



CANCER

Immunotherapy is alive and well, and endometrial cancer may be the prototype



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The authors report no financial relationships relevant to this article.

Each year approximately 60,000 women are diagnosed with endometrial cancer. The majority of the identified tumors will be low grade—cancer found at an early stage that may be treated with surgery alone. Unfortunately, however, too many of the 60,000 patients will have poor prognostic features, such as serous or clear cell histology (high-grade cancer), lymphovascular space invasion, or positive lymph node status.

Advances in technology and the state of science have come a long way since the dichotomy of Type I (endometrioid) and Type II (serous and clear cell) tumors were described by Dr. J. Bokhman in the early 1980s.¹ Our previous *Update* from several years ago stressed the importance of further understanding of the molecular rationale of high-risk, Type II tumors.² To review, The Cancer Genome Atlas project (TCGA) performed a genomic and proteomic characterization in 373 endometrial carcinomas demonstrating the traditional p53 mutations of serous tumors and *PTEN* or *KRAS* genes of endometrioid tumors.³ Most interestingly, they identified numerous other mutations and proposed 4 new genomic categories:

1. polymerase (DNA-directed) epsilon catalytic subunit (*POLE*) ultramutated
2. microsatellite instability (*MSI*) hypermutated
3. somatic copy number alterations high

(serous tumors)

4. somatic copy number alterations low (endometrioid cancer).

In 2016, we are now understanding the molecular basis of disease and how it affects survival; these 4 categories have different survival. But why? Perhaps the answer lies within the endogenous immune system. Tumor-infiltrating lymphocytes are associated with improved survival in multiple types of cancer, including endometrial. Whether these lymphocytes are regulatory or cytotoxic T-cells convolutes the matter further.⁴ To understand these intricacies we need to further categorize how a tumor's genetic mutations affect antigen exposure to the immune system, quantitate the clinical impact of the findings, and selectively target patients with novel therapeutics.

In this *Update*, we look at data on *POLE* mutations, exploring 2 studies that help us to better understand why these types of mutations have uniquely positive prognostic implications (when they logically should not have good survival rates). In addition, we discuss 2 studies that examined mismatch repair defects, in endometrial cancer specifically, and the programmed death (PD)-1 pathway in both endometrial and other cancer types. Are these molecular entities of tumors associated with better or worse prognosis, and why?

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Can the immune system kill *MMR*-deficient endometrial cancer?

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Molecular profiling: Prognostic implications of POLE mutations

Church DN, Stelloo E, Nout RA, et al. Prognostic significance of POLE proofreading mutations in endometrial cancer. J Natl Cancer Inst. 2014;107(1):402.

van Gool IC, Eggink FA, Freeman-Mills L, et al. POLE Proofreading mutations elicit an antitumor immune response in endometrial cancer. Clin Cancer Res. 2015;21(14):3347-3355.

The TCGA identified a subgroup of endometrial carcinomas with mutations of the DNA polymerase POLE. These mutants have a high rate of proofreading error and frequent base pair substitutions. This POLE subgroup (6% to 12% of endometrial tumors) is associated with endometrioid histology and high-grade tumors. Patients with these tumors would be expected to have an aggressive course with poor survival, but often these patients survive without a recurrence. We need more understanding of why.

POLE mutations and prognosis

In a secondary analysis by Church and colleagues of the PORTEC-1 and -2 studies (2 large, randomized controlled trials evaluating postoperative external beam radiation therapy [EBRT] or vaginal brachytherapy), tumors were tested for mutations in POLE (POLE-mutant and POLE wild-type). POLE mutations were detected in 6.1% of tumors overall. Despite their high grade, POLE-

mutant tumors resulted in fewer recurrences (6.2% vs 14.1%) and fewer deaths (2.3% vs 9.7%) than POLE wild-type tumors. In grade 3 tumors, 0 of 15 POLE-mutant tumors recurred.

These results indicate that, even with having poor prognostic features, endometrial cancers with mutations in POLE have an excellent prognosis.⁵

POLE mutations and the immune response

To explain the discrepancy in the results by Church and colleagues, van Gool and colleagues analyzed endometrial cancer specimens from PORTEC-1, -2, and the TCGA studies. Endometrial cancers were categorized as POLE-mutants, POLE wild-type, or microsatellite stable (MSS) tumors. They found that POLE-mutant endometrial cancers have an increased lymphocytic infiltrate (present in 22 of 47 POLE-mutant specimens) as compared with POLE wild-type or MSS tumors.

Also, POLE-mutants had an increased density of cytotoxic T-cells (CD8+) at the tumor center and margin that significantly exceeded that of POLE wild-type or MSS tumors. The proportion of tumors with CD8+ cells exceeding the median were also higher in POLE-mutant (60%) compared with POLE wild-type (31.3%) and MSS (7.2%) tumors. Markers LAG3, TIM-3, TIGI, as well as T-cell inhibitors PD1 and CTLA-4, confirmed evidence of T-cell exhaustion—all of which correlated with CD8 expression.

These findings suggest that POLE mutations lead to hundreds of thousands of DNA fragments stimulating the immune system through prolonged antigenic exposure.⁶ This immune response is so powerful that even these tumors with poor prognostic features will have excellent clinical outcomes.



Endometrial cancers with mutations in POLE exhibit favorable prognosis and excellent clinical outcomes, with the best effects on recurrence and death rates seen in high-grade tumors

WHAT THIS EVIDENCE MEANS FOR PRACTICE

POLE-mutant endometrial cancers have mutations that stimulate the immune system with tremendous amounts of antigenic neopeptides. This robust immune response is demonstrated by tumor infiltrating lymphocytes that enhance antitumor effects and host killing in spite of traditional poor prognostic features.



Mismatch repair and immunology: Targeted therapy for targeted patients

McMeekin DS, Tritchler DL, Cohn DE, et al. Clinicopathologic significance of mismatch repair defects in endometrial cancer: an NRG oncology/gynecologic oncology group study. J Clin Oncol. 2016;34(25):3062–3068.

Le DT, Uram JN, Wang H, et al. PD-1 blockade in tumors with mismatch-repair deficiency. N Engl J Med. 2015;372(26):2509–2520.

The most frequent genetic mutation in endometrial cancer is mismatch repair (MMR) deficiency. Loss of this pathway leads to a failure of repairing replication errors and gives rise to small repeated sequences of DNA, known as MSI. Germline mutations in MMR (Lynch syndrome) occur in only 3% to 5% of endometrial cancers. Somatic mutations in MMR give rise to 10% to 20% of colorectal cancers and upwards of 20% to 40% of endometrial cancers.

Given this high frequency, universal screening utilizing immunohistochemistry of proteins MLH1, MSH2, MSH6, and PMS2 has become the standard of care in tumors to identify MMR deficiency. MMR-deficient endometrial tumors are associated with higher grade and lymphovascular space invasion. The actual clinical prognosis of these tumors, however, has not been well described.⁷ McMeekin and colleagues set out to examine prognosis.

Details of the study by McMeekin and colleagues

In the collaborative study, researchers assessed 1,024 tumors for MMR and categorized them into 1 of 4 groups: normal (62.4%), epigenetic MMR-defective (25.78%), MMR-probable mutation (9.67%), or MSI-low (2.15%). The researchers found that the pathologic features were associated with MMR status. For instance, MMR-

defective tumors were more likely than MMR-normal tumors to be Grade 2 (50% vs 40.7%, respectively). Lymphovascular space invasion also occurred more frequently in MMR-defective than in MMR-normal tumors (32.7% vs 17.13%, respectively). Approximately 22% of patients with MMR-defective tumors had stage III or IV disease, while only 13% to 14% of the other groups presented with such advanced stage.

On univariate analysis, an MMR-defective tumor was associated with worsened progression-free survival (hazard ratio [HR], 1.37). On subsequent multivariate analysis, no difference in survival in MMR-defective vs MMR-normal tumors was found. The authors concluded that MMR status is predictive of response to adjuvant therapy.

An intriguing biologic explanation of how MMR status affects response to adjuvant therapy is that MMR-defective tumors contain lymphocytic infiltrates, consistent with an increased immunologic response.⁸ Similar to the previously discussed POLE mutations, MMR-defective tumors have a tremendous increase in somatic mutations that are on the order of 10 to 100 times that of MMR-proficient tumors. These MMR-defective tumors likely give rise to increased antigen exposure to the immune system.

These immune infiltrates will show signs of exhaustion and upregulate negative feedback systems, which is the point at which the PD-1 pathway becomes critically important. The PD-1 receptor is expressed predominantly on T-cells and its ligands regulate the immune system by inhibition of self-reactive T-cells.⁹

MMR deficiency and anti-programmed death receptor 1

The study by McMeekin and colleagues shows MMR-defective tumors have poor prognostic



MMR-defective endometrial tumors are more likely than MMR-normal tumors to be higher grade and demonstrate lymphovascular space invasion

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features but the same survival as those with MMR proficiency or good prognostic features. Why is this the case? A recent study by Le and colleagues analyzed this question.

Details of the study by Le and colleagues

The investigators performed a phase 2 trial evaluating pembrolizumab (10 mg/kg IV every 14 days), an anti-PD 1 immune checkpoint inhibitor in patients with tumors demonstrating MMR-deficiency. The 3 cohorts included: MMR-defective colorectal cancer (n = 10), MMR-proficient colorectal cancer (n = 18), and MMR-defective noncolorectal cancer (n = 7, including 2 endometrial cancers). Objective response rates were 40%, 0%, and 71% for each group, respectively.

MMR-defective tumors had a striking HR of disease progression or death of 0.04 (95% confidence interval, 0.01–0.21; $P < .001$). Genomic analysis was performed and identified 578 potential mutation-associated neoantigens in the MMR-defective groups (compared with only 21 in the MMR-proficient tumors). These findings promote the concept of a mutation-associated antigen component to the endogenous immune response.¹⁰

Conclusion

The above-stated mutations of mismatch repair and POLE are changing our perspective of endometrial cancer and shedding

WHAT THIS EVIDENCE MEANS FOR PRACTICE

We are on the verge of being able to manipulate the immune system to help us kill cancer. MMR-deficient cells have increased somatic mutations and antigen exposure, with a potential immune response making them excellent candidates for targeted therapy with immune checkpoint inhibition.

These studies support the growing evidence that molecular events have a powerful clinical impact that has the potential to supplant traditional histopathologic staging.

light on the complexities of tumor biology. As future research increasingly incorporates genomic profiling, we anticipate clinical trials may build evidence that adjuvant therapy will be directed by molecular staging, as opposed to traditional surgical or even histologic staging, as these mutations are the root cause of the tumor phenotype.

Key for readers to take away from this Update is that genomic profiling and enrollment in clinical trials is critical to understanding the implications of these mutations and how to best treat our patients. In addition, we should encourage our patients with endometrial cancer to see genetic counselors and have appropriate screening of MMR-deficiency. This will continue to advance our understanding as well as to provide patients with valuable information regarding their diagnosis. 📌



Determining the molecular basis of endometrial tumors may supplant traditional histologic staging

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