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Prognostic and therapeutic implication of molecular classification including L1CAM expression in high-risk endometrial cancer

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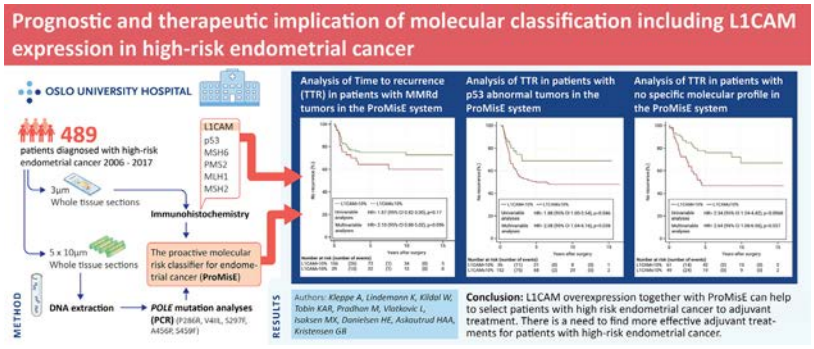
HIGHLIGHTS

- Clearer role of molecular classification and L1CAM in high-risk endometrial cancer.
- ProMisE independently predicted time to recurrence, not cancer-specific survival.
- Patients with *POLE* mutated tumors had an excellent prognosis.
- L1CAM overexpression was a strong, independent marker for recurrence and survival.
- L1CAM overexpression was related to distant recurrences for the p53 and NSMP group.

GRAPHICAL ABSTRACT



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ABSTRACT

Introduction. The role of molecular classification and L1CAM in high-risk endometrial cancer is uncertain. We aimed to determine the association of molecular profiling and L1CAM with patterns of relapse and survival.

Material and methods. This retrospective cohort study included patients referred to Department for Gynecologic Oncology, Oslo University Hospital between January 1, 2006 and December 31, 2017. L1CAM expression and molecular profiling according to ProMisE was performed. Main outcome was time to recurrence (TTR) and cancer specific survival (CSS).

Results. Of 489 patients, 486 could be molecular classified. Thirty-seven (8 %) had *POLE* mutated tumors, 148 (30 %) had MMRd tumors, 189 (39 %) had p53 abnormal tumors, and 112 (23 %) had NSMP tumors. High L1CAM

Abbreviations: L1CAM, L1 cell adhesion molecule; ProMisE, Proactive Molecular Risk Classifier for Endometrial Cancer; EC, endometrial cancer; POLE, DNA polymerase epsilon; MMRd, mismatch repair deficiency; NSMP, no specific molecular profile; TTR, time to recurrence; CSS, cancer specific survival; HR, hazard ratio; CI, confidence interval; LVSI, lymphovascular space invasion; ESMO, European Society for Medical Oncology; FIGO, Federation of Gynecology and Obstetrics; WHO, World Health Organization; OUS, Oslo University Hospital; CT, computer tomography; MR, magnetic resonance imaging; MLH1, MutL homolog 1; MSH2, MutS homolog 2; MSH6, MutS Homolog 6; PMS2, PMS1 Homolog 2; MMRp, mismatch repair proficient; PCR, polymerase chain reaction; REK, Regional Committees for Medical and Health Research Ethics; STROBE, Strengthening the Reporting of Observational Studies in Epidemiology and IQR: inter quartile range.

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Molecular classification
L1CAM expression
Prognosis
Adjuvant therapy
Recurrence location

expression was observed in 256 (53 %), low in 227 (46 %) tumors (6 (1 %) missing). ProMisE was significant for TTR but not for CSS in multivariable analysis. L1CAM was significant in multivariable analysis for both TTR and CSS. In a multivariable model with ProMisE and L1CAM expression in the same multivariable model, ProMisE lost significance while L1CAM remained significant. Patients with *POLE* mutated tumors entailed an excellent prognosis while patients with p53 abnormal or L1CAM overexpressing tumors entailed a poor prognosis with a high frequency of distant recurrences. Patients with MMRd tumors, NSMP and p53 abnormal tumors with low L1CAM had an intermediate prognosis.

Conclusions. L1CAM is an additional adverse factor in the p53 abnormal and NSMP groups. These groups need special attention in studies intensifying adjuvant treatment.

1. Introduction

Endometrial cancer (EC) is the 6th most common female malignancy in the world and the incidence is continuously increasing [1]. The majority of these women present with early-stage disease due to the early onset of symptoms. Based on multiple pathological parameters, patients are further stratified into risk groups designed to reflect their risk of recurrence and tailor adjuvant treatment [2].

The introduction of the Proactive Molecular Risk Classifier for Endometrial Cancer (ProMisE) criteria [3] and the evaluation of lymphovascular space invasion (LVSI) [4] have greatly improved prognostication. These assessments have been implemented in new international guidelines such as the 2022 recommendations from the European Society for Medical Oncology (ESMO) [2] and the 2023 staging from the International Federation of Gynecology and Obstetrics (FIGO) [5]. Due to limited resources, molecular classification is still not widely implemented clinically as it requires the identification of p53 abnormalities, mismatch repair deficiency (MMRd), and DNA polymerase epsilon (*POLE*) mutations.

L1 cell adhesion molecule (L1CAM) expression has also been identified as an important prognostic factor for patients with EC [6–8] and has been found to improve risk assessment beyond ProMisE [9–11]. The role and impact of L1CAM in high-risk EC patients across ProMisE groups remains unclear, although a recent study found L1CAM to be associated with CSS in multivariable analysis of 63 high-risk or advanced EC patients with NSMP tumors [11].

The aim of our study was to investigate survival and localization of recurrence by ProMisE and L1CAM in a large cohort of high-risk EC patients who largely received chemotherapy alone as adjuvant treatment.

2. Material and methods

2.1. Patients

Patients were included from a consecutive series of 1784 patients defined as EC according to the World Health Organization (WHO) 2020 guideline [12], referred to or treated at Department for Gynecologic Oncology, Oslo University Hospital (OUS) 2006–2017 (eFigure 1 in Supplement). The study was approved by the Regional Committees for Medical and Health Research Ethics (REK) in Norway (REK no 2014/701) and the data protection office at OUS. A detailed description of the population is given in Lindemann K and Kildal W et al. [13] Based on the ESMO 2016 guidelines [14], we included a total of 489 high-risk patients consisting of 214 (43.8 %) with endometrioid histology, 273 (55.8 %) with FIGO stage I-III non-endometrioid tumor and 2 (0.4 %) with tumor of unclassifiable histology, In the endometrioid group, 49 (10.0 %) had FIGO stage IB grade 3 regardless of LVSI, 53 (10.9 %) had FIGO stage II, and 112 (22.9 %) had FIGO stage III. All patients underwent surgical treatment without residual tumor. Standard treatment was total hysterectomy, bilateral salpingo-oophorectomy, lymphadenectomy and omentectomy for non-endometrioid tumors. Chemotherapy was recommended as adjuvant treatment. The recommended chemotherapy regimen was carboplatin and paclitaxel during the whole study period. Patients were followed-up regularly. Patterns of recurrence were categorized as either local (vaginal- and central pelvic), extension to the pelvic sidewall including pelvic lymph nodes, paraaortic lymph nodes +/− pelvic lymph nodes, or distant.

For eligible patients a 3 μm section was cut (tissue block), stained with haematoxylin and eosin and examined by a pathologist (MP) to identify blocks with a total tumor area of ≥0.2 cm^2. For patients with mixed cell adenocarcinoma, the block had to contain either a serous or clear cell component.

2.2. Immunohistochemistry

Immunohistochemistry was performed on 3 μm sections. Blinded to clinicopathological- and outcome data, two experienced pathologists scored all sections as described by Köbel M et al. [15] for p53, Zeimet AG et al. [6] for L1CAM, and MLH1, MSH2, MSH6, PMS2 were considered retained if there was normal nuclear protein expression or lost if there was loss of expression [9]. Loss of at least one of the four proteins was considered MMRd, while patients with normal expression were considered mismatch repair proficient (MMRp). For details see eMethods in Supplement.

2.3. POLE mutation analysis

Genomic DNA was extracted and allele-specific polymerase chain reaction (PCR) for the five most common pathogenic *POLE* mutations (P286R, V411L, S297F, A456P, and S459F) accounting for approximately 95 % of pathogenic variants in the *POLE* gene in EC [16,17] was performed by Taqman® Genotyping Assays (Thermo Fisher Scientific). For details see eTable 1 and 2 and eFigure 2 in Supplement.

2.4. Statistical analyses

This study conformed to the Strengthening the Reporting of Observational Studies in Epidemiology (STROBE) guideline (eTable 2 in Supplement). Continuous variables were described with median and interquartile range. Categorical variables were presented with counts and proportions. Differences between categorical variables were assessed by Pearson's χ^2 test, while the Kruskal-Wallis H test was used to assess differences between a categorical and a continuous variable. Univariable survival analyses were performed using the Mantel-Cox log-rank test and Cox regression analysis. Endpoints were cancer-specific survival (CSS) and time to recurrence (TTR), defined as proposed by Punt et al [18]. For TTR, follow-up time was calculated from the date of EC surgery until the date of recurrence, date of death from any cause, or end of follow-up (28th of December 2022). For CSS, follow-up time was calculated from the date of EC surgery until the date of death from EC or end of follow-up. Survival curves were plotted with the Kaplan-Meier method. The multivariable model included the established prognostic variables: Age, FIGO 2009 surgical stage according to the 2009 revision by FIGO [19], histological type with grade

for endometrioid adenocarcinomas, adjuvant treatment, pelvic staging lymphadenectomy, as well as L1CAM expression. Only patients with assessable molecular classification were included in the analyses. A two-sided $P < 0.05$ was considered statistically significant. The analyses were performed using Stata/SE 18.0 (StataCorp, TX).

3. Results

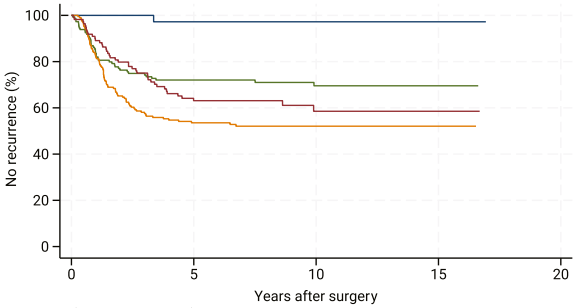
Of the 489 included patients, 279 (57 %) had FIGO stage I or II and 210 (43 %) had FIGO stage III disease (Table 1). Adjuvant treatment was given as platinum-based chemotherapy alone to 357 (73 %)

Table 1
Baseline characteristics of the study cohort and their association with recurrence.

Characteristic	All	No recurrence	Recurred	p*
Patients	489	317	172	
Age at surgery, years	69 (63–77)	69 (61–76)	72 (64–79)	0.0007
Age at surgery				<0.0001
<60 years	84 (17 %)	71 (22 %)	13 (8 %)	
≥60 years	405 (83 %)	246 (78 %)	159 (92 %)	
Surgical stage (FIGO 2009)				<0.0001
IA	103 (21 %)	85 (27 %)	18 (10 %)	
IB	97 (20 %)	70 (22 %)	27 (16 %)	
II	79 (16 %)	47 (15 %)	32 (19 %)	
IIIA	29 (6 %)	19 (6 %)	10 (6 %)	
IIIB	11 (2 %)	7 (2 %)	4 (2 %)	
IIIC1	102 (21 %)	54 (17 %)	48 (28 %)	
IIIC2	68 (14 %)	35 (11 %)	33 (19 %)	
Histological type and grade				0.018
Endometrioid carcinoma G1	67 (14 %)	47 (15 %)	20 (12 %)	
Endometrioid carcinoma G2	56 (11 %)	32 (10 %)	24 (14 %)	
Endometrioid carcinoma G3	91 (19 %)	67 (21 %)	24 (14 %)	
Serous carcinoma	112 (23 %)	63 (20 %)	49 (28 %)	
Clear cell carcinoma	28 (6 %)	22 (7 %)	6 (3 %)	
Mixed with clear cell/serous	58 (12 %)	43 (14 %)	15 (9 %)	
Carcinosarcoma	63 (13 %)	33 (10 %)	30 (17 %)	
Undifferentiated carcinoma	12 (2 %)	9 (3 %)	3 (2 %)	
Unclassifiable carcinoma	2 (0 %)	1 (0 %)	1 (1 %)	
Pelvic lymphadenectomy				0.31
No	69 (14 %)	41 (13 %)	28 (16 %)	
Yes	420 (86 %)	276 (87 %)	144 (84 %)	
Omentectomy				0.95
No	254 (52 %)	165 (52 %)	89 (52 %)	
Yes	235 (48 %)	152 (48 %)	83 (48 %)	
Lymphovascular space invasion				0.0002
No	258 (53 %)	187 (59 %)	71 (41 %)	
Yes	231 (47 %)	130 (41 %)	101 (59 %)	
Adjuvant treatment				0.029
No adjuvant treatment	114 (23 %)	64 (20 %)	50 (29 %)	
Chemotherapy	357 (73 %)	244 (77 %)	113 (66 %)	
External beam radiotherapy	15 (3 %)	8 (3 %)	7 (4 %)	
Chemotherapy and external beam radiotherapy	2 (0 %)	0	2 (1 %)	
Chemotherapy and brachytherapy	1 (0 %)	1 (0 %)	0	
POLE mutated				<0.0001
No	450 (92 %)	279 (88 %)	171 (99 %)	
Yes	37 (8 %)	36 (11 %)	1 (1 %)	
Missing	2 (0 %)	2 (1 %)	0	
Mismatch repair deficient				0.012
No	331 (68 %)	202 (64 %)	129 (75 %)	
Yes	157 (32 %)	114 (36 %)	43 (25 %)	
Missing	1 (0 %)	1 (0 %)	0	
p53 protein expression				0.0027
p53 wild type	261 (53 %)	185 (58 %)	76 (44 %)	
p53 abnormal	228 (47 %)	132 (42 %)	96 (56 %)	
ProMisE				<0.0001
No specific molecular profile	112 (23 %)	71 (22 %)	41 (24 %)	
p53 abnormal	189 (39 %)	101 (32 %)	88 (51 %)	
Mismatch repair deficient	148 (30 %)	106 (33 %)	42 (24 %)	
POLE mutated	37 (8 %)	36 (11 %)	1 (1 %)	
Missing	3 (1 %)	3 (1 %)	0	
L1CAM				<0.0001
<10 %	227 (46 %)	173 (55 %)	54 (31 %)	
≥10 %	256 (52 %)	140 (44 %)	116 (67 %)	
Missing	6 (1 %)	4 (1 %)	2 (1 %)	
Follow-up time, years	6.6 (3.6–10.5)	8.5 (6.1–12.4)	3.3 (1.9–5.9)	<0.0001
Follow-up time of alive patients, years	9.1 (6.6–12.7)	9.1 (6.6–12.6)	9.3 (6.5–13.9)	0.64

Data are median (IQR) or n (%). *Pearson's χ^2 (categorical variables) or Mann-Whitney U (continuous variables) test evaluated using only non-missing values.
Abbreviations: IQR, interquartile range; L1CAM, L1 cell adhesion molecule; NA, not available; *POLE*, DNA polymerase epsilon; ProMisE, Proactive Molecular Risk Classifier for Endometrial Cancer.

A Analysis of TTR by ProMisE

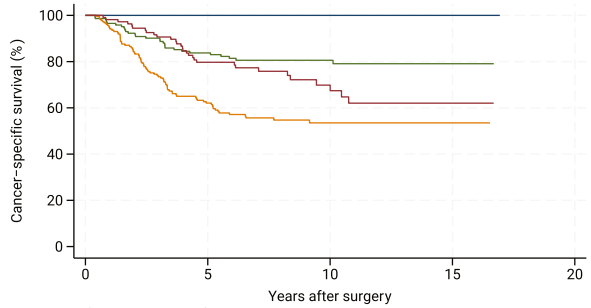


Number at risk (number of events)

MMRd	148	(40)	97	(2)	48	(0)	6	(0)	0
*POLE*mut	37	(1)	34	(0)	19	(0)	5	(0)	0
p53abn	189	(86)	89	(2)	37	(0)	3	(0)	0
NSMP	112	(39)	62	(2)	23	(0)	5	(0)	0

	Univariable analyses			Multivariable analyses		
Variable	HR	95% CI	p-value	HR	95% CI	p-value
MMRd	Ref		<0.0001	Ref		0.027
*POLE*mut	0.07	(0.01-0.54)		0.10	(0.01-0.74)	
p53abn	1.79	(1.24-2.59)		1.50	(0.96-2.36)	
NSMP	1.27	(0.83-1.96)		1.14	(0.73-1.78)	

B Analysis of CSS by ProMisE

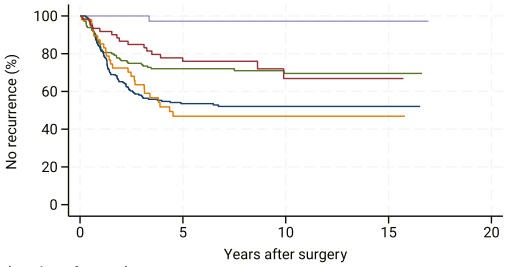


Number at risk (number of events)

MMRd	148	(23)	114	(4)	54	(1)	8	(0)	0
*POLE*mut	37	(0)	35	(0)	20	(0)	5	(0)	0
p53abn	189	(69)	104	(12)	37	(0)	3	(0)	0
NSMP	112	(21)	79	(6)	29	(3)	5	(0)	0

	Univariable analyses			Mulitvariable analyses		
Variable	HR	95% CI	p	HR	95% CI	p
MMRd	Ref		<0.0001	Ref		0.074
POLEmut	0	(0.00-∞)		0	(0.00-∞)	
p53abn	2.75	(1.79-4.23)		2	(1.19-3.38)	
NSMP	1.47	(0.88-2.45)		1.37	(0.80-2.32)	

C Analysis of TTR by ProMisE and L1CAM, with L1CAM added after p53 in ProMisE

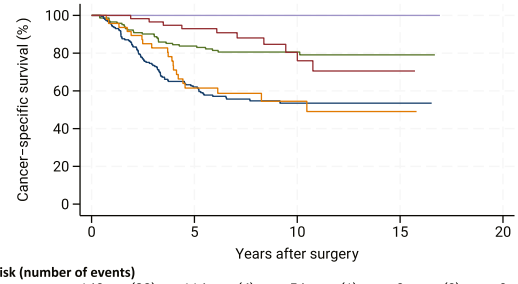


Number at risk (number of events)

MMRd	148	(40)	97	(2)	48	(0)	6	(0)	0
*POLE*mut	37	(1)	34	(0)	19	(0)	5	(0)	0
p53abn	189	(86)	89	(2)	37	(0)	3	(0)	0
NSMP & L1CAM≥10%	49	(24)	19	(0)	9	(0)	2	(0)	0
NSMP & L1CAM<10%	61	(14)	42	(2)	13	(0)	2	(0)	0

	Univariable analyses			Multivariable analyses		
Variable	HR	95% CI	p-value	HR	95% CI	p-value
MMRd	Ref		<0.0001	Ref		0.0084
*POLE*mut	0.07	(0.01-0.54)		0.1	(0.01-0.72)	
p53abn	1.79	(1.24-2.59)		1.64	(1.04-2.58)	
NSMP & L1CAM≥10%	1.88	(1.14-3.10)		1.68	(0.97-2.91)	
NSMP & L1CAM<10%	0.85	(0.48-1.51)		0.77	(0.42-1.42)	

D Analysis of CSS by ProMisE and L1CAM, with L1CAM added after p53 in ProMisE

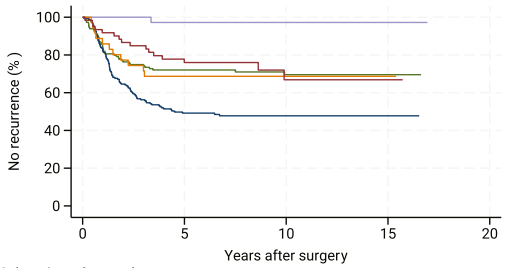


Number at risk (number of events)

MMRd	148	(23)	114	(4)	54	(1)	8	(0)	0
*POLE*mut	37	(0)	35	(0)	20	(0)	5	(0)	0
p53abn	189	(69)	104	(12)	37	(0)	3	(0)	0
NSMP & L1CAM≥10%	49	(17)	25	(2)	10	(1)	2	(0)	0
NSMP & L1CAM<10%	61	(4)	52	(4)	18	(2)	2	(0)	0

	Univariable analyses			Multivariable analyses		
Variable	HR	95% CI	p-value	HR	95% CI	p-value
MMRd	Ref		<0.0001	Ref		0.015
*POLE*mut	0	(0.00-∞)		0	(0.00-∞)	
p53abn	2.76	(1.79-4.24)		2.23	(1.32-3.77)	
NSMP & L1CAM≥10%	2.53	(1.43-4.50)		2.19	(1.18-4.05)	
NSMP & L1CAM<10%	0.83	(0.40-1.70)		0.83	(0.39-1.75)	

E Analysis of TTR by ProMisE and L1CAM, with L1CAM added before p53 in ProMisE

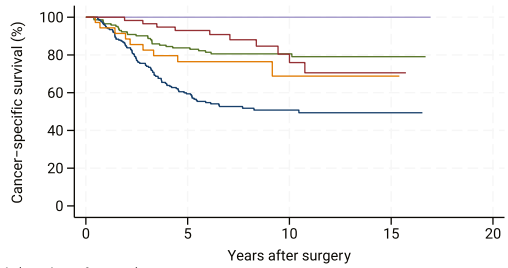


Number at risk (number of events)

MMRd	148	(40)	97	(2)	48	(0)	6	(0)	0
*POLE*mut	37	(1)	34	(0)	19	(0)	5	(0)	0
L1CAM≥10%	201	(99)	87	(2)	38	(0)	4	(0)	0
p53abn & L1CAM<10%	36	(11)	21	(0)	8	(0)	1	(0)	0
NSMP & L1CAM<10%	61	(14)	42	(2)	13	(0)	2	(0)	0

	Univariable analyses			Multivariable analyses		
Variable	HR	95% CI	p-value	HR	95% CI	p-value
MMRd	Ref		<0.0001	Ref		0.0006
*POLE*mut	0.07	(0.01-0.54)		0.1	(0.01-0.72)	
L1CAM≥10%	1.97	(1.37-2.82)		1.9	(1.22-2.97)	
p53abn & L1CAM<10%	1.07	(0.55-2.07)		0.89	(0.44-1.82)	
NSMP & L1CAM<10%	0.85	(0.48-1.51)		0.77	(0.42-1.42)	

F Analysis of CSS by ProMisE and L1CAM, with L1CAM added before p53 in ProMisE



Number at risk (number of events)

MMRd	148	(23)	114	(4)	54	(1)	8	(0)	0
*POLE*mut	37	(0)	35	(0)	20	(0)	5	(0)	0
L1CAM≥10%	201	(78)	106	(13)	39	(1)	4	(0)	0
p53abn & L1CAM<10%	36	(8)	23	(1)	8	(0)	1	(0)	0
NSMP & L1CAM<10%	61	(4)	52	(4)	18	(2)	2	(0)	0

	Univariable analyses			Multivariable analyses		
Variable	HR	95% CI	p-value	HR	95% CI	p-value
MMRd	Ref		<0.0001	Ref		0.0005
*POLE*mut	0.0	(0.00-∞)		0.0	(0.00-∞)	
L1CAM≥10%	2.97	(1.94-4.53)		2.63	(1.58-4.39)	
p53abn & L1CAM<10%	1.46	(0.69-3.10)		1.06	(0.47-2.35)	
NSMP & L1CAM<10%	0.83	(0.40-1.70)		0.84	(0.40-1.79)	

Fig. 1. Kaplan-Meier plots with univariable and multivariable analysis of molecular classifications.
Abbreviations: CI, confidence interval; CSS, cancer-specific survival; HR, hazard ratio; L1CAM, L1 cell adhesion molecule; MMRd, mismatch repair deficient, NSMP, no specific molecular profile; *POLE*, DNA polymerase epsilon; ProMisE, Proactive Molecular Risk Classifier for Endometrial Cancer; TTR, time to recurrence.

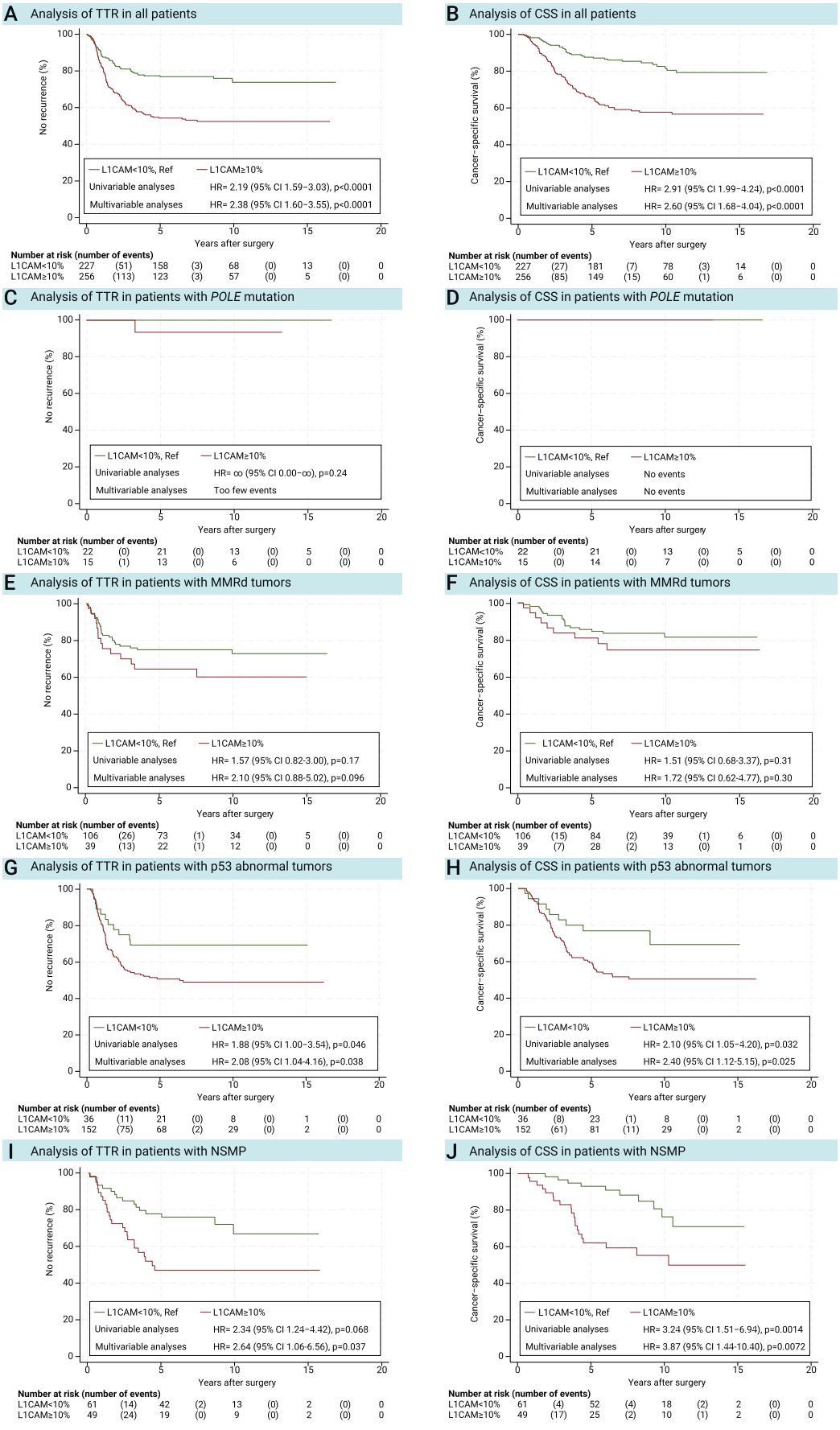


Fig. 2. Kaplan-Meier plots with univariable and multivariable analysis of L1CAM expression in ProMisE subgroups.
Abbreviations: CI, confidence interval; CSS, cancer-specific survival; HR, hazard ratio; L1CAM, L1 cell adhesion molecule; MMRd, mismatch repair deficient, NSMP, no specific molecular profile; *POLE*, DNA polymerase epsilon; ProMisE, Proactive Molecular Risk Classifier for Endometrial Cancer; TTR, time to recurrence.

Table 2
Multivariable analysis with ProMisE, L1CAM expression, and established prognostic variables.

Variable	Analysis of TTR		Analysis of CSS	
	HR (95 % CI)	p	HR (95 % CI)	p
ProMisE		0.077		0.25
No specific molecular profile	ref.		ref.	
p53 abnormal	1.19 (0.77–1.85)		1.28 (0.79–2.08)	
Mismatch repair deficient	1.03 (0.65–1.62)		0.81 (0.47–1.40)	
POLE mutated	0.09 (0.01–0.65)		0.00 (0.00-∞)	
L1CAM		<0.0001		0.0001
≥10 % vs. <10 %	2.38 (1.56–3.61)		2.43 (1.54–3.85)	
Age at surgery		0.013		0.016
≥60 years vs. <60 years	2.13 (1.18–3.87)		2.52 (1.19–5.33)	
Surgical stage (FIGO 2009)		0.0002		0.0014
I	ref.		ref.	
II	1.92 (1.15–3.20)		1.39 (0.76–2.55)	
III	2.52 (1.63–3.90)		2.36 (1.46–3.80)	
Histological type and grade		0.040		0.021
Endometrioid carcinoma G1	ref.		ref.	
Endometrioid carcinoma G2	1.38 (0.72–2.64)		1.51 (0.69–3.30)	
Endometrioid carcinoma G3	1.33 (0.69–2.56)		1.70 (0.79–3.68)	
Serous carcinoma	1.07 (0.55–2.11)		1.45 (0.67–3.17)	
Clear cell carcinoma	0.61 (0.23–1.66)		0.96 (0.33–2.80)	
Mixed with clear cell/serous	0.77 (0.35–1.67)		1.03 (0.42–2.54)	
Carcinosarcoma	2.09 (1.03–4.22)		3.10 (1.37–7.02)	
Undifferentiated carcinoma	1.09 (0.31–3.85)		0.90 (0.20–4.14)	
Unclassifiable carcinoma	2.52 (0.31–20.56)		5.30 (0.62–45.64)	
Pelvic lymphadenectomy		0.24		0.026
Yes vs. No	0.76 (0.48–1.20)		0.56 (0.34–0.93)	
Lymphovascular space invasion		0.012		0.0019
Yes vs. No	1.56 (1.10–2.22)		1.89 (1.27–2.83)	
Adjuvant treatment		<0.0001		0.016
No adjuvant treatment	ref.		ref.	
Chemotherapy	0.36 (0.24–0.53)		0.49 (0.31–0.76)	
External beam radiotherapy	0.71 (0.31–1.64)		0.75 (0.28–2.01)	
Chemotherapy and external beam radiotherapy	1.26 (0.28–5.72)		0.81 (0.10–6.47)	
Chemotherapy and brachytherapy	0.00 (0.00-∞)		0.00 (0.00-∞)	

Abbreviations: CI, confidence interval; CSS, cancer-specific survival; FIGO, International Federation of Gynecology and Obstetrics; HR, hazard ratio; L1CAM, L1 cell adhesion molecule; *POLE*, DNA polymerase epsilon; ProMisE, Proactive Molecular Risk Classifier for Endometrial Cancer; TTR, time to recurrence. 480 had complete data and were included in the multivariable analyses. In TTR analysis, 170 had events, and in CSS analysis, 138 had events.

patients and combined with radiotherapy in 3 (1 %) patients, while 15 (3 %) received external irradiation alone and 114 (23 %) did not receive any adjuvant treatment. Pelvic and para-aortic lymph node staging was performed in 420 (86 %) and in 291 (60 %) patients, respectively. Median follow-up time was 6.6 years (IQR 3.6–10.5).

Classification into ProMisE as recommended by ESMO Clinical Practice Guideline [2], was possible in 486 patients and we identified 37 tumors (8 %) as *POLE* mutated, 148 (30 %) as MMRd, 189 (39 %) as p53 abnormal and 112 (23 %) with no specific molecular profile (NSMP) (eFigure 3). The remaining 3 (1 %) was not evaluable. L1CAM overexpression was observed in 256 (53 %) tumors, low L1CAM expression in 227 (46 %) tumors, and L1CAM expression was not evaluable in 6 (1 %) tumors.

ProMisE was significant for TTR in univariable analysis ($P < 0.0001$) and in multivariable analysis ($P = 0.027$) (Fig. 1A and eTable 3 in Supplement) and for CSS in univariable analysis ($P < 0.0001$) but not in multivariable analysis ($P = 0.074$) (Fig. 1B and eTable 3 in Supplement). Patients with *POLE* mutated tumors had favorable outcome; only 1 out of 37 patients had a recurrence but was still alive 12 years after primary surgery. Patients with p53 abnormal tumors fared worse in univariable and multivariable analysis (Fig. 1A-B). FIGO stage, histological type and grade, age, LVSI, and pelvic lymphadenectomy were statistically significant in multivariable analysis of TTR and CSS (eTable 3 in Supplement). Adjuvant treatment was statistically significantly associated with improved TTR and CSS in both multivariable analyses (eTable 3 in Supplement).

L1CAM expression associated with shorter TTR and CSS in both univariable and multivariable analyses ($P < 0.0001$; Fig. 2A-B, eTable 4 in Supplement). Patients with L1CAM overexpressing tumors tended towards having a worse prognosis independent of ProMisE subgroup, being statistically significant in the p53 abnormal group and the NSMP group of ProMisE (Fig. 2 C-J). Including ProMisE and L1CAM expression in the same multivariable model together with the established prognostic variables resulted in ProMisE loosing statistically significance and L1CAM expression remaining statistically significance (Table 2).

NSMP patients with L1CAM overexpression had similarly poor prognosis as patients with p53 abnormal tumors, while NSMP patients with low L1CAM had similar prognosis as MMRd patients in univariable and multivariable analysis of TTR and CSS (Fig. 1C-D). We explored a different algorithm for molecular classification, by switching the order of p53 and L1CAM classification. Patients were classified based on *POLE* mutation status first, then MMR status, followed by L1CAM expression, and finally p53 abnormality. Univariable and multivariable analysis of TTR and CSS for this alternative molecular classification confirmed that patients with p53 abnormality and low L1CAM expression, performed similarly to patients with MMRp, and no *POLE* mutation (Fig. 1E-F).

Most patients in stage III received chemotherapy according to institutional guidelines. Specifically, 173 (82 %) of 210 stage III patients received adjuvant chemotherapy and only 2 (1 %) received adjuvant chemoradiation. Out of the 279 patients with FIGO stage I or II disease, 85 (30 %) did not receive adjuvant treatment, 184 received adjuvant chemotherapy (66 %), and the remaining 10 (4 %) received adjuvant radiation or chemoradiation. Stratified by ProMisE groups, adjuvant chemotherapy increased TTR and CSS in both univariable and multivariable analysis for patients with MMRd and with p53 abnormal tumors but not in the *POLE* and the NSMP group (Fig. 3 and eFigure 4 in Supplement).

There was a significant positive correlation between p53 abnormality and L1CAM overexpression ($P < 0.0001$) in the 298 tumors without *POLE* mutation and MMRd (Table 3). The effect of chemotherapy was independent of L1CAM expression as the HR of adjuvant chemotherapy

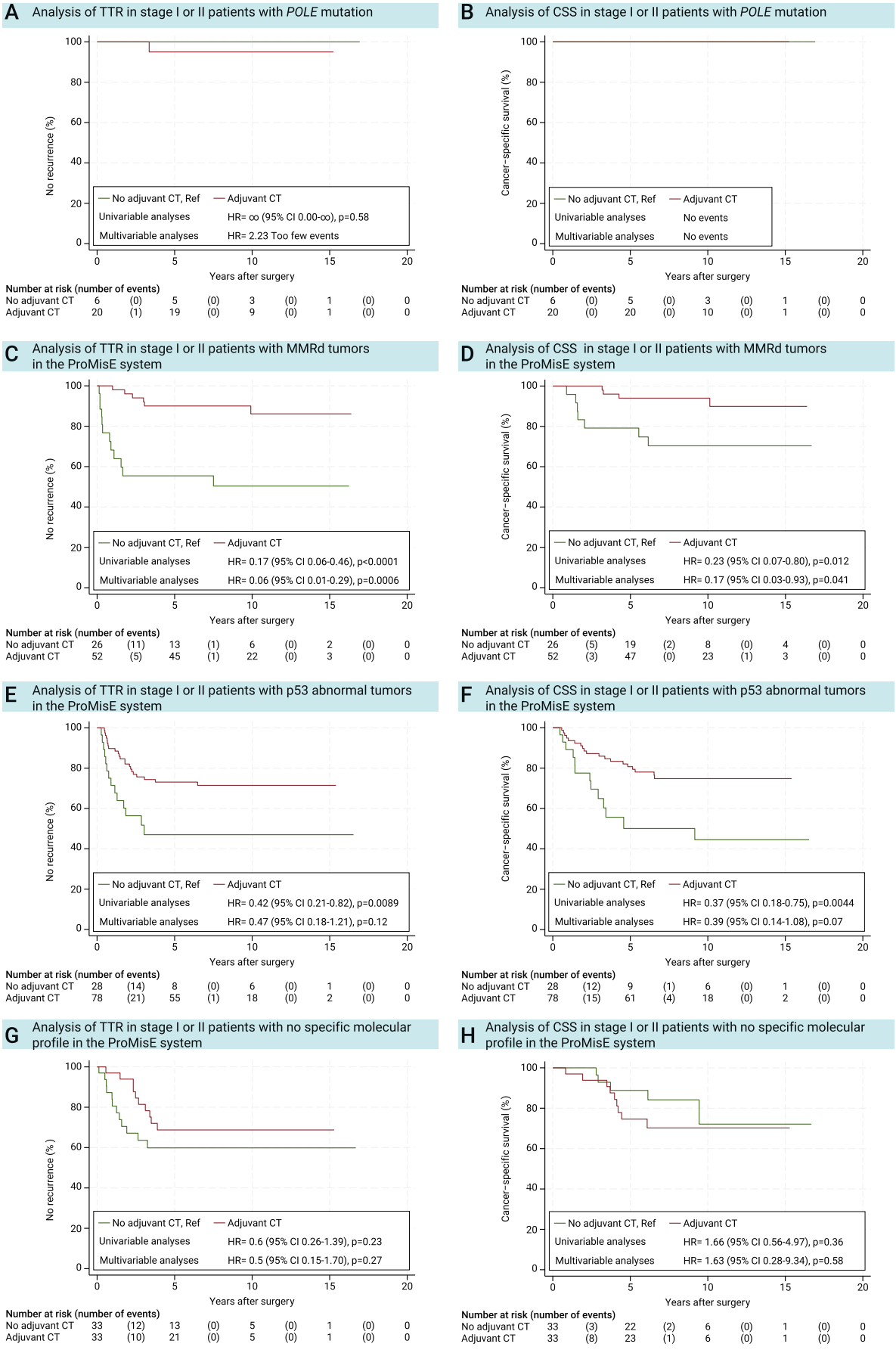


Fig. 3. Kaplan-Meier plots with univariable and multivariable analysis of adjuvant chemotherapy in ProMisE subgroups for patients in stage I and II.
Abbreviations: CI, confidence interval; CSS, cancer-specific survival; CT, chemotherapy; HR, hazard ratio; MMRd, mismatch repair deficient; *POLE*, DNA polymerase epsilon; ProMisE, Proactive Molecular Risk Classifier for Endometrial Cancer and TTR, time to recurrence.

Table 3
Frequency and localization of recurrence by ProMisE with separation of the p53 abnormal and no specific molecular profile group by L1CAM expression irrespective of adjuvant treatment.

	Local	Extension to pelvic side wall including pelvic lymph nodes	Distant including para-aortic lymph nodes	No recurrence	Total
POLE mutated	0	0	1 (3 %)	36 (97 %)	37
Mismatch repair deficient	15 (10 %)	4 (3 %)	23 (16 %)	106 (72 %)	148
p53 abnormal and L1CAM ≥10 %	12 (8 %)	5 (3 %)	60 (40 %)	75 (49 %)	152
p53 abnormal and L1CAM <10 %	2 (6 %)	1 (3 %)	8 (22 %)	25 (69 %)	36
NSMP and L1CAM ≥10 %	8 (16 %)	1 (2 %)	15 (31 %)	25 (51 %)	49
NSMP and L1CAM <10 %	6 (10 %)	0	10 (16 %)	45 (74 %)	61
Missing data	1	0	0	5 (100 %)	6
Total	44 (9 %)	11 (2 %)	118 (24 %)	317 (65 %)	489

Abbreviations: L1CAM, L1 cell adhesion molecule; NSMP, no specific molecular profile; *POLE*, DNA polymerase epsilon; ProMisE, Proactive Molecular Risk Classifier for Endometrial Cancer.

was similar for patients with high and low L1CAM expression (eFigure 5 and eFigure 6).

The localization and frequency of the 172 recurrences by ProMisE and L1CAM expression for the NSMP group are shown in Table 3. The frequency of distant relapse was numerically higher, although not statistically significant, in patients with L1CAM overexpressing tumors explaining a similarly poor prognosis as patients with p53 abnormal tumors.

Considering only patients who received chemotherapy and no radiation as adjuvant treatment, 113 (32 %) out of 357 patients had a recurrence (eTable 5 in Supplement). The proportion of locoregional recurrence was low in all groups. The frequency of distant recurrences was high in the p53 abnormal group, and in the NSMP group with L1CAM overexpression. For patients in the MMRd and the NSMP group in stage I and II who did not receive adjuvant treatment, the proportion of distant recurrences was 10 % (5/52) while the proportion of vaginal recurrences was 29 % (15/52),

4. Discussion

In this large cohort study of high-risk patients, L1CAM expression was a strong prognostic factor together with surgical stage, LVSI, age at surgery, histological type and grade, adjuvant treatment, and pelvic lymph node staging. Molecular classification according to ProMisE was significant in univariable analysis of TTR and CSS, as well as in multivariable analysis of TTR but only when L1CAM was not in the multivariable model. In patients with MMRp tumors, excluding those with *POLE* mutation, a strong positive correlation between p53 abnormality and L1CAM overexpression was observed, consistent with previous studies [8–11,20]. In our study, L1CAM had independent prognostic significance, both in the p53 abnormal and in the NSMP group. Patients with p53 abnormal and overexpressed L1CAM had a frequency of distant metastasis of 40 % compared to 22 % when the tumor had low L1CAM. In the NSMP group, the frequency of L1CAM overexpression was lower but still related to a high frequency of distant relapse. The relationship between L1CAM overexpression and metastasis to pelvic lymph nodes and distant sites is well-known [6,7,9,21,22]. The frequency of relapse on the pelvic sidewall is low in our series, probably due to the high proportion of patient who underwent lymph node staging. Patients with distant metastasis have a very poor prognosis, while patients with vaginal/central pelvic relapse can often be salvaged with radiation [23,24], in particular in patients with an absence of high risk features at primary diagnosis. Due to the metastatic potential, L1CAM expression was a dominating indicator of poor TTR and CSS.

While *POLE* mutation status and L1CAM expression were the molecular features informing the prognosis of patients in this high-risk cohort, adjuvant chemotherapy was observed to benefit patients with MMRd and p53 abnormal tumors. In the randomized PORTEC3 study, chemotherapy added to external beam radiotherapy (EBRT) increased 5-year relapse-free survival in the p53 abnormal group but not in the MMRd and NSMP groups [25]. The high frequency of distant recurrence in some patient groups despite adjuvant chemotherapy indicates a need to improve systemic adjuvant treatment for these patients. Tumors with *TP53* mutations have a high frequency of homolog repair deficiencies [26] indicating a possibility for effect with adjuvant treatment with PARP inhibitors. Several recent phase 3 studies have shown effect of immunotherapy in patients with EC, mostly in MMRd tumors [27–29]. Two of those studies indicated a potential additive benefit of PARP inhibitor given as maintenance therapy in combination with immunotherapy [26,28], particularly for p53 abnormal tumors. Both options are currently being investigated as adjuvant treatment in the RAINBO study [30]. In patients with p53 wild-type tumors maintenance therapy with Selinexor increased progression free survival [31].

In the Nordic-EORTC-Mango randomized study [32], traditional adjuvant treatment with EBRT was compared to EBRT plus adjuvant chemotherapy and found to be inferior in analysis of CSS (HR = 0.55, 95 % confidence interval (CI) 0.35–0.88; $P = 0.01$). In PORTEC3, a positive effect on relapse-free survival was observed for patients treated with chemoradiation compared to EBRT, with the greatest effect for patients in stage III [33]. In our study, almost all patients with stage III EC received chemotherapy. We found the risk of locoregional relapse to be below 10 % in all ProMisE groups considering all stages, supporting the strategy in the Nordic countries to omit adjuvant radiotherapy and rather treat these patients at the time of pelvic relapse [23,24]. The distribution of patients to ProMisE groups in our study is comparable to other reports [3,25,34]. Our study confirms earlier reports showing excellent prognosis for patients with *POLE* mutated tumors irrespective of histomorphologic high-risk factors [10,25]. De-escalation of adjuvant treatment for *POLE*-mutated tumors is currently being investigated [29].

This study has limitations owing to its retrospective design. We have used multivariable analysis throughout to reduce bias as much as possible. Further, the original diagnosis was not subject to a second pathology review but all diagnoses were made by expert gynecological pathologists at the time of primary diagnosis, making the study applicable to daily clinical practice.

5. Conclusions

L1CAM was a strong prognostic marker for high-risk EC patients, especially in the NSMP group. Patients with *POLE* mutations have a very good prognosis and are candidates for a wait-and-see policy in well-designed studies. Patients with MMRd or p53 abnormal tumors benefitted from adjuvant chemotherapy. Patients receiving adjuvant chemotherapy had a low risk of locoregional recurrence, but still a considerable risk of distant recurrence, especially in patients with p53 abnormal or L1CAM overexpressing tumors. There is an unmet need to find more effective systemic adjuvant therapies tailored to these subgroups of EC.

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CRediT authorship contribution statement

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Data availability

Individual patient-level data can be made available to other researchers upon reasonable request by contacting the corresponding author, subject to approval by the relevant persons or review boards at the institutions that provided the original data and material.

Declaration of competing interest

KL reports the following conflicts of interest outside the submitted work: Participation on data safety monitoring or advisory boards of Eisai, MSD, Nykode, AstraZeneca, GSK and Karyopharm (honoraria paid to institution); and research funding paid to institution from GSK. All other authors declare no competing interests.

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Appendix A. Supplementary data

Supplementary data to this article can be found online at https://doi.org/10.1016/j.ygyno.2024.11.005.

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