Gynecologic Oncology 192 (2025) 15–23

Contents lists available at ScienceDirect

Gynecologic Oncology

journal homepage: www.elsevier.com/locate/ygyno

Hormonal biomarkers remain prognostically relevant within the molecular subgroups in endometrial cancer

Stephanie W. Vrede [a,b,*], Willem Jan Van Weelden [a,b], Johan Bulten [c], C. Blake Gilks [d], Steven Teerenstra [e], Jutta Huvila [f], Xavier Matias-Guiu [g], Antonio Gil-Moreno [h,i], Jasmin Asberger [j], Sanne Sweegers [c], Louis J.M. van der Putten [a], Heidi V.N. Küsters-Vandevelde [k], Casper Reijnen [l], Eva Colas [i], Jitka Hausnerová [m], Vit Weinberger [n], Marc P.L.M. Snijders [b], Petra Vinklerova [n], Antonella Ravaggi [o], Franco Odicino [o], Eliana Bignotti [o], Jessica N. McAlpine [p], Roy Kruitwagen [q,a], Johanna M.A. Pijnenborg [a]

[a] Department of Obstetrics and Gynecology, Radboud University Medical Center, Nijmegen, the Netherlands
[b] Department of Obstetrics and Gynecology, Canisius-Wilhelmina Hospital, Nijmegen, the Netherlands
[c] Department of Pathology, Radboud university Medical Center, Nijmegen, the Netherlands
[d] Department of Pathology and Laboratory Medicine, University of British Columbia and BC Cancer Agency, Vancouver, Canada
[e] Department of Health Evidence, Radboud university medical center, Nijmegen, the Netherlands
[f] Department of Pathology, University of Turku, Turku, Finland
[g] Department of Pathology and Molecular Genetics and Research Laboratory, Hospital Universitari Arnau de Vilanova, University of Lleida, IRBLleida, CIBERONC, Lleida, Spain
[h] Gynecological Department, Vall Hebron University Hospital, CIBERONC, Barcelona, Spain
[i] Biomedical Research Group in Gynecology, Vall Hebron Institute of Research, Universitat Autònoma de Barcelona, CIBERONC, Barcelona, Spain
[j] Department of Obstetrics and Gynecology, Medical Center – University of Freiburg, Freiburg, Germany
[k] Department of Pathology, Canisius-Wilhelmina Hospital, Nijmegen, the Netherlands
[l] Department of Radiation Oncology, Radboud University Medical Center, Nijmegen, the Netherlands
[m] Department of Pathology, University Hospital in Brno and Masaryk University, Brno, Czech Republic
[n] Department of Obstetrics and Gynecology, University Hospital Brno and Masaryk University, Brno, Czech Republic
[o] Angelo Nocivelli' Institute of Molecular Medicine, Division of Obstetrics and Gynecology, ASST Spedali Civili di Brescia, University of Brescia, Brescia, Italy
[p] Department of Gynecology and Obstetrics, Division of Gynecologic Oncology, University of British Columbia, Vancouver, Canada
[q] Department of Obstetrics and Gynecology and GROW – School for Oncology and Developmental Biology, Maastricht University Medical Center, Maastricht, the Netherlands

HIGHLIGHTS

- ER/PR expression is prognostic within the molecular subgroups.
- Within MMRd and NSMP EC the three-tiered risk classification of ER expression is prognostically significantly.
- Within p53mut and NSMP EC the three-tiered risk classification of PR expression is prognostically significantly.
- In the entire cohort, PR 0–10 % expression and p53mut are independent prognostic factors for decreased DSS.
- In the entire cohort, PR 90–100 % and *POLE*-mutant are independent prognostic factors for an improved DSS.

ARTICLE INFO

Article history:
Received 4 December 2023
Received in revised form 18 August 2024
Accepted 25 October 2024
Available online xxxx

Keywords:
Endometrial carcinoma
Prognosis
Biomarkers

ABSTRACT

Objective. The prognostic relevance of hormonal biomarkers in endometrial cancer (EC) has been well-established. A refined three-tiered risk model for estrogen receptor (ER)/progesterone receptor (PR) expression was shown to improve prognostication. This has not been evaluated in relation to the molecular subgroups. This study aimed to evaluate the ER/PR expression within the molecular subgroups in EC.

Methods. A retrospective multicenter cohort study was performed and data from the European Network for Individualized Treatment centers and Vancouver, Canada were used. ER/PR immunohistochemical expression was grouped as: ER/PR 0–10 %, 20–80 % or 90–100 %. Molecular subgroups were determined with full next-generation sequencing or combined with immunohistochemistry: *POLE*mut, mismatch repair deficient (MMRd), p53mut and no-specific molecular profile (NSMP).

* Corresponding author at: Department of Obstetrics and Gynaecology, Radboud University Medical Center, Geert Grooteplein Zuid 10, P.O. Box 9101, 6500 HB Nijmegen, the Netherlands.
E-mail address: stephanie.vrede@radboudumc.nl (S.W. Vrede).

https://doi.org/10.1016/j.ygyno.2024.10.028
0090-8258/© 2024 The Authors. Published by Elsevier Inc. This is an open access article under the CC BY license (http://creativecommons.org/licenses/by/4.0/).

Pathology
Molecular classification
Immunohistochemical

Results. A total of 739 patients were included (median follow-up 5.0 years). Tumors were classified as *POLE*mut in 9.1 %($N = 67$), MMRd in 27.6 %($N = 204$), p53mut in 20.8 %($N = 154$) and NSMP in 42.5 %($N = 314$). Among all molecular subgroups, patients with ER/PR 90–100 % expression revealed the best disease-specific survival (DSS). Within p53mut, PR 90–100 % expression showed a 5-year DSS of 100.0 %. ER expression is prognostic more relevant in MMRd and NSMP tumors while PR expression in p53mut and NSMP tumors. Across all molecular subgroups, PR 0–10 %, p53mut, lympho-vascular space invasion and FIGO stage III-IV remained independently prognostic for reduced DSS Whereas PR 90–100 % and *POLE*mut remained independently prognostic for improved DSS.

Conclusion. We demonstrated that ER/PR expression remain prognostically relevant within the molecular subgroups, and that a three-tiered cutoff refines prognostication. These data support incorporating routine evaluation of ER/PR expression in clinical practice.

© 2024 The Authors. Published by Elsevier Inc. This is an open access article under the CC BY license (http://creativecommons.org/licenses/by/4.0/).

1. Introduction

Historically, endometrial cancer (EC) was divided into two histopathological subtypes [1]. Type 1 EC includes low-grade (grade 1 and 2) endometrioid EC (EEC), represents the majority (80 %) of patients, and is associated with obesity and good prognosis. Type 1 EC is considered to be hormone driven with high expression of estrogen (ER) and progesterone receptors (PR) [2]. Type 2 EC represents high-grade tumors (grade 3 EEC and non-endometrioid EC (NEEC)), generally have low ER expression and an unfavorable prognosis [1,3]. Despite the overall good prognosis of type 1 EC, mortality in absolute numbers is higher in type 1 compared to type 2 EC [4].

Hormone receptor expression (ER/PR) are prognostic biomarkers that predict lymph node metastasis (LNM) and outcome [5–7]. The current used cutoff for ER/PR expression within EC is not uniform, is adopted from breast cancer studies, and most frequently considered positive if >1 % or > 10 % expression [8,9]. Studies within EC showing the cutoff with the best prognostic impact are lacking, therefore in a previous explorative analysis different cutoff values for ER/PR expression were evaluated and showed the subgroups 0–10 % with unfavorable outcome, 20–80 % with intermediate outcome and 90–100 % with favorable outcome. This revised three-tiered risk classification model was shown to improve prognostication over the mostly used cutoff of 10 % [10]. Prospective validation is very much supported to verify these findings.

The Cancer Genome Atlas (TCGA) classified patients with EC into four important prognostic subgroups based on their genomic molecular signature: I) ultramutated tumors with polymerase epsilon (*POLE*) mutations, II) hypermutated tumors with microsatellite instability (MSI), III) copy-number-high (CNH) tumors with frequent tumor protein (*TP53*) mutations, IV) copy-number-low (CNL) tumors (also known as no-specific molecular profile (NSMP)) [11]. The Proactive Molecular Risk Classifier for Endometrial Cancer (ProMisE) is a surrogate diagnostic algorithm using low cost clinically applicable immunohistochemistry (IHC); mismatch repair deficient (MMRd) instead of MSI and p53 instead of *TP53* [12,13].

The histopathological subtypes (type 1 and 2) are present within all molecular subgroups. Type 1 (EEC histology) is mainly represented by the *POLE*mut, MMRd and NSMP subgroup, with positive ER/PR expression. Type 2 (NEEC histology) is mainly represented by the p53mut subgroup, with generally negative ER/PR expression [11].

In this era of molecular profiling, the relevance of hormonal biomarkers needs to be redefined. Earlier study demonstrated that ER status was still important for the outcome of EC patients regardless of risk class and p53 or MMR status [14]. Within the NSMP subgroup loss of ER and/or PR expression (<1 % and < 10 %) was shown to be an important prognosticators for EC, but this was not found in the other molecular subgroups [15–17]. So far, it has not been investigated whether the previously mentioned three-tiered ER/PR risk model [10], has prognostic impact in the different molecular subgroups. Therefore, we studied the prognostic relevance of the three-tiered ER/PR classification within the molecular subgroups in EC. It is hypothesized that this three-tiered model refines prognostication within all molecular subgroups.

2. Materials and methods

2.1. Patients

A retrospective multicenter cohort study has been performed. Data was used from the European Network for Individualized Treatment (ENITEC) centers and Vancouver Hospital, Canada. Data from four previously published and one unpublished cohort were collected, resulting in 978 patients (flowchart Supplementary Fig. S1) [10,12,13,18,19]. Patients were treated between 1994 and 2019 (median 2007) and data on clinicopathological characteristics and outcome were collected.

Inclusion criteria were: (I) availability of ER/PR immunohistochemistry, (II) patients successfully classified with either full next-generation sequencing (NGS) or NGS combined with IHC according to ProMisE [12]. An exclusion criteria was: missing follow-up. Patients were aligned according to the diagnostic algorithm in Fig. 1 and final classified according to the World Health Organization (WHO) Classification of Female Genital Tumors [20]; *POLE*mut, MMRd, p53mut and NSMP.

This study followed the Strengthening the Reporting of Observational Studies in Epidemiology (STROBE) reporting guideline.

2.2. DNA analysis

The molecular subgroups included in this study were determined by either full NGS or according to ProMisE. Both methods have been described previously [13,21] and details are provided for the different cohorts in the *Supplementary Method S1*. Briefly, for molecular profiling by full NGS, DNA was isolated from formalin-fixed paraffin-embedded (FFPE) tumor blocks. Next, DNA was sequenced by NGS with single-molecule Molecular Inversion Probes (smMIPs) [22]. For the detection of MSI, 55 MSI markers were tested according to the previously published design [23]. Multiple-classifiers were classified as the molecular subgroup with the best prognosis [24]. For the molecular subgroups determined according ProMisE criteria, *POLE*mut analysis was performed by MiSeq, Sanger or NGS [13,19].

2.3. Immunohistochemical analysis

IHC was performed on 4 um FFPE tumor sections for the ENITEC centers and tissue microarrays (TMA) for Vancouver cohort, as described previously and detailed in the *Supplementary method S1* [10,13,17,19]. In brief, antibodies specific to MSH6, PMS2, p53, ER and PR were used. Staining for p53 was considered abnormal when more than 80 % of tumor cell nuclei showed strong expression (*overexpression*) or when there was complete loss of nuclear staining (*null-expression*) with a

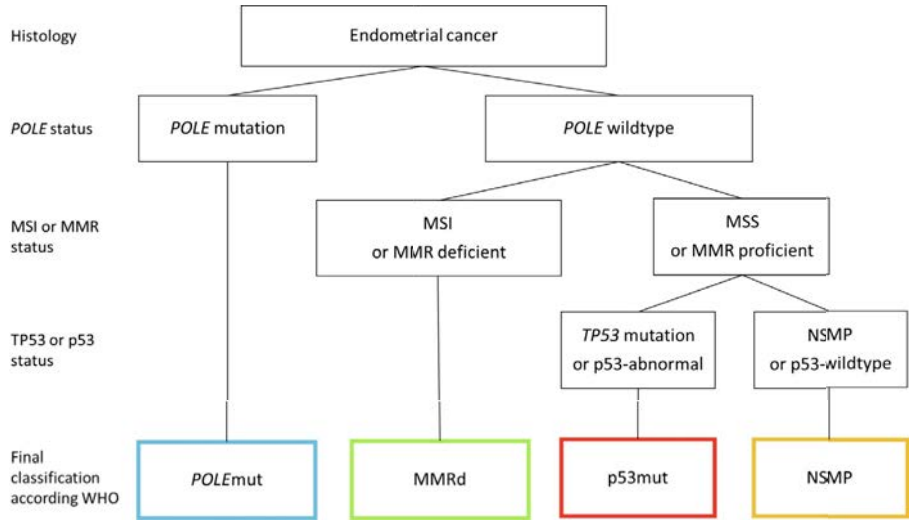

Fig. 1. Diagnostic algorithm of patients diagnosed with full next-generation sequencing or combined with immunohistochemistry, and the final classification according to the World Health organization (WHO) classification of female genital tumors.
Abbreviations: *POLE,* Polymerase epsilon; MSI, Microsatellite instability; MMRd, Mismatch repair deficient; *TP53,* Tumor protein 53; p53mut, p53-mutant; NSMP, No-specific molecular profile.

positive internal control. Mismatch repair deficiency (MMRd) was defined as complete absence of nuclear staining of PMS2 and/or MSH6, in the presence of a positive internal control. For the TMAs, staining for individual MMR proteins and ER/PR was repeated on whole sections whenever there was equivocal, uninterpretable, or aberrant staining. ER and PR expression was determined by estimating the percentage of positive nuclei in the whole invasive tumor area by 'eyeballing'. Scoring for ER and PR expression within the included cohorts was performed by two assessors (pathologists and researchers, who were trained by an expert gynecologic-pathologist), reviewing discrepancies in a consensus meeting. Within the different cohorts, the discrepancies were discussed between the two assessors to find a final agreement for the percentage [10,19]. In our previous study, explorative analysis was performed of the IHC samples with 0, 10, 20, 30, 40, 50, 60, 70, 80, 90, and 100 % of expression. Cutoffs with the strongest prognostic impact were determined. According to this retrospective study the ER/PR risk groups were defined as: ER/PR 0–10 % as high-risk, ERPR 20–80 % as intermediate risk and 90–100 % as low risk, in this study the same cutoffs for the risk groups was used. The Cohen's k value for scoring ER/PR expression as per the three risk groups was 0.703 [10]. Percentages were scored by the pathologist as 0 %, 10 %, 20 %, 30 %, 40 %, 50 %, 60 %, 70 %, 80 %, 90 % and 100 %. In a small amount of cases the percentages were scored in between, e.g. 85 %, these were rounded off into the nearest category (so 85 % was categorized as 90 %). When ER and PR were taken together, the subgroups were defined as ER + PR 0–10 %, ER + PR 20–80 %, ER + PR 90–100 % and discordant. Patients were grouped 'discordant' if the ER and PR percentages were not aligned in the same risk group (e.g. ER 10 % and PR 90 %). These patients were also included in the survival analysis to determine the relevance of this subgroup in clinical practice.

2.4. Primary objective

To study the prognostic relevance of the three-tiered ER/PR risk classification within the molecular subgroups in EC.

2.5. Statistical analysis

The molecular subgroups were compared with the dichotomous clinicopathological characteristics using the χ^2 or Fisher's exact test for categorical data, and the non-parametric Mann-Whitney *U* test for continuous variables. Survival analyses were performed using Kaplan-Meier curves and univariable and multivariable Cox-regression analysis. Associations are shown as hazard ratio (HR), 95 % CI and *P*-value. The including covariates in multivariable analysis are the main known prognostic biomarkers in EC. Myometrial invasion (MI) was excluded because this is already included in FIGO stage. Disease-specific survival (DSS) was defined as time from date of diagnosis to date of death by EC all censored by date of last contact. Patients who died within 1 year of survival were also included, due to this definition. The results were considered significant with *P*-value less than 0.05. Statistical Package for the Social Sciences, version 27.0 (IBM, New York, NY, USA) was used for statistical analyses.

3. Results

In total, 978 patients with known and classifiable ER/PR IHC status were available for molecular analysis. Only patients with a successful molecular analysis were included, resulting in 747 EC patients. In which 8 patients were excluded due to complete missing follow-up, leading to a total of 739 patients included in this study (Flowchart Supplementary Fig. S1). A baseline overview of each included cohort is shown in Supplementary Table 1. The baseline characteristics of the entire cohort are shown in Table 1. Median age was 65.0 (31.0–93.0) years, median BMI 29.0 (15.8–66.2) kg/m^2 and median follow-up 60.0 (1.0–283.0) months. The number of patients with <1 year follow-up ($n = 36$) consisted of patients who died due to EC ($n = 24$), died due to other cause than EC ($n = 5$) or did the rest of their follow-up in another hospital then they were operated ($n = 7$). Exclusion of these 7 cases did not affect results in the multivariable analysis (*data not shown*). The majority of the patients revealed EEC histology 80.4 % ($N = 594$), grade 1–2 EEC 53.5 % ($N = 394$) and FIGO stage I-II 75.5 % ($N = 558$). A minority of patients were diagnosed with ER + PR expression 0–10 % or 90–100 % (respectively, 16.8 % ($N = 124$) and 17.1 % ($N = 126$)). A total of 251 patients (34.0 %) were not aligned to one of the three risk groups and classified as 'discordant'. Most discordant cases are located in patients with ER 20–80 % + PR 0–10 % expression (13.4 %), and PR 20–80 % + ER 90–100 % expression (12.3 %) (*data not shown*).

Tumors were classified as *POLE*mut in 9.1 % ($N = 67$), MMRd in 27.6 % ($N = 204$), p53mut in 20.8 % ($N = 154$), and NSMP in 42.5 % ($N = 314$), in line with the original TCGA paper [11]. The majority

Table 1
Baseline.

		Total N = 739	*POLE*mut N = 67 (9.1)	MMRd N = 204 (27.6)	p53mut N = 154 (20.8)	NSMP N = 314 (42.5)	*P*
Patient characteristics							
Age (years)		65.0 (31.0–93.0)	57.3 (34.0–93.3)	65.1 (42.0–87.0)	72.6 (35.0–93.0)	62.9 (35.0–88.0)	<0.001*
BMI (kg/m^2)		29.0 (15.8–66.2)	27.6 (18.4–58.3)	29.1 (15.8–62.0)	28.4 (17.5–46.2)	29.6 (15.8–66.2)	0.142
Post-operative histology							
Histology	EEC	594 (80.4)	59 (88.1)	187 (91.7)	57 (37.0)	291 (92.7)	<0.001*
	NEEC	145 (19.6)	8 (11.9)	17 (8.3)	97 (63.0)	23 (7.3)	
Grade	1–2	394 (53.3)	30 (44.8)	102 (50.0)	22 (14.3)	240 (76.4)	<0.001*
	3	345 (46.7)	37 (55.2)	102 (50.0)	132 (85.7)	74 (23.6)	
MI[a]	<50 %	400 (54.1)	33 (50.0)	107 (52.5)	75 (50.0)	185 (59.3)	0.172
	>50 %	332 (44.9)	33 (50.0)	97 (47.5)	75 (50.0)	127 (40.7)	
LVSI	No	474 (64.1)	37 (55.2)	115 (56.4)	78 (50.6)	244 (77.7)	<0.001*
	Yes	265 (35.9)	30 (44.8)	89 (43.6)	76 (49.4)	70 (22.3)	
Lymph nodes	N0	231 (31.3)	31 (46.3)	61 (29.9)	38 (24.7)	101 (32.2)	0.170
	N1	54 (7.3)	3 (3.0)	13 (6.4)	15 (9.7)	20 (6.3)	
	Nx	454 (61.4)	34 (50.7)	129 (63.2)	101 (65.6)	190 (39.5)	
FIGO stage[a]	Early	558 (75.5)	60 (90.9)	154 (75.9)	87 (56.5)	257 (82.6)	<0.001*
	Advanced	176 (23.8)	6 (9.1)	49 (24.1)	67 (43.5)	54 (17.4)	
Hormonal receptor expression							
ER	0–10	142 (19.2)	16 (23.9)	39 (19.5)	55 (36.2)	32 (10.2)	<0.001*
	20-80	354 (47.9)	36 (53.7)	98 (49.0)	74 (48.7)	146 (46.6)	
	90–100	236 (31.9)	15 (22.4)	63 (31.5)	23 (15.1)	135 (43.1)	
PR	0–10	241 (32.6)	21 (31.3)	60 (29.7)	101 (66.9)	59 (18.8)	<0.001*
	20-80	344 (46.5)	39 (58.2)	108 (53.5)	38 (25.2)	159 (50.8)	
	90–100	148 (20.0)	7 (10.4)	34 (16.8)	12 (7.9)	95 (30.4)	
ER + PR	0–10	124 (16.8)	13 (19.4)	30 (14.7)	54 (35.1)	27 (8.6)	<0.001*
	20-80	238 (32.2)	29 (43.3)	69 (33.8)	29 (18.8)	111 (35.4)	
	90–100	126 (17.1)	6 (9.0)	29 (14.2)	10 (6.5)	81 (25.8)	
	Discordant	251 (34.0)	19 (28.4)	76 (37.3)	61 (39.6)	95 (30.3)	
Adjuvant treatment[a]							
None		287 (38.8)	24 (36.4)	75 (36.9)	46 (30.1)	142 (45.7)	<0.001*
Radiotherapy		247 (33.4)	25 (37.9)	73 (36.0)	35 (22.9)	114 (36.7)	
Chemotherapy		77 (10.4)	8 (12.1)	19 (9.4)	34 (22.2)	16 (5.1)	
Chemoradiation		122 (16.5)	9 (13.6)	36 (17.7)	38 (24.8)	39 (12.5)	
Outcome							
Recurrence		198 (26.8)	4 (6.1)	58 (29.1)	72 (51.4)	64 (20.5)	<0.001*
Mortality		216 (29.2)	8 (11.9)	57 (27.9)	83 (53.9)	68 (21.7)	<0.001*
EC-related mortality		152 (20.6)	1 (1.5)	38 (19.4)	67 (45.3)	46 (14.9)	<0.001*

Data is presented in number (%), median (IQR).
Abbreviations: *N*, number; *POLE*mut, polymerase epsilon mutant; MMRd, mismatch repair deficient; p53, protein 53; NSMP, No specific molecular profile; EEC, endometrioid endometrial cancer; NEEC, non-endometrioid endometrial cancer; LVSI, lympho-vascular space invasion; N0, negative lymph nodes, N1, positive lymph nodes; Nx, no information about the lymph nodes; FIGO, International Federation of Gynecology and Obstetrics, ER, estrogen receptor; PR, progesterone receptor; EC, endometrial cancer.
[a] Missing data for 7 cases MI, 5 cases for FIGO stage, 6 for adjuvant treatment.
* $P < 0.05$.

of patients within the *POLE*mut, MMRd and NSMP subgroups had EEC histology (respectively, 88.1 %, 91.7 %, 92.7 %), whereas the majority of patients within the p53mut subgroup had NEEC histology (63.0 %).

3.1. Outcome ER or PR expression

Figure 2A-E shows the 5-year DSS curve of the three-tiered ER risk classification within the entire cohort and the molecular subgroups. In the entire cohort, patients with ER 90–100 % expression showed a significantly better DSS when compared to ER 20–80 % ($P < 0.001$) and ER 0–10 % ($P < 0.001$). Patients with ER 20–80 % had a significant higher 5-year DSS compared to ER 0–10 % ($P < 0.001$) (Fig. 2A). Across all molecular subgroups, patients with ER 90–100 % expression showed the most favorable 5-year DSS (Fig. 2B-E). Within *POLE*mut EC, patients with ER 90–100 %, 20–80 % and 0–10 % revealed no significantly different 5-year DSS (respectively, 100.0 %, 100.0 % and 92.0 %) For MMRd tumors, patients with ER 90–100 % and 20–80 % or 0–10 % revealed significantly different 5-year DSS (respectively, 96.0 % vs 80.0 % $P = 0.017$ and 96.0 % vs 71.0 % $P = 0.002$) (Fig. 2C). Within patients with p53mut no significant differences in 5-year DSS were found between the three ER subgroups (Fig. 2D). Within NSMP tumors, patients with ER 0–10 % had an significant worst 5-year DSS of 48.0 % compared to ER 90–100 % (96.0 %, $P < 0.001$) and compared to ER 20–80 % (88.0 %, $P < 0.001$)). (Fig. 2E).

Figure 3A-E shows the 5-year DSS curve of the three-tiered PR risk classification within the entire cohort and the molecular subgroups. In the entire cohort, patients with PR 90–100 % expression showed a significantly better DSS when compared to PR 20–80 % ($P = 0.003$) and PR 0–10 % ($P < 0.001$). Patients with ER 20–80 % had a significant higher 5-year DSS compared to ER 0–10 % ($P < 0.001$) (Fig. 3A). Across all molecular subgroups, patients with PR 90–100 % expression showed the most favorable 5-year DSS and PR 0–10 % the worst (Fig. 3B-E). Within *POLE*mut and MMRd tumors, no significant different 5-year DSS was revealed within the three subgroups of PR expression (Fig. 3B-C). Patients with p53mut EC and PR 90–100 % had a 5-year DSS of 100 %, this was significantly different compared to PR 20–80 % (62.0 %, $P = 0.032$) and PR 0–10 % (48.0 % $P = 0.006$) (Fig. 3D). Within NSMP tumors, patients with PR 90–100 % had an excellent 5-year DSS of 98.0 %, for PR 20–80 % the 5-year DSS was 88.0 % and PR 0–10 % showed the worst 5-year DSS of 56.0 %. All were significantly different from each other (Fig. 3E).

Across all molecular subgroups, PR 0–10 %, p53mut, lympho-vascular space invasion (LVSI) and FIGO stage III-IV remained

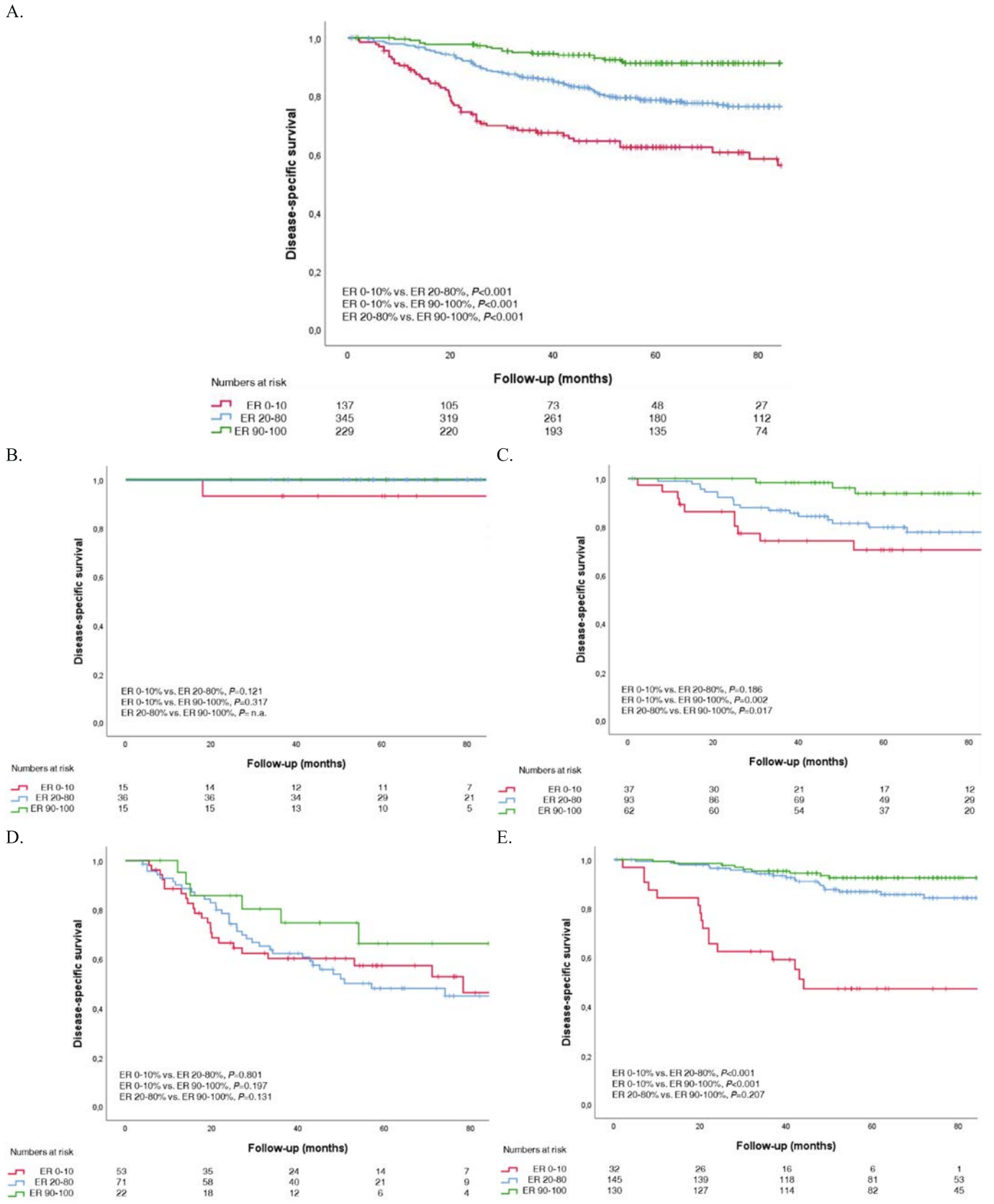

Fig. 2. A. 5-year disease-specific survival (DSS) of the ER three-tiered risk model within the entire cohort. B. 5-year DSS of the ER three-tiered risk model within *POLE*mut patients. C. 5-year DSS of the ER three-tiered risk model within MMRd patients. D. 5-year DSS of the ER three-tiered risk model within p53mut patients. E. 5-year DSS of the ER three-tiered risk model within NSMP patients.
Abbreviations: ER, estrogen receptor; *POLE*, Polymerase epsilon; MMRd, Mismatch repair deficient; *TP53*, p53mut, p53-mutant; NSMP, No-specific molecular profile.

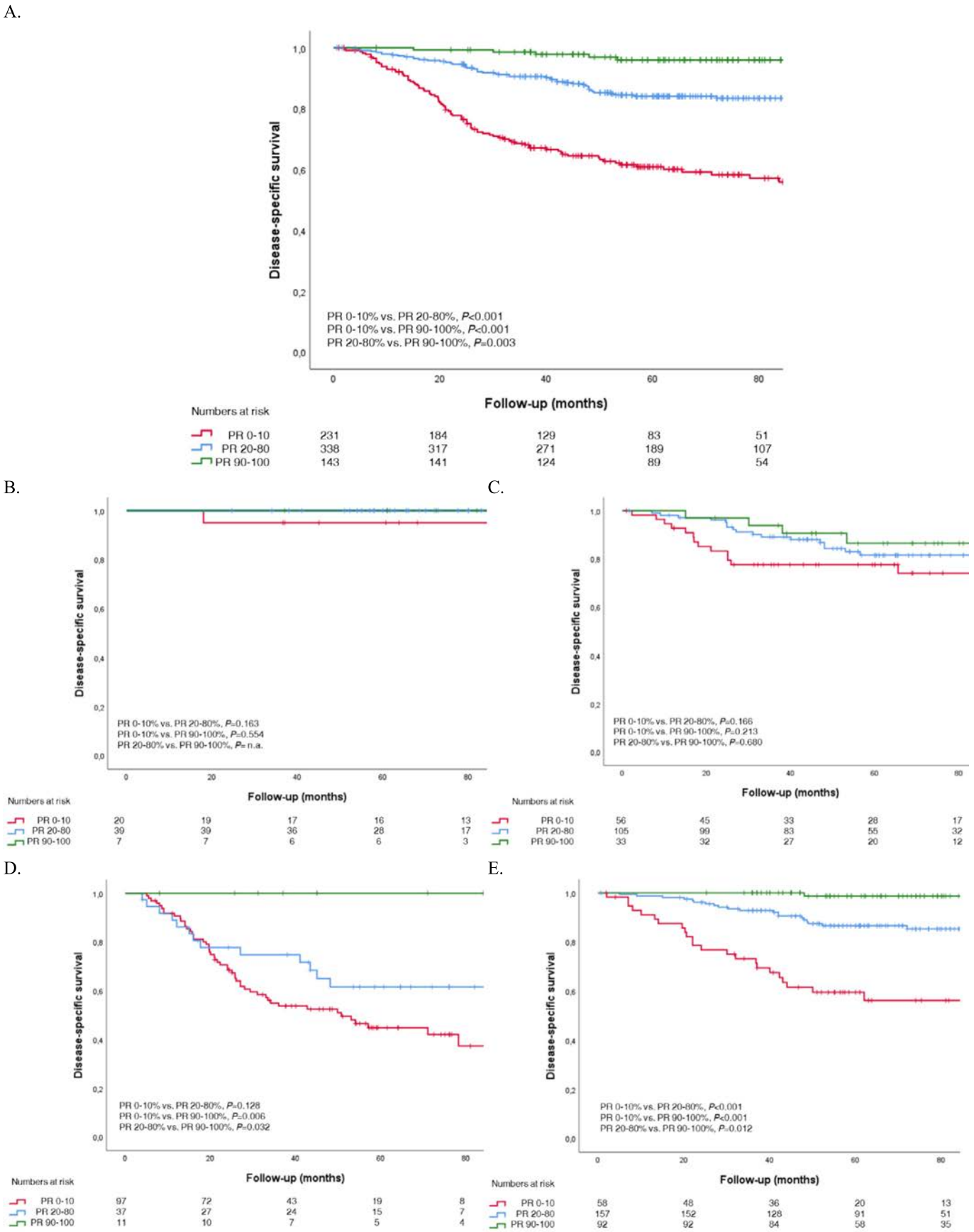

Fig. 3. A. 5-year disease-specific survival (DSS) of the PR three-tiered risk model within the entire cohort. B. 5-year DSS of the PR three-tiered risk model within *POLE*mut patients. C. 5-year DSS of the PR three-tiered risk model within MMRd patients. D. 5-year DSS of the PR three-tiered risk model within p53mut patients. E. 5-year DSS of the PR three-tiered risk model within NSMP patients.

Abbreviations: PR, progesterone receptor; *POLE*, Polymerase epsilon; MMRd, Mismatch repair deficient; *TP53*, p53mut, p53-mutant; NSMP, No-specific molecular profile.

Table 2
Cox regression univariable and multivariable analysis disease-specific survival (DSS).

Variable	Univariable		Multivariable 141 events	
			HR (95 % CI)	*P* value
ER cutoff				
ER 0–10 %	2.16 (1.51–3.08)	<0.001	1.20 (0.80–1.79)	0.378
ER 20–80 %	1		1	
ER 90–100 %	0.41 (0.24–0.69)	<0.001	0.82 (0.48–1.41)	0.463
PR cutoff				
PR 0–10 %	3.09 (2.19–4.35)	<0.001	1.61 (1.04–2.49)	0.030*
PR 20–80 %	1		1	
PR 90–100 %	0.34 (0.16–0.71)	0.005	0.41 (0.17–0.95)	0.039*
Molecular subgroup				
*POLE*mut	0.09 (0.01–0.65)	0.017*	0.06 (0.00–0.50)	0.006*
MMRd	1.37 (0.89–2.10)	0.154	0.79 (0.49–1.26)	0.327
p53mut	4.09 (2.81–5.97)	<0.001*	1.55 (1.00–2.40)	0.046*
NSMP	1		1	
LVSI				
No	1	<0.001*	1	0.025*
Yes	4.14 (2.96–5.78)		1.65 (1.07–2.57)	
FIGO				
Stage I-II	1		1	<0.001*
Stage III-IV	6.17 (4.45–8.55)	<0.001*	2.32 (1.50–3.60)	
Adjuvant treatment				
None	1		1	
Radiotherapy	1.72 (1.04–2.86)	0.035*	1.38 (0.81–2.34)	0.231
Chemotherapy	9.28 (5.65–15.23)	<0.001*	2.25 (1.21–4.17)	0.010*
Chemoradiation	4.27 (2.57–7.09)	<0.001*	1.46 (0.80–2.64)	0.216

Abbreviations: DSS, disease-specific survival; EC, endometrial cancer; HR, hazard ratio; CI, confidence interval; ER, estrogen receptor; PR., progesterone receptor; *POLE*mut, polymerase epsilon mutant; MMRd, mismatch repair deficient; p53, protein 53; NSMP, No specific molecular profile; LVSI, lympho-vascular space invasion; FIGO, Federation International of Gynecology and Obstetrics.

independent prognostic for reduced DSS. Whereas PR 90–100 % and *POLE*mut remained independent prognostic for improved DSS (Table 2).

3.2. Outcome ER + PR expression combined

Supplementary Fig. S2A–E shows the 5-year DSS curve of the three-tiered ER + PR combined risk classification within the entire cohort and the molecular subgroups. The 5-year DSS was significantly different between the three ER + PR risk classification groups (Supplementary Fig. S2A). Patients with p53mut EC and ER + PR 90–100 % had a 5-year DSS of 100 %, and patients with ER + PR 20–80 % and 0–10 % had comparable outcome as ER + PR 0–10 % (respectively, 55.0 % and 44.0 %). The 5-year DSS between ER + PR 0–10 % and 90–100 % was significantly different (Supplementary Fig. S2D). Within NSMP tumors, patients with ER + PR 90–100 % had an excellent 5-year DSS of 98.0 %, for ER + PR 20–80 % the 5-year DSS was 84.0 % and ER + PR 0–10 % showed the worst 5-year DSS of 43.0 %. All were significantly different from each other (Supplementary Fig. S2E). In the entire cohort and within *POLE*mut, MMRd and NSMP subgroup, the patients grouped as 'discordant', showed comparable outcomes as patients with ER + PR 20–80 % expression, Within p53mut EC the outcome was in line with the outcome of ER + PR 0–10 % expression. (Supplementary Fig. S2A–E).

Across all molecular subgroups and ER + PR risk groups, ER + PR 0–10 %, p53mut, lympho-vascular space invasion (LVSI) and FIGO stage III/IV remained independent prognostic factors for reduced DSS. ER + PR 90–100 % and *POLE*mut were independent prognostic factors for improved DSS (*Supplementary Table S2*).

4. Discussion

In this large retrospective multicenter cohort study we confirmed the relevance of using a three-tiered ER/PR risk classification that refined the prognostic relevance across the molecular subgroups. Among all molecular subgroups, patients with ER/PR 90–100 % expression revealed the best 5-year DSS. Interestingly, patients with PR 90–100 % and with p53mut EC revealed an excellent 5-year DSS. In multivariable analyses, PR 0–10 % was an independent prognostic factor for reduced DSS and PR 90–100 % an independent prognostic factor for improved DSS. Combining ER + PR, 0–10 % ER + PR expression was an independent prognostic factor for reduced DSS, while ER + PR 90–100 % for improved DSS.

In EC, numerous studies have already shown the importance of ER and PR expression in relation to predicting LNM and outcome, regardless of risk class [5–7,14,25]. However, no uniform cutoff is applied within EC. In an earlier study, we defined a three-tiered risk classification for ER/PR expression to improve prognostication specifically in patients with EC [10]. The current study confirmed the additional value of using this three-tiered risk classification when compared to the commonly used cutoff of 1 % or 10 %.

The relevance of ER/PR expression within all molecular subgroups was not fully elucidated until this study. Comparable to our data, early studies observed higher PR expression within the NSMP subgroup and low PR expression in p53mut tumors [11,17]. In addition, our study shows the relevance of hormonal biomarkers within the MMRd, p53mut and NSMP subgroups. Vermij et al. confirmed the significance of ER status within the NSMP high-risk EC. Comparable to our study, patients with ER expression <10 % showed the worst outcome compared to ER >10 %. Contrary to our findings, they found no prognostic impact of ER in the other molecular subgroups (especially MMRd) which might be explained by their cut-off of 1–10 % [15]. Jamieson et al. used ER and tumor grade to subclassify the NSMP subgroup. Low-risk NSMP was identified as low-grade EC and ER >1 % with favorable outcome, and high-risk NSMP as high-grade EC and ER <1 % expression with unfavorable outcome [16]. Which confirms the relevance of ER within the NSMP subgroup. Our study revealed also the additional relevance of PR

expression within the NSMP and p53mut subgroup, contrary to the other studies which might again by explained by the use of a three-tiered risk classification [15]. Interestingly, patients with p53mut EC and PR 90–100 % expression showed an excellent 5-year DSS of 100 %, since all these patients had EEC histology, the importance of both morphology and IHC in addition to molecular subgroups within EC is illustrated. Patients within p53mut or NSMP EC and PR 0–10 % show the worst outcome. Early studies indicated that PR <10 % expression was predominantly present in the 'advanced/metastatic' ESGO risk group and predicting disease recurrence in patients and increased risk of death. This is in line with our findings in multivariable regression analysis, were PR expression 0–10 % is more correlated with decreased DSS compared to ER expression 0–10 %. Due to the used cutoffs for ER and PR of 1 % or 10 % the prognostic relevance within the molecular subgroups might have been underestimated when compared with the three-tiered ER/PR risk classification in our study [15,17].

In clinical practice generally both ER and PR IHC expression are determined, therefore, understanding the prognostic relevance of both ER/PR expression within the molecular subgroups is interesting. Early studies indicated that both ER/PR provide additional prognostic information, comparable with our study [5,7,10,17]. Combining ER + PR shows ER + PR 0–10 % as an independent prognostic marker for reduced DSS and ER + PR 90–100 % as an independent prognostic marker for improved DSS. Combining ER + PR expression within the three-tiered risk classification will create a remaining subgroup, in this paper classified as discordant. For clinical practice, when the ER + PR subgroup is discordant in patients with *POLE*mut, MMRd or NSMP EC, the prognosis is in line with an intermediate prognosis. Within p53mut, the prognosis is in line with decreased prognosis (comparable to high risk 0–10 % expression). This decreased prognosis for the discordant group within patients with p53mut, is likely due to more patients with PR 0–10 % (78 %) compared the other molecular subgroups. This is in line with our previous conclusion, showing the relevance of PR expression within the p53mut subgroup.

The strengths of this retrospective study are the large number of included cases from multiple centers, including ER and PR immunohistochemistry and representing all tumor grades and FIGO stages. Second, by including ER/PR expression both and combined these results are highly relevant for clinical practice Furthermore, this is the first study to analyze a three-tried ER/PR risk classification within all molecular subgroups.

Some limitations need to be addressed. First, the mortality rate of *POLE*mut patients is low, possibly hampering interpretation on the impact of ER/PR expression within this specific subgroup. Second, technical allocation of the molecular subgroups differed slightly. However, either full NGS or use of ProMiSe criteria (combination of NGS and IHC) are repeatedly validated as comparable techniques and representative for the daily practice in Europe and Canada [26,27]. Third, a relative high amount of patients were excluded due to unsuccessful molecular profiling, perhaps as an result of using older archival tumor samples for DNA testing, furthermore patients who did the rest of their follow-up within other hospitals ($n = 7$) were included, however exclusion of these 7 cases did not affect results in the multivariable analysis. Fourth, race or ethnicity has not been reported in our study. Although we fully agree that these patients' information might be impact outcome in several diseases [28], within Europe it is not routinely documented in patient files [29]. Fifth, using patients between 1994 and 2019 could have biased the survival because of different treatment strategies over the time. However, the death caused by EC has not been reduced or increased over the 25 years in our study cohort (*data not shown*), therefore we believed this has not biased our results. Finally, according to the ProMiSe criteria the order of molecular subgroup allocation within the Vancouver cohort is different compare to the original TCGA cohort, in which MMRd testing is followed by *POLE* testing [11,13]. The distribution of MMRd that also include *POLE*mut varies, patients with *POLE*mut and MMRd have comparable prognosis to *POLE*mut [30]. Therefore a different allocating order could bias the outcome. However, in the original ProMiSe cohort, no MMRd patients are present with also *POLE*mut. Within the cohorts from the ENITEC centers the order of molecular testing was in line with the original TCGA cohort [10,11,18,19].

This study demonstrates the prognostic importance of ER and PR biomarkers within the era of molecular profiling and the relevance of a three-tiered risk classification. To validate the newly proposed cutoff a large prospective multicenter trial should be performed. Future prospective studies need also focus on response to hormonal treatment within the molecular subgroups, analysis the relevance of ER/PR biomarkers in advanced stage and within adjuvant treatment including patterns of recurrence. Currently, an international randomized control trial has been started to refine the adjuvant treatment in endometrial cancer based on molecular features (RAINBO trial), in which one arm includes patients with NSMP EC (ClinicalTrials.gov Identifier: NCT05255653). Patients with ER positive expression will receive RT and hormonal treatment. However, only the presence of ER expression is part of the inclusion criteria, and the cutoff for positivity is not specified. Furthermore, in order to increase response to hormonal treatment, a different cutoff for ER and PR might be indicated as suggested by a recent paper in which a cutoff of 50 % was suggested [31].

5. Conclusion

Our study demonstrated the prognostic relevance of ER and PR expression within the molecular subgroups of patients with EC and that the use of a three-tiered risk classification refines prognostication. These data support incorporating routine evaluation of ER/PR expression in clinical practice.

Supplementary data to this article can be found online at https://doi.org/10.1016/j.ygyno.2024.10.028.

Tweetable statement

Hormonal biomarkers remain prognostically relevant within the molecular subgroups in endometrial cancer.

Funding

None.

Paper presentation information

The findings of this study were presented on a poster presentation session at the IGCS September 2022 Annual Global meeting in New York.

Ethical approcval

ENITEC centers: This study was approved by the Institutional Review Board of the Radboud University Medical Center and the Institutional Review Boards of all participating centers.

Vancouver cohort: Research ethics approval were present for the tissues.

Data was used from previous published studies and, therefore, informed consent was waived for participants.

CRediT authorship contribution statement

Stephanie W. Vrede: Writing – review & editing, Writing – original draft, Visualization, Validation, Project administration, Methodology, Investigation, Formal analysis, Data curation. **Willem Jan Van Weelden:** Writing – review & editing, Visualization, Resources, Methodology, Investigation, Conceptualization. **Johan Bulten:** Writing – review & editing, Visualization, Supervision, Resources, Investigation. **C. Blake**

Gilks: Writing – review & editing, Resources, Investigation. **Steven Teerenstra:** Writing – review & editing, Formal analysis. **Jutta Huvila:** Writing – review & editing, Resources. **Xavier Matias-Guiu:** Writing – review & editing, Resources. **Antonio Gil-Moreno:** Writing – review & editing, Visualization, Resources, Methodology, Investigation, Conceptualization. **Jasmin Asberger:** Writing – review & editing, Visualization, Resources, Methodology, Investigation, Conceptualization. **Sanne Sweegers:** Writing – review & editing, Investigation. **Louis J.M. van der Putten:** Writing – review & editing, Resources, Investigation. **Heidi V.N. Küsters-Vandevelde:** Writing – review & editing, Resources. **Casper Reijnen:** Writing – review & editing, Visualization, Methodology, Investigation. **Eva Colas:** Writing – review & editing, Resources. **Jitka Hausnerová:** Writing – review & editing, Resources, Investigation. **Vit Weinberger:** Writing – review & editing, Resources. **Marc P.L.M. Snijders:** Writing – review & editing, Visualization, Supervision, Resources. **Petra Vinklerova:** Writing – review & editing, Resources. **Antonella Ravaggi:** Writing – review & editing, Resources, Investigation. **Franco Odicino:** Writing – review & editing, Resources. **Eliana Bignotti:** Writing – review & editing, Resources. **Jessica N. McAlpine:** Writing – review & editing, Resources. **Roy Kruitwagen:** Writing – review & editing, Visualization, Supervision, Methodology. **Johanna M.A. Pijnenborg:** Writing – review & editing, Visualization, Supervision, Methodology, Conceptualization.

Declaration of competing interest

The authors have declared no conflicts of interest.

Acknowledgements

The authors would like to thank Dr. Astrid Eijkelenboom for her assistance and knowledge within the molecular pathology. The authors are grateful to Dr. Germana Tognon and Dr. Laura Ardighieri for their support in performing molecular classification and helpful discussion of results. The molecular analysis of the Brescia cohort (Italy) was supported by “Chiara Andreoli Foundation”.

References

[1] J.V. Bokhman, Two pathogenetic types of endometrial carcinoma, Gynecol. Oncol. 15 (1) (1983) 10–17.
[2] J.J. Kim, E. Chapman-Davis, Role of progesterone in endometrial cancer, Semin. Reprod. Med. 28 (1) (2010) 81–90.
[3] M.E. McDonald, D.P. Bender, Endometrial Cancer: obesity, genetics, and targeted agents, Obstet. Gynecol. Clin. N. Am. 46 (1) (2019) 89–105.
[4] A.C. Rodriguez, Z. Blanchard, K.A. Maurer, J. Gertz, Estrogen signaling in endometrial cancer: a key oncogenic pathway with several open questions, Horm Cancer. 10 (2–3) (2019) 51–63.
[5] L.J.M. van der Putten, N.C.M. Visser, K. van de Vijver, M. Santacana, P. Bronsert, J. Bulten, et al., Added value of estrogen receptor, progesterone receptor, and L1 cell adhesion molecule expression to histology-based endometrial carcinoma recurrence prediction models: an ENITEC collaboration study, Int. J. Gynecol. Cancer 28 (3) (2018) 514–523.
[6] J. Trovik, E. Wik, H.M. Werner, C. Krakstad, H. Helland, I. Vandenput, et al., Hormone receptor loss in endometrial carcinoma curettage predicts lymph node metastasis and poor outcome in prospective multicentre trial, Eur. J. Cancer 49 (16) (2013) 3431–3441.
[7] S.W. Vrede, W.J. van Weelden, N.C.M. Visser, J. Bulten, L.J.M. van der Putten, K. van de Vijver, et al., Immunohistochemical biomarkers are prognostic relevant in addition to the ESMO-ESGO-ESTRO risk classification in endometrial cancer, Gynecol. Oncol. 161 (3) (2021) 787–794.
[8] M. Yi, L. Huo, K.B. Koenig, E.A. Mittendorf, F. Meric-Bernstam, H.M. Kuerer, et al., Which threshold for ER positivity? A retrospective study based on 9639 patients, Ann. Oncol. 25 (5) (2014) 1004–1011.
[9] K.H. Allison, M.E.H. Hammond, M. Dowsett, S.E. McKernin, L.A. Carey, P.L. Fitzgibbons, et al., Estrogen and progesterone receptor testing in breast Cancer: ASCO/CAP guideline update, J. Clin. Oncol. 38 (12) (2020) 1346–1366.
[10] W.J. van Weelden, C. Reijnen, H.V.N. Küsters-Vandevelde, J. Bulten, P. Bult, S. Leung, et al., The cutoff for estrogen and progesterone receptor expression in endometrial cancer revisited: a European network for individualized treatment of endometrial cancer collaboration study, Hum. Pathol. 109 (2021) 80–91.
[11] C. Kandoth, N. Schultz, A.D. Cherniack, R. Akbani, Y. Liu, H. Shen, et al., Integrated genomic characterization of endometrial carcinoma, Nature 497 (7447) (2013) 67–73.
[12] A. Talhouk, M.K. McConechy, S. Leung, W. Yang, A. Lum, J. Senz, et al., Confirmation of ProMisE: a simple, genomics-based clinical classifier for endometrial cancer, Cancer 123 (5) (2017) 802–813.
[13] A. Talhouk, M.K. McConechy, S. Leung, H.H. Li-Chang, J.S. Kwon, N. Melnyk, et al., A clinically applicable molecular-based classification for endometrial cancers, Br. J. Cancer 113 (2) (2015) 299–310.
[14] E. Perrone, I. Capasso, F. De Felice, D. Giannarelli, G. Dinoi, A. Petrecca, et al., Back to the future: the impact of oestrogen receptor profile in the era of molecular endometrial cancer classification, Eur. J. Cancer 186 (2023) 98–112.
[15] L. Vermij, J.J. Jobsen, A. León-Castillo, M. Brinkhuis, S. Roothaan, M.E. Powell, et al., Prognostic refinement of NSMP high-risk endometrial cancers using oestrogen receptor immunohistochemistry, Br. J. Cancer 128 (7) (2023) 1360–1368, https://doi.org/10.1038/s41416-023-02141-0.
[16] A. Jamieson, J. Huvila, D. Chiu, E.F. Thompson, S. Scott, S. Salvador, 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. 36 (4) (2023), 100085.
[17] A.N. Karnezis, S. Leung, J. Magrill, M.K. McConechy, W. Yang, C. Chow, et al., Evaluation of endometrial carcinoma prognostic immunohistochemistry markers in the context of molecular classification, J. Pathol. Clin. Res. 3 (4) (2017) 279–293.
[18] L.J. van der Putten, N.C. Visser, K. van de Vijver, M. Santacana, P. Bronsert, J. Bulten, et al., L1CAM expression in endometrial carcinomas: an ENITEC collaboration study, Br. J. Cancer 115 (6) (2016) 716–724.
[19] A. Ravaggi, D. Capoferri, L. Ardighieri, I. Ghini, F. Ferrari, C. Romani, et al., Integrated biomarker analysis reveals L1CAM as a potential stratification marker for no specific molecular profile high-risk endometrial carcinoma, Cancers (Basel) 14 (21) (2022).
[20] WHO classification of Tumours5th ed, 2020.
[21] S.W. Vrede, J. Kasius, J. Bulten, S. Teerenstra, J. Huvila, E. Colas, et al., Relevance of molecular profiling in patients with low-grade endometrial cancer, JAMA Netw. Open 5 (12) (2022), e2247372.
[22] A. Eijkelenboom, E.J. Kamping, A.W. Kastner-van Raaij, S.J. Hendriks-Cornelissen, K. Neveling, R.P. Kuiper, et al., Reliable next-generation sequencing of formalin-fixed, paraffin-embedded tissue using single molecule tags, J. Mol. Diagn. 18 (6) (2016) 851–863.
[23] E.M.P. Steeghs, L.I. Kroeze, B.B.J. Tops, L.C. van Kempen, A. Ter Elst, A.W.M. Kastner-van Raaij, et al., Comprehensive routine diagnostic screening to identify predictive mutations, gene amplifications, and microsatellite instability in FFPE tumor material, BMC Cancer 20 (1) (2020) 291.
[24] A. León-Castillo, E. Gilvazquez, R. Nout, V.T. Smit, J.N. McAlpine, M. McConechy, et al., Clinicopathological and molecular characterisation of ‘multiple-classifier’ endometrial carcinomas, J. Pathol. 250 (3) (2020) 312–322.
[25] C. Reijnen, E. Gogou, N.C.M. Visser, H. Engerud, J. Ramjith, L.J.M. van der Putten, et al., Preoperative risk stratification in endometrial cancer (ENDORISK) by a Bayesian network model: a development and validation study, PLoS Med. 17 (5) (2020), e1003111.
[26] N. Singh, A.M. Piskorz, T. Bosse, M. Jimenez-Linan, B. Rous, J.D. Brenton, et al., p53 immunohistochemistry is an accurate surrogate for TP53 mutational analysis in endometrial carcinoma biopsies, J. Pathol. 250 (3) (2020) 336–345.
[27] M.K. McConechy, A. Talhouk, H.H. Li-Chang, S. Leung, D.G. Huntsman, C.B. Gilks, et al., Detection of DNA mismatch repair (MMR) deficiencies by immunohistochemistry can effectively diagnose the microsatellite instability (MSI) phenotype in endometrial carcinomas, Gynecol. Oncol. 137 (2) (2015) 306–310.
[28] A. Flanagin, T. Frey, S.L. Christiansen, Updated guidance on the reporting of race and ethnicity in medical and science journals, Jama 326 (7) (2021) 621–627.
[29] A. Sheikh, G. Netuveli, J. Kai, S.S. Panesar, Comparison of reporting of ethnicity in US and European randomised controlled trials, Bmj 329 (7457) (2004) 87–88.
[30] A. León-Castillo, H. Britton, M.K. McConechy, J.N. McAlpine, R. Nout, S. Kommoss, et al., Interpretation of somatic POLE mutations in endometrial carcinoma, J. Pathol. 250 (3) (2020) 323–335.
[31] W.J. van Weelden, R.I. Lalisang, J. Bulten, K. Lindemann, H.J. van Beekhuizen, H. Trum, et al., Impact of hormonal biomarkers on response to hormonal therapy in advanced and recurrent endometrial cancer, Am. J. Obstet. Gynecol. 225 (4) (2021) 407.e1-.e16.