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# Evaluation of prognostic potential of β-catenin and L1CAM expression according to endometrial cancer risk group

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## HIGHLIGHTS

- β-catenin and L1CAM expression showed a mutually exclusive pattern.
- β-catenin was an independent poor prognostic factor for progression-free survival in high-intermediate risk group.
- L1CAM was associated with pathological factors related to poor prognosis but was not an independent prognostic factor.

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## ABSTRACT

*Objective.* We investigate the prognostic role of β-catenin and L1 neuronal cell-adhesion molecule (L1CAM) according to risk groups in endometrial carcinomas (EC).

*Methods.* A total of 335 EC patients were classified according to the Proactive Molecular Risk Classifier for Endometrial Cancer. We evaluated the expression of ß-catenin and L1CAM using immunohistochemistry, and their association with clinicopathological characteristics and survival.

*Results.* The expressions of β-catenin and L1CAM were observed in 10.4% of all patients, respectively, and showed mutually exclusive pattern. While β-catenin expression was associated with endometrioid histology ($p = 0.035$) and low tumor grade ($p = 0.045$), L1CAM expression was associated with non-endometrioid histology ($p < 0.001$), high tumor grade ($p < 0.001$), lymphovascular space invasion ($p = 0.006$), and advanced International Federation of Gynecology and Obstetrics (FIGO) stage ($p = 0.001$). β-catenin expression was most frequent in the no specific molecular (NSMP) group (26/35, 74.3%), followed by the DNA polymerase-ε-mutated (POLE-mut) (6/35, 17.1%), and mismatch repair-deficiency (dMMR) (3/35, 8.6%). L1CAM expression was most frequent in the p53-abnormal group (22/35, 62.9%), followed by the NSMP (6/35, 17.1%), dMMR (4/35, 11.4%), and POLE-mut (3/35, 8.6%). Although both markers did not show statistical significance in multivariate analysis for both progression-free survival (PFS) and overall survival in entire cohort, β-catenin positivity was identified as the sole factor associated with worse PFS in the high-intermediate risk subgroup ($p = 0.001$).

*Conclusion.* The expression of nuclear β-catenin may serve as a potential biomarker for predicting recurrence and guiding therapeutic strategies in high-intermediate risk EC patients.



## 1. Introduction

Endometrial carcinoma (EC) is the most common gynecologic malignancy in developed countries, with increasing incidence and mortality rates over the past years [1]. Most patients present with low-grade early-stage disease and have good outcomes. However, some patients are diagnosed with high-grade advanced stages and have a poor prognosis. Therefore, accurate risk classification is important for selecting an appropriate adjuvant therapy.

In 2013, The Cancer Genome Atlas (TCGA) Research Network classified EC into four prognostically significant subgroups based on genomic characteristics [2]. Inspired by this study, Talhouk et al. developed the Proactive Molecular Risk Classifier for Endometrial Cancer (ProMisE), a clinically applicable molecular-based classification using surrogate

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molecular markers [3]. Subsequent studies consistently demonstrated the prognostic importance of the ProMisE system and its high diagnostic reproducibility [4,5]. The four subgroups are as follows: 1) DNA polymerase-ε (POLE) ultra-mutated (POLEmut) with an excellent prognosis, 2) mismatch repair-deficient (dMMR) with an intermediate prognosis, 3) p53-abnormal (p53abn) with a poor prognosis, 4) no specific molecular profile (NSMP) with an intermediate to excellent prognosis. Because the NSMP group is defined by the exclusion of the signatures of other groups, this group shows a highly heterogeneous prognosis—good-to-intermediate prognosis in low-grade endometrioid endometrial carcinoma (EEC), intermediate outcome in high-grade EEC, and poor prognosis in non-endometrioid carcinomas [6–10]. Considering this heterogeneity and the wide range of prognostic outcomes, new molecular markers are required for risk stratification of this group to adopt the most suited treatment approach.

Recent studies have proposed that mutations in exon3 of *CTNNB1* and expression of L1 neuronal cell-adhesion molecule (L1CAM) may help further stratify the NSMP subgroup [11,12]. A subset of low-grade early-stage EEC has *CTNNB1* exon 3 mutation. Although this mutation is associated with characteristics that showed a good prognosis (endometrioid histology, lower tumor grade, younger patient age, <50% myometrial invasion, and absence of lymphovascular space invasion (LVSI)), it is also associated with decreased recurrence-free survival in EECs [13,14]. L1CAM is a transmembrane glycoprotein belonging to the immunoglobulin family that plays an important role in cell adhesion and migration [15]. Several studies have shown L1CAM expression to be strongly associated with abnormal p53 expression and LVSI [16,17]. Moreover, L1CAM has been shown to stratify the NSMP group [12]. However, contrary to these findings, a recent study published by the TransPORTEC group reported that there was no prognostic value of the expressions of β-catenin and L1CAM in high-risk groups, and only the expression of ER was identified as a favorable prognostic factor [18]. The association between the expression of β-catenin and L1CAM and the prognosis of endometrial cancer remains controversial depending on the cohort. In the ongoing PORTEC-4a trial, in addition to POLE, MMR, and p53 status, *CTNNB1* mutation status and L1CAM expression were included in the risk grouping for high-intermediate-risk endometrial cancer [19]. Therefore, it is necessary to verify the predictive potential of prognostic biomarkers according to the risk group. Thus, we evaluated the expression of β-catenin, a surrogate marker of *CTNNB1* mutation, and L1CAM and investigated their prognostic roles in EEC patients.

## 2. Methods

### 2.1. Patients and samples

We reviewed the medical records and pathological reports of 335 patients with EC who underwent surgery at Seoul National University Bundang Hospital between May 2006 and May 2018. We collected data on age at diagnosis, histologic type, depth of invasion, LVSI, International Federation of Gynecology and Obstetrics (FIGO) grade, FIGO stage, and adjuvant treatment. Hematoxylin and eosin-stained tumor sections were reviewed by two gynaecological pathologists to confirm the diagnosis based on the 2020 WHO classification system [20]. Tumors with endometrioid, serous, clear-cell, or mixed histology were included in this study. None of the patients received any neoadjuvant therapy. This retrospective protocol was approved by the institutional review board, which waived the requirement for informed consent (B-2008/628–304).

### 2.2. Molecular classification

We classified EC patients into four molecular subgroups using surrogate markers as previously described [3–5]. A droplet digital polymerase chain reaction (ddPCR) system (Qx200 Droplet DigitalTM PCR System; Bio-Rad, Hercules, CA, USA) was used to identify five hotspot POLE mutations (P286R, S297F, V411L, A456P, and S459F) [21]. Immunohistochemistry (IHC) was performed using a tissue microarray (TMA) to evaluate p53 and MMR protein (hMLH1, hMSH2, hMSH6, and PMS2) expression. To overcome tumor heterogeneity, paired 2-mm cores were taken from each tissue block and arranged in a new TMA block using a trephine apparatus (SuperBioChips Laboratories, Seoul, Korea). p53 staining was performed using a primary monoclonal antibody (pre-diluted DO-7; Dako, Santa Clara, CA, USA). Strong nuclear positivity (>80% of tumor cells) or complete loss of expression was considered aberrant expression. MMR proteins were stained with primary monoclonal antibodies against MLH1 (G168–728, 1:250; PharMingen, San Diego, CA, USA), MSH2 (FE11, 1:50; Oncogene Research Products, Cambridge, MA, USA), and MSH6 (GRBP·P1/2. D4, 1:200; Serotec Inc., Raleigh, NC, USA) and PMS2 (A16–4, 1:200; PharMingen). A complete loss of nuclear staining for any of the proteins was considered abnormal expression. If more than two molecular signatures were present, we classified the cases into one of four groups based on the presence of the POLE mutation > dMMR > p53abn [22,23].

Moreover, all patients were categorized according to the 2021 European Society for Medical Oncology-European Society of Gynaecological Oncology-European Society for Radiotherapy & Oncology (ESMO-ESGO-ESTRO) guidelines into one of five risk groups, with and without integration of the molecular classification [24].

### 2.3. Assessment of β-catenin and L1CAM expression

β-catenin and L1CAM expression were determined by IHC using a mouse anti-β-Catenin antibody (clone 14, BD Biosciences; 1:750) and a rabbit anti-L1CAM antibody (clone 14.10, BioLegend, 1:100) on TMA. β-catenin expression was scored according to the percentage of positive nuclear staining in tumor cells (0 = no staining, 1 = ≤10%, 2 = 11–50%, and 3 = >50%); we defined β-catenin positivity as >10% of tumor cells showing nuclear staining [25]. L1CAM expression was scored according to the percentage of positive membranous staining in tumor cells (0 = no staining, 1 = ≤10%, 2 = 11–50%, and 3 = >50%); we defined L1CAM positivity as >10% of tumor cells showing membranous staining [12]. Fig. 1 shows representative images of β-catenin and L1CAM expression.

### 2.4. Statistical analysis

Relationships between molecular subgroups and clinicopathological data were evaluated using the $\chi 2$ test and Fisher's exact test. Survival curves were created using the Kaplan–Meier method and compared using the log-rank test. Progression-free survival (PFS) and overall survival (OS) were analyzed using the Cox proportional hazards model. All analyses were two-sided and significance was set at $p < 0.05$. All analyses were performed using the IBM SPSS software (version 25.0; IBM Corp., Armonk, NY, USA).

## 3. Results

### 3.1. Clinicopathologic characteristics

Table 1 shows the clinicopathological characteristics of the 335 patients. The mean age at diagnosis was 55.44 years (range: 24–83 years). The histological subtypes were defined as type I (pure endometrioid) (301/335 patients; 89.9%) and type II or mixed (34/335 patients: 19 serous, 4 clear cell, and 11 mixed; 10.1%). The FIGO stages at diagnosis were stage I (280/335 patients; 83.6%), stage II (6/335 patients; 1.8%), stage III (39/335 patients; 11.6%), and stage IV (10/335 patients; 3.0%). A total of 142 patients (42.4%) received adjuvant therapy (radiotherapy: 50 patients [14.9%]; chemotherapy: 43 patients [12.8%]; chemoradiotherapy: 49 patients [14.6%]). Of 99 patients who received radiation with or without chemotherapy, 82 received pelvic

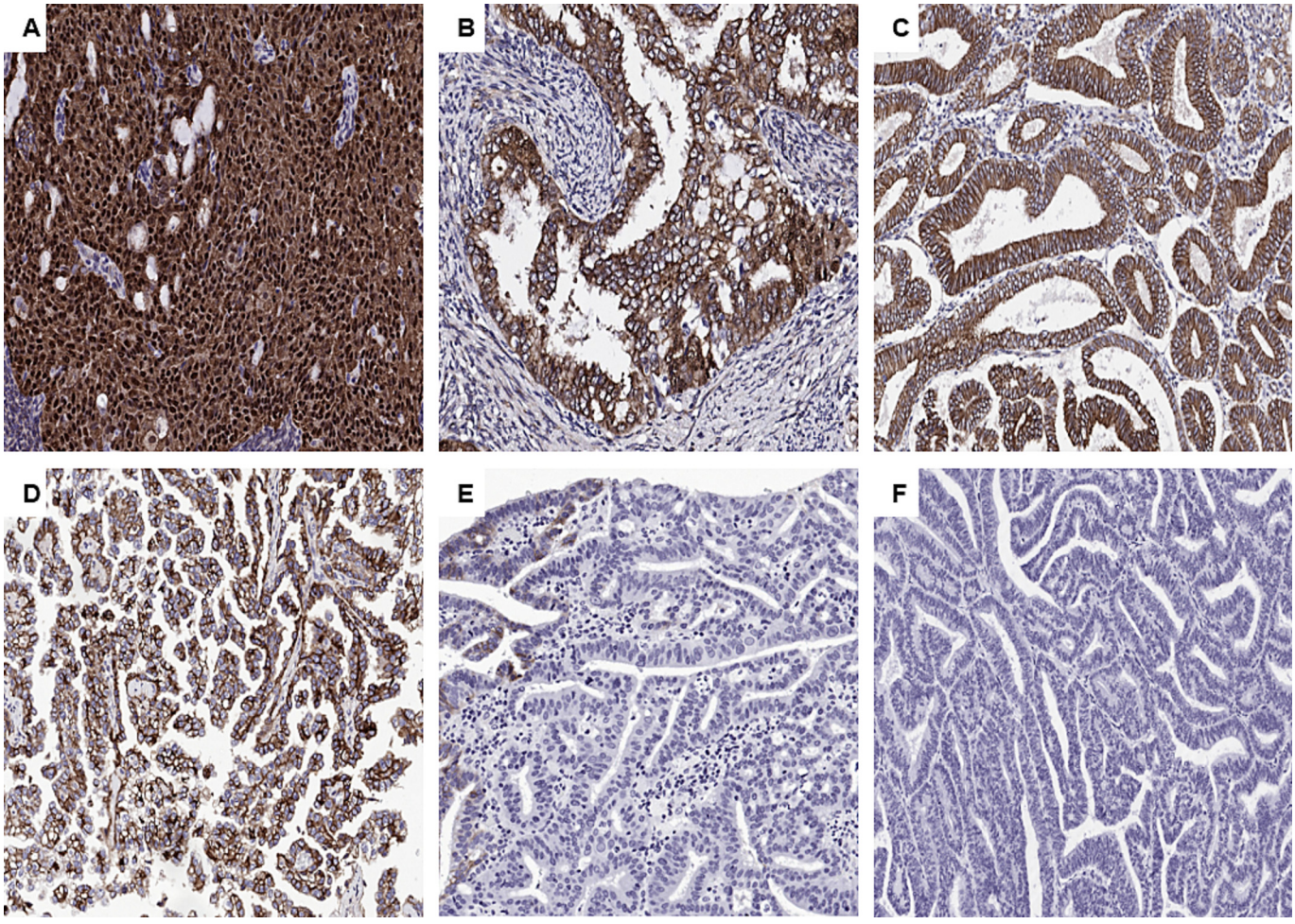


**Fig. 1.** Representative images showing β-catenin (A–C) and L1CAM (D–F) protein expression. (A) Diffuse nuclear staining (Score 3), (B) focal nuclear staining (Score 1), and (C) absence of nuclear staining (Score 0) of β-catenin. (D) Diffuse membranous staining (Score 3), (E) focal membranous staining (Score 1), and (F) absence of membranous staining (Score 0) of L1CAM (200× magnification).

**Table 1**
Clinicopathologic characteristics of patients.

| Characteristics | Total | ß-catenin-negative | ß-catenin-positive | *p*-value | L1CAM-negative | L1CAM-positive | *p*-value |
|---|---|---|---|---|---|---|---|
| **Age at diagnosis (years)** | | | | | | | |
| Mean (±SD) | 55.44(±10.64) | 55.96(±10.31) | 51.00(±12.36) | 0.009* | 54.73(±10.27) | 61.54(±11.85) | <0.001* |
| **Histological subtype** | | | | | | | |
| Type I | 301 (89.9%) | 266 (88.7%) | 35 (100.0%) | 0.035* | 286 (95.3%) | 15 (42.9%) | <0.001* |
| Type II and mixed | 34 (10.1%) | 34 (11.3%) | 0 (0%) | | 14 (4.7%) | 20 (57.1%) | |
| **FIGO grade** | | | | | | | |
| Low (1–2) | 262 (78.2%) | 230 (76.7%) | 32 (91.4%) | 0.045* | 254 (84.7%) | 8 (22.9%) | <0.001* |
| High (3) | 73 (21.8%) | 70 (23.3%) | 3 (8.6%) | | 46 (15.3%) | 27 (77.1%) | |
| **Myometrial invasion** | | | | | | | |
| <1/2 | 238 (71.0%) | 212 (70.7%) | 26 (74.3%) | 0.655 | 220 (73.3%) | 18 (51.4%) | 0.007* |
| ≥1/2 | 97 (29.0%) | 88 (29.3%) | 9 (25.7%) | | 80 (26.7%) | 17 (48.6%) | |
| **LVSI** | | | | | | | |
| Absent | 239 (71.3%) | 211 (70.3%) | 28 (80.0%) | 0.231 | 221 (73.7%) | 18 (51.4%) | 0.006* |
| Present | 96 (28.7%) | 89 (29.7%) | 7 (20.0%) | | 79 (26.3%) | 17 (48.6%) | |
| **FIGO stage** | | | | | | | |
| Early (I–II) | 286 (85.4%) | 254 (84.7%) | 32 (91.4%) | 0.284 | 263 (87.7%) | 23 (65.7%) | 0.001* |
| Advanced (III–IV) | 49 (14.6%) | 46 (15.3%) | 3 (8.6%) | | 37 (12.3%) | 12 (34.3%) | |
| **Adjuvant treatment** | | | | | | | |
| Not done | 193 (57.6%) | 168 (56.0%) | 25 (71.4%) | 0.156 | 187 (62.3%) | 6 (17.1%) | <0.001* |
| Radiation therapy | 50 (14.9%) | 48 (16.0%) | 2 (5.7%) | | 46 (15.3%) | 4 (11.4%) | |
| Chemotherapy | 43 (12.8%) | 41 (13.7%) | 2 (5.7%) | | 25 (8.3%) | 18 (51.4%) | |
| Chemoradiation therapy | 49 (14.6%) | 43 (14.3%) | 6 (17.1%) | | 42 (14.0%) | 7 (20.0%) | |
| **Total** | 335 (100%) | 300 (89.6%) | 35 (10.4%) | | 300 (89.6%) | 35 (10.4%) | |

Abbreviation: SD, standard deviation; FIGO, International Federation of Gynecology and Obstetrics; LVSI, lymphovascular space invasion; L1CAM, L1 cell-adhesion molecule.
* Statistically significant.

external beam radiation and 17 received vaginal brachytherapy. During follow-up, 14.3% of the patients experienced recurrence and 6.0% died because of their disease. The median PFS was 91.7 months (range: 0.6–202.7 months) and the median OS was 96.8 months (range: 3.4–202.7 months).

The expressions of β-catenin and L1CAM were observed in 10.4% of all patients, respectively (Table 1). Both the markers were expressed in mutually exclusive patterns. Of β-catenin-positive tumors, 17/35 (48.6%) showed moderate expression (score 2, 11–50%) and 18/35 (51.4%) showed strong expression (score 3, > 50%). Of L1CAM-positive tumors, 23/35 (65.7%) showed moderate expression (score 2, 11–50%) and 12/35 (34.3%) showed strong expression (score 3, > 50%). β-catenin expression was associated with endometrioid histology ($p = 0.035$) and low tumor grade ($p = 0.045$), however, was not associated with depth of invasion, LVSI and FIGO stage (Table 1). In contrast, L1CAM expression was associated with non-endometrioid histology ($p < 0.001$), high tumor grade ($p < 0.001$), depth of invasion ($p = 0.007$), LVSI ($p = 0.006$) and advanced FIGO stage ($p = 0.001$) (Table 1).

### 3.2. β-catenin and L1CAM expression in ProMisE subgroups

In total, 38 patients (11.3%) were classified as POLEmut, 67 (20.0%) as dMMR, 51 (15.2%) as p53abn, and 179 (53.4%) as NSMP, according to the ProMisE classification (Fig. 2). As shown in Fig. 2, 6/38 (15.8%) patients classified as POLEmut, 3/67 (4.5%) as dMMR and 26/179 (14.5%) as NSMP were β-catenin positive. No patients classified as p53abn showed β-catenin expression. Further, 3/38 (7.9%) patients classified as POLEmut, 4/67 (6.0%) as dMMR, 22/51 (43.1%) as p53abn, and 6/179 (3.4%) as NSMP were L1CAM positive (Fig. 2). ß-catenin expression was most frequent in the NSMP group (26/35, 74.3%), followed by the DNA POLEmut (6/35, 17.1%), and dMMR (3/35, 8.6%) groups. L1CAM expression was most frequent in the p53 abn group (22/35, 62.9%), followed by the NSMP (6/35, 17.1%), dMMR (4/35, 11.4%), and POLEmut (3/35, 8.6%) groups.

### 3.3. β-catenin and L1CAM expression in the ESMO-ESGO-ESTRO risk group

Patients were classified into five risk groups according to the ESMO-ESGO-ESTRO guidelines [24]. When the molecular classification was unknown, 172 patients were classified as low risk (51.3%), 43 (12.8%) as intermediate risk, 59 (17.6%) as high-intermediate risk, 50 (14.9%) as high risk, and 11 (3.3%) as advanced risk. After molecular classification, 182 patients were classified as low-risk (54.7%), 39 (11.7%) as intermediate-risk, 39 (11.7%) as high-intermediate, 62 (18.6%) as high-risk, and 11 (3.3%) as advanced risk (Fig. 3). In total, risk group classification of 35 patients (10.4%) was altered after the molecular classification of tumors was known. Sixteen patients were reclassified as low-risk after molecular classification of POLEmut. Further, 3 and 14 patients were reclassified as intermediate-risk and high-risk, respectively, after molecular classification of p53abn. Two patients with FIGO Stage 3 disease and POLEmut were unclassifiable.

The percentage of patients showing the β-catenin and L1CAM expression in the unknown molecular classification group was as follows: β-catenin positive: 22/172 (12.8%) low, 6/43 (14.0%) intermediate, 4/59 (6.8%) high-intermediate, 2/50 (4.0%) high, and 1/11 (9.1%) advanced; L1CAM positive: 4/172 (2.3%) low, 5/43 (11.6%) intermediate, 5/59 (8.5%) high-intermediate, 18/50 (36.0%) high and 3/11 (27.3%) advanced. After molecular classification, the percentages of patients showing β-catenin and L1CAM expression were changed as follows: β-catenin positive: 25/182 (13.7%) low, 4/39 (10.3%) intermediate, 3/39 (7.7%) high-intermediate, 1/62 (1.6%) high, and 1/11 (9.1%) advanced; L1CAM positive: 4/182 (2.2%) low, 5/39 (12.8%) intermediate, 2/39 (5.1%) high-intermediate, 21/62 (33.9%) high and 3/11 (27.3%) advanced.

### 3.4. Survival analysis

We performed survival analysis based on β-catenin and L1CAM positivity in entire cohort (Table 2). Although L1CAM expression showed an association with worse PFS ($p = 0.014$) and OS ($p = 0.009$) in univariate analysis, it did not show statistical significance in multivariate analysis for both PFS and OS. β-catenin expression did not show statistical significance in risk stratification for both recurrence ($p = 0.767$) and survival ($p = 0.675$). In entire cohort, advanced FIGO stage ($p = 0.025$; HR, 2.220; 95% CI, 1.104–4.464) and adjuvant chemotherapy and chemoradiation therapy ($p = 0.022$; HR, 3.642; 95% CI, 1.210–10.962, and $p = 0.001$; HR, 5.320; 95% CI, 1.898–14.914, respectively) were associated with worse PFS, and tumor grade was associated

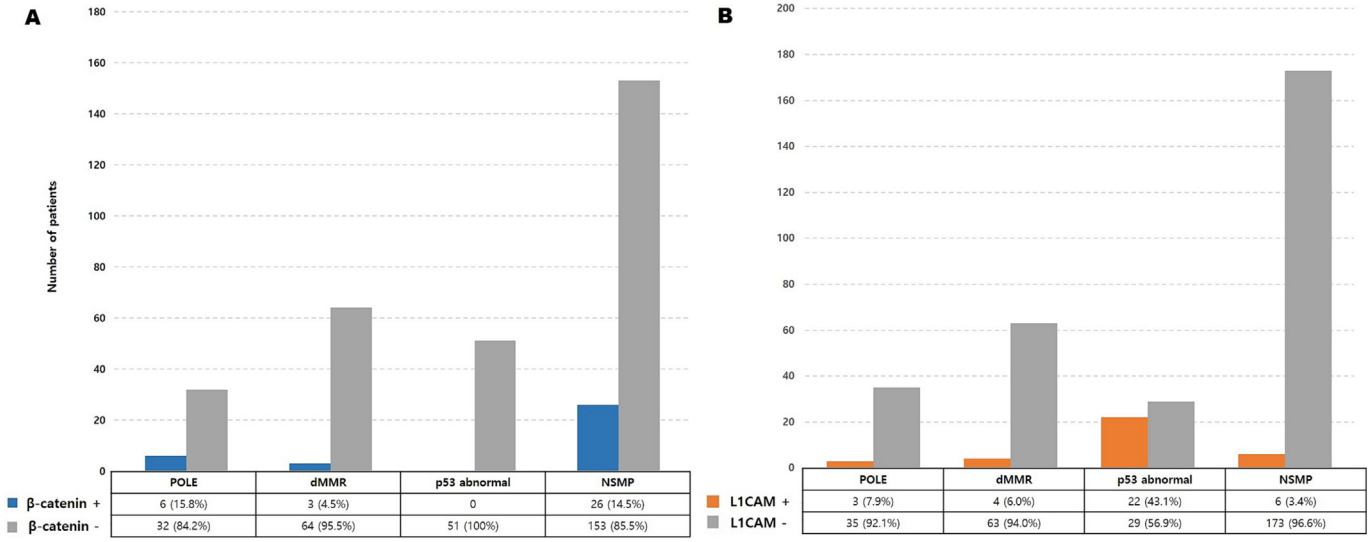


**Fig. 2.** β-catenin (A) and L1CAM (B) expression in the various ProMisE subgroups. Represented as absolute numbers and percentages. Abbreviations: POLE, DNA polymerase ε; dMMR, mismatch repair deficiency; NSMP, no specific molecular profile; L1CAM, L1 cell-adhesion molecule.

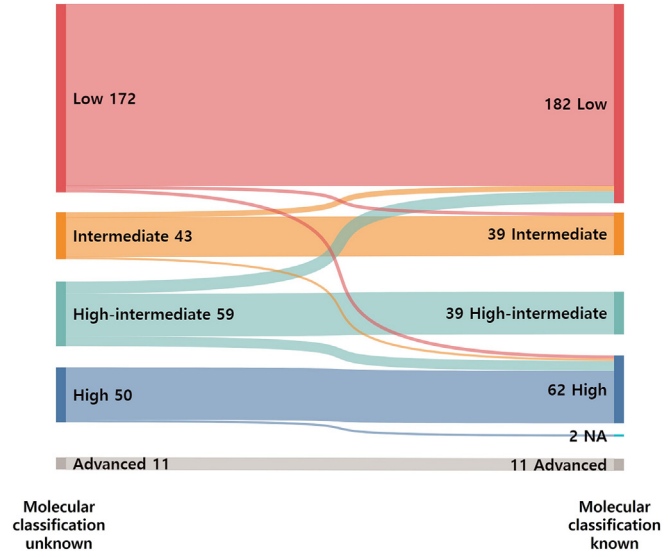


**Fig. 3.** The shifts of patients between molecular unknown and known classification according to 2021 ESMO-ESGO-ESTRO risk groups. Abbreviations: ESMO-ESGO-ESTRO, European Society for Medical Oncology-European Society of Gynaecological Oncology-European Society for Radiotherapy & Oncology; NA, not applicable.

with worse OS ($p = 0.020$; HR, 6.516; 95% CI, 1.337–31.747) in multivariate analysis.

Next, we performed further subgroup analysis to identify factors influencing PFS within each risk group that was reclassified based on molecular classification. β-catenin positivity was identified as the sole factor associated with worse PFS in the high-intermediate risk subgroup ($p = 0.001$) (Table 3). L1CAM positivity did not show statistical significance for both recurrence and survival in any subgroups.

**Table 3**
Factors associated with progression-free survival in high-intermediate risk subgroup ($n = 39$).

| Factors | Univariate ($p$-value) |
|---|---|
| **FIGO grade** | |
| high (3) vs. low (1–2) | 0.146 |
| **LVSI** | |
| present vs. absent | 0.382 |
| **Molecular subgroup** | |
| NSMP vs. dMMR | 0.170 |
| **Adjuvant treatment** | |
| RT vs. not done | 0.856 |
| CT vs. not done | 0.435 |
| CRT vs. not done | 0.723 |
| RT vs. CT/CRT | 0.302/0.564 |
| **ß-catenin** | |
| positive vs. negative | 0.001* |
| **L1CAM** | |
| positive vs. negative | 0.635 |

Abbreviation: FIGO, International Federation of Gynecology and Obstetrics; LVSI, lymphovascular space invasion; NSMP, no specific molecular group; dMMR, mismatch repair-deficiency; RT, radiation therapy; CT, chemotherapy; CRT, chemoradiation therapy; L1CAM, L1 cell-adhesion molecule.
* Statistically significant.

## 4. Discussion

In this study, we evaluated the expression of β-catenin and L1CAM in ECs and explored their potential as prognostic markers. We revealed that β-catenin positivity was identified as the sole biomarker predicting recurrence in the molecular classification known-high-intermediate risk group.

Previous studies reported that *CTNNB1* exon 3 mutations are associated with worse outcomes and intermediate prognoses in patients with low-grade early-stage EC [11,13,26]. Furthermore, some studies have proposed that *CTNNB1*-mutant EC may be regarded as a fifth molecular subgroup of EC [27,28]. β-catenin, encoded by *CTNNB1*, is an adherent junction protein that plays an important role in the Wnt signaling

**Table 2**
Factors associated with survival in the entire cohort ($n = 335$).

| Factors | Progression free survival | | | Overall survival | | |
|---|---|---|---|---|---|---|
| | Univariate | Multivariate | | Univariate | Multivariate | |
| | $p$-value | $p$-value | Hazard ratio (95% CI) | $p$-value | $p$-value | Hazard ratio (95% CI) |
| **Histology** | | | | | | |
| non-endometrioid vs. endometrioid | <0.001* | 0.948 | | <0.001* | 0.840 | |
| **FIGO grade** | | | | | | |
| high (3) vs. low (1–2) | <0.001* | 0.843 | | <0.001* | 0.020* | 6.516 (1.337–31.747) |
| **LVSI** | | | | | | |
| present vs. absent | <0.001* | 0.487 | | 0.008* | 0.601 | |
| **Molecular subgroup** | | | | | | |
| p53-abn vs. POLE | <0.001* | 0.878 | | <0.001* | 0.997 | |
| p53-abn vs. dMMR | <0.001* | 0.860 | | <0.001* | 0.894 | |
| p53-abn vs. NSMP | <0.001* | 0.877 | | <0.001* | 0.900 | |
| NSMP vs. POLE | 0.051 | | | 0.255 | | |
| NSMP vs. dMMR | 0.573 | | | 0.131 | | |
| dMMR vs. POLE | 0.029* | | | NA | | |
| **FIGO stage** | | | | | | |
| high (III, IV) vs. low (I, II) | 0.001* | 0.025* | 2.220 (1.104–4.464) | 0.015* | 0.789 | |
| **Adjuvant treatment** | | | | | | |
| RT vs. not done | 0.031* | 0.202 | | 0.073 | 0.604 | |
| CT vs. not done | <0.001* | 0.022* | 3.642 (1.210–10.962) | <0.001* | 0.328 | |
| CRT vs. not done | <0.001* | 0.001* | 5.320 (1.898–14.914) | 0.002* | 0.156 | |
| RT vs. CT | 0.074 | | | 0.101 | | |
| RT vs. CRT | 0.004* | | | 0.412 | | |
| **L1CAM** | | | | | | |
| positive vs. negative | 0.014* | 0.178 | | 0.009* | 0.351 | |

Abbreviation: FIGO, International Federation of Gynecology and Obstetrics; LVSI, lymphovascular space invasion; p53-abn, p53-abnormal; POLE, DNA polymerase epsilon; dMMR, mismatch repair-deficiency; NSMP, no specific molecular group; RT, radiation therapy; CT, chemotherapy; CRT, chemoradiation therapy; L1CAM, L1 cell-adhesion molecule; CI, confidence interval; NA. not applicable.
* Statistically significant.

pathway. *CTNNB1* exon 3 mutations cause nuclear accumulation of β-catenin protein, and activation of the Wnt/β-catenin signaling pathway, leading to carcinogenesis and progression of EC by inducing the transcription of target genes [29]. Recent studies have attempted to validate nuclear β-catenin expression in place of the identification of *CTNNB1* exon 3 mutations. Kim et al. reported that nuclear β-catenin expression had 100% specificity in detecting *CTNNB1* mutations from wild type, though the low sensitivity (84.9%), and suggested IHC can be used as an initial screening test [25]. Travaglino et al. performed a systematic review of 15 observational studies, and pooled estimates of nuclear β-catenin expression showed 88% sensitivity and 85% specificity in detecting *CTNNB1* exon 3 mutation [27]. In the subsequent meta-analysis results, nuclear β-catenin expression showed a sensitivity and specificity of 85% and 98%, respectively, in predicting *CTNNB1* exon 3 mutations [30]. These results confirm nuclear β-catenin accumulation as an accurate surrogate of CTNNB1 mutation, revealing a considerably excellent specificity. Despite its low sensitivity, β-catenin IHC is less expensive, has a shorter turnaround time than *CTNNB1* sequencing, and is routinely examined in pathological practice. Therefore, although β-catenin IHC may not entirely correlate, it can serve as a useful surrogate marker for predicting the presence of *CTNNB1* mutations in biopsies or resected EC samples.

A recent study reported that abnormal β-catenin expression in the stage I-II NSMP subgroup was significant on multivariate analysis for vaginal recurrence, and adjuvant radiation therapy could significantly decrease the recurrence [31]. These findings are line with our results, demonstrating worse PFS in the molecular classification-known high-intermediate risk subgroup with β-catenin positivity. The study by the TransPORTEC group mentioned earlier focused exclusively on the high-risk group, indicating that the expression of β-catenin is prognostically significant in the early stage, endometrioid, and NSMP subgroups [18]. Taken together, these results suggest that the expression of nuclear β-catenin could serve as a biomarker to determine the implementation of adjuvant therapy in the high-intermediate risk subgroup.

A similar frequency of β-catenin expression was observed in the POLEmut (15.8%) and NSMP (14.5%) groups, but it was rare in the dMMR (4.5%) and the p53abn group (0%), consistent with the 2013 TCGA data [2]. β-catenin expression in the POLEmut group is likely influenced by proofreading errors and ultramutation. Nonetheless, there were no significant prognostic differences based on β-catenin expression in the low-risk subgroup mainly including POLEmut group.

Previous studies have reported that L1CAM expression induces tumor cell migration, invasion, epithelial-mesenchymal transition (EMT), and chemoresistance [15,32]. Some studies have shown the the potential prognostic role of L1CAM expression in predicting recurrence in NSMP EC patients [12,33]. In this study, L1CAM exhibited associations with worse PFS and OS in the univariate analysis of the entire cohort. However, these associations were not demonstrated in the multivariate analysis for PFS and OS. This is believed to be because L1CAM expression is predominantly observed in non-endometrioid histology, high grade, and advanced stage EC, suggesting that it does not act as an independent prognostic factor. These results align with the context that L1CAM was not confirmed as an independent prognostic factor in the TransPORTEC study targeting high-risk EC patients [18]. The prognostic impact of L1CAM expression in the high-intermediate risk group was not confirmed in our cohort, and further validation in larger cohorts is deemed necessary.

Compared with previous studies, L1CAM expression was the highest in the p53abn group and showed similar frequency; however, overall L1CAM expression in the entire EEC cohort was slightly lower than that reported in other studies [12,34,35]. L1CAM expression was observed in 8.6% of the patients in the POLEmut group, 6.0% in the dMMR group, and 3.3% in the NSMP group. This suggests that the tendency of L1CAM to exhibit focal rather than diffuse expression may have led to its underestimation in TMA due to intratumoral heterogeneity.

Previous research in colon cancer has identified L1CAM as a target gene of the β-catenin signaling pathway, and some authors suggest that the transcription factor Slug and β-catenin regulate L1CAM expression, inducing EMT in EC [36,37]. However, in this study, β-catenin and L1CAM were expressed in a mutually exclusive pattern. Similarly, a recent study showed a mutually exclusive pattern of β-catenin and L1CAM expression [38]. This suggests that there are other pathways in the regulation of L1CAM expression that are not associated with β-catenin in EC.

This study was limited by a relatively small cohort study and single-center retrospective design. In addition, β-catenin and L1CAM IHC are known to show intratumoral heterogeneity, which may limit their evaluation in TMA.

In conclusion, we demonstrated that β-catenin and L1CAM were expressed in a mutually exclusive pattern, and β-catenin posiyivity was identified as the sole biomarker predicting recurrence in the molecular classification known-high-intermediate risk group of EC. Therefore, evaluation of nuclear β-catenin expression may serve as a potential biomarker for predicting recurrence and guiding therapeutic strategies in high-intermediate risk endometrial cancer patients.

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## Author contributions

HS Yoon and H Kim: Conceptualization; HS Yoon and H Kim: Study design; HS Yoon, DH Suh, K Kim, JH No, YB Kim, and H Kim: Data acquisition; HS Yoon and H Kim: Data analysis and interpretation; HS Yoon and H Kim: Statistical analysis; HS Yoon and H Kim: Writing - Original Draft; HS Yoon and H Kim: Writing - Review & Editing. All authors approved the final version of the manuscript.

## Data availability

All relevant data are included in the report and associated files.

## Declaration of competing interest

The authors declare no conflicts of interest.

## References

[1] R.L. Siegel, K.D. Miller, N.S. Wagle, A. Jemal, Cancer statistics, 2023, CA Cancer J. Clin. 73 (2023) 17–48, https://doi.org/10.3322/caac.21763.
[2] Cancer Genome Atlas Research Network, C. Kandoth, N. Schultz, A.D. Cherniack, R. Akbani, Y. Liu, H. Shen, A.G. Robertson, I. Pashtan, R. Shen, C.C. Benz, C. Yau, P.W. Laird, L. Ding, W. Zhang, G.B. Mills, R. Kucherlapati, E.R. Mardis, D.A. Levine, Integrated genomic characterization of endometrial carcinoma, Nature 497 (2013) 67–73, https://doi.org/10.1038/nature12113.
[3] A. Talhouk, M.K. McConechy, S. Leung, H.H. Li-Chang, J.S. Kwon, N. Melnyk, W. Yang, J. Senz, N. Boyd, A.N. Karnezis, D.G. Huntsman, C.B. Gilks, J.N. McAlpine, A clinically applicable molecular-based classification for endometrial cancers, Br. J. Cancer 113 (2015) 299–310, https://doi.org/10.1038/bjc.2015.190.
[4] A. Talhouk, M.K. McConechy, S. Leung, W. Yang, A. Lum, J. Senz, N. Boyd, J. Pike, M. Anglesio, J.S. Kwon, A.N. Karnezis, D.G. Huntsman, C.B. Gilks, J.N. McAlpine, Confirmation of ProMisE: a simple, genomics-based clinical classifier for endometrial cancer, Cancer. 123 (2017) 802–813, https://doi.org/10.1002/cncr.30496.
[5] S. Kommoss, M.K. McConechy, F. Kommoss, S. Leung, A. Bunz, J. Magrill, H. Britton, F. Kommoss, F. Grevenkamp, A. Karnezis, W. Yang, A. Lum, B. Krämer, F. Taran, A. Staebler, S. Lax, S.Y. Brucker, D.G. Huntsman, C.B. Gilks, J.N. McAlpine, A. Talhouk, Final validation of the ProMisE molecular classifier for endometrial carcinoma in a large population-based case series, Ann. Oncol. 29 (2018) 1180–1188, https://doi.org/10.1093/annonc/mdy058.
[6] T. Bosse, R.A. Nout, J.N. McAlpine, M.K. McConechy, H. Britton, Y.R. Hussein, C. Gonzalez, R. Ganesan, J.C. Steele, B.T. Harrison, E. Oliva, A. Vidal, X. Matias-Guiu, N.R. Abu-Rustum, D.A. Levine, C.B. Gilks, R.A. Soslow, Molecular classification of grade 3 endometrioid endometrial cancers identifies distinct prognostic subgroups,

Am. J. Surg. Pathol. 42 (2018) 561–568, https://doi.org/10.1097/PAS.0000000000001020.
[7] A. Joehlin-Price, J. Van Ziffle, N.K. Hills, N. Ladwig, J.T. Rabban, K. Garg, Molecularly classified uterine FIGO grade 3 endometrioid carcinomas show distinctive clinical outcomes but overlapping morphologic features, Am. J. Surg. Pathol. 45 (2021) 421–429, https://doi.org/10.1097/PAS.0000000000001598.
[8] S.R. Kim, B.T. Cloutier, S. Leung, D. Cochrane, H. Britton, A. Pina, C. Storness-Bliss, D. Farnell, L. Huang, K. Shum, A. Lum, J. Senz, C.H. Lee, C.B. Gilks, L. Hoang, J.N. McAlpine, Molecular subtypes of clear cell carcinoma of the endometrium: opportunities for prognostic and predictive stratification, Gynecol. Oncol. 158 (2020) 3–11, https://doi.org/10.1016/j.ygyno.2020.04.043.
[9] M.K. McConechy, L.N. Hoang, M.H. Chui, J. Senz, W. Yang, N. Rozenberg, R. Mackenzie, J.N. McAlpine, D.G. Huntsman, B.A. Clarke, C.B. Gilks, C.H. Lee, In-depth molecular profiling of the biphasic components of uterine carcinosarcomas, J. Pathol. Clin. Res. 1 (2015) 173–185, https://doi.org/10.1002/cjp2.18.
[10] A. Santoro, G. Angelico, A. Travaglino, A. Raffone, D. Arciuolo, N. D'Alessandris, F. Inzani, G.F. Zannoni, Clinico-pathological significance of TCGA classification and SWI/SNF proteins expression in undifferentiated/dedifferentiated endometrial carcinoma: a possible prognostic risk stratification, Gynecol. Oncol. 161 (2021) 629–635, https://doi.org/10.1016/j.ygyno.2021.02.029.
[11] E. Stelloo, R.A. Nout, E.M. Osse, I.J. Jürgenliemk-Schulz, J.J. Jobsen, L.C. Lutgens, E.M. van der Steen-Banasik, H.W. Nijman, H. Putter, T. Bosse, C.L. Creutzberg, V.T. Smit, Improved risk assessment by integrating molecular and clinicopathological factors in early-stage endometrial cancer-combined analysis of the PORTEC cohorts, Clin. Cancer Res. 22 (2016) 4215–4224, https://doi.org/10.1158/1078-0432.CCR-15-2878.
[12] F.K. Kommoss, A.N. Karnezis, F. Kommoss, A. Talhouk, F.A. Taran, A. Staebler, C.B. Gilks, D.G. Huntsman, B. Krämer, S.Y. Brucker, J.N. McAlpine, S. Kommoss, L1CAM further stratifies endometrial carcinoma patients with no specific molecular risk profile, Br. J. Cancer 119 (2018) 480–486, https://doi.org/10.1038/s41416-018-0187-6.
[13] K.C. Kurnit, G.N. Kim, B.M. Fellman, D.L. Urbauer, G.B. Mills, W. Zhang, R.R. Broaddus, CTNNB1 (beta-catenin) mutation identifies low grade, early stage endometrial cancer patients at increased risk of recurrence, Mod. Pathol. 30 (2017) 1032–1041, https://doi.org/10.1038/modpathol.2017.15.
[14] M.R. Moroney, K.D. Davies, A.C. Wilberger, J. Sheeder, M.D. Post, A.A. Berning, C. Fisher, C. Lefkowits, S.R. Guntupalli, K. Behbakht, B.R. Corr, Molecular markers in recurrent stage I, grade 1 endometrioid endometrial cancers, Gynecol. Oncol. 153 (2019) 517–520, https://doi.org/10.1016/j.ygyno.2019.03.100.
[15] P. Altevogt, K. Doberstein, M. Fogel, L1CAM in human cancer, Int. J. Cancer 138 (2016) 1565–1576, https://doi.org/10.1002/ijc.29658.
[16] I.C. Van Gool, E. Stelloo, R.A. Nout, H.W. Nijman, R.J. Edmondson, D.N. Church, H.J. MacKay, A. Leary, M.E. Powell, L. Mileshkin, C.L. Creutzberg, V.T. Smit, T. Bosse, Prognostic significance of L1CAM expression and its association with mutant p53 expression in high-risk endometrial cancer, Mod. Pathol. 29 (2016) 174–181, https://doi.org/10.1038/modpathol.2015.147.
[17] D. de Freitas, F.N. Aguiar, C. Anton, C.E. Bacchi, J.P. Carvalho, F.M. Carvalho, L1 cell adhesion molecule (L1CAM) expression in endometrioid endometrial carcinomas: a possible pre-operative surrogate of lymph vascular space invasion, PLoS One 13 (2018), e0209294, https://doi.org/10.1371/journal.pone.0209294.
[18] L. Vermij, J.J. Jobsen, A. León-Castillo, M. Brinkhuis, S. Roothaan, M.E. Powell, S.M. de Boer, P. Khaw, L.R. Mileshkin, A. Fyles, A. Leary, C. Genestie, I.M. Jürgenliemk-Schulz, E.J. Crosbie, H.J. Mackay, H.W. Nijman, R.A. Nout, V.T.H.B.M. Smit, C.L. Creutzber, N. Horeweg, T. Bosse, TransPORTEC Consortium, Prognostic refinement of NSMP high-risk endometrial cancers using oestrogen receptor immunohistochemistry, Br. J. Cancer 128 (7) (2023) 1360–1368, https://doi.org/10.1038/s41416-023-02141-0.
[19] A.S.V.M. van den Heerik, N. Horeweg, R.A. Nout, L.C.H.W. Lutgens, E.M. van der Steen-Banasik, G.H. Westerveld, H.A. van den Berg, A. Slot, F.L.A. Koppe, S. Kommoss, J.W.M. Mens, M.E. Nowee, S. Bijmolt, D. Cibula, T.C. Stam, I.M. Jurgenliemk-Schulz, A. Snyers, M. Hamann, A.G. Zwanenburg, V.L.M.A. Coen, K. Vandecasteele, C. Gillham, C. Chargari, K.W. Verhoeven-Adema, H. Putter, W.B. van den Hout, B.G. Wortman, H.W. Nijman, T. Bosse, C.L. Creutzberg, PORTEC-4a: International randomized trial of molecular profile-based adjuvant treatment for women with high-intermediate risk endometrial cancer, Int. J. Gynecol. Cancer 30 (2020) 2002–2007, https://doi.org/10.1136/ijgc-2020-001929.
[20] WHO Classification of Tumours Editorial Board, Female Genital Tumours: WHO Classification of Tumours, 5th edition4, International Agency for Research on Cancer Publications, Lyon, 2020.
[21] G. Kim, S.K. Lee, D.H. Suh, K. Kim, Y.B. Kim, H. Kim, Clinical evaluation of a droplet digital PCR assay for detecting POLE mutations and molecular classification of endometrial cancer, J. Gynecol. Oncol. 33 (2022), e15, https://doi.org/10.3802/jgo.2022.33.e15.
[22] R. Murali, D.F. Delair, S.M. Bean, N.R. Abu-Rustum, R.A. Soslow, Evolving roles of histologic evaluation and molecular/genomic profiling in the management of endometrial cancer, J. Natl. Compr. Cancer Netw. 16 (2018) 201–209, https://doi.org/10.6004/jnccn.2017.7066.
[23] A. León-Castillo, E. Gilvazquez, R. Nout, V.T. Smit, J.N. McAlpine, M. McConechy, S. Kommoss, S.Y. Brucker, J.W. Carlson, E. Epstein, T.T. Rau, R.A. Soslow, R. Ganesan, X. Matias-Guiu, E. Oliva, B.T. Harrison, D.N. Church, C.B. Gilks, T. Bosse, Clinicopathological and molecular characterisation of 'multiple-classifier' endometrial carcinomas, J. Pathol. 250 (2020) 312–322, https://doi.org/10.1002/path.5373.
[24] N. Concin, X. Matias-Guiu, I. Vergote, D. Cibula, M.R. Mirza, S. Marnitz, J. Ledermann, T. Bosse, C. Chargari, A. Fagotti, C. Fotopoulou, A. Gonzalez Martin, S. Lax, D. Lorusso, C. Marth, P. Morice, R.A. Nout, D. O'Donnell, D. Querleu, M.R. Raspollini, J. Sehouli, A. Sturdza, A. Taylor, A. Westermann, P. Wimberger, N. Colombo, F. Planchamp, C.L. Creutzberg, ESGO/ESTRO/ESP guidelines for the management of patients with endometrial carcinoma, Int. J. Gynecol. Cancer 31 (2021) 12–39, https://doi.org/10.1136/ijgc-2020-002230.
[25] G. Kim, K.C. Kurnit, B. Djordjevic, C. Singh, M.F. Munsell, W.L. Wang, A.J. Lazar, W. Zhang, R. Broaddus, Nuclear β-catenin localization and mutation of the CTNNB1 gene: a context-dependent association, Mod. Pathol. 31 (2018) 1553–1559, https://doi.org/10.1038/s41379-018-0080-0.
[26] I. Ruz-Caracuel, Á. López-Janeiro, V. Heredia-Soto, J.L. Ramón-Patino, L. Yébenes, A. Berjón, A. Hernández, A. Gallego, P. Ruiz, A. Redondo, A. Peláez-García, M. Mendiola, D. Hardisson, Clinicopathological features and prognostic significance of CTNNB1 mutation in low-grade, early-stage endometrial endometrioid carcinoma, Virchows Arch. 479 (2021) 1167–1176, https://doi.org/10.1007/s00428-021-03176-5.
[27] A. Travaglino, A. Raffone, G. Saccone, C. De Luca, A. Mollo, M. Mascolo, G. De Placido, L. Insabato, F. Zullo, Immunohistochemical nuclear expression of β-catenin as a surrogate of CTNNB1 exon 3 mutation in endometrial cancer, Am. J. Clin. Pathol. 151 (2019) 529–538, https://doi.org/10.1093/ajcp/aqy178.
[28] A. Santoro, G. Angelico, A. Travaglino, F. Inzani, D. Arciuolo, M. Valente, N. D'Alessandris, G. Scaglione, V. Fiorentino, A. Raffone, G.F. Zannoni, New pathological and clinical insights in endometrial cancer in view of the updated ESGO/ESTRO/ESP guidelines, Cancers (Basel) 13 (2021), https://doi.org/10.3390/cancers13112623.
[29] M.L. Parrish, R.R. Broaddus, A.B. Gladden, Mechanisms of mutant β-catenin in endometrial cancer progression, Front. Oncol. 12 (2022) 1009345, https://doi.org/10.3389/fonc.2022.1009345.
[30] A. Santoro, D. Arciuolo, A. Travaglino, F. Inzani, G.F. Zannoni, A. Raffone, M. Antonio, Immunohistochemical assessment of β-catenin in endometrial carcinoma, Am. J. Clin. Pathol. 157 (5) (2022) 800, https://doi.org/10.1093/ajcp/aqab207.
[31] C. Hui, M.G. Mendoza, J. Snyder, O. Dorigo, B. Litkouhi, M. Renz, K. Amer, D. Kelly, E.H. Brooke, A.K. Elizabeth, Adjuvant radiation therapy in early-stage endometrial cancer with abnormal beta-catenin expression is associated with improved local control, Gynecol. Oncol. 174 (2023) 42–48, https://doi.org/10.1016/j.ygyno.2023.04.018.
[32] J. Chen, F. Gao, N. Liu, L1CAM promotes epithelial to mesenchymal transition and formation of cancer initiating cells in human endometrial cancer, Exp. Ther. Med. 15 (2018) 2792–2797, https://doi.org/10.3892/etm.2018.5747.
[33] J. Kim, S.I. Kim, N.R. Kim, H. Kim, H.S. Kim, H.H. Chung, J.W. Kim, C. Lee, M. Lee, Prognostic significance of L1CAM expression in addition to ProMisE in endometrial cancer, Gynecol. Oncol. 174 (2023) 231–238, https://doi.org/10.1016/j.ygyno.2023.05.062.
[34] A.N. Karnezis, S. Leung, J. Magrill, M.K. McConechy, W. Yang, C. Chow, M. Kobel, C.H. Lee, D.G. Huntsman, A. Talhouk, F. Kommoss, C.B. Gilks, J.N. McAlpine, Evaluation of endometrial carcinoma prognostic immunohistochemistry markers in the context of molecular classification, J. Pathol. Clin. Res. 3 (2017) 279–293, https://doi.org/10.1002/cjp2.82.
[35] C. Romani, D. Capoferri, C. Reijnen, S. Lonardi, A. Ravaggi, M. Ratti, M. Bugatti, L. Zanotti, G. Tognon, E. Sartori, F. Odicino, S. Calza, J.M.A. Pijnenborg, E. Bignotti, L1CAM expression as a predictor of platinum response in high-risk endometrial carcinoma, Int. J. Cancer 151 (2022) 637–648, https://doi.org/10.1002/ijc.34035.
[36] N. Gavert, M. Conacci-Sorrell, D. Gast, A. Schneider, P. Altevogt, T. Brabletz, A. Ben-Ze'ev, L1, a novel target of beta-catenin signaling, transforms cells and is expressed at the invasive front of colon cancers, J. Cell Biol. 168 (2005) 633–642, https://doi.org/10.1083/jcb.200408051.
[37] M. Pfeifer, U. Schirmer, C. Geismann, H. Schäfer, S. Sebens, P. Altevogt, L1CAM expression in endometrial carcinomas is regulated by usage of two different promoter regions, BMC Mol. Biol. 11 (2010) 64, https://doi.org/10.1186/1471-2199-11-64.
[38] J. Victoor, S.V. Borght, L. Spans, S. Lehnert, H. Brems, A. Laenen, I. Vergote, T. Van Gorp, E. Van Nieuwenhuysen, S. Han, S. Timmerman, A.S. Van Rompuy, I. Vanden Bempt, Comprehensive immunomolecular profiling of endometrial carcinoma: a tertiary retrospective study, Gynecol. Oncol. 162 (2021) 694–701, https://doi.org/10.1016/j.ygyno.2021.06.030.