

Brief Report

Association of Polymerase e-Mutated and Microsatellite-Instable Endometrial Cancers With Neoantigen Load, Number of Tumor-Infiltrating Lymphocytes, and Expression of PD-1 and PD-L1

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IMPORTANCE Immune checkpoint inhibitor therapy has shown benefit in various cancers, but their potential in endometrial cancer (EC) is unknown.

OBSERVATIONS Prediction of neoantigen load was performed using sequencing data from the Cancer Genome Atlas data set. Evaluation of tumor-infiltrating lymphocytes (TILs) and PD-1 and PD-L1 expression was performed in 63 patients with EC referred to our institution. The predicted median (range) neoantigen load (predicted neopeptides per sample) was proportional to the mutational load: highest in ultramutated polymerase e (POLE) tumors (8342 [628-20 440]), less in hypermutated MSI (541 [146-8063]; $P < .001$), and lowest in microsatellite-stable tumors (70.5 [7-1877]; $P < .001$). The POLE and MSI ECs exhibited higher numbers of CD3⁺ (44.5 vs 21.8; $P = .001$) and CD8⁺ (32.8 vs 13.5; $P < .001$) TILs compared with microsatellite-stable tumors. PD-1 was overexpressed in TILs (81% vs 28%; $P < .001$) and peritumoral lymphocytes (90% vs 28%; $P < .001$) of POLE and MSI tumors. PD-L1 expression was infrequently noted in tumor cells but was common in intraepithelial immune cells and more frequent in POLE and MSI tumors (39% vs 13%; $P = .02$).

CONCLUSIONS AND RELEVANCE Polymerase e-mutated and MSI ECs are associated with high neoantigen loads and number of TILs, which is counterbalanced by overexpression of PD-1 and PD-L1. Polymerase e-mutated and MSI EC tumors may be excellent candidates for PD-1-targeted immunotherapies.

JAMA Oncol. 2015;1(9):1319-1323. doi:10.1001/jamaoncol.2015.2151
Published online July 9, 2015.

 Supplemental content at jamaoncolology.com

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The Cancer Genome Atlas project identified 2 groups of endometrioid endometrial cancers (ECs) with high mutation frequency: an ultramutated group (7% of all tumors) that harbored mutations in the exonuclease domain of polymerase e (POLE), and a hypermutated group (28% of tumors) with microsatellite instability (MSI), the majority of which harbored MLH1 promoter methylation.¹ The ultramutated POLE group exhibited an extremely high mutation rate (232×10^{-6} mutations/Mb) with a unique nucleotide change spectrum of increased C→A transversion frequency, whereas the hypermutated MSI group exhibited mutation rates of 18×10^{-6} mutations/Mb with variable length of DNA microsatellites due to an underlying deficiency in mismatch DNA repair.¹⁻³ Mismatch DNA repair deficiency induces single-base mismatches that lead to point mutations in coding regions of genes, as well as insertions or deletions that lead to frame-shift mutations.

It has been suggested that hypermutated tumors may harbor more tumor-specific neoantigens and increased amounts of tumor-infiltrating lymphocytes (TILs).³⁻⁷ Therefore, we assessed whether POLE and MSI ECs harbor more neoantigens and TILs than the comparatively hypomutated microsatellite-stable (MSS) ECs.

Methods

Prediction of HLA Type and Neoantigen Load

Inference of HLA type was performed using the POLY-SOLVER (polymorphic loci resolver) tool.⁵ For prediction of neoantigen load, the Sage Bionetworks' Synapse resource (<https://www.synapse.org> and Lawrence et al⁸) and the NetMHCpan tool (version 2.4)⁹ were used (eMethods in the Supplement).

Table. Clinical and Pathologic Characteristics of the Study Cohort

Characteristic	MSI and POLE Mutated (n = 31)	MSS (n = 32)
Patient age, mean (range), y	62 (38-87)	62 (27-87)
Patient race, No. (%)		
White	25 (81)	20 (63)
Black	3 (10)	0
Hispanic	1 (3)	1 (3)
Asian	0	1 (3)
No data	2 (6)	10 (31)
Tumor histotype and grade, No. (%)		
Endometrioid grade 1	16 (52)	28 (88)
Endometrioid grade 2	8 (26)	1 (3)
Endometrioid grade 3	6 (19)	1 (3)
Other	1 (3) ^a	2 (6) ^b
Lymphovascular invasion, No. (%)		
Present	6 (19)	4 (12)
Absent	25 (81)	28 (88)
Depth of invasion, No. (%)		
No myoinvasion	9 (29)	11 (34)
<50% myometrial thickness	15 (48)	12 (38)
≥50% myometrial thickness	7 (23)	9 (28)
FIGO stage, No. (%)		
1A	25 (81)	23 (72)
1B	5 (16)	5 (16)
2	0	2 (6)
3-4	1 (3)	2 (6)

Abbreviations: FIGO, Fédération Internationale de Gynécologie et d'Obstétrique; MSI, microsatellite instable; MSS, microsatellite stable; POLE, polymerase e.

^a Biphasic grade 1/3.

^b Serous.

Tumor Samples

This study included 63 cases (Table) retrieved from the archives of Brigham and Women's Hospital under an institutional review board-approved protocol; a waiver of consent was granted due to the retrospective nature of the study. The POLE-mutated cases were identified by means of Sanger sequencing of 2 mutational hot spots (exons 9 and 13). Microsatellite instability status was determined using mismatch DNA repair immunohistochemical analysis (eTable 1 in the Supplement).

Immunohistochemical Analysis and Evaluation of Tumor-Associated Lymphocytes

Immunohistochemical analysis was performed for CD3, CD4, CD8, CD20, PD-1, and PD-L1 on formalin-fixed paraffin-embedded tissue samples using standard protocols (eMethods in the Supplement).

Statistical Analyses

Staining results were compared using the *t* test (all *P* values were 2 sided), as well as the Fisher exact test.

At a Glance

- Objective was to assess whether hypermutated polymerase e (POLE) and microsatellite-instable (MSI) endometrial cancers (ECs) harbor more neoantigens and tumor-infiltrating lymphocytes (TILs) than the comparatively hypomutated microsatellite-stable ECs.
- The POLE-mutated tumors had an almost 15-fold higher median number of predicted neoepitopes per sample compared with MSI tumors ($P < .001$) and MSI tumors almost 7-fold higher compared with microsatellite-stable (MSS) tumors ($P < .001$).
- The POLE and MSI tumors exhibited a statistically significantly higher number of CD3⁺ ($P = .001$) and CD8⁺ TILs ($P < .001$) compared with MSS tumors but no difference in CD4⁺ or CD20⁺ TILs.
- PD-1 was overexpressed in TILs and peritumoral lymphocytes of POLE and MSI tumors.
- PD-L1 expression was infrequent in tumor cells but common in intraepithelial immune cells and more frequent in POLE and MSI tumors.

Results

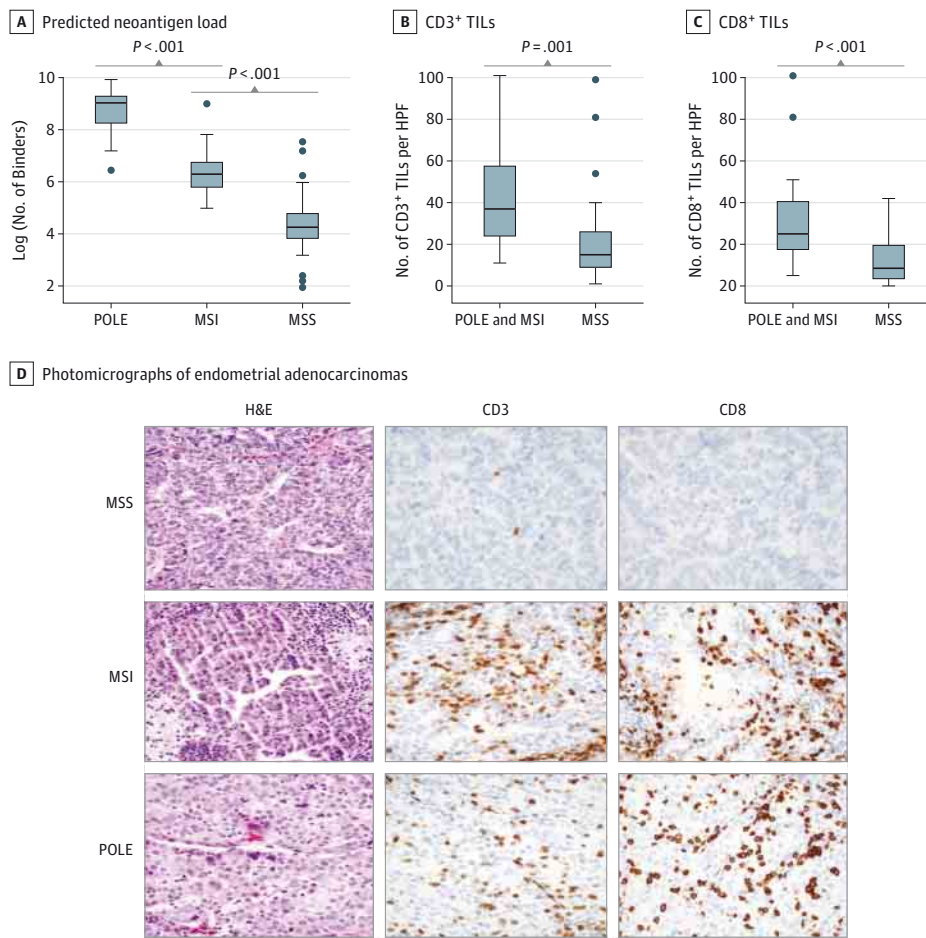
Neoantigen Load of POLE, MSI, and MSS ECs

We accessed whole-exome sequencing data from the 17 POLE-mutated, 65 MSI, and 90 MSS ECs included in the Cancer Genome Atlas EC data set. We performed HLA typing using POLYSOLVER, which was recently reported to infer HLA alleles with 97% accuracy and with a 100% rate of homozygous locus inference calls based on a validation set of 253 HapMap samples. Of these EC cases, 43% (74 of 172) were found to be HLA-A2 positive. Taking into account the 8 most frequent class I major histocompatibility complex (MHC) alleles in whites (ie, A*02:01, C*07:01, A*01:01, A*03:01, C*07:02, C*04:01, B*07:02, C*06:02), 151 of 172 (88%) subjects had at least 1 of these alleles. The neoantigen load for each sample was determined using a pipeline based on the NetMHCpan tool⁹ (version 2.4) that predicts MHC class I binding peptides. For each mutation capable of generating a potential neoantigen (missense and readthrough mutations, as well as in- and out-of-frame insertions and deletions in the coding sequence), binding affinities were predicted for all possible mutation-bearing 9- and 10-mer peptides in the immediate vicinity of the somatic change site. The total number of peptides predicted to have at least a weak binding (<500 nM) with any of the sample's inferred HLA alleles was determined to be the neoantigen load. The POLE-mutated tumors had an almost 15-fold higher median number of predicted neoepitopes per sample (8342 [range, 628-20 440]) compared with MSI tumors (541 [range, 146-8063]; $P < .001$) (Figure 1A) and MSI tumors 7-fold higher compared with MSS tumors (70.5 [range, 7-1877]; $P < .001$) (Figure 1A).

Tumor-Associated Lymphocytes in POLE, MSI, and MSS ECs

Three POLE-mutated (P286R), 28 MSI, and 32 MSS primary ECs from previously untreated patients were evaluated (Table). The

Figure 1. Neoantigen load and CD3⁺ and CD8⁺ Tumor-Infiltrating Lymphocytes (TILs) in Polymerase e (POLE), Microsatellite-Unstable (MSI), and Microsatellite-Stable (MSS) Tumors



A, Predicted neoantigen load. B, Number of CD3⁺ TILs in POLE and MSI vs MSS tumors. C, Number of CD8⁺ TILs in POLE and MSI vs MSS tumors. The horizontal line in the middle of each box indicates the median, while the top and bottom borders of the box mark the 75th and 25th percentiles, respectively. The whiskers above and below the box mark the 90th and 10th percentiles. The points beyond the whiskers are outliers beyond the 90th or 10th percentiles. D, Representative photomicrographs of MSS, MSI, and POLE endometrial adenocarcinomas (original magnification ×40). CD3⁺ TILs are infrequent in MSS tumors but significantly increased in MSI and POLE tumors. Almost all CD3⁺ TILs are CD8⁺ (right column). H&E indicates hematoxylin-eosin, and HPF, high-power field.

POLE and MSI tumors were grouped together as the hypermutated group compared with the hypomutated MSS cancers. The POLE and MSI tumors exhibited a statistically significantly higher mean number of CD3⁺ (44.5 vs 21.8; $P = .001$) and CD8⁺ TILs (32.8 vs 13.5; $P < .001$) compared with MSS tumors (Figures 1B, 1C, and 1D) but no difference in CD4⁺ or CD20⁺ TILs. Furthermore, we did not detect differences in CD3⁺ (44.1 vs 47.3; $P = .86$) and CD8⁺ (48.0 vs 31.2; $P = .29$) TILs between POLE and MSI tumors, respectively. Finally, we observed a statistically significantly higher incidence of presence (ie, 1+, 2+, 3+ staining) of peritumoral CD3⁺ T cells in the stroma surrounding the epithelial component of POLE and MSI tumors compared with MSS tumors (77% of POLE and MSI vs 34% of MSS tumors, respectively; $P < .001$) (eFigure 1 in the Supplement).

PD-1 and PD-L1 Expression in POLE, MSI, and MSS ECs

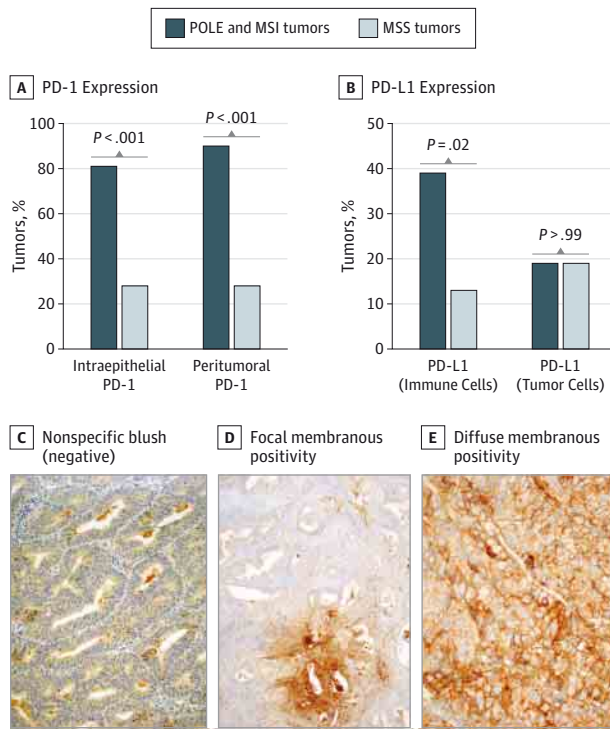
Expression of PD-1 in intraepithelial and peritumoral lymphocytes (presence vs total absence) was significantly more frequent in POLE and MSI compared with MSS ECs ($P < .001$ in both cases) (Figure 2A). PD-L1 expression (presence vs total absence) in intraepithelial immune cells was significantly more frequent in POLE and MSI compared with MSS

tumors ($P = .02$) (Figure 2B). PD-L1 expression in at least 10% of peritumoral immune cells was also more frequent in POLE and MSI tumors (84% vs 56%, $P = .03$). However, PD-L1 expression in tumor cells was not different between POLE and MSI and MSS tumors (Figure 2B); PD-L1 expression in tumor cells followed 3 patterns (Figure 2C): a nonspecific bluish pattern consistent with negative PD-L1 expression (83% of cases), focal membranous positivity (15% of the cases), and extensive membranous positivity (seen in 1 POLE tumor).

Discussion

To our knowledge, we report for the first time that POLE and MSI ECs are associated with significantly increased predicted neoepitopes and numbers of CD3⁺ and CD8⁺ TILs compared with MSS tumors. These observations support the hypothesis that hypermutated tumors also harbor higher neoantigen loads and are associated with increased tumor infiltration by cytotoxic T lymphocytes. PD-1 and PD-L1 were also significantly overexpressed in TILs and peritumoral lymphocytes of POLE and MSI compared with MSS ECs.

Figure 2. PD-1 and PD-L1 Expression in Intraepithelial and Peritumoral Immune and Tumor Cells



A, Percentage of polymerase e (POLE) and microsatellite-instable (MSI) and microsatellite-stable (MSS) tumors expressing PD-1 in tumor-infiltrating lymphocytes and peritumoral lymphocytes. B, Percentage of POLE and MSI and MSS tumors expressing PD-L1 in tumor immune cells and tumor cells. C, D, and E, PD-L1 expression in tumor cells followed 3 patterns: a nonspecific bluish pattern consistent with negative PD-L1 expression, focal membranous positivity, and extensive membranous positivity.

Unlike melanoma, renal cell, and lung cancer,¹⁰ we noted infrequent expression of PD-L1 in tumor cells (with the notable exception of 1 POLE tumor). Instead, PD-L1 expression was noted in intraepithelial immune cells and was significantly more frequent in POLE and MSI tumors. Of note, response to anti-PD-L1 antibody MPDL3280A has been shown to correlate with expression of PD-L1 in tumor-infiltrating immune cells but not in tumor cells.¹¹ Collectively, our findings suggest that POLE and MSI tumors may be excellent candidates for immunotherapies targeting the PD-1 pathway. Of note, there are no prior studies of immunotherapy performed specifically for patients with EC but there is 1 ongoing clinical trial of the PD-1 antibody nivolumab in all MSI cancers (NCT01876511).

We acknowledge that the number of POLE-mutated cases included in our study was small. However, all POLE cases exhibited consistent results with increased number of TILs and PD-1 and PD-L1 overexpression. It is possible that POLE-mutated tumors may harbor more CD8⁺ TILs, which would be consistent with their higher neoantigen load. However, to formally test this hypothesis, a large number of POLE and MSI cases would be required given that both exhibit high numbers of TILs.

Conclusions

Our findings are in keeping with the hypothesis that high mutational loads are associated with high neoantigen loads and an elevated number of TILs, which is counterbalanced by overexpression of immune checkpoints.^{3,12,13} Besides PD-1 and PD-L1, other immune checkpoints¹⁴ such as CTLA-4, LAG-3, and IDO may also be upregulated in POLE and MSI ECs.

ARTICLE INFORMATION

Accepted for Publication: May 20, 2015.

Published Online: July 9, 2015.
doi:10.1001/jamaoncol.2015.2151.

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Author Contributions: Drs Howitt and Konstantinopoulos had full access to all of the data in the study and take responsibility for the integrity of the data and the accuracy of the data analysis. *Study concept and design:* Howitt, Stover, D'Andrea, Wu, Konstantinopoulos. *Acquisition, analysis, or interpretation of data:* Howitt, Shukla, Sholl, Ritterhouse, Watkins, Rodig, Strickland, Wu, Matulonis, Konstantinopoulos.

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Statistical analysis: Shukla, Wu, Konstantinopoulos.

Obtained funding: D'Andrea, Konstantinopoulos.

Administrative, technical, or material support: Howitt, Ritterhouse, Watkins, Rodig, Strickland, Matulonis, Konstantinopoulos.

Study supervision: Howitt, Wu, Konstantinopoulos.

Conflict of Interest Disclosures: Dr Sholl has served as a member of the scientific advisory board for Genentech. Dr Rodig has received research funding from Bristol-Myers Squibb and Roche-Ventana. No other disclosures are reported.

Funding/Support: Dr Wu acknowledges support from the Blavatnik Family Foundation and National Institutes of Health/National Cancer Institute (1R01CA155010-04) for management and analysis of this study. Dr Konstantinopoulos acknowledges support from the Susan Smith Center for Women's Cancers and the Department of Defense Ovarian Cancer Academy Award W81XWH-10-1-0585 for management and analysis of this study. This work was supported, in part, by the Center for Immunology at the Dana-Farber Cancer Institute.

Role of the Funder/Sponsor: The funders had no role in the design and conduct of the study; collection, management, analysis, and interpretation of the data; preparation, review, or approval of the manuscript; and decision to submit the manuscript for publication.

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CORRECTION

Data Errors in Table: In the Original Investigation titled "Risk Adjusting Survival Outcomes in Hospitals That Treat Patients With Cancer Without Information on Cancer Stage,"¹ published online October 8, 2015, erroneous κ values were reported in 8 of the 12 categories of Table 2. The correct values also exceed the thresholds for high levels of agreement and thus do not alter the article's conclusions. For 3- and 5-year mortality in the specified cancer types, the correct respective κ values are as follows: lung/bronchus, 0.84 and 0.84; prostate, 0.93 and 0.91; breast, 0.94 and 0.92; and colorectal, 0.89 and 0.90. This article was corrected online.

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