Tumor Mutational Burden and Mismatch Repair Deficiency Discordance as a Mechanism of Immunotherapy Resistance

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ABSTRACT

Lynch syndrome is a heritable cancer syndrome caused by a heterozygous germline mutation in DNA mismatch repair (MMR) genes. MMR-deficient (dMMR) tumors are particularly sensitive to immune checkpoint inhibitors, an effect attributed to the higher mutation rate in these cancers. However, approximately 15% to 30% of patients with dMMR cancers do not respond to immunotherapy. This report describes 3 patients with Lynch syndrome who each had 2 primary malignancies: 1 with dMMR and a high tumor mutational burden (TMB), and 1 with dMMR but, unexpectedly, a low TMB. Two of these patients received immunotherapy for their TMB-low tumors but experienced no response. We have found that not all Lynch-associated dMMR tumors have a high TMB and propose that tumors with dMMR and TMB discordance may be resistant to immunotherapy. The possibility of dMMR/TMB discordance should be considered, particularly in less-typical Lynch cancers, in which TMB evaluation could guide the use of immune checkpoint inhibitors.

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Background

Lynch syndrome, also known as hereditary nonpolyposis colorectal cancer, is a heritable cancer syndrome predisposing to colorectal and other cancers, including endometrial, gastric, and ovarian cancer.^{1–3} Lynch syndrome is caused by an autosomal dominant heterozygous germline mutation in the DNA mismatch repair (MMR) genes. MMR deficiency (dMMR) increases the likelihood of acquiring somatic genetic mutations, particularly in short repetitive sequences, leading to varying lengths of these regions, termed *microsatellite instability*.^{1,4} However, dMMR is a double-edged sword, because although it predisposes to malignancy, it leads to cancers with more mutations, particularly frameshift alterations, which are associated with “non-self” immunogenic antigens, high lymphocyte infiltration, and immune response.^{5,6} Because of the high immunogenicity of these tumors, microsatellite instability–high (MSI-H) or dMMR cancers have been shown to respond well to immunotherapy, in contrast to their microsatellite-stable (MSS) counterparts.^{7–10} However, despite the promising response of immunotherapy in dMMR cancers, approximately 15% to 30% of patients do not derive significant benefit with single or combination immunotherapy.^{7,8}

This report describes 3 patients with Lynch syndrome who developed a dMMR MSI-H cancer with a high tumor mutational burden (TMB) and a second primary malignancy that was dMMR but with a low TMB. All tumors were assessed using a next-generation sequencing (NGS) panel comprising >300 cancer-associated genes.¹¹ MSI status was evaluated with the MSIsensor algorithm, with MSI-H status defined by MSIsensor scores ≥ 10 .¹² This cutoff for MSI status has been validated against MSI PCR testing and/or MMR immunohistochemistry performed on 138 colorectal cancers (114 MSS and 24 MSI-H) and 40 uterine endometrioid cancers (25 MSS, 15 MSI-H), with a concordance of 99.4%.¹²

Case Reports

The first patient was a man aged 43 years who initially presented with multiple primary colon masses. At total

colectomy, the most advanced lesion was stage IIB. Pathology was notable for tumor-infiltrating lymphocytes (TILs) and the absence of MLH1 and PMS2 staining on immunohistochemistry (Figure 1A). Germline genetic testing revealed a deleterious *MLH1* likely pathogenic

variant confirming Lynch syndrome, and tumor genomic analysis showed an MSI-H phenotype with an MSIsensor score of 40.67 and a TMB of 54.4 mutations per megabase (mt/Mb). This tumor had driver mutations in *APC* and *TP53*, a genomic profile common for colorectal tumors

A

B

Figure 1. Comparison of histopathologic and genomic features across paired primary dMMR tumors. (A) H&E and IHC stains of each tumor pair. In patients 1 and 2, IHC showed normal staining for MSH2 and MSH6 and loss of staining for MLH1 and PMS2 in both tumors. In patient 3, IHC showed normal staining for MLH1 and PMS2 and loss of staining for MSH2 and MSH6 in both tumors. (B) Spectrum of oncogenic mutations and differences in MSIsensor scores, TMB, and FGA, along with depiction of MMR protein status from IHC. The paired tumors from the 3 patients appear genomically distinct, harboring few shared oncogenic mutations. The patients' secondary primary malignancies all have lower TMBs and MSIsensor scores. Abbreviations: CHOL, cholangiocarcinoma; COAD, colorectal adenocarcinoma; dMMR, deficient mismatch repair; FGA, fraction of genome altered; H&E, hematoxylin-eosin; IHC, immunohistochemistry; MMR, mismatch repair; MSIsensor, microsatellite instability sensor; OCCC, ovarian clear cell carcinoma; PNEC, pancreatic neuroendocrine carcinoma; TMB, tumor mutational burden; UCEC, uterine corpus endometrial carcinoma.

(Figure 1B). Three years later, imaging showed a pancreatic tail mass consistent with a new primary, which was completely resected. Surgical pathology on the resected mass showed a poorly differentiated neuroendocrine carcinoma (60% of tumor cells staining for the proliferation marker Ki-67). Immunohistochemistry confirmed the loss of MLH1 and PMS2 in the neuroendocrine carcinoma (Figure 1A). Surprisingly, despite the patient's Lynch syndrome status, NGS revealed an MSS tumor with an MSIsensor score of 1.8 and a low TMB (9.7 mt/Mb; Figure 1B). Unlike in the patient's colon tumor, increased TILs were not seen in the neuroendocrine carcinoma. This second tumor also had a completely different set of somatic mutations, with a truncating *RB1* driver mutation consistent with neuroendocrine carcinoma. Unfortunately, within 6 months after resection, the patient was found to have multiple hypervascular metastatic lesions in the liver, consistent with his pancreatic primary. He was started on pembrolizumab given the dMMR status of his primary tumor. His initial scan after 3 cycles showed a mixed response; however, a subsequent scan showed definitive disease progression with the development of new lesions.

The second patient was a man aged 54 years with 2 resected colon primaries that were dMMR with loss of MLH1/PMS2 on immunohistochemistry (Figure 1A) and germline NGS confirming an *MLH1* likely pathogenic variant. He later presented with a new liver lesion on surveillance imaging. Before it was clear that the patient had a second primary, he received ipilimumab and nivolumab for presumed liver metastasis from colon cancer, with clear progression (the mass grew from 5.7×5.4 cm to 9.0×8.4 cm) after 3 cycles of therapy. Biopsy of the mass showed a morphologically different tumor from the prior colon cancer that was poorly differentiated, suggestive of a new gastrointestinal primary. Subsequent resection of the mass showed a cholangiocarcinoma with positive staining for albumin in situ hybridization. The cholangiocarcinoma was MMR-deficient (absent MLH1/PMS2) and MSI-H, with an MSIsensor score of 13.51 but a low TMB of 7 mt/Mb (Figure 1B). The colorectal tumor had driver mutations in *APC*, *SOX9*, and *NF1*. No somatic mutations were shared between the 2 tumors from this patient. Despite treatment using ipilimumab and nivolumab, no lymphocytic infiltrate was seen in the resected tumor.

The third patient was a woman aged 40 years with an *MSH2* pathologic germline mutation who had synchronous primary endometrial and ovarian cancers found on screening. Both tumors showed a loss of MSH2/MSH6 by immunohistochemistry (Figure 1A). The endometrial adenocarcinoma was evaluated as MSI-intermediate (MSIsensor score, 7.16) with a high TMB of 35.1 mt/Mb.

However, sequencing of the patient's ovarian clear cell carcinoma showed an MSS phenotype (MSIsensor score, 0.12) and a low TMB (7.9 mt/Mb; Figure 1B). Hematoxylin-eosin stains identified increased TILs in the primary endometrial adenocarcinoma, compared with the ovarian clear cell carcinoma without TILs. Consistent with what is expected in these cancer types, both tumors from this patient harbored an *ARID1A* mutation; however, these mutations were in different positions and private to each of their respective tumors.

Discussion

We have reported 3 patients with Lynch syndrome who each developed 2 primary tumors with confirmed dMMR status. Unexpectedly, despite confirmed dMMR within each tumor pair, one tumor had an expected high TMB, but the other had a lower TMB. In agreement with their TMB status, the 2 patients treated with immunotherapy for their TMB-low tumors did not experience a response to the therapy. Using FACETs, an allele-specific copy number algorithm, we assessed ploidy and loss of heterozygosity of *MLH1* in the paired cancers from the first 2 patients discussed herein.¹³ Both paired cancers showed a loss of the normal MMR allele. However, the germline *MLH1* mutant allele was enriched in the first TMB-high cancer compared with the second cancer.

Although research has shown that patients with Lynch syndrome can develop sporadic cancers with retained MMR machinery, Lynch syndrome-associated tumors with confirmed dMMR but with a discordantly low TMB have not been well described. It is known that some MMR variants are associated with dMMR on immunohistochemistry but not MSI-H—but in this case, the same germline MMR variant in each tumor pair led to an MSI-H and TMB-high phenotype in one tumor and a TMB-low phenotype in the other tumor.¹⁴ Consistent with our data, Georgiadis et al¹⁵ reported that although TMB and MSI status were highly correlated, TMB and MSI discordance did occur, and 6 of 7 MSI-H tumors with progressive disease on immunotherapy had low TMB. Similarly, Schrock et al¹⁶ found that within MSI-H colorectal cancer, TMB was predictive of immunotherapy response.

We acknowledge that the immunogenic response in Lynch syndrome tumors is complicated because Lynch syndrome polyps with a low mutational rate still elicit an immune response.¹⁷ In the 3 patients analyzed herein, high levels of TILs were not detected in the TMB-low tumors, in contrast to the TMB-high tumors. In addition, in patient 2, no immune activation was seen after the dual immune checkpoint blockade.

Conclusions

These case reports reveal an important practical clinical point: patients with Lynch syndrome can develop dMMR tumors with a low TMB. Although we cannot draw general conclusions from a 3-person case series, these results, in combination with previous evidence showing that TMB is associated with a response to immunotherapy within MSI-H tumors, suggest that dMMR tumors with a low TMB would not respond to immunotherapy.^{15,16} We propose that dMMR/TMB discordance should be considered in patients with Lynch syndrome, particularly those who develop secondary, less-typical Lynch syndrome cancers, and that TMB should be evaluated in

these tumors to inform the use of immune checkpoint inhibitors.

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