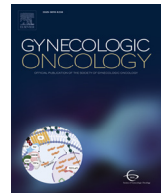




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Immunohistochemical biomarkers are prognostic relevant in addition to the ESMO-ESGO-ESTRO risk classification in endometrial cancer

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HIGHLIGHTS

- Abnormal expression of p53/L1CAM/ER/PR is strongly correlated with higher ESMO-ESGO-ESTRO risk classification groups.
- Within the ‘high-advanced/metastatic’ risk group, abnormal expression of p53/L1CAM/ER/PR was most predictive for outcome.
- p53-abn, ER/PR- and ‘high-advanced/metastatic’ risk group were independently associated with reduced DSS.
- IHC biomarkers have important additional prognostic relevance in both patients with and without lymph node metastasis.

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ABSTRACT

Objective. Pre-operative immunohistochemical (IHC) biomarkers are not incorporated in endometrial cancer (EC) risk classification. We aim to investigate the added prognostic relevance of IHC biomarkers to the ESMO-ESGO-ESTRO risk classification and lymph node (LN) status in EC.

Methods. Retrospective multicenter study within the European Network for Individualized Treatment of Endometrial Cancer (ENITEC), analyzing pre-operative IHC expression of p53, L1 cell-adhesion molecule (L1CAM), estrogen receptor (ER) and progesterone receptor (PR), and relate to ESMO-ESGO-ESTRO risk groups, LN status and outcome.

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Biomarker
Outcome
Lymph node metastasis

Results. A total of 763 EC patients were included with a median follow-up of 5.5-years. Abnormal IHC expression was present for p53 in 112 (14.7%), L1CAM in 79 (10.4%), ER- in 76 (10.0%), and PR- in 138 (18.1%) patients. Abnormal expression of p53/L1CAM/ER/PR was significantly related with higher risk classification groups, and combined associated with the worst outcome within the 'high and advanced/metastatic' risk group. In multivariate analysis p53-abn, ER/PR- and ESMO-ESGO-ESTRO 'high and advanced/metastatic' were independently associated with reduced disease-specific survival (DSS). Patients with abnormal IHC expression and lymph node metastasis (LNM) had the worst outcome. Patients with LNM and normal IHC expression had comparable outcome with patients without LNM and abnormal IHC expression.

Conclusion. The use of pre-operative IHC biomarkers has important prognostic relevance in addition to the ESMO-ESGO-ESTRO risk classification and in addition to LN status. For daily clinical practice, p53/L1CAM/ER/PR expression could serve as indicator for surgical staging and refine selective adjuvant treatment by incorporation into the ESMO-ESGO-ESTRO risk classification.

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1. Introduction

Endometrial cancer (EC) is the most common gynecologic malignancy in industrialized countries and the incidence is rising due to advanced life expectancy and obesity [1]. In general, patients diagnosed at an early stage have a favorable prognosis. Yet, about 20% of patients with clinical early stage disease have a poor outcome [2,3]. ECs are histologically classified into type 1, comprising endometrioid EC (EEC) with a favorable prognosis, and type 2, comprising of non-endometrioid EC (NEEC) most commonly with serous-, carcinosarcoma- or clear cell histology and unfavorable prognosis [4].

Currently used risk classifications systems are based on clinicopathological risk factors, and guide primary- and/or adjuvant treatment. Different EC risk classifications are used in clinical practice: the European Society for Medical Oncology - European Society of Gynaecological Oncology -European Society for Radiotherapy & Oncology (ESMO-ESGO-ESTRO), Post-operative Radiation Therapy for Endometrial Carcinoma (PORTEC) and Gynecologic Oncology Group (GOG) criteria [1,5–7]. All these risk classifications stratify into 'low, low-intermediate, intermediate, high-intermediate, high or advanced/metastatic' based on tumor grade, stage, histology, and age (GOG and PORTEC) [5–8]. The ESMO-ESGO-ESTRO risk classification can be used pre-operatively to guide the need for lymph node (LN) directed surgery, and post-operatively to define adjuvant treatment. Recently, we published the ENDORISK model showing improved pre-operative risk classification in EC with easy accessible biomarkers integrated in a Bayesian network [9]. This personalized network included immunohistochemical (IHC) expression of p53, L1 cell-adhesion molecule (L1CAM), estrogen receptor (ER), progesterone receptor (PR), and clinical preoperative biomarkers and was established to predict lymph node metastasis (LNM) and outcome pre-operatively.

The Cancer Genome Atlas (TCGA) identified four important prognostic molecular subgroups based on integrated genomic data [10], in which patients with p53-abn had the poorest outcome [11–13]. Integration of molecular profiling according to the TCGA in the ESMO risk classification was evaluated by Talhouk et al. and showed high prevalence of p53-abn in the ESMO 'high' risk group. Yet, for the other ESMO risk groups molecular profiling was not discriminative [11].

The integration of molecular profiling appears promising in guiding adjuvant treatment [14]. However, routine molecular profiling in each patient is expensive, and as most patients have a good outcome with hysterectomy only, a cost-effective stepwise approach might be a suitable alternative. It is hypothesized that the use of pre-operative IHC biomarkers such as p53, L1CAM and ER/PR, is not only valuable in guiding primary surgical approach (e.g. ENDORISK), yet also adjuvant treatment in daily clinical practice. Despite their prognostic relevance for LNM and survival in EC, none of these were studied in relation to the post-operative ESMO-ESGO-ESTRO risk classification groups or to LN status [13,15–19]. Therefore, our primary aim was to investigate the added prognostic relevance of pre-operative IHC biomarkers, p53/L1CAM/ER/

PR, to the post-operative ESMO-ESGO-ESTRO risk classification groups in EC. Secondary, the added prognostic relevance of these IHC biomarkers to LN status in EC.

2. Materials and methods

2.1. Study cohort

Within the European Network for Individualized Treatment of Endometrial Cancer (ENITEC), a retrospective multicenter cohort study was performed. The patients were surgically treated between February 1995 and August 2013 at one of the 10 participating ENITEC centers and were identified from a previously published cohort [9,20]. Only patients diagnosed by an expert gynaecological pathologist with complete clinical and pathological data and follow-up of at least 36 months were included, yielding 1199 patients out of ten European hospitals.

2.2. Pathological characteristics

Pre-operative tumor grade and histology were used for analysis, combined with IHC staining of p53, L1CAM, ER and PR according to ENDORISK [9]. Detailed information on tissue processing and IHC analysis is shown in *Supplementary S1 method*.

Scoring of the IHC was performed twice by assessors blinded to pathological and clinical characteristics (N.V., H.K., J.B., K.v.d.V., C.R.). Disagreements in scoring were solved in a consensus meeting with all assessors. For p53, staining was considered abnormal/aberrant (p53-abn) when more than 80% of tumor cell nuclei showed strong expression (over-expression) or when there was complete absence of nuclear staining (null-expression). For L1CAM, the number of tumor cells showing membranous expression was scored and dichotomized, using 10% as a cut-off value. For ER and PR, the number of stained tumor nuclei was scored. Cases were also dichotomized, using 10% as a cut-off value. L1CAM expression was considered abnormal when >10% of tumor cells were positive (L1CAM+), ER and/or PR expression was considered abnormal when <10% nuclear staining was present (ER/PR-).

2.3. Post-operative ESMO-ESGO-ESTRO risk classification

Five subgroups were identified based on post-operative tumor stage, tumor histology, grade, myometrial invasion (MI) and presence of lymphovascular space invasion (LVS): low, intermediate, high-intermediate, high and advanced/metastatic risk group [5].

2.4. Lymph node status

For LN status three subgroups were defined: histologically confirmed LNM (N1), LN sampled by lymphadenectomy and histologically negative (N0), and LN status unknown (Nx) if no lymphadenectomy was performed. Sentinel lymph node (SLN) procedure was allowed

but not performed in this study cohort. For the relation of IHC biomarkers and LN status, including the survival analysis, patients with LN status unknown (Nx) were excluded for analysis.

2.5. Outcome measurements

Our primary aim was to define the added prognostic relevance of pre-operative IHC biomarkers, p53/L1CAM/ER/PR, to the ESMO-ESGO-ESTRO risk classification groups in EC. Secondary, the added prognostic relevance of these IHC biomarkers to LN status in EC.

2.6. Statistical analysis

For statistical analyses, Statistical Package for the Social Sciences (SPSS), version 25.0 (IBM, New York, NY, USA) was applied. The results were considered significant if *P*-value was less than 0.05 ($P < 0.05$). For the association of IHC expression with the ESMO-ESGO-ESTRO risk classification groups, the Mantel-Haenszel χ^2 test for trend was used.

Survival analyses were performed using the Kaplan Meier curves (first 10 years after diagnosis) and univariate and multivariate Cox-regression. Recurrence-free survival (RFS) was defined as time from surgery to time of recurrence from EC disease, and disease-specific survival (DSS) was defined as time from date of surgery to date of death from EC, all censored by date of last contact. The definition of ER/PR was defined as either ER or PR negative and/or positive. The ESMO-ESGO-ESTRO risk classification in the survival analysis was dichotomized: 'low, intermediate and high-intermediate' and 'high and advanced/metastatic'. This dichotomy was used, as the 'high and advanced/metastatic' ESMO-ESGO-ESTRO risk classification groups included all cases with LNM. Associations were calculated as hazard ratio (HR) with corresponding 95% confidence interval (CI) and *P*-value.

2.7. Ethics approval

This study was approved by the Institutional Review Board Radboud University Medical Center and the Institutional Review Boards of all participating center. No informed consent was obtained because all data were analyzed anonymously.

3. Results

3.1. Study cohort

A total of 1199 patients were included from ten European hospitals. For the current study only patients with available pre-operative endometrial biopsies were included. Samples with insufficient tumor tissue were excluded, resulting in 763 patients with a median follow-up of 5.5 years [9]. Baseline patient- and tumor characteristics of included patients were not significantly different when compared with excluded patients (*data not shown*).

Clinicopathological characteristics of the study cohort are shown in Table 1. Mean patient age in the study population was 65 years. Most patients presented pre-operatively with low-grade (1–2) EC and endometrioid histology, 71.4% and 89.4% respectively. Pre-operative IHC expression of p53-abn was present in 112 (14.7%), L1CAM+ in 79 (10.4%) and ER/PR- in 151 (19.8%) patients. IHC staining was unsuccessful in $N = 67$ cases for p53, $N = 19$ for L1CAM, $N = 1$ for ER and $N = 6$ for PR. Lymphadenectomy was performed in 493 (64.6%) patients of whom 53 (10.7%) patients had LNM (N1). Adjuvant treatment was administered in 347 (45.6%) patients, of which 81.6% received radiotherapy (RT). A total of 105 (13.8%) patients developed recurrent EC disease and 102 (13.4%) patients died of whom 61 (59.8%) due to EC.

Stratification of the study cohort according to the ESMO-ESGO-ESTRO risk classification is shown in Table 1. A total of 169 (22.1%) EC patients were classified as 'high' risk.

Table 1
Baseline clinicopathological characteristics of study cohort.

		Total N = 763
Patient characteristics		
Age (years)		65.2 ± 10.2
BMI (kg/m ²)		29.9 ± 6.7
Pre-operative histology		
Tumor grade	1–2	545 (71.4)
	3	109 (14.4)
	Not classified	108 (14.2)
Histology	Endometrioid	682 (89.4)
	Non-endometrioid	39 (5.1)
	Not specified	42 (5.5)
Biomarker expression		
	P53-abnormal	112 (14.7)
	L1CAM positive	79 (10.4)
	ER/PR negative	151 (19.8)
Post-operative histology		
Tumor grade	1–2	607 (79.6)
	3	156 (20.4)
Histology	Endometrioid	714 (93.6)
	Non-endometrioid	49 (6.4)
FIGO stage		
	I-II	675 (88.5)
	IA	428 (56.1)
	IB	196 (25.7)
	II	51 (6.7)
	III - IVB	88 (11.5)
	IIIA	20 (2.6)
	IIIB	4 (0.5)
	IIIC	43 (5.6)
	IVA	2 (0.3)
	IVB	19 (2.5)
	Positive (N1)	53 (6.9)
	Negative (N0)	440 (57.7)
	Unknown [†] (Nx)	270 (35.4)
ESMO-ESGO-ESTRO risk classification		
	Low	366 (48.0)
	Intermediate	140 (18.3)
	High-intermediate	68 (8.9)
	High	169 (22.1)
	Advanced/Metastatic	20 (2.6)
Adjuvant treatment		
	None	415 (54.4)
Radiotherapy		
	283 (37.1)	
	VBT	112 (39.6)
	EBRT	104 (36.7)
	EBRT+VBT	93 (32.9)
Chemotherapy		
	38 (5.0)	
Chemoradiation		
	26 (3.4)	
	Not specified	1 (0.1)
Outcome		
Recurrence		
	105 (13.8)	
	Local	25 (23.8)
	Regional	9 (8.6)
	Distant	69 (65.7)
	Not classified	2 (1.9)
Mortality		
	Overall	102 (13.4)
	EC-related	61 (8.0)

Data is presented in number (%), mean ± standard deviation (SD).

EC, endometrial cancer; ER/PR, estrogen receptor/progesterone receptor; ESMO-ESGO-ESTRO, European Society for Medical Oncology - European Society of Gynaecological Oncology - European Society for Radiotherapy & Oncology; EBRT, external beam radiotherapy; FIGO, Federation International Gynaecology Obstetric; L1CAM, L1 cell-adhesion molecule; N, number; VBT, vaginal beam therapy.

[†] No lymphadenectomy performed.

3.2. Immunohistochemical expression in relation to the ESMO-ESGO-ESTRO risk classification

In Fig. 1 abnormal IHC expression of p53, L1CAM, ER and PR is shown in relation to the ESMO-ESGO-ESTRO risk classification groups. Increased abnormal IHC expression was related to higher risk classification groups ($P < 0.001$), with the highest frequency of p53-abn, L1CAM+, ER- or PR- in the ESMO-ESGO-ESTRO high and advanced/metastatic subgroups.

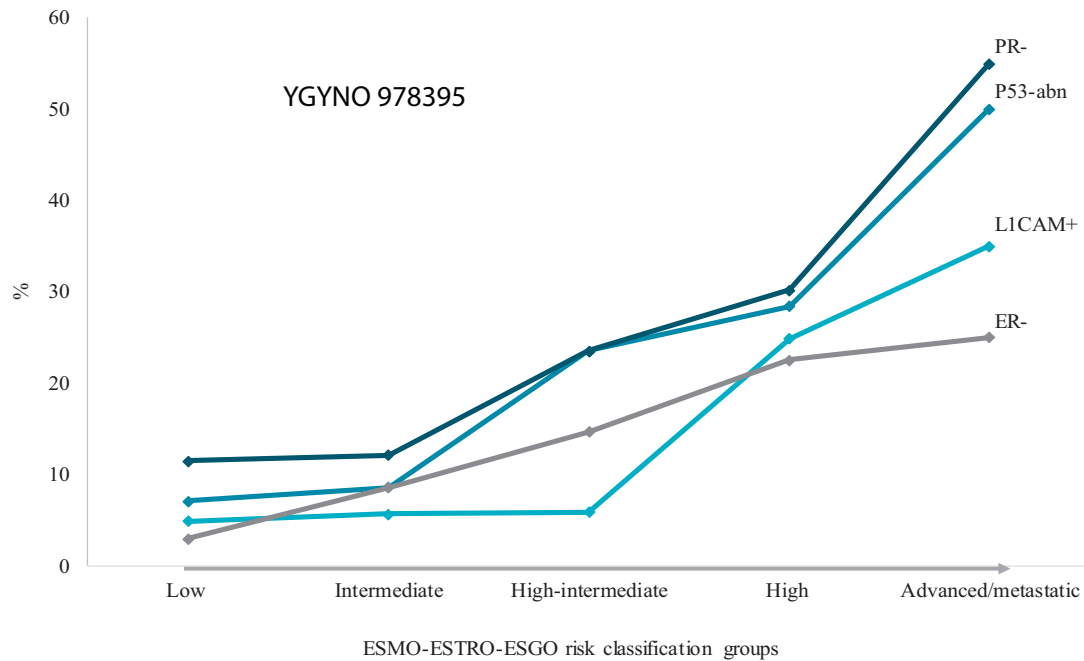


Fig. 1. Abnormal immunohistochemical expression of p53, L1CAM and ER/PR in relation to the ESMO-ESGO-ESTRO risk classification. L1CAM, L1 cell-adhesion molecule; ER/PR, estrogen receptor/progesterone receptor; ESMO-ESGO-ESTRO, European Society for Medical Oncology - European Society of Gynaecological Oncology - European Society for Radiotherapy & Oncology.

3.3. Immunohistochemical expression in addition to ESMO-ESGO-ESTRO risk classification

The RFS according to the ESMO-ESGO-ESTRO risk groups and IHC expression of p53, L1CAM and ER/PR in the ESMO 'high and advanced/metastatic' risk group are shown in Fig. 2. The ESMO-ESGO-ESTRO risk classification group 'high and advanced/metastatic' are significantly associated with poor RFS ($p < 0.001$) (Fig. 2A). Within the 'high and advanced/metastatic' risk group, patients with abnormal IHC expression of; p53, L1CAM and ER/PR, p53 and L1CAM, L1CAM and ER/PR, and only ER/PR have the lowest RFS, compare to patients with abnormal

expression of; p53 and ER/PR, only p53 and only L1CAM (Fig. 2B). Detailed survival curves of the ESMO-ESGO-ESTRO risk groups in relation to IHC expression are demonstrated in Fig. 3A-C. Patients with abnormal IHC expression (p53-abn, L1CAM+ or ER/PR-) and ESMO-ESGO-ESTRO risk group 'high and advanced/metastatic' show the lowest RFS compared with the other subgroups.

The DSS according to the ESMO-ESGO-ESTRO risk classification groups, and detailed survival curves of the ESMO-ESGO-ESTRO risk group in relation to the IHC expression were comparable to the RFS (Supplementary Fig. S1A and Fig. S2A-C). Within the ESMO 'high and advanced/metastatic' risk group, patients with abnormal IHC expression

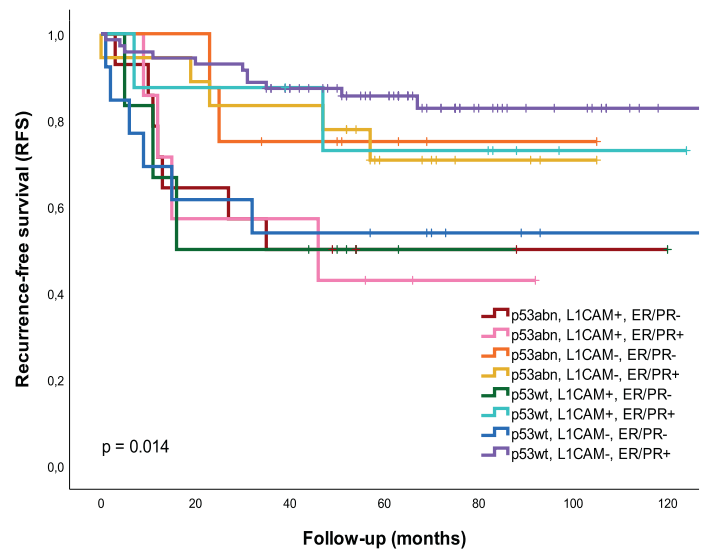
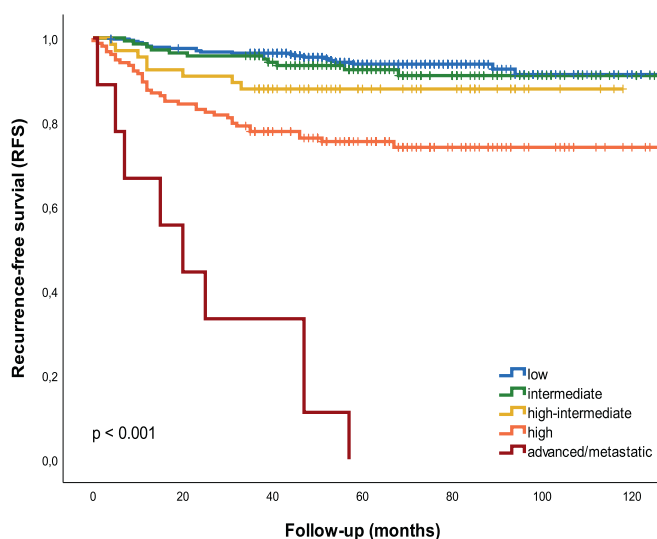


Fig. 2. A. RFS for the ESMO-ESGO-ESTRO risk groups. B. RFS for the ESMO-ESGO-ESTRO 'high and advanced/metastatic' risk group in relation to p53, L1CAM and ER/PR expression. ESMO-ESGO-ESTRO, European Society for Medical Oncology - European Society of Gynaecological Oncology - European Society for Radiotherapy & Oncology; p53abn, p53-abnormal; p53wt, p53-wildtype; L1CAM, L1 cell-adhesion molecule; ER/PR, estrogen receptor/progesterone receptor; RFS, recurrence-free survival.

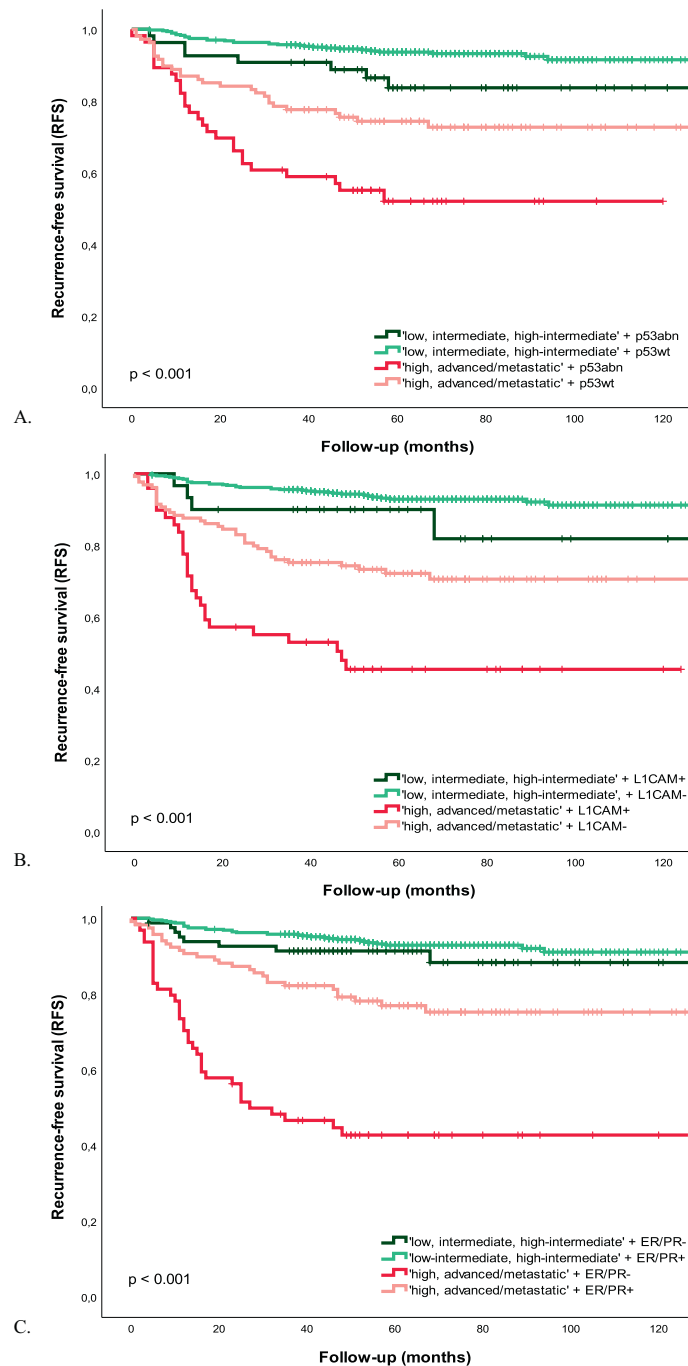


Fig. 3. A. RFS for ESMO-ESGO-ESTRO risk groups and p53-expression. B. RFS for ESMO-ESGO-ESTRO risk groups and L1CAM-expression. C. RFS for ESMO-ESGO-ESTRO risk groups and ER/PR-expression. ESMO-ESGO-ESTRO, European Society for Medical Oncology - European Society of Gynaecological Oncology - European Society for Radiotherapy & Oncology; p53abn, p53-abnormal; p53wt, p53-wildtype; L1CAM, L1 cell-adhesion molecule; ER/PR, estrogen receptor/progesterone receptor; RFS, recurrence-free survival.

of; p53, L1CAM and ER/PR, p53 and L1CAM, p53 and ER/PR and only ER/PR have the lowest DSS compare to patients with abnormal expression of; L1CAM and ER/PR, only p53 and only L1CAM (*Supplementary Fig. S1B*).

3.4. Prognostic relevance of immunohistochemical expression in relation to the ESMO-ESGO-ESTRO risk classification

Multivariate analysis was performed for the prognostic relevance of IHC expression in relation to the ESMO-ESGO-ESTRO risk classification groups. The ESMO-ESGO-ESTRO classification 'high and advanced/

metastatic' risk was independently associated with reduced RFS (HR 3.11 [CI 1.93–5.02] $P < 0.001$) (*Table 2*). P53-abn, ER/PR- and ESMO-ESGO-ESTRO classification 'high and advanced/metastatic' risk were independently associated with reduced DSS (HR 1.88 [CI 1.00–3.51] $P = 0.048$, HR 2.74 [CI 1.48–5.07] $P = 0.001$ and HR 5.69 [CI 3.03–10.67] $P < 0.001$, respectively) (*Table 3*).

3.5. Immunohistochemical expression in relation to lymph node status

The LN status in relation to abnormal IHC expression (p53-abn, L1CAM+ or ER/PR-) is shown in *Supplementary Fig. S3*. LNM was

Table 2
Univariate and multivariate Cox regression analysis of RFS.

Variable	Univariate RFS			Multivariate RFS		
	HR	95% CI	P-value	HR	95% CI	P-value
ESMO-ESGO-ESTRO risk classification						
'Low - Intermediate - High-intermediate' vs 'High - Advanced/metastatic'	3.92	2.52–6.08	<0.001*	3.11	1.93–5.02	<0.001*
Immunohistochemical markers						
p53-abnormal	2.94	1.83–4.72	<0.001*	1.58	0.92–2.71	0.097
L1CAM+	4.27	2.63–6.92	<0.001*	1.78	0.98–3.21	0.058
ER/PR-	3.16	2.03–4.91	<0.001*	1.54	0.90–2.63	0.115

CI, confidence interval; ER/PR, estrogen receptor/progesterone receptor; ESMO-ESGO-ESTRO, European Society for Medical Oncology-European Society for Radiotherapy & Oncology-European Society of Gynaecological Oncology; HR, Hazard ratio; L1CAM, L1 cell-adhesion molecule; RFS, Recurrence-free survival.

* $p < 0.05$.

observed in 21.4% of p53-abn, 31.3% of L1CAM+, and 20% of ER/PR-cases. Survival outcome curves (RFS and DSS) of abnormal and normal IHC expression in relation to LN status (N1/N0) is shown in *Supplementary Fig. S4A-C and Fig. S5A-C*. Patients with LNM (N1) and p53-abn or L1CAM+ had significantly decreased RFS/DSS compared with, patients having LNM (N1) and normal IHC expression (p53-wt or L1CAM-), and patients without LNM (N0) and normal/abnormal IHC expression ($p < 0.001$). No significant reduction in RFS was seen in patients with LNM (N1) and ER/PR- compared with patients having LNM (N1) and ER/PR+ (*Supplementary Fig. S4C*). Patients with LNM (N1) and ER/PR- had significantly reduced DSS compared with patients having LNM (N1) and ER/PR+ (*Supplementary Fig. S5C*). Patients without LNM (N0) and abnormal IHC expression (p53-abn, L1CAM+ or ER/PR-) had similar RFS/DSS compared with patients with LNM (N1) and normal IHC expression (p53-wt, L1CAM- or ER/PR+) (*Supplementary Fig. S4A-C and Fig. S5A-C*).

4. Discussion

In this study the added prognostic relevance of the pre-operative IHC expression of p53, L1CAM and ER/PR, to the ESMO-ESGO-ESTRO risk classification groups is demonstrated. Significantly increased abnormal IHC expression is observed in higher risk classification groups. Within the 'high and advanced/metastatic' risk group, patients with a combination of abnormal IHC expression had the poorest outcome (RFS and DSS). ER/PR-, p53-abn, and ESMO-ESGO-ESTRO 'high and advanced/metastatic' risk group were independently associated with decreased DSS. Furthermore, abnormal IHC expression had added prognostic relevance to LN status. Patients with abnormal IHC expression and LNM had most dismal outcome. Interestingly, patients without LNM and abnormal IHC expression showed comparable RFS/DSS to patients with LNM and normal biomarkers. This indicated that the IHC biomarkers p53, L1CAM and ER/PR have prognostic relevance to the ESMO-ESGO-ESTRO risk classification groups and to patients with and without LNM.

Table 3
Univariate and multivariate Cox regression analysis of DSS.

Variable	Univariate DSS			Multivariate DSS		
	HR	95% CI	P-value	HR	95% CI	P-value
ESMO-ESGO-ESTRO risk classification						
'Low - Intermediate - High-intermediate' vs 'High - Advanced/metastatic'	6.93	3.82–12.57	<0.001*	5.69	3.03–10.67	<0.001*
Immunohistochemical markers						
p53-abnormal	4.44	2.56–7.69	<0.001*	1.88	1.00–3.51	0.048*
L1CAM +	5.43	3.19–9.26	<0.001*	1.17	0.59–2.31	0.656
ER/PR-	5.49	3.30–9.12	<0.001*	2.74	1.48–5.07	0.001*

CI, confidence interval; DSS, disease-specific survival; ER/PR, estrogen receptor/progesterone receptor; ESMO-ESGO-ESTRO, European Society for Medical Oncology-European Society for Radiotherapy & Oncology-European Society of Gynaecological Oncology; HR, Hazard ratio; L1CAM, L1 cell-adhesion molecule.

* $p < 0.05$.

Our findings of p53-abn as important prognosticator is in line with the TCGA data that have been validated by multiple other research groups [10,21]. The percentage of p53-abn in our study cohort (12.0%) in patients with endometrioid histology was comparable to the original TCGA paper (11.4%) [10]. Instead of using p53 sequencing, we used easy accessible p53 IHC staining comparable to the ProMisE classification system, which was shown to be a good surrogate biomarker for p53 mutations [11,13,22]. Talhouk et al. studied the prevalence and prognostic relevance of p53-abn in the ESMO risk classification, and observed a high prevalence of p53-abn in the ESMO 'high' risk group in line with our findings [13]. The fact that integration of ProMisE/TCGA in the ESMO risk classification did not show significant difference in outcome, is contrary to our results in which p53-abn had added prognostic value in the 'high and advanced/metastatic' risk group. This could be explained by the use of the ESMO 2013 guideline in the study of Talhouk et al. compared with the ESMO-ESGO-ESTRO 2016 guideline used in our study [13].

In addition to p53-abn, L1CAM+ is an established prognosticator in EC as observed in our study [16,20,23]. The percentage of L1CAM+ cases was slightly lower in our study compared with other studies [16,19,20,23,24]. This might be related to the fact that pre-operative analysis was used instead of final tumor sections in which L1CAM can be expressed focally and/or at the invasive front predominantly [25]. Our results are in line with a study reporting that patients with L1CAM+ had significantly reduced survival also in the ESMO-ESGO-ESTRO 'high and advanced/metastatic' risk group compared with normal L1CAM expression [24]. A more recent study reported reduced overall survival (OS) and progression-free survival (PFS) in patients with FIGO stage III and L1CAM+ when compared with L1CAM- patients [26]. This is in line with our results since FIGO stage III is included in the 'high' risk group.

Multiple studies have investigated ER/PR expression in relation to outcome in EC reporting conflicting results [18,27,28]. In our study, ER/PR- was not significantly related to RFS in the multivariate analysis, contrary to previous studies [18,27,28]. In line with the study by Trovik et al., ER/PR- was related to DSS and LNM [17]. In a previous ENITEC

study of our study group, mainly loss of PR predicted disease recurrence [18]. Biologically, loss of ER is preceded by loss of PR and therefore PR might be the most relevant to outcome. In the current study, we did not analyze ER/PR separately, interestingly, loss of PR was mainly present in the 'advanced/metastatic' risk group, underlining the possible relevance for distant spread. The expression of ER/PR was studied in relation to the different TCGA groups and although ER and PR biomarkers were both predictive for outcome in the univariate analysis, only the ProMisE subtypes maintained significant association with outcome in the multivariate analysis. It was suggested that the prognostic significance of single biomarkers could be explained by being a covariable with the ProMisE molecular subtype [16]. Similar was shown in the study of Stelloo et al. [29]. Due to the used cut-offs for ER/PR of 5% and 1% respectively in one study, the prognostic value might have been underestimated when compared with the 10% cut-off that was used in our study and the study by Trovik et al. [16,17]. Stelloo et al. did use the 10% cut-off, however they only included early stage EEC patients hampering comparison to our study [29].

There is an ongoing debate about routine surgical staging with LN dissection or sampling in EC, especially after the introduction of molecular profiling. Yet, LN status as determined by either lymphadenectomy or SLN remains an important prognosticator for survival and guiding adjuvant treatment in the current ESMO-ESGO-ESTRO risk classification [5,30–33]. The study of Ouldamer et al. concluded, that even patients within the 'high-intermediate' risk group should receive systematic nodal staging for a significant better survival [34]. This is in line with recent paper of Weelden et al. that demonstrated that patients with FIGO IIIA-B had significant improved outcome if LN were sampled and negative [35]. Our results show the prognostic relevance of IHC expression in addition to LN status, similar to other studies [17,19]. The importance of both, IHC biomarkers and LN status, is shown in our results since patients with LNM and normal IHC expression had comparable RFS/DSS to patients without LNM and abnormal IHC expression.

To our knowledge the prognostic relevance of integrating IHC biomarkers in the ESMO-ESGO-ESTRO risk classification groups has not been studied so far. Yet, there are some limitations that need to be addressed. First, in addition to the IHC biomarkers, we did not include the well-established final histopathological markers related to the prognosis. However, as expected the pre-operative abnormal biomarker expression of p53, L1CAM and ER/PR are significant associated with grade 3, NEEC, LVSI, MI and cervical stromal invasion (CSI) (*data not shown*). Abnormal pre-operative biomarkers could therefore serve as surrogate biomarkers for these final histopathological risk factors. Second, as we used p53 IHC expression as indicator for p53-abn without information on *POLE* or mismatch repair deficient (*MMR-D*) status, we might have slightly overestimated the number of patients with p53-abn. However, as multiple classifiers are only present in 3% of the cases, it is unlikely that this has influenced our findings [36]. Finally, inherent to the retrospective character of the study, differences in outcome might be explained by the fact that adjuvant treatment was not uniformly applied. The majority (80–100%) of the patients with LNM and abnormal IHC expression received chemotherapy (CT) or chemo- and radiotherapy (CTRT) as adjuvant treatment, compared with 55% for patients with LNM and normal IHC expression (*data not shown*). This difference in percentage could be explained by patients being treated according final tumor stage and histology in different ENITEC centers in Europe, i.e. patients that received more often radiotherapy mainly had endometrioid histology, whereas those with adjuvant chemotherapy more often non-endometrioid histology. Thus, patients having worst outcome most frequently received CT or CTRT instead of RT alone, and this does therefore not explain the specifically worse outcome in these patients.

The strength of this multicenter study is the large patient cohort, and well-documented and long-term follow-up. Although primary and adjuvant treatment was not uniformly applied, the current study reflects actual clinical practice facilitating implementation.

For this study we focused on the IHC expression in the pre-operative setting, as the risk of extended disease and LNM appears mainly associated with p53-abn and significantly less with the other TCGA groups [12]. We expect that molecular profiling will be incorporated in future treatment planning [37–40]. The study of Leslie et al. revealed that patients with *TP53* mutation had significant better PFS with adjuvant chemotherapy + bevacizumab when compared to chemotherapy + temsirolimus, this significant difference was not shown in patients with *TP53* wildtype [41]. This illustrated the relevance of TCGA with respect to the adjuvant treatment. However, routine molecular analysis is expensive and requires fully equipped laboratory, therefore a step-wise approach could bridge this gap, and contribute to selective molecular profiling in 'high' risk EC patients [13]. Cosgrove et al. showed that even selective molecular profiling in patients with only EEC histology could provide additional prognostic information. This step-wise approach could be used combined with the Lynch syndrome screening panel and so refining the choice of adjuvant treatment [42]. The recently published ENDORISK model demonstrates that pre-operative identification for patients at risk for LNM can be significantly improved by incorporating clinical and IHC biomarkers into a Bayesian network [9]. Although we fully endorse the integration of both clinical and IHC biomarkers, in clinical practice we often have to deal with incomplete data. This current study showed that the IHC biomarkers could serve as indicator for LN directed surgery and either IHC biomarkers or molecular profiling or a combination, could be used as refinement for selective adjuvant treatment by being incorporated in the ESMO-ESGO-ESTRO risk classification and being used next to LN status. These results should be further validated in an prospective study with an independent cohort.

5. Conclusion

Concluding, pre-operative IHC biomarkers are important prognostic markers within the ESMO-ESGO-ESTRO risk classification groups and in addition to LN status. For daily clinical practice, integrating IHC expression of p53/L1CAM/ER/PR into the ESMO-ESGO-ESTRO risk classification groups may be valuable in guiding surgical staging, and identifying patients who would benefit from specific adjuvant treatment.

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Disclaimers

Nothing to disclaim.

CRediT authorship contribution statement

S.W. Vrede: Conceptualization, Data curation, Formal analysis, Investigation, Methodology, Visualization, Writing - original draft. **W.J. van Weelden:** Conceptualization, Writing - review & editing. **N.C.M. Visser:** Data curation, Investigation, Resources. **J. Bulten:** Data curation, Investigation, Resources. **L.J.M. van der Putten:** Data curation, Investigation. **K. van de Vijver:** Data curation, Investigation, Resources. **M. Santacana:** Data curation, Resources. **E. Colas:** Data curation, Resources. **A. Gil-Moreno:** Data curation, Resources. **C.P. Moiola:** Data curation, Resources. **G. Mancebo:** Data curation, Resources. **C. Krakstad:** Data curation, Resources. **J. Trovik:** Data curation, Resources. **I.S. Haldorsen:** Data curation, Resources. **J. Huvila:** Data curation, Resources. **M. Koskas:** Data curation, Resources. **V. Weinberger:** Data curation, Resources. **M. Bednarikova:** Data curation, Resources. **J. Hausnerova:** Data curation, Resources. **A.A. van der Wurff:** Data curation, Resources. **X. Matias-Guiu:** Data curation, Resources. **F. Amant:** Data curation, Resources. **M.P.L.M. Snijders:** Conceptualization, Supervision, Writing - review & editing. **H.V.N. Küsters-Vandevelde:** Conceptualization, Data curation, Investigation. **C. Reijnen:** Resources, Conceptualization, Data curation, Writing - review & editing. **J.**

M.A. Pijnenborg: Conceptualization, Project administration, Supervision, Writing - review & editing, Funding acquisition.

Declaration of Competing Interest

The authors have declared no conflicts of interest.

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