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L1CAM Predicts Adverse Outcomes in Patients with Endometrial Cancer Undergoing Full Lymphadenectomy and Adjuvant Chemotherapy

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ABSTRACT

Background. L1 cell adhesion molecule (L1CAM) has been established as an important predictor of poor survival of early-stage endometrial cancer patients. We investigated whether L1CAM remains a significant predictor of poor survival of patients with advanced-stage endometrial cancer undergoing extensive surgical staging and adjuvant chemotherapy.

Methods. We prepared tissue microarray (TMA) from surgical tissue specimens of 161 endometrial cancer patients who underwent full lymphadenectomy combined with adjuvant chemotherapy for patients at risk for recurrence, and evaluated expression of L1CAM using immunohistochemistry. The correlation between L1CAM positivity and clinicopathological factors and the prognostic significance of L1CAM expression was investigated.

Results. Among 161 cases who had a follow-up duration of over 3 years, 48 cases (29.8%) showed positive staining for L1CAM. L1CAM positivity was significantly correlated with non-endometrioid histology ($p < 0.0001$), vascular invasion ($p = 0.0157$), and positive cytology ($p = 0.005$), and was a significant predictor of poor survival among advanced-stage patients, but not early-stage patients in our cohort. L1CAM-positive patients showed a higher recurrence rate and frequency of distant failure than L1CAM-negative patients. Multivariate analysis revealed that para-aortic lymph node metastasis (PANM) and L1CAM positivity were independent predictors of poor survival. Overall survival can be stratified into three groups by the combination of PANM and L1CAM positivity.

Conclusion. L1CAM is an independent predictor of poor survival in endometrial cancer patients undergoing full lymphadenectomy and adjuvant chemotherapy, thus indicating that L1CAM can be clinically used as a biomarker to identify those patients at increased risk of recurrence.

L1 cell adhesion molecule (L1CAM) is a transmembrane protein of the immunoglobulin family that has been implicated in promoting cancer cell proliferation, invasion, migration, and metastasis.[1] Molecular classification of endometrial cancer identified by the analysis of The Cancer Genome Atlas (TCGA) data has been widely recognized, and appropriate targeting drugs can be applied based on this classification.[2] Besides this molecular classification, L1CAM has been established as an important predictor of poor survival of early-stage endometrial cancer.[3] L1CAM-

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positive endometrial cancer could be considered a new independent category of four types in the molecular classification of TCGA. A recent analysis reported L1CAM expression as an independent predictor of poor progression-free survival (PFS), overall survival (OS),[4,5] lymph node metastasis (LNM),[6] and distant failure.[7,8]

Regarding the extremely poor prognosis of L1CAM-positive endometrial cancer reported in the literature, two clinical questions still remain: (1) the possibility of inaccurate staging of previous study cohorts; and (2) the efficacy of adjuvant chemotherapy. In the previous studies on the prognostic significance of L1CAM reported from Western countries, all patients did not always undergo full lymph node dissection (LND).[9,10] Surgery without LND or sentinel lymph node biopsy may not be enough for accurate staging of L1CAM-positive patients because of the association between L1CAM positivity and LNM.[4,11] Thus, the prognostic significance of L1CAM expression remains uncertain among patients undergoing extensive surgery, including full LND. Furthermore, a recent publication of immunohistochemistry (IHC) analyses of L1CAM expression from PORTEC-1 and PORTEC-2, which investigated the efficacy of adjuvant radiotherapy (RT) or vaginal brachytherapy for early-stage endometrial cancer patients at high to intermediate risk for recurrence, showed a significantly increased risk of distant failure in patients with high L1CAM expression.[3,7,9] However, the effect of adjuvant chemotherapy on L1CAM-positive endometrial cancer has not been fully evaluated. In our institution, except for patients at low risk for LNM, based on presurgical risk evaluation,[12,13] extensive surgery, including full LND, has been applied for patients, even among early-stage patients with potential risk of recurrence. Moreover, adjuvant chemotherapy has been applied for patients at intermediate risk for recurrence, as well as high-risk patients, as in most Japanese institutions.[14] It remains unclear whether L1CAM serves as a significant prognosticator of endometrial cancer patients from Western countries treated with different strategies. Therefore, in this retrospective analysis we aimed to investigate the prognostic significance of L1CAM among Japanese endometrial cancer patients undergoing extensive surgical staging, including full LND and adjuvant chemotherapy.

METHODS

Patients

A total of 385 patients diagnosed with endometrial cancer, including endometrioid, non-endometrioid, and carcinosarcoma, from 2003 to 2015 in our institution were recruited to this study (Fig. 1). We excluded 176 patients who did not undergo full LND, for several reasons. Because of the potential low risk of LNM, 121 patients were treated by hysterectomy and bilateral salpingo-oophorectomy (BSO) only, 12 patients were treated by hysterectomy and BSO with pelvic LND, 24 young patients were treated by fertility-preserving therapy using 400–600 mg of oral medroxyprogesterone acetate (MPA), 10 patients with a poor general score of 2 or higher on the Eastern Cooperative Oncology Group (ECOG) performance status and/or extremely old age (more than 80 years) did not undergo LND, and 9 patients with distant metastasis underwent palliative therapy. Of the remaining 209 patients who underwent hysterectomy and BSO with full LND (systematic pelvic and para-aortic LND at the level of renal vein), 185 patient specimens were used in preparing tissue microarray (TMA) in their surgically resected specimens of the primary sites, with an additional 24 patients being excluded because of the presence of insufficient lesions for the preparation of TMA, as described below. Finally, an additional 24 patients were excluded as 8 patients underwent neoadjuvant chemotherapy and 16 patients had short follow-up periods of < 36 months. Finally, a total of 161 patients were included in the analysis. Disease stages are reported based on the pathological findings according to the International Federation of Gynecologists and Obstetricians (FIGO) 2008 staging

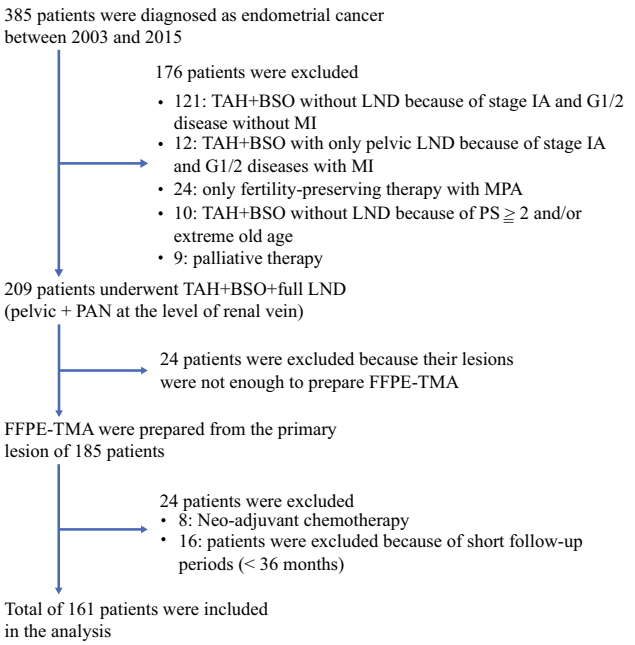

FIG. 1 Study design. We reviewed a total of 385 endometrial cancer patients and analyzed 161 patients retrospectively. *TAH* total abdominal hysterectomy, *BSO* bilateral salpingo-oophorectomy, *LND* lymph node dissection, *MI* myometrial invasion, *PS* performance status, *MPA* medroxyprogesterone acetate, *PAN* para-aortic lymph node, *FFPE* formalin-fixed paraffin-embedded, *TMA* tissue microarray

system.[15] According to a guideline of the Japan Society of Gynecologic Oncology for the treatment of uterine body neoplasms,[16] patients at intermediate or high risk for recurrence diagnosed by postoperative pathological examination (electronic supplementary Table 1) received adjuvant chemotherapy for four to six cycles. An adriamycin and cisplatin combination (AP) regimen, consisting of adriamycin 60 mg/m^2 and cisplatin 50 mg/m^2, or a paclitaxel and carboplatin combination (TC) regimen, consisting of 175 mg/m^2 paclitaxel and carboplatin AUC 5, were used as adjuvant chemotherapy in our institution. The study protocol was approved by the Institutional Review Board at Hokkaido University Hospital (#017-0269).

Preparation of Tissue Microarray

For IHC, we used surgically resected specimens of formalin-fixed paraffin-embedded (FFPE) blocks of primary lesions, and prepared TMA from a total of 185 patients. Archival hematoxylin and eosin (HE) slides of all cases were reviewed to select a representative slide to determine the tumor areas. FFPE-TMA blocks were constructed using a manual tissue microarrayer (JF-4; Sakura Finetek Japan, Tokyo, Japan) with a 1.5-mm diameter needle from two representative tumor areas and one non-tumor area. The finalized array blocks were sliced into 4-μm-thick sections and mounted on glass slides. To assess the pathological diagnosis and adequacy of tissue sampling, a section from each microarray was stained with HE and examined by two certified experienced pathologists (KCH and RM).

Immunohistochemistry (IHC) Staining

Tissue section slides were deparaffinized in xylene and rehydrated using a graded ethanol series. Heat-induced antigen retrieval was conducted in a high-pH antigen retrieval buffer (BenchMark ULTRA; Roche, Basel, Switzerland). Endogenous peroxidase was blocked by incubation at 36 °C in 3% H_2O_2 for 4 min. Sections were labeled using the horseradish peroxidase-labeled polymer method (Ventana ultraView DAB Universal Kit; Roche) and an automated immunostaining system (BenchMark ULTRA; Roche). Immunostained sections were counterstained with hematoxylin, dehydrated in ethanol, and cleared in xylene. Sections were stained with anti-L1CAM antibody (clone 14.10, BioLegend, San Diego, CA, USA) diluted into 1:50.

The specimens were evaluated under light microscopy, using a 10-magnification objective. For assessment of L1CAM staining, only clear staining of the tumor cell membranes was considered positive, and diffuse cytoplasmic or granular staining was diagnosed as negative. Staining was evaluated using the H-score, a semiquantitative approach used to generate a score for each tissue spot of TMA. The percentage of positive tumor area per tumor area (0–100%) was multiplied by the dominant intensity pattern of staining (0, negative or trace; 1+, weak; 2+,

TABLE 1 Patient characteristics

	L1CAM-negative [n = 113]	L1CAM-positive [n = 48]	p-Value
No. of patients (%)	113 (70.2)	48 (29.8)	
Follow-up periods (M)	97 [38–185]	95.5 [39–186]	0.3095
Age (years)	**56 [14–78]**	**64.5 [34–76]**	< **0.0001**
Post-menopause	**83 (74.3)**	**44 (91.7)**	**0.0078**
FIGO 2008			0.4755
I	65 (57.5)	30 (62.5)	
II	16 (14.2)	5 (10.4)	
III	32 (28.3)	13 (27.1)	
Adjuvant therapy			0.6873
None	38 (33.6)	15 (31.3)	
Chemotherapy	75 (66.4)	33 (68.8)	
Adjuvant regimens			0.6595
Taxane–platinum	53 (70.7)	25 (75.8)	
Adriamycin–platinum	21 (28.0)	7 (21.2)	
Others	1 (1.3)	1 (3.0)	

Data are expressed as median [range] or number of patients (%) in each group

P-values were calculated using the Mann–Whitney *U* test or the χ^2 test, and the bolded data had $p < 0.05$

L1CAM L1 cell adhesion molecule, *FIGO* International Federation of Gynecologists and Obstetricians, *M* months

moderate; 3+, intense), and the scores of each tumor spot were calculated using the following formula: [1 × (% area 1+) + 2 × (% area 2+) + 3 × (% area 3+)].[17] The average score of two tumor spots was reported as the H-score, and the overall score ranged from 0 to 300. The H-score was evaluated by two observers (HA and KCH).

Statistical Analysis

In this study, OS was defined as the time from surgery to death from any cause, while PFS was defined as the time from surgery to recurrence. We used the χ^2 test or Fisher's exact test for categorical variables, and the Mann–Whitney *U* test and Spearman's correlation test for continuous variables. The accuracy of the potential variables in predicting the recurrence was summarized using the area under the receiver operating characteristic (AUROC) curves, and the H-score cut-off points were determined using the Youden Index. OS and PFS were estimated using the Kaplan–Meier method, and patients known to be alive or lost to follow-up at the time of analysis were censored at their most recent follow-up. The significance of survival difference was examined using the log-rank test. Multivariate survival analysis was performed using the Cox regression model, with PFS or OS as the outcome measure. The forward-step procedure was used to select the independent variables. A p value < 0.05 was considered statistically significant. Statistical analyses were performed using JMP Pro, version 14.3.0 (SAS Institute Inc., Cary, NC, USA).

RESULTS

Evaluation of L1 Cell Adhesion Molecule (L1CAM) Expression by IHC

The expression level of L1CAM was evaluated using IHC. Representative photos of different L1CAM positivity are shown in Fig. 2a. In previous studies, L1CAM staining was evaluated by the staining area regardless of staining intensity, and over 10% area of positive staining was determined as the cut-off value. Since the range of staining intensity for L1CAM was wide, and most cases were weakly stained, we evaluated staining using the H-score, a scoring system of staining area weighted by staining intensity (described above), in order to analyze more accurately. The median H-score was 12.5 (range 0–300). Representative histograms are shown in Fig. 2b, and the AUROC curve for recurrence is shown in Fig. 2c (AUROC was 0.74584). We determined the H-score cut-off value using the Youden Index (H-score = 35), i.e. L1CAM-negative patients had an H-score of < 35, and L1CAM-positive patients had an H-score of ≥ 35. Based on the H-score, 113 patients (70.2%) were evaluated as L1CAM-negative and 48 patients (29.8%) as L1CAM-positive. The cumulative recurrence rate is shown in Fig. 2d; L1CAM-negative (H-score < 35, blue line) versus L1CAM-positive (H-score ≥ 35, yellow line) [$p = 0.0007$]. In comparison with the previous cut-off value (over 10% of positive area in any staining intensity), 141 cases (87.6%) had the same evaluations in the H-score system (electronic supplementary Fig. 1). Further analyses were performed based on the classification evaluated using the H-score.

Patient Characteristics According to L1CAM Positivity

Patient characteristics according to L1CAM expression are listed in Table 1. The median age was older in L1CAM-positive patients than L1CAM-negative patients (56 vs. 64.5 years; $p < 0.0001$), and the prevalence of postmenopausal women was more frequent in L1CAM-positive women than L1CAM-negative women (74.3% vs. 91.7%; $p = 0.0078$). However, there was no significant difference between the two groups in the median follow-up period for censored cases (97 months vs. 95.5 months; $p = 0.3095$), stage migration ($p = 0.4755$), percentage of patients undergoing adjuvant chemotherapy (65.5% vs. 68.8%; $p = 0.6873$), and chemotherapy regimens.

Correlation Between L1CAM Expression and Pathological Risk Factors

We correlated L1CAM expression with various pathological risk factors, as shown in Table 2. The prevalence of Grade 3 endometrioid and non-endometrioid subtype (G3/Non-endo, 20.4% vs. 56.3%; $p < 0.0001$), vascular invasion (6.2% vs. 18.8%, $p = 0.0157$), and positive peritoneal washing cytology (PWC) [3.5% vs. 22.9%; $p = 0.0001$] was significantly more frequent in L1CAM-positive women than L1CAM-negative women. However, in our cohort, there were no statistically significant differences in postoperative high-risk patients (47.8% vs. 58.3%; $p = 0.3650$), deep myometrial invasion (MI; 45.1% vs. 58.3%; $p = 0.1254$), lymphatic invasion (39.8% vs. 39.6%; $p = 0.9773$), cervical stromal invasion (22.1% vs. 16.7%; $p = 0.4612$), serosal invasion (8.0% vs. 12.5%; $p = 0.3551$), and LNM (22.1% vs. 25.0%; $p = 0.6915$).

Failure Pattern According to L1CAM Expression

Ten of 113 cases (8.8%) in L1CAM-negative patients, and 14 of 48 cases (29.2%) in L1CAM-positive patients finally developed recurrent disease. Details regarding the recurrence pattern are shown in Fig. 3a, b, and electronic supplementary Table 2. Notably, the frequency of distant

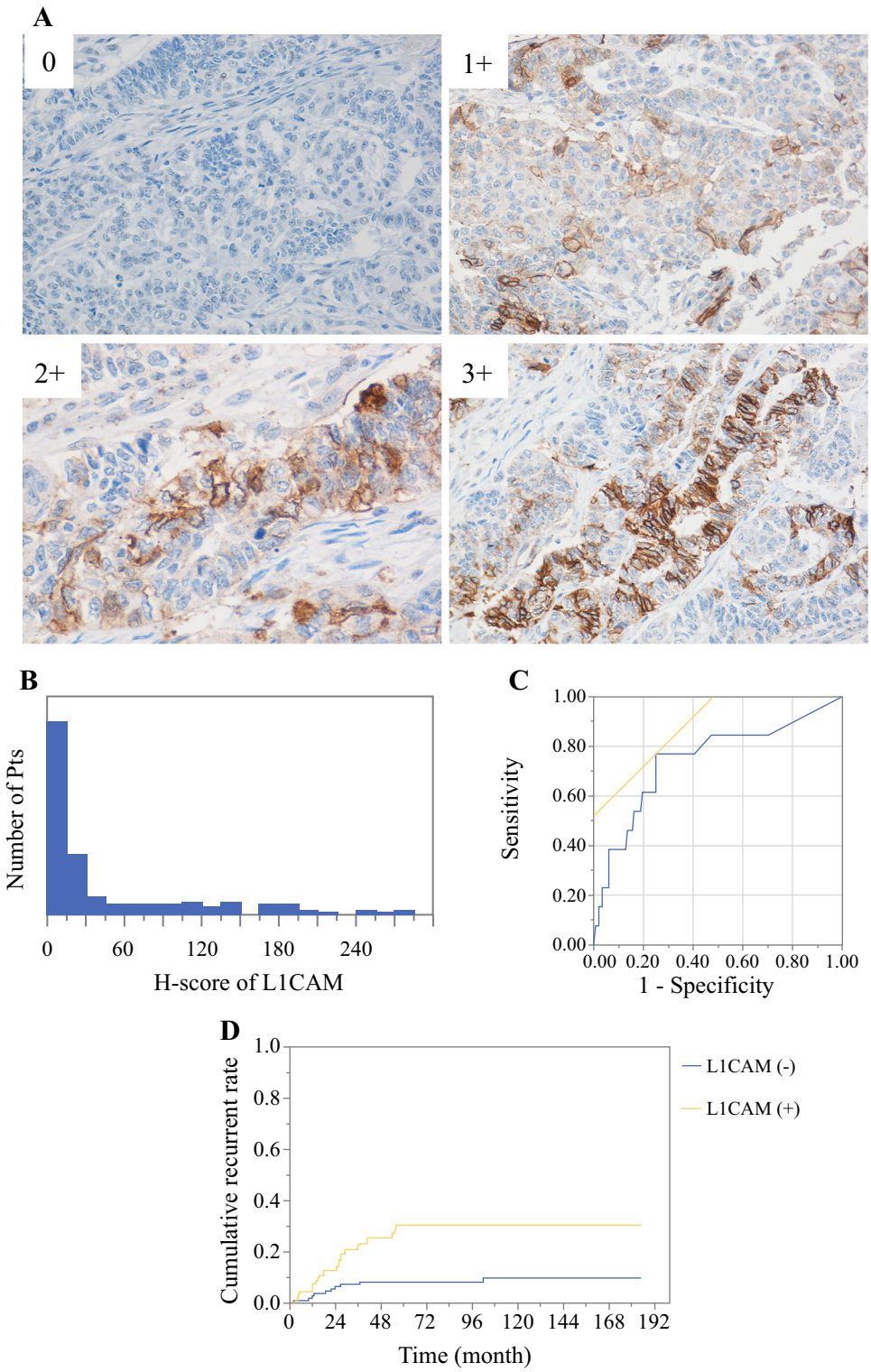

FIG. 2 Evaluation of L1CAM expression by IHC. Expression of L1CAM was evaluated using IHC for FFPE-TMA slides. For assessment of L1CAM, we considered only clear staining of the positive tumor cell membranes, and intensities of staining were divided into four categories: **a** 0 (upper left), 1+ (upper right), 2+ (lower left), and 3+ (lower right). The scores for each tumor spot were calculated using the following formula: [1 × (% area 1+) + 2 × (% area 2+) + 3 × (% area 3+)]. The average score of two tumor spots was reported as the H-score. **b** Histogram of the L1CAM H-score. **c** AUROC curve for recurrence. The AUROC was 0.74584, and the Youden Index yielded an H-score of 35. We determined the H-score cut-off value using the Youden Index (H-score = 35). **d** Cumulative recurrence rate, when the H-score cut-off value was 35; L1CAM-negative (H-score $<$ 35, blue line) versus L1CAM-positive (H-score $\geq$ 35, yellow line) [*p*-value = 0.0007 in a log-rank test]. *L1CAM* L1 cell adhesion molecule, *IHC* immunohistochemistry, *FFPE* formalin-fixed paraffin-embedded, *TMA* tissue microarray, *AUROC* area under the receiver operating characteristic, *Pts* patients

failure was significantly higher in L1CAM-positive patients than L1CAM-negative patients (30% vs. 71.4%; $p = 0.0420$), and the median time to second relapse of L1CAM-positive patients was significantly shorter than that of L1CAM-negative patients, as shown in Fig. 3c (24 vs. 15 months; $p = 0.0402$).

TABLE 2 Correlation between L1CAM expression and pathological risk factors

	L1CAM-negative [n = 113]	L1CAM-positive [n = 48]	p-Value
Risk classification			0.3650
Low risk	32 (28.3)	9 (18.8)	
Intermediate risk	27 (23.9)	11 (22.9)	
High risk	54 (47.8)	28 (58.3)	
Histology			
Endometrioid	**102 (90.3)**	**29 (60.4)**	**< 0.0001**
Grade 1	53 (46.9)	9 (18.8)	
Grade 2	37 (32.7)	12 (25.0)	
Grade 3	12 (10.6)	8 (16.7)	
Non-endometrioid	**11 (9.7)**	**19 (39.6)**	
Serous	1 (0.9)	6 (12.5)	
CCC	1 (0.9)	4 (8.3)	
CS	7 (6.2)	7 (14.6)	
Others	2 (1.8)	2 (4.2)	
MI $\geq$ 1/2	51 (45.1)	28 (58.3)	0.1254
Ly	45 (39.8)	19 (39.6)	0.9773
V	**7 (6.2)**	**9 (18.8)**	**0.0157**
CSI	25 (22.1)	8 (16.7)	0.4612
SI	9 (8.0)	6 (12.5)	0.3551
LNM	25 (22.1)	12 (25.0)	0.6915
No. of metastatic nodes	3 [1–51]	3 [1–40]	0.5748
PLNM alone	6 (24)	3 (25)	0.9046
PANM alone	8 (32)	3 (25)	
PLNM + PANM	11 (44)	6 (50)	
PWC	**4 (3.5)**	**11 (22.9)**	**0.0001**

Data are expressed as number of patients (%) in each group, or median number of metastatic nodes [range]

P-values were calculated using the χ^2 test, and the bolded data had $p < 0.05$

L1CAM L1 cell adhesion molecule, *CCC* clear cell carcinoma, *CS* carcinosarcoma, *MI* myoendometrial involvement, *Ly* lymphatic invasion, *V* vascular invasion, *CSI* cervical stromal invasion, *SI* serosal invasion, *LNM* lymph node metastasis, *PLNM* pelvic lymph node metastasis, *PANM* para-aortic lymph node metastasis, *PWC* peritoneal washing cytology

Effect of L1CAM Expression on Survival

First, we evaluated the effect of L1CAM positivity on PFS and OS in patients with early- and advanced-stage, separately. In early-stage patients, there were no statistically significant differences in either L1CAM-positive or L1CAM-negative patients, both in PFS (p = 0.1550) and OS (p = 0.0996), whereas there were statistically significant differences in L1CAM-positive and L1CAM-negative patients with advanced disease, both in PFS (p = 0.0223) and OS (p = 0.0011), as shown in Fig. 4a, b. Furthermore, PFS and OS, in both L1CAM-positive endometrioid and non-endometrioid carcinomas, were significantly shorter than those of L1CAM-negative patients (electronic supplementary Fig. 2). We then evaluated the effect of L1CAM positivity on adjuvant chemotherapy for the intermediate- and high-risk patients who received adjuvant chemotherapy, as shown in Fig. 4c, d. In comparison with the prognosis of the low-risk patients who did not receive any adjuvant therapy, both OS and PFS were not significantly worse in L1CAM-negative intermediate- or high-risk patients who received adjuvant chemotherapy (5-year OS: 100% vs. 98.6%, p = 0.1741; 5-year PFS: 95.1% vs. 90.3%, p = 0.2470), but were significantly worse in L1CAM-positive intermediate- or high-risk patients who received adjuvant chemotherapy (5-year OS: 100% vs. 82.1%, $p < 0.0001$; 5-year PFS: 95.1% vs. 62.2%, $p < 0.0001$). Furthermore, in comparison with the L1CAM-positive and L1CAM-negative groups, L1CAM-positive patients had significantly worse prognosis, both in OS (p = 0.0008) and PFS (p = 0.0002).

Finally, we correlated L1CAM expression with PFS and OS in the Cox regression model. As shown in Table 3, we excluded surgical stage in the analysis because other

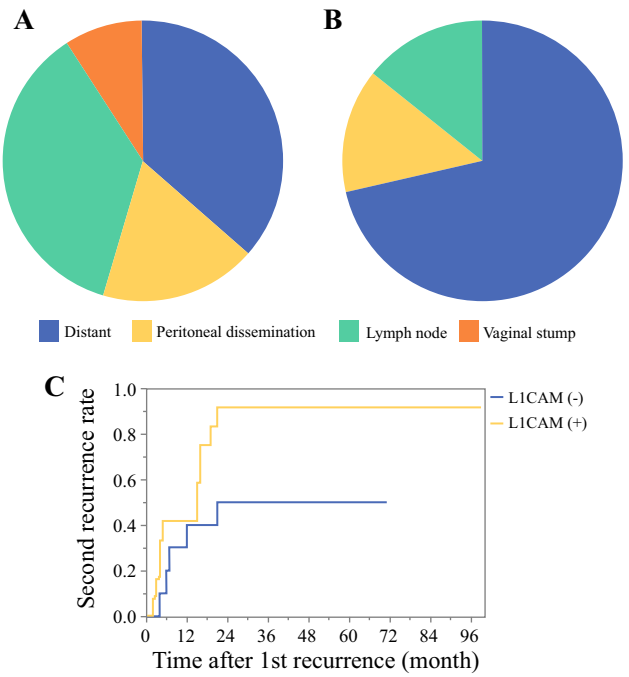

FIG. 3 Failure pattern according to L1CAM expression. Ten L1CAM-negative patients and 14 L1CAM-positive patients finally developed recurrent disease. We divided dominant portions of recurrent diseases into distant failure (blue), peritoneal dissemination (yellow), lymphatic failure (green), and vaginal stump recurrence (red). Pie charts show the distributions of recurrence in **a** L1CAM-negative patients and **b** L1CAM-positive patients. **c** Cumulative second recurrence rate. Median time to second recurrence from the first recurrence was 24 months in L1CAM-negative patients (blue line), and 12 months in L1CAM-positive patients (yellow line) [$p = 0.0402$ in a log-rank test]. *L1CAM* L1 cell adhesion molecule

pathological risk factors were strongly affected on surgical staging, although surgical stage was significantly related to poor PFS (hazard ratio [HR] 2.836, 95% confidence interval [CI] 1.273–6.318; $p = 0.0107$) and OS (HR 3.439, 95% CI 1.153–10.26; $p = 0.0267$) in univariate analysis, as expected. Multivariate analysis revealed that LNM, including para-aortic LNM (PANM; $p = 0.0256$), deep MI ($p = 0.0237$), and L1CAM positivity ($p = 0.0285$) were independent predictors of poor PFS. PANM ($p = 0.0150$) and L1CAM positivity ($p = 0.0107$) were also independent predictors of poor OS.

We divided this study cohort into three groups using a combination of L1CAM positivity and PANM: Group A: L1CAM-negative; Group B: L1CAM-positive and PANM-negative; Group C: L1CAM-positive and PANM-positive. OS could be stratified into three groups, as shown in Fig. 4c. Estimated 5-year OS was 99.1% for Group A, 94.1% for Group B, and 53.3% for Group C. The difference in OS between each group was statistically significant ($p = 0.0413$, Group A vs. Group B; $p = 0.0008$, Group B vs. Group C; and $p < 0.0001$, Group A vs. Group C).

DISCUSSION

In this retrospective analysis, we first demonstrated that L1CAM remains an independent predictor of poor PFS, deep MI, and LNM, and is also an independent predictor of poor OS with LNM. Moreover, the prognosis of advanced-stage endometrial cancer patients, but not early-stage patients with L1CAM positivity, was significantly worse when patients were uniformly treated with extensive surgery, including full lymphadenectomy and adjuvant chemotherapy, but not RT for patients at risk for recurrence.

In the literature, most of the previous reports concluded that L1CAM was a significant predictor of poor survival in early-stage endometrial cancer, and chemotherapy should be applied for L1CAM-positive cases to improve their survival.[3] Chemotherapy is widely applied as an adjuvant therapy for patients at intermediate risk for recurrence and those at high risk for recurrence in Japanese institutions, as we showed in our study.[14] Although no significant difference of survival effect has been reported in JGOG2033 between chemotherapy and RT for endometrial cancer patients at intermediate risk for recurrence, subset analysis demonstrated that chemotherapy significantly improved survival of endometrial cancer patients at high to intermediate risk for recurrence, including those with deep MI, over 70 years of age, and those with cervical stromal invasion, suggesting that some intermediate-risk patients may benefit from adjuvant chemotherapy, but not RT.[18] Since RT has been mainly used as an adjuvant therapy for endometrial cancer patients at intermediate risk for recurrence in Western countries, from which most previous studies on L1CAM have been reported, patients' background seems more heterogenous than our cohort because we uniformly treated patients at risk for recurrence using adjuvant chemotherapy.

We demonstrated that L1CAM positivity was significantly associated with non-endometrioid histology (serous, clear cell, carcinosarcoma) and PWC, as previously described.[19,20] L1CAM-positive patients had shorter PFS and OS than L1CAM-negative patients, regardless of histological type. Moreover, non-endometrioid histology was not an independent predictor of worse prognosis. Because we treated patients at intermediate and high risk for recurrence with adjuvant chemotherapy, but not RT, the result of our study suggests that adjuvant chemotherapy may not efficiently reduce the risk of distant failure of L1CAM-positive patients. One of the possible explanations for this result is that L1CAM-positive cancer might have resistance to conventional chemotherapy, such as TC and AP regimens, as used in our study. Transforming growth factor (TGF)-β signaling lead to Slug- and TWIST1-dependent upregulation of L1CAM expression,[21,22] which

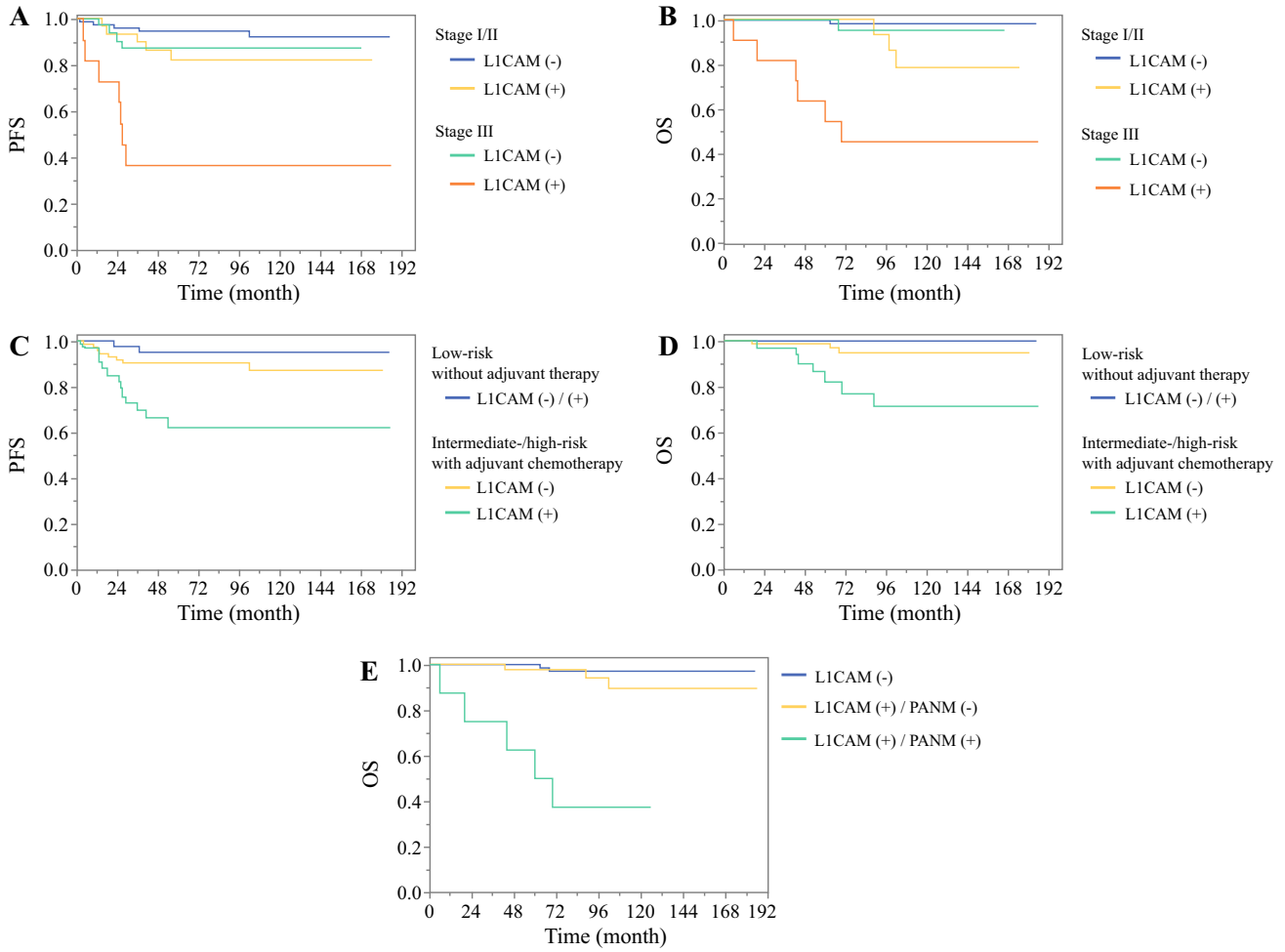

FIG. 4 Prognostic and risk stratification value of surgical stage and L1CAM expression. The effect of L1CAM positivity on early- versus advanced-stage patients in **a** PFS and **b** OS; blue line indicates early/L1CAM-negative patients; yellow line indicates early/L1CAM-positive patients; green line indicates advanced/L1CAM-negative patients; and red line indicates advanced/L1CAM-positive patients. **c** PFS and **d** OS of the intermediate- or high-risk groups for recurrence with adjuvant chemotherapy, compared with the low-risk group without adjuvant therapy; blue line indicates the low-risk group who did not receive adjuvant therapy; the intermediate- or high-risk group for recurrence who received adjuvant chemotherapy were divided into L1CAM-negative (yellow line) and L1CAM-positive (green line) patients. Multivariate analysis, excluding surgical stage, revealed that the independent predictors for OS were PANM and L1CAM positivity. We divided this study cohort into three groups according to the combination of PANM and L1CAM positivity: **e** Group A (blue line), L1CAM-negative; Group B (yellow line), L1CAM-positive and PANM-negative; Group C (green line), L1CAM-positive and PANM-positive. OS was estimated using the Kaplan–Meier methods and *p*-values were calculated using the log-rank test ($p = 0.0413$, Group A vs. B; $p = 0.0008$, Group B vs. C; $p < 0.0001$, Group A vs. C). *PFS* progression-free survival, *OS* overall survival, *L1CAM* L1 cell adhesion molecule, *PANM* para-aortic lymph node metastasis

were suppressed by miR-34a[21] and also strongly upregulated by miR-21-5p (miR-21).[23] Furthermore, the expression of L1CAM was associated with epithelial mesenchymal transition (EMT), and AKT, JNK and ERK signaling, resulting in tumor cell survival, migration, invasion, and resistance for cisplatin and paclitaxel.[24–26]

We also showed that L1CAM positivity was correlated with vascular invasion but not lymphatic invasion or LNM, while several studies reported that L1CAM was associated with LNM. Because the recurrence pattern of L1CAM-positive patients was mainly distant metastasis as in previous studies, and because L1CAM was an independent predictor for worse prognosis as well as LNM in this study, it was considered that L1CAM-positive tumor cells had a higher affinity for tumor vessels than lymph vessels. Although we could not evaluate the expression of L1CAM in tumor vessels because of the use of TMA in IHC analysis, several studies reported that the expression of L1CAM has been found in the vasculature of various solid tumors,[27] and L1CAM expressed in tumor vessels interacted with a binding partner of L1CAM expressed in tumor cells, including integrin, neuropilin-1, or L1CAM itself, resulting in tumor cell migration via cell–cell interactions.[28] Because L1CAM expression of tumor vessels was

TABLE 3 Univariate and multivariate analysis for PFS and OS

	PFS		OS	
	HR [95% CI]	*p*-Value	HR [95% CI]	*p*-Value
Univariate analysis				
Stage	**2.836 [1.273–6.318]**	**0.0107**	**3.439 [1.153–10.26]**	**0.0267**
G3/non-endo	**2.053 [1.012–4.584]**	**0.0486**	**3.352 [1.064–10.57]**	**0.0389**
MI $\geq$ 1/2	**4.333 [1.617–11.61]**	**0.0035**	2.493 [0.766–8.115]	0.1294
Ly	1.644 [0.739–3.660]	0.2234	1.367 [0.459–4.073]	0.5741
V	**2.815 [1.045–7.583]**	**0.0407**	2.689 [0.586–12.34]	0.2034
CSI	1.379 [0.547–3.477]	0.4958	0.835 [0.184–3.785]	0.8146
SI	**3.855 [1.528–9.725]**	**0.0043**	**3.757 [1.025–13.76]**	**0.0457**
LNM	**2.711 [1.203–6.107]**	**0.0161**	**3.365 [1.126–10.06]**	**0.0299**
PANM+	**2.665 [1.140–6.233]**	**0.0237**	**4.032 [1.270–12.81]**	**0.0180**
PWC	**3.675 [1.455–9.285]**	**0.0059**	**6.027 [1.842–19.72]**	**0.0030**
L1CAM	**3.701 [1.641–8.342]**	**0.0016**	**9.187 [2.522–33.48]**	**0.0008**
Multivariate analysis				
G3/non-endo	1.115 [0.433–2.875]	0.8213	1.274 [0.341–4.753]	0.7183
MI $\geq$ 1/2	**3.332 [1.174–9.457]**	**0.0237**	–	–
V	1.439 [0.530–4.493]	0.5308	–	–
SI	1.551 [0.623–3.862]	0.3458	3.974 [0.943–16.75]	0.0601
PLNM alone	0.931 [0.289–2.999]	0.9051	1.401 [0.301–6.800]	0.6523
PANM+	**3.508 [1.166–10.56]**	**0.0256**	**5.612 [1.211–26.01]**	**0.0150**
PWC	1.564 [0.494–4.951]	0.4467	1.562 [0.341–7.168]	0.5659
L1CAM	**3.158 [1.128–8.839]**	**0.0285**	**7.987 [1.654–38.58]**	**0.0107**

Univariate and multivariate analyses for each risk factor were performed using the Cox regression model and show the hazard ratio and 95% confidence interval data, and the bolded data had $p < 0.05$

PFS progression-free survival, *OS* overall survival, *HR* hazard ratio, *CI* confidence interval, *non-endo* non-endometrioid histological subtype, *MI* myometrial involvement, *Ly* lymphatic invasion, *V* vascular invasion, *CSI* cervical stromal invasion, *SI* serosal invasion, *LNM* lymph node metastasis, *PLNM* pelvic lymph node metastasis, *PANM+* para-aortic lymph node metastasis with or without PLNM, *PWC* peritoneal washing cytology

reported to be regulated by angiogenic and inflammatory cytokines, including vascular endothelial growth factor-A, bevacizumab may prevent distant recurrence of L1CAM-positive endometrial cancer.

Because multivariate analysis demonstrated that OS can be stratified by the combination of L1CAM and LNM, we should develop a new treatment strategy for L1CAM-positive patients. To achieve favorable outcomes for L1CAM-positive patients with poor survival, we need to consider using molecular targeting therapy in combination with chemotherapy. Although molecular classification can propose appropriate treatment options according to the type of classification, such as immune checkpoint inhibitor for polymerase-ε ultramutated and microsatellite instability-high cancer, the molecular profile of L1CAM-positive endometrial cancer remains to be elucidated. We should therefore further investigate the biological role of L1CAM related to possible chemoresistance mechanisms in the near future.

We conclude that L1CAM is an independent predictor of poor survival of patients with advanced-stage endometrial cancer undergoing full LND and adjuvant chemotherapy. Limitations of this study include that it was a retrospective study, and the number of samples was relatively small compared with previous large-scale studies. However, our findings clearly indicated that adjuvant chemotherapy did not effectively rescue L1CAM-positive patients undergoing extensive surgery. We should develop new treatment strategies using known targeting agents or new targeting drugs for L1CAM-positive endometrial cancer patients with poor survival.

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REFERENCES

1. Kiefel H, Bondong S, Hazin J, et al. L1CAM: a major driver for tumor cell invasion and motility. *Cell Adh Migr.* 2012;6(4):374–84.
2. Cancer Genome Atlas Research N, Kandoth C, Schultz N, et al. Integrated genomic characterization of endometrial carcinoma. *Nature.* 2013;497(7447):67–73.
3. Stelloo E, Nout RA, Osse EM, et al. Improved risk assessment by integrating molecular and clinicopathological factors in early-stage endometrial cancer-combined analysis of the PORTEC cohorts. *Clin Cancer Res.* 2016;22(16):4215–24.
4. Zeimet AG, Reimer D, Huszar M, et al. L1CAM in early-stage type I endometrial cancer: results of a large multicenter evaluation. *J Natl Cancer Inst.* 2013;105(15):1142–50.
5. Fogel M, Gutwein P, Mechtersheimer S, et al. L1 expression as a predictor of progression and survival in patients with uterine and ovarian carcinomas. *Lancet.* 2003;362(9387):869–75.
6. Suh DH, Kim MA, Kim HS, et al. L1 cell adhesion molecule expression is associated with pelvic lymph node metastasis and advanced stage in diabetic patients with endometrial cancer: a matched case control study. *J Cancer Prev.* 2014;19(3):231–9.
7. Wortman BG, Creutzberg CL, Putter H, et al. Ten-year results of the PORTEC-2 trial for high-intermediate risk endometrial carcinoma: improving patient selection for adjuvant therapy. *Br J Cancer.* 2018;119(9):1067–74.
8. Bosse T, Nout RA, Stelloo E, et al. L1 cell adhesion molecule is a strong predictor for distant recurrence and overall survival in early stage endometrial cancer: pooled PORTEC trial results. *Eur J Cancer.* 2014;50(15):2602–10.
9. Vizza E, Mancini E, Laquintana V, et al. The prognostic significance of positive peritoneal cytology in endometrial cancer and its correlations with L1-CAM biomarker. *Surg Oncol.* 2019;28:151–7.
10. Fadare O, Roma AA, Desouki MM, et al. The significance of L1CAM expression in clear cell carcinoma of the endometrium. *Histopathology.* 2018;72(3):532–8.
11. Geels YP, Pijnenborg JM, Gordon BB, et al. L1CAM expression is related to non-endometrioid histology, and prognostic for poor outcome in endometrioid endometrial carcinoma. *Pathol Oncol Res.* 2016;22(4):863–8.
12. Mitamura T, Watari H, Todo Y, et al. Lymphadenectomy can be omitted for low-risk endometrial cancer based on preoperative assessments. *J Gynecol Oncol.* 2014;25(4):301–5.
13. Todo Y, Okamoto K, Hayashi M, et al. A validation study of a scoring system to estimate the risk of lymph node metastasis for patients with endometrial cancer for tailoring the indication of lymphadenectomy. *Gynecol Oncol.* 2007;104(3):623–8.
14. Watanabe Y, Kitagawa R, Aoki D, et al. Practice pattern for postoperative management of endometrial cancer in Japan: a survey of the Japanese Gynecologic Oncology Group. *Gynecol Oncol.* 2009;115(3):456–9.
15. Pecorelli S. Revised FIGO staging for carcinoma of the vulva, cervix, and endometrium. *Int J Gynecol Obstet.* 2009;105(2):103–4.
16. Ebina Y, Katabuchi H, Mikami M, et al. Japan Society of Gynecologic Oncology guidelines 2013 for the treatment of uterine body neoplasms. *Int J Clin Oncol.* 2016;21(3):419–34.
17. Mazieres J, Brugger W, Cappuzzo F, et al. Evaluation of EGFR protein expression by immunohistochemistry using H-score and the magnification rule: re-analysis of the SATURN study. *Lung Cancer.* 2013;82(2):231–7.
18. Susumu N, Sagae S, Udagawa Y, et al. Randomized phase III trial of pelvic radiotherapy versus cisplatin-based combined chemotherapy in patients with intermediate- and high-risk endometrial cancer: a Japanese Gynecologic Oncology Group study. *Gynecol Oncol.* 2008;108(1):226–33.
19. Kommoss FK, Karnezis AN, Kommoss F, et al. L1CAM further stratifies endometrial carcinoma patients with no specific molecular risk profile. *Br J Cancer.* 2018;119(4):480–6.
20. Dellinger TH, Smith DD, Ouyang C, Warden CD, Williams JC, Han ES. L1CAM is an independent predictor of poor survival in endometrial cancer: an analysis of The Cancer Genome Atlas (TCGA). *Gynecol Oncol.* 2016;141(2):336–40.
21. Schirmer U, Doberstein K, Rupp AK, et al. Role of miR-34a as a suppressor of L1CAM in endometrial carcinoma. *Oncotarget.* 2014;5(2):462–72.
22. Roberts CM, Tran MA, Pitruzzello MC, et al. TWIST1 drives cisplatin resistance and cell survival in an ovarian cancer model, via upregulation of GAS6, L1CAM, and Akt signalling. *Sci Rep.* 2016;6:37652.
23. Doberstein K, Bretz NP, Schirmer U, et al. miR-21-3p is a positive regulator of L1CAM in several human carcinomas. *Cancer Lett.* 2014;354(2):455–66.
24. Chen J, Gao F, Liu N. L1CAM promotes epithelial to mesenchymal transition and formation of cancer initiating cells in human endometrial cancer. *Exp Ther Med.* 2018;15(3):2792–7.
25. Kim H, Hwang H, Lee H, Hong HJ. L1 cell adhesion molecule promotes migration and invasion via JNK activation in extrahepatic cholangiocarcinoma cells with activating KRAS mutation. *Mol Cells.* 2017;40(5):363–70.
26. Ito T, Yamada S, Tanaka C, et al. Overexpression of L1CAM is associated with tumor progression and prognosis via ERK signaling in gastric cancer. *Ann Surg Oncol.* 2014;21(2):560–8.
27. Angiolini F, Cavallaro U. The pleiotropic role of L1CAM in tumor vasculature. *Int J Mol Sci.* 2017;18(2): pii: E254.
28. Issa Y, Nummer D, Seibel T, et al. Enhanced L1CAM expression on pancreatic tumor endothelium mediates selective tumor cell transmigration. *J Mol Med (Berl).* 2009;87(1):99–112.