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Time to first recurrence, pattern of recurrence, and survival after recurrence in endometrial cancer according to the molecular classification

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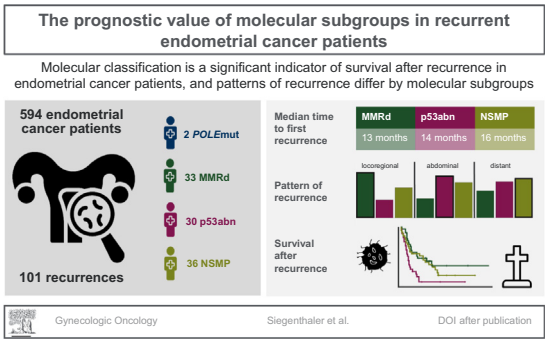
HIGHLIGHTS

- The pattern of recurrence in endometrial cancer differs among the molecular subgroups.
- Molecular classification of the primary tumor in endometrial cancer is a significant predictor of survival after recurrence.
- MMRd endometrial cancer patients experience more locoregional recurrences and show the best survival after recurrence.
- p53abn endometrial cancer patients have the worst survival rate after recurrence.

GRAPHICAL ABSTRACT



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ABSTRACT

Objective. Despite its generally favorable prognosis at primary diagnosis, recurrence of endometrial cancer remains an important clinical challenge. The aim of this study was to analyze the value of molecular classification in recurrent endometrial cancer.

Methods. This study included patients with recurrent endometrial cancer who underwent primary surgical treatment between 2004 and 2015 at the Karolinska University Hospital, Sweden and the Bern University Hospital, Switzerland (KImBer cohort) with molecular classification of the primary tumor.

Results. Out of 594 molecularly classified endometrial cancer patients, 101 patients experienced recurrence, consisting of 2 *POLE*mut, 33 MMRd, 30 p53abn, and 36 NSMP tumors. Mean age at recurrence was 71 years and mean follow-up was 54 months. Overall, median time to first recurrence was 16 months (95% CI 12–20); with the shortest median time in MMRd patients, with 13 months (95% CI 5–21). The pattern of recurrence was distinct among molecular subgroups: MMRd tumors experienced more locoregional, while p53abn cases showed more abdominal recurrences ($P = .042$). Median survival after recurrence was best for MMRd cases (43 months, 95% CI 11–76), compared to 39 months (95% CI 21–57) and 10 months (95% CI 7–13) for the NSMP and p53abn cases respectively (log-rank, $P = .001$).

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Conclusion. Molecular classification is a significant indicator of survival after recurrence in endometrial cancer patients, and patterns of recurrence differ by molecular subgroups. While MMRd endometrial cancer show more locoregional recurrence and the best survival rates after recurrence, p53abn patients experience abdominal recurrence more often and had the worst prognosis of all recurrent patients.

1. Introduction

Endometrial cancer is the most common gynecological tumor in developed countries. It has a generally favorable prognosis, with an overall 5-year survival rate of 80% [1]. Its incidence is increasing due to greater prevalence of its risk factors, including obesity, metabolic syndrome, and age [2]. Over the past years, the management of endometrial cancer has become considerably more personalized, mainly as a result of the introduction of the new molecular classification [3] and of sentinel lymph node mapping [4]. Prospective studies taking the molecular classification into account are finally on the way to optimize treatment strategies.

About 18% of endometrial cancer patients experience recurrence, the majority during the first two years after primary surgical treatment [5,6]. In these patients, treatment options are limited and mortality remains high. Distant recurrences constitute the most common dissemination pathway and survival after recurrence rarely exceeds two years [5,7–10]. Few previous studies have investigated the relationship between the pattern of recurrence and clinicopathological characteristics of the primary disease [5,11]. Current data demonstrate that the pattern of recurrence is one of the most important prognostic factors in recurrent endometrial cancer [5,6,12–16]. Nevertheless, there is little evidence regarding surveillance after primary treatment, and the rationale for postoperative follow-up in the standard management of endometrial cancer remains a topic of continuing debate [17–20]. A more detailed understanding of the mechanisms of recurrence and its prognosis is needed in order to tailor adjuvant treatment and surveillance as well as treatment at recurrence.

The understanding of endometrial cancer at the molecular level has seen an incredible evolution over the past decade. In 2013, The Cancer Genome Atlas (TCGA) collaborative endometrial project developed an integrated classification of endometrial cancer into four genomic subgroups [3]. This was further developed into a simplified molecular classifier called the Proactive Molecular Risk Classifier for Endometrial Cancer (ProMisE), which identifies four molecular subtypes: polymerase epsilon ultramutated (*POLE*mut), mismatch repair deficient (MMRd), p53 abnormal (p53abn), and non-specific molecular profile (NSMP) [21]. Since then, the clinical applicability and the prognostic significance of the molecular classification of endometrial cancer have been investigated by numerous research groups [22–25]. With their integration in the 5th edition of the WHO Classification of Female Genital Tumors, molecular classification found its definitive place in endometrial cancer diagnosis [26]. In addition, in 2021 the European Society of Gynecological Oncology (ESGO), the European Society for Radiotherapy and Oncology (ESTRO), and the European Society of Pathology (ESP) published updated guidelines for the determination of risk groups in endometrial cancer, integrating molecular classification into risk classification and recommendations for adjuvant treatment [17].

In our study, we aimed to gain a deeper understanding of recurrent endometrial cancer by analyzing the association between time to first recurrence, pattern of recurrence, and survival after recurrence and the molecular subgroups in a clinically well-annotated cohort of endometrial cancer patients with complete molecular classification of the primary tumor.

2. Materials and methods

2.1. Patient cohort and clinical data

This is a retrospective cohort study of endometrial cancer patients who underwent primary treatment between 2004 and 2015 consisting of 344 patients from the Karolinska University Hospital, Sweden and 250 patients treated at the Bern University Hospital, Switzerland (known as the KimBer cohort [24]). All pathology slides were reviewed by reference pathologists as previously described [24]. Follow-up data on recurrence and survival were available through standardized databases and follow-up controls in both clinics. Ethical approval was obtained from the local ethics committees in Stockholm and in Bern (reference numbers: 2016/362 and 2018–00479 respectively). All patients provided written informed consent for the use of their biobanked tissue and clinical data for research purposes. The analysis of the whole study cohort including oncological outcomes of all 594 patients has already been published [24] and we focus in this work on the patients of this cohort who developed recurrence.

2.2. Molecular classification

Molecular analysis was performed on the primary tumor according to the WHO Classification of Tumors, 5th Edition [26], and cases were classified as either *POLE*mut, MMRd, NSMP, or p53abn. Immunohistochemistry (IHC) for p53 and MMR proteins was performed on a tissue microarray (TMA). The analysis of TMA was carried out in triplicates, the details of TMA construction were published previously [24,27]. MMRd was defined as loss of nuclear staining in at least of the four MMR proteins (MLH1, MSH2, MSH6, and PMS2). p53abn was defined as either complete loss of nuclear protein expression or strong homogenous nuclear overexpression. In case of unclear staining, IHC staining was repeated on whole slide images to clarify the MMR and p53 status. All patients were analyzed for mutations of POLE gene (NM.006231) exons 9–14 by Sanger sequencing. A tumor was considered *POLE*mut if sequencing proved the existence of a hotspot mutation in the exonuclease domain *POLE*. Multiple classifiers harboring more than one molecular classifying feature were categorized as recommended by Leon-Castillo et al. [28,29] MMRd-p53abn cases as MMRd and *POLE*mut-p53abn cases as *POLE*mut. Detailed description of the TMA construction and IHC interpretation are under supplementary materials (S1).

2.3. Outcomes

Recurrences were classified as locoregional, abdominal, or distant recurrences, according to the first site of recurrence. Locoregional recurrences included vaginal and pelvic recurrences (including pelvic lymph nodes and local spread to rectum and bladder); recurrences outside the pelvis consisting of peritoneal carcinomatosis or omental metastasis were classified as abdominal recurrences; distant recurrences include lung, liver, bone, and brain metastases as well as lymph node involvement other than pelvic or paraaortic. Simultaneous locoregional and abdominal recurrence was classified as abdominal recurrence; simultaneous abdominal and distant recurrence was considered to be distant recurrence; and simultaneous locoregional and distant recurrence was

considered to be distant recurrence. In a separate analysis, we studied dissemination pathways and defined mixed recurrences as recurrences with multiple dissemination pathways (simultaneous locoregional, abdominal, and/or distant recurrence). Recurrence-free survival was calculated for the whole study population, defined as time from primary staging surgery to recurrence or death. Time to first recurrence was defined as the time from primary staging surgery to recurrence. Survival after recurrence was defined as time from recurrence until death due to any cause; patients with residual tumor or metastatic disease at primary diagnosis were excluded from this survival analysis because many of these cases had progressive disease. Surviving patients were censored at the date of their last follow-up.

2.4. Statistical analysis

Statistical calculations were performed using the Statistical Package for Social Sciences (IBM SPSS Statistic Version 25.0). Categorical variables were reported as frequencies and percentages, while continuous variables were reported as means and standard deviations or medians and range. Patient, tumor, and treatment characteristics were analyzed using Chi-square statistics (X^2) or Fisher's exact test in case of categorical variables and *t*-test or analysis of variance (ANOVA) for continuous variables. Survival curves were generated using the Kaplan-Meier method and compared using log-rank test. Univariable Cox regression analyses were conducted to assess the relationship between risk of recurrence or deathand other prognostic factors. Any variables significant in the univariable analysis were included in the multivariable analysis. A *P*-value of less than 0.05 was considered statistically significant.

3. Results

3.1. General characteristics of the whole study cohort

In the whole cohort of 594 molecularly classified endometrial cancer patients, 38 (6%) were classified as *POLE*mut, 199 (34%) MMRd, 86 (14%) p53abn, and 271 (46%) NSMP. 342 (58%) received no adjuvant treatment, 90 (15%) were treated with adjuvant radiotherapy, 41 (7%) with chemotherapy, 107 (18%) with chemoradiation, and one patient with endocrine therapy. More information on adjuvant treatment by molecular subgroup is provided in supplementary material, Table S2. 101 (17%) patients experienced recurrence during mean follow-up of 54 months (95% CI 44–64): 2/38 (5%) *POLE*mut, 33/199 (17%) MMRd, 30/86 (35%) p53abn, and 36/271 (13%) NSMP (P <.001). The two recurrent *POLE*mut tumors showed both hotspot mutations (c.857C > G, p.P286R and c.1376C > T, p.S459F). Mean recurrence-free survival was 110 months (95% CI 103–117) with best survival in *POLE*mut (144 months, 95% CI 130–159) patients, followed by NSMP (113 months, 95% CI 102–124), MMRd (107 months, 95% CI 94–120), and p53abn patients (80 months, 95% CI 64–95), $P < .001$ (Fig. 1). FIGO stage > I (HR 2.17–24.17, 95% CI 1.12–42.06), adjuvant treatment (HR 1.82. 95% CI 1.22–2.71) and p53 abnormality (HR 3.04, 95% CI 1.87–4.94) were significantly associated with a higher risk of recurrence in univariable Cox regression analysis. Multivariable analysis showed increased risk for recurrence in patients with FIGO stage > I (HR 2.04–19.28, 95% CI 1.02–34.98) and in the p53abn molecular subgroup (HR 1.85, 95% CI 1.12–3.07) (supplementary material, Table S3). For a more detailed description of the clinicopathological characteristics and the analysis of the outcomes of the whole study cohort we refer to our previously published article [24].

3.2. Clinicopathological characteristics of the recurrent patient cohort

The 101 recurrent patients had a mean age at recurrence of 71 years and mean BMI was 30 kg/m^2. Surgical approach for primary staging surgery was minimally invasive in 68 patients (40 laparoscopic and 28 robotic). Lymphadenectomy was performed in 64 patients with mean number of 34 (SD ±17) lymph nodes per patient removed. The majority of the patients with recurrence had grade 3 tumors (54%) and 56% had lymphovascular space invasion (LVSI). 54% of the MMRd tumors were FIGO stage I at primary diagnosis compared to 50% of the *POLE*mut, 44% of the NSMP, and 27% of the p53abn tumors ($P = .162$). The majority of patients with recurrence had received adjuvant treatment (68%), including chemoradiation in 33 patients, radiotherapy in 18 patients (of those 13 patients had vaginal brachytherapy only and five patients had vaginal brachytherapy plus external beam radiation), chemotherapy in 17 patients and endocrine therapy in one patient. Table 1 provides a detailed description of the main clinicopathological characteristics and their association with the molecular classification.

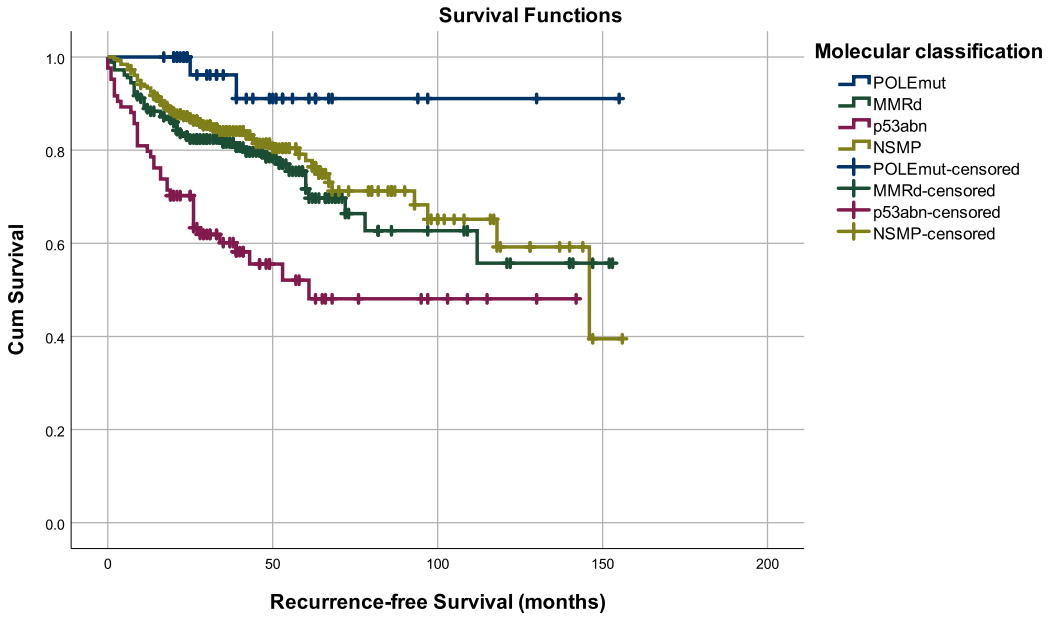


Fig. 1. Recurrence-free survival by molecular subgroup including all 594 molecularly classified endometrial cancer patients (log-rank, $P < .001$). Abbreviations: POLEmut = polymerase epsilon ultramutated, MMRd = mismatch repair deficient, p53abn = p53 abnormal, NSMP = non-specific molecular profile, CI = confidence interval.

Table 1
Association between molecular classifications and demographic and clinicopathological characteristics.

	Total	*POLE*mut	MMRd	p53abn	NSMP	*P*-value
Number of patients, *N (%)*	101 (100)	2 (2.0)	33 (32.7)	30 (29.7)	36 (35.6)	
Age at recurrence, years, *mean ± SD*	70.6 ± 9.0	66.8 ± 14.6	70.3 ± 10.4	70.8 ± 8.0	71.0 ± 8.5	0.924
BMI, kg/m^2, *mean ± SD*	29.8 ± 6.6	24.2 ± 2.5	30.7 ± 7.1	30.0 ± 7.0	29.2 ± 5.9	0.497
Surgical approach for primary staging surgery, *N (%)*						
- minimally invasive	68 (67.3)	2 (100)	24 (72.7)	17 (56.7)	25 (69.4)	
- open	33 (32.7)	0 (0)	9 (27.3)	13 (43.3)	11 (30.6)	0.023
Nodal assessment performed, *N (%)*	64 (63.4)	2 (100)	21 (63.6)	18 (60.0)	23 (63.9)	0.298
Grade, *N (%)*						
- G1	20 (19.8)	0 (0)	9 (27.3)	1 (3.3)	10 (27.8)	
- G2	27 (26.7)	0 (0)	13 (39.4)	3 (10)	11 (30.6)	
- G3	54 (53.5)	2 (100)	11 (33.3)	26 (86.7)	15 (41.7)	0.001
FIGO stage, *N (%)*						
- I	43 (42.6)	1 (50)	18 (54.4)	8 (26.7)	16 (44.4)	
- II	11 (10.9)	0 (0)	3 (9.1)	5 (16.7)	3 (8.3)	
- III	26 (25.7)	1 (50)	8 (24.2)	7 (23.3)	10 (27.8)	
- IV	21 (20.8)	0 (0)	4 (12.1)	10 (33.3)	7 (19.4)	0.445
LVSI positive, *N (%)*	57 (56.4)	2 (100)	18 (54.5)	16 (53.3)	15 (41.7)	0.623
Histological subtype, *N (%)*						
- endometrioid	64 (63.4)	0 (0)	25 (75.8)	11 (36.7)	28 (77.8)	
- serous	14 (13.9)	1 (50)	1 (3)	10 (33.3)	2 (5.6)	
- clearcell	3 (3)	0 (0)	0 (0)	3 (10)	0 (0)	
- carcinosarcoma	3 (3)	0 (0)	1 (3)	2 (6.7)	0 (0)	
- neuroendocrine	1 (1)	0 (0)	1 (3)	0 (0)	0 (0)	
- mucinous	1 (1)	0 (0)	0 (0)	0 (0)	1 (2.8)	
- mixed	15 (14.9)	1 (50)	5 (15.2)	4 (13.3)	5 (13.9)	0.006
Positive lymph nodes, *N (%)*	30 (29.7)	0 (0)	9 (27.3)	9 (30.0)	12 (33.3)	0.685
ESGO/ESTRO/ESP 2021 risk group, *N (%)*						
- Low	10 (9.9)	1 (50)	5 (15.2)	0 (0)	4 (11.1)	
- Intermediate	10 (9.9)	0 (0)	2 (6.1)	0 (0)	8 (22.2)	
- High-intermediate	17 (16.8)	0 (0)	12 (36.4)	0 (0)	5 (13.9)	
- High	39 (38.6)	0 (0)	8 (24.2)	19 (63.3)	12 (33.3)	
- Advanced/metastatic	24 (23.8)	0 (0)	6 (18.2)	11 (36.7)	7 (19.4)	
- unclassifiable	1 (1.0)	1 (50)	0 (0)	0 (0)	0 (0)	<0.001
Adjuvant therapy, *N (%)*						
- None	32 (31.7)	0 (0)	15 (45.5)	4 (13.3)	13 (36.1)	
- Radiotherapy	18 (17.8)	0 (0)	6 (18.2)	2 (6.7)	10 (27.8)	
- Chemotherapy	17 (16.8)	1 (50)	2 (6.1)	12 (40.0)	2 (5.6)	
- Chemoradiation	33 (32.7)	1 (50)	9 (27.3)	12 (40.0)	11 (30.6)	
- Endocrine therapy	1 (1)	0 (0)	1 (3.0)	0 (0)	0 (0)	0.003
Follow-up, months, *mean ± SD*	53.6 ± 1.5	48.0 ± 5.1	53.5 ± 2.6	53.4 ± 4.5	54.4 ± 2.3	0.850
Treatment at recurrence, *N (%)*						
- none	14 (13.8)	0 (0)	3 (9.1)	6 (20.0)	5 (13.9)	
- Surgery	11 (10.9)	0 (0)	5 (15.2)	0 (0)	6 (16.7)	
- Radiotherapy	26 (25.7)	0 (0)	16 (48.5)	2 (6.7)	8 (22.2)	
- Chemoradiation	9 (8.9)	0 (0)	0 (0)	4 (13.3)	5 (13.9)	
- Chemotherapy	35 (34.6)	1 (50)	9 (27.3)	15 (50.0)	10 (27.8)	
- Endocrine therapy	6 (5.9)	1 (50)	0 (0)	3 (10.0)	2 (5.6)	0.002

Abbreviations: N = number, SD = standard deviation, POLEmut = polymerase epsilon ultramutated, MMRd = mismatch repair deficient, p53abn = p53 abnormal, NSMP = non-specific molecular profile, BMI = Body mass index, FIGO = Federation International de Gynecologie et Obstetrique, LVSI = lymphovascular space invasion, ESGO = European Society of Gyneaecological Oncology, ESTRO = European Society for Radiotherapy and Oncology, ESP = European Society of Pathology.

3.3. Time to first recurrence

Median time to first recurrence was 16 months (95% CI 12–20), irrespective of the molecular subgroup. Patients with MMRd endometrial cancers experienced the numerically shortest time to first recurrence, followed by patients classified as p53abn, NSMP, and *POLE*mut, with medians of 13 (95% CI 5–21), 14 (95% CI 10–18), 16 (95% CI 12–20), and 25 (6 and 84 months) months respectively ($P = .224$). Forty-one patients experienced a recurrence during the first year after primary surgical treatment, with no statistically significant difference between molecular subtypes (16 MMRd, 12 p53abn, and 13 NSMP, $P = .471$). In nine patients, the recurrence occurred more than five years after primary surgical treatment consisting of one *POLE*mut, three MMRd, and five NSMP tumors.

3.4. Pattern of recurrence

In total, there were 31 locoregional, 30 abdominal, and 40 distant recurrences (Table 2). There was a tendency towards more locoregional recurrences in the MMRd group (46%), compared to p53abn (20%) and NSMP (28%) tumors ($P = .111$). On the other hand, p53abn patients experienced the most abdominal recurrences (43%) in comparison with the MMRd (18%) and NSMP (31%) subgroup ($P = .131$). In NSMP and *POLE*mut patients, distant recurrences represented the most common dissemination, with 42% and 100% respectively. The pattern of recurrence in MMRd patients differed significantly from that of patients with p53abn tumors ($P = .042$). Isolated vaginal recurrences were detected in 36% of patients in the MMRd group, compared to 10% and

Table 2
Pattern of recurrence by molecular subgroup.

Pattern of recurrence *N (%)*	Total *N* = 101	*POLE*mut *N* = 2	MMRd *N* = 33	p53ab *N* = 30	NSMP *N* = 36
Locoregional	31 (30.7)	0 (0)	15 (45.5)	6 (20.0)	10 (27.8)
Abdominal	30 (29.7)	0 (0)	6 (18.2)	13 (43.3)	11 (30.6)
Distant	40 (39.6)	2 (100)	12 (36.4)	11 (36.7)	15 (41.7)

Abbreviations: N = number, POLEmut = polymerase epsilon ultramutated, MMRd = mismatch repair deficient, p53abn = p53 abnormal, NSMP = non-specific molecular profile.

25% in the p53abn and NSMP group respectively ($P = .083$). On the other hand, non-locoregional recurrences were found most frequently in patients in the p53abn group, with 80% compared to 54% and 72% in the MMRd and NSMP groups respectively ($P = .089$). Thirty-one patients experienced mixed recurrences with multiple dissemination pathways: 13 had simultaneous locoregional and abdominal recurrences, two were combined locoregional and distant recurrences, 12 had abdominal and distant recurrences, and four presented with all three dissemination pathways of recurrences. The seventy patients presenting with only one dissemination pathway consisted of 31 locoregional, 17 abdominal, and 22 distant recurrences. p53abn patients showed the most mixed recurrences (40%) compared to the MMRd group (21%) and the NSMP group (28%, $P = .057$). Pattern of recurrence correlated significantly with the type of adjuvant treatment given in first line: Patients who had received adjuvant radiotherapy or chemoradiation experienced more distant recurrences (51%) compared to radiotherapy-naïve patients who presented with more locoregional recurrences (42%, $P = .001$) (supplementary material, Table S4). Of the 31 patients experiencing locoregional recurrence, 10 patients (32%) had received adjuvant radiotherapy or chemoradiation after primary staging surgery. Divided by molecular subgroup, 80% of the locoregional recurrences each in the MMRd and NSMP subgroup were radiotherapy-naïve compared to 17% in the p53abn subgroup ($P = .012$). As expected, patients with early stage primary tumors experienced significantly more locoregional recurrences (51% in FIGO stage I, 36% in stage II, 15% in stage III, and 5% in stage IV, $P < .001$) (Table S4).

3.5. Survival after recurrence

Eighty-seven patients received treatment for recurrence, with the majority being treated with chemotherapy and radiotherapy (Table 1). Sixty-nine patients died during follow-up: one *POLE*mut case, 19 MMRd cases, 25 p53abn cases, and 24 NSMP cases. Median survival after recurrence was 23 months (95% CI 6–40) among all subgroups, with the best survival for MMRd cases, followed by NSMP, p53abn, and *POLE*mut cases, with medians of 43 months (95% CI 11–76), 39 months (95% CI 21–57), 10 months (95% CI 7–13), and 6 months respectively (log-rank, $P = .001$). The corresponding Kaplan-Meier survival curves for the survival time after recurrence are shown in Fig. 2; patients with residual tumor or metastatic disease and *POLE*mut cases are excluded. The one-year survival rate after recurrence was 78% for MMRd, 66% for NSMP, and 32% for p53abn tumors. In univariable analysis, the following clinicopathological factors were associated with the risk of death after recurrence: non-endometrioid histological subtype, tumor size, high-grade histology, FIGO stage > I, molecular classification (p53abn and *POLE*mut subgroup), adjuvant treatment, and non-locoregional pattern of recurrence. Non-locoregional pattern of recurrence remained a significant independent predictor of risk of death after recurrence in the multivariable analysis (HR 2.99, 95% CI 1.15–7.78, $P = .025$)(Table 3). Locoregional recurrences were significantly associated with an improved survival after recurrence in MMRd and NSMP tumors (log-rank, $P = .034$ and $P = .001$) but not in p53abn patients (log-rank, $P = .495$) (Fig. 3).

4. Discussion

Endometrial cancer recurrence remains an important clinical challenge as the prognosis is inevitably poor in these patients and better tailored approaches in both the adjuvant and relapsed setting are needed. In our large multicenter study, we evaluated for the first time the associations between the molecular subtype at primary diagnosis and time to first recurrence, pattern of recurrence, and survival after recurrence in patients with endometrial cancer. Our results demonstrate different patterns of recurrence among the molecular subgroups: More locoregional recurrences in the MMRd group, more abdominal recurrences in the p53abn group, and more distant recurrences in the NSMP group. MMRd patients presented with the shortest median time to first recurrence but the longest survival after recurrence.

In our study population, 41% of the recurrences occurred during the first year after primary surgical treatment, and 67% occurred in the first two years; this is consistent with current literature reporting that about 70% of recurrences in women with endometrial cancer occur within the first two years after surgery [5,30]. Median time to first recurrence was 16 months in our study population, which was slightly above the

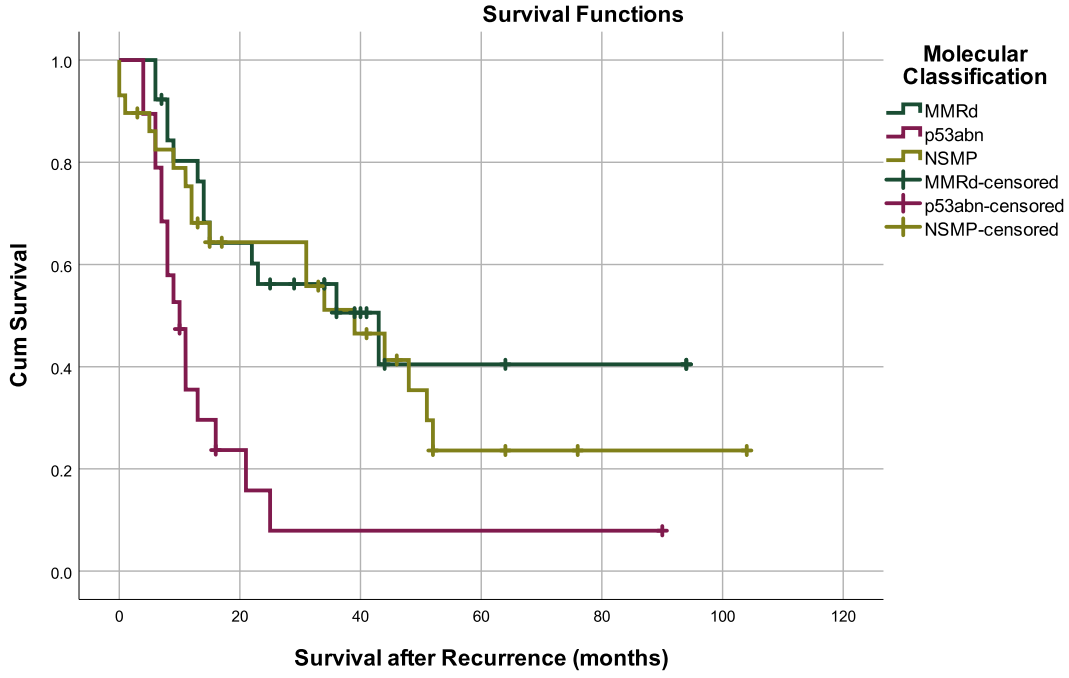


Fig. 2. Kaplan-Meier survival curves for survival after recurrence in different molecular subgroups, *POLE*mut cases and patients with residual tumor or metastatic disease excluded (log-rank, $P < .001$). Abbreviations: MMRd = mismatch repair deficient, p53abn = p53 abnormal, NSMP = non-specific molecular profile.

Table 3
Cox regression for univariable and multivariable analysis for risk of death after recurrence according to clinicopathological features, excluding 24 patients with residual tumor or metastatic disease.

Clinicopathological factor	Univariable analysis			Multivariable analysis		
	HR	95% CI	*P*-value	*HR*	95% CI	*P*-value
Age at recurrence (years)	1.0	0.97–1.04	0.841	–	–	–
Histologic subtype			0.008			0.731
- Endometrioid (ref)	1.0	Reference		1.0	Reference	
- Non-endometrioid	2.23	1.23–4.04		1.17	0.49–2.80	
Tumorsize (mm)	1.02	1.03–1.03	0.001	1.0	0.99–1.02	0.323
LVSI			0.225	–	–	–
- no (ref)	1.0	Reference				
- yes	1.43	0.80–2.54				
Grading			0.002			0.235
- low grade (G1, G2) (ref)	1.0	Reference		1.0	Reference	
- high grade (G3)	2.52	1.40–4.52		1.65	0.72–3.75	
FIGO stage			0.005			0.294
- I (ref)	1.0	Reference		1.0	Reference	
- > I	2.29	1.30–4.06		1.43	0.74–2.77	
Lymph node status			0.494	–	–	–
- negative (ref)	1.0	Reference				
- positive	1.26	0.65–2.42				
Molecular subgroup			0.002			0.359
- *POLE*mut	8.34	1.03–67.8		4.84	0.52–45.4	
- MMRd	0.75	0.37–1.53		0.99	0.47–2.18	
- p53abn	2.60	1.29–5.25		1.84	0.75–4.49	
- NSMP (ref)	1.0	Reference		1.0	Reference	
Adjuvant therapy			0.001			0.969
- none (ref)	1.0	Reference		1.0	Reference	
- yes	3.18	1.60–6.33		1.02	0.39–2.69	
Time to first recurrence (months)	0.99	0.98–1.00	0.157	–	–	–
Pattern of recurrence			<0.001			0.025
- locoregional (ref)	1.0	Reference		1.0	Reference	
- non-locoregional	3.90	1.98–7.65		2.99	1.15–7.78	

Abbreviations: N = number, LSVI = lymphovascular space invasion, FIGO = Federation International de Gynecologie et Obstetrique, HR = Hazard ratio, CI = confidence interval.

previously published 13 months in the study of Bendifallah et al. [6] A remarkable proportion of almost 10% of the patients in our study experienced recurrence after more than five years after primary treatment. This is an interesting finding, as most follow-up investigations end after five years. In our cohort the numerically shortest median time to first recurrence was observed in the MMRd subgroup (13 months) which is in accordance with the current literature: Kim et al. [31] investigated the impact of mismatch repair status on endometrial cancer recurrence, demonstrating a worse recurrence-free survival in MMRd cases compared to mismatch repair proficient (MMRp) cases in univariate analysis. There is also evidence of a shorter time to first recurrence in high-risk endometrial cancer patients and patients with non-endometrioid histologies [30].

In the current study, we could demonstrate distinct patterns of recurrence according to molecular subgroups, with more locoregional recurrences in the MMRd group (Table 2). In the study of Kim et al. [31], MMRd endometrial cancer showed a unique recurrence pattern involving retroperitoneal lymph nodes, compared to more distant recurrences in the MMRp group [31]. Due to the retrospective design of our study, it is challenging to untangle the associations between the adjuvant treatment given based on risk classification and the molecular subgroups, which were retrospectively applied. Along with the GOG-249 [33] and GOG-258 [34] trial showing that radiotherapy reduces the incidence of lymph node recurrences, radiotherapy-naïve patients presented with significantly more locoregional recurrences in our study (Table S4). One may therefore argue that the high number of locoregional reoccurrence occur mainly in radiotherapy-naïve patients, representing 55% of the MMRd group. However, even if the proportion of radiotherapy-naïve patients is similar in the NSMP group (42%), pattern of recurrence in this group differs with the majority of patients presenting with distant recurrence. Together with the fact that the proportion of patients who had received radiotherapy in first line is similar across the molecular groups, we therefore think that the locoregional pattern of recurrence is truly associated with the MMR status. p53abn patients experienced most frequently abdominal recurrences in our cohort, in line with precedent literature demonstrating uterine serous carcinomas- representing the vast majority of p53abn tumors - to relapse commonly with abdominal disease [32].

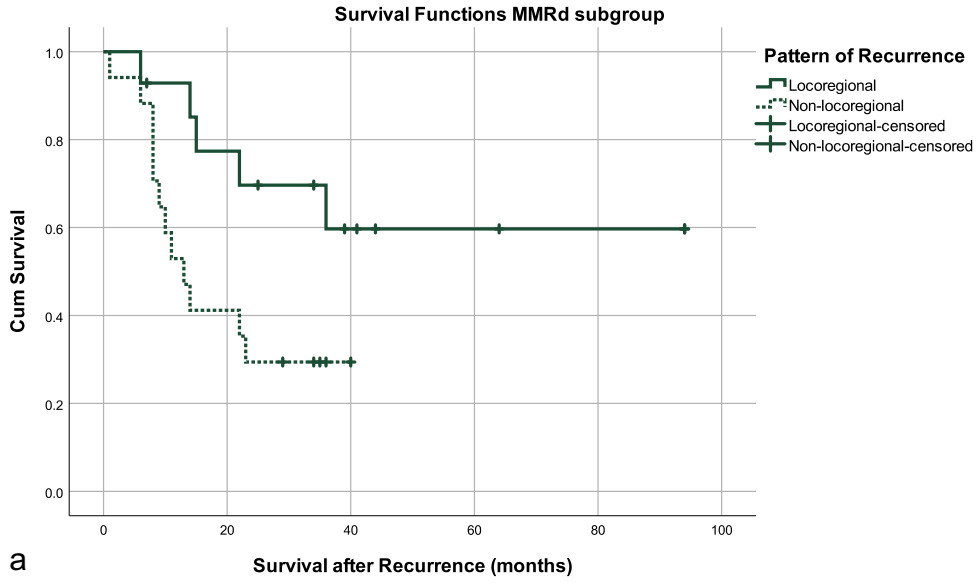
In our study, median survival after recurrence was 23 months (95% CI 6–40), which is in line with the previous literature reporting median survival rates between one and two years after recurrence [5,6,9,14]. With our data, the molecular classification of the primary tumor remained a significant factor for survival after recurrence (Fig. 2), emphasizing the need to consider the molecular groups in tailoring of adjuvant treatment and follow-up. Until now, it is well established that clinicopathological characteristics predict the risk of recurrences and death such as in patients with serous uterine carcinomas who often present with disseminated disease and face poor oncological outcome [9,32]. The short survival after recurrence in the p53abn subgroup underlines the urgent need to improve treatment strategies in patients with abdominal or distant failure. The poor prognosis even of locoregional recurrence in p53abn patients (Fig. 3b) may be explained by the circumstance that only 17% of the p53abn patients experiencing locoregional recurrence were radiotherapy-naïve. The rather favorable prognosis for MMRd endometrial cancer recurrence is presumably supported by the combination of high proportions of locoregional recurrences and treatment-naïve patients in this subgroup, leading to a greater chance of curative treatment with salvage radiotherapy or surgery. This is underlined by the fact that 80% of the MMRd patients with locoregional recurrence were radiotherapy-naïve.

Our study provides evidence of different intrinsic tumor biologies among the molecular subgroups affecting the pattern of recurrence and the survival after recurrence, but the association of molecular subgroup, pattern of recurrence, and oncological outcome should further be studied in prospective studies.

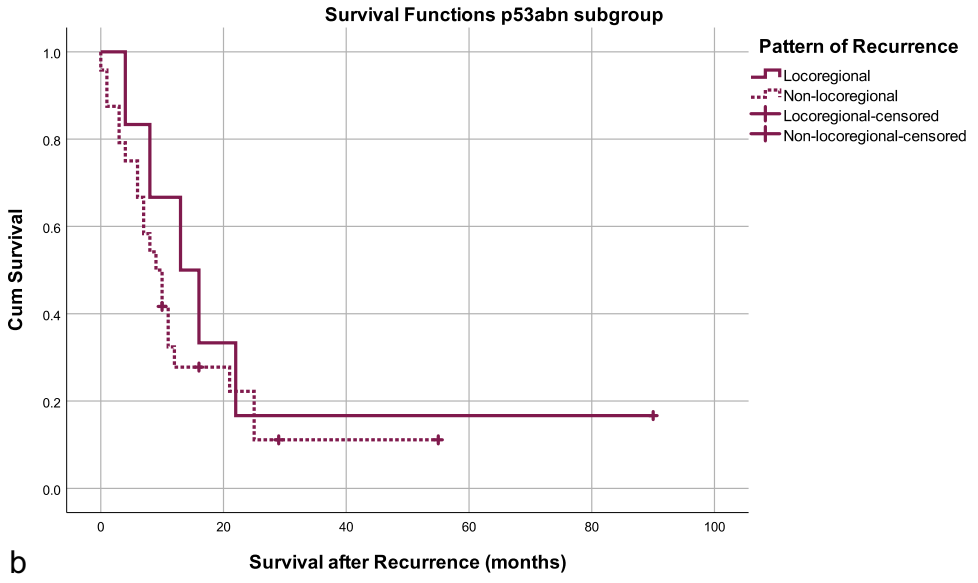
Since *POLE*mut tumors are known to be associated with an excellent prognosis, the two patients of recurrence are of great interest. The recurrent cases both had p53 mutations, had high-grade non-endometrioid histology, LVSI positive and recurred after adjuvant treatment (chemotherapy and chemoradiation). Out of the whole population, ten patients showed simultaneous *POLE* and p53 mutations (double classifiers) and two of those (20%) experienced recurrence. Leon-Castillo et al. [28] investigated 31 *POLE*mut-p53abn endometrial cancer cases, showing an excellent 5-year recurrence-free survival of 91% and therefore concluding that *POLE*mut-p53abn double classifiers should be categorized as *POLE*mut with the potential to de-escalate treatment. Noteworthily, the double classifiers in the population of Leon-Castillo et al. exhibited more morphological features characteristic of *POLE*mut endometrial cancers such as endometrioid histology and early stage, with only 10% showing LVSI. We therefore need better understanding of the double classifiers before we can safely suggest de-escalating adjuvant treatment.

4.1. Clinical relevance of our findings

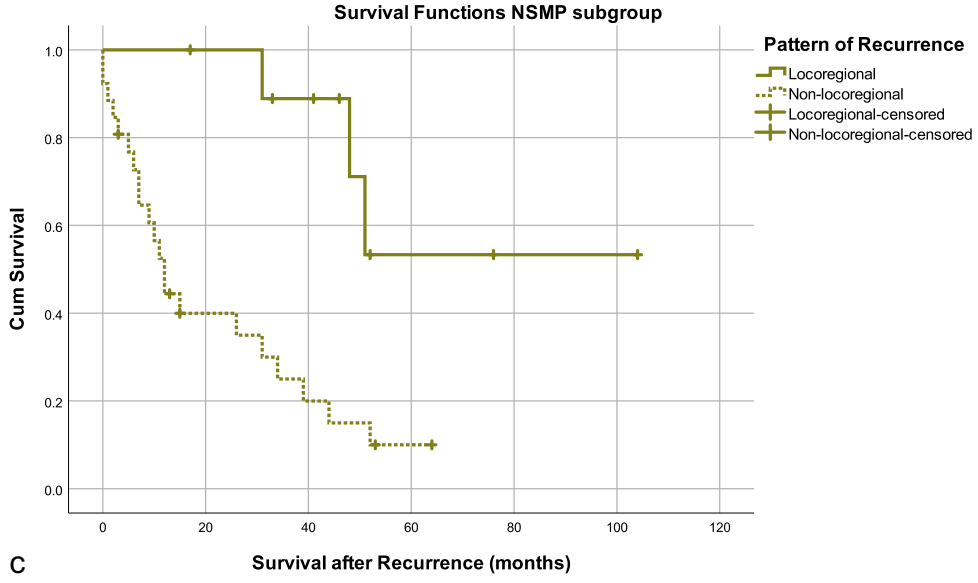
Our study provides additional data to understand the role of molecular classification for endometrial cancer recurrences. Time to first recurrence, pattern of recurrence, and survival after recurrence provide useful information to guide recommendations for follow-up and to tailor adjuvant treatment. Proper follow-up is mandatory, with the goal of an early detection and treatment of recurrences, resulting in an improvement in the survival rate. This is particularly important in patients with locoregional recurrences, offering them a chance for curative treatment with either radiotherapy or surgery. However, data on the frequency and duration of endometrial cancer follow-up after primary treatment are not available.



a



b



c

Fig. 3. Kaplan-Meier survival curves for survival after recurrence according to the pattern of recurrence among different molecular subgroups: a MMRd subgroup (log-rank, $P = .034$); b p53abn subgroup (log-rank, $P = .495$), c NSMP subgroup (log-rank, $P = .001$). Abbreviations: MMRd = mismatch repair deficient, p53abn = p53 abnormal, NSMP = non-specific molecular profile.

Optimization of adjuvant treatment in endometrial cancer is still a subject of debate, and the incorporation of the molecular classification into treatment decision-making algorithms is recommended. The role of immunotherapy in addition to front-line treatment is currently being investigated, and may change outcome particularly in MMRd patients who respond well to immunotherapy in first- or second-line treatment [36,37].The role of adjuvant radiotherapy to prevent locoregional recurrences need to be considered in the context of potentially high cure rates in radiotherapy-naïve patients. On the other hand, p53abn endometrial cancer patients experience recurrence more often abdominally, sustaining the rationale for systemic treatment with adjuvant chemotherapy in this patient cohort, analog to ovarian cancer treatment. [38,39] Given the poor survival in recurrent p53abn endometrial cancer, consideration of more aggressive upfront and second-line treatment in these patients should be considered, as for example with Trastuzumab in Her2/neu positive tumors [40]. Lastly, *POLE*mut-p53abn double classifiers may still have a high chance of recurrence particularly when presenting with typical characteristics of p53abn endometrial cancers. Attempts to de-escalate adjuvant treatment such as the TAPER study (https://clinicaltrials.gov/ct2/show/NCT04705649) do therefore include *POLE*mut/p53wt patients only.

4.2. Strengths and weaknesses

To the best of our knowledge, this is the first study analyzing the association of molecular classification with pattern of recurrence and survival after recurrence in endometrial cancer. The major strengths of the current study include its large sample size, its multicenter design, and the length of follow-up. The most important limitation is the retrospective study design and the missing information on the molecular subtype of the recurrent tumor.

5. Conclusion

In conclusion, endometrial cancer molecular subgroups show distinct patterns of recurrence and different outcomes after recurrence. MMRd endometrial cancers show more locoregional recurrences and had the best survival after recurrence. By contrast, p53abn cancers recur more often abdominally and have the worst prognosis after recurrence. Our results further highlight the different intrinsic tumor biologies among the molecular subgroups and their prognostic importance even in recurrent endometrial cancer.

Supplementary data to this article can be found online at https://doi.org/10.1016/j.ygyno.2022.02.024.

Credit authorship contribution statement

F. Siegenthaler: Conceptualization, Methodology, Validation, Formal analysis. **K. Lindemann:** Conceptualization, Methodology, Validation, Supervision. **E. Epstein:** Data curation, Validation, Investigation, Resources. **T.T. Rau:** Data curation, Validation, Investigation, Resources. **D. Nastic:** Data curation, Validation, Investigation, Resources. **M. Ghaderi:** Methodology, Data curation. **F. Rydberg:** Methodology, Data curation. **M.D. Mueller:** Conceptualization, Methodology, Validation. **J. Carlson:** Data curation, Validation, Investigation, Resources. **S. Imboden:** Conceptualization, Methodology, Validation, Supervision.

Declaration of Competing Interest

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SI participates on advisory board of GSK.

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