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Clinicopathologic characteristics, tumor infiltrating lymphocytes and programed cell death ligand-1 expression in 162 endometrial carcinomas with deficient mismatch repair function

Jesus A Chavez[1], **Lai Wei**[2], **Adrian A Suarez**[1], **Anil V Parwani**[1], **Zaibo Li**[1]

[1]Department of Pathology, Wexner Medical Center at The Ohio State University, Columbus, Ohio, USA

[2]Center for Biostatistics, Department of Biomedical Informatics, The Ohio State University, Columbus, Ohio, USA

Abstract

Objective—Endometrial carcinoma (EC) with deficient mismatch repair (dMMR) protein has been reported to have increased tumor infiltrating lymphocytes (TILs) and programed cell death ligand-1 (PD-L1) expression. TILs and PD-L1 expression are compared between two main types of dMMR ECs (epigenetic dMMR due to MLH1 promoter methylation vs mutated dMMR due to genetic mutation).

Methods—Immunohistochemistry for PD-L1 was performed in triplicate on tissue microarray sections. TILs were semi-quantitatively evaluated on whole-slide images of whole histologic sections. The clinicopathologic characteristics together with PD-L1 expression and TILs were analyzed between mutated and epigenetic dMMR ECs.

Results—Of the 162 dMMR ECs identified, 126 had epigenetic dMMR and 36 had mutated dMMR. Univariate analysis demonstrated mutated dMMR ECs showed younger age, less myometrium invasion of >50%, less lymphovascular invasion, and more TILs than epigenetic dMMR ECs. Multivariate analysis demonstrated significantly younger age and more TILs in mutated dMMR ECs than in epigenetic ECs. PD-L1 expression did not show any significant difference between these two groups. Seventeen (13.5%) patients with epigenetic dMMR EC had recurrence and 13 (10.3%) patients died of disease. In contrast, only one patient with mutated dMMR EC had recurrence (3%) and died of disease (3%).

Conclusion—ECs with mutated dMMR demonstrated significantly increased TILs than ECs with epigenetic dMMR, suggesting a stronger immune reaction and potential response to immunotherapy in these tumors.

Correspondence to: Zaibo Li, Department of Pathology, Wexner Medical Center at The Ohio State University, Columbus, OH 43210, USA; Zaibo.Li@osumc.edu.

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INTRODUCTION

Deficient mismatch repair (dMMR) has been reported in approximately 20%–40% of endometrial carcinomas (ECs), either through genetic mutation (mutated dMMR), or epigenetic silencing (MLH1 promoter methylation, epigenetic dMMR).[12] Tumor infiltrating lymphocytes (TILs) are well-recognized histologic markers associated with dMMR in multiple solid tumors[3–7] including ECs.[8] Beside TILs, dMMR ECs are also commonly associated with programed cell death ligand-1 (PD-L1), a biomarker of the checkpoint immune system.[9–12] Programed cell death-1 (PD-1) and its ligand PD-L1 suppress the CD8 cytotoxic immune response by inducing apoptosis of infiltrating T cells and increasing the amount of regulatory T cells in the tumor microenvironment.[1314] Multiple clinical trials have demonstrated that antibodies against PD-1 or PD-L1 are beneficial for patients with melanoma, pulmonary non-small cell carcinoma, and renal cell carcinoma.[15–21] The underlining mechanism for this association is due to the increased tumor mutation burdens/neoantigens in dMMR tumors, which in turns cause immune response with increased TILs and PD-L1 expression.[2223]

Previously, we have described that PD-L1 is expressed in a significant proportion of ECs and is associated with dMMR in a large EC cohort (700 EC cases).[12] A recent study performed at our institution demonstrated that epigenetic dMMR ECs were associated with advanced stage, higher grade, presence of lymphovascular space invasion, and older age.[24] Herein, we aimed to investigate TILs and PD-L1 expression in dMMR ECs with the goal of finding significant differences between epigenetic dMMR ECs and mutated dMMR ECs for their potential as markers to distinguish the dMMR subtypes.

METHODS

Patients and specimens

This study was approved by the institutional review board of The Ohio State University Wexner Medical Center. A computer-generated search of the institutional pathology database was performed to identify dMMR ECs between April 2012 and January 2015. The diagnoses were confirmed by reviewing selected hematoxylin and eosin (H&E) stained slides and the final pathology reports. The pathologic findings (histologic type, histologic grade, and pathologic tumor, node, metastasis (TNM) stage) were recorded. Follow-up time was calculated from the date of diagnosis to that of endometrial cancer-related death or the end of follow-up.

MMR immunohistochemistry

The MMR status was confirmed by reviewing the previously performed (by institutional protocol) immunohistochemistry for MLH1 (NovoCastra, clone: ES05), PMS2 (BD, clone: A16–4), MSH2 (Calbiochem, clone: FE11), and MSH6 (Epitomics, clone: EP 49) at a dilution of 1:200.

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Classification of dMMR ECs by MLH1 promoter methylation assay and mutation sequencing

Any ECs with MLH1 and PMS2 protein loss were tested with MLH1 promoter methylation assay at our molecular pathology division. ECs with both MLH1/PMS2 loss and MLH1 promoter methylation were classified as ECs with epigenetic dMMR. The other dMMR ECs were classified as ECs with genetic mutation (mutated dMMR) after sequencing-confirmed mutation.

Pd-L1 immunohistochemistry

Tissue microarray (TMA) blocks were constructed from paraffin-embedded tissue blocks to include areas representative of tumor. In an attempt to create a representative sample and avoid sampling bias, 1 mm triplicate cores from each tumor were constructed. PD-L1 immunohistochemistry was performed (clone SP263; Ventana) on 4 μm of the previously constructed TMA sections using an automated stainer (Ventana Medical Systems, Tucson, Arizona) and the slides were independently reviewed by two pathologists (ZL and AAS). Any membranous staining in tumor or stromal cells was considered specific staining. Problematic cases were reviewed by both pathologists to reach consensus.

TIL quantification

In order to quantify and score the TILs, selected slides with representative sections of tumor were digitally scanned (Philips Intel-liSite) and the digital images were reviewed with the Philips Digital Pathology Solutions viewer by two pathologists (JAC and ZL). TILs were scored as a percentage by reviewing the slides at 50x, 100x, and 200x magnifications (Figure 1).

Statistical analysis

Categorical data were summarized as frequency and percentage and continuous variables as medians and ranges. For the univariate analysis, Fisher's exact or χ^2 test was used to examine the associations between mutated/epigenetic and the categorical variables. Wilcoxon rank-sum test was used to compare the continuous variables (ie, age). A multivariable logistic regression model was fitted to mutation status using all the variables with $p<0.15$ in the univariate analysis. Variables with $p>0.05$ were removed sequentially from the multivariable model using the backward selection method. Overall survival was calculated from the date of surgery to the date of death, or censored at the date of last follow-up. The Kaplan-Meier method has been used for overall survival. Log-rank test has been used to compare the overall survival between epigenetic patients and mutated patients.

RESULTS

MMR deficiency

A total of 162 dMMR ECs were identified, including 126 with loss of MLH1 and PMS2 protein expression, four with PMS2 loss, 19 with MSH2 and MSH6 loss, and 13 with MSH6 loss. All 126 ECs with loss of both MLH1 and PMS2 were confirmed as having MLH1

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promoter methylation by MLH1 promoter methylation assay, and the other 36 cases were confirmed to have genetic mutation by sequencing.

Clinicopathologic characteristics were summarized in Table 1. Univariate analysis demonstrated mutated dMMR ECs showed younger age, less myometrium invasion of >50%, and less lymphovascular invasion than epigenetic dMMR ECs. However, only younger age was observed in multivariate analysis (Table 2).

Pd-L1 expression

PD-L1 protein expression in both tumor cells and stromal cells was evaluated as described previously.[12] No significant difference of PD-L1 expression in either tumor cells or stromal cells was found between 126 ECs with epigenetic dMMR and 36 ECs with mutated dMMR (Table 1).

TILs

TILs were evaluated by reviewing digital whole-slide images at different magnifications. An average percentage of TILs was estimated for each case by reviewing multiple fields. Of all 162 cases, the median percentage of TILs was 20.0% (range 0%–100%). The median percentage of TILs was 37.5% (range 1%–80%) in 36 ECs with mutated dMMR, while the median percentage of TILs was 15% (range 0%–100%) in 126 ECs with epigenetic dMMR. Both univariate and multivariate analyzes demonstrated significantly higher TILs in mutated dMMR ECs than in epigenetic dMMR ECs (Tables 1 and 2). One representative case of endometrial serous carcinoma with loss of MSH2 and MSH6 showed PD-L1 expression and TILs in Figure 2.

Overall survival and recurrence follow-up

The median follow-up period was 36 (range 0.5–69.8) months. Seventeen (13.5%) patients with epigenetic dMMR EC had recurrence and thirteen (10.3%) patients died of disease. In contrast, only one patient with mutated dMMR EC had recurrence (3%) and died of disease (3%). However, no statistical significance of overall survival was observed between these two groups. Kaplan-Meier curves of overall survival were plotted as shown in Figure 3. Median survival has not been reached due to a small number of cases with either recurrence or death of disease; therefore, a multivariate analysis with COX-regression model was not performed.

DISCUSSION

Morphologic features such as prominent TILs, heterogeneity, and mucinous differentiation (among others) are currently being used as histologic markers suggestive of MMR deficiency in colorectal carcinoma.[25] In the case of EC, a high TILs count,[8] high-grade morphology,[26,27] as well as the more recently described non-papillary tumor growth and the presence of endometrial hyperplasia, have been reported to be associated with dMMR, but their clinical utility remains to be determined.[8]

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Two molecular subgroups of hypermutated ECs exist, including polymerase epsilon (POLE) and dMMR.[28] Both POLE and dMMR ECs have been reported to harbor higher tumor mutation burdens and neoantigens, more prominent TILs, and better clinical outcomes, when compared with other ECs.[91129] The association between PD-L1 and MMR deficiency has been extensively studied in multiple solid tumors.[1930–34] In our previous study, higher PD-L1 expression in tumor cells was found in dMMR ECs than other ECs.[12] MMR deficiency can be caused by either MLH1 promoter methylation (epigenetic dMMR) or genetic mutation (mutated dMMR). Recent studies have suggested different clinicopathologic characteristics between these two subgroups of dMMR ECs, being that epigenetic dMMR ECs were associated with advanced stage, higher grade, presence of lymphovascular space invasion, and older age.[24] However, rare study has been carried out to explore TILs and PD-L1 expression between these two subgroups of dMMR ECs.

To the best of our knowledge, the current study is the largest study to examine TILs and PD-L1 expression in different subgroups of dMMR ECs, namely ECs with MLH1 promoter hypermethylation (epigenetic) and ECs with genetic mutation. Our results revealed no significant difference between these two subgroups of dMMR ECs for PD-L1 expression, but significantly higher TILs in ECs with mutated dMMR. The current study failed to demonstrate better overall survival in patients with mutated dMMR ECs due to a small number of cases with recurrence/death and relatively short follow-up period (median 36 months); however, a tendency of better survival in patients with mutated dMMR ECs was observed. The clinical significance of higher TILs in mutated dMMR ECs than in epigenetic dMMR ECs is unknown, but it may be associated with a better prognosis of mutated dMMR ECs[24] and may predict a better response to immunotherapy.

Genomic instability caused by impaired DNA repair function, including mismatch repair proteins, not only leads to increased mutagenicity and carcinogenicity, but also increases neoantigen load on tumor cells, resulting in increased immunogenicity. The immunogenicity of tumor cells resulting from neoantigens causes immune reactions, leading to immune attack to tumor cells by CD8 +T lymphocytes. For example, dMMR colorectal cancers not only have 10 to 100 times more somatic mutations compared with MMR-proficient colorectal tumors, but also have more prominent immune reaction with lymphocytic infiltration.[5222335–39] A pivotal study of PD1 blockade by pembrolizumab provided the first compelling evidence that MMR-deficient colorectal cancers are more responsive to immune checkpoint inhibitor therapy compared with MMR-proficient tumors.[31]

Later on, the same research group expanded this study to many other solid tumors with MMR deficiency and reached the same conclusions.[40] In the current study, a higher TIL level was identified in mutated dMMR ECs than in epigenetic dMMR ECs, which may be caused by higher mutation/neoantigen load in mutated dMMR ECs than in epigenetic ECs and potentially serves as a marker to predict response to immunotherapy.

The present study has some limitations, including the abovementioned relatively short follow-up period and usage of tissue microarray. Tissue microarray sections may not represent the whole spectrum of tumor, resulting in false negativity or false high positivity of PD-L1 expression due to intra-tumoral heterogeneity and, in turn, causing statistical

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insignificance for PD-L1 expression. However, TILs were evaluated on whole sections of tumors thus avoiding this misrepresentation caused by tissue microarray.

In summary, we have demonstrated that epigenetic and mutated dMMR ECs showed similar levels of PD-L1 expression, but mutated dMMR ECs had a significantly higher TIL level, which may predict better prognosis and potentially serve as a marker for response to targeted immunotherapy.

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HIGHLIGHTS

- The largest study to examine tumor infiltrating lymphocytes (TILs) and programed cell death ligand-1 (PD-L1) expression in different subgroups of deficient mismatch repair (dMMR) endometrial carcinomas (ECs).
- ECs with mutated dMMR demonstrated significantly more TILs than those with epigenetic dMMR.
- No significant difference of PD-L1 was observed between these two subgroups of dMMR ECs.

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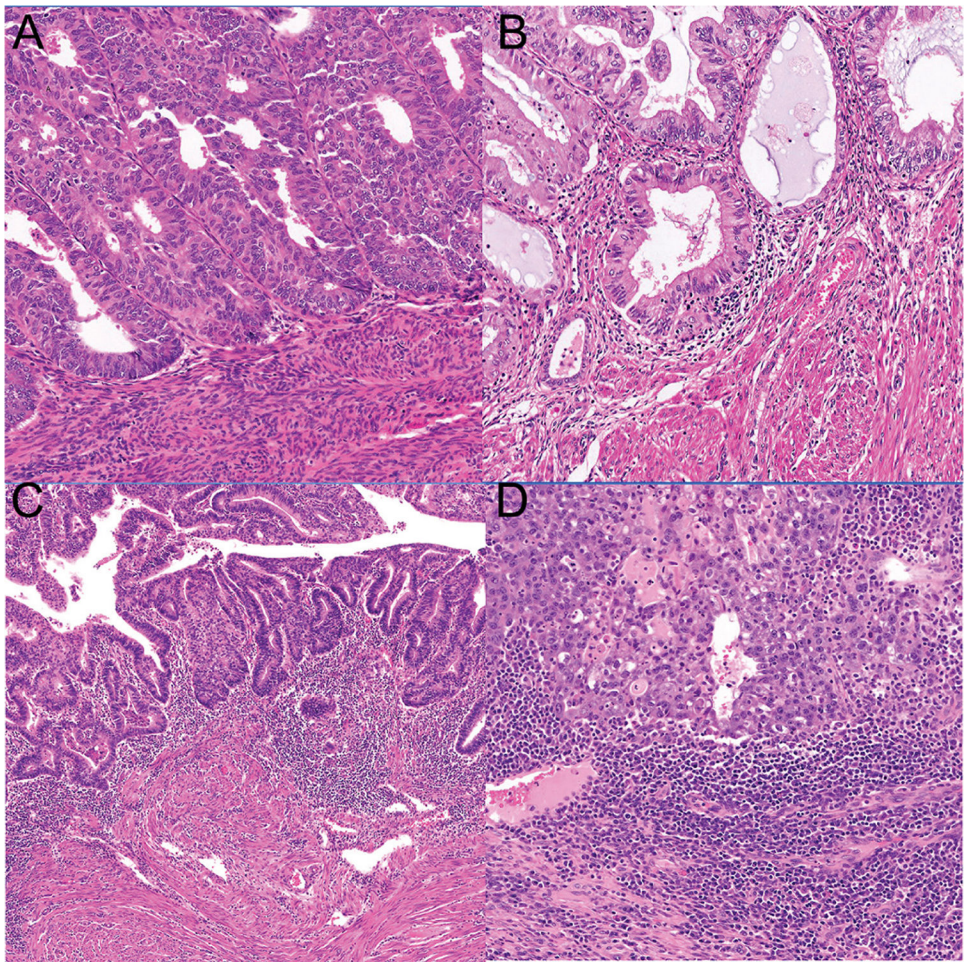

Figure 1.

Tumor infiltrating lymphocytes comparison per percentage estimated. (A) 5%, (B) 25%, (C) 50%, and (D) 100%, respectively; magnification 100x.

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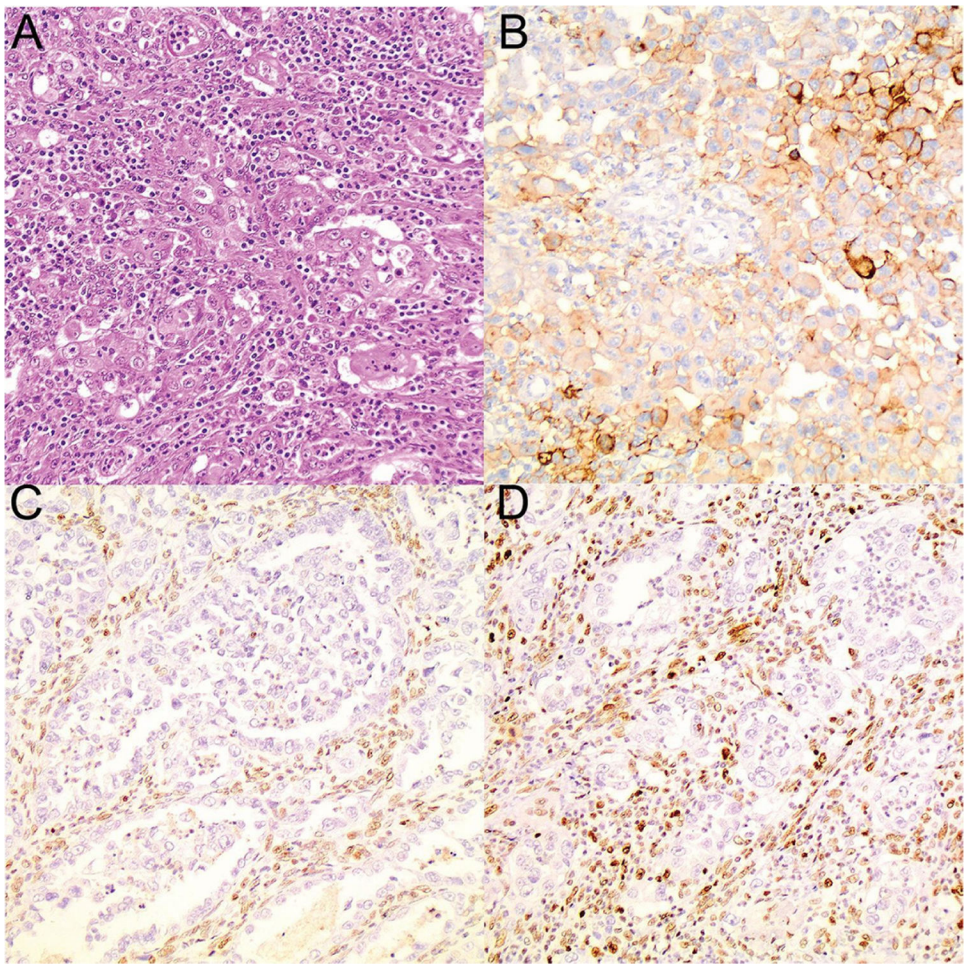

Figure 2.

Representative images of programed cell death ligand-1 (PD-L1) expression in an endometrial serous carcinoma with loss of MSH2 and MSH6. (A) Endometrial serous carcinoma with peri-tumoral lymphocytes, haematoxylin & eosin (H&E) staining, magnification 200x. (B) PD-L1 expression on tumor cells, immunohistochemical staining (IHC) with anti-PD-L1 (SP263, Ventana), magnification 200x. (C) Tumor cells showing loss of MSH2 protein, IHC with anti-MLH1, magnification 200x. (D) Tumor cells showing loss of MSH2 protein, IHC with anti-MSH6; magnification 200x.

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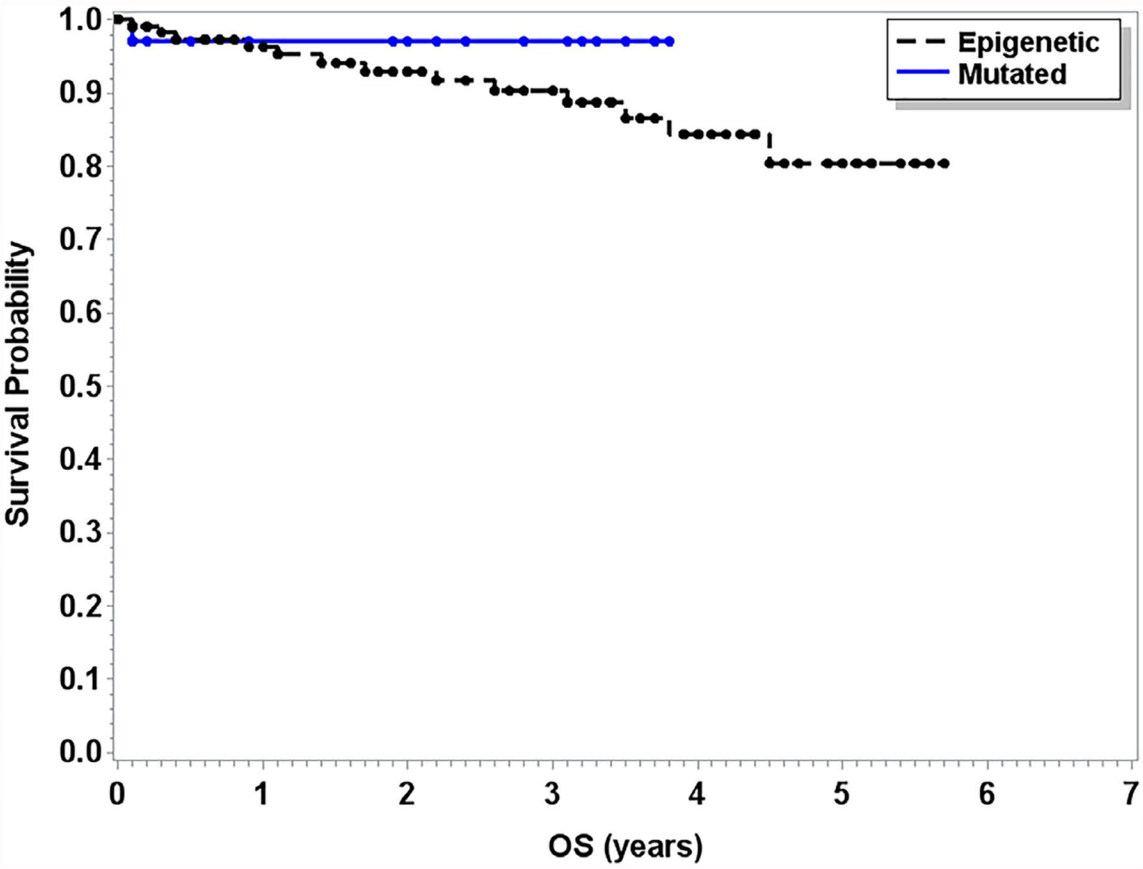

Figure 3.

Overall survival (OS) plots between mutated and epigenetic defective mismatch repair endometrial carcinoma patients.

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Table 1

Univariate analysis of clinicopathologic characteristics, programed cell death ligand-1 expression and tumor infiltrating leucocytes between mutated and epigenetic deficient mismatch repair endometrial carcinomas

Characteristic	Mutated (n=36) n (%)	Epigenetic (126) n (%)	P values
Age (years) (median (range))	55 (36–77)	64 (44–91)	<0.001
Tumor grade			
FIGO 1/2	29 (81%)	98 (78%)	0.721
FIGO 3	7 (19%)	28 (22%)	
Tumor (T) stage			
1	34 (94%)	105 (83%)	0.228
2	1 (3%)	6 (5%)	
≥3	1 (3%)	15 (12%)	
Node (N) stage			
0	22 (88%)	75 (77%)	0.278
≥1	3 (12%)	23 (23%)	
TIL (median (range))	37.5 (1–80)	15 (0–100)	<0.001
Tumorous PD-L1 (median (range))	0 (0–56.7)	0 (0–31.7)	0.569
Stromal PD-L1 (median (range))	0 (0–10)	0 (0–10)	0.411
Myometrium invasion (>50%)	8 (22%)	54 (43%)	0.025
LVI(+)	8 (22%)	54 (43%)	0.025
LUS involvement	10 (28%)	57 (45%)	0.061
Cervical stromal involvement	2 (6%)	9 (7%)	1
Adnexal involvement	2 (6%)	17 (13%)	0.25
Death	1 (3%)	13 (10%)	0.197
Overall survival (years) (median (95% CI))	NR (NR-NR)	NR (NR-NR)	0.127

*Low/intermediate-grade tumors include FIGO 1 and 2 endometrioid carcinoma. High-grade tumors include FIGO 3 endometrioid carcinoma, serous carcinoma, clear cell carcinoma, malignant mixed Müllerian tumor (MMMT), and undifferentiated carcinoma.

FIGO, International Federation of Gynecology and Obstetrics.; LUS, lower uterine segment; LVI, lymphovascular invasion; NR, author to define; PD-L1, programed cell death ligand-1; TIL, tumor infiltrating lymphocytes.

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Table 2

Multivariate analysis of clinicopathologic characteristics and tumor infiltrating leucocytes between mutated and epigenetic deficient mismatch repair endometrial carcinomas

Characteristic	Mutated (n=36) n (%)	Epigenetic (126) n (%)	P values
Age (years) (median (range))	55 (36–77)	64 (44–91)	<0.001
TIL (median (range))	37.5 (1–80)	15 (0–100)	<0.001
Myometrium invasion (>50%)	8 (22%)	54 (43%)	0.724
LVI(+)	8 (22%)	54 (43%)	0.053
LUS involvement	10 (28%)	57 (45%)	0.232

LUS, lower uterine segment; LVI, lymphovascular invasion; TIL, tumor infiltrating lymphocytes.