Efficacy of PD-1 Blockade in Refractory Microsatellite-Stable Colorectal Cancer With High Tumor Mutation Burden

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Clinical Practice Points

- Immune checkpoint inhibitors are currently approved by the US Food and Drug Administration for treatment-refractory metastatic colorectal cancer (mCRC) that is microsatellite instability-high and/or mismatch repair deficient. However, the overwhelming majority (≥ 90%) of mCRC patients have tumors that are microsatellite stable (MSS) or mismatch repair proficient, and thus have disease that is unlikely to respond to checkpoint inhibitors.
- We present a case of treatment-refractory MSS mCRC with high tumor mutation burden (TMB) and unique molecular features that may have contributed to meaningful response to a programmed cell death protein 1 (PD-1) inhibitor.
- In MSS metastatic colorectal tumors, a high TMB and mutations in proteins such as ARID1A, MSH6, and TGF-β that are suggestive of a hypermutated phenotype may predict response to checkpoint blockade.
- Future studies are warranted to comprehensively characterize molecular features indicative of hypermutated and high TMB but MSS colorectal tumors that could guide selection of patients with disease that may best respond to immunotherapy.

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Introduction

On May 23, 2017, the programmed cell death protein 1 (PD-1) inhibitor pembrolizumab represented the first approved immune checkpoint inhibitor by the US Food and Drug Administration (FDA) in metastatic colorectal cancer (mCRC) based on its tumor agnostic indication in treatment-refractory microsatellite instability (MSI)-high (H) and/or mismatch repair deficient (dMMR) solid tumors.[1] This was followed by the approval of another PD-1 inhibitor, nivolumab, in dMMR/MSI-H mCRC refractory to fluoropyrimidine, oxaliplatin, and irinotecan on August 1, 2017, on the basis of the results of the phase 2 CheckMate 142 trial.[2] More recently, the FDA on July 10, 2018, approved the combination of nivolumab and the cytotoxic T-lymphocyte antigen 4 (CTLA-4) inhibitor ipilimumab in dMMR/MSI-H mCRC that has similarly progressed while receiving therapy with fluoropyrimidine, oxaliplatin, and irinotecan on the basis of an updated cohort from CheckMate 142.[3] Although immunotherapy has now been established as a standard option in the treatment paradigm of dMMR/MSI-H mCRC, ≥ 90% of mCRC patients have tumors that are microsatellite stable (MSS) or mismatch repair proficient (pMMR).[4] These patients are unlikely to derive significant clinical benefit from checkpoint inhibitors alone.

We present the case of a patient with MSS mCRC characterized by high tumor mutation burden (TMB) and several

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nonsynonymous mutations (including within *MSH6*, *ARID1A*, and *TGF-β*) who experienced a durable response to pembrolizumab in the treatment-refractory setting.

Case Report

A 61-year-old man was originally treated with concurrent capecitabine and radiotherapy for stage III rectal adenocarcinoma (cT3N1M0) that was found to be pMMR by immunohistochemical (IHC) testing. The patient delayed definitive surgery after neoadjuvant chemoradiation for insurance reasons and eventually experienced local disease progression, prompting administration of 10 cycles of FOLFOX (5-fluorouracil, leucovorin, and oxaliplatin). A palliative colostomy was performed for obstruction; computed tomographic scans revealed new and multiple liver metastases.

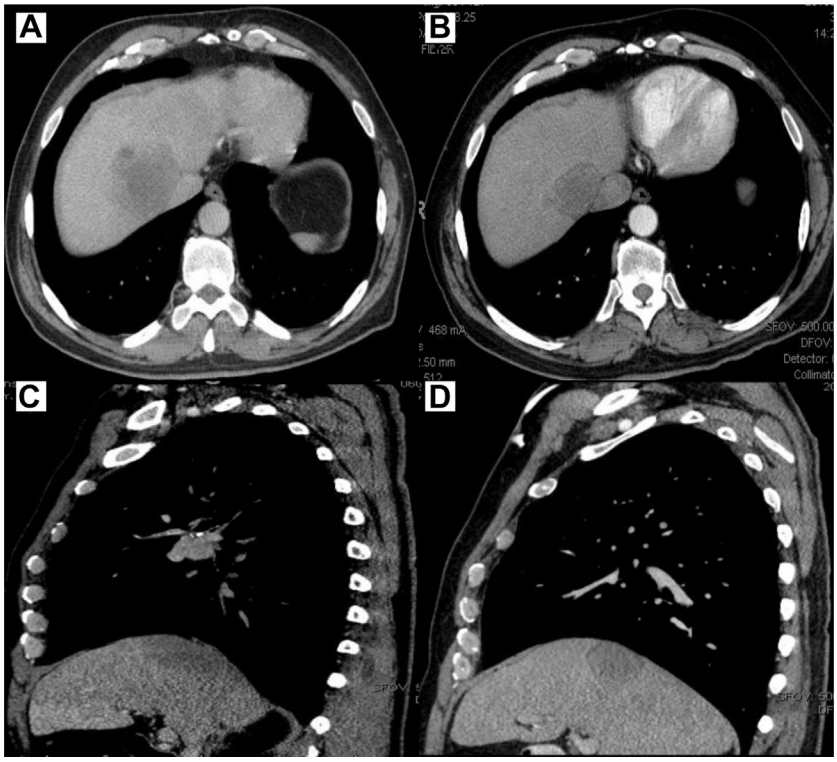
Next-generation sequencing (NGS) (Foundation Medicine, Cambridge, MA) was performed on archival tumor tissue samples taken from the primary colorectal cancer at diagnosis and showed the following mutation profile: *KRAS/NRAS* wild type (exons 2, 3, and 4), $BRAF^{R444W}$, MSS, TMB of 32 mutations (mut)/megabase (Mb), and the following pathogenic mutations: $MSH6^{R1035*}$, $PIK3CA^{E542K}$, $NOTCH1^{R1663W}$, $MEN1^{R521fs*43}$, $ERBB2^{R678Q}$, $ACVR1B^{R375,R485}$, $APC^{R876*,R1450*,R332*}$, $ARID1A^{W1670*,D1850fs*4}$, $FBXW7^{R505C}$, $HSD381^{T353M}$, and $TGFBR2^{R528C}$.

The case was reviewed at the local tumor board, and in the absence of available clinical trials, the consensus recommendation was to proceed with palliative immunotherapy with pembrolizumab, given the NGS result with high TMB and rapid disease progression while receiving chemotherapy. The patient initiated therapy with pembrolizumab 200 mg provided intravenously every 3 weeks. Imaging studies revealed radiographic disease regression at the first posttreatment computed tomographic scan at 2 months. This met the definition of Response Evaluation Criteria in Solid Tumors response at 2 months with response that remained sustained at 9 months, the time of this report (Figure 1). No significant adverse events were observed during pembrolizumab therapy.

Discussion

In mCRC patients with disease that is refractory to standard cytotoxics (ie, fluoropyrimidine, oxaliplatin, and irinotecan), checkpoint inhibitors have provided promising and durable responses (overall response rate, 55%; complete response rate, 3%; 12-month progression-free survival rate, 71% [95% confidence interval, 61.4-78.7]; 12-month overall survival rate, 85% [95% confidence interval, 77.0-90.2]) in dMMR/MSI-H tumors.[3] For the remaining ≥ 90% of mCRC patients, who receive pMMR/MSS approved agents, including regorafenib and TAS-102, very low

Figure 1 **Computed Tomographic Scan of Patient With Rectal Adenocarcinoma at Baseline and 9 Months After Pembrolizumab Treatment. Treatment With Pembrolizumab in Patient With Microsatellite-stable but High Tumor Mutation Burden Metastatic Colorectal Cancer Resulted in Radiographic Reduction in Size of 4.2 × 3.2 cm Metastasis Within Medial Right Hepatic Dome and Ill-defined 2.8 cm Hypodense Metastatic Lesion in Left Hepatic Lobe Identified at Baseline (A, Axial; C, Sagittal) that was Sustained 9 Months Later (B, Axial; D, Sagittal)**



overall response rates of 1% to 1.6% are evident, with a median progression-free survival and overall survival improvement over placebo of 0.2 to 0.3 months and 1.4 to 1.8 months, respectively.[5,6] There is a unmet need to identify subsets of patients with pMMR/MSS mCRC who may experience clinically meaningful responses to therapy with checkpoint inhibitors.

Higher TMB has been associated with response to checkpoint inhibitors in several tumor types, including urothelial cancer (median, 12.4 mut/Mb), melanoma (median, 37.1 mut/Mb), and non–small-cell lung cancer ($\geq$ 10 mut/Mb).[7-9] In a large cohort of 6004 colorectal cancer patients, the median TMB was 4.5 mut/Mb (range, 0-746.9 mut/Mb), where 95% of cases were MSS and 5% of cases were MSI-H.[10] Notably, 99.7% (301/302) of MSI-H cases were classified as high TMB (defined as TMB $\geq$ 11.7 mut/Mb), whereas only 2.9% (164/5702) of MSS cases fulfilled the criteria for high TMB (range, 11.7-703.6 mut/Mb). Despite confirming the mismatch repair proficiency status by IHC and an MSS status by NGS (Foundation One), the hypermutated profile (TMB of 32 mut/Mb) predicted a higher likelihood of response to checkpoint inhibition and to a successful clinical outcome with pembrolizumab therapy in our case.

Notably, our patient's tumor had a nonsense mutation in *MSH6*^{R1035*}, which was identified on NGS despite pMMR/MSS status by IHC at initial diagnosis and MSS status on NGS. The *MSH6* mutation in this case was not under loss of heterozygosity. Instances of mutations in *MSH6* have been described where protein function other than truncation and antigenicity are affected, thereby showing intact staining on IHC.[11,12] MSI status as determined by PCR using the standard 2 mononucleotide repeats and 3 dinucleotide repeats has been shown to detect a MSS phenotype despite MSH6 deficiency by IHC, given that these tumors are generally stable at dinucleotide repeats.[13] MSI testing via the NGS assay in this case is based on a genome-wide analysis of 95 microsatellite loci, and reports have shown that approximately 35% of cases with mutations in MMR genes are MSS.[14,15] However, it has also been observed that even in the MSS setting, cases of MMR mutations are enriched for high TMB, as seen in this case.[10]

Regardless of our patient's MSS status, there are several genomic findings that corroborate a hypermutated phenotype in this instance. Deficiency in ARID1A, a component of the SWI/SNF chromatin remodeling complex that recruits the MMR protein MSH2 to chromatin during replication, has been shown to compromise MMR, resulting in increased mutation load, higher numbers of tumor-infiltrating lymphocytes, and programmed cell death ligand 1 expression that predicted response to checkpoint inhibition in preclinical cancer models.[16] Increased TGF-β signaling has been shown to represent a primary mechanism of immune evasion and T-cell exclusion, while its blockade renders metastatic colorectal mouse tumors susceptible to checkpoint blockade.[17] Our case had a known inactivating nonsense and frameshift mutation in *ARID1A*, an *MSH6* nonsense mutation, and a known kinase-dead mutation in *TGF-βR2*^{R528C}. These alterations, taken together with the setting of high TMB, suggest a hypermutated phenotype that may underlie the clinical response observed with pembrolizumab in this MSS tumor.

Conclusion

Efforts are ongoing to characterize patients with MSS mCRC who may derive meaningful clinical benefit from immunotherapy, similar to dMMR/MSI mCRC. We described a case of a patient with treatment-refractory MSS mCRC who experienced prolonged response to pembrolizumab with a tumor mutation profile consistent with a hypermutated phenotype, as demonstrated by high TMB and mutations in genes associated with high TMB like *ARID1A* and *TGF-β*. The presence of high TMB and molecular alterations associated with hypermutated phenotypes may identify subsets of MSS mCRC patients whose disease may respond to checkpoint blockade.

Disclosure

The authors have stated that they have no conflict of interest.

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