The Clinical Impact of the Genomic Landscape of Mismatch Repair–Deficient Cancers

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ABSTRACT

The mismatch repair (MMR) system which detects and corrects base mismatches and insertions and deletions that occur during DNA synthesis is deregulated in approximately 20% of human cancers. MMR-deficient tumors have peculiar properties, including early-onset metastatic potential but generally favorable prognosis, and remarkable response to immune therapy. The functional basis of these atypical clinical features has recently started to be elucidated. Here, we discuss how the biological and clinical features of MMR-deficient tumors might be traced back to their ability to continuously produce new somatic mutations, leading to increased levels of neoantigens, which in turn stimulate immune surveillance.

Significance: Tumors carrying defects in DNA MMR accumulate high levels of mutations, a feature linked to rapid tumor progression and acquisition of drug resistance but also favorable prognosis and response to immune-checkpoint blockade. We discuss how the genomic landscape of MMR-deficient tumors affects their biological and clinical behaviors. Cancer Discov; 8(12); 1–11. ©2018 AACR.

INTRODUCTION

Cancer genes are commonly classified into two major groups: oncogenes and tumor suppressors. The majority of oncogenes control key nodes of signaling pathways and are modified by genomic alterations that constitutively activate their protein counterparts, leading to increased cell proliferation. Tumor-suppressor genes typically harbor molecular alterations, such as deletions or loss-of-function mutations that inactivate their function. Many tumor-suppressor genes are involved in repairing DNA replication errors that occur during cell division. Alterations in DNA-repair genes do not directly promote cell proliferation but are thought to fuel tumorigenesis by increasing mutation rates, thus contributing to cancer progression.

In human cells, postreplicative DNA mismatch repair (MMR) is performed by protein complexes consisting of MutL homolog 1 (MLH1), MutS homolog 2 (MSH2), MutS homolog 6 (MSH6), and PMS1 homolog 2 (PMS2). Other elements of the MMR machinery include the exonuclease1 (EXO1) and polymerases capable of proofreading activity, such as Polymerase ε and δ (POLE and POLD; Fig. 1). MMR proficiency is required for the detection and replacement of single-nucleotide mismatches that might escape proofreading during replication. In addition, MMR is essential for correcting small insertions and deletions that may occur when replication complexes move across repetitive sequences, so-called microsatellites (see ref. 1 for a detailed description of the molecular mechanisms). Loss of MMR activity, due to the lack of function of any of its key players, is associated with tumor development and microsatellite instability (MSI tumors). At the genomic level, MMR-deficient (MMRd) tumors accumulate large numbers of frameshifts (FS) and single-nucleotide variants (SNV), and are therefore characterized by high mutational burden (2). MMRd tumors are uniquely represented across tumor types: Systematic analyses by exome-wide identification of microsatellite loci showed high prevalence of MSI in endometrial (~30%), gastric (~20%), and colorectal (~15%) cancers, and low proportions in other tumor types (refs. 3, 4; Table 1). Germline mutations in MMR genes are associated with cancer disorders such as Lynch syndrome, if only one allele is inactivated in the germline (5), and constitutional MMR deficiency (cMMRd), a rare disease caused by biallelic inactivation of MMR genes in the germline. Individuals affected by Lynch syndrome have an increased lifetime risk of colorectal tumors and other neoplasms of the gastrointestinal (GI) system, and usually develop tumors earlier than patients with corresponding sporadic tumors. Furthermore, Lynch syndrome is associated with the development of endometrial, urinary tract, ovarian, pancreatic, and breast tumors; gliomas, and skin neoplasms (Muir–Torre syndrome; refs. 5–7). In addition, cMMRd confers a strong increase in the incidence of lymphoproliferative malignancies and childhood cancers (8). Genetic but also epigenetic events are involved in the onset of MMRd status and the emergence of...
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of MSI. Indeed, somatic biallelic methylation of the MLH1 promoter is an essential mechanism of gene inactivation in MSI-positive colorectal cancer and endometrial cancer (9, 10). MLH1 methylation occurs in approximately 19% of sporadic and approximately 16% of Lynch syndrome colorectal cancers (11). Nearly all (92%) endometrial cancers with MSI show MLH1 promoter hypermethylation (12). Associations between MLH1 promoter methylation and gender, tumor location, MLH1 protein expression, and BRAF mutations have been reported (11).

Notably, MMRd cancers often have a more favorable prognosis compared with their MMR-proficient counterparts (13); however, MSI tumors can also progress to the metastatic stage and respond poorly to chemotherapies (14, 15). The unique biological features of MSI tumors have long been recognized, but only recently have their genomic properties been linked to the observed clinical phenotypes. This review summarizes current knowledge of the mechanisms that contribute to the peculiar clinical behaviors of MSI tumors.

MMR-DEFICIENT TUMORS: CLINICAL FEATURES AND THERAPEUTIC RESPONSE

MMR pathway alterations affect the development, clinicopathologic characteristics, and therapeutic response of the tumors in which they occur. Colorectal and endometrial carcinomas, in particular, are held as “proof of principle” of the MSI paradigm. MMRd colorectal cancers are strongly enriched in the early stages of diagnosis (stage I ~10%, stage II ~20%, stage III ~12%; ref. 16), and only about 3% to 5% of stage IV colorectal cancers are MSI (17, 18). However, such correlation is less clear in endometrial cancer, possibly because of the high prevalence (up to 75%) of non-MSI patients diagnosed with stage I and II disease (19). Interestingly, the distribution of MSI is not equal among endometrial cancer histotypes: Endometrioid adenocarcinomas are enriched for MMR deficiency (up to 40% of tumors), whereas nonendometrioid carcinomas are rarely MSI (20).

Enigmatically, almost 90% of MMRd colorectal cancers are found in the right colon (21), suggesting that yet-to-be-discovered contributions from the microenvironment play

Figure 1. Molecular mechanisms of DNA MMR. MMR ensures error-free DNA replication through the activity of multiprotein complexes: MutSα together with MutLα recognize base-base mismatches and small insertions/deletions; MutSβ together with MutLα recognize large indels; EXO1 catalyzes excision in the presence of a mismatch. POLE and POLD generate error-free resynthesis of DNA after nucleotide excision.
a fundamental role in the pathogenesis of these neoplasms. Furthermore, the positive correlation between MSI status and survival after recurrence, although evident for proximal colorectal cancer, is lost in distal MSI tumors (22). This implies that additional features play a role in the clinical phenotype of MMRd colorectal cancers. Detection of MMR deficiency is feasible with cost-effective techniques using formalin-fixed samples, and multiple retrospective analyses have highlighted the prognostic and predictive impact of MMRd (23). In colorectal cancer, MSI status correlates with favorable prognosis, and MSI tumors have an advantage in disease-free survival (DFS) after primary tumor resection and overall survival (OS) compared with microsatellite-stable (MSS) tumors, irrespective of tumor stage (24, 25). MSI status is also correlated with other prognostic features such as invasiveness and high prevalence of immune cell infiltrates (26). The impact of MMR deficiency on prognosis has also been investigated in endometrial cancer, and a positive association with survival was reported in a study of 191 patients with endometrial cancer (all stages, all histologies; ref. 27). However, although this association is observed among nonendometrioid endometrial cancers (a more aggressive histotype, rarely MSI) undergoing adjuvant therapy (28), MMR status did not correlate with outcome in retrospective analysis on endometrioid endometrial cancers, of which about 15% to 30% are MSI (29, 30). This also suggests that although MSI status is associated with more indolent behavior in endometrial cancer, additional variables are likely to contribute to the clinical phenotype of MSI tumors.

MMRd might have an influence on the prognostic impact of other molecular variables. For example, the \textit{BRAF} V600E mutation, which is highly prevalent in sporadic but not Lynch syndrome–related MSI colorectal cancers, is a negative prognostic factor in colorectal cancer (31); however, a recent analysis of more than 4,000 patients who underwent adjuvant therapy demonstrated that \textit{BRAF} status has no correlation with prognosis for MSI patients (32).

In cellular models, MMR deficiency confers a 100-fold increase in resistance to methylating agents, such as temozolomide and N-methyl-N’-nitro-N-nitrosoguanidine (33), and up to 10-fold increased resistance to the purine analogue 6-thioguanine (34). Moreover, secondary resistance to these agents is associated with MMR inactivation as observed in patients with temozolomide-resistant glioblastomas, in which loss of function of MSH6 has been reported (35). The emergence of resistance to temozolomide is also correlated with acquisition of MMR deficiency in colorectal cancer mouse cell models (36). Furthermore, immunosuppression by azathioprine, a 6-thiopurine prodrug, is correlated with secondary MMRd acute myeloid leukemia/myelodysplastic syndrome (37) and is a risk factor for MSI lymphomas in mice (38). High frequency of MSI was reported in therapy-related secondary pediatric neoplasms (39). The MMRd context also offers opportunities for molecularly directed therapies based on the concept of synthetic lethality. For example, treatment with oxidative damage-inducing drugs, such as methotrexate and other agents, in an \textit{MSH2}-deficient background has been proposed as a strategy for selective killing of MMRd tumors (40–42).

Of note, MMRd tumors display unique patterns of response and resistance to anticancer therapies. In colorectal cancer, the antimetabolite 5-fluorouracil (5-FU) is the backbone of primary treatment regimens and is often administered together with the topoisomerase I inhibitor irinotecan.

### Table 1. Percentage of patients with MSI in multiple cancer types according to refs. 3, 4

<table>
<thead>
<tr>
<th>Tumor</th>
<th>%MSI-H</th>
</tr>
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<tbody>
<tr>
<td>Uterine corpus endometrial carcinoma</td>
<td>28.30%–29.75%</td>
</tr>
<tr>
<td>Stomach adenocarcinoma</td>
<td>18.71%–21.92%</td>
</tr>
<tr>
<td>Colon adenocarcinoma</td>
<td>16.61%–19.05%</td>
</tr>
<tr>
<td>Rectal adenocarcinoma</td>
<td>3.13%–5.26%</td>
</tr>
<tr>
<td>Ovarian cancer</td>
<td>1.59%–3.21%</td>
</tr>
<tr>
<td>Hepatocellular carcinoma</td>
<td>0.59%–2.93%</td>
</tr>
<tr>
<td>Renal clear cell carcinoma</td>
<td>1.06%–2.15%</td>
</tr>
<tr>
<td>Breast cancer</td>
<td>0%–1.74%</td>
</tr>
<tr>
<td>Head and neck squamous cell carcinoma</td>
<td>0.59%–1.19%</td>
</tr>
<tr>
<td>Glioblastoma multiforme</td>
<td>0.38%–1.27%</td>
</tr>
<tr>
<td>Lung squamous cell carcinoma</td>
<td>0.45%–1.23%</td>
</tr>
<tr>
<td>Prostate adenocarcinoma</td>
<td>0.6%–0.65%</td>
</tr>
<tr>
<td>Urothelial bladder cancer</td>
<td>0.4%–0.54%</td>
</tr>
<tr>
<td>Lung adenocarcinoma</td>
<td>0.21%–0.63%</td>
</tr>
<tr>
<td>Papillary kidney carcinoma</td>
<td>0%–0.7%</td>
</tr>
<tr>
<td>Low-grade glioma</td>
<td>0.19%–0.58%</td>
</tr>
<tr>
<td>Cutaneous melanoma</td>
<td>0%</td>
</tr>
<tr>
<td>Thyroid cancer</td>
<td>0%</td>
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**NOTE:** Prevalence of MSI-high (MSI-H) below 1% is highlighted in yellow, between 1% and 10% in blue, and more than 10% in violet.
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in situ analysis of IL17-positive cell

In contrast, high expression of IL17A and RORC associated with infiltrating lymphocytes [cytotoxic T cells, Type 1 helper (Th1) cells, and memory T cells] in the center and the invasive margins are positive predictors of better survival (63). For other cancers, preferentially infiltrate triple-negative tumors, and such phenotypes correlate with better DFS (56). A trend toward longer progression-free survival (PFS) was observed in patients with MMRd but did not reach statistical significance (56). The PETACC-3 trial, in which 2,333 patients with stage III colorectal cancer were randomly assigned to adjuvant treatment with biweekly infusion of 5-FU either alone or with biweekly irinotecan, analysis of the MSI population did not show significant difference in 3-year DFS or OS compared with the MSS population (57). These results were later confirmed (58) and, to date, MSI status does not have implications for irinotecan treatment in colorectal cancer.

THE IMMUNOLOGIC LANDSCAPE OF MMRd CANCERS

Among the clinicopathologic features attributed to the MSI phenotype, an increased immune infiltration has been consistently reported across different histologies and tumor types. In recent years, it has become apparent that immune infiltration represents a trait d’union to explain the peculiar clinical features shared by MSI tumors. Distinct molecular subtypes of human cancers are associated with distinct immune microenvironments. In breast cancer, CD8+ T cells preferentially infiltrate triple-negative tumors, and such phenotypes correlate with better DFS (59, 60). For other cancers, such as colorectal cancer, glioblastoma, and head and neck cancer, similar type-specific immune landscapes have been observed (61, 62). The adaptive immunity compartment, and in particular cytotoxic T-cell infiltration, is associated with a better outcome in colorectal, breast, and lung cancers (63–65). In colorectal cancer, the type, density, and localization of infiltrating lymphocytes [cytotoxic T cells, Type 1 helper (Th1) cells, and memory T cells] in the center and the invasive margins are positive predictors of better survival (63).

In contrast, high expression of IL17A and RORC associated with Th17 subtype and in situ analysis of IL17-positive cell density correlates with poor prognosis (66). The presence of high numbers of tumor-infiltrating lymphocytes (TIL) has long been recognized as a hallmark of microsatellite-unstable colorectal cancer tumors (67).

The peculiar genomic landscape of MSI tumors uniquely contributes to the quality of the neoantigen profiles (depending on whether they derive from SNV or insertion/deletion; see next section for details). Assuming that even a single immunogenic antigen can trigger an immune response, the occurrence of FSs (resulting from insertion/deletion) increases the number of putative neoantigens per alteration and, accordingly, the likelihood of generating immunogenic epitopes. The concept is summarized by the “neoantigen roulette”: tumors with fewer mutations are less likely to contain “winning neoantigens” and are more likely to be unresponsive to immunotherapy (68). Conversely, cancers with a greater number of neoantigens are more prone to immune surveillance and have increased propensity of responding to immunotherapy (68). Giannakis and colleagues showed that a higher neoantigen load was positively associated with overall lymphocytic infiltration, TILs, memory T cells, and colorectal cancer–specific survival (69).

The link between MSI status and high prevalence of FSs is highlighted by the correlation between FS mutations and the density of TILs positive for the following markers: CD3, CD8, and FOXP3. Furthermore, CD8+ TIL density correlates with the number and spectrum of FS mutations in patients with colorectal cancer (67). The distribution of CD3+ lymphocytes and CD8+ cytotoxic T cells in the tumor core and in the invasive margins is relevant to calculating the so-called immunoscore (70). In this regard, when compared with their MSS counterpart, MSI tumors typically have (i) higher density of Th1 and effector-memory T cells, (ii) more in situ proliferating T cells, (iii) upregulated expression of immune checkpoint molecules, including PD-1 and PD-L1 (71), and (iv) higher infiltration by mutation-specific cytotoxic T cells (72). High expression of IFNγ, a cytokine secreted by Type 1 helper and cytotoxic T cells (Th1/Tc1), occurs more frequently in MMRd than in MMR-proficient tumors (71). A distinctive cytokine expression profile that includes CCL5, CXCL10, and CXCL9, which are involved in Th1 response and in the recruitment of memory CD45RO+ T cells, is found in MSI patients (73). Furthermore, the immune-checkpoint molecules PD-1, PD-L1, CTLA4, LAG3, and IDO, which play key roles in immune suppression, are upregulated in MSI colorectal cancer tumors when compared with MSS tumors (71). Notably, expression of immune-checkpoint molecules in the tumor microenvironment is not a definitive marker of response (74, 75).

The correlation between MSI and increased immune infiltration extends beyond colorectal tumors. For example, in endometrial cancers, MSI positively correlates with high levels of immune infiltration. Indeed, in a cohort of 63 patients with endometrial cancer, MSI status was associated with increased predicted neoepitopes and number of CD3+ and CD8+ TILs when compared with MSS tumors (76). Highly infiltrated endometrial cancers are more likely to respond to immunotherapy, as reported by Santin and colleagues, who described a remarkable clinical response to the anti–PD-1 antibody nivolumab in 2 patients with hypermutated endometrial cancer (77). Positive results in patients with endometrial cancer...
were also reported by Le and colleagues (78). The concordance between MMR deficiency and immune infiltration has also been evaluated in ovarian cancers. Xiao and colleagues analyzed 419 ovarian cancers for MSI status, number of TILs, and expression of PD-1 and PD-L1 (79). They found that ovarian cancer with MMR alterations exhibited increased numbers of TILs and PD-L1-positive intratumoral immune cells. Patients with ovarian cancer with negative MMR protein expression had significantly better PFS than MMR-proficient patients. MSI clear-cell ovarian carcinomas (CCOC) also have higher numbers of infiltrating CD8+ lymphocytes and increased CD8+/CD4+ ratio; moreover, an enrichment for PD-1+ TILs was found in comparison with MSS CCOCs (80, 81).

**USING GENOMIC MAPS TO DEFINE THE NEOANTIGEN LANDSCAPE OF MMRd CANCERS**

In tumor cells, alterations of the MMR machinery result in the accumulation of mutations at higher rates than in normal cells (82). Some of these variants are transcribed, translated, and processed to generate neoantigens that are subsequently presented on the MHC and eventually recognized by T cells. Although it has long been recognized that MSI tumors are hypermutated, the impact of MMR deficiencies on neoantigen profiles has only recently been examined in great detail. MMR and other DNA-repair mechanisms differentially affect the emergence of SNVs, indels, and other genetic variations. SNVs, which affect an individual amino acid, are common in cancer and have been widely investigated, as they are relatively easy to identify using next-generation sequencing (NGS) and bioinformatic algorithms. In contrast, small insertions or deletions, which lead to FSs (new amino acid frame), are more difficult to detect with NGS strategies and require sophisticated bioinformatic tools for proper recognition (83). MMR deficiency affects the entire genome, including intronic, transcribed but not translated, and gene-encoding regions (84). MSI of coding regions influences not only cell growth (when they occur in certain oncogenes and tumor-suppressor genes) but also the generation of new peptides (neopeptides; ref. 84). At the protein level, FSs drive the emergence of new peptides that vary greatly from their wild-type counterparts and, therefore, may be highly antigenic. Epitopes are processed and presented by both HLA class I and II, stimulating the activation of CD8+ T cells (class I) and the “helper” function of CD4+ T cells (class II; ref. 85). Due to its highly polymorphic structure, HLA expression is specific to individual patients. Prediction of tumor neoantigens is performed by identifying DNA alterations (86, 87) and using HLA-binding prediction software (refs. 88, 89; Fig. 2). However, these tools have limited accuracy, and functional validation of predicted neopeptides is necessary to confirm the outputs of *in silico* algorithms (90). Large datasets of high-quality NGS data are being used to develop the next generation of neopeptide prediction algorithms and might soon be capable of overcoming these challenges. In summary, despite remarkable advances in bioinformatics and computational methodologies, neopeptides cannot be unequivocally identified using *in silico* analysis, and functional experiments are still required to confirm that a somatic DNA variant translates into a neoantigen.

**RESPONSE AND RESISTANCE OF MMRd TUMORS TO IMMUNOTHERAPY**

The remarkable clinical effectiveness of immune-modulatory molecules in the treatment of MMRd tumors prompted careful examination of the molecular features of patients with MSI cancer (91). A seminal observation that stimulated the field was that response to checkpoint inhibitors correlated with mutational burden across tumor types (92). Recent advances in the characterization of the genomic landscape of MSI tumors revealed a high mutational burden phenotype (93). MSI tumors are thought to produce immunogenic neoantigens as a direct consequence of the increased number of SNVs and FSs (2), and in the clinic microsatellite-unstable tumors exhibit high response rates to immune-checkpoint inhibitors. Pembrolizumab, an anti-PD-1 agent, is highly effective in metastatic MSI patients who failed two or more lines of treatments (78). This therapy is also very effective in patients with MSI tumors of non–colorectal cancer histology (from 11 tumor types; ref. 94). Objective responses were observed in 53% of the patients, half of whom had Lynch syndrome, and response rates were similar between Lynch syndrome and sporadic MSI cases. Moreover, response rates were comparable in colorectal cancer and non–colorectal cancer tumor types. Notably, therapy-induced lymphocyte expansion was observed in responsive patients, and immunogenic neoantigens were identified and proven to result from FS mutations (94). On the basis of the outstanding clinical results, in 2017 pembrolizumab was approved by the FDA for the treatment of any solid tumor with MSI, followed by approval of nivolumab for MSI mCRC. Although extensive and durable responses have been reported in patients with MSI tumors, it is worth considering that mutations not only increase immune response but can conversely impair the antigen processing/presentation pathways. Further clinical evidence indicates that primary and secondary (post therapy) resistance occurs in MSI patients treated with checkpoint inhibitors (94–96). Molecular analysis of tumor samples from MSI patients displaying resistance to checkpoint blockade highlighted the role of genes controlling the antigen-presenting machinery and response to IFNy in driving immune evasion (97, 98). Clonal selection has also been reported in association with secondary resistance to immunotherapy in this setting (99), and alteration in JAK1 (an IFNy response gene) has been identified in a patient with colorectal cancer with primary resistance to pembrolizumab (97). In addition, alterations of HLA class I and II were reported in MSI colorectal cancer (100, 101), and a Beta-2 Microglobulin (*B2M*) mutation was detected in an MSI patient treated with pembrolizumab who developed brain metastasis under treatment, whereas another patient whose primary tumor harbored a *B2M* mutation developed a second *B2M* variant in a brain lesion (94). Although these data are provocative, only systematic studies of large cohorts of MSI patients treated with checkpoint blockade will clearly define the most prevalent mechanisms of primary and acquired resistance to immunotherapy in MMRd cancers.

Although resistance to checkpoint inhibitors does occur in patients with MSI colorectal cancer, its frequency appears remarkably lower than what is reported for other tumor types. For example, among patients with advanced, pretreated
non–small cell lung cancer who received nivolumab, 68% had treatment discontinuation because of disease progression, and 44% were primary resistant (102). In contrast, only 14% of patients with MSI colorectal cancer treated with pembrolizumab, 23% of those treated with nivolumab, and 12% of patients receiving the combination nivolumab and ipilimumab (anti-CTLA4) were primary resistant to treatment (94, 95, 103).

**PRECLINICAL MODELS OF MMRd CANCERS**

Preclinical mouse models designed to recapitulate the molecular landscape of human cancers have been instrumental in understanding the molecular basis of tumor progression and provide the rationale for therapeutic development (104). Several mouse models were generated to recapitulate MMR deficiency in human cancers. For example, mice carrying either heterozygous or homozygous null mutations in the Mlh1 gene were shown to be prone to development of GI tract tumors, lymphomas, and other cancer types. The first Mlh1 knockout (KO) models did not properly recapitulate Lynch syndrome, as the mice developed tumors predominantly in the small intestine. Nevertheless, these models were extremely useful for investigating the impact of MMR in colorectal cancer pathogenesis. They include strains with inactivated Mlh1, Msh2, Msh3, Msh6, or Pms2 as well as combination mutant mice lacking both Msh2 and Msh3 or Msh3 and...
The phenotype associated with inactivation of individual MMR genes in mouse models can be summarized as follows: the absence of Mlh1, Msh2, Msh6, and Pms2 (that participate in the MutL and MutS complexes) tends to have highly penetrant phenotype and predisposition to cancer. In contrast, Msh3 and Mlh3 that are involved in other MMR pathways have a milder phenotype. Mlh4 and Msh5 have an essential role in meiotic recombination but not in cancer (106).

Hegan and colleagues used transgenic reporter genes (supFG1 and cII) to monitor the impact of MMR gene inactivation, and found that mean mutation frequencies of all MMRd mice were significantly higher than wild-type mice. Notably, Mlh1-deficient mice exhibited the highest mutation frequencies among the supFG1 single nullizygous mice (>72 times the mutation frequencies of wild-type mice), whereas Msh2-deficient mice showed a 65-fold increase in mutation frequencies compared with wild-type. Interestingly, the mutation frequencies observed in the Msh2/Msh3 and in Msh3/Msh6 double KO mice were significantly greater (over 90-fold and 100-fold, respectively) than those occurring in single KO mice (105). Mice homozygous null for Mlh1 showed tumors along the whole GI tract. The incidence of tumors was higher in mice with homozygous loss of Mlh1 (72%) than in mice with heterozygous alleles (32%; ref. 107). Similar results were reported in mice with Msh2 loss. Mice with homozygous loss of Msh2 develop lymphoid tumors with MSI with high frequency starting at 2 months of age (108). Overall, Mlh1- and Msh2-deficient models showed an increased number of lesions, faster tumor growth, and shorter median survival times than mice deficient in other MMR genes (109).

To develop mouse models that are more representative of patient tumors, mouse lines with Apc, Trp53, and Kras mutations were crossed with MMRd mice. Mice with heterozygous germine mutation in Apc (a gene that is mutated in the majority of patients with colorectal cancer) showed accelerated cancer progression when crossed with Mlh1 KO mice (107). The same was obtained when Trp53 cII mice were crossed with Msh2 Δ/Δ (110) or Msh6 Δ/Δ (111) and when Kras cII/Ab-Cre transgenic mice acquired homozygous Msh6 loss (112).

The models described resemble patients that carry mutations in MMR genes in both alleles and in all tissues. Several conditional and tissue-specific knockouts with heterozygous alterations have also been generated to understand the role of MMR genes in distinct organs and tissues. For example, mice with Msh2 Δ/Δ Villin-Cre recombinase system allow for intestinal loss of Msh2 expression. This model was pivotal not only in determining MMR gene function during intestine development but also in understanding the roles of these genes in cancer. Within the first year of life, Msh2 Δ/Δ mice developed intestinal adenocarcinomas that histologically resembled Lynch syndrome (113). Furthermore, the availability of Mlh1 Δ/Δ mice enabled analysis of MMR inactivation in specific cells or tissues (114). Iterations of genetically engineered mouse models carrying alterations commonly found in human colorectal cancer progression are also available. For example, the Msh2 Δ/Δ allele and the Apc Δ/Δ allele were combined, and tumorigenesis was induced by viral infusion of Adenoaviral-Cre into the colon (115). This strategy leads to the development of multiple adenomas and, less frequently, adenocarcinomas.

Mouse models of MMR deficiency were also used to test the efficacy of anticancer drugs. Notably, treatment with FOLFOX therapy (5-FU, leucovorin, oxaliplatin) was not effective in conditional Villin-Cre Msh2 Δ/Δ null mice (113). Together with data obtained in colorectal cancer cell models, these findings suggest that MMR alterations might influence the efficacy of commonly utilized chemotherapeutic agents in colorectal cancer.

The impact of MMR inactivation has also been assessed in mouse cellular models and tumor-derived organoids. CRISPR/Cas9 was recently used to delete the Mlh1 gene in colon, pancreatic, and breast murine cancer cell lines (36). The absence of MMR led to accumulation of SNVs and FSSs, and to the progressive generation of neoantigens. MMR inactivation increased the mutational burden and led to dynamic mutational profiles, resulting in persistent renewal of neoantigens in vitro and in vivo, whereas MMR-proficient cells exhibited stable mutational loads. Although Mlh1 KO had no consequences on the growth of tumor cells in immune-compromised mice, MMRd cells were largely unable to form tumors in syngeneic mice owing to CD8+ T cell-mediated immune surveillance (36). Indeed, tumor growth of Mlh1 KO cells in mice was accompanied by T-cell receptor expansion, as measured by circulating peripheral blood mononuclear cells, suggesting antigen recognition by specific T lymphocytes. These data imply that alterations of the MMR machinery in cancer can trigger immune surveillance and control tumor growth through the generation of new neoantigenic peptides (36). Genome editing was also recently used to systematically decipher the mutational consequences of DNA-repair deficiency in human intestinal organoid cultures; for example, MLH1 KO organoids were shown to accumulate mutations due to replication errors, and genomic analysis identified an MSI signature (116).

## CONCLUSIONS

Genomic instability has long been considered a hallmark of cancer cells and has been linked to the aggressiveness of human tumors (117). Furthermore, the ability to accumulate new mutations and molecularly evolve has been related to the acquisition of drug resistance, which often restricts the effectiveness of anticancer drugs such as kinase inhibitors (118, 119). Yet, MMRd cancers, which continuously generate new mutations and rapidly evolve, often display favorable clinical outcomes when compared with MSS tumors of the same histology. How the molecular features of MMRd cancers lead to the unique phenotypes described above has long remained unexplored and unexplained. The high response rates and long-term responses of MSI tumors to therapies based on immune-checkpoint blockade triggered renewed interest in the genomic landscapes of MMRd cancers. The prevalent view states that the response of MSI cancer to immunotherapy is associated with the levels of neoantigens, which are the result of the accumulation of new somatic mutations. However, several aspects remain to be elucidated, including the extraordinarily long responses of patients bearing MSI tumors. If MMRd cancers continually mutate, why do they less frequently develop resistance to checkpoint inhibitors? Perhaps not only the number of mutations, but also the fact that...
these are continuously produced contributes to the clinical behavior of MSI tumors. The unique molecular and biological features of MM Rd cancers will likely provide a strong foundation for major discoveries.

Disclosure of Potential Conflicts of Interest

G. Germano has ownership interest (including stock, patents, etc.) in Neophere. A. Bardelli has ownership interest (including stock, patents, etc.) in Phoremost and Neophere and is a consultant/advisory board member for Phoremost and Neophere. No potential conflicts of interest were disclosed by the other authors.

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