Pancreatic Cancer Progression in a Patient With Lynch Syndrome Receiving Immunotherapy: A Cautionary Tale

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ABSTRACT

Pancreatic ductal adenocarcinomas (PDACs) with DNA mismatch repair deficiency (MMRd) respond preferentially to immune checkpoint inhibitors (ICIs). However, a subset of MMRd PDACs does not respond to these agents. This report describes a patient with PDAC who experienced rapid disease progression suggestive of hyperprogressive disease. The case involved a 63-year-old man carrying a pathogenic germline *PMS2* mutation who developed metastatic PDAC. His tumor showed isolated loss of *PMS2* expression by immunohistochemistry (IHC). He was treated with pembrolizumab, but his disease rapidly progressed. Whole-genome and transcriptome sequencing of a liver metastasis biopsy, acquired at disease progression, showed a retained wild-type *PMS2* allele and hallmarks of microsatellite stability, including low tumor mutational burden and low MSI sensor score. PCR-based microsatellite instability (MSI) testing of the treatment-naïve tumor showed microsatellite stability. The ICI-treated tumor had a lower density of CD8+ T-cell infiltration than the treatment-naïve tumor, which is contrary to the expected evolution with ICI responsiveness. Through this case and a review of the literature, we highlight the low penetrance of *PMS2* germline mutations in PDAC and discuss pitfalls in ascertaining MMRd and MSI based on IHC testing alone. An orthogonal confirmatory assay is warranted in the presence of uncommon immunophenotypes, such as isolated *PMS2* loss, to optimize selection of patients with PDAC for immunotherapy.

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Pancreatic ductal adenocarcinoma (PDAC) is a difficult-to-treat malignancy, with a 5-year survival rate of 9%. Treating PDAC with therapies that target specific molecular aberrations may be more effective than empirical chemotherapeutic strategies.

The DNA mismatch repair (MMR) genes (*MLH1*, *MSH2*, *MSH6*, *PMS2*) correct DNA replication errors. We and others have shown that approximately 1% of PDACs are related to MMR deficiency (MMRd).¹ MMRd tumors typically arise in patients with Lynch syndrome (LS) who carry a pathogenic germline mutation in one of the MMR genes. In the absence of LS, MMRd can also be caused by biallelic somatic inactivation of an MMR gene, either by mutations or by epigenetic silencing.

MMRd tumors accumulate insertion and deletion mutations, particularly within microsatellite regions, leading to high microsatellite instability (MSI-H). This increased mutational burden generates neoantigens that are recognized by cytotoxic CD8+ T lymphocytes.¹ One mechanism by which MMRd tumors evade immune-mediated killing is through upregulating immune checkpoints, such as PD-1 and CTLA-4. As a result, MMRd tumors are preferentially sensitive to immune checkpoint inhibitors (ICIs), which reactivate the antitumor immune response. Pembrolizumab (PD-1 monoclonal antibody) has shown a 62% objective response rate in MMRd/MSI-H PDAC.²

However, a subset of patients treated with ICIs experience a paradoxical phenomenon of accelerated disease progression, termed *hyperprogressive disease* (HPD). This report presents a patient with LS and PDAC who developed rapid disease progression while receiving pembrolizumab, with accompanying molecularly characterized longitudinal tumor biopsies.

Case Report

Clinical Presentation

A 63-year-old man of French-Canadian ancestry presented with a pancreatic head mass and multiple liver hypodensities. Biopsy of a liver mass revealed a poorly

differentiated adenocarcinoma, consistent with metastasis from a pancreatic primary. The patient was treated with FOLFIRINOX (5-FU/folinic acid/irinotecan/oxaliplatin), but his disease progressed after 6 cycles (Figure 1A). He received gemcitabine/nab-paclitaxel as second-line treatment and experienced a partial response. However, he developed regimen-limiting peripheral neuropathy and was switched to gemcitabine monotherapy.

Immunohistochemistry (IHC) for the MMR proteins was performed on the liver biopsy, showing isolated loss of PMS2 expression, with intact nuclear expression of MLH1, MSH2, and MSH6 (Figure 2A). IHC for the MMR proteins was repeated, and isolated PMS2 loss was observed again. Because these IHC results were suggestive of LS, we performed germline testing for 86 cancer predisposition genes (Invitae Multi-Cancer Panel) using peripheral blood. Germline testing showed a pathogenic mutation in *PMS2* (c.2117delA), confirming LS. Notably, the patient did not meet Bethesda guidelines for LS. Based on the loss of PMS2 expression, therapy was switched to pembrolizumab.

Progressive Disease During Pembrolizumab Treatment

After 4 cycles of pembrolizumab, restaging tomographic scans showed rapid progression of the liver metastases present at the start of immunotherapy. There was a >2-fold increase in the rate of tumor growth on pembrolizumab compared with that on gemcitabine/nab-paclitaxel, meeting criteria for HPD (Figure 1B).³ In addition, there was interval appearance of innumerable new liver and lung metastases. A progression biopsy was obtained from the dominant segment 8 liver metastasis. The patient experienced rapid clinical deterioration and died 2 months later.

Molecular Characterization of the Diagnostic and Progression Biopsies

To examine the molecular mechanisms underlying the disease progression in this patient, we performed MMR IHC on the progression biopsy. Surprisingly, IHC for the MMR proteins showed intact nuclear expression of all 4 MMR proteins (Figure 2A). Furthermore, we performed whole-genome sequencing (WGS) of the tumor and matched lymphocyte DNA to depths of 86× and 62×, respectively, using methods we have described previously.⁴ The germline *PMS2* c.2117delA mutation was identified by WGS. However, there was no evidence of a second somatic hit, and the wild-type *PMS2* allele was retained (Figure 2B, C). The tumor was microsatellite stable with a low MSI sensor score (0.79). The tumor mutational burden (TMB) was 7.44 mutations per megabase, which is less than what has been reported for MMRd tumors.¹ Furthermore, the COSMIC Mutational Signatures findings were 16, 5, and 12, which are not associated with MMRd.¹ Common driver genes were mutated, including *KRAS*, *TP53*, and *SMAD4*.¹ We did not identify a *BRAF*V600E mutation, which is associated with *MLH1* promoter hypermethylation. We also performed tumor whole-transcriptome sequencing. Wild-type *PMS2* transcripts were identified. The tumor was classified as basal-like based on unsupervised clustering against 164 PDACs from the COMPASS trial (ClinicalTrials.gov identifier: NCT02750657).⁴

Fresh frozen tumor was not available from the initial biopsy to perform WGS. However, we evaluated the initial biopsy for MSI. After tumor macrodissection, MSI testing was performed using the clinical-grade Idylla MSI Test (Biocartis), which features a panel of 7 monomorphic microsatellite markers (*ACVR2A*, *BTBD7*, *DIDO1*, *MRE11*, *RYR11*, *SEC31A*, *SULF2*). The tumor was found to be microsatellite stable, with none of the 7 MSI markers showing instability.

Immune Microenvironment of the Diagnostic and Progression Biopsies

To examine differences in the tumor immune microenvironment after progression, the diagnostic and progression

Figure 1. Clinical evolution of the patient. (A) Graph showing changes in serum CA 19-9 (black y axis and dots) and RECIST sum of diameters (red y axis and diamonds) while receiving therapy. The red and green dotted lines indicate the thresholds for progressive disease (+20%) and partial response (−30%), respectively. (B) CT scans obtained before initiation of pembrolizumab (left; corresponds to red diamond labeled “4” in panel A) and after 4 cycles of pembrolizumab (right; corresponds to red diamond labeled “5” in panel A), showing marked tumor progression consistent with hyperprogressive disease. Abbreviations: FOLFIRINOX, 5-FU/folinic acid/irinotecan/oxaliplatin; gem, gemcitabine.

Figure 2. Molecular characterization of diagnostic and progression biopsies. (A) Mismatch repair immunohistochemistry of the diagnostic biopsy showing isolated PMS2 loss (top) and progression biopsy showing intact expression of all 4 proteins (bottom). (B) Integrative Genomics Viewer tracks of germline DNA (top), tumor DNA (middle), and tumor RNA (bottom), showing the germline *PMS2* c.2117delA mutation and retention of second wild-type *PMS2* allele in the tumor. (C) Circos plot depicting whole-genome sequencing of the progression biopsy. (D) Comparison of the tumor immune microenvironment of the diagnostic biopsy (top) versus the progression biopsy (bottom). In the first column, CD8 (brown) and panCK (teal) are dually stained. Abbreviations: CNV, copy number variation; DEL, deletion; DUP, duplication; INDEL, insertion or deletion mutation; INV, inversion; log2R, log 2 ratio; panCK, pan-cytokeratin; SNV, single-nucleotide variant; SV, structural variant; TRL, translocation.

biopsies were stained for CD8 (cytotoxic T lymphocytes), FOXP3 (T-regulatory lymphocytes), and PD-L1 (Figure 2D). Compared with the diagnostic biopsy, the progression biopsy had a lower density of CD8+ T cells (1.7 vs 380.4 cells/mm² tumor) and FOXP3+ T-regulatory cells (26.2 vs 184.7 cells/mm² tumor). Both biopsies were negative for PD-L1.

Discussion

This report describes a patient with LS and PDAC who had rapid disease progression while receiving pembrolizumab. The isolated loss of PMS2 expression on IHC, in the

presence of a germline *PMS2* mutation, was suggestive of LS-associated MMRd. However, the initial tumor was microsatellite stable on MSI testing. One explanation that could reconcile the discordant IHC and MSI findings is that a second somatic mutation in *PMS2* may have been acquired as a later passenger event, rather than a causative driver. This could result in loss of protein expression while the tumor retains microsatellite stability. To this end, Alpert et al⁵ reported that 7.1% of colorectal cancers with isolated PMS2 loss by IHC were not MSI-H by PCR testing. In addition to the passenger mutation hypothesis, functional

redundancy of PMS2 with MLH3 and PMS1 may play a role in rescuing MMR function.

The progression biopsy showed a retained wild-type *PMS2* allele and genomic hallmarks of microsatellite stability, including a low TMB comparable to that of MMR-proficient PDACs.^{1,6} Furthermore, the tumor harbored typical driver mutations in *KRAS*, *TP53*, and *SMAD4*, which are less prevalent in MMRd PDACs.¹ Together, these findings show the absence of an MMR “genomic scar” and suggest that this PDAC was unlikely MMRd or *PMS2*-driven, despite the initial *PMS2* loss on IHC. The discrepancy between the MMR IHC results of the diagnostic biopsy and the progression biopsy may also be explained by intratumoral heterogeneity, wherein a subclonal population exhibiting focal *PMS2* loss may have been sampled on the diagnostic biopsy. Indeed, patterns of MMR protein loss may be heterogeneous within a given tumor. Tachon et al⁷ described a case of colorectal cancer in which the primary tumor exhibited areas of MLH1/*PMS2* loss, but a lymph node metastasis retained intact expression of all MMR proteins and was microsatellite stable. It is also plausible that this subclone with *PMS2* loss may have been eliminated under the selective pressure of therapy. This illustrates potential pitfalls of interpreting MMR IHC based on small biopsies that may not be representative of the staining patterns across the entire tumor.

Although *PMS2* is conventionally grouped with the 3 other MMR genes, *PMS2* carriers have a lower risk of extracolonic tumors than carriers of other MMR gene mutations. Across 1,213 patients with PDAC, we did not identify any MMRd PDACs that were driven by a germline *PMS2* mutation.¹ Furthermore, *PMS2* is the only MMR gene for which a germline mutation is not an indication for PDAC surveillance according to the NCCN Clinical Practice Guidelines in Oncology (NCCN Guidelines) for Genetic/Familial High-Risk Assessment: Breast, Ovarian, and Pancreatic.⁸ In the MSK-IMPACT experience, 2 of 3 patients with a germline *PMS2* mutation developed MMR-proficient PDAC, characterized by intact *PMS2* protein expression, a low TMB, and a low MSI sensor score.⁶ One of these patients received a combination of PD-L1 inhibitor and C-C chemokine receptor 4 inhibitor and developed progressive disease after 3 months. These findings underscore that PDACs in individuals with *PMS2* germline mutations may arise sporadically, without being driven by biallelic inactivation of *PMS2*. Furthermore, they highlight the rarity of bona fide *PMS2*-driven MMRd PDAC.

Cohen et al⁹ reported a cohort of patients who were assessed locally as having MMRd and MSI-H colorectal cancer and received ICIs. Of 5 patients who had primary resistance to ICIs, 3 (60%) were found to be MMR proficient after central retesting for MMR IHC and MSI PCR. Indeed,

misdiagnosis of MMRd and MSI-H status may underlie a subset of resistance to ICI. Currently, ascertainment of MMRd and MSI-H status using a single diagnostic method (IHC or PCR) is sufficient for enrollment in most ICI clinical trials. Although this practice is dictated by practical considerations, the negative impacts of a misdiagnosis cannot be understated. We propose that for any case lacking an undebatable IHC profile (ie, simultaneous loss of *PMS2* and MLH1 or of MSH6 and MSH2), use of an orthogonal assay to confirm MMRd and MSI-H is warranted before initiating ICI treatment. In particular, the trigger for orthogonal confirmatory testing should include cases with isolated loss of one of the minor partners (MSH6, *PMS2*), which are rare immunophenotypes. This caution is particularly important in cancers that are less common in the spectrum of LS-associated malignancies, such as PDAC.¹⁰ With the advent of next-generation sequencing, genomic hallmarks of MSI can also serve as a complementary test.

There is an emerging body of literature suggesting that a subset of patients treated with ICIs experience a distinct phenomenon of accelerated tumor growth. In clinical practice, distinguishing HPD from “conventional” progressive disease is challenging, especially in the context of multiple failed lines of therapy and an aggressive malignancy such as PDAC. Several definitions of HPD have been proposed, all of which aim to show tumor growth that markedly exceeds what is expected of the natural history of the disease. Based on these definitions, the rate of HPD in patients receiving ICIs ranges between 7% and 29% across multiple tumor types.^{11,12} The clinical importance of the HPD phenotype is that it is associated with significantly worse survival outcomes. However, the molecular mechanisms underlying HPD remain poorly understood. Older age and presence of liver metastases have been proposed as risk factors for HPD.^{11,13} At a genomic level, *MDM2/4* amplification, *EGFR* mutation, and *B2M* mutation have been associated with HPD, but these were not identified in our case.¹⁴ An increase in CD8+ density in ICI-treated tumors has been associated with radiologic response.¹⁵ In our case, the ICI-treated tumor displayed lower CD8+ T-lymphocyte infiltration.

Conclusions

Altogether, the absence of robust biomarkers of HPD further underscores the importance of integrating orthogonal MMRd and MSI-H testing strategies to select patients who are most likely to benefit from immunotherapy.

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