REPRINTED FROM JULY 2026

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Hypermutated stage IIC colon carcinoma with a rare *POLE* exonuclease variant and co-occurring *MSH6* and *PIK3CA* alterations

CAP TODAY *and the Association for Molecular Pathology have teamed up to bring molecular case reports to* CAP TODAY *readers. AMP members write the reports using clinical cases from their own practices that show molecular testing's important role in diagnosis, prognosis, and treatment. The following report comes from Yale School of Medicine. If you would like to submit a case report, please send an email to the AMP at* amp@amp.org. *For more information about the AMP and all previously published case reports, visit* www.amp.org.

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Colorectal carcinoma (CRC) with hypermutation is most commonly associated with DNA mismatch repair (MMR) deficiency; however, defects in DNA polymerase proofreading represent an important alternative mechanism. Recognition of *POLE*-driven hypermutation has diagnostic and therapeutic implications, particularly in microsatellite-stable CRC.

Case. A 54-year-old man presented with weight loss, rectal bleeding, and diarrhea. Colonoscopy revealed a mass in the transverse colon, and biopsy demonstrated a moderately differentiated adenocarcinoma. As part of universal colorectal cancer screening, MMR protein immunohistochemistry showed retained nuclear expression of MLH1, PMS2, MSH2, and MSH6, consistent with proficient MMR (pMMR) status (**Fig.1**).[1]

The patient underwent laparoscopic extended left colectomy. Final pathology demonstrated tumor invasion into Gerota's fascia without lymph node metastases (pT4bN0, AJCC eighth edition), corresponding to stage IIC disease. Adjuvant FOLFOX chemotherapy was initiated, and comprehensive next-generation sequencing was requested to further characterize tumor biology. In addition, the patient's oncologist requested NGS to assess for PI3K pathway mutations in light of the ALASCCA trial.[2]

Molecular findings. Paired tumor-germline DNA sequencing (Oncomine Comprehensive Assay v3) identified 45 somatic variants with allelic frac-

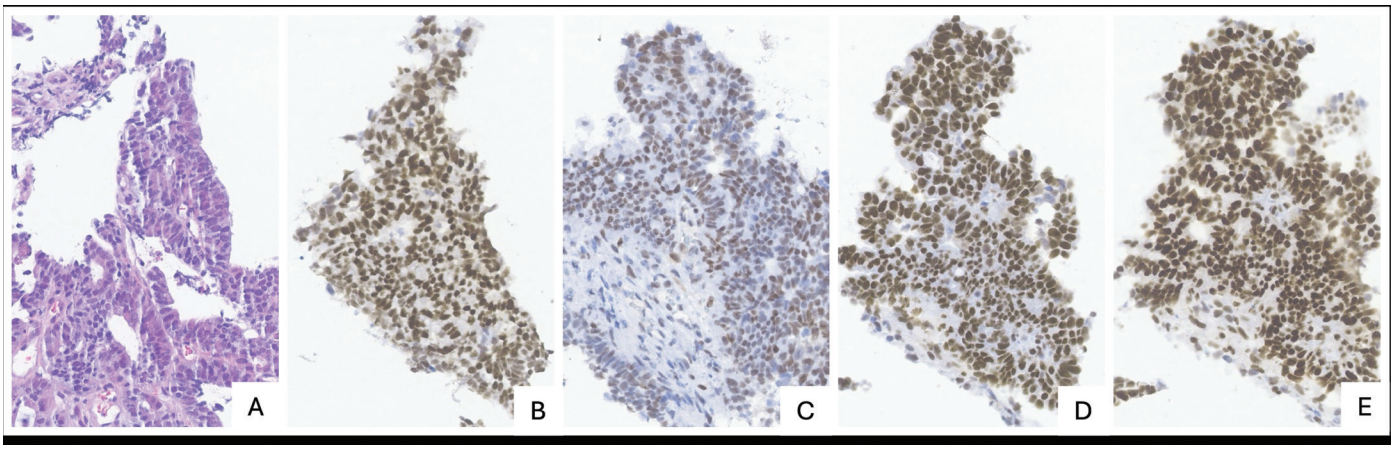

Fig. 1. Colon adenocarcinoma (transverse colon, biopsy): H&E and MMR IHC. **A**) H&E, 40×: neoplastic colonic epithelium. **B–E**) MMR IHC, 40×; MLH1, PMS2, MSH2, and MSH6—panels B–E, respectively—show retained nuclear expression in tumor cells. Interpretation: no abnormal loss of MMR proteins, consistent with proficient MMR status (pMMR).

Gene	Variant (cDNA, RefSeq transcript)	Variant (protein)	Classification	VAF
POLE	NM_006231.4:c.1331T>A	p.Met444Lys	Likely oncogenic	26%
MSH6	NM_000179.3:c.3964G>T	p.Glu1322Ter	Pathogenic	21%
PIK3CA	NM_006218.4:c.263G>A	p.Arg88Gln	Oncogenic	21%

Table 1. Tier 1 (clinically actionable) somatic variants identified by the paired tumor germline sequencing using the Oncomine Comprehensive Assay v3; VAF (variant allele fraction, ≥5 percent) is defined as the proportion of sequencing reads supporting the variant allele relative to the total reads at that genomic position. Estimated tumor purity after manual microdissection was approximately 50 percent.

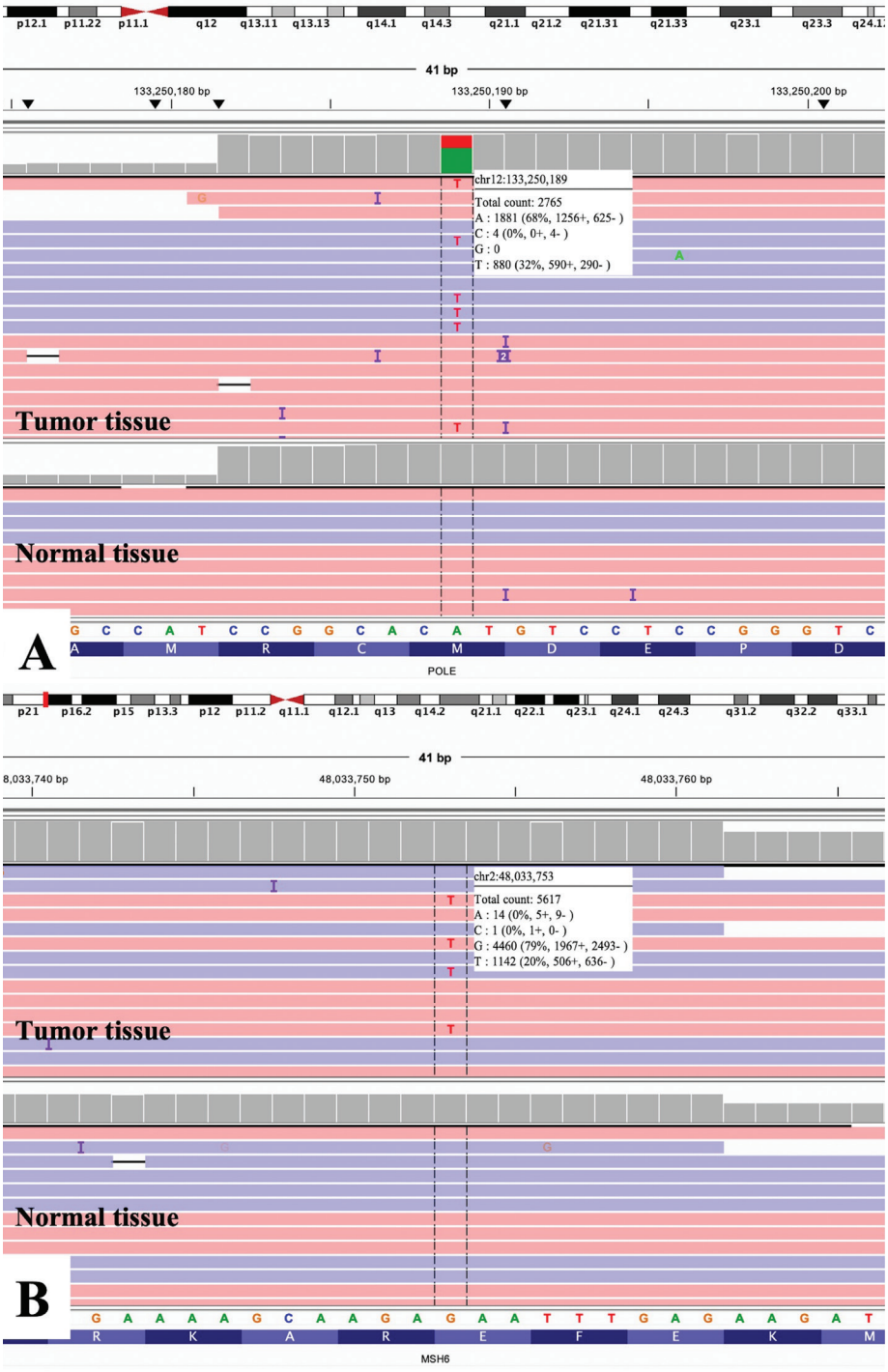

Fig. 2. Integrative Genomics Viewer (IGV) view of *POLE* and *MSH6* variants: **A**) *POLE* c.1331T>A (p.Met444Lys) (minus-strand gene) variant present in tumor and absent in the patient's normal tissue (benign lymph node). Note: IGV shows VAF ≈ 32 percent, whereas the validated pipeline reports VAF = 26 percent after quality filters. **B**) *MSH6* c.3964G>T (p.Glu1322Ter) variant present in tumor and absent in the patient's normal tissue.

tions greater than five percent.Three clinically significant somatic variants were detected in this tumor: *POLE* p.Met444Lys, *MSH6* p.Glu1322Ter, and *PIK3CA* p.Arg88Gln. These three variants are classified as tier I (clinically actionable) based on the AMP/ASCO/CAP somatic variant interpretation guidelines (**Table 1**).[3] The remaining 42 somatic variants were classified as variants of uncertain significance and correspond to AMP tier III.[3] No pathogenic germline variants were detected. Representative Integrative Genomics Viewer images of the *POLE* and *MSH6* variants are shown in **Fig. 2**.

Discussion. A notable feature of this case is the coexistence of *POLE* p.Met444Lys and *MSH6* p.Glu1322Ter variants in a tumor with a high number of somatic mutations. The number of somatic variants detected in this tumor is significantly (i.e. more than 10-fold) greater than what is typically identified in colorectal cancers analyzed on this gene panel. Although the *POLE* and *MSH6* alterations are both pathogenic and associated with hypermutation, the hypermutation arises through fundamentally distinct molecular mechanisms.[4] Discriminating between these mechanisms is critical, as they carry different biological, diagnostic, and therapeutic implications.

POLE encodes the catalytic subunit of DNA polymerase epsilon and contains an exonuclease ("proofreading") domain essential for maintaining replication fidelity. Hotspot missense variants affecting this domain disrupt proofreading activity and act in a dominant manner to promote extreme hypermutation. Such exonuclease-domain mutations define a distinct molecular subtype of hypermutated CRC characterized by very high tumor mutational burden, enhanced immune infiltration, and a favorable prognosis.[4-6] The *POLE* p.Met444Lys variant identified in this tumor lies within the exonuclease domain and has been rarely described in association with hypermu-

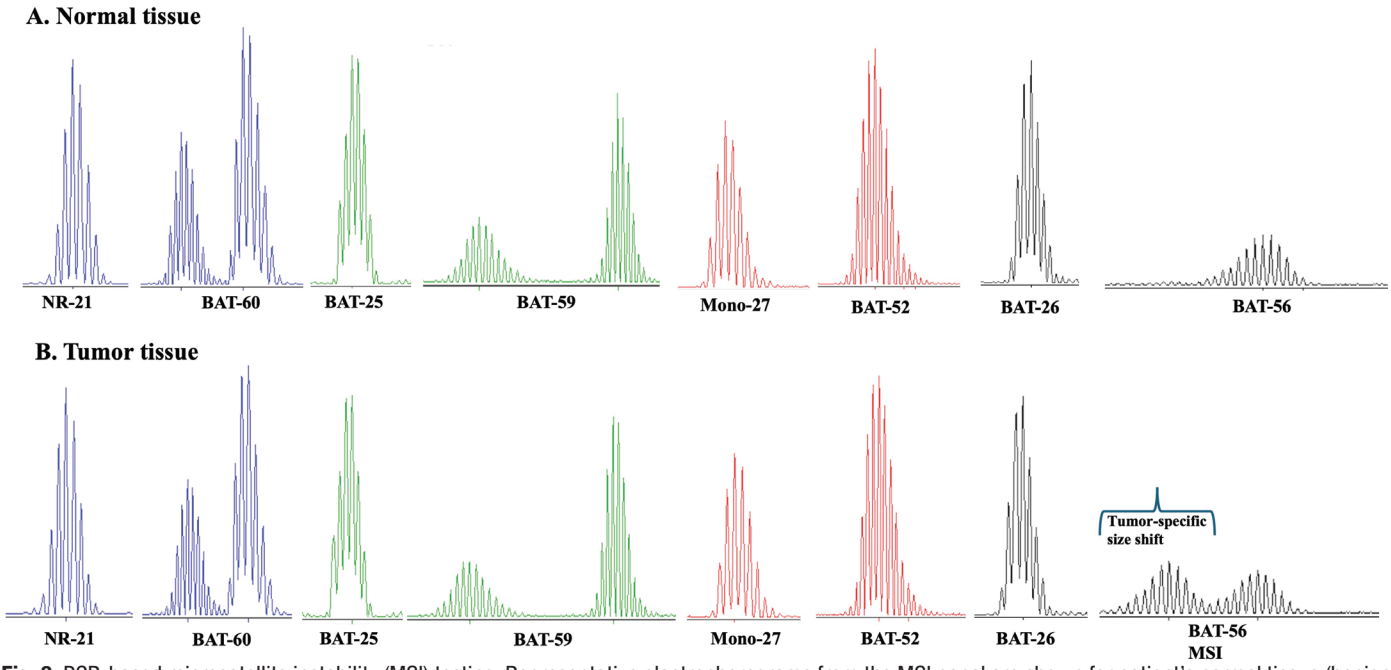

Fig. 3. PCR-based microsatellite instability (MSI) testing. Representative electropherograms from the MSI panel are shown for patient's normal tissue (benign tissue from large intestine **A**) and tumor tissue **B**). Seven loci (NR-21, BAT-60, BAT-25, BAT-59, Mono-27, BAT-52, BAT-26) demonstrate identical allele patterns between normal and tumor, consistent with microsatellite stability (MSS). The BAT-56 locus shows a tumor-specific size shift, consistent with instability at this single marker. Overall, the tumor does not meet criteria for MSI-high and is classified as microsatellite stable with one reproducibly unstable locus.

tated endometrial cancer and CRC.[7-9] Although this variant has not been reported in the ClinVar database, a patient with a constitutional *POLE* p.Met444Lys variant and hypermutated CRC has been previously described.[10] In addition, the orthologous yeast mutation results in an increased spontaneous mutation rate supporting a pathogenic role for this alteration.[11]

In contrast, hypermutation driven by DNA MMR deficiency requires biallelic inactivation of an MMR gene, resulting in defective repair of mismatched bases generated during DNA replication. In this tumor, only a single, truncating *MSH6* p.Glu1322Ter variant was detected, and the tumor was pMMR by IHC (Fig. 1). Evidence for defective MMR was also sought by performing PCR-based microsatellite instability testing. The findings showed instability in only one of eight tested loci and argue against defective DNA MMR as the primary driver of hypermutation in this tumor (**Fig. 3**).[12,13] These findings were further supported by bioinformatic analysis of the NGS data using MSIsensor2, which showed no evidence of microsatellite instability.

Taken together, the molecular, immunohistochemical, and MSI PCR findings strongly support *POLE* exonuclease-domain dysfunction as the predominant mechanism underlying the hypermutated phenotype in this patient's tumor.

Clinical significance. This case illustrates the critical importance of identifying *POLE*-driven hypermutation in CRC. While CRC hypermutation is most often attributed to MMR deficiency, *POLE* hypermutated CRC represents a distinct and much rarer clinically relevant molecular subtype. In contrast to the well-established screening protocols for MMR deficiency, standardized guidelines for assessing for *POLE* hypermutation are lacking.

Hypermutated CRC are clinically significant because these tumors are expected to be particularly sensitive to immune checkpoint therapy due to increased neoantigen load, as reflected in the NCCN Clinical Practice Guidelines in Oncology for Colon Cancer.[1] Consistent with this, advanced CRC with pathogenic *POLE* exonuclease-domain mutations have been associated with responses to immune checkpoint inhibitor therapy, even in pMMR CRC.[14,15] Moreover, in a recent multicenter study, patients with POLE- or POLD1-proofreading-deficient metastatic colorectal cancer showed high response rates to immune checkpoint inhibitors, in some cases exceeding those typically reported for mismatch-repair–deficient colorectal cancer.[16]

In light of these data, phase three data from the ATOMIC trial demonstrated improved disease-free survival with the addition of atezolizumab, an anti-PD-L1 immune checkpoint inhibitor, to adjuvant FOLFOX in patients with stage III MSI-H/dMMR CRC, findings that have informed recent NCCN guideline updates.[1,17] For high-risk stage II disease, including stage IIC MSI-H/dMMR or POLE/POLD1-mutated CRC, NCCN guidelines allow consideration of adjuvant systemic therapy analogous to low-risk stage III disease, including fluoropyrimidine-based chemotherapy with atezolizumab.[1]

In addition, identification of a concurrent activating *PIK3CA*

p.Arg88Gln variant provided another actionable finding based on emerging clinical trial data showing that *PIK3CA* mutations are associated with improved outcomes in CRC patients receiving adjunctive low-dose aspirin therapy, as demonstrated in observational studies and the ALASCCA trial. [2,18]

Following comprehensive review at a multidisciplinary precision medicine tumor board, atezolizumab was added to adjuvant FOLFOX chemotherapy, and low-dose aspirin was also initiated.

From a broader perspective, this case highlights an opportunity for evolution in clinical testing strategies. We believe broad NGS testing should be incorporated routinely for stage II to IV MMR-proficient CRC. This approach has the practical advantage of capturing *POLE* exonuclease-domain pathogenic variants and hypermutation in the same workflow used to assess several other clinically relevant alterations, including RAS/RAF, ERBB2, and PI3K pathway changes.[19,20] Although current NCCN guidelines do not explicitly mandate universal *POLE* testing in CRC, we view routine NGS-based tumor profiling as an important step toward consistent and equitable access to molecularly informed care. Integrating these complementary approaches is essential to ensure accurate tumor classification and to guide optimal, personalized therapy for patients with CRC. □

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Test yourself

Here are three questions taken from the case report. Answers are online now at www.amp.org/casereports *and will be published next month in* CAP TODAY.

1. Which molecular finding most strongly supports *POLE*-driven hypermutation in this case?

a. Presence of a truncating *MSH6* variant.
b. Instability at one microsatellite locus.
c. Retained expression of all MMR proteins by immunohistochemistry.
d. Finding of *POLE* exonuclease variant with functional evidence of polymerase proofreading deficiency in the setting of hypermutated tumor.

2. Why is identification of *POLE* exonuclease-domain mutations clinically important in colorectal cancer?

a. They predict resistance to chemotherapy.
b. They are associated with low tumor mutational burden.
c. They may predict responsiveness to immune checkpoint inhibitor therapy.
d. They exclude the need for microsatellite instability testing.

3. Which statement best describes the relationship between hypermutation and mismatch repair deficiency in colorectal cancer?

a. All hypermutated colorectal cancers are mismatch repair deficient.
b. Hypermutation may result from either mismatch repair deficiency or polymerase proofreading defects.
c. Hypermutation occurs only in microsatellite-unstable tumors.
d. Hypermutation is independent of DNA repair mechanisms.

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