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Ultra-hypermutated colorectal adenocarcinoma identified at relapse

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 Quest Diagnostics Nichols Institute. 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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We present the case of a 75-year-old woman with a history of a pT4 colorectal adenocarcinoma who was status-post right hemicolectomy two years prior to the current care episode. She returned for urgent medical evaluation of recurrent abdominal pain, was diagnosed with a small bowel obstruction, and underwent exploratory laparotomy with small bowel resection and primary anastomosis. Gross review of the resection specimen revealed serosal abnormalities, including numerous adhesions and a solid mass lesion that extended into peri-intestinal adipose tissue. Histologic evaluation confirmed the presence of persistent/recurrent colorectal adenocarcinoma (**Fig. 1**). Initial immunohistochemical profiling demonstrated loss of nuclear staining for MLH1 and PMS2 but preserved MSH2 and MSH6 staining, suggestive of an abnormality of DNA mismatch repair (MMR-deficiency), possibly affecting MLH1. Comprehensive genomic profiling was performed on a representative section of the tumor, estimated to contain about 60 percent tumor nuclei.

The patient's sample was analyzed using the Solid Tumor Expanded Panel (Quest Diagnostics, San Juan Capistrano), which targets 523 genes using a hybrid-capture–based next-generation sequencing system for sequence alterations, gene fusion, copy number alterations, tumor mutational burden, and microsatellite instability. NGS revealed 37 pathologic (AMP tier I/II) alterations, 155 variants of uncertain significance (VUS, AMP tier III) alterations, an extremely high tumor mutational burden of 182.9 mutations/Mb, and a high microsatellite instability score of 82.6 percent unstable sites. The five tier I variants included the following: *POLD1* (p.P116Hfs*53, variant allele frequency 36.8 percent), *MLH1* (p.R497Pfs*6, VAF 58.6 percent), *MSH2* (p.A230Lfs*16, VAF

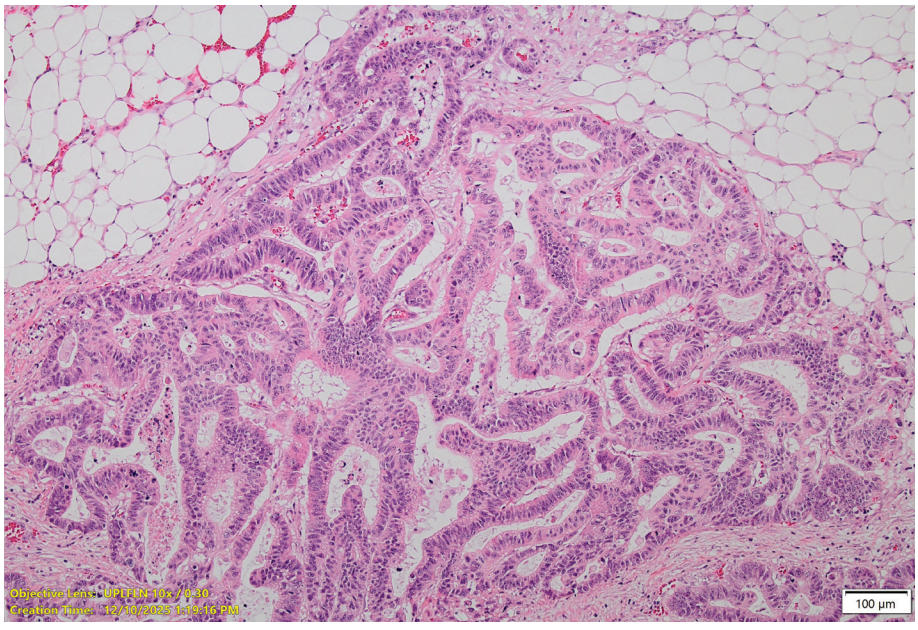

Fig. 1. Hematoxylin and eosin stain, 200×. Representative image from recurrent CRC serosal mass lesion.

22.9 percent), *MSH6* (p.G1105Wfs*3, VAF 31.3 percent), and *PIK3CA* (p.Y1021C, VAF 25.3 percent). Sequencing findings for the frameshift truncating alterations in MMR-related genes are visually represented in **Fig. 2**. No copy number alterations or fusions were detected.

The World Health Organization genomically classifies colorectal carcinoma into two main groups by mutation rate: hypermutated and non-hypermutated cancers, which match well with the MSI and chromosomal instability pathways. About 15 percent of CRCs are hypermutated and have high MSI related to defective DNA MMR, typically related to either *MLH1* promoter hypermethylation or gene mutation(s) of mismatch repair genes. Two to three percent of CRC cases are ultra-hypermutated, caused by inactivating alterations of *POLE* or rarely *POLD1*, which encode proteins that "proofread" and correct mispaired bases incorporated during DNA replication. This case had functionally deleterious alterations in *POLD1* as well as in MMR genes *MLH1*, *MSH2*, and *MSH6*, and had corresponding extreme increases in TMB and MSI. It is noted that immunohistochemical stains showed preserved expression of MSH2 and MSH6, but this likely represents a false-negative result, which can occur in five to 10 percent of mismatch repair-deficient cases, possibly caused by retained protein expression despite sequence mutation, subclonality, and/or tumor heterogeneity.[1,2]

The molecular findings permitted classification of this patient's tumor into the NCCN "dMMR/MSI-H or *POLE/POLD1* mutation with ultra-hypermutated phenotype [e.g. TMB >50 mut/Mb]" category, which has specific treatment algorithms for disease presentation at both initial presentation and recurrence. Patients with *POLE/POLD1*-mutation positive ultra-hypermutated CRC generally have a more favorable prognosis, thought to be related to immune responses stimulated by numerous neoantigens arising from the DNA proofreading deficit, and their disease generally responds well to immune checkpoint inhibitor therapy.[3]

Further workup will be necessary to confirm the germline or somatic status of the *POLD1* variant and to

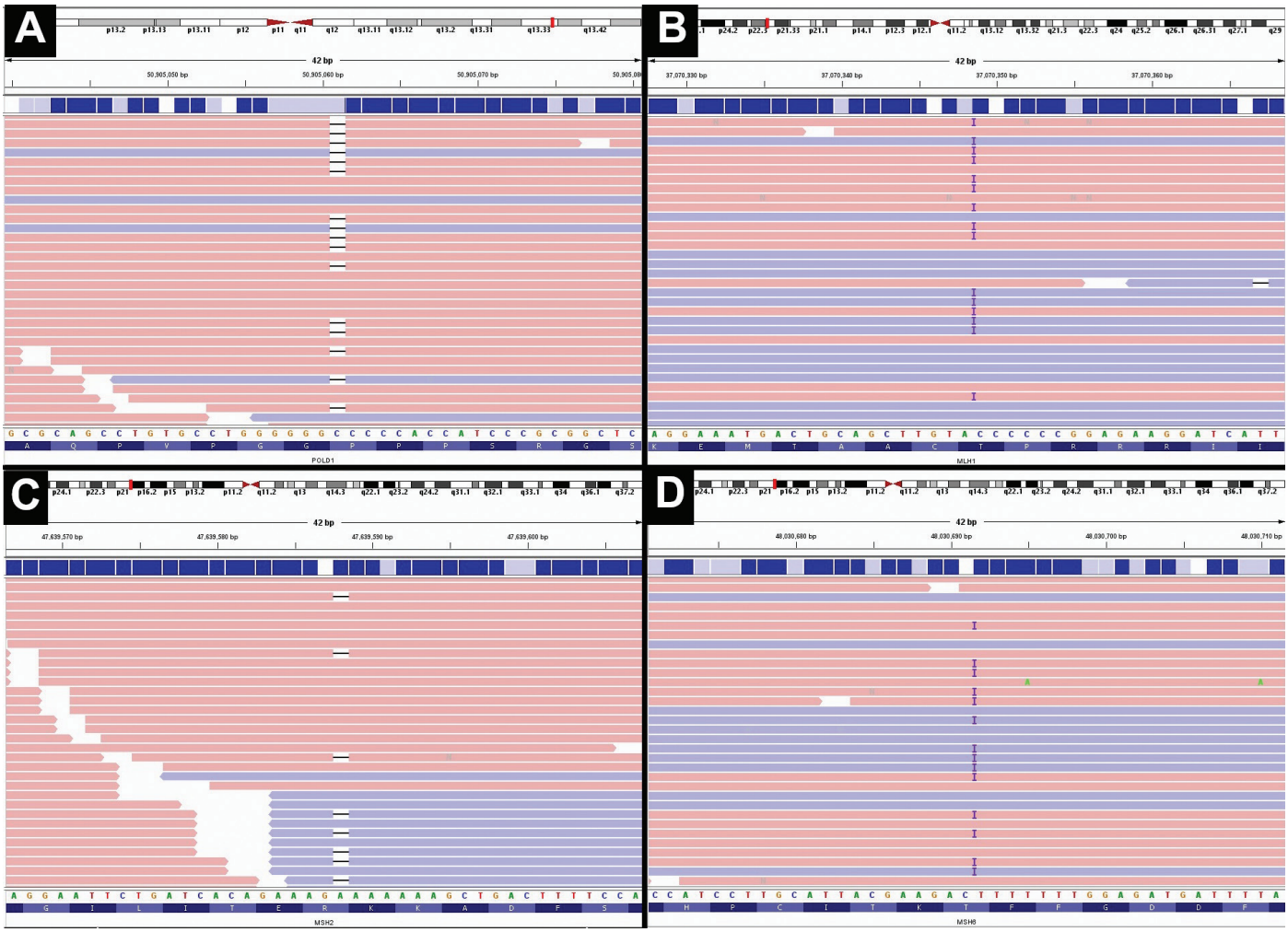

Fig. 2. Integrative Genomics Viewer (IGV; Broad Institute, Cambridge, Mass.) showing truncating frameshift sequence alterations in DNA MMR-associated genes c.347delC (A), *MLH1* c.1489dupC (B), *MSH2* c.687delA (C), and *MSH6* c.3312dupT (D).

determine if there are other germline deficits of DNA MMR, given the relatively high variant allele frequencies associated with DNA MMR genes *MLH1, MSH2,* and *MSH6*. Lynch syndrome and constitutional mismatch repair deficiency (CMMRD) are both inherited DNA repair disorders with mutations in the same MMR genes, but they differ fundamentally in inheritance pattern, age of onset, cancer spectrum, and disease severity. Lynch syndrome is an autosomal dominant disorder caused by monoallelic germline mutations in MMR genes or *EPCAM*, where the single wild-type allele provides adequate MMR function until a second somatic hit occurs in tumor tissue. CMMRD is an autosomal recessive disorder caused by biallelic germline pathogenic variants in MMR genes, resulting in complete absence of DNA MMR activity in all tissues from birth, frequent café-au-lait macules, earlier onset of cancer development, and a broader spectrum of malignancies compared with Lynch syndrome.[4] Parents of children with CMMRD typically each carry one MMR mutation and may themselves have Lynch syndrome. IHC staining of associated tumors in both syndromes will demonstrate loss of normal nuclear expression of specific MMR proteins (MLH1, MSH2, MSH6, or PMS2), but in CMMRD the loss of staining impacts both tumor and normal tissues and PMS2 and MSH6 are typically involved, while MLH1 and MSH2 loss/variants are less frequent but associated with more aggressive phenotypes.

An additional germline concern lies with potential pathogenic alterations of *POLE* and/or *POLD1*, which can predispose patients to adenomatous polyps, CRC, endometrial tumors, and other malignancies; the condition is referred to as polymerase proofreading-associated polyposis (PPAP).[3,5] Furthermore, constitutional defects of DNA polymerase genes (*POLE* and *POLD1*) are known to have the potential to lead to biallelic somatic MMR gene inactivation, which can mimic Lynch syndrome.[1,6]

In summary, molecular characterization added significant value in this patient's case. In addition to identifying a rare alteration permitting subcategorization of disease with specific treatment guidance and therapy, the clinical team was also alerted to the possibility of germline considerations that could alter follow-up management for the patient and her family members. □

1. National Comprehensive Cancer Network. NCCN Clinical Practice Guidelines in Oncology: Genetic/Familial High-Risk Assessment: Colorectal, Endometrial, and Gastric. Version 1.2025. www.nccn.org

2. Sepulveda AR, Hamilton SR, Allegra CJ, et al. Molecular biomarkers for the evaluation of colorectal cancer: guideline from the American Society for Clinical Pathology, College of American Pathologists, Association for Molecular Pathology, and American Society of Clinical Oncology. *Arch Pathol Lab Med*. 2017;141(5):625–657.

3. National Comprehensive Cancer Network. NCCN Clinical Practice Guidelines in Oncology: Colon Cancer. Version 5.2025. www.nccn.org

4. Ercan AB, Aronson M, Fernandez NR, et al. Clinical and biological landscape of constitutional mismatch-repair deficiency syndrome: an International Replication Repair Deficiency Consortium cohort study. *Lancet Oncol*. 2024;25(5):668–682.

5. Mur P, García-Mulero S, Del Valle J, et al. Role of *POLE* and *POLD1* in familial cancer. *Genet Med*. 2020;22(12):2089–2100.

6. Morak M, Heidenreich B, Keller G, et al. Biallelic *MUTYH* mutations can mimic Lynch syndrome. *Eur J Hum Genet*. 2014;22(11):1334–1337.

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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. What is the function of the proteins encoded by the *POLE/POLD1* genes?

a. Helps trigger apoptosis or cell cycle pauses.
b. Catalytic subunits of DNA polymerases, necessary for proofreading function and mismatch repair.
c. Part of the TGF-beta signaling pathway is involved in regulation of cell growth/proliferation and development of many body systems.
d. Histone methyltransferase that mediates epigenetic gene regulation.
e. Hormone-inducible transcriptional repressor.

2. Why are colorectal cancer patients with *POLD1* thought to have a more favorable prognosis?

a. Creation of numerous neoantigens produced from aberrant proofreading function causes an immune response and is favorable to immune checkpoint inhibitor therapy.
b. Patients with TGF-beta/SMAD pathway alterations may benefit from investigational therapies targeting TGF-beta signaling.
c. High ERBB3 expression may correlate with better overall survival in cases of *RAS*-wild-type metastatic colorectal cancer.
d. dMMR status is associated with a decreased likelihood of tumor metastasis and a favorable outcome in patients with stage II disease.
e. Functionally deleterious/truncating alterations of *POLD1* may be related to enhanced radiosensitivity.

3. Which of the following tumor mutational burdens (reported in mutations/megabase) would be considered high enough to be suggestive of the ultra-hypermutated phenotype associated with *POLE/POLD1* gene mutation?

a. 53
b. 7
c. 25
d. 40
e. 10