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The prognosis of endometrial cancers stratified with conventional risk factors and modified molecular classification

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Abstract

This study aimed to validate the Proactive Molecular Risk Classifier for Endometrial Cancer, a modified version of The Cancer Genome Atlas, using data from 184 patients with endometrial cancer (median age: 57.5 years; median follow-up period: 109 months) who had undergone radical surgery (including systemic lymphadenectomy) and subsequent adjuvant chemotherapy (patients with intermediate or high recurrence risk) from 2003 to 2015. Tissue microarrays were prepared from surgical specimens and classified using the conventional clinical risk classifier. Immunohistochemistry was used to detect mismatch repair proteins, L1 cell adhesion molecule, and p53. Direct sequencing was used to identify hotspot mutations in the polymerase-epsilon gene. Forty-five patients were identified as having high L1 cell adhesion molecule expression, 41 as low risk, 34 as mismatch repair-deficient, 13 as polymerase-epsilon gene-mutated, five as having abnormal p53, and 46 as other. Patients were stratified into significantly different prognostic groups ($p < 0.0001$): favorable (low risk and polymerase-epsilon gene-mutated), intermediate (mismatch repair-deficient and other), and unfavorable (high L1 cell adhesion molecule expression and abnormal p53) with 5-year disease-specific survival rates of 100%, 93.8%, and 75.1%, respectively (Kaplan–Meier method). The combination of conventional recurrent risk classification, sequencing for polymerase-epsilon gene mutations and immunohistochemistry for L1 cell adhesion molecule, p53, and mismatch repair proteins can be used to determine the prognoses of patients with endometrial cancer.

KEYWORDS
adjuvant chemotherapy, endometrial cancer, lymphadenectomy, molecular classification, survival

Abbreviations: CN, copy number; DSS, disease-specific survival; EC, endometrial cancer; FFPE-TMAs, formalin-fixed, paraffin-embedded tissue microarray; IHC, immunohistochemistry; LNM, lymph node metastasis; LVSI, lymphovascular space invasion; MMR, mismatch repair; PFS, progression-free survival; *POLE*, polymerase-epsilon; POLE-EDM, *POLE* exonuclease domain mutation; PORTEC, Post-Operative Radiation Therapy in Endometrial Carcinoma; ProMisE, Proactive Molecular Risk Classifier for Endometrial Cancer; TCGA, The Cancer Genome Atlas.

1 | INTRODUCTION

Endometrial cancer is the sixth most common malignancy in women, and an estimated 417,000 cases were newly diagnosed and 97,370 deaths occurred worldwide in 2020.[1] Despite recently improved treatment strategies, such as minimally invasive surgeries, the numbers of morbidities and deaths are increasing in developed countries. Therefore, the development of a treatment method tailored to individual tumors is necessary.

There are two primary carcinogenic mechanisms of EC classified according to estrogen dependence. Type 1 ECs are estrogen-dependent, low-grade, endometrioid carcinomas with favorable prognoses. Type 2 ECs are estrogen-independent and aggressive phenotypes with poor prognoses that are typically high-grade, non-endometrioid subtypes, including serous carcinoma, clear cell carcinoma, and carcinosarcoma.[2,3] The histological subtype reflects the cancer grade, and the degree of progression is evaluated using stages, which mainly depend on morphological findings. However, identifying these characteristics is not always useful for individual treatment as various genomic features can be used as therapeutic targets in tumors of the same histological subtype.

Molecular profiles of EC types have been identified using TCGA. ECs can be classified into four integrated clusters based on genomic characteristics: *POLE* gene (*POLE*, ultramutated), microsatellite instability (MSI, hypermutated), CN low (endometrioid), and CN high (serous-like).[4–7] TCGA molecular classification stratifies patients with EC based on prognosis, regardless of the conventional clinicopathological features. Patients in the *POLE* cluster have good prognoses, patients in the MSI and CN low clusters have intermediate prognoses, and those classified as CN high have poor prognoses. As each cluster has its own characteristic gene signatures and alterations, the use of these clusters may allow the application of precision medicine. However, the original TCGA classification required whole genomic analyses of fresh-frozen tumor specimens, which was expensive and labor intensive; therefore, it was not directly introduced into daily clinical practice, although it has been used in several clinical trials.[8]

ProMisE is a clinically applicable modified classification system that utilizes a surrogate approach of a limited panel of IHC and POLE-EDM analyses and alters the order of the classification steps.[6,7,9–12] ProMisE has been verified for use in several patient cohorts, and some current clinical trials are examining its potential to determine appropriate treatment methods. The prognostic significances of TCGA and ProMisE molecular classification systems have been reported in western countries.[4–11,13–15] Furthermore, studies regarding TCGA molecular classification system have focused on patients in the Post-Operative Radiation Therapy in Endometrial Carcinoma (PORTEC) trials, which investigated the efficacy of adjuvant radiotherapy or vaginal brachytherapy for patients with early-stage cancers who had an intermediate or high risk of recurrence.[8,13,14] In addition, high expression of L1 cell adhesion molecule (L1CAM) is reported to predict poor prognosis in patients with various cancers, including EC,[16,17] and to stratify the prognosis of EC patients without specific molecular profile in TCGA classification.[18] Therefore, it has been enlisted in the PORTEC-4a study as an independent prognostic factor.[19] However, the pathological staging of most patients in these previous studies was not completely confirmed, and radiation therapy is the first choice for adjuvant therapy in the real world. Therefore, the prognostic significance of the ProMisE classification system remains uncertain for patients undergoing radical surgery, including systematic lymphadenectomy and adjuvant chemotherapy.

Although the therapeutic significance of radical surgery, including para-aortic lymphadenectomy, and chemotherapy as adjuvant therapy is under investigation in clinical trials, radical surgery and post-operative chemotherapy are the standard treatments in the Japanese guidelines.[20–22] At most Japanese institutions, radical surgery, including systematic lymphadenectomy, is performed for patients with early-stage disease who have a risk of recurrence and for those with advanced disease, but not for patients with a low risk of LNM based on preoperative evaluations.[23,24] Adjuvant chemotherapy is the first choice for patients with an intermediate or high risk of recurrence. Also, in these cases, we reported that positive L1CAM immunostaining predicted adverse outcomes.[25]

This retrospective study investigated the prognostic significance of ProMisE for Japanese patients with EC who had undergone complete staging, including pelvic and para-aortic lymphadenectomy and adjuvant chemotherapy. The study also explored a new classification system, which comprises conventional risk criteria based on pathological findings and immunohistochemical markers in ProMisE with the addition of L1CAM immunostaining. The newly modified classification system adequately stratified patient survival, thereby identifying those patients with an extremely favorable prognosis who are candidates for skipping further molecular examinations.

2 | MATERIALS AND METHODS

2.1 | Study design

This retrospective observational study included patients who were pathologically diagnosed with EC and who were treated at our institution from 2003 to 2015. We proposed radical surgeries, including lymphadenectomy, for patients who were at intermediate or high risk of LNM based on a preoperative scoring system.[23,24] Patients who underwent a total abdominal hysterectomy, bilateral salpingo-oophorectomy, and systematic lymphadenectomy up to the renal vein level were included in this study. Patients without sufficient primary lesions to prepare FFPE-TMAs and those with less than 36 months of follow-up data were excluded from the study (Figure 1). Biomarker analyses were conducted and correlated with the patients' clinical course. This study was approved by the institutional review board of Hokkaido University Hospital (protocol code 017-0269; January 17, 2018) and was conducted according to the principles of the Declaration of Helsinki.

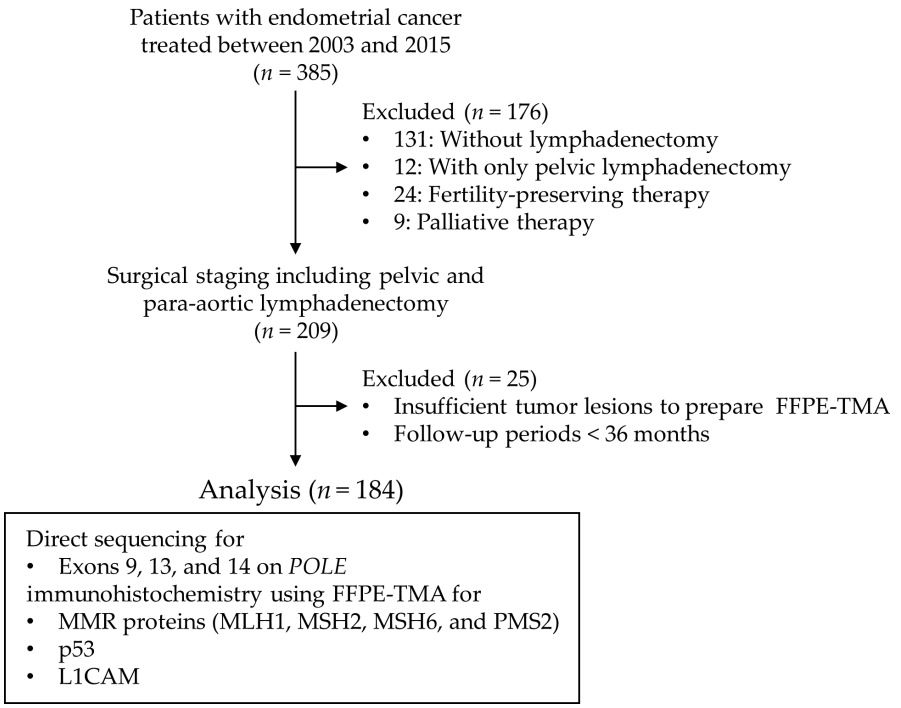

FIGURE 1 Flow chart of patient selection. FFPE-TMA, formalin-fixed paraffin-embedded tissue microarray; L1CAM, L1-cell adhesion molecule; MLH1, mutL homolog 1; MMR, mismatch repair; MSH2, mutS homolog 2; MSH6, mutS homolog 6; PMS2, PMS1 homolog 2; *POLE*, polymerase-epsilon gene

2.2 | Diagnosis and treatment

The surgical specimens were pathologically staged, and the stages of all samples were classified according to the 2008 International Federation of Gynecologists and Obstetricians staging system.[26] All patients were treated according to the Japan Society of Gynecologic Oncology guidelines.[21] Briefly, adjuvant therapy was not administered to patients at low risk of recurrence, which was defined as Stage IA Grade 1 or 2 endometrioid carcinoma (endo G1/2) without LVSI. Patients at intermediate or high risk of recurrence underwent adjuvant chemotherapy using adriamycin and cisplatin or paclitaxel and carboplatin.

2.3 | Direct sequencing for hotspot mutations on the POLE gene

Direct sequencing was performed using the Sanger method to detect hotspot mutations on exons 9, 13, and 14 of the *POLE* gene. Genomic DNA was extracted from tumor specimens of 10-μm FFPE slices resected during radical surgery using the QIAamp DNA FFPE Tissue Kit (Qiagen) according to the manufacturer's protocol. The samples were enriched using the GoTaq Colorless Master Mix (Promega). The primers used for PCR amplification and the PCR conditions are listed in Tables S1 and S2. An Applied Biosystems 3130xl Genetic Analyzer (Thermo Fisher Scientific) was used to analyze the samples. Two researchers (H.Y and Y.H.) evaluated the results using Chromas Version 2.6.6 (Technelysium Pty Ltd) and confirmed suspected mutations using reverse primers.

2.4 | IHC

IHC was conducted using the FFPE-TMA slides, which included two tumor sites and one nontumor site for each patient,[25] to identify p53 and the MMR proteins MLH1, MSH2, MSH6, and PMS2. IHC for p53 was conducted using a mouse monoclonal anti-human p53 antibody (clones DO-7, 1:200; Dako) for 30 min at room temperature, and was visualized using the Dako Envision FLEX system.[27] IHC for MMR proteins was performed using mouse monoclonal anti-human MLH1, MSH2, MSH6, and PMS2 antibodies (clones ES05, FE11, EP49, and EP51, respectively, prediluted; Dako) for 30 min at room temperature, and was visualized using the Dako Envision FLEX system. IHC results were evaluated by two researchers (H.Y. and K.C.H.). MMR deficient (MMR-D) samples were defined as negative staining for at least one MMR protein. A 2+ pattern (>50% positive staining tumor cells) and a null pattern (completely negative staining in tumor cells) were defined as an abnormal expression of p53 (p53 abn). The IHC procedure for L1CAM was conducted as previously described,[25] and an H-score > 35 was considered high expression of L1CAM (L1CAM+) in this study. Tissues that were difficult to evaluate due to poor quality staining were treated as missing data.

2.5 | ProMisE classification system

We classified the patients using the ProMisE algorithm, in which we extracted the patients in the order MMR-D, POLE-EDM, normal expression of p53 (p53 wt, 1+ pattern), p53 abn (null pattern, 2+ pattern), and unclassifiable (patients without a profile).[12,15] As patients were classified in the order of each molecular marker, groups that

were classified earlier were more susceptible to overlapping prognostic factors. Therefore, we examined the association of each molecular marker using a Venn diagram (Figure 3).

2.6 | Statistical analysis

PFS and DSS were defined as the time from radical surgery to disease recurrence and death from EC (death of disease, DOD), respectively, up to October 2021. PFS and DSS were estimated using the Kaplan–Meier method. The Kruskal–Wallis rank test and Fisher's exact test were used to compare continuous variables and categorical variables, respectively. The log-rank test with Bonferroni correction was used to determine survival differences, and the Cox proportional hazard model with Firth's penalized maximum likelihood bias reduction method was used for survival analysis using R version 4.1.0 software (R Core Team) using the coxphf, survivplot, survival, and survminer libraries. Statistical significance was set at $p < 0.05$. In multivariate analyses, we included the statistically significant risks in the univariable analysis for adjustment.

3 | Results

3.1 | Characteristics of patients with molecular markers

In total, 385 patients with EC were treated at our institution from 2003 to 2015, including 209 who underwent radical surgery. Twenty-five patients were excluded; therefore, 184 patients were included in the final analysis (Figure 1). The median follow-up period was 102.5 months (range: 2–214 months), and the median age was 58 years (range: 14–78 years). To investigate the features of each molecular marker, Table 1 shows the characteristics of the patients with positive marker and includes 32 (27.1%) overlapping cases with more than one molecular marker. Twenty-three suspected pathogenic mutations were confirmed in 22 patients, including 19 patients (10.3%) with pathogenic mutations in the *POLE* gene (Table S3), in which more than 40% had non-endo G1/2 histological type and over two-thirds (68.4%) were classified as having intermediate or high risk of recurrence. Fifty-eight patients (31.5%) were identified as MMR-D (Table S4), in which 69% were classified

TABLE 1 Characteristics of patients with molecular markers

	Total ($n = 184$)	POLE-EDM ($n = 19$)	MMR-D ($n = 58$)	p53 abn ($n = 20$)	L1CAM+ ($n = 55$)	No markers ($n = 66$)
Follow-up period (months)	102.5 (2–214)	147 (44–209)	103.5 (16–214)	93 (5–198)	93 (5–211)	112 (2–202)
Age (years)	58 (14–78)	57 (34–68)	58 (24–73)	61.5 (21–42)	63 (34–76)	57 (14–78)
2008 FIGO stage						
I	99 (53.8%)	14 (73.7%)	32 (55.2%)	12 (60%)	33 (60%)	34 (51.5%)
II	23 (12.5%)	1 (5.2%)	8 (13.8%)	2 (10%)	6 (10.9%)	8 (12.1%)
III	51 (27.7%)	4 (21.1%)	15 (25.9%)	3 (15%)	15 (27.3%)	19 (28.8%)
IV	11 (6.0%)	0 (0%)	3 (5.2%)	3 (15%)	1 (1.8%)	5 (7.5%)
Histological subtype						
Endo G1/2	123 (66.8%)	11 (57.9%)	40 (69.0%)	8 (40%)	23 (41.8%)	55 (83.3%)
Endo G3	26 (14.1%)	6 (31.6%)	11 (19.0%)	3 (15%)	11 (20%)	4 (6.1%)
Serous	9 (4.9%)	0 (0%)	1 (1.7%)	5 (25%)	7 (12.7%)	0 (0%)
CCC	8 (4.3%)	0 (0%)	1 (1.7%)	1 (5%)	5 (9.1%)	3 (4.5%)
CS	15 (8.2%)	1 (5.3%)	3 (5.2%)	2 (10%)	7 (12.7%)	4 (6.1%)
Others	3 (1.6%)	1 (5.3%)	2 (3.4%)	1 (5%)	2 (3.6%)	0 (0%)
Risk of recurrence						
Low	41 (22.3%)	6 (31.6%)	11 (19.0%)	2 (10%)	9 (16.4%)	20 (30.3%)
Intermediate	41 (22.3%)	5 (26.3%)	16 (27.6%)	5 (25%)	13 (23.6%)	12 (18.2%)
High	102 (55.4%)	8 (42.1%)	31 (53.4%)	13 (65%)	33 (60%)	34 (51.5%)
5-year DSS with vs. without biomarker	90.1%	100% vs. 90%	91.3% vs. 91.3%	79.7% vs. 91.3%	81.1% vs. 93.7%	95.5% vs. 87.1%
10-year DSS with vs. without biomarker	85.3%	100% vs. 84.2%	89.3% vs. 83.8%	73.0% vs. 86.6%	68.7% vs. 92.0%	93.8% vs. 80.6%
p-value for the DSS	–	0.08	0.5	0.08	<0.001	0.03

Note: Data are presented as medians (ranges) or numbers (percentages). The log-rank test was used to determine the *p*-value for the DSS.

Abbreviations: CCC, clear cell carcinoma; CS, carcinosarcoma; DSS, disease-specific survival rate; Endo G1/G2, Grade 1 or 2 endometrioid carcinoma; Endo G3, Grade 3 endometrioid carcinoma; FIGO, Federation of Gynecologists and Obstetricians; L1CAM+, high expression of L1 cell adhesion molecule; MMR-D, MMR deficiency; p53 abn, abnormal expression of p53; POLE-EDM, polymerase-epsilon endonuclease domain mutation.

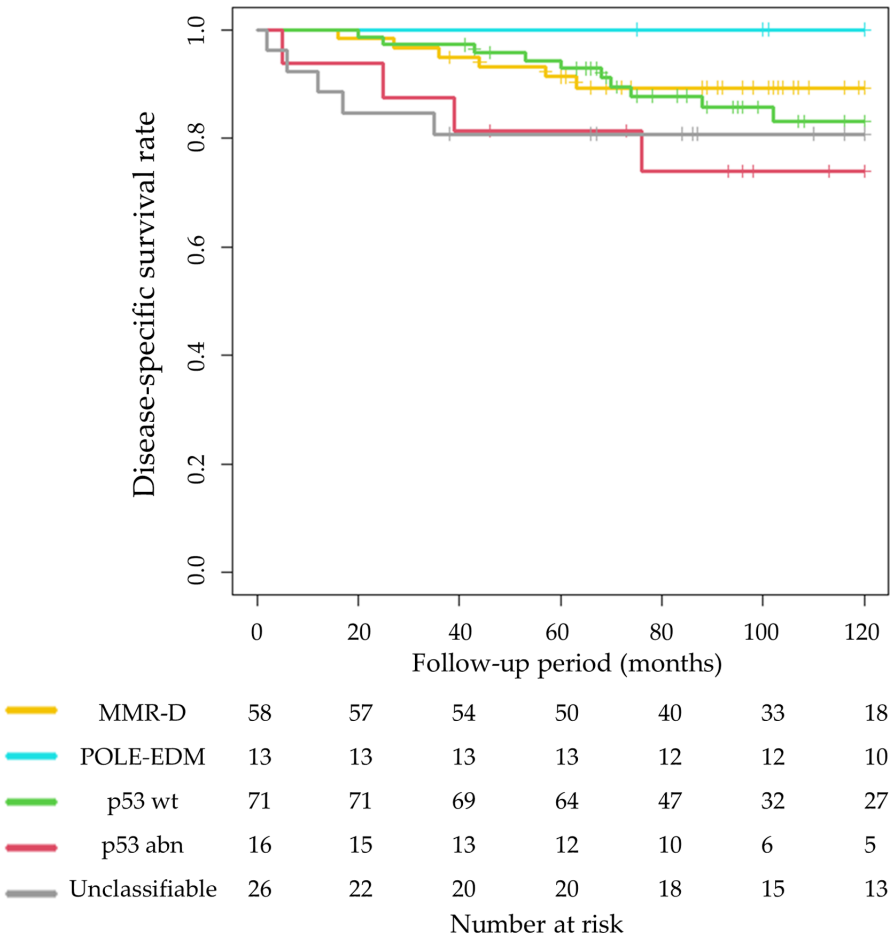

FIGURE 2 Disease-specific survival rates of patients according to the Proactive Molecular Risk Classifier for Endometrial Cancer classification system. MMR-D, MMR deficiency; p53 abn, abnormal expression of p53; p53 wt, normal expression of p53; POLE-EDM, polymerase-epsilon endonuclease domain mutation

as endo G1/G2, and 81% of them classified as having intermediate or high risk of recurrence. Twenty patients (10.9%) had p53 abn, including 18 with a 2+ pattern and two with a null pattern. Of these patients, 15% had Stage IV disease, 25% had serous carcinoma, and 90% were classified as having an intermediate or high risk of recurrence. Fifty-five patients (29.9%) had L1CAM+, of which 38.2% had non-endometrioid carcinoma, including 12.7% with serous carcinoma.

Overall, a total of 118 patients (64.1%) had at least one molecular marker. Thirteen tissue samples could not be analyzed for POLE-EDM due to insufficient tumor specimens (seven samples) or low-quality sequences (six samples), and IHC could not be conducted on two samples due to too few tumor cells on the TMA. Of the included patients whose specimens could not be analyzed, 66 patients (34.8%) did not have any molecular markers (no markers). Among these molecular markers and clinicopathological findings, L1CAM+ had a positive relationship to p53 abn and a negative to endo G1/2 (Table S5).

The DSS of patients with any biomarker was significantly worse than that of patients with no biomarkers ($p = 0.03$). The DSS rate of patients with L1CAM+ was significantly worse than that of patients with low expression of L1CAM (L1CAM-) (5-year DSS: 81.1% vs. 93.7%; 10-year DSS: 68.7% vs. 92.0%; $p < 0.001$).

3.2 | ProMisE classification system

In total, 58 patients (31.5%) were classified as MMR-D, 13 (7.0%) as POLE-EDM, 71 (38.6%) as normal p53 (p53 wt), and 16 (8.7%) as p53 abn in the order according to the ProMisE classification system (Figure S1). Twenty-six patients were unclassifiable due to missing IHC results for MMR protein (21 patients) or missing sequencing results for the *POLE* gene (5 patients). As shown in Figure 2, the 5-year DSS was 91.3% in the MMR-D group, 100% in the POLE-EDM group, 94.3% in the p53 wt group, 81.2% in the p53 abn group, and 80.8% in the unclassifiable group. The 10-year DSS was 89.3% in the MMR-D group, 100% in the POLE-EDM group, 83.0% in the p53 wt group, 73.9% in the p53 abn group, and 80.8% in the unclassifiable group. The prognosis was not well stratified; the survival rates of MMR-D and p53 wt were reversed at 10 years, and POLE-EDM may have to be first distinguished because of the quite favorable prognosis.

Potential confounding factors of the classification systems

The overlap of these molecular biomarkers was also investigated (Figure 3). Duplicating molecular features were found in six patients (31.6%) in POLE-EDM, 16 (27.6%) in MMR-D, 15 (75%) in p53 abn, and 29 (52.7%) in L1CAM+. Fourteen patients showed both p53 abnormal and positive L1CAM, which amounted to 70.0% for p53 abn and 25.5% for L1CAM+.

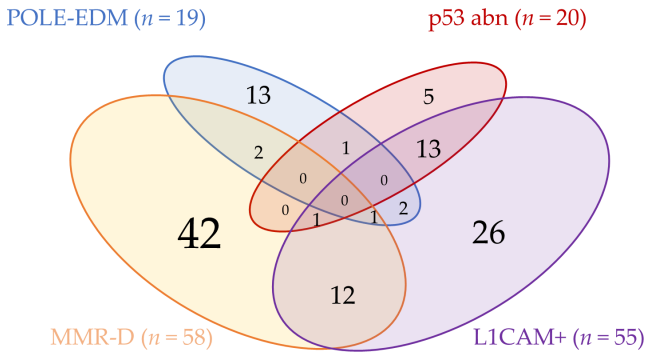

FIGURE 3 Venn diagram of the four molecular markers. The number in each area represents the number of patients with the corresponding molecular markers. L1CAM+, high expression of L1 cell adhesion molecule; MMR-D, MMR deficiency; p53 abn, abnormal expression of p53; POLE-EDM, polymerase-epsilon endonuclease domain mutation

The prognostic impact of the conventional risk classification system for recurrence was investigated among 41 patients classified as having a low risk of recurrence (low risk) who did not undergo adjuvant therapy. Of these patients, 28 (68.3%) expressed some molecular markers, including 11 (39.3%) with MMR-D, nine (32.1%) with L1CAM+, six (21.4%) with POLE-EDM, and two (7.1%) with p53 abn. The median follow-up period of patients in the low-risk group was 116 months (range: 41–214 months). No patients in this group died of EC, although two (5.3%) patients experienced disease recurrence that was controlled by secondary treatment for more than 3 years. Both patients had none of molecular features examined in this study.

Patients with POLE-EDM had a favorable prognosis and no recurrences or deaths due to EC. Of the 19 patients with POLE-EDM, five were in the intermediate-risk group and eight were in the high-risk groups; 13 (68.4%) had conventional risk factors for recurrence and underwent adjuvant chemotherapy. The 5-year and 10-year DSS rates for patients with POLE-EDM were 100%, including even the patients at intermediate and high risk for recurrence.

Overall, 55 patients (29.9%) had L1CAM+. When patients with a low risk of recurrence and those who had POLE-EDM at intermediate or high risk for recurrence were excluded, L1CAM+ was identified as the worst prognostic factor, with a hazard ratio of 3.83 (range: 1.718–8.53; $p = 0.0045$) (Table 2). Half of the deaths that occurred in patients with L1CAM+ occurred more than 5 years after the initial treatment (Figure S2). When low-risk patients, those with POLE-EDM, and those with L1CAM+ were excluded, there was no overlap between patients with p53 abn and those with MMR-D (Figure 3); the DSS rate was worse in patients with p53 abn than in those with MMR-D.

3.3 | Combination of the molecular biomarkers with clinicopathologic findings

We reordered the steps of the molecular biomarkers in the ProMisE classification system, adding L1CAM and a low risk of recurrence based on the conventional risk classification system as factors. Patients were extracted in the following order: low risk ($n = 41$), POLE-EDM ($n = 13$), L1CAM+ ($n = 45$), p53 abn ($n = 5$), MMR-D ($n = 34$), and others ($n = 46$), as shown in Figure 4.

The median patient age was 57.2 years (range: 14–76 years) in the low-risk group, 56.7 years (range: 48–66 years) in the POLE-EDM group, 62.3 years (range: 41–76 years) in the L1CAM+ group, 57.6 years (range: 42–70 years) in the p53 abn group, 55.1 years (range: 24–73 years) in the MMR-D group, and 55.0 years (range: 32–78 years) in the others group (all $p < 0.01$) (Table 3). The histological subtypes and rates of LVSI were significantly different between each group, with the exception of the low-risk group (all $p = 0.026$). Among the 13 patients with POLE-EDM who were not in the low-risk group, six patients (46.2%) had endo G3 and eight patients (61.5%) had a high risk of recurrence. Among the 34 patients with MMR-D, 26 patients (76.5%) had endo G1/2 and 13 patients (38.2%) had LNM. LVSI occurred in 79.4%, 61.6%, 57.8%, and 40% of patients with MMR-D, POLE-EDM, L1CAM+, and p53 abn, respectively. The conventional substantial risk factors, such as the stage, risk classifications for recurrence, positive rate of LNM, deep myometrial invasion, and peritoneal cytology, were not significantly different between the groups.

The PFS of the POLE-EDM group was more favorable than that of patients with a low risk of recurrence (5-year PFS: 100% vs. 95.1%; 10-year PFS: 100% vs. 95.1%; $p = 0.42$) (Figure 5A). The PFS rate of the MMR-D group (5-year PFS: 79.4%; 10-year PFS: 79.4%) was worse than that of the others group (5-year PFS: 89.1%; 10-year PFS: 85.4%), although they were not significantly different ($p = 0.35$). No patient in the low-risk group or the POLE-EDM group died during the study period (Figure 5B). The 5-year and 10-year DSS rates of the MMR-D group were 94.1% and 90.6%, respectively, and those of the other group were 93.5% and 91.1%, respectively. The 5-year and 10-year DSS rates of the L1CAM+ group were 76.8% and 61.7%, respectively, and those of the p53 abn group were 40% and 40%, respectively ($p = 0.22$).

3.4 | Survival analysis of modified molecular classification system

The univariate analysis revealed that the modified classification system was significantly associated with PFS (Table 4A). Stage, myometrial invasion, histology, LNM, recurrent risk classification, POLE-EDM and L1CAM+ were significant variables ($p = 0.0019$, 0.0295, 0.0059, 0.0184, 0.0092, likelihood ratio test, respectively). The modified classification system was identified as an independent prognostic factor for DSS (Table 4B).

The modified classification system was able to stratify patients based on PFS (Figure 6). PFS was significantly different between the favorable and intermediate groups ($p = 0.024$), the intermediate and unfavorable groups ($p = 0.0025$), and the favorable and unfavorable groups ($p < 0.0001$). The 5-year PFS rate was 96.3% in the favorable group, 85.0% in the intermediate group, and 59.4% in the

TABLE 2 Cox regression analysis of molecular features

Marker	Results	Number of events/ total number of patients	HR	95% CI	LRT *p*-value
L1CAM	H-score < 35 or missing	10/85	1		
	H-score > 35	15/45	3.08	(1.42–6.96)	0.0045
MMR	Proficient or missing	19/85	1		
	Deficient	6/45	0.60	(0.23–1.39)	0.2458
p53	Normal or missing	20/113	1		
	Abnormal	5/17	2.12	(0.74–5.08)	0.1480

Abbreviations: CI, confidence interval; HR, hazard ratio; L1CAM, L1 cell adhesion molecule; LRT, likelihood ratio test; MMR, mismatch repair.

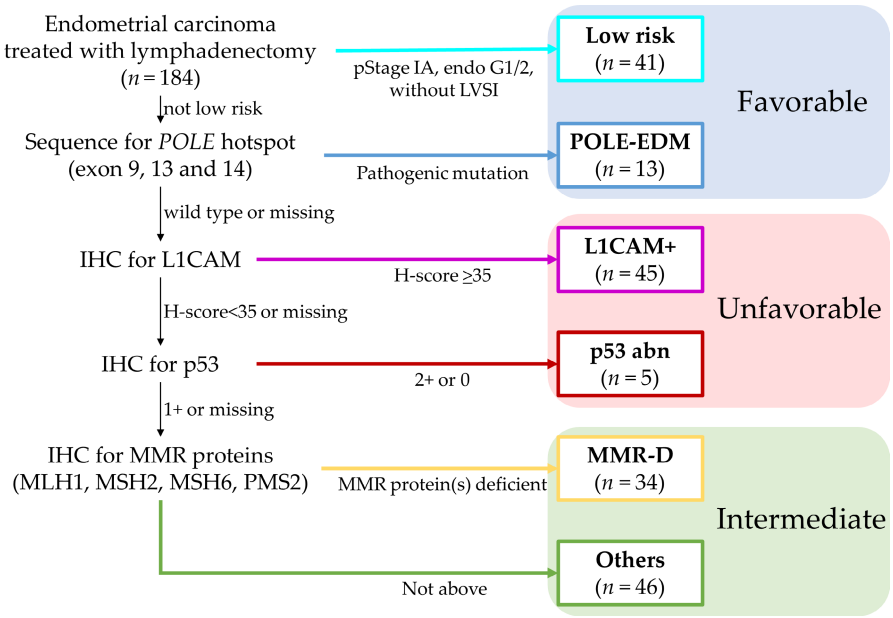

FIGURE 4 Modified molecular classification system combined with clinicopathologic findings and L1 cell adhesion molecule (L1CAM). Endo G1/G2, Grade 1 or 2 endometrioid carcinoma; IHC, immunohistochemistry; L1CAM+, high expression of L1CAM; LVSI, lymphovascular space invasion; MLH1, mutL homolog 1; MMR, mismatch repair; MMR-D, mismatch repair deficiency; MSH2, mutS homolog 2; MSH6, mutS homolog 6; p53 abn, abnormal expression of p53; POLE-EDM, polymerase-epsilon exonuclease domain mutation; PMS2, PMS1 homolog 2;

unfavorable group. The 10-year PFS rate was 96.3% in the favorable group, 83.0% in the intermediate group, and 59.4% in the unfavorable group. The DSS rate was significantly different between the favorable and intermediate groups ($p = 0.026$), the intermediate and unfavorable groups ($p = 0.0001$), and the favorable and unfavorable groups ($p = 0.0001$). The 5-year DSS was 100% in the favorable group, 93.8% in the intermediate group, and 73.2% in the unfavorable group. The 10-year DSS rate was 100% in the favorable group, 91.0% in the intermediate group, and 59.9% in the unfavorable group.

4 | DISCUSSION

In this study, we analyzed EC patients who had undergone radical surgery, including complete lymphadenectomy, and adjuvant chemotherapy in patients with an intermediate or high risk of recurrence based on pathological findings. We revealed three novel findings: (1) patients with low risk of recurrence in conventional risk classification had a favorable prognosis regardless of molecular features. In patients with intermediate or high risk of recurrence, (2) the prognosis of POLE-EDM-positive patients was extremely favorable, but (3) that of patients with L1CAM+, which was not included in the ProMisE classification system, was extremely poor when adjuvant chemotherapy was administrated. Based on these findings, we proposed combining conventional clinicopathological risk factors with IHC for L1CAM, MMR, and p53 and with sequencing for *POLE* mutations. The resulting system may be more suitable than ProMisE classification system for stratifying patients with EC.

The ProMisE classification system has been proposed as an alternative to the original TCGA molecular classification by a surrogate approach of a limited panel of IHC and POLE-EDM analyses. However, the order of classification steps is also important. TCGA classification system screens *POLE* first, whereas the ProMisE classification system prioritizes MMR-D. In our newly modified classification system, we first sorted patients at low risk of recurrence. In this study, the patients with low risk of recurrence had favorable prognosis, while 53.7% (22/41) of patients in the low-risk group had additional unfavorable molecular features. This finding suggests that patients who had a low risk of recurrence based on surgical staging may not need to undergo molecular examinations due to their highly favorable prognoses regardless of their molecular features. These patients accounted for 22.3% of patients (41/184) in this study. Of note, TCGA molecular classification

TABLE 3 Patient characteristics based on the modified molecular classification system

	Low risk (*n* = 41)	POLE-EDM (*n* = 13)	L1CAM+ (*n* = 45)	p53 abn (*n*-5)	MMR-D (*n* = 34)	Others (*n* = 46)	*p*-value
Follow-up period (months)	116 (41–214)	110 (44–190)	88 (5–211)	39 (12–163)	105 (16–207)	112 (2–202)	0.0068
Age (years)	57.2 (14–76)	56.7 (48–66)	62.3 (41–76)	57.6 (42–70)	55.1 (24–73)	55.0 (32–78)	0.0021
2008 FIGO stage							
I	41 (100%)	8 (61.5%)	23 (51.1%)	1 (20%)	12 (35.3%)	14 (30.4%)	0.20
II	–	1 (7.7%)	6 (13.3%)	–	8 (23.5%)	8 (17.4%)	
III	–	4 (30.8%)	15 (33.3%)	2 (40%)	11 (32.4%)	19 (41.3%)	
IV	–	–	1 (2.2%)	2 (40%)	3 (8.8%)	5 (10.9%)	
Histological subtype							
Endo G1/2	41 (100%)	5 (38.5%)	14 (31.1%)	2 (40%)	26 (76.5%)	35 (76.1%)	<0.01
Endo G3	–	6 (46.2%)	10 (22.2%)	1 (20%)	5 (14.7%)	4 (8.7%)	
Serous	–	–	6 (13.3%)	1 (20%)	1 (2.9%)	–	
CCC	–	–	5 (9.1%)	–	–	3 (6.5%)	
CS	–	1 (7.7%)	7 (12.7%)	1 (20%)	2 (3.1%)	4 (8.7%)	
Others	–	1 (7.7%)	2 (3.6%)	–	–	–	
Myometrial invasion							
≥50%	0 (0%)	9 (69.2%)	32 (71.1%)	4 (80%)	22 (64.7%)	26 (56.5%)	0.65
LVSI							
Positive	0 (0%)	8 (61.5%)	26 (57.8%)	2 (40%)	27 (79.4%)	21 (45.7%)	0.026
Peritoneal cytology							
Positive	3 (7.3%)	0 (0%)	11 (24.4%)	0 (0%)	3 (8.8%)	3 (6.5%)	0.06
LN metastasis							
Positive	0 (0%)	2 (15.4%)	15 (33.3%)	2 (40%)	13 (38.2%)	16 (34.8%)	0.67
Risk of recurrence							
Low	41 (100%)	–	–	–	–	–	0.64
Intermediate	–	5 (38.5%)	13 (28.9%)	–	11 (32.4%)	12 (26.1%)	
High	–	8 (61.5%)	32 (71.1%)	5 (100%)	23 (67.6%)	34 (73.9%)	
Adjuvant therapy							
None	38 (92.7%)	–	6 (13.3%)	–	3 (8.8%)	7 (15.2%)	0.63
Chemotherapy	3 (7.3%)	13 (100%)	38 (84.4%)	5 (100%)	29 (85.3%)	39 (84.8%)	
CT + RT	–	–	1 (2.2%)	–	2 (5.9%)	–	

Note: Data are presented as median (range) or number (percent). The Kruskal–Wallis rank test is used to compare continuous variables and Fisher's exact test is used to compare categorical variables.

Abbreviations: CCC, clear cell carcinoma; CS, carcinosarcoma; CT, chemotherapy; Endo G1/G2, Grade 1 or 2 endometrioid carcinoma; Endo G3, Grade 3 endometrioid carcinoma; FIGO, Federation of Gynecologists and Obstetricians; L1CAM+, high expression of L1 cell adhesion molecule; LN, lymph node; LVSI, lymphovascular space invasion; MMR-D, mismatch repair deficiency; p53 abn, abnormal expression of p53; POLE-EDM, polymerase-epsilon exonuclease domain mutation; RT, radiotherapy.

system cannot always be utilized in clinical practice due to its high cost.[5] Although the ProMisE classification system limits sequencing to hotspot mutations in the *POLE* gene combined with IHC for MMR proteins and p53,[9,12,15] hotspot sequencing remains time consuming and costly. Furthermore, the present study results highlight the clinical significance of conventional pathological findings for the favorable prognosis and diagnostic significance of surgical staging. The European Societies of Gynecological Oncology, Radiotherapy and Oncology, and Pathology guidelines state that molecular classification is encouraged, especially in patients with high-grade tumors, and that *POLE* mutation analysis may not be necessary for patients with low-risk or intermediate-risk EC with low-grade histology.[6,28]

If patients with POLE-EDM are not extracted first, their favorable prognosis may confound the prognosis of the other groups. This is because the patients with POLE-EDM showed 5-year and 10-year DSS rates of 100%, even although 68.4% (13/19) of them had conventional risk factors for recurrence. In contrast, a high expression of L1CAM was associated with worse outcomes.[16,17,25] Half of the deaths in patients with L1CAM+ occurred more than 5 years after the initial treatment (Figure S1), suggesting the clinical importance of extending follow-up periods for these patients and underscoring

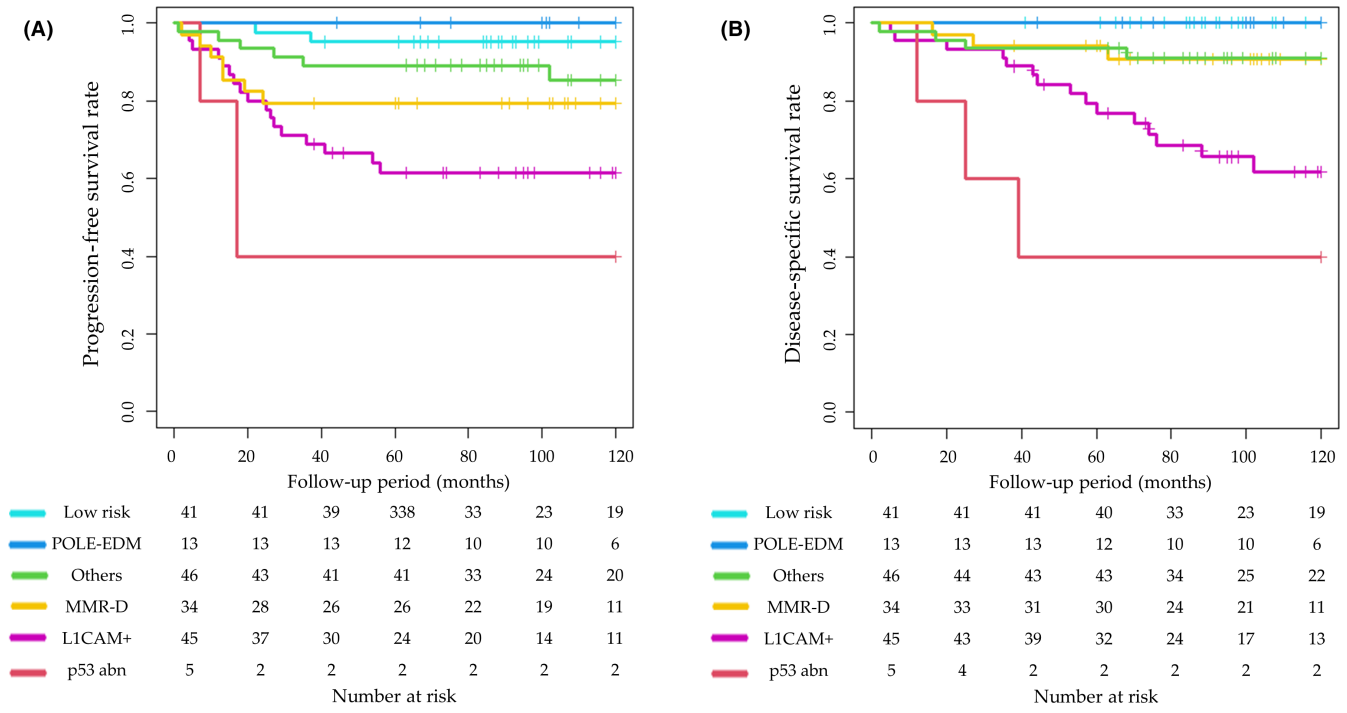

FIGURE 5 Progression-free survival rate (A) and disease-specific survival rate (B) of each group. FIGO, Federation of Gynecologists and Obstetricians; L1CAM+, high expression of L1 cell adhesion molecule; Low risk, low risk recurrence; MMR-D, mismatch repair deficiency; p53 abn, p53 abnormal expression; POLE-EDM, polymerase-epsilon exonuclease domain mutation

the need to modify the ProMisE. Patients with L1CAM+ were extracted before those with p53 abn or MMR-D to identify patients who should be monitored in the long term regardless of the IHC results for p53 and MMR proteins. In this study, p53 immunostaining did not accurately stratify the patients based on prognosis prior to the extraction of patients with L1CAM+. The prognoses of patients with L1CAM+ and p53 abn were more favorable than those of patients who were L1CAM− and p53 abn, which may be influenced by patient characteristics. In this study, 14 patients were L1CAM+ and p53 abn, including two patients in the low-risk group. Of the remaining 12 patients, 10 (83.3%) had Stage I/II disease. In contrast, of the five patients who were L1CAM− and p53 abn, one (20%) had Stage I/II disease (excluding one patient with POLE-EDM).

The present study suggests the ProMisE classification system failed to stratify patients with EC by prognosis. Fewer patient deaths were observed in the POLE-EDM and MMR-D than in previous ProMisE cohorts. Because we excluded patients at low risk in the preoperative LNM score, patients in our cohort tended to have more unfavorable characteristics, including LVSI, deep myometrial invasion, and LNM.[26,29] These discrepancies in the prognostic stratifications could be explained by the different treatment strategies used between Japan and western countries. The ProMisE classification system has been proposed as an alternative to the original TCGA molecular classification. It has been reported to be helpful in determining patient prognosis in western countries that use radiotherapy as a standard adjuvant therapy. Based on the results of the PORTEC-1 and PORTEC-2 trials, vaginal brachytherapy is a standard adjuvant therapy for intermediate-risk or high-risk patients in European countries.[30,31] Chemoradiotherapy (radiotherapy followed by systemic chemotherapy) was reported to improve overall survival compared with external beam radiotherapy alone for patients at high risk of recurrence in the PORTEC-3 trial.[13,14] The results of the PORTEC trials also suggested that TCGA molecular classification system stratified patients at high–intermediate risk and high risk of recurrence based on prognosis.[8,14,19] In contrast, surgical staging, including lymphadenectomy, is typically used to identify patients with a risk of recurrence in Japan, even in patients with early-stage disease. Adjuvant chemotherapy, but not radiotherapy, has been used for patients with an intermediate or a high risk of recurrence, as suggested by the Japanese guidelines for uterine body neoplasms.[22] Patients with POLE-EDM and MMR-D may have benefited from conventional chemotherapy via an enhancement of their immunogenic backgrounds. Previous studies have reported that the number of tumor-infiltrating lymphocytes (TILs) is a favorable prognostic factor for patients with EC, as well as an association between TILs and hypermutated tumors, such as POLE-EDM and MMR-D.[32–35] Lymphocytes are one of the most vulnerable cells for radiotherapy. However, the favorable prognosis of patients with POLE-EDM may result from the immunogenicity of this biomarker.[36]

These molecular classification systems also help to determine how treatment strategies can be personalized. The advantages of adjuvant chemotherapy over radiotherapy are controversial. Patients with abnormal p53 expression were reported to have the worst prognosis in European studies where adjuvant radiotherapy is widely used for all patients with EC.[13,29] In this study, chemotherapy was the standard adjuvant treatment. The PORTEC-4a

TABLE 4 Cox proportional hazard model. (A) Univariate and multivariate analyses for progression-free survival. (B) Univariate and multivariate analyses for disease-specific survival

	Univariate analysis			Multivariate analysis		
	HR	95% CI	LRT *p*-value	HR	95% CI	LRT *p*-value
(A)						
Age (years)						
<58	1			–	–	–
>58	1.21	0.63–2.41	0.5732			
2008 FIGO Stage						
I	1			1		
II/III/IV	2.90	1.47–6.07	0.0019	1.90	0.88–4.44	0.1021
Histological subtype						
Endo G1/2	1			1		
Endo G3/non-Endo	2.09	1.08–4.03	0.0295	1.15	0.51–2.59	0.7110
Risk of recurrence						
Low	1			1		
Intermediate/high	4.31	1.44–21.06	0.0059	0.22	0.001–44.40	0.4836
Myometrial invasion						
Without	1			1		
With	3.55	1.71–8.15	0.0004	1.89	0.80–5.07	0.1528
LVSI						
Without	1			1		
With	3.11	1.60–6.01	0.0010	1.02	0.50–2.12	0.9669
LN metastasis						
Without	1			–	–	–
With	2.19	1.00–5.61	0.0510			
Adjuvant therapy						
None	1			1		
Chemotherapy	4.09	1.90–8.17	0.0006	1.05	0.44–3.14	0.9216
Modified classification						
Favorable	1			1		
Intermediate	3.96	1.20–20.17	<0.0001	4.31	0.55–556.46	0.1569
Unfavorable	11.02	3.53–54.84	<0.0001	23.47	2.39–3140.79	0.0042
POLE-EDM						
Without	1			1		
With	0.11	0.00–0.76	0.0184	1.11	0.00–13.62	0.9488
MMR-D						
Without	1			–	–	–
With	0.90	0.42–1.80	0.7758			
p53						
wt (1+)	1			–	–	–
abn (0 or 2+)	1.97	0.77–4.33	0.1460			
L1CAM						
Low	1			1		
High	2.42	1.25–4.69	0.0092	0.47	0.16–1.66	0.2177

(Continues)

TABLE 4 (Continued)

	Univariate analysis			Multivariate analysis		
	HR	95% CI	LRT *p*-value	HR	95% CI	LRT *p*-value
(B)						
Age (years)						
<58	1			–	–	–
>58	1.43	0.66–3.32	0.3703			
2008 FIGO stage						
I	1			1		
II/III/IV	3.28	1.46–8.18	0.0037	2.11	0.68–6.44	0.1891
Histological subtype						
Endo G1/2	1			1		
Endo G3/non-Endo	2.37	1.09–5.18	0.0297	1.18	0.48–2.88	0.7110
Risk of recurrence						
Low	1			–	–	–
Intermediate/high	>10,000	0–infinite	<0.0001			
Myometrial invasion						
Without	1			1		
With	3.13	1.36–8.27	0.0066	1.01	0.39–2.87	0.9910
LVSI						
Without	1			–	–	–
With	1.37	0.63–3.00	0.4227			
LN metastasis						
Without	1			1		
With	2.92	1.34–6.35	0.0080	1.16	0.44–3.35	0.7616
Adjuvant therapy						
None	1			–	–	–
Chemotherapy	2.24	0.89–7.13	0.0910			
Our classification						
Favorable	–			–		
Intermediate	1			1		
Unfavorable	4.77	1.99–11.44	0.0005	11.66	2.75–40.89	0.0022
POLE-EDM						
Without	1			–	–	–
With	<0.0001	0–infinite	0.02			
MMR-D						
Without	1			–	–	–
With	0.72	0.28–1.87	0.5			
p53						
wt (1+)	1			–	–	–
abn (0 or 2+)	2.35	0.88–6.26	0.1			
L1CAM						
Low	1			1		
High	3.83	1.72–8.53	<0.0001	0.45	0.15–1.76	0.2226

Abbreviations: CI, Confidence interval; Endo G1/G2, Grade 1 or 2 endometrioid carcinoma; Endo G3, Grade 3 endometrioid carcinoma; FIGO, Federation of Gynecologists and Obstetricians; HR, hazard ratio; L1CAM, L1 cell adhesion molecule; LN, lymph node; LRT, likelihood ratio test; LVSI, lymphovascular space invasion; MMR-D, mismatch repair-deficient; p53 abn, p53 abnormal; p53 wt, p53 wild type; POLE-EDM, polymerase-epsilon exonuclease mutation.

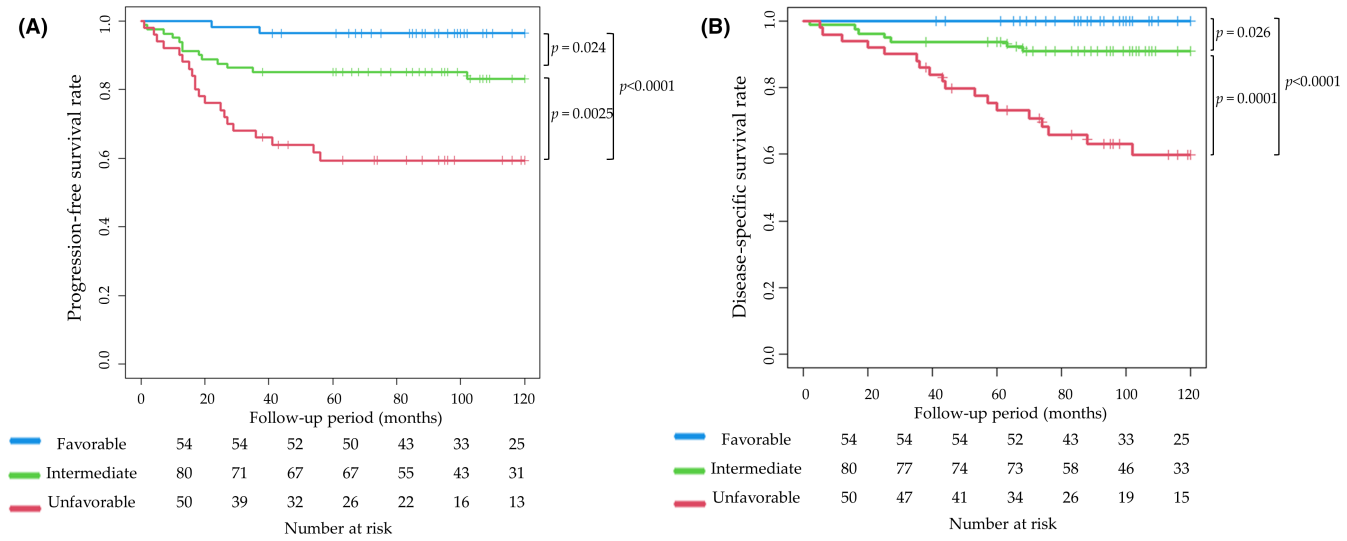

FIGURE 6 Progression-free survival rate (A) and disease-specific survival rate (B) curves based on the modified classification system. The *p*-values of the DSS data are determined using the log-rank test

trial is an ongoing study focusing on personalizing adjuvant therapy for patients with an intermediate or high risk of recurrence.[19] Patients with recurrent EC and MSI-high can be treated with pembrolizumab, an anti-PD-1 monoclonal antibody. Results of the KEYNOTE-158 trial demonstrated that patients with recurrent or unresectable EC responded best to pembrolizumab.[37,38] The clinical efficacy of adjuvant chemotherapy combined with pembrolizumab followed by maintenance pembrolizumab is currently being investigated.[39] Pembrolizumab and lenvatinib, a multityrosine kinase inhibitor, may be a promising target therapy as a recent clinical trial reported a significant survival benefit for patients with platinum-resistant, recurrent EC regardless of the MSI status.[40] These novel treatments may improve patient outcomes; however, no specific treatment options for L1CAM+ and p53 abn patients have been established. The prognostic classifications may need to be distinguished from the methods of personalizing treatments because the prognostic outcomes are the results of the different treatments. Investigating the differences between our classification system and TCGA and ProMisE classification systems could be a step toward addressing the treatment approach that improves the prognosis of POLE-EDM and MMR-D patients or worsens the prognosis of L1CAM+ and p53 abn patients.

This study has some limitations. In addition to the retrospective nature of the study, patients who underwent radical surgery, including systemic lymphadenectomy, were enrolled; this may have created a selection bias. We preoperatively estimated the risk of LNM with a scoring system using magnetic resonance imaging, serum CA125 level, and tumor histology.[23] Based on the preoperative scoring system, we omitted lymphadenectomy in patients with low risk of LNM; additionally, on pathological examination, most of these patients were at low risk of recurrence,[41] and no lymphatic failure was observed during median follow-up periods of 60.5 months.[42] In this study, we excluded patients who did not undergo lymphadenectomy. Due to the preoperative scoring system, the overall proportion of patients with a low risk of recurrence in the present study should be low. A validation study is needed to clarify whether patients treated without lymphadenectomy, or with sentinel lymph node biopsy or adjuvant radiotherapy can be classified according to this new classification for prognosis. As IHC for L1CAM, MMR proteins, and p53 were performed using TMA, a partial expression of tumors may be represented. *POLE* hotspot mutations were not analyzed in seven patients because of the poor quality of sequencing results, which may have deteriorated during long-term storage of surgical specimens. Additionally, the small number of p53 abn cases was a limitation. It is necessary to increase the number of p53 abn available for follow-up on the results. Therefore, a new retrospective study should be conducted.

In conclusion, the prognosis of patients with EC treated with complete staging surgery and adjuvant chemotherapy could be stratified by modified molecular classification proposed in this study based on prognosis of EC using the combination of conventional recurrent risks; IHC for L1CAM, MMR proteins, and p53; and sequencing for *POLE* hotspot mutations. Patients diagnosed with a low risk of recurrence based on surgical staging and *POLE* mutations could be suitable candidates for omitting further molecular examinations due to their highly favorable prognoses.

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DATA AVAILABILITY STATEMENT

The data are not publicly available due to patient privacy issues.

DISCLOSURE

The authors declare no conflicts of interest.

ETHICAL APPROVAL

The study was approved by the Institutional Review Board of Hokkaido University Hospital (protocol code 017-0269, January 17th, 2018). The study was conducted according to the principles of the Declaration of Helsinki. Informed consent was obtained from all patients involved in the study.

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SUPPORTING INFORMATION

Additional supporting information can be found online in the Supporting Information section at the end of this article.

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