# Mismatch repair deficient colorectal cancer in the era of personalized treatment

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**Abstract** | The molecular and genetic subtyping of cancer has allowed the emergence of individualized therapies. This approach could potentially deliver treatments that have both increased efficacy as well as reduced toxicity. A well-defined subtype of colorectal cancer (CRC) is characterized by a deficiency in the mismatch repair (MMR) pathway. MMR deficiency not only contributes to the pathogenesis of a large proportion of CRC, but also determines the response to many of the drugs that are frequently used to treat this disease. In this Review we describe the MMR deficient phenotype and discuss how a deficiency in this DNA repair process may impact on the management of CRC, including surgery, adjuvant chemotherapy and the choice of systemic agents for the palliation of advanced disease. We also discuss how the DNA repair defect in MMR deficient CRC could be exploited in the development of novel therapeutic strategies.

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## Introduction

Colorectal cancer (CRC) is the third most common cancer type and the third leading cause of cancer deaths in developed countries. More than 145,000 people are diagnosed with CRC each year in the US alone.[1] It is understandable, therefore, that major efforts have been directed at dissecting the underlying pathogenesis of this disease. Pivotal to these studies has been the growing appreciation of the genetic heterogeneity that exists within CRC. One of the most studied genotypic subtypes of CRC is that characterized by a deficient mismatch repair (dMMR) pathway, usually found in combination with microsatellite instability (MSI). This Review provides some background to dMMR in CRC before outlining its impact on standard management. We also discuss how the dMMR phenotype could be exploited in developing novel therapeutic strategies. These issues are currently of renewed interest due to the publication of new clinical data that suggests a differential response to chemotherapeutics in the dMMR population.

## The mismatch repair system

The DNA content of each cell is replicated prior to cell division. This is an error-prone process that may lead to the introduction of incorrect DNA bases into newly synthesized DNA (base-base mismatches) or the formation of unmatched DNA loops (insertion–deletion loops). If left unrepaired, these errors may result in permanent mutations that could change the behavior of a cell and foster tumorigenesis. Cells have evolved a number of DNA repair mechanisms to counter these errors, including the MMR system. In brief, this pathway involves four key processes: recognition of the erroneous bases or insertion–deletion loops, excision of these lesions, substitution of the lesion with the correct sequence, and religation of the DNA (Figure 1). Instrumental to this process are the two protein dimer complexes hMutS and hMutL. The initial recognition of mismatches is performed by hMutS, which is found in two major forms, as hMutSα (a hMSH2/hMSH6 dimer) or hMutSβ (a hMSH2/hMSH3 dimer). hMutSα recognizes base-base mismatches and short insertion–deletion loops, whereas hMutSβ detects larger mismatches. When hMutS binds to a DNA lesion it initiates the recruitment of hMutL, a second protein complex normally comprised of MLH1 and PMS2. hMutL coordinates the recruitment of additional proteins that complete DNA repair. MMR proteins also affect mitotic and meiotic recombination, cell-cycle control at the G2 checkpoint, and apoptosis in response to particular types of DNA damage.[2]

The repair process fails when MMR proteins are deficient or defective and unrepaired mutations become scattered throughout the genome, a situation known as the mutator phenotype. For example, mutation rates are 100–1,000-fold greater in human tumor cells with dMMR than in normal cells.[3,4] In CRC, dMMR and the mutator phenotype can lead to loss-of-function mutations in tumor suppressor genes, such as *TGFBR2*, *IGF2R* and *PTEN*, as well as causing gain-of-function mutations in a number of oncogenes (Figure 2).[5–13] These secondary mutations are thought to drive the oncogenic process, so the inheritance of loss-of-function MMR gene mutations

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**Key points**

- Deficient MMR (dMMR) in colorectal cancer (CRC) occurs as a result of inherited or sporadic abnormalities
- Phenotypic characteristics, such as proximal anatomical location, mucinous features, lymphocytic infiltration, and pushing margins help to identify dMMR tumors
- The presence of dMMR in a tumor may be of relevance to the surgical management and systemic treatment of a patient
- Preclinical investigations suggest that cancer cells with dMMR show resistance to 5-fluorouracil, but are sensitive to irinotecan and mitomycin C; clinical data corroborate these findings although further studies are required
- Heterogeneity exists within the dMMR CRC subtype, which could be explained by the presence or absence of secondary mutations that occur as a consequence of the dMMR-associated mutator phenotype
- dMMR and the mutations that arise as a result of this deficiency could be exploited as novel therapeutic targets

predisposes patients to a range of tumor types, including CRC, gastric, endometrial and bladder cancers.[14,15]

Improvements in molecular techniques have allowed the routine assessment of dMMR status in tumors as part of clinical care. Furthermore, a number of dMMR cell models exist, which means that therapeutic approaches to treating this particular subset of CRC can be assessed *in vitro* and readily translated into the clinical setting. Published work suggests that dMMR tumor cells have a distinct response to standard treatments and also to many emerging therapies for CRC.

## dMMR in CRC

Current estimates suggest that dMMR is present in 15–17% of all primary CRC.[16,17] This deficiency may arise from the inheritance of a mutation in an MMR gene, somatic (non-inherited) MMR gene alterations, epigenetic suppression of MMR gene expression, or a combination of these factors.[15,16] MMR genes generally function as classical tumor suppressor genes, where loss-of-function of both alleles is required for loss of the tumor suppressive effect, which conforms to the Knudson two-hit hypothesis.[18]

The heritable disorder associated with dMMR in adults is Lynch syndrome, also known as hereditary non-polyposis colon cancer. Lynch syndrome is an autosomal dominant condition, associated with a predisposition to multiple cancers, and accounts for approximately 3% of all CRC cases (Table 1).[14,19] Lynch syndrome results from a germline loss-of-function mutation in either *MLH1, MSH2, PMS2* or *MSH6* (the first hit). The causative germline abnormality in an MMR gene is usually a nonsense or frameshift mutation. This can lead to a truncated protein, although gross genomic alterations that cause MMR gene loss have been identified in a minority of cases.[20] The second hit normally happens somatically. Loss of heterozygosity, mutations, gene conversion, and promoter methylation have all been described as causative mechanisms for the loss-of-function of the remaining allele.[16,20] Other mechanisms that lead to Lynch syndrome have also been described, such as hemiallelic methylation of *MLH1*[21,22] or *MSH2*.[23] Biallelic inherited

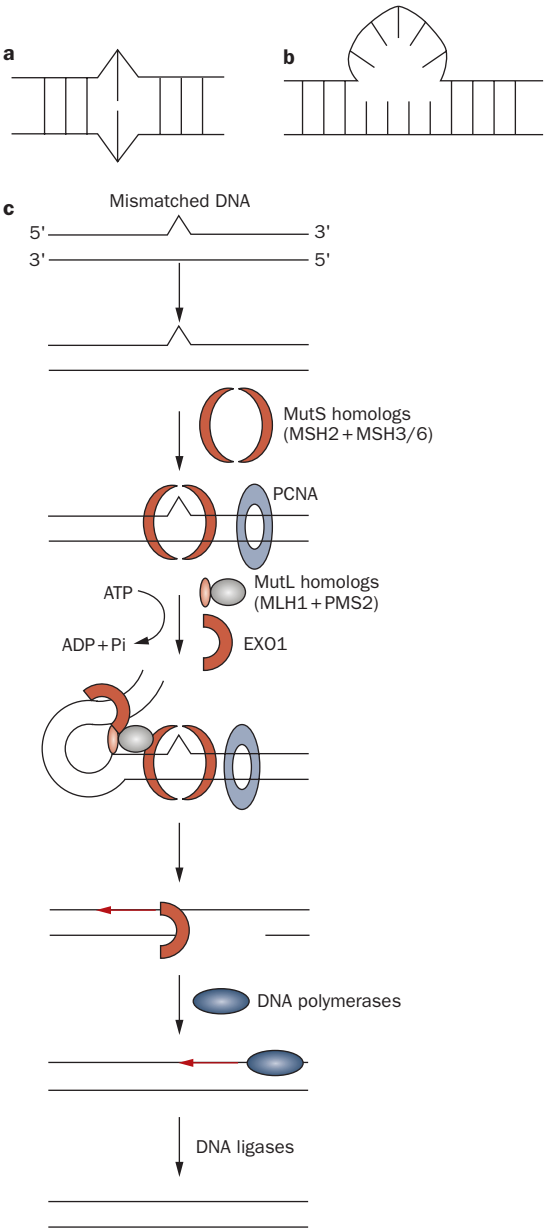


**Figure 1** | Schematic representation of mismatches and the MMR pathway. The MMR system recognizes **a** | a base–base mismatch or **b** | an insertion–deletion loop. **c** | MutS homologs bind to the affected site of DNA, which triggers ATP-dependent conformational changes and the binding of MutL homologs. These in turn recruit other proteins, including PCNA and exonucleases with the subsequent excision of the damaged strand. The interactions of the bound proteins trigger DNA looping, which brings the two sites together. The resultant gap in the strand is then filled by DNA polymerases and the break is removed by DNA ligase.[2,127] Abbreviations: EXO1, exonuclease 1; PCNA, proliferating-cell-nuclear-antigen.

dysfunction in MMR genes is found in the constitutional mismatch repair-deficiency syndrome. This syndrome generally presents in childhood with multiple malignancies, including CRC, leukemias and lymphomas.[24,25] Sporadic dMMR constitutes the majority of dMMR CRC (12–13% of all CRC cases), and the cause is frequently

suppression of *MLH1* transcription owing to biallelic hypermethylation of the *MLH1* promoter.[9,16] The association between MMR defects and Lynch syndrome is summarized in Table 1.

## The dMMR phenotype

Although there are typical clinicopathological features associated with the dMMR phenotype (Box 1), 40% of Lynch syndrome cases cannot be reliably diagnosed based on morphological criteria alone.[26] Debate persists regarding the degree of morphological heterogeneity between Lynch syndrome tumors and CRC associated with sporadic dMMR.[26–28] Whereas some groups have provided evidence that Lynch syndrome tumors are more closely linked with medullary and signet-ring subtypes and mucinous features, others have suggested that mucinous features and poor differentiation are more frequent in sporadic dMMR tumors.[27,28] A strong relationship exists between sporadic dMMR CRC and the CpG island methylator phenotype (CIMP) subtype of CRC. CIMP is characterized by the regional hypermethylation of CpG islands in DNA and can result in loss of *MLH1* expression,[29] thus leading to dMMR. CIMP CRC is usually found in serrated polyps rather than classical adenomas and is strongly associated with oncogenic *BRAF* V600E mutations, older age and female sex, so some heterogeneity in phenotype between Lynch syndrome cancers and sporadic dMMR CRC might be expected.[27–29]

## Identification of dMMR

Complementary approaches exist that enable the identification of dMMR in tumors. Mutations in MMR genes can be identified by DNA sequencing, and the presence or absence of MMR proteins can be assessed by immunohistochemistry on tumor samples (Table 1).[14,30] In addition, the mutator phenotype can be measured by assessing MSI status. Microsatellites are short (1–6 base pairs), repetitive DNA sequences that are interspersed throughout the genome.[31,32] The repetitive nature of microsatellites results in a susceptibility to replication errors caused by the slippage of DNA polymerases over tandem repeats. These replicative errors can normally be corrected by MMR; in dMMR, however, the errors become fixed and the length of microsatellites is altered. MSI can be easily measured in extracted tumor DNA[31,32] and this analysis is now internationally standardized.[33] Tumors are defined as MSI (interchangeable with MSI-High or MSI+) if ≥ two microsatellite markers within a defined panel of five markers show instability, MSI-Low where one of the five markers are positive, and microsatellite stable (MSS) where none of the five markers show instability. Some classify MSI-Low tumors as a unique clinical phenotype, whilst others group MSI-Low with MSS.[29] Whereas MSI correlates well with the biallelic loss of *MLH1*, *MSH2* and *PMS2*,[16,32] a large proportion of cases with *MSH6* deficiency do not exhibit MSI, presumably due to a degree of functional redundancy in the role of hMutSα.[30,34] Additional analysis would, therefore, be required if MSI was not present and *MSH6* deficiency was suspected from the clinical or family history.

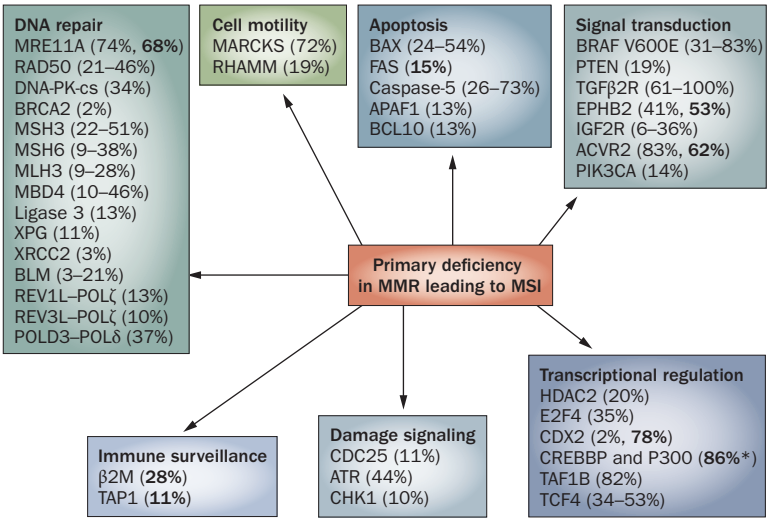


**Figure 2** | Genes mutated in dMMR cells and tumors. dMMR commonly results in the development of a mutator phenotype and the accumulation of mutations in microsatellites throughout the genome. Genes affected include those with critical roles in tumor biology. Where known, the mutational frequency in tumor tissue is shown, as well as loss of expression frequency (in bold). The asterisk (*) indicates where frequency was assessed only in cell lines derived from MSI tumors and not directly in tumor tissue.[5–13,128,129] Abbreviations: dMMR, deficient mismatch repair; MMR, mismatch repair; MSI, microsatellite instability.

**Table 1** | MMR genes associated with Lynch syndrome[14,30,34,132]

| Affected gene | Contribution to Lynch syndrome cases (%) | Median age at presentation (years) | Features of IHC | Sensitivity of IHC in germline mutation detection (%) | Sensitivity of MSI testing in germline mutation detection (%) |
|---|---|---|---|---|---|
| *MLH1* | 32 | 45 | Loss of PMS2 expression | 92 | 92 |
| *MSH2* | 39 | 45 | Loss of MSH6 expression | 93 | 93 |
| *MSH6* | 14 | 56 | Isolated protein loss | 100 | 25 |
| *PMS2* | 15 | 59 | Isolated protein loss | 100 | 67 |
| All | 100 | 40–60 | – | 83–94 | 83–85 |

Abbreviations: IHC, immunohistochemistry; MMR, mismatch repair; MSI, microsatellite instability.

The Revised Bethesda Guidelines were developed to identify those at risk of Lynch syndrome and indicate tumors that should be tested for dMMR and MSI (Box 2).[33] There have been attempts to improve these guidelines,[35–37] and other parameters, such as *BRAF* V600E mutational analysis and methylation status of *MLH1* can also be used to exclude patients with sporadic dMMR.[9,28,38]

## Heterogeneity within dMMR

Heterogeneity in the clinical phenotype of dMMR CRC is expected as a result of the different mechanisms by which it occurs (Figure 2). The median age at presentation (45 years) of Lynch syndrome cases associated with

**Box 1** | Features associated with dMMR tumors[15,28]

- Proximal (70% proximal to the splenic flexure)
- Poorly differentiated
- Large and lymph-node-negative
- Excess of mucinous (15%), signet cell and medullary subtypes (highly characteristic)
- Excess of tumor-infiltrating lymphocytes and Crohn-like reaction
- Accelerated carcinogenesis from tubulovillous adenoma to carcinoma within 2–3 years in Lynch syndrome cases

Abbreviation: dMMR, deficient mismatch repair.

**Box 2** | Revised Bethesda Guidelines for dMMR testing[33]

1. CRC diagnosed in a patient aged <50 years
2. Presence of synchronous, metachronous colorectal or other Lynch syndrome-related tumor*, regardless of age
3. CRC with MSI phenotype‡ diagnosed in a patient aged <60 years
4. Patient with CRC and a first-degree relative with a Lynch syndrome-related tumor, with one of the cancers diagnosed in a patient aged <50 years
5. Patient with CRC with two or more first-degree or second-degree relatives with a Lynch syndrome-related tumor, regardless of age

*Lynch syndrome-related tumors include colorectal, endometrial, stomach, ovarian, pancreas, ureter, renal pelvis, biliary tract and brain tumors, sebaceous gland adenomas and keratoacanthomas, and carcinoma of the small bowel. ‡MSI phenotype includes the presence of tumor-infiltrating lymphocytes, a Crohn-like lymphocytic reaction, and mucinous, signet-ring, or medullary features. Abbreviations: CRC, colorectal cancer; dMMR, deficient mismatch repair; MSI, microsatellite instability.

MLH1 or MSH2 deficiency is considerably younger than those with MSH6 deficiency (56 years, Table 1). Similarly, the lifetime risk of developing a particular cancer varies—*MLH1* mutations are associated with a higher risk of colonic cancers, and *MSH2* mutations with a higher incidence of extra-colonic disease. *MSH6* mutations are particularly associated with adenocarcinoma of the endometrium.[14] The spectrum of secondary mutations that occur as a result of dMMR may affect tumor biology (*TGFB2R*, *ACVR2*) and therapeutic response (*MRE11A*, *hRAD50*, *PIK3CA*),[5] and their presence likely determines the nature of the disease and outcome of the patient.

## The management of dMMR CRC

dMMR CRC represents a distinct cancer subtype in terms of pathogenesis and this phenotype may also determine the response to therapy. How the management of CRC may be modified by the presence of dMMR is outlined below.

### Management and surveillance in Lynch syndrome

The recommended surveillance of patients with Lynch syndrome includes more frequent colonoscopic examination (every 1–2 years) than the usual standard of care following primary CRC (every 3 years) (Table 2). Although subtotal colectomy has been advocated as an alternative to regular colonoscopies, it may be associated with a poorer quality of life and rectal surveillance is still required.[15,39,40] Neoadjuvant strategies are now being used in the management of CRC,[41] which could provide a window to test diagnostic material so that surgical management may be appropriately informed (Figure 3).

### dMMR as a prognostic marker

Although some studies have produced contradictory results, a meta-analysis of available data confirms dMMR with MSI as an independent prognostic marker associated with a favorable outcome in CRC.[17] In this study, MSI CRC was associated with a significantly improved overall survival regardless of stage (combined hazard ratio (HR) 0.65, 95% CI 0.59–0.71).[17] Patients treated with adjuvant 5-fluorouracil (5-FU) had a better prognosis in the presence of MSI (HR 0.72, 95% CI 0.61–0.84).[17] Additional evidence in support of a good outcome for dMMR tumors can be found in three large-scale studies assessing dMMR prevalence in metastatic disease.[42–44] Only approximately 4% of metastatic tumors exhibited MSI compared with 15–17% of primary cancers, reinforcing the view that dMMR is associated with a less aggressive natural history.

Caveats remain, however. First, CRC with a *BRAF* V600E mutation has been associated with an adverse outcome.[45,46] Given the association between *BRAF* V600E and sporadic dMMR, it might be expected that these cases would have a worse prognosis than familial cases.[9] This has been addressed in a study that analyzed MSI and *BRAF* V600E status in tumor tissue from patients treated with adjuvant chemotherapy. MSI without a *BRAF* V600E mutation was associated with a significantly better prognosis ($P = 0.002$), whereas patients with tumors that exhibited both MSI and *BRAF* V600E had a similar disease-free survival to those without MSI.[47] The second caveat concerns chromosomal instability. It is well known that MSS CRC is associated with chromosomal instability and aneuploidy, where gross genomic and chromosomal alterations are observed. Although MSI and chromosomal instability are not mutually exclusive,[48,49] there is an inverse association between the two. Chromosomal instability is an independent marker of poor prognosis[50] and in agreement with this, one study has shown that the prognostic effect of MSI disappears when chromosomal instability, or ploidy, is also taken into account.[51,52]

### dMMR as a marker of response to chemotherapy

Preclinical studies that have assessed the response of dMMR cells to chemotherapy generally fall into one of two categories. One approach uses a matched, isogenic pair of cell lines, where the difference between the cell lines is the loss-of-function of a particular MMR gene. The second approach treats MSI cancers as a whole and compares treatment responses in a panel of MSI cell lines with a panel of MSS cancer cell lines. While both yield

valuable information, the first approach highlights the function of specific MMR genes, whereas the latter provides more information regarding the relative therapeutic sensitivites of MSI tumors as a whole.

### *5-FU and related compounds*

The fluorinated pyrimidine analog 5-FU and its derivatives form the basis of most chemotherapy regimens for CRC. 5-FU is commonly administered with leucovorin (also known as folinic acid) as a biomodulator to improve efficacy, although levimasole and methotrexate have also been used with 5-FU historically.[53] The oral prodrug capecitabine and the folate inhibitor of thymidylate synthetase, raltitrexed, are sometimes used in place of 5-FU.[54,55] 5-FU is metabolized to a series of different derivatives that may elicit antitumor activity. FdUMP is one derivative that inhibits thymidylate synthetase, which is essential for the generation of nucleotides required for DNA replication, whereas 5FdUTP is incorporated directly into DNA and 5FUTP into RNA.[56] 5FUTP incorporation is thought to be cytotoxic as it interferes with RNA processing, whilst a 5FdUTP lesion in DNA may be lethal by causing DNA strand breaks or triggering apoptosis.

Although not all studies agree,[57] most suggest that dMMR is associated with resistance to treatment at clinically achievable steady-state plasma concentrations of 5-FU.[58] These observations have been reproduced using a range of MLH1, MSH2 and MSH6-deficient models and assay systems.[55,59,60] dMMR tumor cells grown *in vitro* are approximately 18-fold more resistant to 5-FU and its analogs compared with MMR proficient cells.[61] The resistance of a cell line to 5-FU that is MLH1-deficient due to a methylated *MLH1* promoter, was reversed by re-expressing MLH1 with the use of the demethylating agent 5-azacytidine.[62] Xenograft experiments confirm that MSI CRC shows resistance to 5-FU.[63]

It seems that this resistance of dMMR cancer cells to 5-FU is due to the incorporation of 5-FU metabolites into DNA, rather than their effects on thymidylate synthetase or incorporation into RNA.[55,62,64] Whilst dMMR cells show resistance to 5-FU, they are not resistant to raltitrexed, which is a specific inhibitor of thymidylate synthetase and does not become incorporated into DNA.[55] A number of models have been proposed to explain the tolerance of dMMR tumor cells to 5-FU, most notably the so-called futile cycling model (Figure 4).

A meta-analysis published in 2005 suggested that patients with MSI tumors did not show significant benefit from adjuvant 5-FU-based chemotherapy (HR 1.24, 95% CI 0.72–2.14);[17] this finding has been confirmed by most subsequent studies (Table 3). Adjuvant chemotherapy only improves overall survival by approximately 3% in patients with stage II disease[65] and these patients do not seem to benefit from 5-FU-based treatment.[66] It has been recommended, therefore, that stage II CRC should be analyzed for dMMR to guide decisions regarding treatment.[66] Although these studies have been prone to confounding variables, possible biological reasons for the reported differences between studies include the inherent heterogeneity within dMMR and the effect of the biomodulators used.[66,67] Leucovorin potentiates the effects of 5-FU as a thymidylate synthetase inhibitor,[67] whereas levamisole acts as an immune modulator. Levamisole has been used to treat conditions associated with an excessive cytotoxic T-cell response.[68,69] Since MSI tumors are particularly immunogenic with increased levels of tumor-infiltrating leukocytes,[70] levamisole might reduce the beneficial immune response associated with dMMR tumors. Based on these data, clinical trials are underway to assess the effects of MSI prospectively. The US Intergroup study E5202[71] is determining the likely risk of relapse after adjuvant treatment for stage II CRC based on initial stratification by MSI status and loss of heterozygosity at 18q; low-risk patients in this trial are subject to observation whereas high-risk cases receive a combination of leucovorin, 5-FU, oxaliplatin (FOLFOX) and bevacizumab.[71]

The interpretation of data in the metastatic setting is made difficult by the low prevalence of dMMR in

**Table 2** | Surveillance recommendations in Lynch syndrome[14,133]

| Primary tumor site | Recommendation | Frequency |
|---|---|---|
| Colorectal | Colonoscopy from age 20–25, or age 30 (*MSH6* mutation) | Every 1–2 years |
| Endometrium | Transvaginal ultrasound and endometrial biopsy from age 30–35 | Every 1–2 years |
| Gastric | Endoscopy of the upper gastrointestinal tract from age 35 if there is a family history of gastric cancer | Every 1–2 years |
| Urothelial | Ultrasound and cytology from age 25–35 | Every 1–2 years |

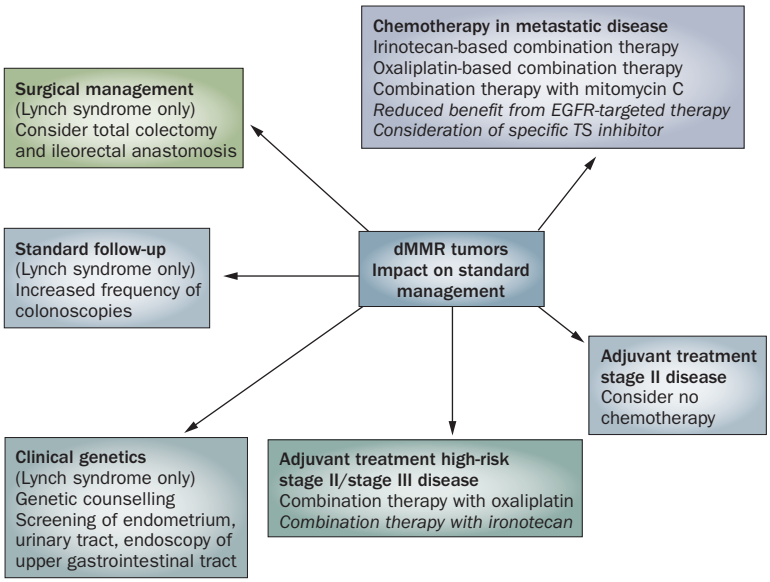


**Figure 3** | The impact of dMMR on the management of colorectal cancer. The presence of dMMR in a tumor sample may affect many aspects of management. This includes screening, the systemic therapy given and patient follow-up. Recommendations for where there is a reasonable evidence-base are shown. Interpretations from *in vitro* data (in italics) require further examination in clinical studies. Abbreviations: dMMR, deficient mismatch repair; TS, thymidylate synthetase.

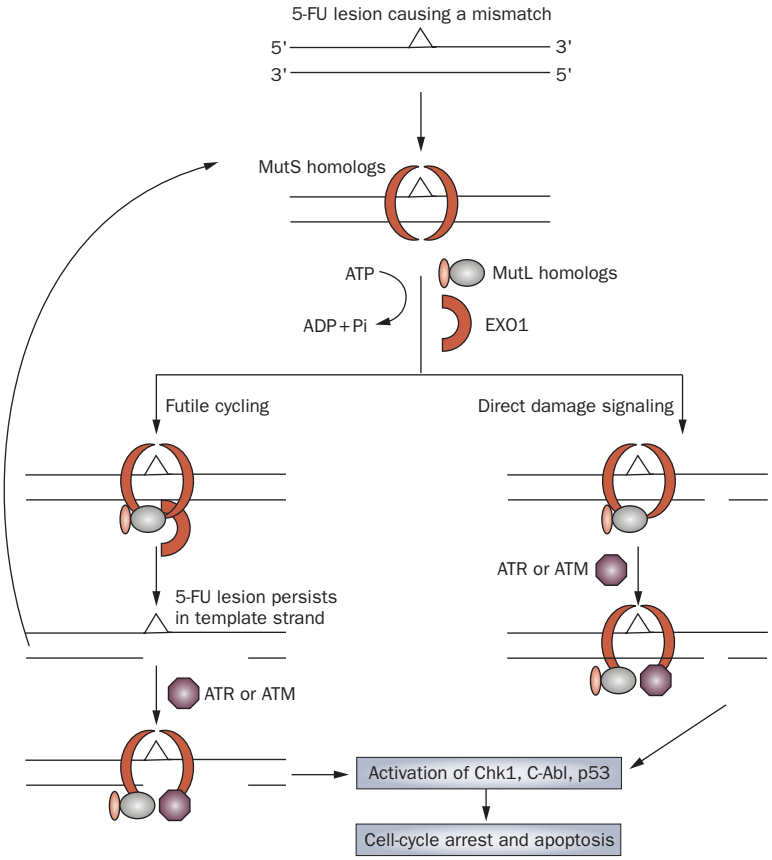


**Figure 4** | The futile cycling and direct damage signaling models.[2,119] The presence of a mismatch, such as that produced by the insertion of 5FdUTP into DNA, is detected by MutS homologs and results in the recruitment of MutL homologs. In the futile cycling model, MutL removes part of the newly synthesized strand, and the remaining strand that still contains 5FdUTP is used as a template to resynthesize DNA, which leads to a futile cycle of mismatches. The persistence of strand breaks hinder the progression of replication forks, which leads to the recruitment of ATM/ATR and ultimately cell-cycle arrest and apoptosis. In dMMR cells these mismatches are not recognized and the cells survive. Alternatively, the direct signaling model proposes that in a normal cell with many 5dUTP-containing mismatches, MutS and MutL directly interact with ATM/ATR and cause cell-cycle arrest and apoptosis, rather than mediating repair.[130] Abbreviations: ATM, ataxia telangiectasia mutated; ATR, ataxia telangiectasia and Rad3 related; EXO1, exonuclease 1; 5-FU, 5-fluorouracil; MMR, mismatch repair.

patients with metastasis. A small meta-analysis assessed MSI as a predictive marker of response to chemotherapy in metastatic CRC and confirmed that MSI status did not significantly predict the effect of chemotherapy (HR 0.95, 95% CI 0.65, $P = 0.11$).[72]

How the findings from *in vitro* models can be translated into the clinical setting depends on additional variables. Confounding factors include thymidylate synthetase levels and the function of this enzyme, as high levels confer resistance to 5-FU.[73] Most published data, including a study of 320 samples from adjuvant trials,[74] suggest no relationship between thymidylate synthetase and MMR status.[75,76] Another confounding factor is the overlap between CIMP and MSI, and between CIMP, MSI and *BRAF* V600E. Regardless of MSI and *BRAF* status, CIMP has been associated with a favorable prognosis.[45] The data supporting CIMP as a predictive marker of response to chemotherapy are very limited, however, and inconclusive at present.[77,78] Alternative panels of CIMP markers are in use,[79,80] which require standardization before meaningful conclusions can be reached regarding the interaction of CIMP, MSI and response to chemotherapy.

### *Platinum compounds in CRC*

Repair mechanisms associated with the platinum salts oxaliplatin, cisplatin and carboplatin differ as a result of the distinct molecular shapes of their DNA adducts. Oxaliplatin, a third-generation platinum salt, contains a bulky 1,2-diaminocyclohexane moiety that becomes incorporated into DNA via cytotoxic intrastrand and interstrand adducts. This creates similar, though larger DNA lesions to cisplatin and carboplatin.[81] These adducts cause cytotoxicity by disrupting cellular processes, such as replication and transcription, which ultimately trigger apoptosis when adequate repair is not possible.[82]

It has been established from several studies that dMMR is associated with resistance to cisplatin and carboplatin treatment, which may be explained by the role of MMR in recognizing cisplatin and carboplatin adducts in DNA.[82,83] Since MMR proteins do not recognize oxaliplatin-related adducts, resistance to oxaliplatin does not occur with dMMR. Oxaliplatin should on this basis provide equivalent benefit to patients regardless of the MMR status of their tumors.[57,84]

### *Topoisomerase inhibitors*

DNA is normally tightly packaged in a supercoiled structure, which must be relaxed for replication and transcription to occur. Topoisomerase I covalently binds to double-stranded DNA and induces transient single-strand breaks, which allow the passage of one strand of DNA around the other as it unwinds. The single-strand breaks are then repaired and transcription can ensue. Camptothecin and its derivative irinotecan are topoisomerase I inhibitors that cause cytotoxicity by either blocking the unwinding of DNA ahead of replication forks or by causing a persistence of single-strand breaks.[85,86]

Studies examining topoisomerase inhibitor response and MMR status are conflicting; some groups suggest that dMMR is associated with resistance to topoisomerase inhibitors,[87,88] whereas others have found no differential response.[86,89] Further studies observed that both MSH2 and MLH1-deficient cell lines were more sensitive to irinotecan and etoposide when given continuously.[90,91] This apparent disparity can be explained by the cell line model used. dMMR paired cell lines are often created using a tumor cell line that possesses a MMR defect together with established MSI and secondary mutations, and then correcting the MMR gene defect alone.[84,87] These secondary mutations are, however, not present in most MSS CRC cell lines.[91] The paired MMR deficient/proficient models may not consider the effects of secondary mutations whereas the panel of MSI versus MSS cell lines will. Most MSI CRC tumors and cell lines possess secondary mutations in *MRE11A* and *hRAD50*,[7,91] which are constituent parts of the

**Table 3** | Trials assessing the effect of dMMR on 5-FU-based treatment outcomes

| Reference | Tissue resource | Analyzed/ total | MSI frequency | Stage | Treatment | Result |
|---|---|---|---|---|---|---|
| Kim *et al.* (2007)[134] | NSABP trials between 1977 and 1990 | 542/ 5,555 | 18.1% | Dukes B & C | 5-FU-based, portal or systemic vs no treatment | No predictive value of MSI |
| Jover *et al.* (2009)[135] | EPICOLON project | 505/754 | 10.1% | Stage II & III | 5-FU-based vs no treatment | Benefit of 5-FU restricted to pMMR alone (log rank $P=0.00001$ pMMR, $P=0.7$ dMMR) |
| Sargent *et al.* (2008)[66] | NCCTG, GIVIO, ECOG and data from Ribic *et al.*[136] | 512 total | 15% | Stage II & III | 5-FU+levamisole, 5-FU+FO vs no treatment | Benefit of 5-FU restricted to pMMR alone (pMMR OS HR 0.69, $P=0.047$, dMMR OS HR 1.26, $P=0.68$). 5-FU treatment associated with inferior outcome in stage II disease HR 2.8, $P=0.05$ |
| Tejpar *et al.* (2009)[137] | PETACC 3 trial | 1,254/ 3,278 | 22% stage II 12% stage III | Stage II & III | 5-FU+FO vs 5-FU +FO+irinotecan | Prognostic effect of MSI in patients treated with 5-FU (HR 0.05, $P=0.0077$) |

Abbreviations: dMMR, deficient mismatch repair; ECOG, Eastern cooperative oncology group; 5-FU, 5-fluorouracil; FO, folinic acid; GIVIO, interdisciplinary group for cancer care evaluation; HR, hazard ratio; MSI, microsatellite instability; NCCTG, North central cancer treatment group; NSABP, national surgical adjuvant breast and bowel project; OS, overall survival; PETACC, pan-European trials in adjuvant colon cancer; pMMR, proficient mismatch repair.

double-strand break repair complex MRE11A–hRAD50–NBS1 (MRN). Since double-strand break repair genes sensitize cells to topoisomerase 1 inhibitors,[86,92] the sensitizing effect will only be seen when MSI cells are compared with MSS cells. Xenograft data also demonstrate that MSI is more sensitive to irinotecan than MSS.[93] The clinical data on dMMR and irinotecan is inconclusive, possibly because irinotecan is often used in combination with 5-FU, to which dMMR cells are resistant (Table 4). One study does suggest, however, that in the adjuvant setting, the MSI population derives comparatively more benefit from 5-FU in combination with irinotecan than the MSS population.[94]

*Mitomycin C*

The hypersensitivity of dMMR cells to mitomycin C has been demonstrated in several cell line models.[95,96] Given that mitomycin C in combination with capecitabine is an effective regimen in the third-line metastatic setting,[97] this combination could be equally successful for patients with dMMR.

*Anti-EGFR targeted therapy*

CRC with *KRAS* or *BRAF* V600E mutations (reportedly mutually exclusive events in CRC pathogenesis), is associated with a poor response to EGFR-targeted therapy.[98] MSI CRC, and in particular sporadic dMMR CRC, has much higher rates of *BRAF* mutations than MSS.[99] Although *KRAS* mutations occur in both MSS and MSI phenotypes,[99] they are particularly associated with some subtypes of CIMP.[29] Metastatic dMMR CRC, therefore, are probably less likely to respond to anti-EGFR targeted therapy.

*Anti-VEGF therapy*

Tumors with increased angiogenesis are associated with a poor prognosis. The more-favorable outcome observed with MSI tumors compared with MSS tumors may be due in part to reduced levels of VEGF and lower microvessel density. Mucinous tumors with MSI have been found to have both low microvessel density and low VEGF expression levels in one study,[100] and low rates of VEGF expression in another.[101] It is possible, therefore, that MSI tumors might benefit comparatively less from VEGF-targeted therapy, such as bevacizumab compared with other CRC tumor subtypes.

## Novel therapeutic strategies

The presence of dMMR might modulate the standard current management of CRC, but of equal importance is how dMMR tumors might be managed more successfully with novel treatment strategies in the future. Potential approaches to treating dMMR cancers include targeting the primary mutation in the MMR genes by exploiting synthetic lethal interactions, or targeting the secondary mutations that occur as a result of dMMR. Although this latter strategy is clearly limited to a small fraction of the dMMR population, certain secondary mutations are ubiquitous in dMMR CRC. A combination of treatments that target both primary and secondary mutations might also be feasible (Figure 5).

The *in vitro* data supporting the differential response of dMMR cancer cells to chemotherapy is highly dependent on the model used, and different *in vitro* approaches can produce opposing results. An understanding of the variety and limitations of the models in current use is, therefore, essential.

The development of new technologies has made high-throughput drug and RNA interference screening a practical reality. These technologies are important for the *in vitro* discovery of new drugs and genetic targets for the treatment of tumors. Complementary to this is the use of high-throughput array technology for the molecular profiling of tumor tissue, and bioinformatic analysis and systems biology approaches to identify genes and pathways that might be of functional importance in this tumor subset. These techniques have already been adopted in the study of MSI CRC and results suggest that this tumor subset may be particularly sensitive to compounds inhibiting the PI3K/AKT/mTOR pathway.[102]

The identification of a new treatment strategy that has been validated *in vitro* requires consideration of

**Table 4** | Assessing the impact of dMMR on response to irinotecan

| Reference | Trial | Analyzed/ total | MSI frequency | Disease stage | Treatment | Result |
|---|---|---|---|---|---|---|
| Fallik *et al.* (2003)[138] | Single trial | 44/75 | 9.7% | Metastatic disease | Irinotecan | Improved response rate in MSI ($P=0.009$) |
| Charara *et al.* (2004)[139] | Single study rectal cancer | 57 | 23% | Early stage disease | 5-FU, irinotecan and radiotherapy | 3/5 tumors with complete response were MSI, 10/36 with partial response |
| Tejpar *et al.* (2009)[137] | PETACC 3 trial | 1254/3278 | 22% stage II 12% stage III | Stage II–III | 5-FU+FO vs 5-FU+FO +irinotecan | No benefit for MSI patients when irinotecan added to 5-FU |
| Bertagnolli *et al.* (2009)[94] | CALGB 89803 | 723/1264 | 13.3% | Stage III | 5-FU+FO vs weekly 5-FU+FO +irinotecan | dMMR patients receiving irinotecan had improved survival compared with pMMR (0.76, 95% CI 0.64–0.88 vs 0.59, 95% CI 0.53–0.64, $P=0.03$). No difference in 5-FU+FO treated patients |

Abbreviations: CALGB, cancer and leukemia group B; dMMR, deficient mismatch repair; 5-FU, 5-fluorouracil; FO, folinic acid; MSI, microsatellite instability; PETACC, pan-European trials in adjuvant colon cancer; pMMR, proficient mismatch repair.

the optimal strategy to test the hypothesis *in vivo*. Demonstration of target inhibition *in vivo* in the context of a clinical trial is essential and the concurrent development of companion biomarkers *in vitro* is vital. Likewise, the biological effects of the agents should inform the most appropriate study end points and the scheduling of the drugs in a combination study. The success of these therapeutic approaches will depend on the commitment of the clinical and academic oncology community to coordinate multicenter studies, studies involving multiple tumor types, and the accompanying translational analysis.

## Exploiting sensitivities to chemotherapy

The combination of camptothecin or irinotecan with thymidine has been proposed as a therapeutic approach for dMMR tumors based on impressive *in vitro* results.[103] A preclinical study demonstrated that camptothecin combined with thymidine suppressed colony formation of dMMR tumor cells by up to 3,000-fold compared with camptothecin alone. dMMR cell lines containing a frameshift mutation in *MRE11A* were most sensitive to this combination, and correction of the MMR defect did not result in the reversal of sensitivity to camptothecin or thymidine. This result implies that the selective effects occurred as a result of targeting secondary mutations and defective double-strand break repair. Similar results were found in mouse xenografts. Thymidine is known to increase cellular thymidine triphosphate and reduce cytosine triphosphate, which slows DNA replication and causes an accumulation of cells in S phase of the cell cycle. Cells deficient in homologous recombination are sensitive to thymidine as it triggers an ataxia telangiectasia mutated (ATM)-mediated cascade through CHK2 and MRN, a complex containing MRE11A.[103] By prolonging S phase, cells may be increasingly subjected to double-strand breaks at replication forks, which could result in increased apoptosis if left unrepaired.[86]

Iododeoxyuridine is a halogenated analog of thymidine that may be of benefit in the treatment of dMMR tumors. It accumulates in dMMR cells over time as the resulting mismatches are not corrected.[104] This drug also acts as a radiation sensitizer,[105] so a combination of iododeoxyuridine with radiation therapy has been proposed. This could be potentiated by methoxyamine, an inhibitor of base-excision repair.[105,106] Since methoxyamine is thought to elicit its effect on base-excision repair by inhibiting poly-(ADP-ribose) polymerase (PARP), potent and selective PARP inhibitors may serve some utility here.[107]

The issue of whether MMR status can be altered by chemotherapy or radiotherapy has been inadequately assessed in CRC. In one small series of 30 patients undergoing chemoradiation for rectal cancer, 5 of 18 patients had dMMR on tumor biopsy prior to treatment, 4 of 11 had a change of MSI status during treatment, with 3 of 11 becoming MSI-High.[108] If this outcome is observed in larger studies and is not an artefact of radiation-induced tissue necrosis, the utility of strategies targeting dMMR tumors might be broader than currently appreciated.

### Synthetic lethal strategies

Restoring tumor suppressor gene function is technically difficult and, therefore, problematic as a therapeutic strategy. An alternative approach is to exploit synthetic lethal relationships. Two genes, proteins or pathways are synthetic lethal if loss of one is compatible with cellular viability, but loss of both leads to cell death.[109,110] As MMR genes function as classical tumor suppressor genes,[111] treatment of dMMR tumors may be particularly suited to a synthetic lethal strategy. This approach is already showing considerable promise in the clinic in the treatment of *BRCA1* or *BRCA2*-deficient breast and ovarian cancers with PARP inhibitors.[112] PARP inhibition leads to blockade of base-excision repair, which in turn results in the persistence of single-strand breaks, stalled replication forks and ultimately may lead to the formation of lethal double-strand breaks. As the major mechanism for repairing replication forks and double-strand breaks involves *BRCA1* and *BRCA2*, loss of either of these tumor suppressor genes results in profound

sensitivity to PARP inhibitors.[113,114] Since loss-of-function of *BRCA1* or *BRCA2* leads to a deficiency in homologous recombination and a reliance on less accurate forms of double-strand break repair, PARP inhibition in combination with *BRCA1* and *BRCA2* deficiency leads to genomic instability and cell death.[110,113] This approach has the potential to deliver a large therapeutic window between the dose required to produce toxic effects in a tumor cell and that required to produce toxic effects in normal tissue that still has both copies of the *BRCA1* or *BRCA2* gene.

Our laboratory has shown that a synthetic lethal relationship exists between MSH2 deficiency and methotrexate treatment.[115] A screen to identify drugs that caused selective cell death in MSH2-deficient tumor cells identified methotrexate amongst other drugs known to cause oxidative damage. The selective lethality was characterized by accumulation of 8-OHdG, a DNA lesion associated with oxidative stress.[115] Moreover, inhibition of dihydrofolate reductase using RNA interference led to increased cell death in MSH2-deficient cells, suggesting that methotrexate was causing lethality via its known substrate, dihydrofolate reductase. A phase II, non-randomized clinical trial of methotrexate in MSH2-deficient metastatic CRC (MESH, NCT00952016) is currently underway, incorporating measurement of 8-OHdG as a biomarker.

Given the presence of secondary mutations in double-strand break repair genes, such as *MRE11A* and *hRAD50* in the majority of dMMR tumors,[7] it would also be interesting to test PARP inhibitors for the treatment of these cancers. To date, however, *in vitro* data demonstrating the selectivity of dMMR CRC cell lines to PARP inhibition compared with proficient MMR CRC cell lines has not been impressive.[116]

### Exploiting the chromosomal stability of dMMR

Chromosomal instability is associated with taxane resistance.[117] Since MSI is inversely correlated with chromosomal instability, it has been proposed that MSI tumors might exhibit increased sensitivity to taxane therapy, which is ineffective in the treatment of unselected CRC.[118] Based on these observations, the CINATRA trial[119] has been initiated, which includes a cohort of patients with MSI tumors treated with patupilone, a microtubule-stabilizing compound.

### Restoring MMR function

MMR function could potentially be restored in sporadic dMMR CRC by the use of demethylating agents to induce re-expression of the *MLH1* promoter. Decitabine is currently being trialled in combination with carboplatin in ovarian cancer on this basis, with the aim of restoring platinum sensitivity.[120] This approach could, however, result in generalized demethylation and the consequences of this are unpredictable.

### Targeting gain-of-function mutations

Since a large proportion of dMMR tumors acquire gain-of-function mutations in oncogenes, inhibitors of these could also be used to target subsets of dMMR CRC (Figure 2). Examples include inhibitors of PIK3CA,[102] (and the downstream components AKT and mTOR), and *BRAF* V600E[121] (and the downstream component MEK).[122]

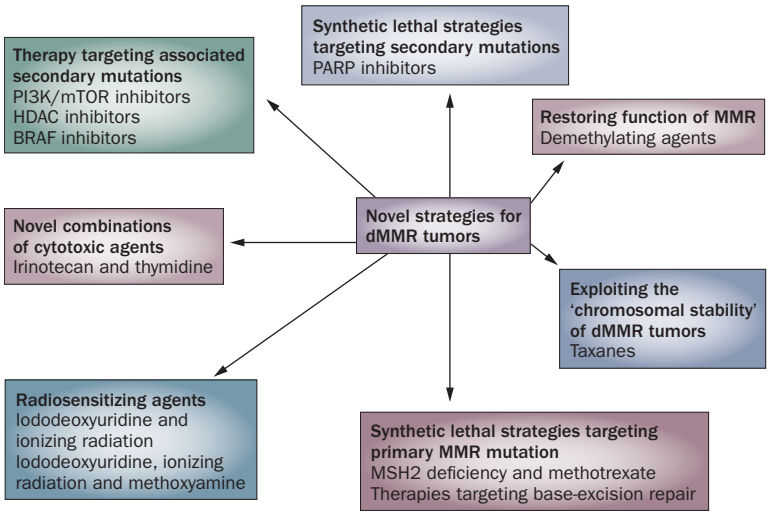


**Figure 5** | Novel strategies for the treatment of dMMR tumors. Novel strategies include exploiting the sensitivity of dMMR tumor cells to standard chemotherapies, synthetic lethal strategies targeting the primary tumor, and targeting secondary mutations.[86,104,105,115,116,119,131] Abbreviations: dMMR, deficient mismatch repair; HDAC, histone deacetylase; mTOR, mammalian target of rapamycin; PARP, poly-(ADP-ribose) polymerase; PI3K, phosphatidylinositol 3-kinase.

## Conclusions

Tumors with dMMR are clinically distinct from other subtypes of CRC and may respond differently to treatment. Although there may be value in treating dMMR tumors as a whole, there is increasing evidence that dMMR tumors are a heterogeneous group. This is due to the etiology of the dMMR and the secondary mutations that occur as a consequence of MSI. Since pathways that are key to tumor biology are often affected by frameshift mutations, this not only impacts on tumor morphology and behavior, but also on treatment response.

Discrepancies between the preclinical observations of treatment response in dMMR CRC can be partly explained by the differences in the models used to investigate dMMR. The optimum approach from a mechanistic perspective is the use of matched cell lines and other homogeneous models to elucidate what is a genuine consequence of dMMR. From a therapeutic perspective, it is important to include additional models or data sets that more closely match the *in vivo* setting. These models should not only possess dMMR, but also MSI and secondary mutations. A comparison of these models with cells or tumors without those mutations would identify differences between tumors and normal tissue. It may be equally important to target the secondary mutations as the primary dMMR mutation, and targeting primary and secondary mutations in combination should also be exploited therapeutically.

Therapeutic strategies that target dMMR are currently limited to a small proportion of tumors, but it is

likely that this subset will increase as a result of ongoing studies. It has been observed that chemotherapy or radiotherapy can induce dMMR,[123] although this has not been tested for CRC as this cancer is not routinely re-biopsied on relapse. Downregulation of MMR genes has also been observed with certain tumor-associated phenotypes, such as inflammatory bowel disease, hypoxia, oxidative stress and other inflammatory conditions.[124–126] It would be pertinent to investigate whether phenotypes associated with a relative MMR deficiency may also benefit from strategies that selectively target dMMR.

**Review criteria**

Searches were performed on PubMed and on Google Scholar for articles relating to "mismatch repair" or "microsatellite instability" and "colorectal cancer". Only articles published in English were considered. From these, articles were selected that either provided information on *in vitro* studies, the mechanistic basis of MMR, or how dMMR CRC might modify the natural history of CRC in the clinical setting, or response to treatment. The ASCO website was also searched for abstracts from 2008 and 2009 that were not published in another format.


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