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Endometrial Tumor Immune Response: Predictive Biomarker of Response to Immunotherapy

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The tumor-specific immune response in endometrial cancer is variable within molecular subtypes. Thus, the tumor immune response offers a novel biomarker, separate from molecular subtypes, to predict which patients might respond to immunotherapy.

See related article by Talhouk et al., p. 2537

In this issue of *Clinical Cancer Research*, Talhouk and colleagues (1) demonstrate pronounced variation in the immune response within endometrial cancer molecular subtypes. Accordingly, this study offers an opportunity to more discretely classify endometrial cancers and proposes potential novel methods to identify high-risk patients likely to respond to immunotherapy.

By implementing a comprehensive genomic analysis, The Cancer Genome Atlas (TCGA) project was the first to identify four molecular subtypes of endometrial cancer based on survival outcomes (2): *POLE* ultramutated, microsatellite instability (MSI) hypermutated, copy-number (CN) low, and copy-number (CN) high. Prior to this study, endometrial tumor grade and subtype assignment were largely subjective and often biased with poor interobserver reproducibility (3). The TCGA not only provided a reproducible, objective method to predict prognosis, but also offered informative data regarding potential treatment response and the opportunity to group tumors for clinical trials and drug development. While the TCGA was groundbreaking, it was not suited for clinical practice. Talhouk and colleagues identify a more practical technique, the Proactive Molecular Risk Classifier for Endometrial Cancer (ProMise). This tool identifies molecular subgroups with similar survival curves to the TCGA: p53 mutation (p53 abnormal), MSI, POLE, and no specific molecular profile or p53 wild type. It utilizes IHC to identify MMR and p53 expression and DNA sequencing to identify *POLE* mutations (4). By utilizing more straightforward methods, this classification system lends itself to clinical application.

Approximately 10% of endometrial cancers evaluated in the TCGA were comprised of an ultramutated group that possessed exceedingly high mutation rates (232×10^{-6} mutations/Mb). These tumors all carried mutations in the exonuclease domain of *POLE*, a subunit of DNA polymerase, and thus are classified as such. While these patients often demonstrate aggressive histopathologic features correlated with predicted poor survival, they possess the most favorable survival curves (4). The reason for this might lie in the immune environment of these tumors. POLE tumors have been noted to be enriched with lymphocytic infiltration, high neoantigen load, and upregulation of cytotoxic T-cell effector markers (5). This strong association led to the conjecture that the robust immune response of these tumors may be the driving force behind superior survival. Talhouk and colleagues demonstrate similar findings with increased $CD3^+CD8^+$ tumor-infiltrating lymphocytes (TIL) and $CD3^+CD8^-$ TILs in POLE tumors and higher B cells and plasma cells in POLE tumor stroma. POLE tumors also expressed the highest levels of PD-L1 and encompassed the most TIL^{high} tumors (89.5%). Given these findings, it is likely these patients would be ideal candidates for immunotherapy, however, given their excellent prognosis, this adjuvant therapy is not often warranted and difficult to study in this population.

The second group of tumors identified were hypermutated (18×10^{-6} mutation/Mb) and characterized by MSI arising from defective postreplicative DNA mismatch repair (MMR). These deleterious mutations were germline, somatic, or epigenetic events. MMR defective tumors, like POLE tumors, are associated with high-grade tumors and lymphovascular space invasion, yet outcomes are similar to patients with MMR normal tumors (6). Similar to POLE tumors, previous studies have demonstrated increased TILs in MSI-positive tumors suggesting this as a mechanism for improved survival (7). Talhouk and colleagues illustrate increased $CD3^+CD8^+$ TILs and $CD3^+CD8^-$ TILs in MMR-deficient tumors, as well as increased B cells and plasma cells in the tumor stroma compared with p53 mutant or p53 abnormal, but to a lesser extent than POLE tumors. Fifty-six percent of MMR-deficient tumors expressed PD-L1 and 78% of tumors were TIL^{high} indicating increased immunogenicity of these tumors. Interestingly, there were no significant differences in survival outcomes between TIL^{high} and TIL^{low} tumors.

The last subgroups analyzed were CN-high and CN-low tumors, comprising >50% of all endometrial tumors. Ninety-one percent of the CN-high tumors had *TP53* mutations correlating this group with the "p53-mutant" group described by Talhouk and colleagues (4). These patients tend to have type II cancers. Hence, a much more aggressive disease. Conversely, CN-low or p53 wild-type tumors more commonly have a better prognosis; however, when these tumors do recur or metastasize, treatment options are limited. While the immune environment was overall less pronounced in these two tumor types, there were still a fair number of tumors that were TIL^{high}. Traditionally,

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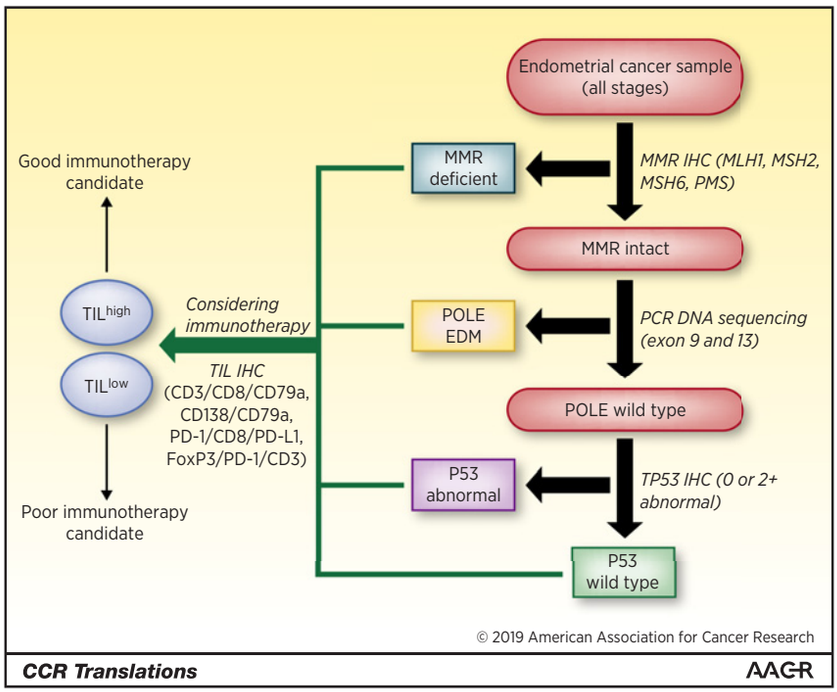

Figure 1.
Methods of molecular and immunologic classification of endometrial cancers. EDM, exonuclease domain mutation.

immunotherapy has not been utilized in these tumors, but these data suggest that the immune response is potentially predictive of a response to targeted therapy. This is especially important given we are in dire need for additional treatment options, specifically for p53-mutant tumors.

In the wake of immunotherapy and personalized medicine, it is necessary to identify biomarkers to predict treatment response. While stratifying tumors as TIL^{high} and TIL^{low} does not independently predict outcomes, it is possible, and in fact likely, that this type of grading system will identify patients who might respond to innovative immunotherapy. For example, the checkpoint inhibitor pembrolizumab has been approved for recurrent MSI-high solid tumors. However, despite identifying this specific group, these tumors demonstrate a diverse array of responses with response rates around 13% (8). Notably, the patients who do respond tend to have pronounced and prolonged responses, and greatly benefit from this drug. High mutational burden has been associated with improved response to immune checkpoint inhibitors in melanoma and non–small cell lung cancer. This has been attributed to the immune environment of these tumors, specifically the presence of increased neoantigens and tumor-specific cytotoxic activity (9). It is likely that this phenomenon could be replicated in TIL^{high} endometrial cancers. In Fig. 1, we propose a novel classification system integrating TILs with traditional molecular subtypes to determine which patients would be the best immunotherapy candidates.

The importance of TILs in ovarian cancer has been demonstrated with improved overall survival in patients whose tumors expressed T cells (10). Patients with intratumoral T cells had an overall survival 2.8 times longer than those without T cells indicating a noteworthy prognostic reliance on tumor-specific cytologic activity. In this study, patient tumors with any number of T cells were compared with patient tumors without T cells. This differs from Talhouk and colleagues who compare TIL^{high} endometrial tumors with TIL^{low} tumors and did not demonstrate a prognostic difference. It is possible that further stratifying the expression of TILs as Zhang and colleagues did would reveal differing outcomes.

Previous research demonstrates the importance of cellular immune response in cancer treatment. This study by Talhouk and colleagues provides a novel foundation for the use of immunotherapy in all subsets of endometrial cancers based on the immune response profile. It not only addresses cell-mediated immunity, but also evaluates the role of humoral immunity, which has been suggested to be extremely important in tumor progression and treatment. The knowledge that p53 abnormal and p53 normal tumors demonstrate the TIL^{high} phenotype is exceedingly important. It suggests that immune blockade may play an important role in treatment of these tumors. This study simply characterized the tumors and did not test the hypothesis that TIL^{high} tumors are more responsive to immunotherapy. Further studies are necessary to evaluate this premise. Nonetheless, this study offers valuable insight into the most common gynecologic malignancy and specifically sheds light on difficult to treat subtypes. The authors should be congratulated on leading the charge in understanding immune response in endometrial cancer.

Disclosure of Potential Conflicts of Interest

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