Mismatch repair and clinical response to immune checkpoint inhibitors in endometrial cancer

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LAY SUMMARY:

- Endometrial cancer is common, and a subset recurs and requires additional treatment.
- Some of these are recognized as being susceptible to immune therapies and are said to have mismatch repair deficiency (dMMR).
- However, this clinical trial highlights which cases are more likely to respond well: those containing mutations in genes known as Lynch genes and also some with mutations in POLE/POLD1 ("ultra-hypermutation" genes).
- In contrast, the majority of dMMR endometrial cancers have silencing or DNA methylation of one of these genes, *MLH1*, and do not seem to be as responsive to single-agent immune therapy.
- The availability of combination therapies may be important to consider for these women.

Endometrial cancer (EC), the most common gynecological cancer affecting women in developed countries, is rising in incidence, partly because of increasing obesity and our aging population.[1] On the basis of genomic, proteomic, and epigenomic evaluations, 4 distinct molecular subtypes of EC have been defined: polymerase ε (*POLE*)–hypermutated, microsatellite instability, copy number–low/p53 wild type, and copy number–high/p53-mutated.[2]

Up to 30% of all ECs are associated with DNA mismatch repair deficiency (dMMR).[3] As for other tumor types, dMMR in EC may be either acquired or due to inherited defects in 1 of 4 DNA mismatch repair (MMR) genes (*MLH1*, *MSH2*, *MSH6*, or *PMS2*) or in *EPCAM* (causing downstream silencing of *MSH2*).[4-9] The majority of dMMR in ECs (~75% of dMMR and ~20% of all ECs) is caused by acquired hypermethylation of the *MLH1* gene promoter.[3] The remaining causes of dMMR in ECs are attributed to either double somatic MMR mutations or germline MMR pathogenic variants.[10] Cancers with dMMR typically have a microsatellite instability–high (MSI-H) phenotype due to uncorrected errors that occur in repetitive DNA sequence during DNA replication, which results in a high somatic mutation frequency. When these dMMR-related mutational events occur in coding regions, the generation of high levels of novel frameshift peptide antigens occurs. The abundance and "foreign" nature of these frameshift peptide neoantigens in dMMR cancers likely explain the strong CD3+ and CD8+ T-cell responses, which are predictive of sensitivity to immune checkpoint inhibitor (ICI) therapy.[11]

Most patients are diagnosed at an early stage and cured with surgery and/or local therapies. Chemotherapy (carboplatin combined with paclitaxel) has remained the first-line systemic therapy beyond endocrine therapy for women with advanced or recurrent EC. Therapeutic options after this are associated with poor outcomes with response rates of 20% or less.[12] Chemotherapy resistance has been reported in dMMR tumors, and this may explain the worse prognosis in the advanced setting in comparison with MMR-proficient tumors.[3,12,13]

Immunotherapy using a single-agent ICI may be a highly effective treatment for dMMR/MSI-H ECs with reported overall tumor response rates (ORRs) between 27% and 57%.[12] However, these studies are small and include single-arm

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phase 2 studies and basket studies. A recent presentation of the 309/KEYNOTE-775 clinical trial, which included the combination of pembrolizumab and lenvatinib, a multikinase inhibitor, in a second or subsequent line of therapy for EC, indicated a median ORR of 40.0% versus 12.3% for a physician's choice of chemotherapy with median progression-free survival of 10.7 months versus 3.7 months (hazard ratio, 0.36; 95% CI, 0.23-0.57; $P < .001$).[14] Pembrolizumab was granted accelerated Food and Drug Administration approval for use in any MSI-H or dMMR tumors, including ECs, but this has not yet resulted in widespread reimbursement elsewhere. In March 2020, the National Comprehensive Cancer Network recommended The Cancer Genome Atlas Research Network molecular typing of EC for the first time and included it in the guidelines for the diagnosis and treatment of EC; this was reflective of the impact of ICI therapy based on tumor microenvironment and genotype.

In this issue of *Cancer*, Bellone et al[15] report potential prognostic differences in responses to ICI therapy by differing underlying mechanisms of dMMR in a prospective study.[16] In this small phase 2 study of 24 patients with a median follow-up of 25.8 months, the observed ORR of 58% (95% CI, 36.6%-77.9%) is typical of those demonstrated in other single-agent ICI therapy studies.[12] However, marked differences in response rates were observed according to whether dMMR was related to a somatic mutation in an MMR gene (Lynch-like; 6 patients; ORR, 100%) or the EC was associated with *MLH1* promoter hypermethylation (19 patients; ORR, 44%; $P = .024$). Three-year progression-free survival and overall survival outcomes also differed similarly: 100% versus 30% ($P = .017$) and 100% versus 43% ($P = .043$), respectively. There were no germline MMR pathogenic variant carriers detected in this study. Additionally, the median time to a partial/complete response was shorter—62 days (interquartile range [IQR], 53-75 days) among patients classified as Lynch-like and 177 days (IQR, 86-460 days) among those with *MLH1* hypermethylation ($P = .020$)—with reportedly similar tumor sizes at the baseline.

To understand the marked difference in outcomes between the Lynch-like/MMR gene–mutated cohort and the *MLH1*-methylated cohort, a range of translational analyses were performed to determine the differences at immunological and molecular levels. Differences were observed in genetic (*POLE*/*POLD1*), genomic (tumor mutation burden [TMB]), and immunologic parameters (macrophage markers). Three of the 6 Lynch-like patients (and the only 3 patients overall) demonstrated TMBs that were ultra-hypermutated and driven by somatic exonuclease domain mutations in either *POLE* or *POLD1*; 1 tumor's TMB exceeded 400 mutations/Mb and was related to a known *POLE* somatic hotspot mutation (p.V411L). Although individual TMBs were not reported, further investigation of tumor sequencing data may reveal more subtle differences in TMB or mutated genes disrupted by dMMR that differ between dMMR subtypes or, more specifically, between responders and nonresponders. Further delineation of ICI responses may be determined from a tumor mutational signature analysis of whole exome sequencing (WES) data, an analysis that proved useful in this study for confirmation of the dMMR status and the exclusion of a false-positive dMMR tumor.

Only half of the 6 Lynch-like dMMR ECs demonstrated double somatic MMR mutations in the gene that was indicated to be defective by the pattern of loss of MMR protein expression by immunohistochemistry (IHC; PEM23, PEM14, and PEM25). Among the remaining 3 Lynch-like tumors, PEM05 showed no somatic mutations in *MLH1* despite MMR IHC showing a loss of expression of MLH1 and PMS2. PEM02's EC showed solitary loss of PMS2 expression and a somatic copy number variant across *PMS2*, and notably, patient PEM06, classified as Lynch-like and demonstrating a complete response, had *MLH1* promoter hypermethylation and a somatic *MSH6* mutation and could have alternatively been classified as a sporadic *MLH1*-methylated tumor. The pattern of loss observed in this tumor (*MLH1*/*PMS2* and *MSH6*) is not uncommon where MSH6 loss via somatic mutation is considered secondary to dMMR caused by *MLH1* methylation.[17,18]

PD-L1 expression is a proven biomarker for predicting ICI responses in several cancer types and has approved companion platforms for assessment in some tumor types, including cervical cancer, but such validity is lacking for EC. Several studies have indicated higher expression in dMMR/MSI-H tumors, whereas a more recent review reported very low rates in ECs (3.1%).[19] In the clinical setting, outcomes for ICI therapy in EC according to PD-L1 staining have been variable with no consensus on a standardized approach to clinically relevant cut points.[19] In this small study, no difference in PD-L1 staining was observed between the Lynch-like and *MLH1*-methylated cohorts either in tumor cells, despite significant tumor expression of PD-L1, or in associated immune cells.

One of the hallmarks of dMMR tumors is a higher rate of tumor-infiltrating lymphocytes (TILs), which include a range of lymphocytes exerting various influences on immune interactions. ECs with high TILs are

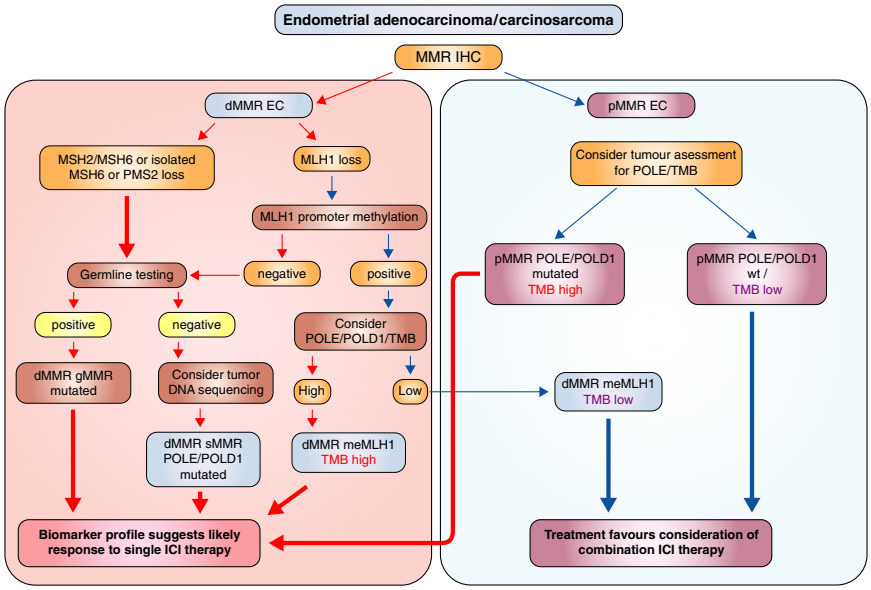

Figure 1. Proposed pathway for the investigation of endometrial adenocarcinoma/carcinosarcoma with MMR IHC, tumor and matched germline sequencing, and *MLH1* promoter methylation. MMR IHC is performed and results in either deficient (dMMR) or proficient (pMMR) categorization. For dMMR cases showing a loss of MLH1/PMS2 protein expression, *MLH1* promoter methylation testing should then be performed, and an assessment of TMB may be considered by DNA sequencing (eg, by WES). Germline MMR (gMMR) testing is performed for dMMR cases (and if MLH1 methylation is negative), and if it is positive for a pathogenic variant, EC would be categorized as dMMR gMMR mutated (Lynch syndrome). If gMMR testing is negative, then DNA sequencing of the tumor may be performed as part of a panel or WES to categorize EC as dMMR somatic MMR (sMMR) mutated. EC on a pink background is favored to receive single-agent ICI therapy. EC on the blue background is favored to be considered for combination ICI therapy. In the pink background, a transition from dark pink to pale pink indicates an immunologically hot tumor to a less warm tumor; the blue background indicates an immunologically cold tumor. The MMR genes are *MSH2*, *MSH6*, *PMS2*, and *MLH1*. dMMR indicates mismatch repair deficiency; EC, endometrial cancer; gMMR, germline mismatch repair mutation; ICI, immune checkpoint inhibitor; IHC, immunohistochemistry; meMLH1, tumor hypermethylation of the *MLH1* gene promoter; MMR, mismatch repair; pMMR, mismatch repair proficiency; POLE, polymerase ε; POLE/POLD1, somatic mutation in the exonuclease domain of either the *POLE* or *POLD1* gene; sMMR, somatic mismatch repair mutation; TMB, Tumor Mutational Burden; WES, whole exome sequencing; wt, wild type.

associated with improved overall outcomes. Variation in the immune environment has been reported between subtypes of dMMR tumors, with germline MMR pathogenic variant carrier tumors having reduced macrophages and increased CD8+ cells with activated cytotoxic tumor lymphocytes in comparison with those with acquired *MLH1* methylation or somatic mutations.[20] In this study, no differences were reported for TILs. However, higher infiltration of CD68+ macrophages in both tumor and adjacent stroma was observed for the Lynch-like dMMR cohort versus the *MLH1*-methylated cohort (IHC scores, 2.8 vs 2.1; $P = .022$). The small number of cases and some variability in the literature require some caution in interpreting this finding.

A genetic analysis was performed by WES, which allowed additional useful information to be derived, including documentation of pathogenic mutations in genes driving ultra-hypermutated phenotypes such as *POLE* and *POLD1*. Two pathogenic somatic mutations were reported in *POLE*, and 2 were reported in *POLD1* (all in Lynch-like cases), although only 2 of these were in the exonuclease domain and were associated with the ultra-hypermutated phenotype (*POLE* p.V411L and *POLD1* p.D316N); both resulted in good outcomes. Further interrogation of the tumor mutational signatures in the remaining 2 *POLE*/*POLD1* putative somatic-mutated tumors, specifically the presence of single-base substitution signatures associated with defective *POLE* or *POLD1*, could clarify their pathogenic status.

A high TMB is thought to be one of the key indicators of ICI sensitivity. Cancers with dMMR typically have an MSI-H phenotype and a high mutational frequency. As such, they have a high predicted neoantigen load and thus generate CD3+ and CD8+ T-cell responses, which predict for a higher response to ICI therapy. TMB was significantly higher in cases with somatic MMR gene mutations (mean ± SD, 4386 ± 5045 mutations/Mb [median, 2939 mutations/Mb; IQR, 867-5108 mutations/Mb] vs 608 ± 241 mutations/Mb [median, 604 mutations/Mb; IQR, 411-798 mutations/Mb]; $P = .0076$). Thus,

although TMB appears to reflect the clinical outcomes of ICI therapy in these 2 cohorts in this small study, the driving influence of somatic mutations in *POLE* or *POLD1* and the resultant ultra-hypermutated phenotype was likely to have influenced the overall cohort result.

This study by Bellone and colleagues[15] highlights the need for a transition to tumor sequencing–based detection of the dMMR status. The assessment of multiple tumor-based features as evidence of defective MMR, namely microsatellite instability, tumor mutational signatures, and TMB, has the potential to improve dMMR detection accuracy, and when it is considered together with the ability to classify a tumor's etiology as either double somatic MMR mutations or germline MMR pathogenic variants from the same assay, it has significant potential for precision ICI therapy. For a study of this size, displaying individual response data (eg, duration-of-response data, including a swimmer's plot with relevant molecular annotations) and providing individual values for TMB would have been extremely valuable.

The consideration of potential mechanisms underlying the significantly better ORR for Lynch-like patients versus *MLH1*-methylated patients is important, and although both subtypes display dMMR and high levels of somatic mutations, there is a key difference in biology between these 2 groups. *MLH1* gene promoter hypermethylation is a key mechanism that inactivates *MLH1* transcription and leads to dMMR, but there are significant DNA methylation changes occurring beyond the *MLH1* locus at a genome-wide level in *MLH1*-methylated tumors. The CpG island phenotype (CIMP) has been reported in several cancer types, including colorectal cancer and glioblastoma, and it is strongly associated with *MLH1* hypermethylation in EC (MC1 cluster in The Cancer Genome Atlas analysis[21]). The inactivation of tumor suppressor genes by DNA methylation may target a different set of genes that are not altered in tumors with double somatic MMR pathogenic variants (Lynch-like) or even in Lynch syndrome ECs that are devoid of these genome-wide DNA methylation or CIMP changes.

The authors also report 2 patients with *MLH1* promoter hypermethylated ECs, one with primary resistance and another with secondary resistance, with long durable responses on continued pembrolizumab provided off study after localized therapies successfully treated single sites of resistant (oligometastatic) disease. As described for hypermutated *POLE* ECs,[22] neoantigens may play a role underpinning immunity and may also provide a rationale for the *MLH1*-methylated cases described here.[22]

This hypothesis-driving study highlights the need for further evaluation of the interaction between ICI responses and the mechanism of dMMR (see the recommended pathway for the investigation of EC in Fig. 1). Although germline MMR carriers and Lynch-like tumors with double somatic loss are highly likely to respond to a single-agent ICI, those with *MLH1* hypermethylation may benefit from additional agents to induce an ICI response. Building these translational aspects into clinical trials is important because they provide a greater understanding of when minimal immune-based treatments are required and when combination therapy is appropriate. It is worth considering that reporting dMMR EC clinical trials of ICI therapy without reporting the MMR gene mutation versus *MLH1* methylation status for all individual patients from this point onward would be like reporting an ovarian cancer PARP inhibitor clinical trial without reporting the BRCA1/2 status from 2015 onward—challenging, but not impossible, and essential for enabling the field to move forward effectively.

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CONFLICT OF INTEREST DISCLOSURES

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