Title page

Impact of molecular profile on prognosis and relapse pattern in low and intermediate risk endometrial cancer

Kristina Lindemann, MD, PhD[1,2*], Wanja Kildal, PhD[3*], Andreas Kleppe, PhD[3,4,5], Kari Anne R Tobin, PhD[3], Manohar Pradhan, PhD[3], Maria X. Isaksen[3], Ljiljana Vlatkovic[3], Håvard E. Danielsen, PhD[3,6**], Gunnar B. Kristensen, MD, PhD[1,3], Hanne A. Askautrud, PhD[3]

1. Department of Gynecological Oncology, Division of Cancer Medicine, The Norwegian Radium Hospital, Oslo University Hospital, Oslo, Norway.

2. Institute of Clinical Medicine, Faculty of Medicine, University of Oslo, Norway

3. Institute for Cancer Genetics and Informatics, Oslo University Hospital, Oslo, Norway

4. Department of Informatics, University of Oslo, Oslo, Norway

5. Centre for Research-based Innovation Visual Intelligence, UiT The Arctic University of Norway, Tromsø, Norway

6. Nuffield Division of Clinical Laboratory Sciences, University of Oxford, Oxford OX3 9DU, UK.

* Joint first authors with equal contribution
** Author has deceased

Corresponding author:
Prof. K. Lindemann, Department of Gynecological Oncology, Division of Cancer Medicine, The Norwegian Radium Hospital, Oslo University Hospital, PB 4953 Nydalen 0424 Oslo, Norway; Institute of Clinical Medicine, University of Oslo, Faculty of Medicine.
Phone: (47) 22934000 fax: (47) 22934469
Email: klinde@ous-hf.no

Key Words: Endometrial cancer, low and intermediate risk, adjuvant chemotherapy, molecular classification, recurrence

Acknowledgements

We would like to acknowledge the contribution of The Department of Pathology, Oslo University Hospital, Norway. We also thank the laboratory and technical personnel at the Institute for Cancer Genetics and Informatics, Oslo University Hospital, Norway for their skillful assistance.

ABSTRACT

Introduction The role of molecular classification in patients with low/intermediate risk endometrial cancer (EC) is uncertain. Higher precision in diagnostics will inform the unsettled debate on optimal adjuvant treatment. We aimed to determine the association of molecular profiling with patterns of relapse and survival.

Material and methods This retrospective cohort study included patients referred to The Norwegian Radium Hospital, Oslo University Hospital from 2006-2017. Patients with low/intermediate risk EC were molecularly classified as pathogenic polymerase epsilon (*POLE*)-mutated, mismatch repair deficient (MMRd), p53 abnormal, or no specific molecular profile (NSMP). The main outcomes were time to recurrence (TTR) and cancer-specific survival (CSS).

Results Of 626 patients, 610 could be molecularly classified. Fifty-seven patients (9%) had POLE-mutated tumors, 202 (33%) had MMRd tumors, 34 (6%) had p53 abnormal tumors and 317 (52%) had NSMP tumors. After median follow-up time of 8.9 years, there was a statistically significant difference in TTR and CSS by molecular groups. Patients with p53 abnormal tumors had poor prognosis, with 10 of the 12 patients with relapse presenting with para-aortic/distant metastases. Patients with *POLE* mutations had excellent prognosis. In the NSMP group, L1CAM expression was associated with shorter CSS but not TTR.

Conclusions The differences in outcome by molecular groups are driven by differences in relapse frequency and -patterns and demand a higher precision in diagnostics, also in patients with low/intermediate risk EC. Tailored adjuvant treatment strategies need to consider systemic treatment for patients with p53 abnormal tumors and de-escalated treatment for patients with *POLE* mutated tumors.

Abbreviations

CI - confidence interval, CSD – cancer-specific death, CSS – Cancer-specific survival, CT - computer tomography, DFS - disease free survival, EC - Endometrial carcinoma, ESMO - European Society for Medical Oncology, FIGO - International Federation of Gynecology and Obstetrics, FFPE - formalin-fixed paraffin-embedded, HR - hazard ratio, L1CAM - L1 cell adhesion molecule, LVSI - lymphovascular space invasion, MLH1- mutL homolog 1, MMRd - Mismatch repair deficiency, MMRp - Mismatch repair proficiency, MR - magnetic resonance, MSH2- mutS homolog 2, MSH6 - MutS Homolog 6, NSMP - no specific molecular profile, OUH - Oslo University Hospital, OS - overall survival, PCR - Polymerase chain reaction, PMS2 - PMS1 homolog 2, mismatch repair system component, REK- Regional Committees for Medical and Health Research Ethics, TTR - Time to recurrence, WHO - World Health Organization,

INTRODUCTION

Endometrial cancer (EC) is the most common gynecological malignancy in the Western world with increasing incidence due to the higher prevalence of risk factors such as obesity. Most patients present with early-stage disease and based on histomorphology, patients are stratified into groups to estimate their risk of relapse and to tailor adjuvant treatment. In the Nordic countries, radiotherapy has been omitted from first-line treatment for most patients with low or intermediate risk in the absence of a proven survival benefit[1,2], and the fact that the majority of patients can be cured with radiotherapy when diagnosed with a vaginal/pelvic relapse[3]. This strategy has resulted in no detriment in survival[4] but has been challenged by a better understanding of the prognosis by applying molecular classification[5]. Since 10-15% of low or intermediate risk patients still relapse, further optimization of treatment is warranted.

Recent guidelines have incorporated molecular classification as a tool for escalation of treatment for patients with aggressive features such as p53 abnormal tumors[6,7,8]. Still, molecular classification is not widely implemented, particularly due to requirements in resources associated with POLE mutation detection. We therefore need solid data on the association with prognosis in patients with low/intermediate early-stage disease and how molecular classification may inform their management. In a recent analysis of patients with low grade EC, 73.8% of the patients were classified as early-stage[9]. Patients with low-grade EC of any stage had a favorable 5-year disease-specific survival independent of the molecular subgroups, questioning the added value of non-targeted molecular profiling in clinical practice. However, 75% of the patients had received adjuvant treatment and 57% had received adjuvant radiotherapy[9]. Classification of endometrial cancer has further evolved and L1 cell adhesion molecule (L1CAM) has been reported as a prognostic marker for patients with endometrial carcinoma with no specific molecular profile[10]. It remains unclear how molecular profiling plays out in a predominantly radiotherapy-naive population of low/intermediate risk EC.

A better refinement of adjuvant treatment is crucial to avoid under treatment of early-stage patients, but also to choose the best modality based on the expected localization of relapse and survival. The aim of this study was to examine survival by molecular groups and localization of relapse in a large cohort of low and intermediate risk EC patients.

MATERIAL AND METHODS

Patients and follow-up

We included 1784 patients with endometrial cancer (WHO 2020)[11] from a consecutive cohort referred to The Norwegian Radium Hospital, Oslo University Hospital (OUH) from January 2006-December 2017. The study was approved by the Regional Committees for Medical and Health Research Ethics (REK) in Norway (REK no 2014/701) and by the data protection office at The Oslo University Hospital. All specimens were reviewed by a pathologist specialized in gynecologic pathology at primary diagnosis. Lymphovascular space invasion (LVSI) was described as present or absent. For the 1353 eligible patients (eFigure 1), a 3 µm section was cut from each tissue block, the hematoxylin and eosin-stained sections were examined by a pathologist (MP), and a random block with a total tumor area of at least 0.2 cm^2 was chosen for each of 1228 patients. The ESMO guidelines (2016) were used for risk classification[12]. Low risk and (high-) intermediate risk patients included (i) stage I endometrioid, grade 1-2, <50% myometrial invasion, LVSI negative (low risk), (ii) stage I endometrioid grade 1-2 ≥50% myometrial invasion, LVSI negative (intermediate risk), (iii) stage IA endometrioid grade 3 regardless of LVSI (high-intermediate risk) and iv) stage I endometrioid grade 1-2, independent of myometrial invasion, positive LVSI (high-intermediate risk). We included 626 low and (high)intermediate risk patients.

Institutional guidelines considered standard treatment to be total hysterectomy and bilateral salpingo-oophorectomy in all cases and lymphadenectomy in stage IB grade 1-2 and stage IA grade 3. No adjuvant treatment was considered standard of care. Patients were monitored every three months during the first two years, every six months for the following three years, and then annually at OUH or the local hospital. Patterns of relapse were categorized as either vaginal- and central pelvic relapse (local), extension to the pelvic side wall including pelvic lymph nodes, paraaortic lymph nodes +/-pelvic lymph nodes and distant.

Immunohistochemistry and scoring

Immunohistochemistry for p53, L1CAM, MLH1, MSH2, MSH6 and PMS2 was done on 3 µm sections from each tumor block of the hysterectomy specimen. Blinded to clinicopathological- and outcome data, two experienced pathologists (MP or LV) scored all sections according to the description by Köbel M *et al.*[13] for p53, Zeimet AG *et al.*[14] for L1CAM, and MLH1, MSH2, MSH6, PMS2 were considered retained if there was normal nuclear protein expression or lost if there was loss of protein expression. For details see eMethods.

Pole mutation analyses

Five 10µm scrolls were cut from the tumor area from the selected tissue blocks, and genomic DNA was extracted using the RecoverAll Total NucleicAcid Isolation kit (Thermo Fischer Scientific).

Genotyping assays was used to perform allele-specific 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 endometrial cancer[15] (eTable 1 and eFigure 2). For P286R, V411L, S297F we used primers and probes as previously described[16], for A456P and S459F we designed probes and primers based on the known context sequence. For details see eMethods.

Molecular profiling

Classification into molecular groups was according to the ESMO Clinical Practice Guidelines[8] recommendations.

Statistical analyses

Continuous variables were described with median and interquartile range. Categorical variables were presented with counts and proportions. Differences between categorical variables were assessed by the Pearson's χ^2 test. The Kruskal-Wallis H test was used to assess differences between categorical and continuous variables. 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)[17].

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 on December 28th, 2022. For CSS, follow-up time was calculated from the date of EC surgery until the date of death from any cause or end of follow-up. Survival curves were plotted with the Kaplan-Meier method. The cumulative incidence function was estimated with death from other causes than EC as competing event in competing risk analysis of both cancer-specific death (CSD) and TTR. The multivariable model included age, surgical stage according to the 2009 revision by FIGO[18], histological type with grade for endometrioid adenocarcinomas, adjuvant treatment, pelvic lymphadenectomy, as well as L1CAM expression. A two-sided $p<0.05$ was considered statistically significant. The analyses were performed using Stata/SE 18.0 (StataCorp, TX).

RESULTS

We included 626 low and intermediate risk patients with a median age of 68 years (range 59-74 years) (Table 1). The majority were diagnosed with FIGO stage IA (n=432, 69%) and well differentiated endometrioid adenocarcinoma (n=375, 60%). In total 356 were considered low risk, 138 as low/intermediate and 132 as high/intermediate risk patients. Pelvic lymphadenectomy was part of the primary surgery in 286 (46%) patients. Only 24 patients (4%) received adjuvant treatment, all with platinum-based chemotherapy. Molecular subgroup distribution in the 610 patient who could be

molecularly classified is displayed in Table 1 and eFigure1. The remaining 16 patients (3%) could not be classified according to ProMisE.

Table 1: Baseline characteristics of the study cohort

Baseline characteristic		All patients	Patients without cancer-specific death	Patients with cancer-specific death	P-value
Age at surgery, years		68 (59-74)	67 (59-74)	73 (67-80)	0.0005
FIGO stage (2009)	IA	432 (69%)	403 (69%)	29 (63%)	0.36
	IB	194 (31%)	177 (31%)	17 (37%)	
Histology	Endometrioid, G1	375 (60%)	360 (62%)	15 (33%)	<0.0001
	Endometrioid, G2	204 (33%)	182 (31%)	22 (48%)	
	Endometrioid, G3	47 (8%)	38 (7%)	9 (20%)	
Lymphovascular space invasion	No	534 (85%)	504 (87%)	30 (65%)	<0.0001
	Yes	92 (15%)	76 (13%)	16 (35%)	
Pelvic lymphadenectomy	Yes	286 (46%)	261 (45%)	25 (54%)	0.22
Adjuvant treatment	No	340 (54%)	319 (55%)	21 (46%)	0.16
	None	602 (96%)	556 (96%)	46 (100%)	
	Chemotherapy	24 (4%)	24 (4%)	0	
ProMisE classification	*POLE* mutated	57 (9%)	57 (10%)	0	<0.0001
	Mismatch repair deficient	202 (32%)	180 (31%)	22 (48%)	
	p53 abnormal	34 (5%)	23 (4%)	11 (24%)	
	No specific molecular profile	317 (51%)	306 (53%)	11 (24%)	
L1CAM expression	Missing	16 (3%)	14 (2%)	2 (4%)	0.014
	<10%	565 (90%)	528 (91%)	37 (80%)	
	≥10%	50 (8%)	42 (7%)	8 (17%)	
	Missing	11 (2%)	10 (2%)	1 (2%)	

For the analyses of clinical outcomes by molecular groups, 610 patients with ProMisE classification were included. Median follow-up time was 8.9 years (95% CI: 6.2-12.6 years). For the entire cohort, there was a statistically significant difference in TTR ($p<0.001$) and CSS ($p<0.001$) by molecular groups (Figure 1a-b). The 5-year cumulative incidence for recurrence and CSD for patients with *POLE* mutated tumors was 3.5% (95% CI: 0.7-10.7%) and 0%, with MMRd tumors 17.8% (95% CI: 12.9-23.4%) and 8.4% (95% CI: 5.1-12.8%), with p53 abnormal tumors 32.4% (95% CI: 17.6-48.0%) and 29.4% (95% CI: 15.4-44.9%), and with NSMP tumors 8.5% (95% CI: 5.8-11.9%) and 2.5% (95% CI: 1.2-4.7%).
The difference in TTR and CSS by molecular groups remained significant in separate analyses of patients with grade 1/2 tumors (TTR, $p=0.0010$ and CSS, $p<0.0001$), but not in grade 3 tumors (TTR, $p=0.10$ and CSS, $p=0.098$) (eFigure 3).

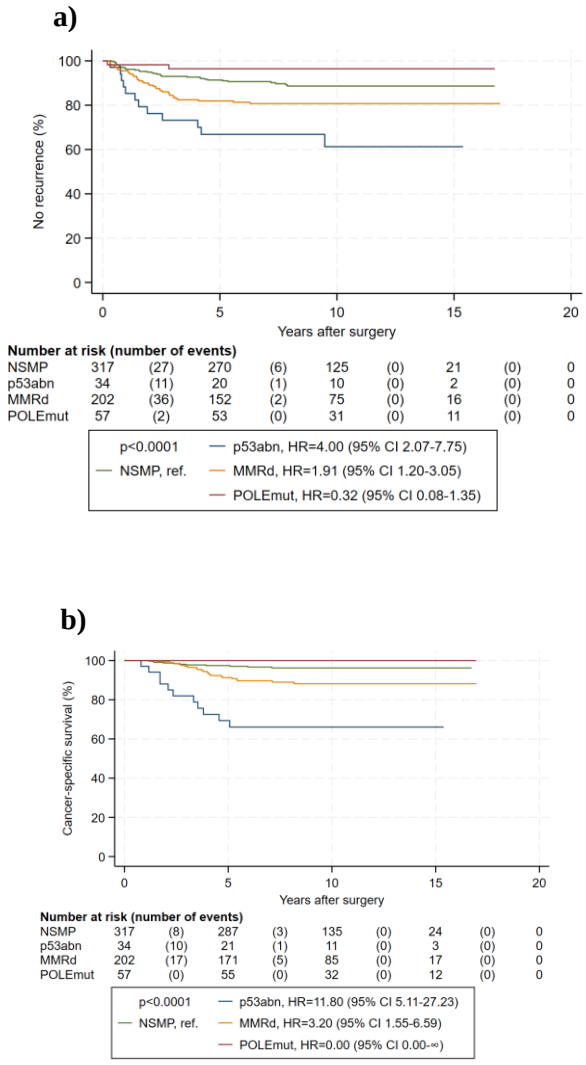

Figure 1a-b: Survival analysis of ProMisE.
(a) Analysis of time to recurrence. (b) Analysis of cancer-specific survival.

In separate analyses of patients who had not received adjuvant chemotherapy, molecular groups were still associated with TTR (p<0.0001) and CSS (p<0.0001), and the estimated 5-year cumulative incidences remained largely unchanged (eFigure 4, eTable 2).

In multivariable Cox regression analyses molecular groups remained significantly associated with TTR (p=0.005) and CSD (p=0.0002) (Table 2).

Table 2: Multivariable analysis of time to recurrence and cancer-specific survival

Group	Risk of recurrence		Risk of cancer-specific death	
	HR (95% CI)	p-value	HR (95% CI)	p-value

ProMisE classification	No specific molecular profile	ref.	0.0050	ref.	0.0002
	p53 abnormal	2.89 (1.41-5.94)		7.14 (2.83-18.04)	
	Mismatch repair deficient	1.57 (0.96-2.58)		2.25 (1.03-4.89)	
	POLE mutated	0.33 (0.08-1.39)		0.00 (0.00-∞)	
Age at surgery	≥60 years vs. <60 years	1.78 (0.95-3.34)	0.071	1.53 (0.59-3.97)	0.38
Histology			0.30		0.021
	Endometrioid, G1	ref.		ref.	
	Endometrioid, G2	1.31 (0.79-2.16)		2.21 (1.05-4.65)	
	Endometrioid, G3	1.72 (0.84-3.55)		3.81 (1.44-10.04)	
L1CAM expression	≥10% vs. <10%	1.02 (0.50-2.09)	0.95	1.55 (0.69-3.48)	0.28
Lymphovascular space invasion	Yes vs no	1.80 (1.06-3.04)	0.028	3.31 (1.70-6.46)	0.0004
Adjuvant treatment	Chemotherapy vs. No adjuvant treatment	0.42 (0.10-1.73)	0.23	0.00 (0.00-∞)	1.0
Pelvic lymphadenectomy	No vs. yes	1.27 (0.80-2.01)	0.61	0.79 (0.41-1.52)	0.48

CI: confidence interval

The highest risk estimates for CSD were estimated in the group of patients with MMRd tumors and p53 abnormal tumors with HR of 2.25 (95% CI: 1.03-4.89) and 7.14 (95% CI: 2.83-18.04), respectively. LVSI was independently associated with risk of relapse (HR 1.80, 95% CI 1.06-3.04) and CSD (HR 3.31, 95% CI: 1.70-6.46), whereas L1CAM overexpression was not associated with either risk of relapse or CSD (Table 2).

In the NSMP group, L1CAM expression was significantly associated with shorter CSS (HR 12.52, 95% CI: 3.53-44.42, p<0.0001) but not TTR (HR 2.7, 95% CI: 0.95-7.7, p=0.053) (Figure 2).

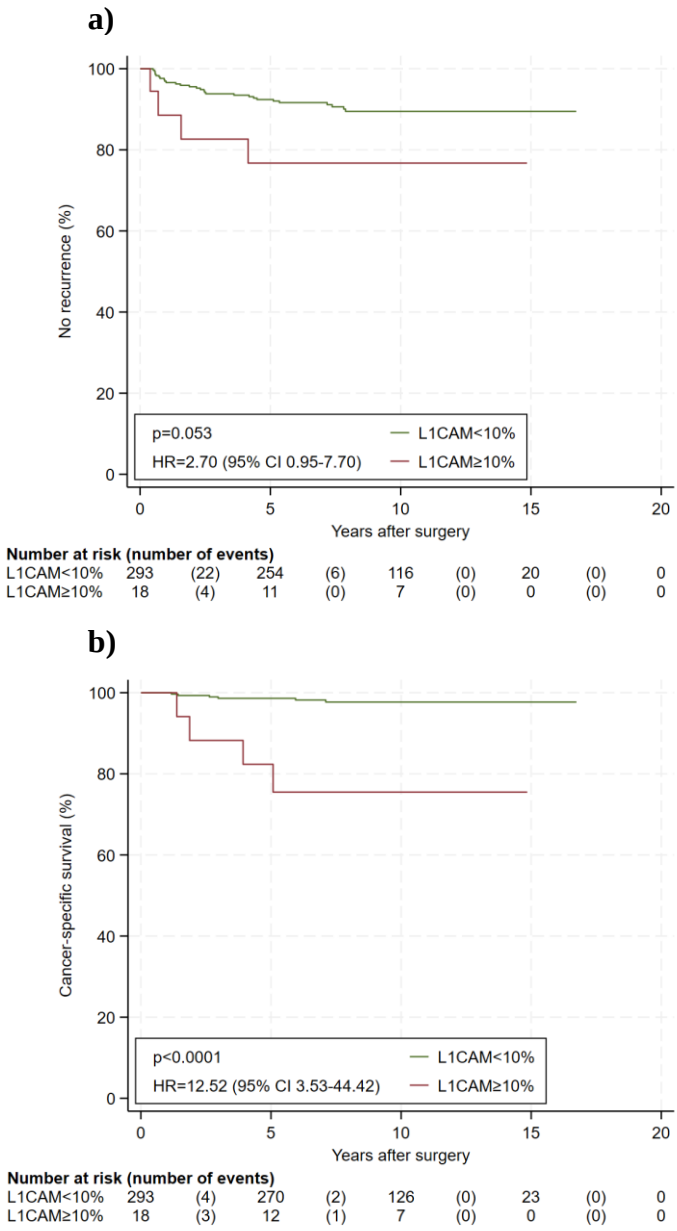

Figure 2a-b: Survival analysis of L1CAM expression in patients with NSMP tumors. (a) Analysis of time to recurrence. (b) Analysis of cancer-specific survival.

In total, 85/626 (13.6%) patients had recurrent disease, of these 37 were locoregional relapses. The localization of relapse differed by molecular group (Table 3). While patients with MMRd showed equal distribution of local/pelvic and para-aortic/distant relapse, the vast majority of patients with p53 abnormal tumors developed para-aortic/distant relapse. In the NSMP group with L1CAM ≥10%, all four relapses were distant metastases, but in the NSMP group with L1CAM <10%, 15 of 27 recurrences were localized to the vagina/central pelvis (p=0.015).

Table 3: Frequency and localization of relapse by ProMisE group

	Local	**Extension to pelvic side wall pelvic including lymph nodes**	**Para-aortic lymph nodes**	**Distant**	**No relapse**	**Total**
POLE mutated	0	1 (1.8%)	0	1 (1.8%)	55 (96.5%)	57
Mismatch repair deficient	14 (6.9%)	4 (1.9%)	5 (2.4%)	15 (7.4%)	164 (81.2%)	202
p53 abnormal	2 (5.9%)	0	1 (2.9%)	9 (26.5%)	22 (64.7%)	34
L1CAM ≥10% (in ProMisE no specific molecular profile)	0	0	0	4 (22.2%)	14 (77.8%)	18
L1CAM <10% (in ProMisE no specific molecular profile)	15 (5.1%)	1 (0.3%)	2 (0.7%)	10 (3.4%)	265 (90.4%)	293
Missing data	2	0	1	2	17	22
Total	33 (5.3%)	6 (0.9%)	15 (2.4%)	41 (6.5%)	537 (85.8%)	626

DISCUSSION

In this large cohort study of patients with low and intermediate risk early-stage EC, molecular classification was associated with TTR and CSS, independently of other clinical and histomorphologic parameters. Even though the prevalence of p53 abnormal tumors was low, the risk of para-aortic/distant relapse was high resulting in increased risk of CSD.

We here report on the prognostic impact of molecular classification in patients largely untreated after primary surgery. In particular, none of the patients in our cohort had received adjuvant radiotherapy, and the results therefore add to the ongoing debate on the optimization of adjuvant treatment in early-stage EC. A recent report on low grade EC challenged the necessity of molecular classification as no significant association by molecular subgroups with survival was found[9]. However, the analysis may have been underpowered to detect such a difference in early-stage disease as 26% of the patients had

advanced disease. Still, a significant association between p53 abnormal tumors and risk of disease-specific death was reported, underlining that molecular classification is important independent of histomorphology.

We confirm that p53 abnormality is a rare event in low/intermediate risk endometrial cancer[19]. The PORTEC group reported that in these patients, pelvic radiation was associated with longer locoregional recurrence-free survival compared to vaginal brachytherapy/observation. This benefit in terms of locoregional control may however be driven by the fact that patients were not systematically staged. In our cohort, about half of the patients underwent pelvic staging, which may have contributed to a low risk of locoregional relapse. Patients with p53 abnormal tumors had a particularly high risk of distant recurrence, which has also been previously reported[20]. This relapse pattern is driving the poor survival in patients with p53 abnormal tumors as shown by the particularly high HRs in analysis of CSD. Adjuvant pelvic radiotherapy will not prevent these relapses, does not prolong survival[1] and is associated with considerable morbidity[21,22]. It is therefore reasonable to reserve this treatment for the few low/intermediate risk patients with pelvic relapse who have excellent survival with salvage radiation[3]. Preventing distant relapses in patients with p53 abnormal tumors, appear to require systematic treatment also in early-stage, in line with data in high risk patients where p53 abnormal tumors derived particular benefit when chemotherapy was added to radiotherapy[23]. Some of these tumors may also harbor defects in the homologous recombination pathway and maintenance strategies with PARP inhibition need to be explored.

Our study confirms the excellent prognosis in patients with *POLE* mutated tumors also for adjuvant treatment naïve patients[24-26]. De-escalation of treatment in patients with *POLE* mutated tumors is currently being investigated in the RAINBO blue study[27]. *POLE* mutation status in EC is commonly determined by DNA sequencing, a method that is time-consuming, expensive, and unavailable in hospitals without specialized equipment and expertise, thereby slowing down *POLE* mutation testing in clinical practice. As a result, women with *POLE* mutations are currently being overtreated. We established a fast and inexpensive allele-specific PCR for the 5 most common *POLE* mutations, which cover around 95% of the pathogenic *POLE* mutations in EC[15,28]. A similar approach, QPOLE[29], demonstrate an accuracy of 98.6%, a sensitivity of 95.2% and a specificity of 100% as compared to sequencing. Our results confirm that PCR mutation analysis identifies patients with excellent prognosis in a clinical setting, and we are convinced that these methods will facilitate faster implementation in clinical practice.

Almost a third of the patients in our cohort had MMRd tumors, highlighting the need to screen for Lynch syndrome in all patients with endometrial cancer[30]. For patients with MMRd both TTR and CSS were shorter compared to patients in the NSMP group, but more favorable than for patients with p53 abnormal

tumors. Almost 20% of patients with MMRd tumors had a recurrence, calling for optimization of postoperative treatment. The equal distribution of locoregional and para-aortic/distant relapses has also been shown in cohorts with higher rates of adjuvant radiotherapy[21]. In the PORTEC studies, radiotherapy did not improve locoregional recurrence-free survival in patients with MMRd tumors[19]. As <10% of the patients in our cohort developed locoregional relapse, this needs to be balanced with the expected toxicity associated with pelvic radiation. One may argue that salvage radiation at the time of locoregional recurrence is the best approach for patients with MMRd tumors, instead of adjuvant radiotherapy to all patients in first line. Surveillance and patient awareness remain crucial to detect locoregional recurrences early, and patients should be counselled on the risk of relapse when followed with observation alone after surgery. Patients with MMRd recurrent disease face improved survival when chemotherapy is combined with a checkpoint inhibitor[31], and immunotherapy alone in the metastatic/recurrent setting is currently explored in the ongoing phase 3 trials (NCT05201547, NCT05173987). However, advancing treatment in first line will have a greater potential to cure patients.

Finally, we confirm high L1CAM expression as a biomarker for poor outcome in patients with NSMP tumors[10]. These patients face a high risk of CSD due to their high risk of distant recurrence calling for evaluation of systemic treatment strategies. Some of those, in our cohort 5 of the 18 L1CAM positive cases, may also show other features of aggressive behaviour such as LVSI, illustrating the need for a better characterization before we can recommend systematic assessment of L1CAM. Other biomarkers such as hormone receptor expression have been explored in NSMP tumors[32] underlining that this is a heterogeneous patient group which need better characterization.

This study has some limitations, including its retrospective design. Further, the original diagnosis was not subject to a second pathology review and LVSI was not assessed according to the most recent WHO classification[11]. However, all primary diagnoses were made by expert gynecological pathologists, thus making the study applicable to clinical practice.

The differences in patterns of relapse, TTR and CSS by molecular groups demand a higher precision in diagnostics in patients with low/intermediate risk EC. These results call for a more individualized approach to adjuvant treatment already in first line. While observation alone still is a reasonable choice for most patients with low/intermediate risk EC, patients with p53 abnormal tumors and NSMP patients with high L1CAM expression need to be considered for adjuvant treatment strategies.

Funding

This work was supported by the Norwegian Cancer Society [grant number 198168].

Conflict of interest statement

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 remaining authors have declared no conflicts of interest.

CRediT author statement

Kristina Lindemann: Conceptualization, Data Curation, Writing - Original Draft
Wanja Kildal: Conceptualization, Investigation, Visualization, Writing - Original Draft
Andreas Kleppe: Methodology, Formal analysis, Visualization, Data Curation
Kari Anne R Tobin: Methodology, Investigation
Manohar Pradhan: Investigation
Maria X. Isaksen: Investigation
Ljiljana Vlatkovic: Investigation
Håvard E. Danielsen: Resources
Gunnar B. Kristensen: Conceptualization, Data Curation
Hanne A. Askautrud: Conceptualization, Resources

All authors: Writing - Review & Editing

References:

1. 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. *British journal of cancer*. Oct 2018;119(9):1067-1074. doi:10.1038/s41416-018-0310-8
2. Creutzberg CL, Nout RA, Lybeert ML, et al. Fifteen-year radiotherapy outcomes of the randomized PORTEC-1 trial for endometrial carcinoma. Randomized Controlled Trial
Research Support, Non-U.S. Gov't. *International journal of radiation oncology, biology, physics*. Nov 15 2011;81(4):e631-8. doi:10.1016/j.ijrobp.2011.04.013
3. Lindemann K, Smogeli E, Smastuen MC, et al. Salvage Radiation for Pelvic Relapse after Surgically Treated Endometrial Cancer. *Cancers (Basel)*. Mar 18 2021;13(6)doi:10.3390/cancers13061367
4. Ortoft G, Hansen ES, Bertelsen K. Omitting adjuvant radiotherapy in endometrial cancer increases the rate of locoregional recurrences but has no effect on long-term survival: the Danish Endometrial Cancer Study. *International journal of gynecological cancer : official journal of the International Gynecological Cancer Society*. Oct 2013;23(8):1429-37. doi:10.1097/IGC.0b013e3182a5e77d
5. Talhouk A, McConechy MK, Leung S, et al. Confirmation of ProMisE: A simple, genomics-based clinical classifier for endometrial cancer. *Cancer*. Mar 1 2017;123(5):802-813. doi:10.1002/cncr.30496
6. Concin N, Creutzberg CL, Vergote I, et al. ESGO/ESTRO/ESP Guidelines for the management of patients with endometrial carcinoma. *Virchows Arch*. Feb 19 2021;doi:10.1007/s00428-020-03007-z
7. Berek JS, Matias-Guiu X, Creutzberg C, et al. FIGO staging of endometrial cancer: 2023. *International journal of gynaecology and obstetrics: the official organ of the International Federation of Gynaecology and Obstetrics*. Aug 2023;162(2):383-394. doi:10.1002/ijgo.14923
8. Oaknin A, Bosse TJ, Creutzberg CL, et al. Endometrial cancer: ESMO Clinical Practice Guideline for diagnosis, treatment and follow-up. *Annals of oncology : official journal of the European Society for Medical Oncology / ESMO*. Sep 2022;33(9):860-877. doi:10.1016/j.annonc.2022.05.009
9. Vrede SW, Kasius J, Bulten J, et al. Relevance of Molecular Profiling in Patients With Low-Grade Endometrial Cancer. *JAMA Netw Open*. Dec 1 2022;5(12):e2247372. doi:10.1001/jamanetworkopen.2022.47372
10. Kommoss FK, Karnezis AN, Kommoss F, et al. L1CAM further stratifies endometrial carcinoma patients with no specific molecular risk profile. *British journal of cancer*. Aug 2018;119(4):480-486. doi:10.1038/s41416-018-0187-6
11. Board WCoTE. *2020 WHO Classification of Female Genital Tumors*. 5. ed. vol 4. International Agency for Research on Cancer (IARC); 2020.
12. Colombo N, Creutzberg C, Amant F, et al. ESMO-ESGO-ESTRO Consensus Conference on Endometrial Cancer: diagnosis, treatment and follow-up. *Annals of oncology : official journal of the European Society for Medical Oncology / ESMO*. Jan 2016;27(1):16-41. doi:10.1093/annonc/mdv484
13. Kobel M, Ronnett BM, Singh N, Soslow RA, Gilks CB, McCluggage WG. Interpretation of P53 Immunohistochemistry in Endometrial Carcinomas: Toward

Increased Reproducibility. *Int J Gynecol Pathol*. Jan 2019;38 Suppl 1(Iss 1 Suppl 1):S123-S131. doi:10.1097/PGP.0000000000000488
14. Zeimet AG, Reimer D, Huszar M, et al. L1CAM in Early-Stage Type I Endometrial Cancer: Results of a Large Multicenter Evaluation. *Journal of the National Cancer Institute*. Aug 7 2013;105(15):1142-1150. doi:10.1093/jnci/djt144
15. Leon-Castillo A, Britton H, McConechy MK, et al. Interpretation of somatic POLE mutations in endometrial carcinoma. *The Journal of pathology*. Mar 2020;250(3):323-335. doi:10.1002/path.5372
16. Domingo E, Freeman-Mills L, Rayner E, et al. Somatic POLE proofreading domain mutation, immune response, and prognosis in colorectal cancer: a retrospective, pooled biomarker study. *Lancet Gastroenterol Hepatol*. Nov 2016;1(3):207-216. doi:10.1016/S2468-1253(16)30014-0
17. Punt CJ, Buyse M, Kohne CH, et al. Endpoints in adjuvant treatment trials: a systematic review of the literature in colon cancer and proposed definitions for future trials. *Journal of the National Cancer Institute*. Jul 4 2007;99(13):998-1003. doi:10.1093/jnci/djm024
18. Pecorelli S. Revised FIGO staging for carcinoma of the vulva, cervix, and endometrium. Introductory. *International journal of gynaecology and obstetrics: the official organ of the International Federation of Gynaecology and Obstetrics*. May 2009;105(2):103-4.
19. Horeweg N, Nout RA, Jurgenliemk-Schulz IM, et al. Molecular Classification Predicts Response to Radiotherapy in the Randomized PORTEC-1 and PORTEC-2 Trials for Early-Stage Endometrioid Endometrial Cancer. *Journal of clinical oncology : official journal of the American Society of Clinical Oncology*. Jul 24 2023:JCO2300062. doi:10.1200/JCO.23.00062
20. Siegenthaler F, Lindemann K, Epstein E, et al. Time to first recurrence, pattern of recurrence, and survival after recurrence in endometrial cancer according to the molecular classification. *Gynecologic oncology*. May 2022;165(2):230-238. doi:10.1016/j.ygyno.2022.02.024
21. Nout RA, Smit VT, Putter H, et al. Vaginal brachytherapy versus pelvic external beam radiotherapy for patients with endometrial cancer of high-intermediate risk (PORTEC-2): an open-label, non-inferiority, randomised trial. Comparative Study
Multicenter Study
Randomized Controlled Trial
Research Support, Non-U.S. Gov't. *Lancet*. Mar 6 2010;375(9717):816-23. doi:10.1016/S0140-6736(09)62163-2
22. Nout RA, van de Poll-Franse LV, Lybeert ML, et al. Long-term outcome and quality of life of patients with endometrial carcinoma treated with or without pelvic radiotherapy in the post operative radiation therapy in endometrial carcinoma 1 (PORTEC-1) trial. *Journal of clinical oncology : official journal of the American Society of Clinical Oncology*. May 1 2011;29(13):1692-700. doi:10.1200/JCO.2010.32.4590
23. Leon-Castillo A, de Boer SM, Powell ME, et al. Molecular Classification of the PORTEC-3 Trial for High-Risk Endometrial Cancer: Impact on Prognosis and Benefit From Adjuvant Therapy. *Journal of clinical oncology : official journal of the American Society of Clinical Oncology*. Aug 4 2020:JCO2000549. doi:10.1200/JCO.20.00549
24. Cancer Genome Atlas Research N, Kandoth C, Schultz N, et al. Integrated genomic characterization of endometrial carcinoma. *Nature*. May 02 2013;497(7447):67-73. doi:10.1038/nature12113

25. Stasenko M, Tunnage I, Ashley CW, et al. Clinical outcomes of patients with POLE mutated endometrioid endometrial cancer. *Gynecologic oncology*. Jan 2020;156(1):194-202. doi:10.1016/j.ygyno.2019.10.028
26. Kommoss S, McConechy MK, Kommoss F, et al. Final validation of the ProMisE molecular classifier for endometrial carcinoma in a large population-based case series. *Annals of oncology : official journal of the European Society for Medical Oncology / ESMO*. May 1 2018;29(5):1180-1188. doi:10.1093/annonc/mdy058
27. Consortium RR. Refining adjuvant treatment in endometrial cancer based on molecular features: the RAINBO clinical trial program. *International journal of gynecological cancer : official journal of the International Gynecological Cancer Society*. Dec 20 2022;33(1):109-17. doi:10.1136/ijgc-2022-004039
28. McAlpine JN, Chiu DS, Nout RA, et al. Evaluation of treatment effects in patients with endometrial cancer and POLE mutations: An individual patient data meta-analysis. *Cancer*. Jul 15 2021;127(14):2409-2422. doi:10.1002/cncr.33516
29. Van den Heerik A, Ter Haar NT, Vermij L, et al. QPOLE: A Quick, Simple, and Cheap Alternative for POLE Sequencing in Endometrial Cancer by Multiplex Genotyping Quantitative Polymerase Chain Reaction. *JCO Glob Oncol*. May 2023;9:e2200384. doi:10.1200/GO.22.00384
30. Crosbie EJ, Ryan NAJ, Arends MJ, et al. The Manchester International Consensus Group recommendations for the management of gynecological cancers in Lynch syndrome. *Genet Med*. Oct 2019;21(10):2390-2400. doi:10.1038/s41436-019-0489-y
31. Mirza MR, Chase DM, Slomovitz BM, et al. Dostarlimab for Primary Advanced or Recurrent Endometrial Cancer. *The New England journal of medicine*. Jun 8 2023;388(23):2145-2158. doi:10.1056/NEJMoa2216334
32. Vermij L, Jobsen JJ, Leon-Castillo A, et al. Prognostic refinement of NSMP high-risk endometrial cancers using oestrogen receptor immunohistochemistry. *British journal of cancer*. Mar 2023;128(7):1360-1368. doi:10.1038/s41416-023-02141-0

Figure legends

Figure 1: Survival analysis of ProMisE. (a) Analysis of time to recurrence. (b) Analysis of cancer-specific survival.

Figure 2: Survival analysis of L1CAM expression in patients with NSMP tumors. (a) Analysis of time to recurrence. (b) Analysis of cancer-specific survival.