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ARTICLE

L1CAM in Early-Stage Type I Endometrial Cancer: Results of a Large Multicenter Evaluation

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Background Despite the excellent prognosis of Fédération Internationale de Gynécologie et d'Obstétrique (FIGO) stage I, type I endometrial cancers, a substantial number of patients experience recurrence and die from this disease. We analyzed the value of immunohistochemical L1CAM determination to predict clinical outcome.

Methods We conducted a retrospective multicenter cohort study to determine expression of L1CAM by immunohistochemistry in 1021 endometrial cancer specimens. The Kaplan–Meier method and Cox proportional hazard model were applied for survival and multivariable analyses. A machine-learning approach was used to validate variables for predicting recurrence and death.

Results Of 1021 included cancers, 17.7% were rated L1CAM-positive. Of these L1CAM-positive cancers, 51.4% recurred during follow-up compared with 2.9% L1CAM-negative cancers. Patients bearing L1CAM-positive cancers had poorer disease-free and overall survival (two-sided Log-rank $P < .001$). Multivariable analyses revealed an increase in the likelihood of recurrence (hazard ratio [HR] = 16.33; 95% confidence interval [CI] = 10.55 to 25.28) and death (HR = 15.01; 95% CI = 9.28 to 24.26). In the L1CAM-negative cancers FIGO stage I subdivision, grading and risk assessment were irrelevant for predicting disease-free and overall survival. The prognostic relevance of these parameters was related strictly to L1CAM positivity. A classification and regression decision tree (CRT)identified L1CAM as the best variable for predicting recurrence (sensitivity = 0.74; specificity = 0.91) and death (sensitivity = 0.77; specificity = 0.89).

Conclusions To our knowledge, L1CAM has been shown to be the best-ever published prognostic factor in FIGO stage I, type I endometrial cancers and shows clear superiority over the standardly used multifactor risk score. L1CAM expression in type I cancers indicates the need for adjuvant treatment. This adhesion molecule might serve as a treatment target for the fully humanized anti-L1CAM antibody currently under development for clinical use.

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Endometrial cancer is the most common gynecologic cancer in developed countries and the second most common gynecologic cancer worldwide (1). From clinical and epidemiological studies, a dualistic classification of endometrial cancers was proposed, namely type I and type II tumors, which have different patterns of molecular alterations that underlie their pathogenesis and clinical outcome (2,3). Type I carcinomas accounting for 80% of the cancers, typically display low-grade endometrioid histology, and frequently develop on the basis of premalignant hyperplastic lesions. By contrast, type II cancers are most frequently of nonendometrioid serous or clear-cell histology, often arise in older women displaying an atrophic endometrium, and are unrelated to hyperestrogenism (1). These tumors are more likely to present in advanced stages and have a poorer prognosis than stage-matched type I endometrial cancers. However, beyond this dualistic model, there are endometrial cancers that exhibit overlapping histopathological, immunohistochemical, and clinical features of the various subtypes and are classified as carcinomas of mixed cell type. This category includes tumors with 10% or more of the second cell type, and by convention endometrioid carcinomas with squamous elements are not placed in that group of mixed cancers. For this mixed-type cancer the literature is ambiguous on how much serous component is needed for a tumor to exhibit the same adverse clinical manner as a purely serous cancer (4). In two studies, the behavior was found to be equal to that of purely serous cancers if the serous component accounted for more than 25% (4–6).

In general, prognosis of early-stage type I endometrial cancer is excellent, with a 10-year overall survival rate exceeding 80% (1). Surprisingly, despite optimal risk-adapted treatment, a small but substantial number of patients exhibit recurrence and poor survival. Obviously in such cases, available risk factors are not able to reliably predict poor clinical course.

There is some evidence showing that immunohistochemical demonstration of the L1 cell adhesion molecule (L1CAM; CD171) in either the majority of tumor cells or even in small areas of tumors classified as endometrioid cancers in routine histology is able to discriminate a subset of highly aggressive tumors with adverse clinical outcome (7,8). L1CAM is a 200 to 220 kDa membrane glycoprotein of the immunoglobulin superfamily and is crucially involved in processes of neurogenesis (9–11). Moreover, L1CAM is expressed in a variety of tumors, where its presence is associated with poor clinical outcome (7,11–15). However, despite some progress in elucidating the signaling of L1CAM, it is presently not clear by what molecular mechanisms L1CAM confers the highly malignant phenotype to cancer cells (16). Because *L1CAM* gene is located on the X chromosome, its overexpression might reflect a partial or complete loss of X chromosome inactivation (17).

In this international, multicenter trial, we aimed to validate the immunohistochemical determination of L1CAM in paraffin-embedded samples as a reliable tool for identifying a subgroup of Fédération Internationale de Gynécologie et d'Obstétrique (FIGO) stage I, type I endometrial cancers exhibiting a highly malignant phenotype.

Methods

Patients

Patients included in this multicenter study had a FIGO stage I endometrial cancer classified as type I endometrioid carcinoma in routine histopathological examination. Histopathological diagnosis was confirmed by central review of the hematoxylin-eosin slides. Patients were staged after definitive histology according to the 2009 FIGO classification (18) and randomly assigned to the three classical risk groups: low risk (myometrial invasion <50%, grade I and II, no lymph space or vascular invasion); intermediate risk (same as low risk but grade III); and high risk (myometrial invasion ≥50%, any grade, lymph space or vascular invasion) (19).

In the 10 centers, surgery and adjuvant radiotherapy were performed in accordance with local treatment policy. In general, surgery was risk-adapted and comprised extrafascial hysterectomy and bilateral salpingo-oophorectomy alone for low-risk and was supplemented with pelvic and para-aortic lymph node dissection for intermediate- and high-risk tumors. Intermediate- and high-risk cancers were generally treated with adjuvant brachytherapy of the vaginal cuff (Table 1).

Written informed consent regarding tissue and data use for scientific purposes was obtained from all participating patients. Data transfer and use for statistical analyses were done in a pseudo-anonymized manner. The retrospective study was approved by the ethics committees of the participating centers.

Immunohistochemical Staining and Evaluation

Immunohistochemical staining was performed as previously described (7,8). Briefly, 3- to 4-μm thick paraffin sections were cut and mounted on Superfrost Plus slides that were exposed in a pressure cooker to EDTA buffer, pH 8.0, for antigen retrieval. An automated immunohistochemistry procedure was performed using the I6000 immunostainer (Biogenics, San Ramos, CA). Endogenous peroxidase activity was blocked by 10 minutes of treatment with 3% hydrogen peroxide in methanol. Primary L1CAM antibody (clone L1-40.10) was obtained after immunization of mice with human L1-Fc protein comprising the ectodomain of L1CAM (20). Slides were incubated with primary antibodies for 45 minutes, and immunoperoxidase staining was accomplished using the Supersensitve Detection Kit with AEC or DAB (Zymed Labs, San Francisco, CA) as substrates, then counterstained with hematoxylin before coverslipping and reading by light microscopy. Omission of the primary antibody was used as a negative control and a highly L1CAM-expressing serous ovarian cancer as a positive control. If 10% or more of the tumor cells showed L1CAM staining, the cancer was rated positive. This threshold was determined by unpruned classification and regression decision tree (CRT), and the classifier was verified using 10-fold cross-validation (Supplementary Figure 1, A and B, available online). The stained sections were examined by two pathologists (M. Huszar and E. Müller-Holzner) who were blinded to patients' clinical outcome (inter-rater reliability: κ-coefficient = 0.933). Disagreement occurred in 21 cancers and was resolved by consensus. Distribution of the L1CAM staining results is given in Supplementary Figure 2 (available online), which also shows that in 14 cancers (1.4%), the percentage of immunostained cells was near the 10% threshold.

Statistical Analysis

The study population was characterized using statistical descriptive analyses overall as well as differentiating between L1CAM-positive and L1CAM-negative patients. Therefore, cross-table statistics were performed and evaluated using Fisher exact test for categorical and the Wilcoxon rank (Mann–Whitney U) test for quantitative factors. Survival and multivariable analyses were performed using the Kaplan–Meier method and Cox proportional hazard model, respectively. Tests for a zero slope of the scaled Schoenfeld residuals over time were used to check the proportional hazards assumption. In cases in which the assumption was violated, stratification was used. "Death" was defined as any death, and "recurrence" was related to an unequivocal clinical diagnosis (histologically confirmed in 83%). Survival distributions were compared by the Mantel–Haenszel approach with the Peto modification of the Gehan–Wilcoxon test (21). Center adjustment was obtained by stratification. To check the proportional hazards assumption, we tested for a zero slope of the scaled Schoenfeld residuals over time and used stratification in cases in which the assumption was violated. All variables revealing prognostic significance in univariate analysis were included in the mulivariable evaluations. In addition, we also used the CRT decision tree as a machine learning approach to predict recurrence and death within the next 5 years using the various parameters available in the study. Here, we selected samples having an event within 5 years as case samples and samples having no event within an observation period of at least 5 years as control samples (used cost factor = 5). All applied methods are described in detail in the Supplementary Methods (available online). The analyses were performed using

Table 1. Clinicopathologic parameters according to L1CAM expression (N = 1021)

Variable, No. (%)	Σ	L1CAM positive*	L1CAM negative†	*P*‡
Age at diagnosis				.22
<64 years	488 (47.8)	79 (16.2)	409 (83.8)	
≥64 years	533 (52.2)	102 (19.1)	431 (80.9)	
FIGO Stage				<.001
FIGO Ia	722 (70.7)	104 (14.4)	618 (85.6)	
FIGO Ib	299 (29.3)	77 (25.8)	222 (74.2)	
Assessed risk§				<.001
Low	657 (64.3)	87 (13.2)	570 (86.8)	
Intermediate	306 (30.0)	72 (23.5)	234 (76.5)	
High	58 (5.7)	22 (37.9)	36 (62.1)	
Grading				<.001
Grade I	530 (51.9)	58 (10.9)	472 (89.1)	
Grade II	366 (35.8)	83 (22.7)	283 (77.3)	
Grade III	125 (12.2)	40 (32.0)	85 (68.0)	
Histology				.003
Pure endometrioid	984 (96.4)	165 (16.8)	819 (83.2)	
Areas (<10%) of other diff.	37 (3.6)	16 (43.2)	21 (56.8)	
Squamous	21 (2.0)	8 (38.1)	13 (61.9)	
Mucinous	4 (0.4)	1 (25.0)	3 (75.0)	
Serous	9 (0.9)	5 (55.6)	4 (44.4)	
Clear Cell	3 (0.3)	2 (66.7)	1 (33.3)	
Myometrial invasion				<.001
None	130 (12.7)	9 (6.9)	121 (93.1)	
<50%	592 (58.0)	95 (16.0)	497 (84.0)	
≥50%	299 (29.3)	77 (25.8)	222 (74.2)	
Lymphadenectomy				.87
No	529 (51.8)	95 (18.0)	434 (82.0)	
Yes	492 (48.2)	86 (17.5)	406 (82.5)	
PCTHǁ				.79
No	996 (97.6)	176 (17.7)	820 (82.3)	
Yes	25 (2.4)	5 (20.0)	20 (80.0)	
Brachytherapy				<.001
No	694 (68.0)	98 (14.1)	596 (85.9)	
Yes	327 (32.0)	83 (25.4)	244 (74.6)	

* L1CAM positive: ≥10%.

† L1CAM negative: <10%.

‡ *P* value of two-sided Fisher exact test.

§ Classical multifactor risk assessment (as defined in "Methods").

ǁ Adjuvant polychemotherapy.

R 2.14 (RDevCoreTeam, Vienna, Austria) and SPSS for Windows 20.0 software (SPSS, Chicago, IL). Statistical significance was defined as *P* less than .05 for all tests. All statistical tests were two-sided. Because 98.2% of the study population was white, we did not perform analysis in the context of racial differences. A recent paper by Elshaikh et al. from Detroit showed that race is not a relevant factor for the clinical outcome in early endometrioid endometrial cancer (22). Of our cohort, 12.1% was Jewish, and with respect to this context, we know that between this small group of patients and the rest of our cohort there were no statistical differences in terms of clinicopathologic characteristics, rate of L1CAM expression or clinical outcome (data not shown).

Results

Of the 1021 investigated FIGO stage I endometrioid endometrial carcinomas, 181 (17.7%) were found to be L1CAM-positive. One hundred thirty-seven cancers (75.7%) exhibited focal staining of cell clusters, and 44 (24.3%) showed diffuse immunostaining in more than 50% of cancer cells. No special staining pattern, such as isolated or particularly intensive L1CAM expression at the myoinvasive front, was revealed. Of the included cancers, 96.4% were purely endometrioid carcinomas, whereas the remaining 3.6% showed areas of nonendometrioid differentiation (all comprising <10% of the tumor). Cancers containing a second cell type were more frequently L1CAM-positive than were purely endometrioid carcinomas ($P = .003$) (Table 1). Stage Ib cancers were more frequently L1CAM-positive than were stage Ia carcinomas ($P < .001$).

Median age of the entire study population at diagnosis was 64 years (range = 34–96 years). L1CAM status was not related to patient age at diagnosis or to the classical epidemiological risk factors for type I endometrial cancer such as diabetes, obesity, nulliparity, hypertension, and unopposed estrogen exposure (Supplementary Table 1, available online).

Classical risk assessment for the entire cohort is listed in Table 1 together with the corresponding L1CAM-positive rates. In

the low-risk group, 13.2% of the cancers were L1CAM-positive, whereas in intermediate- and high-risk cancers the positive rate was 25.8% ($P < .001$). L1CAM positivity was associated with histopathological grade ($P < .001$) and increasing depth of myometrium infiltration ($P < .001$).

During a median follow-up of 5.3 years, 117 patients (11.5%) experienced recurrence. Of these recurrences, 69.2% (n = 81) and 94.9% (n = 111) occurred during the first 2 and 5 years, respectively. With regard to L1CAM status, 51.4% (n = 93) of the L1CAM-positive tumors and 2.9% (n = 24) of the L1CAM-negative tumors recurred. As depicted in Figure 1A, in L1CAM-positive and L1CAM-negative cancers, 69.9% (n = 65) and 66.7% (n = 16) of the observed recurrences, respectively, occurred within 2 years after initial treatment. Time to recurrence, when subdivided into early (≤2 years), intermediate (>2 and ≤5 years) and late (>5 years) relapses, was unrelated to L1CAM status. For the 117 observed recurrences, the crude L1CAM-positive rate was 79.5%, and the crude L1CAM-negative rate was 20.5%. Moreover, regarding distant recurrences the L1CAM-positivity rate was even higher, namely 85.7% (66 of 77 events). In Table 2, observed study events were compared with the estimates generated by the Peto-modified Mantel–Haenzel approach. Whereas for L1CAM-positive cancers this approach estimated that the "observed" frequencies exceed by far the "expected" frequencies of recurrences and deaths, the diametric opposite was assessed for L1CAM-negative tumors ($P < .001$). This was true for the whole study population and for the separated cancer risk classes.

In univariate survival analyses, disease-free and overall survival were poorer in patients with L1CAM-positive cancers than in patients with tumors lacking relevant L1CAM expression ($P < .001$) (Figure 1). Median disease-free and overall survival in patients with L1CAM-positive tumors were 4.5 years and 8.9 years, respectively, whereas median disease-free and overall survival were not reached in L1CAM-negative cancers. Furthermore, it should be stated that disease-free and overall survival were poorer for cancers with diffuse L1CAM immunostaining in more than 50% of the tumor cells than for cancers exhibiting focal (10%–50%) L1CAM expression ($P < .001$) (Supplementary Figure 3, available online).

Furthermore, differences in disease-free and overall survival between L1CAM-positive and -negative cancers were more prominent in tumors classified as intermediate- and high-risk than in low-risk tumors. A similar adverse impact of L1CAM positivity was seen in FIGO stage Ib (Figure 2). Interestingly, in L1CAM-negative cancers, disease-free and overall survival did not differ statistically between FIGO stages Ia and Ib, between the various grades of differentiation, or between the conventional risk classes. As depicted in Figure 3 and Supplementary Figure 4 (available online), only when tumors were L1CAM-positive did the mentioned variables exhibit statistically significant relevance for patient survival (Supplementary Table 2, available online).

In the multivariable Cox model, L1CAM expression retained independent prognostic significance for disease-free as well overall survival ($P < .001$). It is noteworthy that L1CAM-positivity exhibited the most impressive hazard ratios (HRs) for recurrence (HR = 16.33; 95% confidence interval [CI] = 10.55 to 25.28) and death (HR = 15.01; 95% CI = 9.28 to 24.26), as compared with the other prognostic variables included in the multivariable calculations (Table 3). In L1CAM-positive cancers, the hazard ratios were 13.37 (95% CI = 6.71 to 26.67) for predicting locoregional recurrence and 34.07 (95% CI = 17.06 to 68.04) for predicting distant recurrence (Table 4). Removal cancers containing small (<10%) serous or clear-cell components from the calculations did not substantially affect the study outcome (Supplementary Table 3, available online).

Additionally, when the CRT decision tree was used as a classifier, only L1CAM was selected for the final model to predict recurrence and death within 5 years, with a sensitivity of 0.74 and a

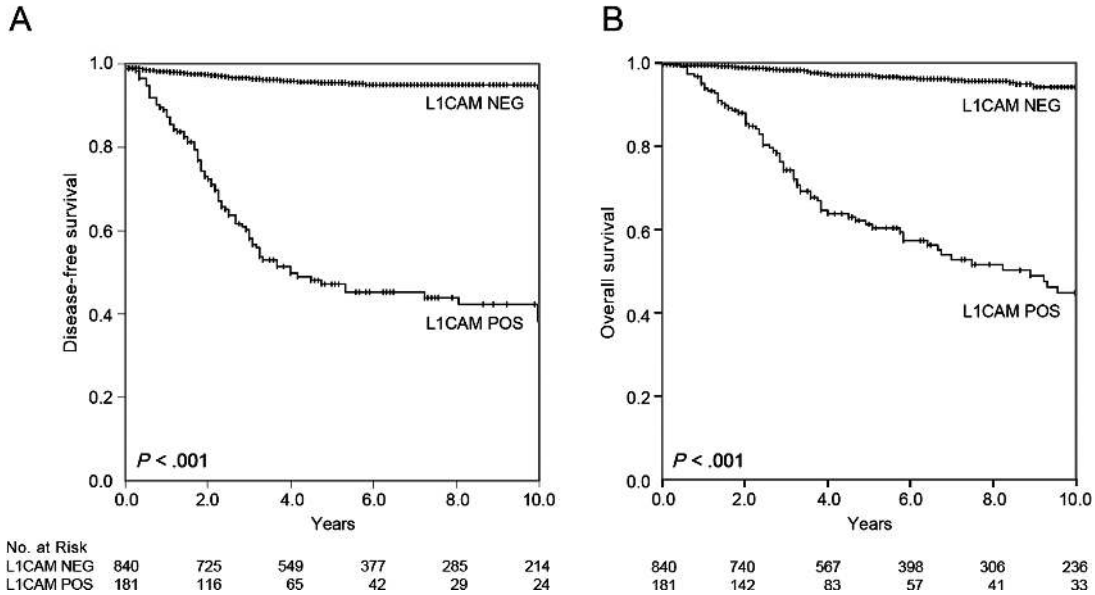

Figure 1. Univariate survival analyses according to L1CAM expression in 1021 patients with Fédération Internationale de Gynécologie et d'Obstétrique (FIGO) stage I, type I endometrial cancers. **A**) Disease-free survival. **B**) Overall survival. Differences in survival between L1CAM positive (pos; ie, immunostaining in ≥10% of the tumor cells) and L1CAM negative (neg; ie, immunostaining in <10% of the tumor cells) groups were assessed by the two-sided log-rank test. The numbers of patients at risk are given below the graphs.

Table. 2. Estimates for clinical outcomes and observed study events with respect to L1CAM status

		Observed	Estimates*			
	No.*	Events	O†	E‡	(O–E)2/E	(O–E)2/V
All cancers (N = 1021)						
Death						
L1CAM negative§	840	27	25.0	77.8	35.7	245
L1CAM positive‖	181	72	67.5	14.8	187.8	
Recurrence total						
L1CAM negative	840	24	29.2	92.4	43.1	306
L1CAM positive	181	93	80.0	16.9	235.6	
Distant recurrence						
L1CAM negative	840	11	16.1	60.8	32.8	217
L1CAM positive	181	66	56.6	11.9	167.3	
Local recurrence						
L1CAM negative	840	15	14.7	36.4	13.0	90.1
L1CAM positive	181	29	28.2	6.5	72.4	
Low-risk cancers (n = 657)						
Death						
L1CAM negative	570	14	13.7	28.7	7.9	63.2
L1CAM positive	87	20	19.4	4.3	52.4	
Recurrence total						
L1CAM negative	570	16	15.5	38.7	13.9	116
L1CAM positive	87	30	28.8	5.6	95.7	
Intermediate- and high-risk cancers (n = 364)						
Death						
L1CAM negative	270	13	10.8	44.7	25.7	137
L1CAM positive	94	52	46.5	12.6	90.7	
Recurrence total						
L1CAM negative	270	15	13.3	49.4	26.4	143
L1CAM positive	94	56	49.6	13.5	96.5	

* Testing the difference of survival curves by using a Mantel–Haenszel approach with the Peto modification of the Gehan–Wilcoxon test (adjusted for the centers by stratification).

† Observed frequencies of clinical outcomes in group (with weights on each outcome of S(t), where S is the Kaplan–Meier estimate of survival).

‡ Expected frequencies of clinical outcomes in group; degree of freedom = 1 in each case.

§ L1CAM positive, ≥10%.

‖ L1CAM negative <10%. For all listed comparisons, *P* value was <.001.

specificity of 0.91 (accuracy = 87.90%) and a sensitivity of 0.77 and a specificity of 0.89 (accuracy = 87.60%), respectively.

Discussion

Risk-adapted treatment achieves excellent prognosis for stage I endometrioid uterine carcinomas with 10-year overall survival exceeding 80% (1). Nonetheless, some patients with that favorable prognostic background unexpectedly experience recurrence and may ultimately die from the disease. This retrospective, multicenter investigation aimed to identify a subset of cancers that are at high risk for recurrence and exhibit poor survival. For this discrimination at the molecular level, immunohistochemical demonstration of L1CAM was used in 1021 paraffin-embedded type I endometrial carcinomas.

The 11.5% recurrence rate reported here for FIGO stage I, type I cancers observed over a median follow-up of 5.3 years, as well as the time to relapse, are in accordance with the reports from other series (23–25). Of the 1021 investigated type I endometrial cancers, 17.7% were rated L1CAM-positive in this study. L1CAM expression in 10% or more of the tumor cells was associated with an overwhelming increase in the likelihood of distal or local recurrence and moreover was independently related to poor overall survival. Although, Blagoev et al. recently emphasized the limited informative power of hazard ratios when considered separately, those ascertained for L1CAM positivity, namely 16.33 and 15.01 for recurrence and death, respectively, are nonetheless extraordinary for a single predictive biomarker (26). By comparison, in endometrial cancer the hazard ratio of 4.5 for DNA-ploidy and 5.02 for positive peritoneal cytology to predict recurrence and death, respectively, are among the best published (27,28). Similarly, for other disorders such as myelodysplastic syndromes, hazard ratios for death ranging from 1.38 to 2.48 were recently highlighted for point mutations in five various genes (29). Furthermore, we found that discrimination between FIGO stages Ia and Ib, between histopathological grades, and between low- and intermediate- and high-risk cancers is irrelevant for survival prediction when tumors lack L1CAM expression. Only in L1CAM-positive cancers do FIGO stage I subdivision, grading, and standard risk classification achieve prognostic significance for disease-free as well as overall survival. Thus, statistically significant differences in survival observed in the entire patient cohort for the mentioned clinicopathological variables appear to have some relationship to L1CAM overexpression. It is of crucial relevance to understand why a minority of early endometrioid uterine cancers

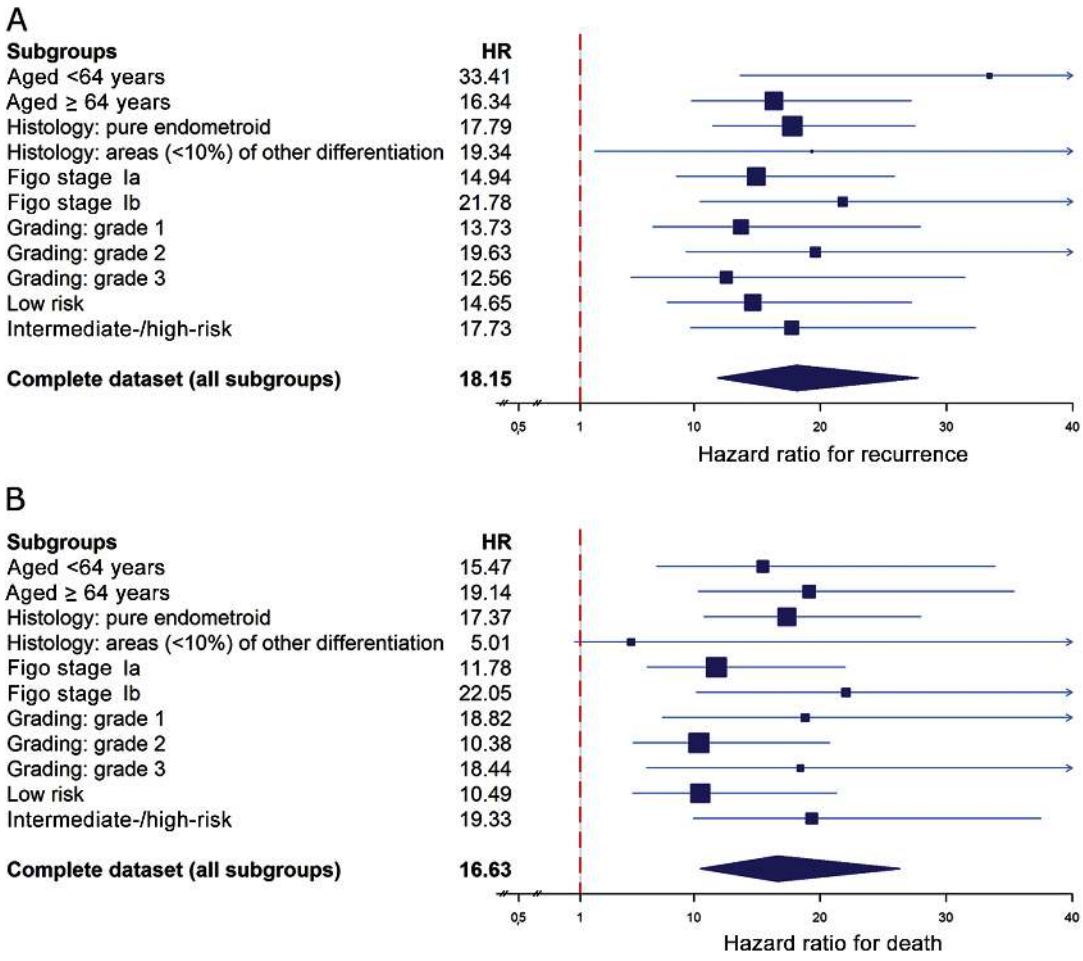

Figure 2. Forest plot based on univariate hazard ratios (HRs) from Cox regression for all subgroups after adjustment for centers. The independent variable is L1CAM status. **A**) Disease-free survival, **B**) Overall survival. **Squares** represent hazard ratios. **Bars** represent 95% confidence intervals. Square size is proportional to weights used in the analysis. **Diamonds** represent overall hazard ratios (**center**) with associated 95% confidence intervals (**lateral tips**). FIGO = Fédération Internationale de Gynécologie et d'Obstétrique.

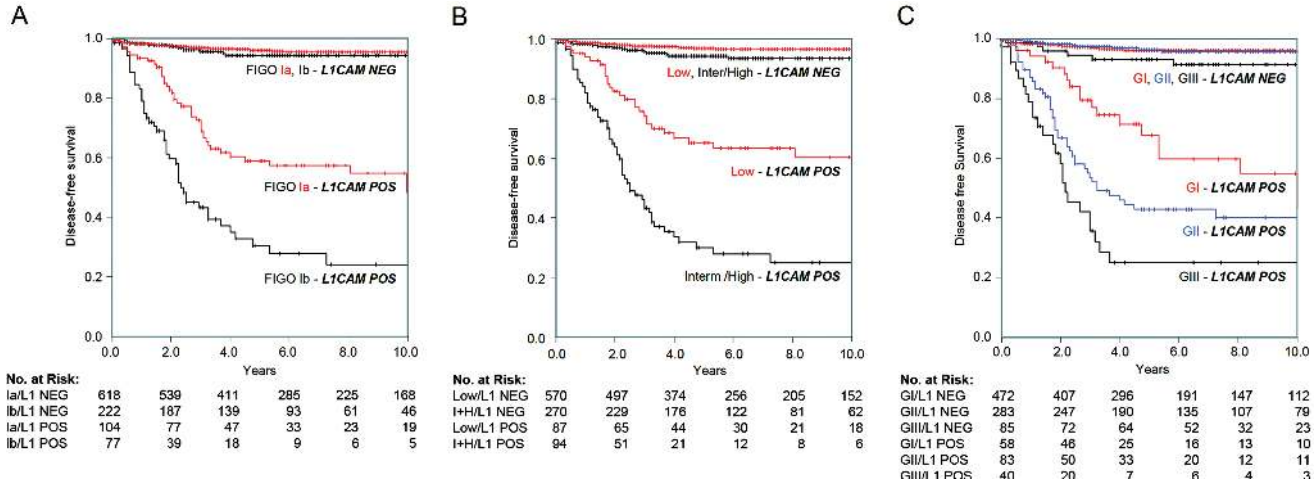

Figure 3. Univariate disease-free survival analyses for Fédération Internationale de Gynécologie et d'Obstétrique (FIGO) stage, risk assessment, and grading according to the L1CAM status. **A**) FIGO stage: Ia (**red**), Ib (**black**). **B**) Risk assessment: low risk (**red**), intermediate (Interm)/high Risk (**black**). **C**) Histopathological grading: grade I (GI) (**red**), grade II (GII) (**blue**), grade III (GIII) (**black**). Similar results were obtained for overall survival (shown in Supplementary Figure 4, A–C, available online). Differences between clinicopathologic groups were assessed by the log-rank test. The numbers of patients at risk are given below the graphs. G = grade; H = high risk; I = intermediate risk; Ia and Ib = FIGO; L1 = L1CAM; stages; Low = low risk.

Table 3. Multivariable survival analysis (Cox regression model) (N = 1021)

	Disease-free survival			Overall survival		
Variables*	**HR†**	**95% confidence interval**	***P***	**HR†**	**95% confidence interval**	***P***
L1CAM						
Negative	1.00			1.00		
Positive	16.33	(10.55 to 25.28)	<.001	15.01	(9.28 to 24.26)	<.001
Histology						
Pure endometrioid	1.00			1.00		
Areas of other differentiation	1.15	(0.57 to 2.32)	.69	0.99	(0.42 to 2.29)	.97
Age at diagnosis						
<64 years	1.00			1.00		
≥64 years	2.03	(1.35 to 3.05)	<.001	1.79	(1.15 to 2.81)	.01
FIGO Stage						
FIGO Ia	1.00			1.00		
FIGO Ib	1.20	(0.57 to 2.51)	.64	1.61	(0.73 to 3.56)	.24
Risk						
Low	1.00			1.00		
Intermediate/high	1.54	(0.64 to 3.67)	.33	1.42	(0.56 to 3.64)	.46
Grading						
Grade I	1.00			1.00		
Grade II	1.12	(0.67 to 1.81)	.64	1.06	(0.61 to 1.83)	.84
Grade III	1.27	(0.63 to 2.59)	.51	1.65	(0.77 to 3.52)	.46

* Variables statistically significant in the univariate evaluations were included into the model. Statistical tests were two-sided.

† HR = hazard ratio. Adjusted for centers by stratification.

Table 4. Multivariable Cox regression model related to the site of recurrence (N = 1021)

	Locoregional recurrence			Distant recurrence		
Variables*	**HR†**	**95% confidence interval**	***P***	**HR†**	**95% confidence interval**	***P***
L1CAM						
Negative	1.00			1.00		
Positive	13.37	(6.71 to 26.67)	<.001	34.07	(17.06 to 68.04)	<.001
Histology						
Pure endometrioid	1.00			1.00		
Areas of other differentiation	0.74	(0.34 to 4.61)	.74	1.22	(0.54 to 2.75)	.27
Age at diagnosis						
<64 years	1.00			1.00		
≥64 years	4.49	(2.09 to 9.67)	<.001	1.42	(0.84 to 2.39)	.19
FIGO Stage						
FIGO Ia	1.00			1.00		
FIGO Ib	1.18	(0.30 to 4.69)	.81	0.81	(0.33 to 2.00)	.64
Risk						
Low	1.00			1.00		
Intermediate/high	0.98	(0.21 to 4.63)	.98	2.67	(0.89 to 7.97)	.08
Grading						
Grade I	1.00			1.00		
Grade II	2.03	(0.91 to 4.52)	.08	0.93	(0.49 to 1.79)	.84
Grade III	1.94	(0.51 to 6.63)	.35	1.08	(0.43 to 2.68)	.88

* Variables statistically significant in the univariate evaluations were included into the model. All statistical tests were two-sided.

† HR = hazard ratio. Adjusted for centers by stratification.

exhibit L1CAM, which appears to confer this highly aggressive phenotype.

There are three lines of interpretation for the occurrence of L1CAM-positive areas in cancers classified as purely endometrioid in routine histology. First, these positive foci may simply represent hidden or missed cell clusters exhibiting serous or clear-cell differentiation, corresponding to a histopathological misdiagnosis. This line of interpretation would fit the idea that a subset of nonendometrioid uterine cancers may evolve from preexisting purely endometrioid carcinomas as a result of dedifferentiation and progression (30–32) and would also go along with our findings that uterine type II carcinomas are more frequently L1CAM-positive than are endometrioid cancers (7,33). Nonetheless, reducing L1CAM positivity to admixtures of clearcell or serous elements missed in routine diagnosis may be an oversimplification, as in cancers containing areas of squamous cell differentiation

L1CAM-positivity was detected particularly in the squamous elements. Recently, even small serous or clear-cell components admixed with endometrioid cancers were found to adversely affect prognosis (34). Therefore in a subanalysis, the cancers with any serous or clear-cell elements were excluded from our calculations. However, probably because of the small number of cancers, the study results were not considerably affected.

The second line of interpretation relates to reports showing that in endometrioid cancers epithelial–mesenchymal transition plays a crucial role during myometrial invasion and is substantiated by histomorphological alterations in terms of "microcystic, elongated and fragmented" (MELF) glands. MELF areas, as compared with conventional glandular tumor areas, are usually hormone receptor negative and exhibit reduced E-cadherin expression (35). This is in accordance with our previous observations that in endometrial cancers L1CAM expression is inversely related to the expression of E-cadherin, estrogen receptors, and progesterone receptors and has therefore been implicated in the epithelial–mesenchymal transition phenomenon (8). Furthermore, epithelial–mesenchymal transition induction by transforming growth factor β1 in endometrial cancer cells led to a drastic upregulation of L1CAM and vimentin together with a downregulation of E-cadherin through a mechanism dependent on the epithelial–mesenchymal transition–transcription factor Slug (8).

The third line of interpretation pertains to the functional background of L1CAM in neurogenesis and its involvement in neurodevelopmental disorders (10,11,36). Without having direct molecular evidence, we cannot completely rule out that L1CAM-expressing cells do not exhibit traits of neuroendocrine differentiation and L1CAM-positive areas may represent morphologically hidden aggressive neuroendocrine elements, worsening clinical outcome (31). This would agree with the very high expression of L1CAM in 85% of the neuroendocrine-differentiated oat cell carcinomas of the lung (37).

Routine immunohistochemical L1CAM determination should be required for all type I endometrial cancers because L1CAM positivity showed superiority over classical risk assessment, histopathological grading, and FIGO stage I subdivision in predicting clinical outcome. It is worth noting that L1CAM-based risk assessment can be obtained from curettage material before major surgery (7). However, the reliability of this procedure, especially for sampling errors, requires validation in prospective approaches.

The most intriguing question is what treatment is most beneficial for patients with L1CAM-positive type I endometrial cancers. Do these patients need more radical surgery or adjuvant radio- and/or chemotherapy? More aggressive surgery, especially in conventionally low-risk patients, does not appear to considerably improve outcome as observed in intermediate- and high-risk patients, who have already had additional lymph node dissection without showing tremendous benefit in the subset of L1CAM-positive tumors. The value of adjuvant radiotherapy also seems questionable, considering the demonstrated superiority of chemotherapy over whole abdominal irradiation as an adjuvant approach for FIGO stage III/IV patients (38) and the fact that L1CAM shows the highest hazard ratio in predicting distant recurrences. Even the efficacy of chemotherapy is uncertain in view of the limited chemosensitivity of endometrial cancers, including type II carcinomas (39). However, Hogberg's recent report showed statistically significant benefit for disease-free survival in high-risk profile cancers when adjuvant chemotherapy is sequentially added to radiotherapy. This tempts us to speculate that chemotherapy could be the most valid option for adjuvant treatment in L1CAM-positive cancers (40). Therefore, it is of paramount importance that the ongoing clinical trials evaluating the value of adjuvant chemotherapy in type I endometrial cancers rated conventionally as "high-risk" urgently introduce L1CAM expression as a main stratification criterion.

As typical for retrospective investigations, this study is limited by potential biases, such as patient selection, incomplete data acquisition, and unstandardized adjuvant treatment of included patients. Furthermore, the semiquantitative centralized immunohistochemical L1CAM evaluation and the uneven intratumoral distribution of L1CAM-positive cells may represent additional limitations. Therefore, a prospective randomized trial will be the highest priority next step in validating the real clinical usefulness of that biomarker.

Another very promising approach could use the transmembrane L1CAM itself as a target for antibody-mediated therapy (41). Xenograft mouse models for ovarian and pancreatic cancer have shown that antitumor action is provided predominantly by immunological mechanisms (42–44). Furthermore, a fully humanized anti-L1CAM antibody has been successfully synthesized and tested (42), and members of our research group are developing this antibody for clinical use.

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