Prognostic value of molecular classification in stage IV endometrial cancer

Uijterwaal, M.H.; Dijk, D. van; Lok, C.A.R.; Kroon, C.D. de; Kasius, J.C.; Zweemer, R.; ... ; Nooij, L.S.

Prognostic value of molecular classification in stage IV endometrial cancer

Margot H Uijterwaal,[1] Dione van Dijk,[2] Christianne A R Lok ,[3] Cor D De Kroon,[4] Jenneke C Kasius,[5] Ronald Zweemer,[6] Cornelis G Gerestein,[6] Nanda Horeweg ,[7] Tjalling Bosse,[2] Jacolien van der Marel,[8] Linda S Nooij [4]

For numbered affiliations see end of article.

Correspondence to
Dr Linda S Nooij, Department of Gynecological Oncology, Leiden University Medical Center, Leiden 2300 RC, The Netherlands; l.s.nooij@lumc.nl

MHU, JvdM and LSN contributed equally.

Received 23 October 2023
Accepted 11 April 2024

To cite: Uijterwaal MH, van Dijk D, Lok CAR, *et al. Int J Gynecol Cancer* Published Online First: [please include Day Month Year]. doi:10.1136/ijgc-2023-005058

ABSTRACT

Objectives Multiple studies have proven the prognostic value of molecular classification for stage I–III endometrial cancer patients. However, studies on the relevance of molecular classification for stage IV endometrial cancer patients are lacking. Hypothetically, poor prognostic molecular subtypes are more common in higher stages of endometrial cancer. Considering the poor prognosis of stage IV endometrial cancer patients, it is questionable whether molecular classification has additional prognostic value. Therefore, we determined which molecular subclasses are found in stage IV endometrial cancer and if there is a correlation with progression-free and overall survival.

Methods A retrospective multicenter cohort study was conducted using data from five Dutch hospitals. Patients with stage IV endometrial cancer at diagnosis who were treated with primary cytoreductive surgery or cytoreductive surgery after induction chemotherapy between January 2000 and December 2018 were included. Exclusion criteria were age <18 years or recurrent disease. The molecular classification was performed centrally on all tumor samples according to the World Health Organization 2020 classification (including *POLE* and estrogen receptor status). The Kaplan–Meier method was used to calculate progression free and overall survival in the molecular subclasses, for the different histological subtypes and for estrogen receptor positive versus estrogen receptor negative tumors. Groups were compared using the log-rank test.

Results 164 stage IV endometrial cancer patients were molecularly classified. Median age of the patients was 67 years (range 33–86). Most patients presented with a non-endometrioid histological subtype (58%). Intra-abdominal complete cytoreductive surgery was achieved in 60.4% of the patients. 101 tumors (61.6%) were classified as p53 abnormal, 35 (21.3%) as no specific molecular profile, 21 (12.8%) as mismatch repair deficient, and 6 (3%) as *POLE* mutated. Molecular classification had no significant impact on progression free (p=0.056) or overall survival (p=0.12) after cytoreductive surgery. Overall survival was affected by histologic subtype (p<0.0001) and estrogen receptor status (p=0.013).

Conclusion The distribution of the molecular subclasses in stage IV endometrial cancer patients differed substantially from the distribution in stage I–III endometrial cancer patients, with the unfavorable subclasses being more frequently present. Although the molecular classification was not prognostic in stage IV endometrial cancer, it could guide adjuvant treatment decisions.

WHAT IS ALREADY KNOWN ON THIS TOPIC

⇒ Cytoreductive surgery might be beneficial for stage IV endometrial cancer patients if complete cytoreductive surgery can be achieved.
⇒ Molecular subclass might also influence prognosis and choice of (adjuvant) treatment in this patient group.

WHAT THIS STUDY ADDS

⇒ This is the first study to investigate the value of molecular classification on overall and progression free survival in surgically treated stage IV endometrial cancer patients.
⇒ This study showed that the distribution of molecular classification was different compared with earlier stages of endometrial cancer.
⇒ Molecular subtype was not associated with differences in progression free or overall survival.

HOW THIS STUDY MIGHT AFFECT RESEARCH, PRACTICE OR POLICY

⇒ This study can form the basis for further prospective research on the influence of molecular classification on prognosis and treatment choice in this rare patient group with a poor prognosis.

INTRODUCTION

Most endometrial cancer patients present with an early, curable stage. On rare occasions, endometrial cancer patients present with distant metastases at diagnosis.[1] This is a challenging clinical scenario, often resulting in a poor prognosis and a high risk of endometrial cancer related death.[2 3] For patients with lymph node metastases (stage III), primary and adjuvant treatment guidelines are well established.[1 4 5] In contrast, the optimal treatment is not very clear in stage IV disease at diagnosis. The European Society of Gynaecological Oncology–European Society for Radiotherapy and Oncology–European Society of Pathology guideline recommends that cytoreductive surgery should be considered for all stage IV endometrial cancer patients if complete resection is deemed feasible.[6] This is, however, controversial because the benefit of cytoreductive surgery in stage IV endometrial cancer has not been studied in randomized controlled trials. Only small, retrospective studies on the role of cytoreductive surgery in stage

IV endometrial cancer patients are available.[5 7–9] A meta-analysis from 2010 includes a large but heterogeneous group of stage III and IV primary and recurrent endometrial cancer patients. The results from this meta-analysis suggest that complete cytoreductive surgery is associated with superior overall survival, compared with optimal and incomplete cytoreductive surgery.[3]

According to the Cancer Genome Atlas, endometrial cancer is classified into four molecular subclasses,[10] using clinically implementable surrogate markers[11]: *POLE* mutated (*POLE*mut), mismatch repair deficient (MMRd), p53 abnormal (p53abn), and no specific molecular profile (NSMP).[12] This classification has strong and independent prognostic value in International Federation of Gynecology and Obstetrics (FIGO) stages I–III endometrial cancer[13–15] and guides adjuvant treatment choices.[14 16]

Given the aforementioned studies on the prognostic value of the molecular classification in stages I–III endometrial cancer, we questioned the value of the molecular classification in stage IV endometrial cancer patients. Therefore, we retrospectively determined the molecular classification of the tumor of stage IV endometrial cancer patients who underwent cytoreductive surgery and determined whether molecular classification correlated with progression-free and overall survival.

METHODS

Patient Selection and Study Design

After ethics approval, clinicopathological patient data and formalin fixed, paraffin embedded tumor blocks of all patients with stage IV endometrial cancer at diagnosis (FIGO 2009)[17] were retrospectively collected. Patients had undergone primary cytoreductive surgery or cytoreductive surgery after induction chemotherapy, between 2000 and 2018 in one of the five participating hospitals. Four tertiary referral centers and one general district hospital in the Netherlands contributed patients to the study. Exclusion criteria were age <18 years or recurrent disease.

Clinical characteristics, including age at diagnosis, World Health Organization (WHO) performance status, extent of the primary tumor, surgical result, (neo)adjuvant treatment and progression, and survival data, were collected from the medical records. In accordance with Dutch guidelines, endometrial cancer patients had a preoperative chest X-ray. Patients with high grade endometrial cancer underwent CT of the thorax and abdomen. Complete cytoreductive surgery was defined as no visible macroscopic residual tumor after surgery, optimal as visible residual tumor <1 cm after surgery, and incomplete as visible residual tumor ≥1 cm after surgery. Pathology data on histological classification and grade were collected from local pathology reports and centrally revised by one gynecopathologist.

Molecular Classification

All available tumor samples were molecularly classified according to the WHO classification algorithm. Immunohistochemistry was performed on 4 µm whole tumor slides for p53 and mismatch repair proteins (MLH1, PMS2, MSH2, and MSH6). P53 immunohistochemistry was scored as either wild-type or abnormal expression pattern.[18] Abnormal p53 expression included strong positive p53 expression in >80% of the tumor nuclei, significant cytoplasmic p53 expression in >80% of the tumor, or complete absence of p53 expression (with positive internal control).[18 19] Mismatch repair deficiency was confirmed if >10% of the tumoral nuclei showed lack of nuclear staining in one or more of the mismatch repair proteins with the presence of a positive internal control. *POLE* mutational status was assessed with the Q-*POLE* method.[20] If the Q-*POLE* method was unequivocal or failed, the KASPar competitive allele specific polymerase chain reaction was performed (LGC Genomics, Berlin, Germany). Tumors were considered *POLE*mut if a previously described pathogenic somatic mutation in *POLE* was established.[21] Estrogen receptor status was determined using immunohistochemistry; tumors were considered positive if ≥10% of tumor cells showed positive estrogen receptor staining.[22] In addition, next generation sequencing was performed on all tumors using the Ampliseq Cancer Hotspot Panel version 6, covering the complete *TP53* gene as well as hotspot sites of frequently mutated genes in endometrial cancer.

Statistical Analysis

Differences between the molecular subclasses in non-normally distributed continuous variables (age) and ordinal variables (WHO performance status) were tested for significance using the Kruskal–Wallis H test, and differences between nominal variables were assessed using the Fisher–Freeman–Halton test.

Time to death was defined as the time from cytoreductive surgery to death due to any cause, with censoring at the last follow-up in alive patients. Time to progression was defined as time from cytoreductive surgery to recurrence or progression of residual tumor, with censoring at the last follow-up or death. Actuarial survival times were estimated according to Kaplan–Meier's methodology and compared between groups using log rank tests. Median follow-up was calculated by the reverse Kaplan–Meier method.

To investigate the independence of any prognostic impact of clinicopathological and molecular tumor features, univariable and subsequently multivariable Cox's proportional hazards models for overall and progression free survival were built, including the features with a significant association with the two outcomes, while limiting the number of covariates to prevent overfitting. All p values were two sided, and statistical significance was accepted at $p<0.05$. Statistical analyses were performed using SPSS V.25 and R V.4.2.3 (http://www.r-project.org/). R packages used in this study included survival, rms, ggPlot2, and Survminer.

RESULTS

A total of 219 patients with stage IV endometrial cancer at diagnosis underwent cytoreductive surgery during the study period. Formalin fixed, paraffin embedded tissue blocks for molecular classification were available from 168 patients, and these patients were included in this study. All required analyses were completed successfully in 164 patients and their tumors could be molecularly classified.

Patient Characteristics

Patient and tumor characteristics are shown in Table 1. Most patients presented with intra-abdominal metastases (124/164, 76%). This was equally distributed between the four molecular subclasses. Localisation of the most severe metastases is described in Table 1. A total of 86 of 164 patients (52.4%) received

Table 1 Patient and tumor characteristics by molecular classification

Characteristics	Molecular subclass					
	*POLE*mut	MMRd	p53abn	NSMP	Total	
	No (%)	No (%)	No (%)	No (%)	No (%)	P value
No of patients	6 (3.7)	21 (12.8)	101 (61.6)	35 (21.3)	164 (100)	
Age (years) (median (range))	62 (48–78)	65 (45–80)	70 (33–86)	65 (40–85)	67 (33–86)	0.023*
WHO status†						
0	5 (83.3)	13 (61.9)	57 (56.4)	20 (57.1)	90 (54.9)	0.34*
1	0 (0.0)	2 (9.5)	18 (17.8)	11 (31.4)	30 (18.3)	
2	0 (0.0)	1 (4.8)	1 (1.0)	1 (2.9)	4 (2.4)	
3	0 (0.0)	2 (9.5)	1 (1.0)	0 (0.0)	3 (1.8)	
Definition of stage IV						
Only intra-abdominal metastases‡	6 (100)	17 (80.9)	71 (70.3)	30 (85.7)	124 (75.6)	0.87§
Bladder invasion	1 (16.7)	2 (9.5)	1 (1.0)	0 (0.0)	4 (2.4)	0.26§
Bowel invasion	1 (16.7)	3 (14.3)	5 (5.0)	2 (5.7)	11 (6.7)	
Pelvic peritoneal metastasis	1 (16.7)	2 (9.5)	6 (5.9)	5 (14.3)	14 (8.5)	
Intra-abdominal peritoneal metastasis	3 (50.0)	10 (47.6)	59 (58.4)	23 (65.7)	95 (57.9)	
Intra- and extra-abdominal metastases	0 (0.0)	3 (14.3)	16 (15.8)	5 (14.3)	24 (14.6)	
Liver metastasis	0 (0.0)	0 (0.0)	1 (1.0)	0 (0.0)	1 (0.6)	
Pulmonary metastasis	0 (0.0)	2 (9.5)	10 (9.9)	3 (8.6)	15 (9.1)	
Bone metastasis	0 (0.0)	0 (0.0)	1 (1.0)	2 (5.7)	3 (1.8)	
Supraclavicular lymph node metastasis	0 (0.0)	1 (4.8)	4 (4.0)	0 (0.0)	5 (3.0)	
Histotype and grade¶						
Endometrioid grades 1–2	0 (0.0)	9 (42.9)	7 (6.9)	15 (42.9)	31 (18.9)	<0.001§
Endometrioid grade 3	4 (66.7)	10 (47.6)	13 (12.9)	8 (22.9)	35 (21.3)	
Serous	1 (16.7)	0 (0.0)	61 (60.4)	1 (2.9)	63 (38.4)	
Clear cell	1 (16.7)	2 (9.5)	7 (6.9)	9 (25.7)	19 (11.6)	
Undifferentiated	0 (0.0)	0 (0.0)	3 (3.0)	1 (2.9)	4 (2.4)	
Carcinosarcoma	0 (0.0)	0 (0.0)	8 (7.9)	1 (2.9)	9 (5.5)	
Estrogen receptor expression (>10%)	2 (33.3)	17 (81.0)	39 (38.6)	19 (54.3)	77 (46.9)	0.002§
Cytoreductive surgery						
Complete	5 (83.3)	16 (76.2)	57 (56.4)	21 (60.0)	99 (60.4)	0.018§
Optimal (<1 cm residual disease)	1 (16.7)	0 (0.0)	18 (17.8)	11 (31.4)	30 (18.3)	
Incomplete (>1 cm residual disease)	0 (0.0)	5 (23.8)	26 (25.7)	3 (8.6)	34 (20.7)	
Other therapies**						
None	1 (16.7)	4 (19.0)	19 (18.8)	9 (25.7)	33 (20.1)	0.48§
Neoadjuvant chemotherapy	0 (0.0)	0 (0.0)	3 (3.0)	1 (2.9)	4 (2.4)	
Neoadjuvant and adjuvant chemotherapy	0 (0.0)	3 (14.3)	17 (16.8)	1 (2.9)	21 (12.8)	
Adjuvant chemotherapy and/or radiotherapy	3 (50.0)	6 (28.6)	35 (34.7)	17 (48.6)	61 (37.2)	

*Kruskal–Wallis test.
†In 31 patients World Health Organization (WHO) performance status was unknown.
‡In 15 patients, localization of the most severe metastases was missing.
§Fisher–Freeman–Halton test.
¶In 2 patients, histotype could not be assessed.
**In 44 patients, no information on therapies other than surgery was available.
MMRd 1, mismatch repair deficient; NSMP, no specific molecular profile; p53abn, p53-abnormal; POLEmut, POLE mutant.

(neo)adjuvant chemotherapy or radiotherapy. Data on treatment with immunotherapy or targeted therapy were unavailable.

Tumor Characteristics

Sixty-six (66/164, 40.2%) tumors were endometrioid and 95 (95/164, 58%) were non-endometrioid endometrial cancer. Histotype was missing for two tumors. In total, 61.6% of tumors (101/164) were classified as p53abn, 21.3% as NSMP (35/164), 12.8% as MMRd (21/164), and 3.7% as *POLE*mut (6/164). *POLE*mut, NSMP, and MMRd subclasses were correlated with endometrioid histology (4/6 (66.7%); 23/35 (65.7%); and 19/21 (90.5%), respectively, p<0.001). Serous carcinoma was found most frequently in p53abn tumors: 61/101 (60.40%, p<0.001). Eight of nine (88.9%) carcinosarcomas and three of four (75%) undifferentiated tumors were p53abn. Estrogen receptor expression was found in all molecular subclasses. Estrogen receptor positive tumors were found in 81% of MMRd (17/21), 33.3% of *POLE*mut (2/6), and 38.6% of the p53abn (39/101) tumors.

Cytoreductive Surgery

Intra-abdominal complete cytoreductive surgery, defined as no visible macroscopic residual tumor after surgery, was achieved in 60.4% (99/164) of all patients. There was a significant difference in the incidence of complete cytoreductive surgery between the molecular subclasses (p=0.018). In five of six patients with a *POLE*mut tumor, complete cytoreductive surgery was accomplished. In the MMRd group, complete cytoreductive surgery was achieved in 16/21 patients (76.2%). In contrast, the p53abn and NSMP groups had the lowest rate of complete cytoreductive surgery. Complete cytoreductive surgery was achieved in 66.7% of patients with an estrogen receptor positive tumor (52/78) and in 54.7% of patients with an estrogen receptor negative tumor (47/86).

Prognostic Factors

Median overall survival was 35 months for patients with NSMP tumors, 30 months for patients with *POLE*mut tumors, 17 months for patients with p53abn tumors, and 24 months for patients with MMRd tumors. Overall survival did not differ between the four molecular subclasses (Figure 1, curve A). Median progression free survival was 12 months in patients with p53abn tumors, compared with 25 and 26 months for patients with MMRd and NSMP tumors. Median progression free survival in patients with *POLE*mut tumors was not reached. Two patients with a *POLE*mut tumor died of non-cancer related causes. One patient with a *POLE*mut tumor had disease progression 13 months after cytoreductive surgery and died after 30 months. One patient with a *POLE*mut tumor had recurrence after 7 months and was still alive 14 months after primary treatment. The remaining two patients were alive without disease progression at 38 and 70 months after primary treatment.

Univariable analysis showed a difference in overall and progression free survival for histologic subtype and grade (Figure 2). Low grade endometrioid endometrial cancer was associated with better overall and progression free survival compared with high grade endometrioid endometrial cancer and non-endometroid endometrial cancer. Although the number of carcinosarcomas and undifferentiated endometrial cancers was low in this study, they seemed to have the worst prognosis.

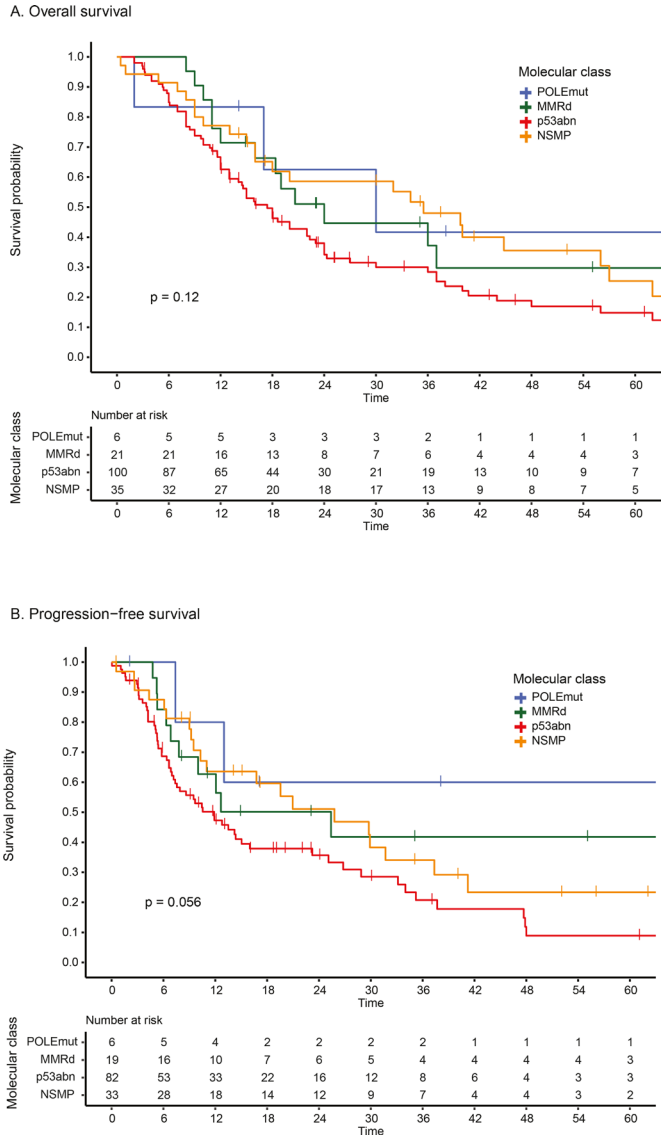

Figure 1 Clinical outcomes of stage IV endometrial cancer by molecular subclass. EC, endometrial cancer; MMRd, mismatch repair deficient; NSMP, no specific molecular profile; POLEmut, *POLE* mutant; p53abn, p53 abnormal.

Patients with an estrogen receptor positive tumor had better overall and progression free survival compared with estrogen receptor negative endometrial cancer (31 months vs 16 months, p=0.013, and 27 months vs 9 months, p=0.005, respectively) (Figure 3). Estrogen receptor status did not affect overall (p=0.72) or progression free survival (p=0.11) in the NSMP subclass (Figure 4).

Multivariate Analysis

Multivariate analysis (Table 2) showed that complete cytoreductive surgery was associated with improved progression free and overall survival. Non-endometrioid histotype was associated with decreased progression-free survival, but not with decreased overall survival. Molecular classification and estrogen receptor expression did not influence overall and progression free survival in this multivariable analysis.

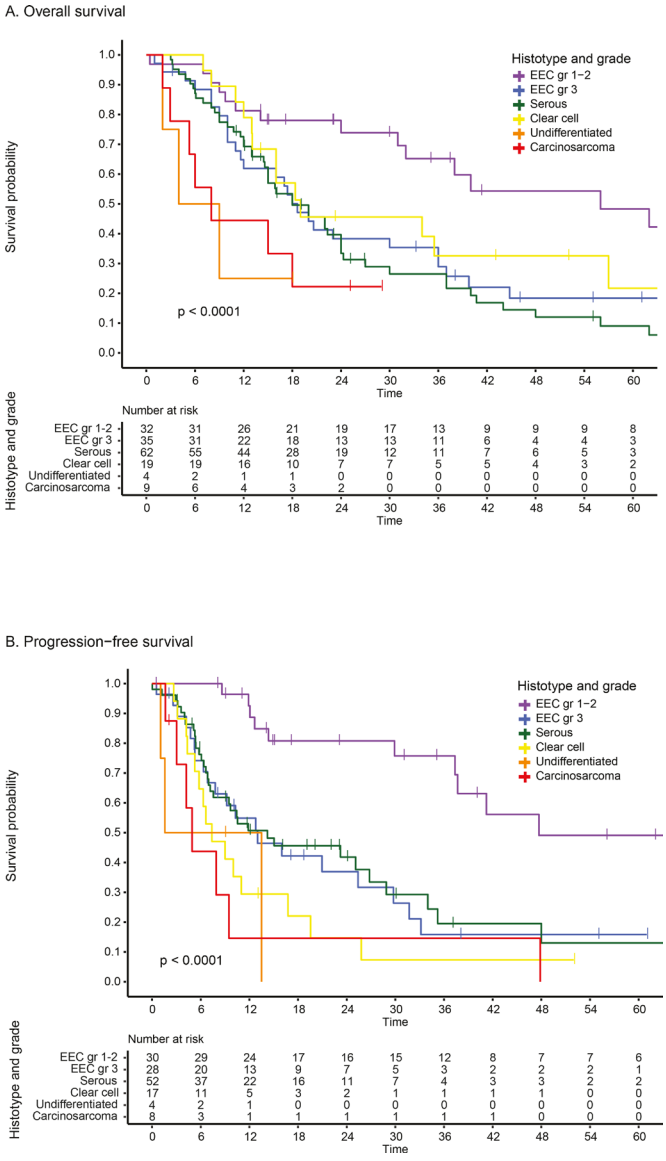

Figure 2 Clinical outcomes of stage IV endometrial cancer by histotype and tumor grade. EEC, endometrioid endometrial cancer; gr, grade.

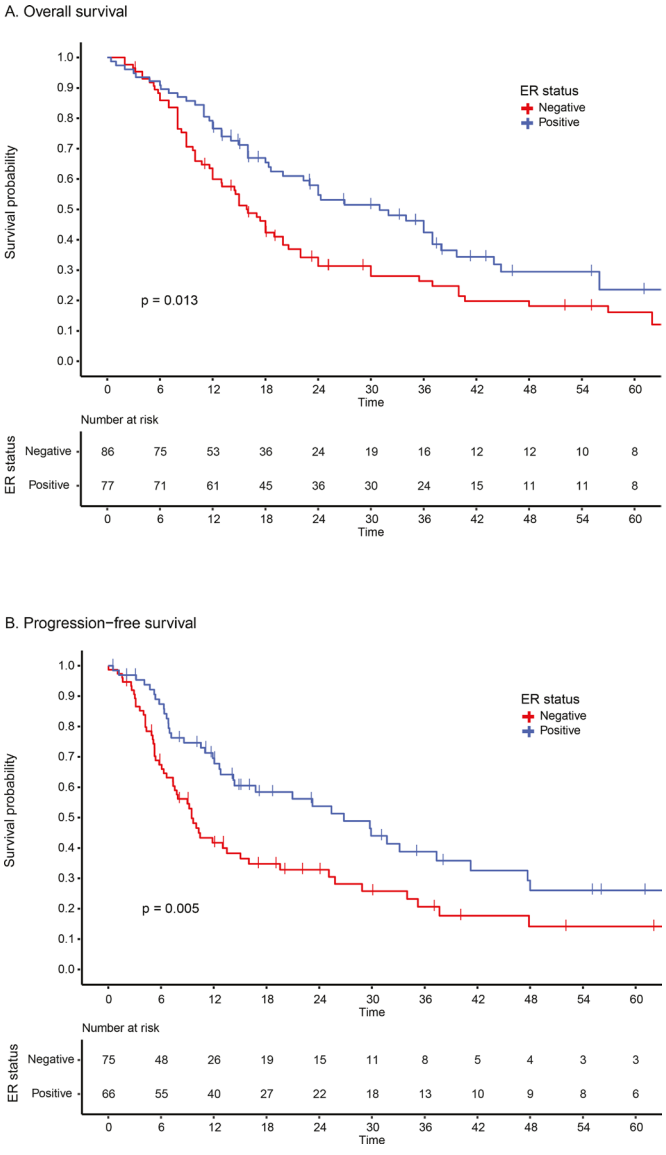

Figure 3 Clinical outcomes of stage IV endometrial cancer by estrogen receptor (ER) status.

DISCUSSION

Summary of Main Results

Our study showed that the distribution of the molecular classification was different compared with earlier stage endometrial cancer, with poor prognostic types being more common. In contrast, molecular classification had limited prognostic influence in this study. There was no difference in progression free and overall survival in univariable and multivariable analysis.

Results in the Context of Published Literature

This study showed a predominance of p53abn endometrial cancer (61.6%). Previous studies investigating molecular classification in endometrial cancer showed 7.3% *TP53* mutations in low risk endometrial cancer and 22.7% *TP53* mutations in high risk endometrial cancer.[13 14 23–25] Because high risk endometrial cancer will metastasize and lead to stage IV disease, it is not surprising that we found an even higher percentage of p53abn tumors. MMRd and NSMP endometrial cancer were found more frequently in early stage endometrial cancer than in our stage IV cohort. The molecular analysis of the PORTEC-1 (Post Operative Radiation Therapy in Endometrial Carcinoma) and PORTEC-2 (Vaginal Brachytherapy versus Pelvic External Beam Radiation Therapy for patients with Endometrial Cancer of High-intermediate risk) trials (n=695 early stage endometrioid endometrial cancer) found 29.6% MMRd and 57.6% NSMP.[13] The molecular analysis of the PORTEC-3 (Adjuvant Chemoradiotherapy versus Radiotherapy alone for women with high-risk Endometrial Cancer) trial (n=410 high risk endometrial cancer) showed 33.4% MMRd and 31.5% NSMP.[14] These results differ from the results in this study where we found 12.8% MMRd and 21.3% NSMP endometrial cancer.

In our study, we found six patients with *POLE*mut endometrial cancer. This was an unexpected finding because most reports showed very few *POLE*mut tumors in higher stages of endometrial cancer.[14 23–26] None of the patients with a *POLE*mut tumor presented with extra-abdominal disease or received neoadjuvant chemotherapy. Five *POLE*mut patients had complete and one

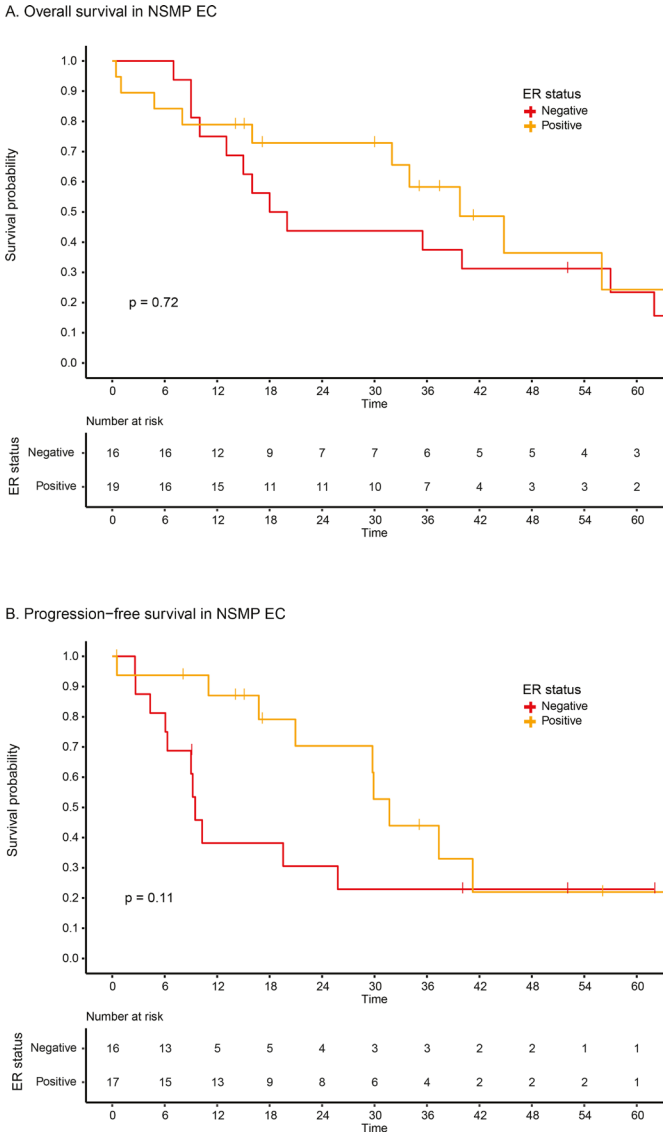

Figure 4 Clinical outcomes of stage IV no specific molecular profile endometrial cancer by ER status.

optimal cytoreductive surgery. Two patients with a *POLE*mut tumor were alive without disease progression at the end of follow-up, 38 and 70 months after primary treatment. One patient developed a recurrence, but was still alive at the end of follow-up. However, *POLE*mut did not impact overall survival. Two of six patients died of non-cancer related causes. One patient died shortly after surgery due to surgical complications. Another patient died 17 months after complete cytoreductive surgery while having no indication of clinical or radiological disease recurrence. In the other molecular subclasses, non-cancer related deaths were also present, but never as substantial as in the *POLE*mut group (33.3%).

Consequently, the overall survival analyses by molecular subclass must be interpreted with caution. However, it remains intriguing that *POLE*mut tumors can evolve towards stage IV endometrial cancer. In this study, three patients with a *POLE*mut tumor presented with intra-abdominal peritoneal metastases, while one patient had peritoneal metastases that were limited to the pelvic area. The other two patients presented with bladder or bowel invasion. Siegenthaler et al molecularly classified 101 recurrent endometrial cancer patients and found two patients with *POLE*mut endometrial cancer (2.0%). Both patients presented with distant recurrences. Further analysis of these patients showed that their tumors also had *TP53* mutations and high grade endometrioid histology.[26] In our cohort, there were no double classifiers (*POLE*mut and p53abn). Four patients with a *POLE*mut tumor had a high grade endometrioid, one serous and one clear cell histology. Future studies should be directed at identifying factors that may contribute to progression in this generally so favorable molecular subclass.

Histotype was correlated with molecular classification. Histotypes traditionally associated with poor prognosis (serous and carcinosarcomas) were most frequently found in p53abn cancers. This study confirmed the correlation of non-endometrioid histotype with decreased progression free and overall survival in univariate analysis and with decreased progression free survival in multivariate analysis. The distribution of estrogen receptor positive tumors in this study was in line with studies regarding lower stage endometrial cancer.[13 15] Patients with positive estrogen receptor tumors had

Table 2 Multivariable analysis of progression free survival and overall survival

	Progression free survival			Overall survival		
	Hazard ratio	95% CI	P value	Hazard ratio	95% CI	P value
Completeness of CRS (complete vs optimal/incomplete)	2.080	1.347 to 3.213	<0.001	2.517	1.721 to 3.680	<0.001
Histotype and grade (endometrioid vs non-endometrioid)	1.842	1.037 to 3.275	0.037	1.306	0.819 to 2.083	0.262
ER status (ER negative vs ER positive)	0.763	0.467 to 1.246	0.280	0.744	0.487 to 1.135	0.170
Molecular classification						
MMRd		Reference			Reference	
*POLE*mut	0.441	0.092 to 2.111	0.306	0.784	0.215 to 2.858	0.713
p53abn	1.033	0.476 to 2.242	0.936	1.168	0.611 to 2.232	0.639
NSMP	0.945	0.429 to 2.080	0.888	0.9350.981	0.492 to 1.959	0.957

CRS, cytoreductive surgery; ER, estrogen receptor; MMRd, mismatch repair deficient; NSMP, no specific molecular profile; p53abn, p53 abnormal; *POLE*mut, *POLE* mutant.

more favorable overall survival compared with estrogen receptor negative tumors. However, multivariate analysis did not confirm this correlation.

Molecular classification did not influence overall survival in univariate and multivariate analyses. It is unexpected that this study showed limited prognostic stratification between the different molecular subclasses. Previous studies showed that there was a clear difference in survival between patients with NSMP and MMRd compared with p53abn and *POLE*mut endometrial cancer. Patients with NSMP and MMRd tumors have an intermediate prognosis compared with the unfavorable outcome of p53abn and favorable outcome of *POLE*mut.[13 14 27] In this cohort, there was no significant difference between the molecular subclasses in progression free and overall survival in univariate and multivariate analyses. One explanation for these differences in prognostic impact could be that this cohort represents patients with the most aggressive tumors of their molecular subclass and that prognosis is influenced by this aggressive behavior. Another explanation could be that patient characteristics influence outcomes (age and comorbidities). In addition, this difference might be influenced by the result of cytoreductive surgery in the different molecular subclasses and due to the fact that two of the six patients with a *POLE*mut tumor died of other causes. Lastly, we suggest that the fact that all women in this study had metastasized endometrial cancer is a possible cause for the poor survival rates, irrespective of molecular classification of the tumor. It could be hypothesized that in advanced stage endometrial cancer the extent of disease outweighs the overall survival rates, rather than the molecular classification.

A previous study showed that estrogen receptor status can refine risk stratification in patients with high risk NSMP endometrial cancer. Positive estrogen receptor status had a clear beneficial influence on prognosis.[22 28] In this study, further stratification of the NSMP subclass based on estrogen receptor expression showed no difference in progression free and overall survival (Figure 3B). In general, we might assume that where molecular stratification states a clear prognostic difference in low stage endometrial cancer, this does not seem to be the case in stage IV endometrial cancer patients.

Currently, there are developments in systemic treatment options for stage IV endometrial cancer, consisting of chemotherapy and targeted therapy (poly(ADP-ribose) polymerase inhibitors, tyrosine kinase inhibitors, and immunotherapy such as programmed death ligand 1 inhibitors). Some treatments are registered, while others are still under investigation.[29–32] Molecular classification of stage IV endometrial cancer will help to guide future choices in adjuvant systemic treatment. This will improve the prognosis for stage IV endometrial cancer patients and influence clinical practice.

Strengths and Weaknesses

To our knowledge, this study is the first to investigate molecular classification and correlation with progression free and overall survival in patients with stage IV endometrial cancer at diagnosis. Due to the rarity of stage IV endometrial cancer at diagnosis, and the retrospective nature of this study, the results may be affected by information bias and confounding. For example, WHO performance status and data on (neo)adjuvant therapy were not well documented. Moreover, confounding by indication may have been present in this study because the extent of the disease could influence treatment decisions. Selection of patients eligible for cytoreductive surgery and (neo)adjuvant treatment regimen might have influenced our study results. This is especially true for stage IV endometrial cancer due to the high heterogeneity in disease extent and treatment choices. This information bias and confounding implies that the results of this study should be interpreted with caution.

CONCLUSIONS

This study assessed molecular classification in a large group of surgically treated patients with stage IV endometrial cancer at diagnosis. Our findings with regard to the impact and relevance of molecular classification were different compared with earlier stage endometrial cancer. The distribution differed, with a higher prevalence of p53abn endometrial cancer, which is explained by the more aggressive behavior of p53abn endometrial cancer. Molecular classification did not influence progression free and overall survival in univariate and multivariate analyses. This study can form the basis for further prospective research on the influence of molecular classification on prognosis in this rare patient group with a poor prognosis.

Author affiliations
[1]Department of Gynecology, Rijnstate Hospital, Arnhem, The Netherlands
[2]Department of Pathology, Leiden University Medical Center, Leiden, The Netherlands
[3]Department of Gynecologic Oncology, Netherlands Cancer Institute, Amsterdam, The Netherlands
[4]Department of Gynecologic Oncology, Leiden University Medical Center, Leiden, The Netherlands
[5]Department of Gynecologic Oncology, Amsterdam UMC Locatie AMC, Amsterdam, The Netherlands
[6]Department of Gynecologic Oncology, UMC Utrecht, Utrecht, The Netherlands
[7]Department of Radiation Oncology, Leiden University Medical Center, Leiden, The Netherlands
[8]Department of Gynecology, Roosevelt Clinics, Leiden, The Netherlands

Correction notice This article has been corrected since it was first published. The spelling of author nameDione van Dijk has been corrected.

X Nanda Horeweg @NandaHoreweg

Contributors MU: investigation and writing. DvD: writing–original draft. CARL: conceptualization, resources, and writing–review and editing. CDK: resources, and writing–review and editing. JCK: resources and writing–review and editing. RZ: resources. CGG: resources. NH: methodology, formal analysis, data curation, and writing–review and editing. TB: conceptualization, investigation, resources, and writing–review and editing. JvdM: conceptualization, methodology, investigation, writing and guarantor. LN: conceptualization, methodology, investigation, writing and guarantor.

Funding The authors have not declared a specific grant for this research from any funding agency in the public, commercial, or not-for-profit sectors.

Competing interests NH is the co-inventor, not owner, of a patent in preparation on an artificial intelligence model on endometrial cancer, unrelated to the current work; member of the DSMB of the Apollo study (EudraCT No 2022-002500-21); and member of the steering committee of the RAINBO Research Consortium.

Patient consent for publication Not applicable.

Ethics approval The study was approved by the medical ethics committees of the Antoni van Leeuwenhoek Hospital, Amsterdam UMC, UMCU, and LUMC (NKI-AvL, IRBDm20-020).

Provenance and peer review Not commissioned; externally peer reviewed.

Data availability statement Data are available in a public, open access repository.

ORCID iDs
Christianne A R Lok http://orcid.org/0000-0001-8693-7299
Nanda Horeweg http://orcid.org/0000-0002-8581-4753
Linda S Nooij http://orcid.org/0000-0001-5741-4595

REFERENCES

1 Makker V, MacKay H, Ray-Coquard I, *et al*. Endometrial cancer. *Nat Rev Dis Primers* 2021;7:88.
2 Creasman WT, Odicino F, Maisonneuve P, *et al*. Carcinoma of the corpus uteri. FIGO 26th annual report on the results of treatment in gynecological cancer. *Int J Gynaecol Obstet* 2006;95 Suppl 1:S105–43.
3 Barlin JN, Puri I, Bristow RE. Cytoreductive surgery for advanced or recurrent endometrial cancer: a meta-analysis. *Gynecol Oncol* 2010;118:14–8.
4 Amant F, Moerman P, Neven P, *et al*. Endometrial cancer. *Lancet* 2005;366:491–505.
5 Eto T, Saito T, Kasamatsu T, *et al*. Clinicopathological prognostic factors and the role of cytoreduction in surgical stage IVB endometrial cancer: a retrospective multi-institutional analysis of 248 patients in Japan. *Gynecol Oncol* 2012;127:338–44.
6 Concin N, Matias-Guiu X, Vergote I, *et al*. ESGO/ESTRO/ESP guidelines for the management of patients with endometrial carcinoma. *Int J Gynecol Cancer* 2021;31:12–39.
7 Bristow RE, Duska LR, Montz FJ. The role of cytoreductive surgery in the management of stage IV uterine papillary serous carcinoma. *Gynecol Oncol* 2001;81:92–9.
8 Ayhan A, Taskiran C, Celik C, *et al*. The influence of cytoreductive surgery on survival and morbidity in stage IVB endometrial cancer. *Int J Gynecol Cancer* 2002;12:448–53.
9 McEachron J, Zhou N, Hastings V, *et al*. Optimal cytoreduction followed by chemoradiation in stage IVB uterine serous carcinoma. *Cancer Treat Res Commun* 2022;33:100631.
10 Cancer Genome Atlas Research N, Kandoth C, Schultz N. Integrated genomic characterization of endometrial carcinoma. *Nature* 2013;500:242.
11 McAlpine J, Leon-Castillo A, Bosse T. The rise of a novel classification system for endometrial carcinoma; integration of molecular subclasses. *J Pathol* 2018;244:538–49.
12 Vermij L, Smit V, Nout R, *et al*. Incorporation of molecular characteristics into endometrial cancer management. *Histopathology* 2020;76:52–63.
13 Stelloo E, Nout RA, Osse EM, *et al*. Improved risk assessment by integrating molecular and clinicopathological factors in early-stage endometrial cancer-combined analysis of the PORTEC cohorts. *Clin Cancer Res* 2016;22:4215–24.
14 León-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. *J Clin Oncol* 2020;38:3388–97.
15 Karnezis AN, Leung S, Magrill J, *et al*. Evaluation of endometrial carcinoma prognostic immunohistochemistry markers in the context of molecular classification. *J Pathol Clin Res* 2017;3:279–93.
16 Horeweg N, Nout RA, Jürgenliemk-Schulz IM, *et al*. The molecular classification predicts response to radiotherapy in the randomized PORTEC-1 and -2 trials for early-stage endometrioid endometrial cancer. *J Clin Oncol* 2023;41:4369–80.
17 Pecorelli S. Revised FIGO staging for carcinoma of the vulva, cervix, and endometrium. *Int J Gynaecol Obstet* 2009;105:103–4.
18 Vermij L, Léon-Castillo A, Singh N, *et al*. P53 immunohistochemistry in endometrial cancer: clinical and molecular correlates in the PORTEC-3 trial. *Mod Pathol* 2022;35:1475–83.
19 Singh N, Piskorz AM, Bosse T, *et al*. P53 immunohistochemistry is an accurate surrogate for Tp53 mutational analysis in endometrial carcinoma biopsies. *J Pathol* 2020;250:336–45.
20 Van den Heerik ASVM, 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* 2023;9:e2200384.
21 León-Castillo A, Britton H, McConechy MK, *et al*. Interpretation of somatic POLE mutations in endometrial carcinoma. *J Pathol* 2020;250:323–35.
22 Vermij L, Jobsen JJ, León-Castillo A, *et al*. Prognostic refinement of NSMP high-risk endometrial cancers using oestrogen receptor immunohistochemistry. *Br J Cancer* 2023;128:1360–8.
23 van Gool IC, Bosse T, Church DN. POLE proofreading mutation, immune response and prognosis in endometrial cancer. *Oncoimmunology* 2016;5:e1072675.
24 Jumaah AS, Al-Haddad HS, McAllister KA, *et al*. The clinicopathology and survival characteristics of patients with POLE proofreading mutations in endometrial carcinoma: a systematic review and meta-analysis. *PLoS One* 2022;17:e0263585.
25 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* 2021;127:2409–22.
26 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. *Gynecol Oncol* 2022;165:230–8.
27 Kolehmainen AM, Pasanen AM, Koivisto-Korander RL, *et al*. Molecular characterization in the prediction of disease extent in endometrial carcinoma. *Eur J Obstet Gynecol Reprod Biol* 2021;256:478–83.
28 Jamieson A, Huvila J, Chiu D, *et al*. Grade and estrogen receptor expression identify a subset of no specific molecular profile endometrial carcinomas at a very low risk of disease-specific death. *Mod Pathol* 2023;36:100085.
29 Fader AN, Roque DM, Siegel E, *et al*. Randomized phase II trial of carboplatin-paclitaxel compared with carboplatin-paclitaxel-trastuzumab in advanced (stage III-IV) or recurrent uterine serous carcinomas that overexpress Her2/neu (NCT01367002): updated overall survival analysis. *Clin Cancer Res* 2020;26:3928–35.
30 Eskander RN, Sill MW, Beffa L, *et al*. Pembrolizumab plus chemotherapy in advanced endometrial cancer. *N Engl J Med* 2023;388:2159–70.
31 Makker V, Colombo N, Casado Herráez A, *et al*. Lenvatinib plus pembrolizumab for advanced endometrial cancer. *N Engl J Med* 2022;386:437–48.
32 Mirza MR, Chase DM, Slomovitz BM, *et al*. Dostarlimab for primary advanced or recurrent endometrial cancer. *N Engl J Med* 2023;388:2145–58.