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Original Article

Evaluating the prognostic value of estrogen receptor status in stage III MMR-proficient endometrial cancer: real-world data from an Asian population

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Abstract: To evaluate the prognostic value of estrogen receptor (ER) expression in mismatch repair-proficient (MMRp) stage III endometrial cancer (EC) in an Asian cohort using an immunohistochemistry (IHC) - based molecular classification, we retrospectively analyzed patients with stage III EC classified by IHC into three molecular subtypes: mismatch repair-deficient (MMRd), TP53-mutant, and MMRp. The MMRp group was further stratified by ER expression (10% cutoff) into ER-positive and ER-negative subgroups, yielding four molecular subtypes. Clinicopathological features and survival outcomes were compared. Ninety-five patients were included: 7 (7.4%) with MMRp ER-negative, 34 (35.8%) with MMRp ER-positive, 27 (28.4%) with MMRd, and 27 (28.4%) with TP53-mutant tumors. With a median follow-up of 61 months, progression-free survival (PFS) and cancer-specific survival (CSS) differed significantly across four IHC-based subtypes (log-rank P = 0.0013 and P<0.0001, respectively). In multivariate Firth's penalized Cox regression, MMRp ER-negative status was an independent predictor of poor PFS (HR 4.91; 95% CI, 1.43-15.80) and CSS (HR 7.44; 95% CI, 1.85-30.80), independent of histologic grade. Even within low-risk MMRp endometrioid grade 1-2 tumors, ER-negativity identified a high-risk subset (12.1%, 4/33). To our knowledge, this is among the first studies to evaluate the prognostic relevance of ER expression in stage III MMRp EC in an Asian cohort using a pragmatic IHC-based approach. Low ER expression identified a distinct high-risk subgroup whose outcomes approached those of TP53-mutant tumors.

Keywords: Estrogen receptor, molecular subtype, immunohistochemistry, endometrial cancer, prognosis

Introduction

The Cancer Genome Atlas stratifies endometrial cancer (EC) into four molecular categories with distinct prognostic implications: POLE-ultramutated (POLEmut), mismatch repair-deficient (MMRd), TP53-mutant, and no specific molecular profile (NSMP). Although the prognostic profiles of the POLEmut, MMRd, and TP53-mutant subgroups are well-established, patients with NSMP EC exhibit heterogeneous outcomes, reflecting the diverse molecular landscape within this category. To address this heterogeneity, the 2025 ESGO/ESTRO/ESP guidelines now recommend incorporating estrogen receptor (ER) expression to stratify NSMP tumors, using a 10% cutoff [1]. Specifically, NSMP tumors with negative ER expression are reclassified as high-risk, a strategy supported by recent pivotal studies [2, 3].

However, a critical knowledge gap remains. Supportive evidence for ER status as a prognostic stratifier has, to date, been derived predominantly in Western populations. Whether these findings apply to Asian populations, which

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may differ in genetic background and clinicopathological characteristics, has not been rigorously evaluated. Evaluating the prognostic utility of ER expression in an Asian cohort may help assess the broader applicability of the updated ESGO recommendations across different populations. Moreover, comprehensive genomic profiling, such as next-generation sequencing for POLE mutations, is not routinely available in many Asian clinical settings owing to its high cost and limited infrastructure. To date, data directly evaluating the prognostic value of ER expression within a fully IHC-based framework - the most clinically feasible approach in these settings - remain limited.

To address these unmet needs, this study aimed to evaluate the prognostic value of ER expression within the MMR-proficient (MMRp) subtype using an IHC-based molecular classification in an Asian cohort of stage III EC.

Material and methods

Patient recruitment

Patients with International Federation of Gynecology and Obstetrics (FIGO) stage III EC who were diagnosed and treated at Kaohsiung Chang Gung Memorial Hospital between January 2013 and December 2021 were retrospectively enrolled. This study was approved by the Chang Gung Memorial Hospital Institutional Review Board (IRB No. 202201399B0) on September 20, 2022, and conducted in accordance with the Declaration of Helsinki. The IRB granted a waiver for participant consent. All patients underwent complete staging surgery including total hysterectomy, bilateral salpingo-oophorectomy, and pelvic lymphadenectomy with or without para-aortic lymphadenectomy and were staged according to the 2009 FIGO system. Exclusion criteria were: (1) incomplete medical records; (2) no adequate specimens available for immunohistochemistry (IHC) analysis; (3) refusal to receive adjuvant treatment; (4) follow-up time less than 3 months; and (5) incomplete adjuvant treatment (**Figure 1**). Clinical and pathological data were retrieved from medical records.

Immunohistochemistry analysis

IHC analysis was performed on formalin-fixed, paraffin-embedded tissue sections. All of the tissues were obtained from hysterectomy specimens, with the exception of two cases which were obtained from metastatic sites (ovary, omentum and lymph nodes) during restaging surgery. Tumor tissues were fixed in 10% neutral buffered formalin for 6-72 hours following College of American Pathologists (CAP) guidelines and subsequently processed into paraffin blocks. Paraffin-embedded tumor tissues were sectioned at 3 μm thickness, and all staining procedures were performed using a Ventana BenchMark Ultra Autostainer (Roche Diagnostics Ltd., Taipei, Taiwan) according to the manufacturer's instructions.

MMR and TP53 staining: The primary antibodies used to assess MMR status were those specific for MLH1 (clone GM011, 1:100; Genemed, South San Francisco, CA, USA), MSH2 (clone ZR260, 1:100; Zeta, Arcadia, CA, USA), MSH6 (clone GM024, 1:100; Genemed, South San Francisco, CA, USA) and PMS2 (clone A16-4, 1:50; Zytomed Systems GmbH, Berlin, Germany). The protocols used to process the specimens have been described previously [4]. IHC testing for TP53 (clone DO-7, 1:200; Leica, Newcastle Upon Tyne, United Kingdom) was classified as either mutation-type or wild-type. The mutation-type was defined by strong staining in more than 80% of tumor cell nuclei or a complete absence of nuclear staining, and the wild-type was defined by variable staining intensity with a wide distribution among tumor cell nuclei [5].

Estrogen receptor staining and interpretation: Currently, the CAP does not specifically outline protocols for assessing ER IHC staining in endometrial cancer; therefore, we adopted a protocol similar to that recommended for breast cancer based on CAP guidelines. The primary antibody used for ER was clone SP1 (ready-to-use; Roche Diagnostics Ltd.) with overnight incubation at 4°C, and the detection system used was Roche Ventana Ultraview DAB Detection 760-500. Sections were counterstained with hematoxylin and mounted with aqueous mounting media. Slides were visualized at 200× magnification. Previously tested breast cancer specimens with known positive and negative ER immunostaining results were used as positive and negative controls for each staining batch. ER expression was assessed across the whole tumor section. ER positivity was defined as more than 10% of tumor cell

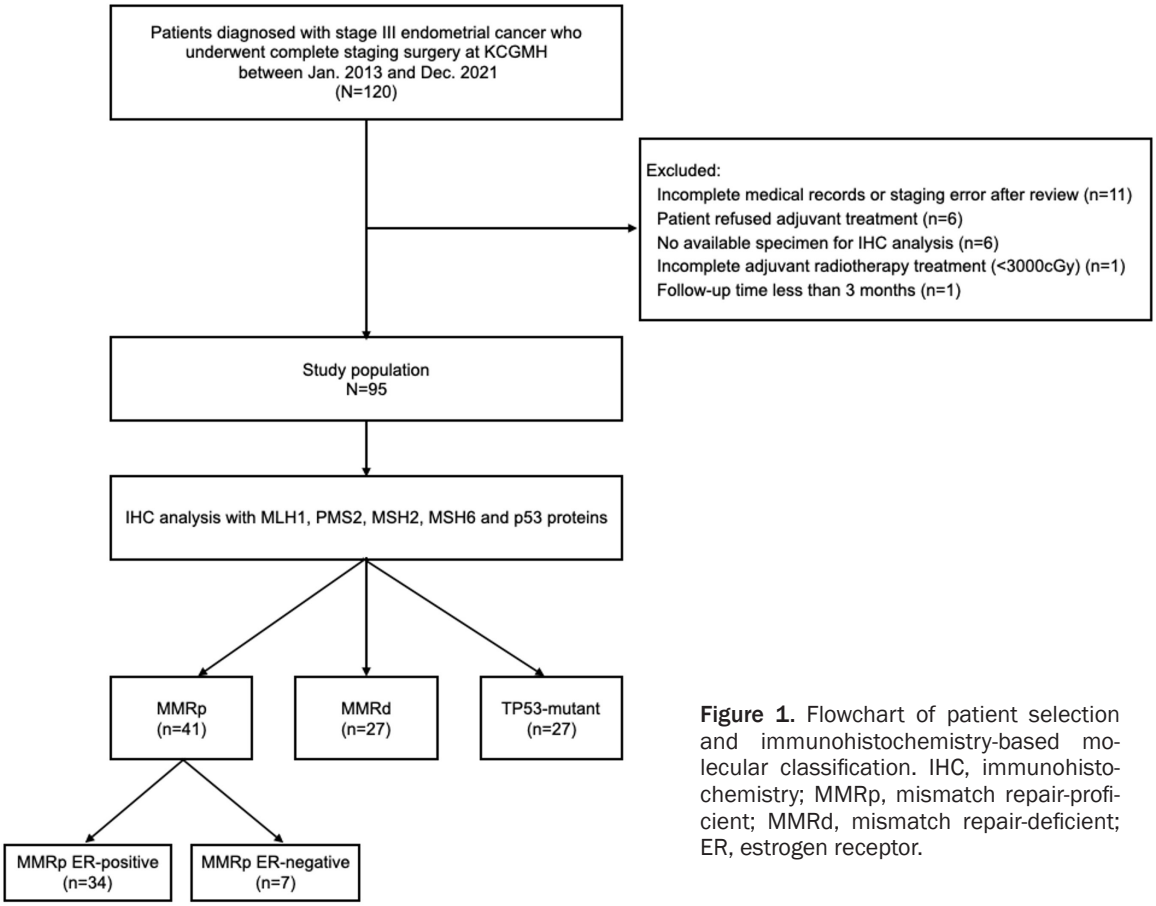

Figure 1. Flowchart of patient selection and immunohistochemistry-based molecular classification. IHC, immunohistochemistry; MMRp, mismatch repair-proficient; MMRd, mismatch repair-deficient; ER, estrogen receptor.

nuclei showing positive staining, based on the threshold recommended by Vermij et al. for prognostic stratification in NSMP endometrial carcinoma [2].

Quality control and pathological review

All cases were independently interpreted by two experienced gynecological pathologists who were blinded to the clinical outcomes. In case of discrepant interpretations, the pathologists reviewed the slides together to reach a consensus. If the consensus could not be reached, a third expert pathologist made the final determination. Our pathology laboratory is accredited under the CAP Laboratory Accreditation Program (CAP number 7232636), demonstrating compliance with rigorous quality and performance standards.

Molecular subtype classification

The enrolled patients were first classified into three subgroups according to the results of IHC analysis: MMR proficient (MMRp) group (MMRp status and TP53 wild-type), MMRd group (MMR protein loss with or without TP53 mutation-type), and TP53-mutant group (with TP53 mutation-type and MMRp status). The co-existence of MMRd and TP53-mutant in tumors was considered as the MMRd subgroup [6]. The MMRp group was further stratified by ER status into MMRp ER-positive and MMRp ER-negative subgroups (**Figure 1**).

Survival analysis and pattern of recurrence

PFS was calculated from staging surgery to the first event of recurrence, progression, death, or last follow-up for those without recurrence or progression. CSS was calculated from staging surgery to death from EC or last follow-up.

Statistical analysis

Clinicopathological features among the four molecular subtypes were compared using the chi-square or Fisher's exact test, as appropriate. PFS and CSS were estimated using Kaplan-Meier analysis. To complement the hazard

ratio-based analysis and quantify absolute between-group differences in survival without relying on the proportional hazards assumption, we performed restricted mean survival time (RMST) analysis. RMST, defined as the area under the survival curve up to a prespecified time point, represents the average event-free survival time within a given follow-up interval [7]. A time point of 36 months was selected for PFS analysis because most recurrence or progression events in endometrial cancer occur within this period [8]. For CSS analysis, 36 months was selected to allow comparison with PFS, and an additional time point of 55 months was evaluated, corresponding to the maximum follow-up duration commonly shared by all four IHC-molecular groups.

Because of the limited number of patients and events, Firth's penalized likelihood Cox regression was performed for multivariate survival analysis to reduce finite-sample bias [9]. The events-per-variable ratios were 6.4 for PFS analysis and 4.8 for CSS analysis. In addition to molecular subtype, histologic grade and age were included as covariates in the multivariate models, as both have been reported as independent prognostic factors beyond molecular classification in previous studies [10, 11]. A *P*-value <0.05 was considered to indicate statistical significance. Analyses were conducted using R version 4.6.1 and SAS version 9.4 (SAS Institute, Cary, NC, USA).

Results

Prevalence and clinicopathological features across four IHC-based molecular subtypes within this stage III EC cohort

A total of 95 patients were included in the analysis: 7 (7.4%) had MMRp ER-negative tumors, 34 (35.8%) had MMRp ER-positive tumors, 27 (28.4%) had MMRd tumors, and 27 (28.4%) had TP53-mutant tumors (**Figure 1**). Within the overall MMRp group, ER-negative tumors accounted for 17.1% (7/41). Clinicopathological characteristics across the four molecular subtypes are summarized in **Table 1**.

When comparing clinicopathological characteristics between the MMRp ER-negative and MMRp ER-positive groups, no significant differences were observed in age, FIGO substage, or histologic type. The MMRp ER-negative group exhibited a higher proportion of high-grade tumors (42.9% vs. 8.8%) and lymphovascular invasion (100% vs. 61.8%) compared with the MMRp ER-positive group. In addition, the MMRp ER-negative group tended to receive adjuvant chemoradiotherapy more frequently than the MMRp ER-positive group (42.9% vs. 23.5%); however, these differences did not reach statistical significance in the two-group comparison (grade, P = 0.051; lymphovascular invasion, P = 0.077; chemoradiotherapy, P = 0.361; Fisher's exact test) (**Table 1**).

Survival across four IHC-based molecular subtypes

With a median follow-up of 61 months (range: 3-119 months), the 5-year PFS and CSS rates for the entire cohort were 65.4% and 70.3%, respectively. Kaplan-Meier analysis demonstrated significant differences in both PFS and CSS across the four molecular subtypes (**Figure 2A** and **2B**). The median PFS was 21 months for MMRp ER-negative, not reached for MMRp ER-positive, not reached for MMRd, and 39 months for TP53-mutant tumors (log-rank *P* = 0.0013). The corresponding median CSS were 41 months (MMRp ER-negative), not reached (MMRp ER-positive), not reached (MMRd), and 53 months (TP53-mutant) (log-rank *P*<0.0001).

To quantify absolute between-group differences, we performed RMST analysis with MMRp ER-positive as the reference (**Table 2**). At a 36-month horizon, the RMST for PFS was significantly shortened in the MMRp ER-negative (-13.46 months; P = 0.004) and TP53-mutant (-7.88 months; P = 0.003) groups, whereas the MMRd group did not differ significantly (-2.96 months; P = 0.209). For CSS, the 36-month RMST difference in the MMRp ER-negative group was not significant (-1.78 months; P = 0.330), but at a 55-month horizon it became significant (-11.25 months; P = 0.015), indicating that most cancer-specific deaths in this group occurred after 36 months. The TP53-mutant group showed significantly shorter CSS at both horizons (36 months: -5.25 months, P = 0.007; 55 months: -12.78 months, P<0.001), whereas the MMRd group did not differ at either horizon.

Independent prognostic value of ER status

In multivariate Firth's penalized Cox regression adjusting for age and histologic grade, MMRp ER-negative status remained an independent

Table 1. Clinicopathological characteristics of patients according to molecular subtypes

Variable	Total	MMRp ER-negative	MMRp ER-positive	MMRd*	TP53-mutant	*P*-value
No. of patients	95	7 (7.4)	34 (35.8)	27 (28.4)	27 (28.4)	
Age						0.004
≤60 years	65 (68.4)	5 (71.4)	28 (82.4)	21 (77.8)	11 (40.7)	
>60 years	30 (31.6)	2 (28.6)	6 (17.6)	6 (22.2)	16 (59.3)	
FIGO stage						0.668
IIIA/IIIB	28 (29.5)	3 (42.9)	13 (38.2)	6 (22.2)	6 (22.2)	
IIIC1	36 (37.9)	2 (28.6)	13 (38.2)	11 (40.7)	10 (37.0)	
IIIC2	31 (32.6)	2 (28.6)	8 (23.5)	10 (37.0)	11 (40.7)	
Histology						<0.0001
Endometrioid G1-2	52 (54.7)	4 (57.1)	29 (85.3)	16 (59.3)	3 (11.1)	
Endometrioid G3	10 (10.5)	1 (14.3)	2 (5.9)	1 (3.7)	6 (22.2)	
Mucinous	3 (3.2)	0 (0)	2 (5.9)	1 (3.7)	0 (0)	
Serous	9 (9.5)	0 (0)	0 (0)	0 (0)	9 (33.3)	
Clear cell	2 (2.1)	1 (14.3)	0 (0)	1 (3.7)	0 (0)	
Carcinosarcoma	11 (11.6)	0 (0)	0 (0)	3 (11.1)	8 (29.6)	
Mixed	4 (4.2)	1 (14.3)	1 (2.9)	2 (7.4)	0 (0)	
Dedifferentiated	3 (3.2)	0 (0)	0 (0)	2 (7.4)	1 (3.7)	
Squamous cell	1 (1.1)	0 (0)	0 (0)	1 (3.7)	0 (0)	
Grade						<0.0001
Low	57 (60.0)	4 (57.1)	31 (91.2)	19 (70.4)	3 (11.1)	
High	38 (40.0)	3 (42.9)	3 (8.8)	8 (29.6)	24 (88.9)	
Lymphovascular invasion						0.160
Negative	25 (26.3)	0 (0)	13 (38.2)	6 (22.2)	6 (22.2)	
Positive	70 (73.7)	7 (100)	21 (61.8)	21 (77.8)	21 (77.8)	
Adjuvant treatment						0.204
RT	6 (6.3)	1 (14.3)	2 (5.9)	2 (7.4)	1 (3.7)	
CT	66 (69.5)	3 (42.9)	24 (70.6)	16 (59.3)	23 (85.2)	
CTRT	23 (24.2)	3 (42.9)	8 (23.5)	9 (33.3)	3 (11.1)	

Values are n (%). *23 patients in the MMRd subgroup showed concurrent MLH1 and PMS2 loss, 3 had concurrent MSH2 and MSH6 loss, and 1 had isolated MSH6 loss. Notably, only 1 patient had both MMR loss and TP53 mutation; this patient was classified into the MMRd subgroup. MMRp, mismatch repair-proficient; MMRd, mismatch repair-deficient; ER, estrogen receptor; FIGO, International Federation of Gynecology and Obstetrics; G1, 2, 3, FIGO grade 1, 2, 3; low grade, FIGO grade 1-2; high grade, FIGO grade 3; RT, radiotherapy; CT, chemotherapy; CTRT, chemotherapy and radiotherapy.

adverse prognostic factor for both PFS (HR 4.91; 95% confidence interval (CI), 1.43-15.80; P = 0.013) and CSS (HR 7.44; 95% CI, 1.85-30.80; P = 0.006). TP53-mutant status showed a borderline association with CSS (HR 3.95; 95% CI, 0.98-17.80; P = 0.054). Older age (>60 years) was independently associated with worse CSS (HR 2.44; 95% CI, 1.03-5.94; P = 0.042) only. High histologic grade was not significantly associated with either PFS or CSS in the multivariate model (**Table 3**).

To further assess whether the prognostic effect of ER-negativity was independent of histology and grade, we conducted the survival analysis within MMRp endometrioid grade 1-2 tumors (n = 33) (**Figure 3**). Even within this relatively homogeneous, molecularly and histologically low-risk subgroup, ER-negative tumors had significantly worse PFS (median 18.5 vs. not reached; log-rank P = 0.003) and CSS (median 36.5 months vs. not reached; log-rank P< 0.0001). The 36-month RMST difference was significant for PFS (-13.38 months; P = 0.018) but not for CSS (-3.49 months; P = 0.205), the latter reflecting the relatively late occurrence of cancer-specific deaths and the small number of ER-negative patients.

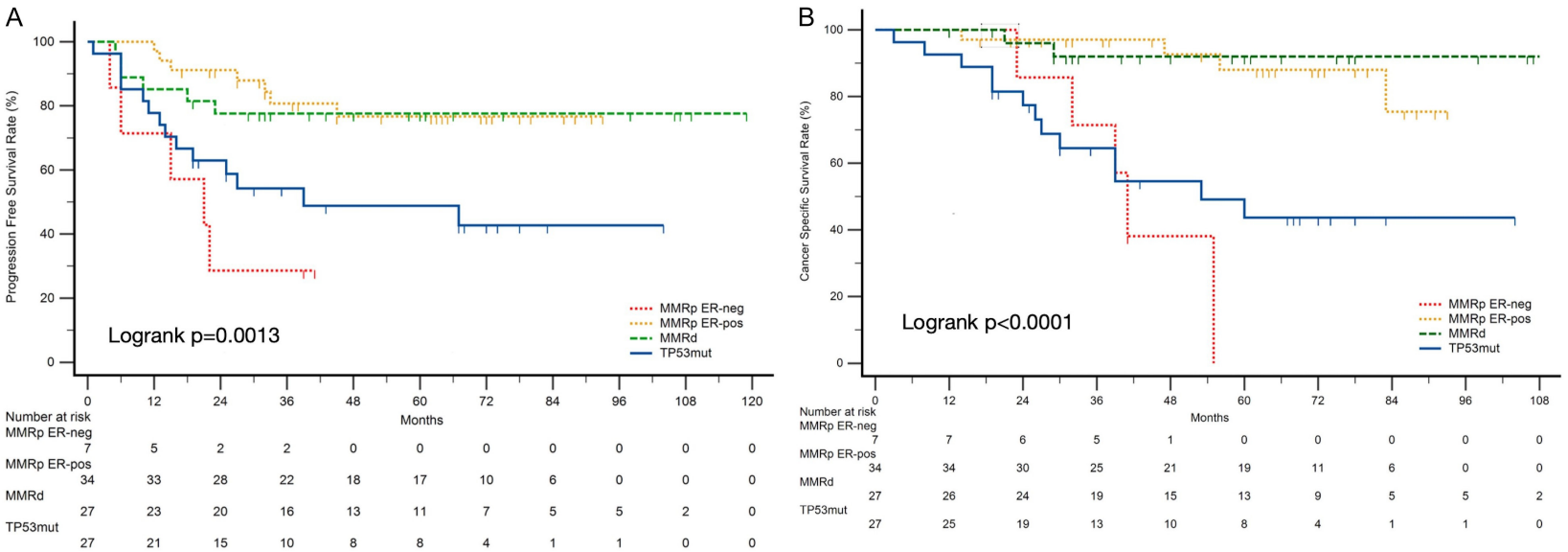

Figure 2. Kaplan-Meier curves comparing (A) progression-free survival and (B) cancer-specific survival across the four molecular subtypes. MMRp, mismatch repair-proficient; ER, estrogen receptor; neg, negative; pos, positive; TP53mut, TP53-mutant.

Table 2. Restricted mean survival time analysis of progression-free survival and cancer-specific survival across molecular subtypes

Molecular subtype	MMRp ER-positive (n = 34)	MMRp ER-negative (n = 7)	MMRd (n = 27)	TP53-mutant (n = 27)
Progression-free survival				
No. of events	7	5	6	14
Median PFS, mo	NR	21	NR	39
RMST analysis at 36 months				
RMST, mo (95% CI)	33.46 (31.25-35.66)	20.00 (11.17-28.83)	30.50 (26.44-34.56)	25.58 (20.83-30.32)
Difference vs reference (95% CI)	Ref.	-13.46 (-22.55 to -4.36)	-2.96 (-7.58 to 1.66)	-7.88 (-13.11 to -2.65)
RMST *P-value*	Ref.	0.004	0.209	0.003
Cancer-specific survival				
No. of events	4	5	2	13
Median CSS, mo	NR	41	NR	53
RMST analysis at 36 months				
RMST, mo (95% CI)	35.35 (34.10-36.60)	33.57 (30.21-36.93)	35.12 (33.87-36.37)	30.10 (26.51-33.70)
Difference vs reference (95% CI)	Ref.	-1.78 (-5.36 to 1.80)	-0.23 (-2.00 to 1.54)	-5.25 (-9.06 to -1.44)
RMST *P-value*	Ref.	0.330	0.796	0.007
RMST analysis at 55 months				
RMST, mo (95% CI)	53.44 (51.04-55.85)	42.19 (33.43-50.96)	52.60 (49.38-55.82)	40.66 (33.95-47.37)
Difference vs reference (95% CI)	Ref.	-11.25 (-20.34 to -2.16)	-0.84 (-4.86 to 3.18)	-12.78 (-19.91 to -5.65)
RMST *P-value*	Ref.	0.015	0.682	<0.001

MMRp, mismatch repair-proficient; MMRd, mismatch repair-deficient; ER, estrogen receptor; PFS, progression-free survival; mo, month; RMST, restricted mean survival time; 95% CI, 95% confidence interval; NR, not reached; CSS, cancer-specific survival. Ref., reference.

Table 3. Multivariate Firth's penalized Cox regression analysis for progression-free survival and cancer-specific survival

Variable	Progression-free survival			Cancer-specific survival		
	Adjusted HR	95% CI	*P-value*	Adjusted HR	95% CI	*P-value*
Molecular subtype						
MMRp ER-positive	Ref.	-	-	Ref.	-	-
MMRp ER-negative	4.91	1.43-15.80	0.013	7.44	1.85-30.80	0.006
MMRd	1.10	0.36-3.24	0.861	0.74	0.13-3.40	0.705
TP53-mutant	2.01	0.63-6.82	0.243	3.95	0.98-17.80	0.054
Age						
≤60 years	Ref.	-	-	Ref.	-	-
>60 years	1.22	0.56-2.63	0.609	2.44	1.03-5.94	0.042
Grade						
Low grade	Ref.	-	-	Ref.	-	-
High grade	1.60	0.61-4.07	0.331	1.00	0.31-3.29	0.995

MMRp, mismatch repair-proficient; MMRd, mismatch repair-deficient; ER, estrogen receptor; HR, hazard ratio; 95% CI, 95% confidence interval. Ref., reference.

The aggressive behavior of MMRp ER-negative subgroup was further illustrated at the case-level (**Table 4**). All seven patients with MMRp ER-negative tumors exhibited ER expression of ≤5%. Four had low-grade endometrioid carcinomas, whereas the remaining three had aggressive histologic types (one clear cell carcinoma, one mixed carcinoma, and one grade 3 endometrioid carcinoma). Only two patients remained alive at the time of analysis, and neither had reached 60 months of follow-up (39 and 41 months). Among these cases, poor outcomes were observed across different histologies and adjuvant regimens.

Alternative ER cut-offs do not improve prognostic stratification

We evaluated the prognostic value of alternative ER stratifications within the MMRp group.

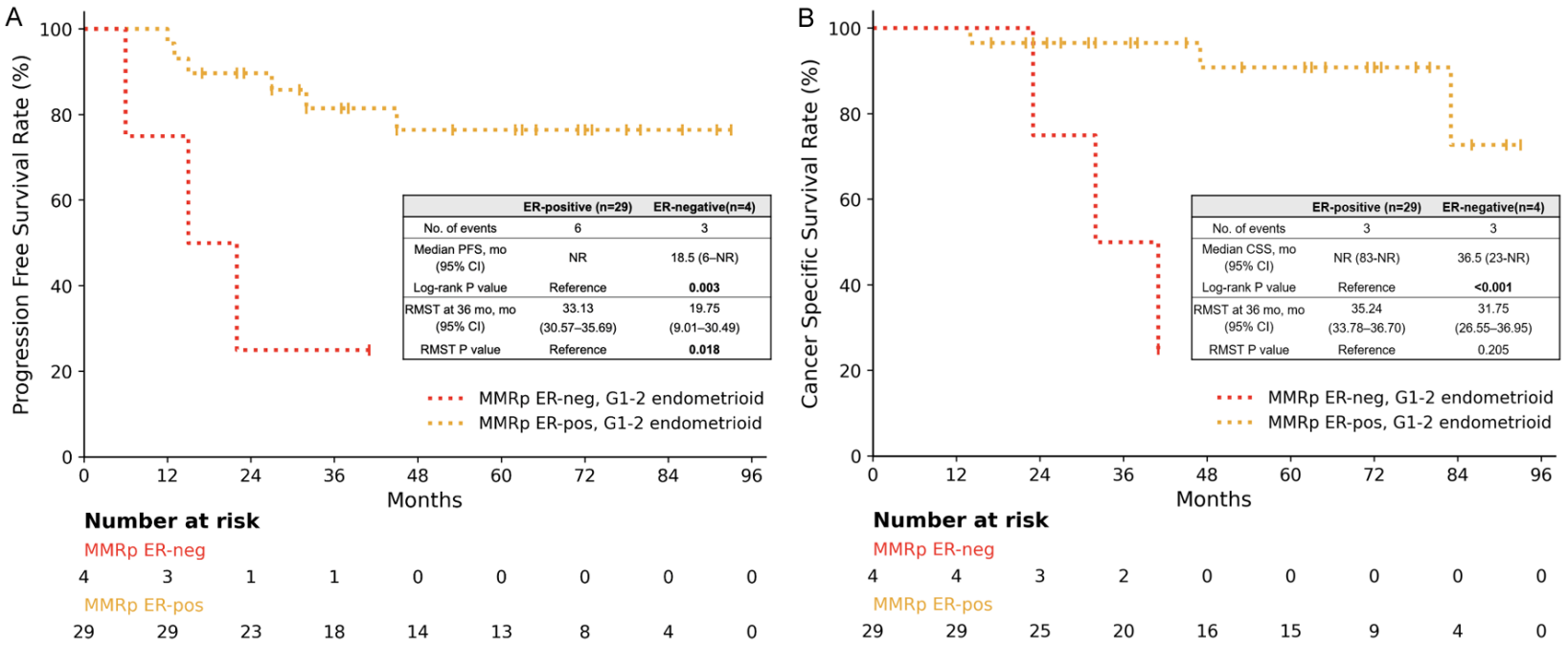

Figure 3. Kaplan-Meier curves comparing (A) progression-free survival and (B) cancer-specific survival in mismatch repair-proficient endometrioid grade 1-2 tumors, stratified by estrogen receptor status. MMRp, mismatch repair-proficient; ER, estrogen receptor; neg, negative; pos, positive; PFS, progression-free survival; mo, month; CI, confidence interval; RMST, restricted mean survival time; NR, not reached; CSS, cancer-specific survival.

Table 4. Characteristics and survival of seven patients in the mismatch repair-proficient estrogen receptor-negative group

Case	Age	Histology	FIGO grade	ER (%)	Stage	Adjuvant treatment	Follow-up (months)	Died of EC
1	69	Endometrioid	1	0	IIIC1	CTRT	41	No
2	53	Endometrioid	2	0	IIIA	RT	23	Yes
3	36	Endometrioid	2	5	IIIB	CT	32	Yes
4	56	Endometrioid	2	5	IIIC2	CTRT	41	Yes
5	51	Endometrioid	3	1	IIIB	CTRT	39	No
6	71	Mixed	High grade	0	IIIC1	CT	55	Yes
7	58	Clear cell	High grade	0	IIIC2	CT	39	Yes

FIGO, International Federation of Gynecology and Obstetrics; ER, estrogen receptor; EC, endometrial cancer; RT, radiotherapy; CT, chemotherapy; CTRT, chemotherapy and radiotherapy.

Using a three-tier cut-off of ≤10% vs. 11-50% vs. >50%, no significant survival difference was detected between the 11-50% and >50% subgroups (CSS P = 0.743; PFS P = 0.685). Similarly, using a cut-off of ≤10% vs. 20-80% vs. ≥90%, no significant difference was found between the 20-80% and ≥90% subgroups (CSS P = 0.482; PFS P = 0.478) (Figures S1, S2, S3, S4).

Patterns of adjuvant treatment and outcomes within each molecular subtype are shown in Figure S5.

Discussion

Our study suggests that integrating ER status into the IHC-based molecular classification may improve risk stratification within MMRp endometrial cancer. Specifically, we identified the MMRp ER-negative group as a clinically distinct high-risk subset with a poor survival outcome approaching those of TP53-mutant tumors, albeit following a delayed pattern of cancer-specific mortality.

Support for the prognostic value of the 10% ER cutoff in an Asian cohort

The MMRp or NSMP subtype constitutes the largest proportion of EC cases but is characterized by marked clinical heterogeneity. Although the College of American Pathologists recommends a >1% threshold for ER positivity based on breast cancer guidelines initially [12], emerging evidence suggests that this cutoff is suboptimal for endometrial cancer. Recent landmark studies, including the TransPORTEC consortium post-hoc analysis of the PORTEC-3 trial by Vermij et al. [2] and the multicenter analysis by Vrede et al. [3], have demonstrated that a 10% cutoff offers superior prognostic discrimination in NSMP endometrial cancers.

In contrast to Vermij et al., which evaluated molecular classification using genomic testing in patients enrolled in a randomized controlled trial [2], the ENDOESTRO study evaluated both IHC-based and genomic-based molecular classification, with retrospective and prospective cohorts, respectively [13]. However, participants in the ENDOESTRO study were predominantly from the Western populations. Our findings extend these observations by demonstrating that the 10% ER cutoff also provides meaningful prognostic stratification when integrated with an IHC-based molecular classification in a Taiwanese cohort of patients with stage III endometrial cancer. Notably, all seven MMRp ER-negative tumors in our cohort expressed ER in ≤5% of tumor cells, with no case falling in the 5-10%; our exploratory data indicate that ER expression above 10% provides no additional prognostic discrimination, supporting binarization at a low ER threshold but cannot precisely resolve whether 5% or 10% is the optimal boundary for defining ER-negativity.

The prevalence, characteristics and poor prognosis of MMRp ER-negative tumors

The prevalence of ER-negative tumors within the MMRp or NSMP subtype varies significantly across studies and appears to correlate with disease risk and stage. Studies focusing on early-stage or generalized populations typically report lower prevalence rates. For instance, the PORTEC-4a trial, which focused on high-intermediate risk disease, reported a prevalence of approximately 2% (7/331) of ER-negative sta-

tus within the NSMP group [14]. Similarly, the ENDOESTRO study, based on a generalized cohort, reported that ER-negative tumors accounted for 6.5% (50/772) of the IHC-based MMRp group in their retrospective cohort [13].

In contrast, in the high-risk PORTEC-3 trial, the proportion of ER-negative tumors rose to 11% (13/117) within the NSMP group, although the authors noted that this figure might be underestimated owing to missing data [15]. The notably higher proportion of ER-negativity (17.1%, 7/41) observed in our IHC-based MMRp tumors compared with these studies likely reflects the enrichment of aggressive phenotypes in our exclusively stage III cohort. This cross-study pattern is consistent with the possibility that ER loss is more frequently observed in higher-risk disease, although differences in study design and case mix should be considered.

In the ENDOESTRO all-staged EC retrospective cohort, multivariate analysis identified ER expression <10%, older age (≥60 years), and advanced 2021 ESGO/ESTRO risk classification as three independent factors associated with worse disease-free survival within IHC-based MMRp EC [13]. In our stage III EC cohort, multivariate analysis identified MMRp ER-negative tumor as an independent adverse prognostic factor for both PFS and CSS, whereas age over 60 years was independently associated with worse CSS only; neither association was attributable to histologic grade. Therefore, both the ENDOESTRO retrospective analysis and our findings indicate that the prognostic value of ER status within MMRp EC is not explained by histologic type or grade.

We further observed that the 10% ER cut-off could separate high-risk cases even within the relatively homogenous MMRp endometrioid G1-2 subgroup. This finding highlights that a non-negligible subset (12.1%, 4/33) of prognostically high-risk cases may be concealed within tumors that otherwise exhibit favorable clinicopathologic features. Given the small number of ER-negative cases (n = 4) in this subgroup, this observation should be considered exploratory and hypothesis-generating and warrant validation in larger independent cohorts.

Although the MMRp ER-negative subgroup was small, its poor prognosis was consistent across all analytic approaches - Kaplan-Meier analysis of the four IHC-based molecular subtypes, RMST estimation, multivariate Firth's penalized Cox regression, and case-level review - and it was clearly distinguished from the MMRp ER-positive subgroup. Descriptively, the between-group differences (MMRp ER-negative vs. ER-positive) were approximately one year for both PFS at 36 months (-13.46 months) and CSS at 55 months (-11.25 months). Because of the limited sample size, the uniformly poor outcomes observed across different adjuvant modalities in the case-level review should be interpreted as a descriptive, hypothesis-generating observation rather than as evidence of treatment resistance. Collectively, these exploratory data may offer clinicians additional insight into the clinical behavior of MMRp ER-negative EC.

Study limitations

This study has several limitations. First, its retrospective design and limited sample size may restrict the generalizability of our findings. Nevertheless, the survival differences between the ER-positive and ER-negative groups reached statistical significance despite the limited number of patients and events, suggesting that the observed association may be clinically meaningful. However, the corresponding wide confidence intervals (e.g., CSS HR, 7.44; 95% CI, 1.85-30.80) indicate considerable statistical uncertainty, and the magnitude of the estimated effect should therefore be interpreted with caution and validated in larger independent cohorts. In addition, patients who refused or did not complete adjuvant treatment were excluded; this may have biased the cohort toward better-treated patients, and the treatment-related observations should therefore be regarded as exploratory.

Second, we did not perform POLE mutational analysis. However, a recent meta-analysis estimated that only 2.77% of FIGO stage II-IV ECs harbor POLE mutations, a relatively low prevalence [16]. Given this low prevalence in advanced disease, together with the high cost and limited accessibility of next-generation sequencing in many Asian clinical settings, our pragmatic IHC-based approach may still be informative for routine practice, particularly in settings where comprehensive molecular testing is not readily available.

Conclusions

To the best of our knowledge, this is among the first studies to examine the prognostic utility of the 10% ER cutoff in Asian patients with stage III MMRp EC using a pragmatic IHC-based approach. Our findings support the clinical relevance of distinguishing MMRp ER-negative tumors from their ER-positive counterparts. The MMRp ER-negative subtype was associated with particularly poor outcomes in this cohort. Consequently, identifying this subgroup may help refine prognostic counseling and support consideration of enrollment in future clinical trials evaluating novel therapeutic strategies.

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Disclosure of conflict of interest

None.

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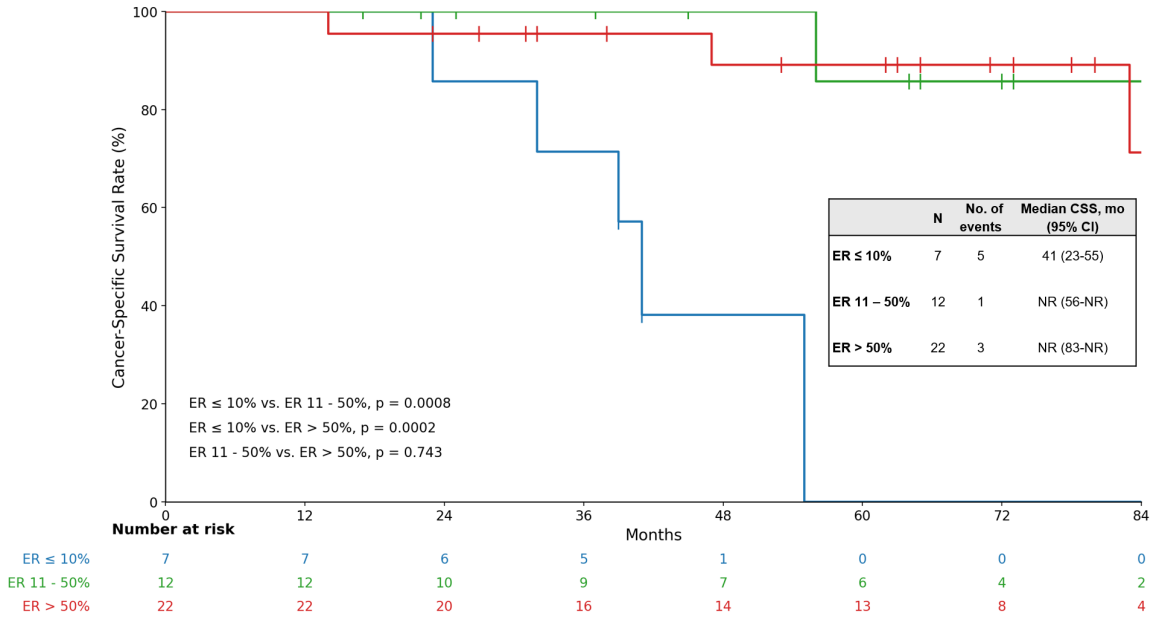


Figure S1. Kaplan-Meier curves of cancer-specific survival stratified by estrogen receptor expression using ≤10%, 11-50% and >50% cutoffs in patients with mismatch repair-proficient endometrial cancer. ER, estrogen receptor; CSS, cancer-specific survival; NR, not reached; mo, month; 95% CI, 95% confidence interval.

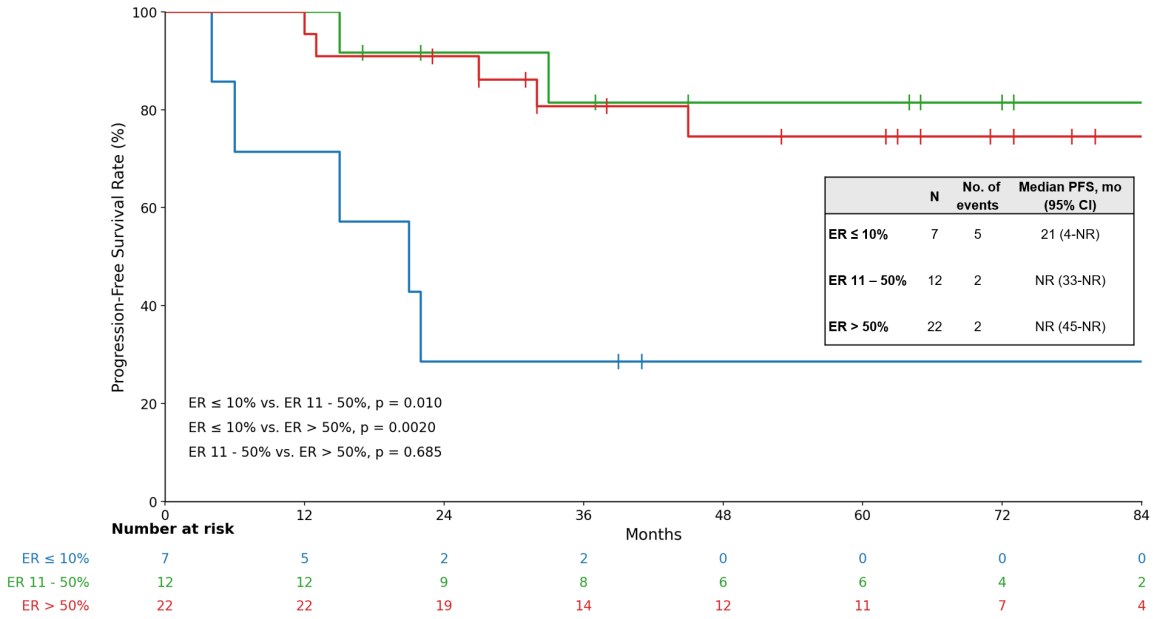
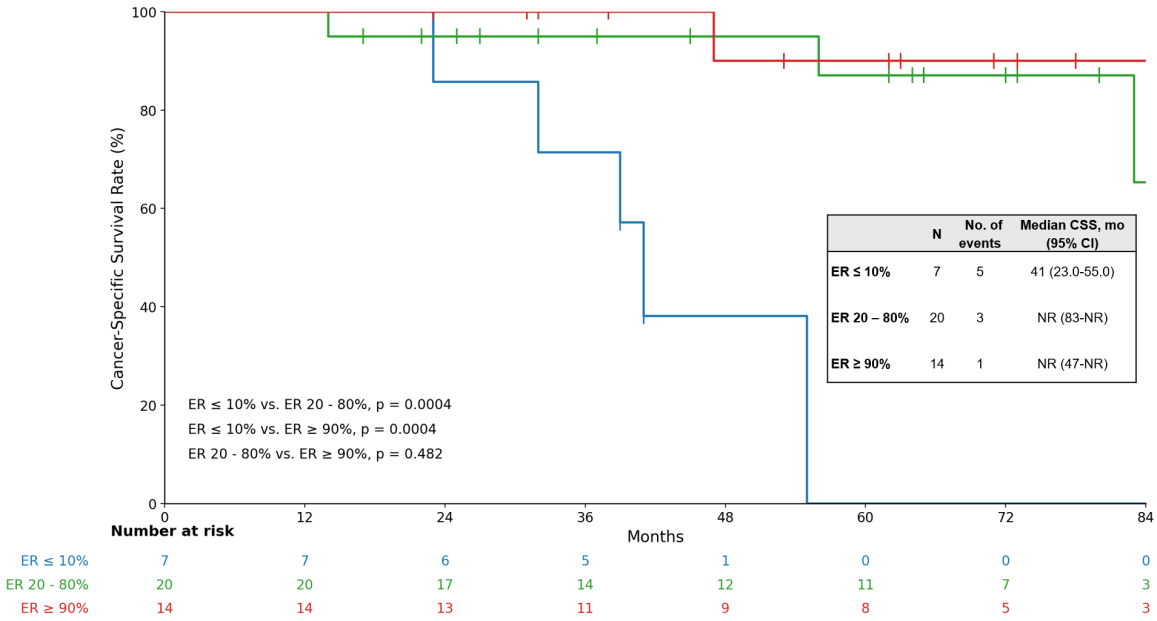


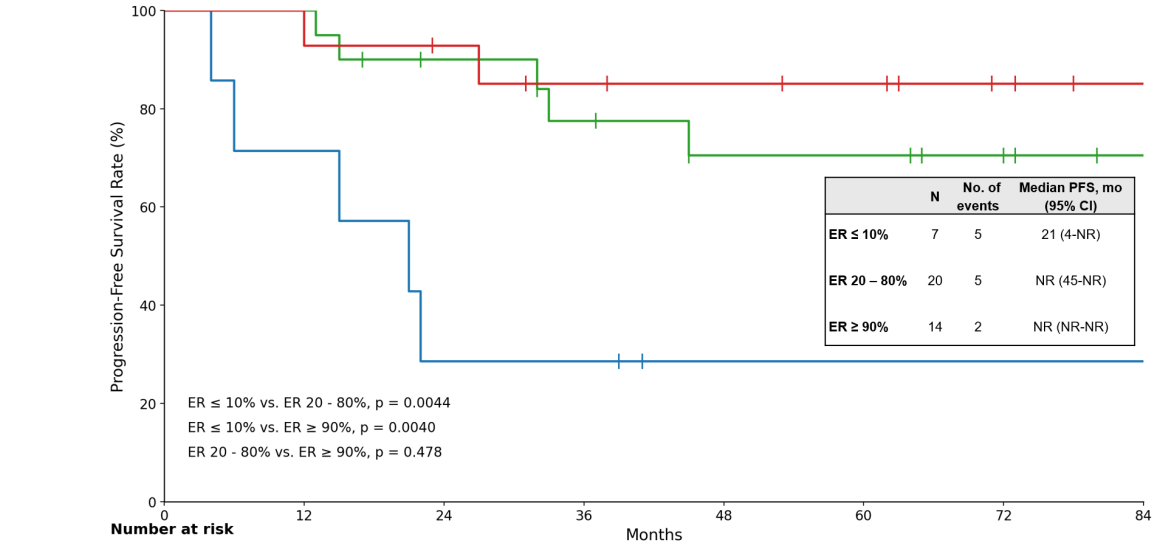
Figure S2. Kaplan-Meier curves of progression-free survival stratified by estrogen receptor expression using ≤10%, 11-50% and >50% cutoffs in patients with mismatch repair-proficient endometrial cancer. ER, estrogen receptor; PFS, progression-free survival; NR, not reached; mo, month; 95% CI, 95% confidence interval.



Number at risk

ER ≤ 10%	7	7	6	5	1	0	0	0
ER 20 - 80%	20	20	17	14	12	11	7	3
ER ≥ 90%	14	14	13	11	9	8	5	3

Figure S3. Kaplan-Meier curves of cancer-specific survival stratified by estrogen receptor expression using ≤10%, 20-80% and ≥90% cutoffs in patients with mismatch repair-proficient endometrial cancer. ER, estrogen receptor; CSS, cancer-specific survival; NR, not reached; mo, month; 95% CI, 95% confidence interval.



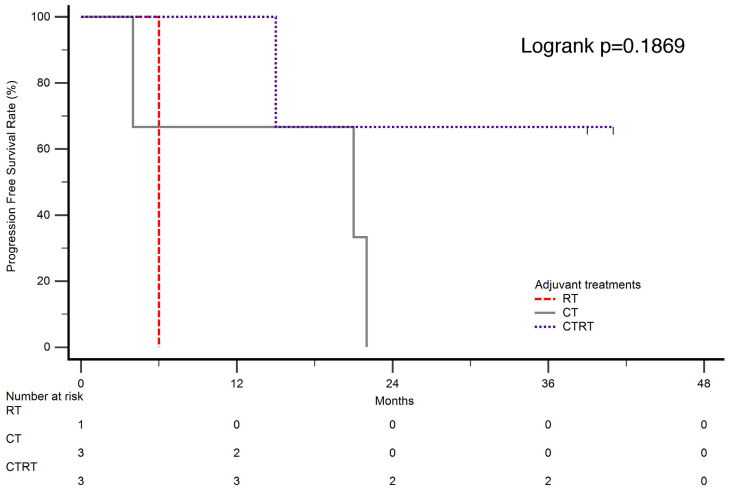
Number at risk

ER ≤ 10%	7	5	2	2	0	0	0	0
ER 20 - 80%	20	20	16	12	9	9	6	3
ER ≥ 90%	14	14	12	10	9	8	5	3

Figure S4. Kaplan-Meier curves of progression-free survival stratified by estrogen receptor expression using ≤10%, 20-80% and ≥90% cutoffs in patients with mismatch repair-proficient endometrial cancer. ER, estrogen receptor; PFS, progression-free survival; NR, not reached; mo, month; 95% CI, 95% confidence interval.

ER status for risk stratification in Asian MMRp EC

A



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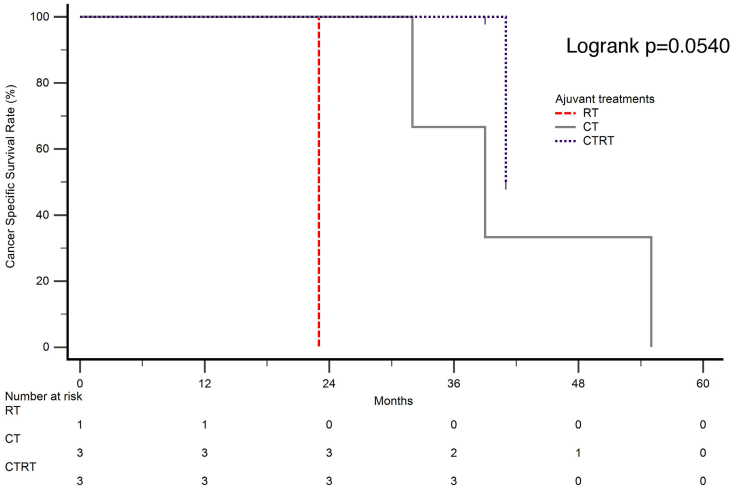

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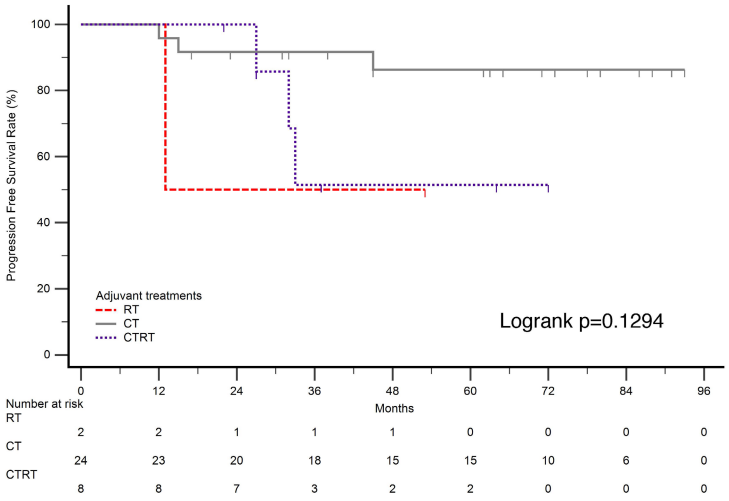

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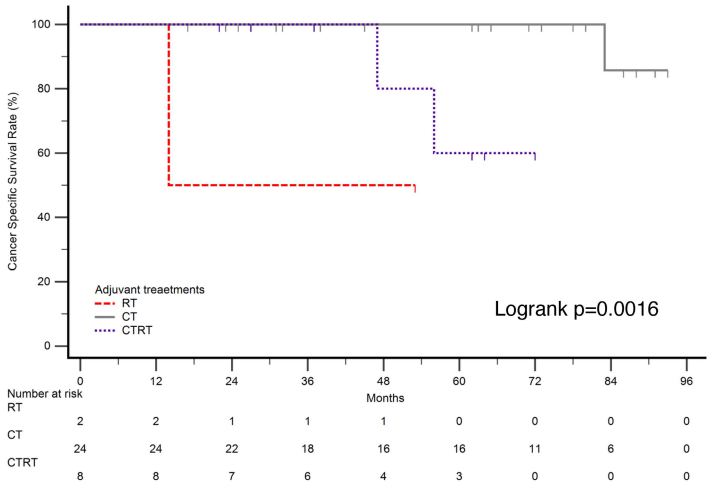

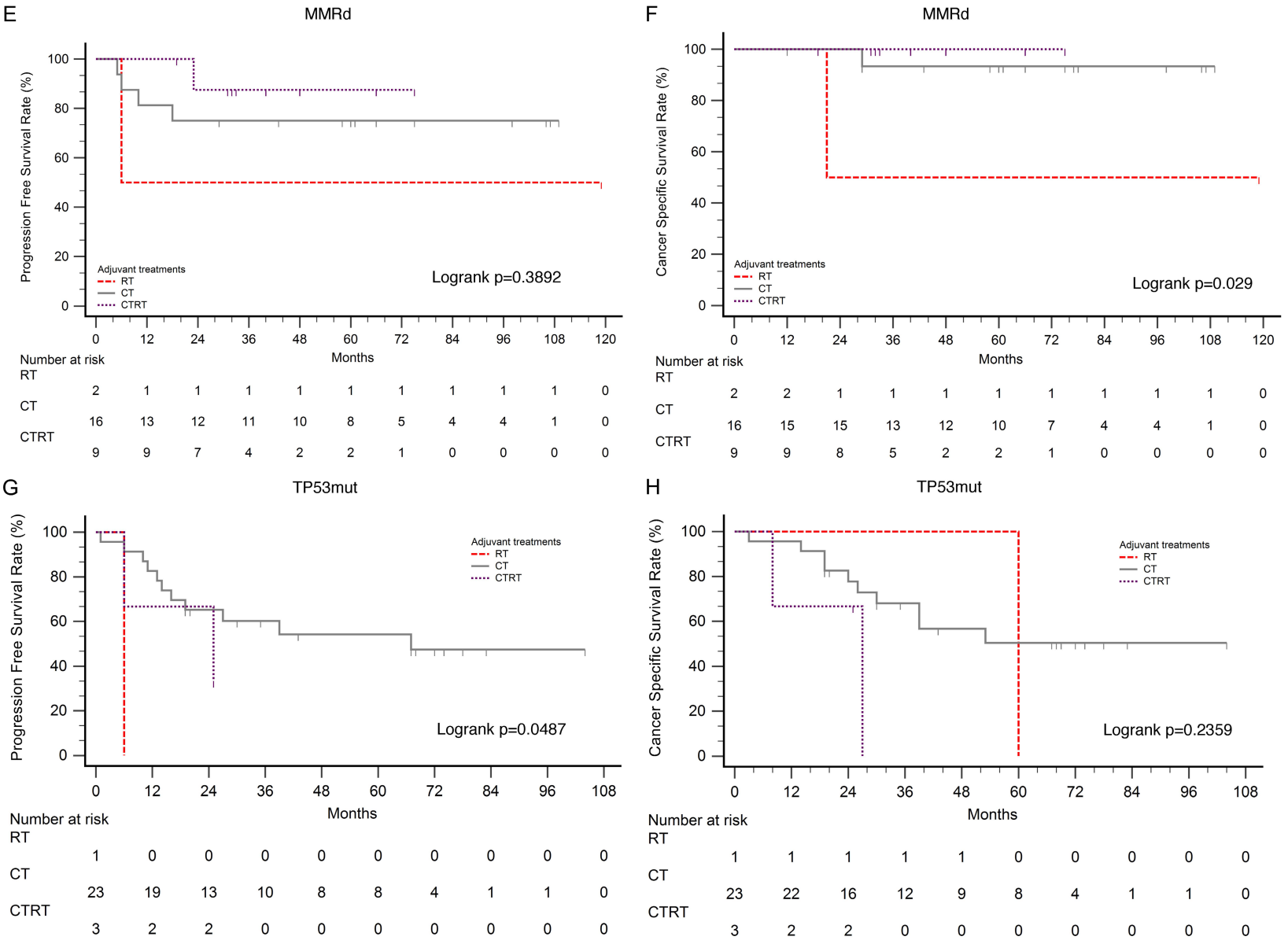

Figure S5. Progression-free survival and cancer-specific survival stratified by adjuvant treatment strategies within each molecular subtype. A, B. MMRp ER-negative; C, D. MMRp ER-positive; E, F. MMRd; G, H. TP53-mutant. RT, radiotherapy; CT, chemotherapy; CTRT, chemotherapy plus radiotherapy.