Clinical and molecular characteristics of high-risk, recurrent, or metastatic endometrial cancer that Is human epidermal growth factor receptor 2-Low

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Clinical and Molecular Characteristics of High-Risk, Recurrent, or Metastatic Endometrial Cancer That Is Human Epidermal Growth Factor Receptor 2–Low

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ABSTRACT

PURPOSE Recent success of human epidermal growth factor receptor 2 (HER2)–targeted antibody-drug-conjugate trastuzumab-deruxtecan in HER2-low and HER2-positive tumors has sparked interest in examining the HER2 status of tumors not traditionally associated with HER2 amplification. Despite the increasing number of systemic treatment options, patients with advanced endometrial cancer (EC) still face a poor prognosis. This study evaluates HER2-low status in over 800 EC, correlating HER2 with both molecular and clinical features.

METHODS HER2 status was determined by immunohistochemistry (IHC) and dual in situ hybridization (DISH) on four studies of previously classified high-risk EC (PORTEC-3 and Medical Spectrum Twente cohort), recurrent or metastatic EC (DOMEC), and a primary stage IV cohort. EC was classified as HER2-negative (IHC 0), HER2-low (IHC 1+/2+ without amplification), or HER2-positive (IHC 3+ or DISH-confirmed amplification). Survival analysis was performed using the Kaplan-Meier method. Cox proportional hazards models assessed the independence of any prognostic impact of HER2 status.

RESULTS HER2 status was determined in 806 EC: 74.8% were HER2-negative, 17.2% HER2-low, and 7.9% HER2-positive. HER2-low was found across all molecular classes and histotypes. The highest rates of HER2-low and HER2-positive tumors were in recurrent or metastatic EC (35.6% and 15.6%), followed by primary stage IV EC (29.9% and 12.4%) and high-risk EC (14.2% and 6.8%). HER2 status had no independent prognostic value.

CONCLUSION A quarter of high-risk, metastatic, or recurrent EC exhibited HER2 overexpression. The presence of HER2 overexpression in all clinical and molecular categories highlights the need for broad testing and offers treatment options for a wide range of patients.

ACCOMPANYING CONTENT

Appendix

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INTRODUCTION

Endometrial cancer (EC) is the most common gynecological cancer in Europe, with an estimated 124,874 new cases and 30,272 deaths in 2022.[1] EC is often curable by surgery alone. However, in patients with high-risk, recurrent, or metastatic disease, survival is poor.[2] For women with advanced disease, or patients with high risk of recurrence on the basis of clinicopathological or molecular features, adjuvant radiotherapy and/or chemotherapy is recommended.[3] Despite newer systemic therapies resulting in better disease control, there is still an unmet need for novel effective systemic treatment options for this patient group.

A promising druggable target in other solid malignancies is the human epidermal growth factor receptor 2 (HER2). HER2-targeting monoclonal antibodies (trastuzumab and pertuzumab) have been well established in the treatment of HER2-positive (3+ score on immunohistochemistry [IHC] or 2+ with confirmed amplification of *ERBB2*) breast and gastric cancers.[4,5] Positive results have also been published for HER2-positive recurrent or advanced uterine

CONTEXT

Key Objective

How does human epidermal growth factor receptor 2 (HER2)–low status correlate with molecular and clinical features in high-risk, recurrent, and advanced endometrial cancer (EC)?

Knowledge Generated

HER2 overexpression was identified in a quarter of high-risk, metastatic, or recurrent EC (17.2% HER2-low and 7.9% HER2-positive). HER2-low overexpression was observed across all molecular classes and histotypes and has no independent prognostic value in high-risk EC.

Relevance *(G. Fleming)*

HER2 status in EC should not be used for decision-making other than patient selection for HER2 targeted therapies. HER2 positive status is primarily seen in p53 abnormal ECs, but screening of ECs for HER2-low status for potential targeted therapies should be performed across molecular and histologic subtypes.*

*Relevance section written by *JCO* Associate Editor Gini Fleming, MD.

serous carcinomas in a phase II trial, where trastuzumab was added to standard chemotherapy.[6] A phase II/III trial is currently conducted (ClinicalTrials.gov identifier: NCT05256225).

Tumors with faint (IHC 1+) or moderate (IHC 2+) overexpression of HER2 in the absence of *ERBB2* amplification, referred to as HER2-low, do, however, not benefit from these HER2-targeted monoclonal antibodies.[8] New potent HER2-targeting antibody-drug conjugates (ADCs), trastuzumab-deruxtecan (T-DXd) in particular, have shown to significantly prolong survival in both HER2-positive and HER2-low breast and gastric cancers, which swiftly led to Food and Drug Administration and European Medicines Agency approval.[9-12] Compared with older ADCs, such as trastuzumab-emtansine, T-DXd has an increased efficacy due to an increased drug-to-antibody ratio of 8:1. T-DXd also has tolerable toxicity due to the tumor-selective linker that only dissolves in the tumor environment[13] and a proven bystander effect: inducing endocytosis of surrounding tumor cells, including HER2-negative tumor cells.[14]

Exciting results of phase II studies with T-DXd were published recently. Interim results of the DESTINY-PanTumor02 study showed promising overall response rates in locally advanced or metastatic EC: 84.6% in the HER2 IHC 3+ population and 47.1% in the HER2 IHC 2+ group.[15] In uterine carcinosarcomas, response rates were 54.5% (HER2-positive) and 70% (HER2-low).[16] Median progression-free survival (PFS) was 6.2 months in the HER2-positive group and 6.7 months in the HER2-low population.

Molecular characteristics of HER2-low EC have not been studied yet. According to The Cancer Genome Atlas (TCGA), EC is classified into four subgroups: *POLE* ultra-mutated (*POLE*mut) with excellent prognosis, mismatch repair–deficient (MMRd) with intermediate prognosis, p53 mutant (p53abn) with poor prognosis, and nonspecific molecular profile (NSMP) with variable prognosis.[17] HER2 amplification is almost exclusively found in the p53abn subclass, but lacks independent prognostic impact in HER2-positive high-risk EC.[18] The distribution and prognostic value of HER2-low across molecular classes are still unknown.

In addition to HER2-low overexpression, *ERBB2* mutations can be targeted with HER2 ADCs.[19] *ERBB2*-mutated tumors were targeted effectively with T-DXd in a phase II study including non–small-cell lung cancer.[20] *ERBB2* mutations have been described in gynecologic cancers, especially in EC.[21] In the TCGA, 7.2% of EC show *ERBB2* mutations.[22,23] However, large studies on clinicopathological implications of *ERBB2* mutations in EC and the relation to HER2 overexpression are lacking. Since these mutations could be additional targets for HER2 ADCs in EC, this study also investigated the prevalence of *ERBB2* mutations in EC.

For the design of future clinical trials and allocation of HER2-directed therapy in EC, more insight into the prevalence and clinical and molecular correlations of HER2-status in high-risk and advanced EC is crucial. The aim of this study was to investigate the prevalence of overexpression levels of HER2 in a large cohort of high-risk, recurrent, and advanced EC including all histotypes and molecular classes. In addition, we studied the correlations of HER2 expression with *ERBB2* mutation and the prognostic value of HER2 status (low and positive) in high-risk EC. We correlated our findings with clinical, pathological, and outcome parameters.

METHODS

Patient and Tissue Selection

Eligible patient data were retrieved from two published clinical trials and one prospective and one retrospective cohort. The randomized PORTEC-3 trial included 660 patients with high-risk disease (FIGO 2009 stage I, endometrioid-type grade 3 with deep myometrial invasion or lymphovascular space invasion [LVSI; or both], endometrioid-type stage II or III, or stage I-III EC with serous or clear cell histology). This trial showed improved overall survival (OS) and failure-free survival in women who received adjuvant chemoradiotherapy compared with women who underwent radiotherapy alone. The study design and results have been described previously.[24] The second study is the DOMEC trial conducted from 2019 to 2020. This phase II trial studied the efficacy and safety of durvalumab with olaparib in metastatic and recurrent EC after one line of platinum-based therapy.[25] Durvalumab and olaparib were tolerated well, but efficacy outcomes did not meet the prespecified survival outcomes; the median PFS was 3.4 months. The Medical Ethics Committee (MEC) of all participating centers gave approval, and written informed consent was obtained from all patients. The third study was a prospective cohort from Medical Spectrum Twente (MST), Enschede, the Netherlands.[26] This cohort consists of 270 patients with high-risk EC who meet the same inclusion criteria as the PORTEC-3 population. Permission for the use of the data and tumor materials was obtained from the MEC of Leiden University Medical Center (LUMC), and a waiver for written informed consent was given. The fourth cohort is a retrospective multicenter cohort study of 219 patients who underwent cytoreductive surgery for primary stage IV EC in five hospitals in the Netherlands.[27] Permission to use tumor material and anonymized clinical data was granted by the LUMC MEC.

Formalin-fixed paraffin-embedded (FFPE) hysterectomy tumor samples were used for HER2 staining. In patients from the DOMEC trial with recurrent EC, material from the primary tumor was used for HER2 staining. Molecular classification according to the WHO 2020 classification had been performed previously on all tumors for all four studies.[28]

Procedure Immunohistochemistry/Dual In Situ Hybridization

HER2 status was examined by IHC on all samples, and dual in situ hybridization (DISH) was performed for all cases with an IHC 2+ or 3+ score. For both the IHC and DISH, we used the Ventana BenchMark GX (Roche Diagnostics, Basel, Switzerland). FFPE tumor blocks were cut into 4 μm slides and stained for HER2 using the anti-HER2/neu (4B5) Rabbit Monoclonal Primary Antibody and the Ventana ultraView DAB Detection Kit (Roche Diagnostics). An external on-slide HER2 IHC 3+ control was added to all cases. To detect amplification, the INFORM HER2 DISH DNA Probe Cocktail assay was used (Roche Diagnostics). Since tumor material from various hospitals in the world was used, different fixation methods were used to embed the tumor tissue. To improve IHC and DISH staining results, the standard manufacturer's protocols were adjusted as described before.[18]

All HER2 IHC slides were scored by an expert gynecopathologist (T.B.), blinded for clinicopathological and molecular data, using the methodology described by Vermij et al[29] (Fig 1). Tumors with any membranous staining (complete and incomplete) in <10% of the cells were considered HER2-negative. Tumors with faint (in)complete membranous staining in ≥10% of tumor cells were scored as IHC 1+, with moderate (in)complete membranous staining in ≥10% of the tumor as IHC 2+, and with strong (in)complete membranous staining in ≥10% of the tumor cells as IHC 3+. DISH was performed on tumors with IHC 2+ or 3+ scores. HER2 (black) and chromosome 17 (red) signals were counted in ≥20 tumor cells by two researchers (D.v.D./T.B.). An HER2 ratio ≥2 indicated amplification.[30] HER2 heterogeneity in EC was addressed by scoring areas with the highest IHC scores. Discordant results were resolved by consensus between researchers or by consulting two expert gynecopathologists (T.B./V.S.). Tumors with <10% HER2 staining were designated HER2-negative. HER2-low was defined as IHC 1+ or IHC 2+ without amplification, whereas IHC 2+/3+ with amplification was considered HER2-positive.

Next-Generation Sequencing

Targeted next-generation sequencing was previously performed on the PORTEC-3 and MST cases using the AmpliSeq Cancer Hotspot Panel versions 5 and 6, including hotspot loci in exons 8 and 17-21 of the *ERBB2* gene. Details on DNA isolation and sampling have been previously described.[31] For samples with a failed DISH assay, HER2 status was determined by assessing normalized copy number plots for *ERBB2* amplification. Samples with low median coverage or high standard deviation were deemed ineligible for *ERBB2* amplification assessment. Pathogenic *ERBB2* mutations were evaluated blind to HER2 status, considering only those with a variant allele frequency >0.10 and coverage >100 reads. The pathogenicity of nonsynonymous mutations was confirmed by a clinical molecular biologist or the ClinVar database.[32]

Statistical Analysis

The correlations of HER2 status with clinicopathological characteristics were tested using the chi-square test or Fisher-Freeman-Halton exact test for nominal variables and the Kruskal-Wallis test for ordinal and continuous variables. The length of follow-up was estimated using the reversed Kaplan-Meier method. Time-to-event analyses for OS, recurrence-free survival (RFS), and PFS were performed according to Kaplan-Meier's methodology, and groups were compared using the log-rank test. Multivariable

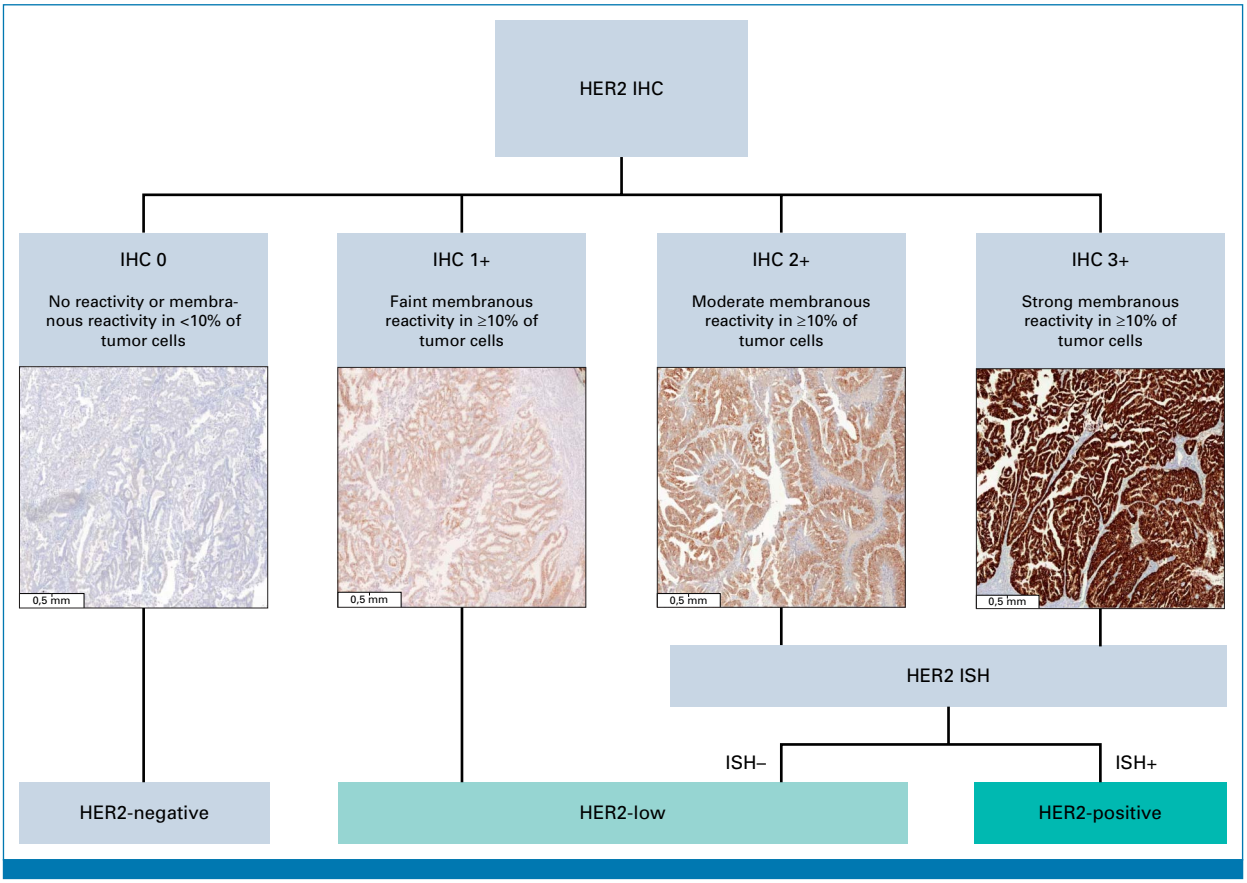

FIG 1. Endometrial cancer–specific HER2 scoring algorithm according to Vermij et al.[29] HER2, human epidermal growth factor receptor 2; IHC, immunohistochemistry; ISH, in situ hybridization.

time-to-event analysis for OS was performed on the merged cohort of PORTEC-3 and MST using a Cox's proportional hazards model. Covariates were predefined and included age, molecular class, histotype and grade, stage, and LVSI. Statistical significance was accepted at $P < .05$, and all tests were two-sided.

Survival outcomes of the four cohorts were analyzed by HER2 status. Since PORTEC-3 and MST were conducted in the adjuvant setting, RFS and OS were recorded. The DOMEC trial and stage IV cohort were conducted in patients with advanced disease, where PFS and OS were registered. Therefore, the survival analyses were conducted for the adjuvant and advanced setting separately. Since the DOMEC and stage IV cohort had included substantially different populations, survival analyses were performed for each study individually. Multivariable analysis was performed correcting for age, stage, histotype and grade, LVSI, and molecular classification.

RESULTS

The four studies included a total of 1,204 patients with EC, with FFPE tumor samples available for testing in 899 (74.7%) cases (high-risk, n = 684; recurrent/metastatic, n = 48; and primary stage IV, n = 167). HER2 status was successfully determined in 806 (89.7%) of these 899 tumors (Fig 2). In 93 cases (10.3%), HER2 status could not be determined due to failed IHC (n = 86, 9.6%) or DISH (n = 7, 0.8%). Patient and tumor characteristics of the 806 patients by study are provided in Appendix Table A1 (online only). Patient and tumor characteristics for the four cohorts combined by HER2 status are shown in Table 1. The median age in the total cohort was 65 years. Patients with HER2-positive EC were significantly older than those with HER2-negative and HER2-low EC. The median follow up time was 5.2 years for the PORTEC-3 population, 5.9 years for the MST cohort, 8.4 months for the DOMEC study patients, and 18 months for the stage IV cohort.

In the majority of all tumor samples, HER2 expression was absent and considered HER2-negative (n = 603, 74.8%). This included a few EC with intense nuclear staining of HER2 in the absence of any membranous staining (Appendix Fig A1A). Any HER2 overexpression (IHC 1+/2+/3+) was found in 25.2% (n = 203/806) of all tumors (Fig 1). Sixty-four cases (7.9%) were HER2-positive (n = 36/806 [4.5%] IHC 3+ and n = 28/806 [3.5%] IHC 2+ with amplification), and 139 (17.2%) were HER2-low. This group of HER2-low encompassed 81 (10%) EC with HER2 IHC 1+ and 58 (7.2%) EC with IHC 2+ without amplification. We observed substantial

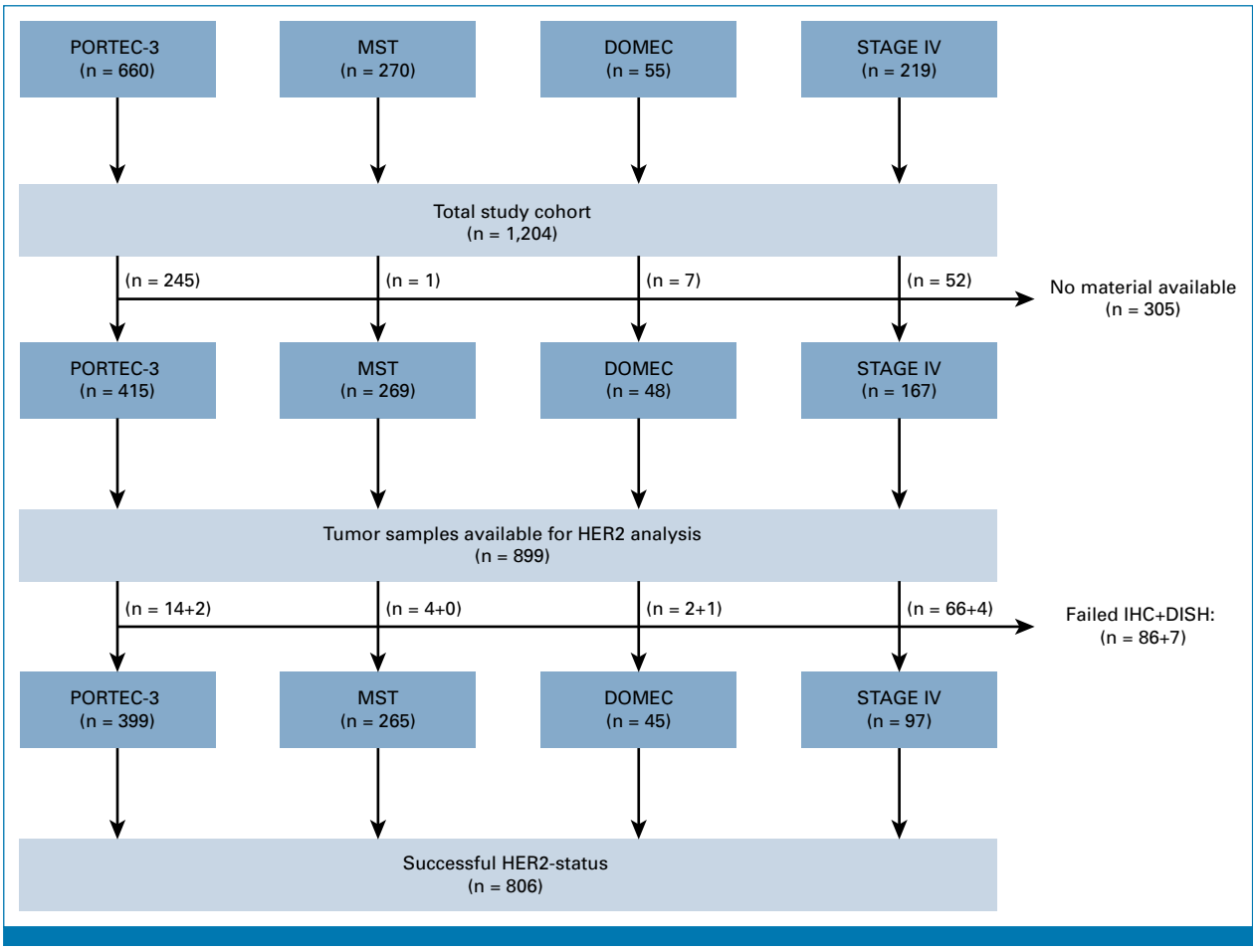

FIG 2. Flowchart of HER2 testing of all four included cohorts. DISH, dual in situ hybridization; HER2, human epidermal growth factor receptor 2; IHC, immunohistochemistry.

heterogeneity in tumor areas expressing membranous HER2, mostly among IHC 1+ and 2+ tumors (Appendix Fig A1B). The distribution of HER2 status varied among the included cohorts and trials ($P < .001$, Table 2).

Correlation With Clinicopathological and Molecular Characteristics

Primary tumor stage was not significantly associated with HER2 overexpression (HER2-low and HER2-positive). In addition, no association was observed between HER2-low expression and histotype or molecular class. HER2-low overexpression was observed in 23.2% (n = 52/224) of p53abn EC, 19.9% (n = 44/221) of MMRd EC, and 14.5% (n = 34/234) of NSMP EC. Only in *POLE*mut, EC HER2-low overexpression was rare (n = 4/67, 6%). In contrast, HER2-positivity was predominantly observed in nonendometrioid EC (n = 46, 72.9%) and was almost exclusive to the p53abn molecular class (n = 60, 98.4%).

Pathological Characteristics of *ERBB2* Mutations

We identified 14 (1.5%) EC with a pathogenic *ERBB2* mutation in the combined cohort of PORTEC-3 and MST (n = 930, Appendix Table A2). Only one *ERBB2*mut tumor showed HER2-low expression (IHC 2+ and DISH−). In 12 *ERBB2*mut tumors, HER2 expression was absent. In one case, HER2 amplification status could not be determined because there was insufficient tumor tissue left. The majority of *ERBB2*mut EC (n = 10/14, 71.4%) were MMRd, two were *POLE*mut (14.3%), and two tumors were NSMP (14.3%).

Associations of HER2 Status With Clinical Outcome

No significant differences in OS were observed between HER2-negative, HER2-low, and HER2-positive EC within the MST and stage IV cohorts, and DOMEC trial (Figs 3B and 3D). Within the PORTEC-3 trial population (Fig 3A), significantly worse OS was observed for HER2-positive EC compared with HER2-low and HER2-negative EC (5-year OS 51.6% *v* 80.2% *v* 80.9%, respectively). Within the combined high-risk cohort (PORTEC-3 and MST), HER2-positive EC showed lowest OS and RFS with 5-year rates of 48.6% (95% CI, 35.4 to 66.8) and 53.9% (95% CI, 40.8 to 71.2), respectively (Fig 4). Stratified by molecular class, no statistically significant differences in OS were observed between HER2-negative, HER2-low, and HER2-positive EC in the combined PORTEC-3/MST cohort, DOMEC trial, and

TABLE 1. Patient and Tumor Characteristics by HER2-Status

Characteristics	Total	HER2-Negative	HER2-Low	HER2-Positive	*P*
No. of patients	n = 806 (100%)	n = 603 (74.8%)	n = 139 (17.2%)	n = 64 (7.9%)	
Age, years, median (range)	64.5 (25-92)	64 (25-92)	65 (33.5-86)	70.7 (55.8-86)	<.001[a]
Stage, No. (%)					.12[a]
IA	83 (10.8)	52 (9.1)	17 (12.3)	14 (22.6)	
IB	138 (17.9)	102 (17.8)	23 (16.7)	13 (21)	
II	183 (23.7)	145 (25.3)	29 (21)	9 (14.5)	
IIIA	97 (12.6)	82 (14.3)	9 (6.5)	6 (9.7)	
IIIB	46 (6)	35 (6.1)	8 (5.8)	3 (4.8)	
IIIC	122 (15.8)	98 (17.1)	19 (13.8)	5 (8.1)	
IV	103 (13.3)	58 (10.1)	33 (23.9)	12 (19.4)	
Histotype and grade, No. (%)					<.001[b,*]
Grade 1-2 EEC	277 (35.9)	235 (41.2)	35 (25.4)	7 (11.1)	
Grade 3 EEC	199 (25.8)	153 (26.8)	36 (26.1)	10 (15.9)	
SEC	145 (18.8)	76 (13.3)	41 (29.7)	28 (44.4)	
CCC	65 (8.4)	42 (7.4)	16 (11.6)	7 (11.1)	
Carcinosarcoma	33 (4.3)	22 (3.9)	4 (2.9)	7 (11.1)	
Undifferentiated	21 (2.7)	18 (3.2)	3 (2.2)	0 (0)	
Other	32 (4.1)	25 (4.4)	3 (2.2)	4 (6.3)	
LVSI, No. (%)					.51[b]
Present	301 (47)	231 (46)	46 (48.9)	24 (54.5)	
Absent	399 (53)	271 (54)	48 (51.1)	20 (45.5)	
Molecular classification, No. (%)					<.001[b]
*POLE*mut	67 (8.7)	63 (11)	4 (3)	0 (0)	
MMRd	221 (28.9)	177 (31)	44 (32.8)	0 (0)	
p53abn	224 (31.9)	132 (23.1)	52 (38.8)	60 (98.4)	
NSMP	234 (30.5)	199 (34.9)	34 (25.4)	1 (1.6)	
ER expression, No. (%)					<.001[b]
ER-positive	520 (69.1)	405 (73)	84 (62.2)	51 (50)	
ER-negative	232 (30.9)	150 (27)	51 (37.8)	51 (50)	
Received treatment, No. (%)					.013[c]
VBT	22 (3.5)	14 (2.8)	2 (2.1)	6 (13.6)	
EBRT	394 (61.9)	317 (63.5)	53 (56.4)	24 (54.5)	
EBRT + CT	221 (34.7)	168 (33.7)	39 (41.5)	14 (31.8)	

Abbreviations: CCC, clear cell carcinoma; CT, chemotherapy; EBRT, external beam radiotherapy; EEC, endometrioid endometrial cancer; ER, estrogen receptor; HER2, human epidermal growth factor receptor 2; LVSI, lymphovascular space invasion; MMRd, mismatch repair–deficient; NSMP, no specific molecular profile; p53abn, p53 abnormal; *POLE*mut, *POLE* mutant; SEC, serous endometrial cancer; VBT, vaginal brachytherapy.
[a]Kruskal-Wallis test.
[b]Chi-square test.
[c]Fisher-Freeman-Halton exact test.
**P* value tested by chi-square test with four categories (grade 1-2 EEC, grade 3 EEC, SEC, and other).

stage IV cohort (Appendix Figs A2-A4). However, in the combined PORTEC-3/MST cohort, HER2-low MMRd EC had a higher 5-year OS compared with HER2-negative MMRd EC (86.7% [95% CI, 76.5 to 98.3] *v* 71.2% [95% CI, 64.6 to 78.5], $P = .039$, respectively; Appendix Fig A2B). Multivariable analysis also did not show any independent prognostic value of HER-low and HER2-positivity for OS in the combined PORTEC-3/MST cohort (hazard ratio [HR], 0.67 [95% CI, 0.43 to 1.03] and HR, 0.81 [95% CI, 0.51 to 1.32]; Appendix Table A3).

DISCUSSION

In this large study of high-risk, recurrent, and metastatic EC, we found a prevalence of HER2-low expression of 17.2%. This study showed that HER2-low is not restricted to a specific histotype or molecular class, and has no independent prognostic value in high-risk EC. HER2 overexpression (low or positive) was observed more commonly in recurrent/metastatic and primary advanced disease, compared with the stage I-III high-risk population.

TABLE 2. Distribution of HER2-Status in High-Risk EC, Primary Stage IV EC, and Recurrent or Metastatic EC

	Total	High-Risk	Primary Stage IV	Recurrent/Metastatic	
HER2-Status	n = 806 (100%)	n = 664 (100%)	n = 97 (100%)	n = 45 (100%)	*P*
HER2-negative, No. (%)	603 (74.8)	525 (79.1)	56 (57.7)	22 (48.9)	<.001
HER2-low, No. (%)	139 (17.2)	94 (14.2)	29 (29.9)	16 (35.6)	
HER2-positive, No. (%)	64 (7.9)	45 (6.8)	12 (12.4)	7 (15.6)	

Abbreviations: EC, endometrial cancer; HER2, human epidermal growth factor receptor 2.

Large studies specifically focusing on HER2-low EC are lacking. Some data on HER2-low can be deducted from our previous study directed at identifying HER2-positive high-risk EC in the PORTEC-3 trial population.[18] As we added another cohort of high-risk patients (MST cohort), we could further validate the prevalence of HER2-low. One abstract on different levels of HER2 expression in serous EC and uterine carcinosarcomas (stage unknown) reported IHC 1+ or 2+ scores in approximately 60% of the cases.[33] We performed similar analysis on our four cohorts separately and found comparable IHC 1+/2+ rates only in the DOMEC trial population with recurrent/metastatic disease (n = 14/23, 61%). This patient group is also most in need of novel systemic treatment options. New trials with HER2-directed ADCs, T-DXd in particular, should therefore prioritize those with recurrent/metastatic EC. The promising interim results in patients with locally advanced or metastatic EC of the

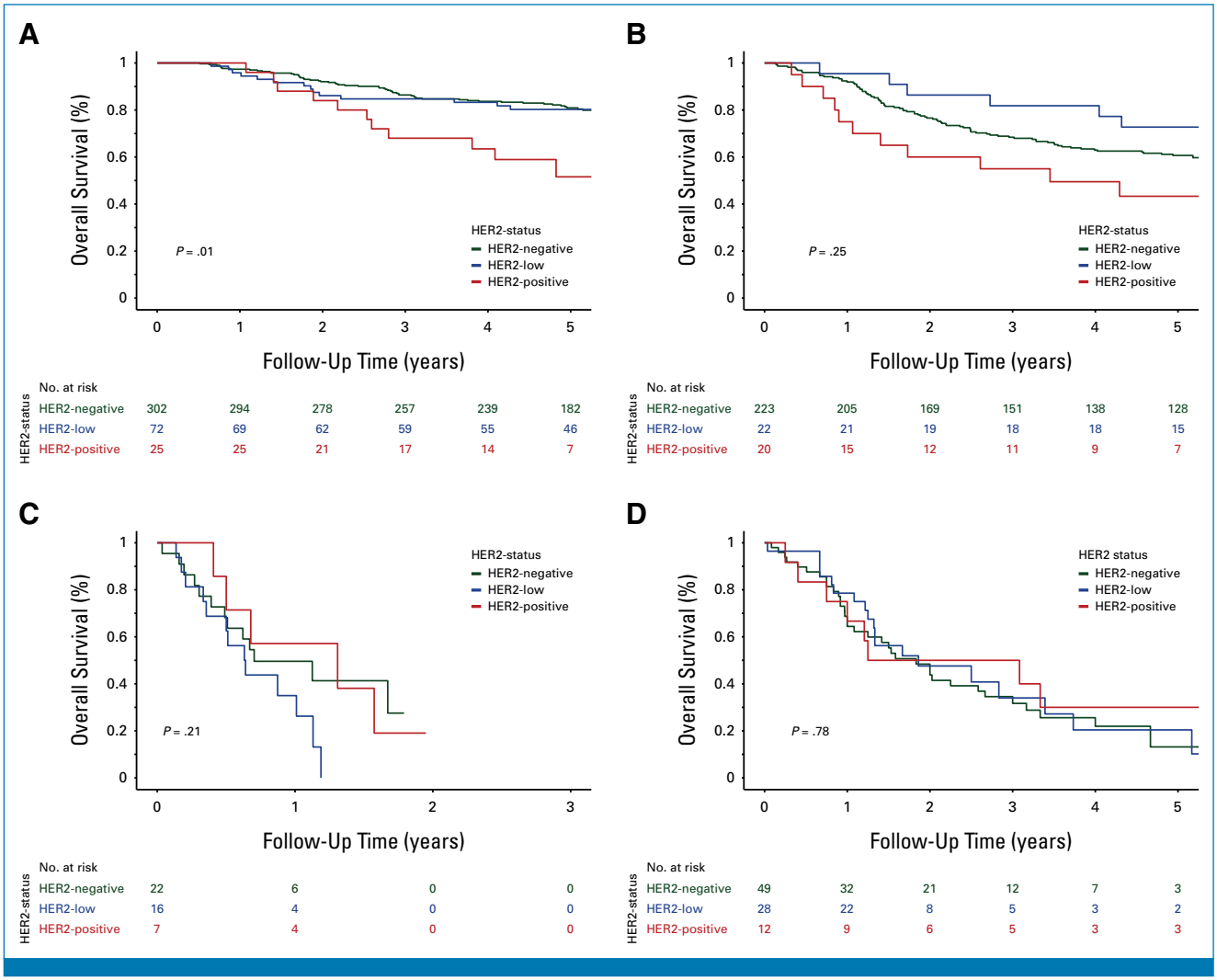

FIG 3. Kaplan-Meier survival curves for overall survival stratified by HER2-status: (A) PORTEC-3 trial, (B) MST cohort, (C) DOMEC trial, and (D) stage IV cohort. HER2, human epidermal growth factor receptor 2; MST, Medical Spectrum Twente.

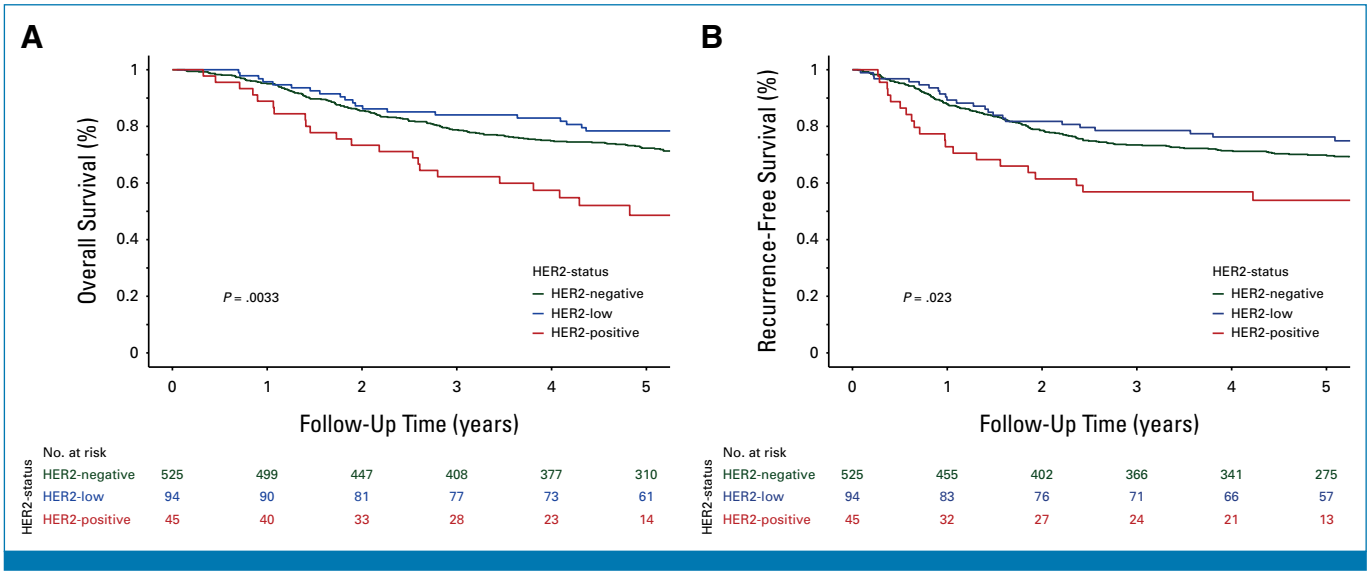

FIG 4. Kaplan-Meier survival curves for high-risk endometrial cancer stratified by HER2-status: (A) overall survival and (B) recurrence-free survival. HER2, human epidermal growth factor receptor 2.

DESTINY-PanTumor02 study[15] advocate for more clinical trials in this population.

Our study showed that HER2-positive and HER2-low EC represent a different group of tumors. HER2-positive tumors are mainly of the p53abn molecular class and often have a serous histotype,[18] whereas HER2-low can be observed in all molecular and histological subtypes. Given the strong association between HER2-positivity and p53abn EC, HER2 amplification may act as an oncogenic driver in these tumors. In contrast, HER2-low expression less likely plays a role in tumor progression. Nevertheless, HER2-low expression has gained clinical relevance as it may represent a druggable target. Testing for HER2-low expression in future studies investigating the efficacy of T-DXd in EC should be performed on all EC, regardless of histotype or molecular class.

To our knowledge, this is the first study to investigate the prognostic relevance of HER2-low in EC. In line with our expectations, we did not identify a prognostic relevance for HER2-low. This was also supported by previous work demonstrating no significant independent prognostic value of HER2-positivity in EC.[18] Similarly, a lack of prognostic significance has been observed for HER2-low in other malignancies. In breast cancer, HER2-low generally has no prognostic value, but some studies reported a marginal prognostic benefit of HER2-low in hormone receptor–negative breast cancer.[34-36] In early-stage gastric cancer and colorectal cancer, HER2-low did not independently influence prognosis either.[37,38]

In preparation of potentially more routine HER2 testing in EC, pathologists will need to be educated about the EC-specific challenges of HER2 scoring. The most prominent issue is the heterogeneity that we have observed, which is clearly more common than in the breast cancer literature and more comparable with what has been described in gastric cancer.[39] We found both heterogeneity in distribution and intensity of HER2 expression. Here, we applied the methodology recently published by Vermij et al, which incorporates a minimum threshold of 10% HER2 staining of any intensity regardless of the completeness of membranous staining. This implies that cases with HER2 staining in tiny foci (<10%) were scored as IHC 0. Geographical/subclonal overexpression ranging from 10% to 90% of HER2 expression was more commonly observed in the context of HER2-positive ECs, in which the positive areas are easily identified by their strong and diffuse staining. In HER2-low tumors, the expression was often more diffuse, but with heterogeneity in intensity, resulting in a patchy pattern in which tumor areas transitioned from 1+ to 2+. Theoretically, this observed heterogeneity in EC should not influence the efficacy of treatment with T-DXd significantly due to the described bystander effect.[14]

As a result of the HER2 staining heterogeneity, in addition to variation in testing protocols and preanalytical factors, the interobserver agreement on HER2 0 and HER2 1+ scores is poor.[40] HER2 IHC assays were originally designed to act as a surrogate marker for HER2 gene amplification. The assays were never optimized for distinguishing IHC 0 from IHC 1+, as both were considered HER2-negative and thus not considered eligible for anti-HER2 therapies. Future research should focus on the development of reproducible testing methods to reliably identify non-amplified HER2 protein–expressing tumors that may benefit from T-DXd.

In conclusion, this is the largest study to date to describe HER2-status, and in particular HER2-low expression, in EC in relation to clinicopathological and molecular characteristics. This study shows that the HER2-positive (7.9%) and HER2-low (17.2%) EC collectively count for a quarter of all high-risk, recurrent, and metastatic EC. HER2-low was observed in all histotypes and across all molecular classes without independent prognostic value. *ERBB2* mutations were rare in high-risk EC (1.5%), and no association was observed with HER2 overexpression. These findings are important for the allocation of HER2-targeted treatments and the design of clinical trials, and imply that a quarter of patients with high-risk or advanced EC can potentially benefit from T-DXd therapy.

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AUTHORS' DISCLOSURES OF POTENTIAL CONFLICTS OF INTEREST

Clinical and Molecular Characteristics of High-Risk, Recurrent, or Metastatic Endometrial Cancer That Is Human Epidermal Growth Factor Receptor 2–Low

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APPENDIX

TABLE A1. Patient and Tumor Characteristics by HER2-Status of All Four Included Trial Populations and Cohorts

	PORTEC-3	MST	DOMEC[a]	Stage IV
Characteristic	No. (%)	No. (%)	No. (%)	No. (%)
No. of patients	399 (100)	265 (100)	45 (100)	97 (100)
Age, years, median (range)	62.2 (26.7-83.5)	70 (25-92)	69 (51-85)	67.5 (33-86)
Stage				
IA	50 (12.5)	24 (9.9)	9 (26.5)	0 (0)
IB	73 (18.3)	59 (24.4)	6 (17.6)	0 (0)
II	101 (25.3)	78 (32.2)	4 (11.8)	0 (0)
IIIA	43 (10.8)	50 (20.7)	4 (11.8)	0 (0)
IIIB	29 (7.3)	17 (7)	0 (0)	0 (0)
IIIC	103 (25.8)	14 (5.3)	5 (14.7)	0 (0)
IV	0 (0)	0 (0)	6 (17.6)	97 (100)
Histotype and grade				
Low-grade EEC	160 (40.1)	91 (37.6)	6 (13.6)	20 (23)
High-grade EEC	110 (27.6)	68 (28.1)	6 (13.6)	15 (17.2)
Serous	66 (16.5)	25 (10.3)	17 (38.6)	37 (42.5)
Clear cell	37 (9.3)	13 (5.4)	6 (13.6)	9 (10.3)
Carcinosarcoma	0 (0)	24 (9.9)	6 (13.6)	3 (3.4)
Undifferentiated	7 (1.8)	8 (3.3)	3 (6.8)	3 (3.4)
Other	3 (0.8)	13 (4.9)	0 (0)	0 (0)
Molecular classification				
*POLE*mut	49 (12.5)	16 (6.6)	0 (0)	2 (2.2)
MMRd	135 (34.5)	69 (28.5)	6 (13.6)	11 (12.4)
p53abn	92 (23.5)	68 (28.1)	28 (63.6)	56 (62.9)
NSMP	115 (29.4)	89 (36.8)	10 (22.7)	20 (22.5)
LVSI				
Present	249 (62.4)	52 (21.6)	—	—
Absent	150 (37.6)	189 (78.4)	—	—
ER status				
ER-positive	283 (76.6)	172 (68.8)	20 (55.6)	45 (51.1)
ER-negative	86 (23.3)	78 (31.2)	20 (44.4)	43 (48.9)
Received treatment				
RT (VBT or EBRT)	195 (48.9)	221 (92.8)	—	—
RT (VBT or EBRT) + CT	204 (51.1)	17 (7.1)	—	—

Abbreviations: CT, chemotherapy; EBRT, external beam radiation therapy; EEC, endometrioid endometrial carcinoma; ER, estrogen receptor; HER2, human epidermal growth factor receptor 2; LVSI, lymphovascular space invasion; MMRd, mismatch repair–deficient; MST, Medical Spectrum Twente; NSMP, no specific molecular profile; p53abn, p53 abnormal; *POLE*mut, *POLE* mutant; RT, radiotherapy; VBT, vaginal brachytherapy.
[a]Incomplete data on tumor stage (n = 11), histotype and grade (n = 1), molecular classification (n = 1), and LVSI (n = 5).

TABLE A2. Tumor Characteristics of Pathogenic *ERBB2* Mutated Tumors

Sample	Molecular Subgroup	Histotype and Grade	VAF	DNA Mutation	Protein	IHC/DISH[a]
1	MMRd	Low-grade EEC	0.371	c.2524G>A	V842I	0
2	MMRd	Low-grade EEC	0.261	c.2524G>A	V842I	0
3	NSMP	Low-grade EEC	0.340	c.2584A>G	T862A	0
4	MMRd	High-grade EEC	0.244	c.929C>A	S310Y	0
5	MMRd	High-grade EEC	0.379	c.2524G>A	V842I	2+/DISH−
6	MMRd	—	0.260	c.2524G>A	V842I	—[b]
7	MMRd	Low-grade EEC	(1) 0.418	(1) c.2524G>A	(1) V842I	0
			(2) 0.386	(2) c.2305G>A	(2) D769N	
8	MMRd	Low-grade EEC	0.234	c.2524G>A	V842I	0
9	MMRd	Low-grade EEC	0.304	c.2524G>A	V842I	0
10	MMRd	SEC	0.554	c.2524G>A	V842I	0
11	*POLE*mut	Low-grade EEC	0.357	c.929C>T	S310F	0
12	NSMP	Low-grade EEC	0.693	c.2524G>A	V842I	0
13	MMRd	High-grade EEC	0.537	c.2305G>A	D769N	0
14	*POLE*mut	High-grade EEC	0.391	c.2305G>A	D769N	0

Abbreviations: DISH, dual in situ hybridization; EC, endometrial cancer; EEC, endometrioid endometrial carcinoma; IHC, immunohistochemistry; MMRd, mismatch repair–deficient; NSMP, no specific molecular profile; *POLE*mut, *POLE* mutant; SEC, serous EC; VAF, variant allele frequency.
[a]DISH only performed on IHC 2+/3+ EC.
[b]No material available for IHC.

TABLE A3. Multivariable Analysis of the Prognostic Impact of HER2-Status on Overall Survival in High-Risk Endometrial Cancer, Corrected for Age, Molecular Class, Histotype and Grade, Stage, and Lymphovascular Space Invasion

HER2-Status	HR	95% CI	*P*
HER2-negative			
HER2-low	0.67	0.44 to 1.03	.066
HER2-positive	0.81	0.50 to 1.32	.410

Abbreviations: HER2, human epidermal growth factor receptor 2; HR, hazard ratio.

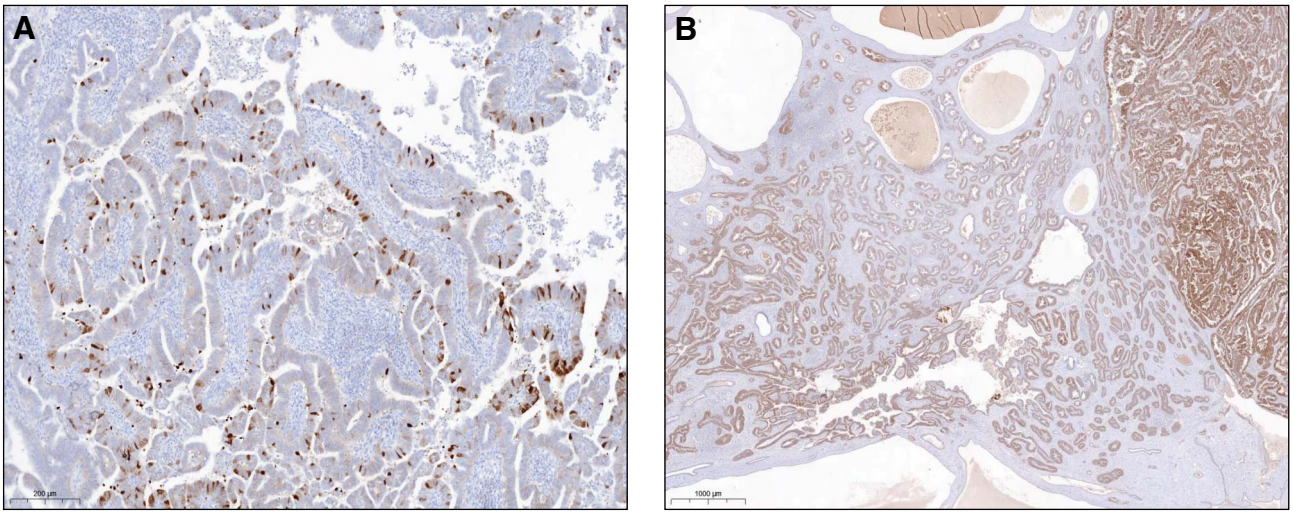

FIG A1. (A) Nuclear staining of HER2 IHC staining and (B) heterogeneity of HER2 overexpression on IHC. HER2, human epidermal growth factor receptor 2; IHC, immunohistochemistry.

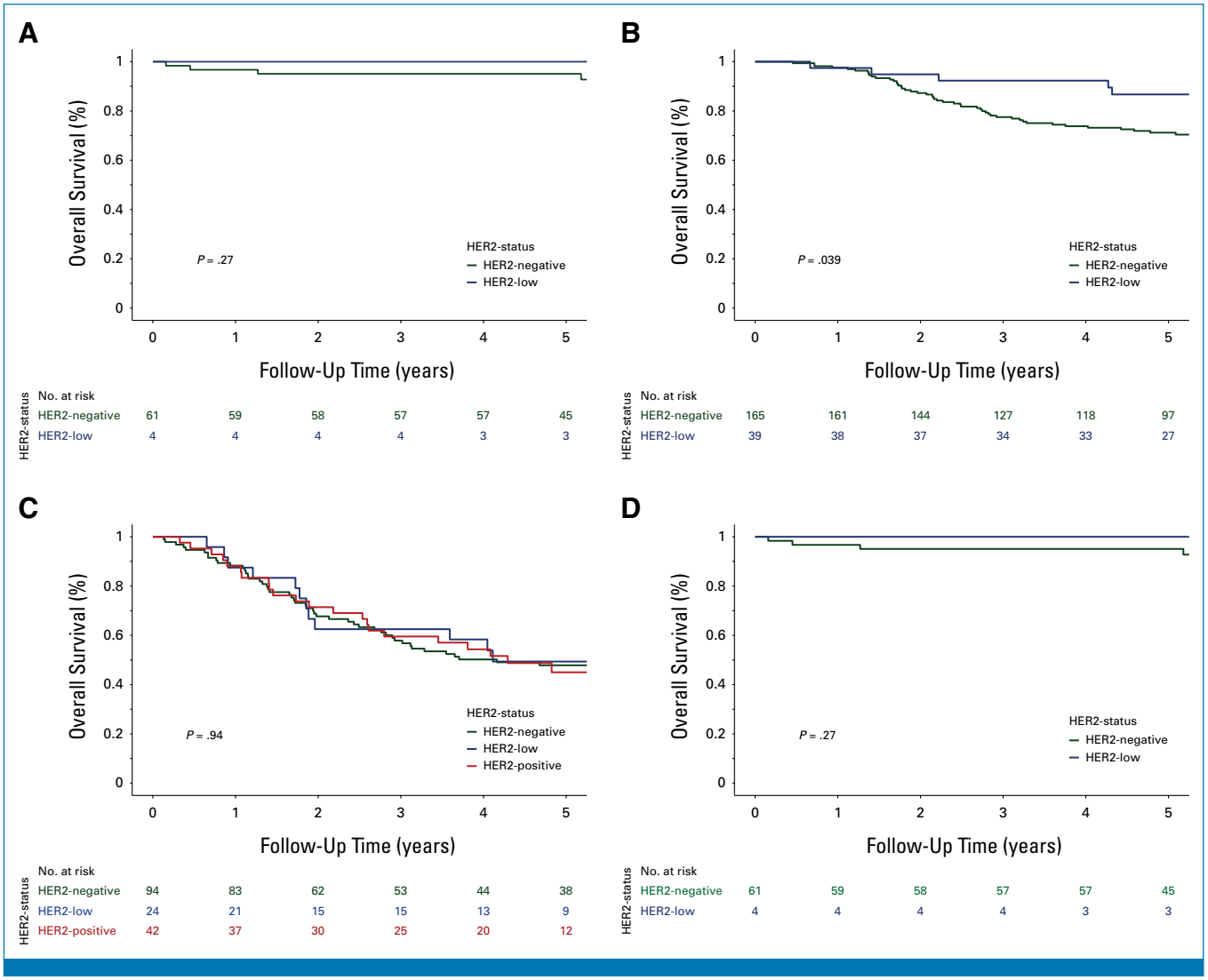

FIG A2. Kaplan-Meier survival curves of the combined PORTEC-3 trial and MST cohort with high-risk EC stratified by HER2-status, per molecular classification: (A) *POLE*mut, (B) MMRd, (C) p53abn, and (D) NSMP. EC, endometrial cancer; HER2, human epidermal growth factor receptor 2; MMRd, mismatch repair–deficient; MST, Medical Spectrum Twente; NSMP, no specific molecular profile; p53abn, p53 abnormal; *POLE*mut, *POLE* mutant.

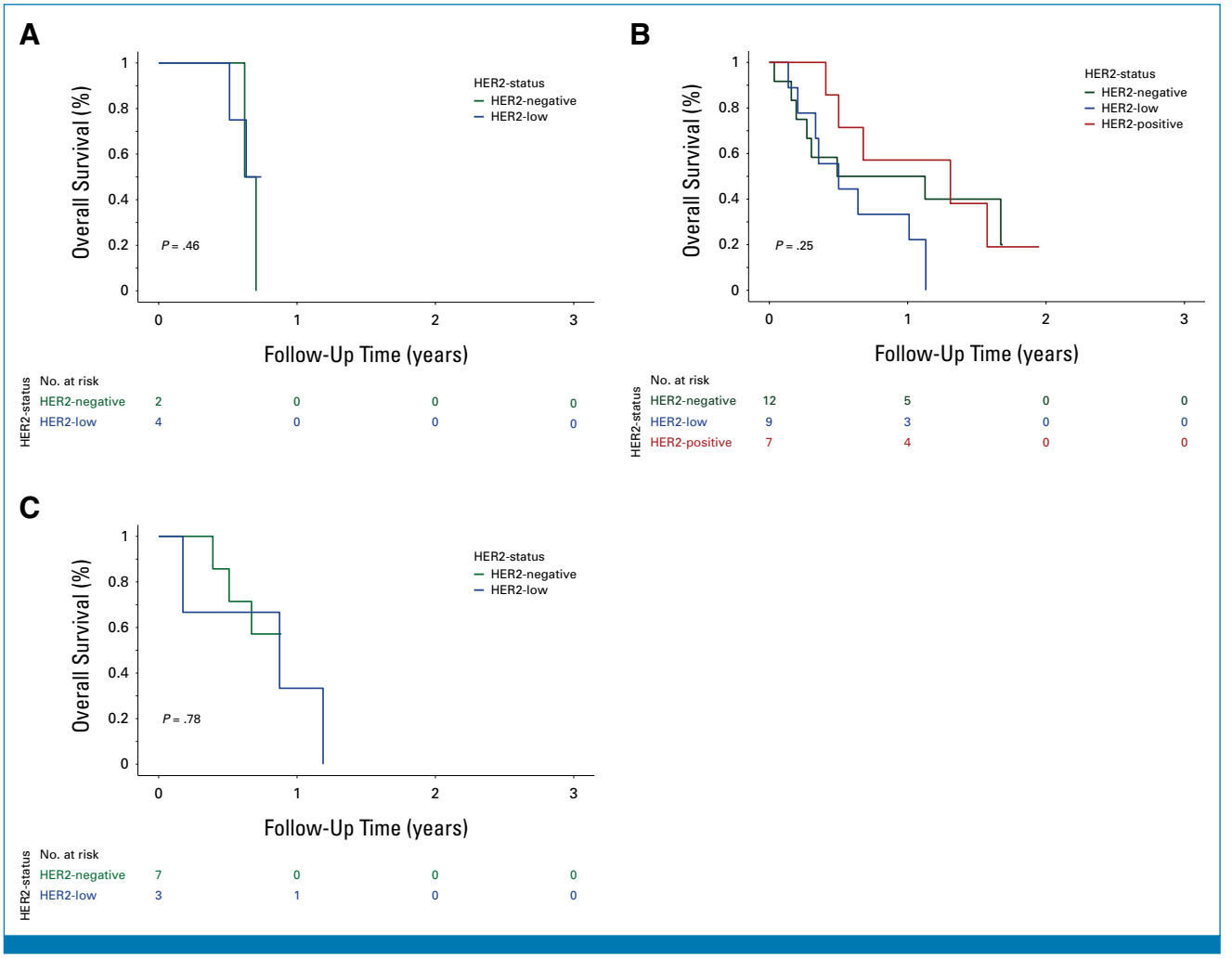

FIG A3. Kaplan-Meier survival curves of the DOMEC trial (recurrent or metastatic EC) stratified by HER2-status, per molecular classification: (A) MMRd, (B) p53abn, and (C) NSMP. EC, endometrial cancer; HER2, human epidermal growth factor receptor 2; MMRd, mismatch repair–deficient; NSMP, no specific molecular profile; p53abn, p53 abnormal.

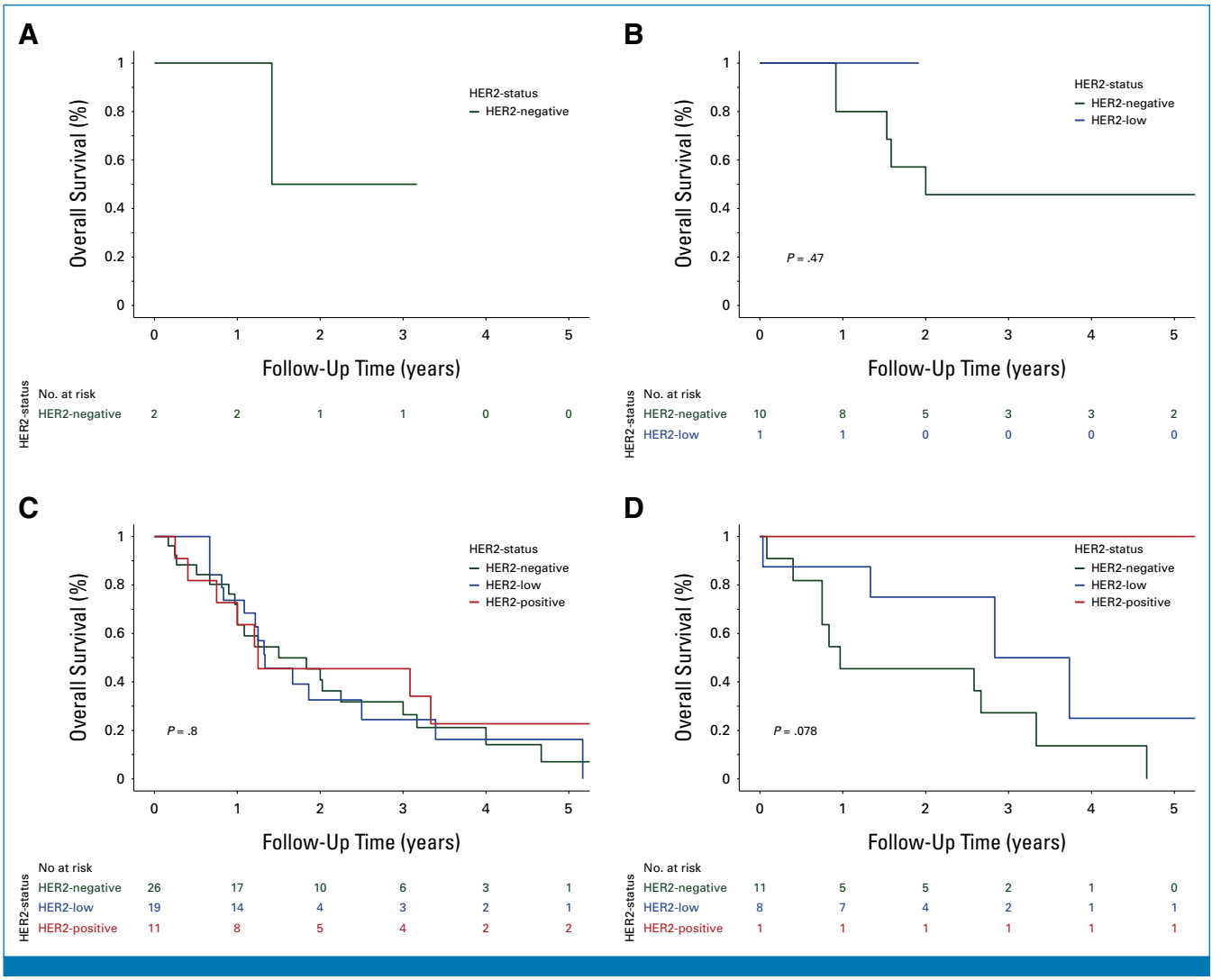

FIG A4. Kaplan-Meier survival curves of the primary stage IV EC cohort stratified by HER2-status, per molecular classification: (A) *POLE*mut, (B) MMRd, (C) p53abn, and (D) NSMP. EC, endometrial cancer; HER2, human epidermal growth factor receptor 2; MMRd, mismatch repair–deficient; NSMP, no specific molecular profile; p53abn, p53 abnormal; *POLE*mut, *POLE* mutant.