483) *vs.* surgery alone (n=1,480) (38). Among all patients with known MMR status, the risk of recurrence of dMMR tumors was reduced by half compared to pMMR tumors [11% (25 of 218) *vs.* 26% (438 of 1,695) recurred; risk ratio (RR) =0.53; 95% CI, 0.40-0.70; P<0.001]. However, MMR status did not predict benefit from chemotherapy (HR =0.97, P=0.92) (39). More recently, the prognostic impact of dMMR in stage II and III CRC patients was further examined using pooled data analysis from 17 adjuvant trials in the ACCENT database (40). This analysis involved 7,803 patients of which 571 received surgery alone and 3,878 patients received 5-FU monotherapy. Among stage II patients, dMMR *vs.* pMMR was strongly associated with increased TTR (HR =0.27; 95% CI, 0.10-0.75; P=0.01) and improved OS (HR =0.27; 95% CI, 0.10-0.74; P=0.01) in patients treated with surgery alone. However, such advantage of dMMR over pMMR was attenuated in patients treated with adjuvant 5-FU (TTR: HR =0.81, 95% CI, 0.55-1.19; P=0.29; OS: HR =0.87; 95% CI, 0.61-1.26; P=0.47). Among stage III patients receiving surgery alone, those with dMMR tumors were also found to have better outcome (TTR: HR =0.59; 95% CI, 0.28-1.23; P=0.16; OS: HR =0.69; 95% CI, 0.35-1.36; P=0.28)

vs. pMMR cases. In stage III CRC patients, a significant survival benefit for 5-FU monotherapy *vs.* surgery alone was seen in patients with pMMR tumors (5-year TTR =64% *vs.* 47%), but also in patients with dMMR tumors (5-year TTR =72% *vs.* 60%). These findings support the current and recommended management of non-metastatic CRC whereby stage II patients with dMMR tumors are spared adjuvant 5-FU due to lack of efficacy, whereas all stage III patients received adjuvant chemotherapy irrespective of MMR status.

In a study that evaluated 2,141 stage II and stage III colon cancers from 5-FU-based adjuvant therapy trials, patients with dMMR colon cancers were shown to have reduced rates of tumor recurrence, delayed TTR, and improved survival rates compared with patient with pMMR cancers (41). Furthermore, an exploratory subset analysis suggested that dMMR tumors with suspected germline mutations (i.e., LS) had improved disease-free survival (DFS) after 5-FU-based treatment (DFS: HR =0.26; 95% CI, 0.09-0.77; P=0.009) compared with sporadic dMMR tumors where no benefit was observed (DFS: HR =0.79; 95% CI, 0.35-1.80; P=0.58). These preliminary findings raise the possibility that the utility of MMR status as a predictive factor for 5-FU treatment might differ according to the molecular mechanism underlying dMMR/MSI, which awaits further evaluation.

Treatment with standard 5-FU plus oxaliplatin adjuvant therapy

At present, the use of oxaliplatin in combination with adjuvant 5-FU chemotherapy is the standard of care for stage III colon cancer patients (42-44). Preclinical studies have shown that dMMR tumor cells are susceptible to oxaliplatin despite displaying resistance to 5-FU (45). To date, limited data are available for the prognostic/predictive impact of MMR on chemosensitivity to oxaliplatin-based treatment (46-49). In a retrospective study that included 303 unselected stage III colon cancer patients who received adjuvant FOLFOX, MMR status was a prognostic factor conferred a better DFS for patients with dMMR compared to pMMR tumors (50). Gavin *et al.* reported an analysis of 2,299 stage II and stage III colon cancers from participants in National Surgical Adjuvant Breast and Bowel Project (NSABP) adjuvant studies, including C-07 (5-FU plus LV ± oxaliplatin) and C-08 (FOLFOX ± bevacizumab) trials (51). The authors reported that dMMR was associated with better prognosis for recurrence in patients treated with FOLFOX

compared with pMMR (TTR: HR =0.58; 95% CI, 0.35-0.96; P=0.03). However, MMR status was not predictive of oxaliplatin efficacy, since the interaction test between MMR status and treatment was not statistically significant. Flejou *et al.* reported the results of MMR status in 986 of the 2,240 patients enrolled in the Multicenter International Study of Oxaliplatin/5-FU LV in the Adjuvant Treatment of Colon Cancer (MOSAIC). The authors found that the DFS benefit from FOLFOX compared with 5-FU alone was also evident in patients with dMMR colon cancers (52). Taken together, available data suggest a potential benefit for oxaliplatin in node-positive dMMR colon cancers and therefore, do not support any change in the current therapy of these patients.

Patients treated with 5-FU plus irinotecan-based adjuvant therapy

It is important to emphasize that two randomized phase III studies [Cancer and Leukemia Group B (CALGB) 89803 (53) and Pan-European Trials in Alimentary Tract Cancers 3 (PETACC-3) trials (54)], failed to show the benefit of adding irinotecan to 5-FU as adjuvant chemotherapy in the treatment of stage III colon cancer patients. Thus unlike oxaliplatin, irinotecan is not used in the adjuvant setting. Preclinical studies including *in vitro* and xenograft model systems found that dMMR tumor cells exhibited sensitivity to irinotecan (55-57). In a retrospective analysis of 702 stage III colon cancer patients included in the CALGB 89803 trial, those with dMMR (n=96) who were treated with IFL (irinotecan, 5-FU and LV) had significantly improved 5-year DFS as compared with IFL-treated pMMR patients (n=606) (5-year DFS: 76% *vs.* 59%; HR =0.53; 95% CI, 0.29-0.96; log-rank P=0.03) (58) that was not observed among patients treated with 5-FU/LV. However, this finding was not supported by an analysis of 1,254 patients included in the PETACC-3 study (59) where the addition of irinotecan to 5-FU/LV did not show significantly improved survival in patients with dMMR tumors.

Patients treated with targeted therapies in an adjuvant setting

Recent success of biologic agents in the metastatic setting such as the use of antibodies directed against vascular endothelial growth factor (VEGF) or epidermal growth factor receptor (EGFR), resulted in the evaluation of these agents in the adjuvant setting. However, phase III adjuvant

trials failed to show a significant survival benefit for anti-VEGF (60,61) or anti-EGFR antibodies (62-64) combined with adjuvant chemotherapy in patients with stage III colon cancer. In the NSABP C-08 trial where no benefit for the addition of bevacizumab to FOLFOX therapy was observed (60), a post hoc analysis found that patients with dMMR tumors derived a statistically significant survival benefit from the addition of bevacizumab (HR =0.52; 95% CI, 0.29-0.94; P=0.02) compared with patients with pMMR tumors (HR =1.03; 95% CI, 0.84-1.27; P=0.78) (65). The mechanism responsible for bevacizumab benefit in dMMR tumors is unknown, and confirmation of these data is needed. The North Central Cancer Treatment Group (NCCTG) N0147 trial tested the addition of cetuximab to FOLFOX (63) or FOLFIRI (irinotecan, 5-FU and LV) (64) adjuvant chemotherapy in the treatment of stage III colon cancer. The FOLFIRI-containing arms were discontinued (64) when other contemporary trials demonstrated no benefit to using irinotecan as adjuvant therapy (53,54). The addition of cetuximab to FOLFOX failed to improve DFS, the primary endpoint of this study compared to FOLFOX alone (63). Several biomarker analyses have been conducted using prospectively collected biospecimens from this study (25,66-69), where the treatment arms were combined based on the finding of no interaction between MMR status and treatment (FOLFOX ± cetuximab). In the N0147 trial, dMMR was detected in 314 (12%) of 2,580 stage III colon cancer patients and was not prognostic overall for DFS (HR =0.82; 95% CI, 0.64-1.07; 225 P=0.14) (66). Interestingly, favorable DFS was observed for dMMR *vs.* pMMR tumors in the proximal colon (HR =0.71; 95% CI, 0.53-0.94; P=0.018), but not in the distal colon (HR =1.71; 95% CI, 0.99-2.95; P=0.056), after adjustment for *KRAS* and *BRAF*^{V600E} mutations and relevant covariates (66).

Conclusions

Abundant evidence suggests that MMR status is a valuable prognostic and predictive biomarker for non-metastatic CRC. Tumors with dMMR/MSI have a distinct phenotype and consistent data support dMMR as a biomarker of better stage-adjusted survival. While the majority of dMMR CRCs are sporadic, one-third arises in the setting of LS that has critical implications for patients and family members. To improve the identification of these patients in clinical practice, it has been recommended that all resected CRC be analyzed for MMR status. The excellent prognosis of

resected stage II colon cancers with dMMR and evidence of lack of 5-FU benefit supports the recommendation to not administer adjuvant 5-FU chemotherapy in this population. In stage III CRC patients in whom oxaliplatin-based adjuvant chemotherapy is the current standard of care, there remain no convincing evidence to exclude such patients with dMMR tumors from receiving adjuvant FOLFOX. Accordingly, MMR status does not influence chemotherapy decisions in stage III patients. Recent and emerging data underscore molecular heterogeneity in CRCs and in the subset of dMMR tumors. Studies in pooled data from similar clinical trials may help to further explore this tumor heterogeneity and to decipher its impact on patient prognosis and on the efficacy of current chemotherapy regimens.

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Footnote

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