Microcystic elongated and fragmented (MELF) pattern of invasion: molecular features and prognostic significance in the PORTEC-1 and -2 trials

Heerik, A.S.V.M.V.; Aiyer, K.T.S.; Stelloo, E.; Juergenliemk-Schulz, I.M.; Lutgens, L.C.H.W.; Jobsen, J.J.; ... ; Bosse, T.

Gynecologic Oncology 166 (2022) 530–537

Contents lists available at ScienceDirect

Gynecologic Oncology

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

Microcystic elongated and fragmented (MELF) pattern of invasion: Molecular features and prognostic significance in the PORTEC-1 and -2 trials

A.S.V.M. van den Heerik [a], K.T.S. Aiyer [b], E. Stelloo [b], I.M. Jürgenliemk-Schulz [c], L.C.H.W. Lutgens [d], J.J. Jobsen [e], J.W.M. Mens [f], E.M. van der Steen-Banasik [g], C.L. Creutzberg [a], V.T.H.B.M. Smit [b], N. Horeweg [a], T. Bosse [b,*]

[a] Department of Radiation Oncology, Leiden University Medical Center, Leiden, the Netherlands
[b] Department of Pathology, Leiden University Medical Center, Leiden, the Netherlands
[c] Department of Radiation Oncology, University Medical Center Utrecht, Utrecht, the Netherlands
[d] Department of Radiation Oncology, Maastricht University Medical Centre+, Maastricht, the Netherlands
[e] Department of Radiation Oncology, Medisch Spectrum Twente, Enschede, the Netherlands
[f] Department of Radiotherapy, Erasmus MC Cancer Institute, University Medical Center Rotterdam, Rotterdam, the Netherlands
[g] Department of Radiation Oncology, Radiotherapy Group, Arnhem, the Netherlands

HIGHLIGHTS

- MELF pattern of invasion is identified in 13.1% of early-stage endometrial cancer.
- MELF invasion is associated with low-grade endometrial cancer.
- MELF invasion is predominantly seen in *CTNNB1* wild type no-specific-molecular-profile endometrial cancer.
- MELF invasion has no independent prognostic impact.

ARTICLE INFO

Article history:
Received 25 April 2022
Received in revised form 24 June 2022
Accepted 26 June 2022
Available online 13 July 2022

Keywords:
Endometrial cancer
MELF pattern of invasion
Molecular endometrial cancer classification

ABSTRACT

Objective. Microcystic, elongated fragmented (MELF) pattern of myometrial invasion is a distinct histologic feature occasionally seen in low-grade endometrial carcinomas (EC). The prognostic relevance of MELF invasion was uncertain due to conflicting data, and it had not yet appropriately been studied in the context of the molecular EC classification. We aimed to determine the relation of MELF invasion with clinicopathological and molecular characteristics, and define its prognostic relevance in early-stage low/intermediate risk EC.

Methods. Single whole tumor slides of 979 (85.8%) out of 1141 (high)intermediate-risk EC of women who participated in the PORTEC-1/−2 trials were available for review. Clinicopathological and molecular features were compared between MELF invasion positive and negative cases. Time-to-event analyses were done by Kaplan-Meier method, log-rank tests and Cox' proportional hazards models.

Results. MELF invasion was found in 128 (13.1%) cases, and associated with grade 1–2 histology, deep myometrial invasion and substantial lymph-vascular space invasion (LVSI). 85.6% of MELF invasion positive tumors were no-specific-molecular-profile (NSMP) EC. NSMP EC with MELF invasion were *CTNNB1* wild type in 92.2% and *KRAS* mutated in 24.4% of cases. Risk of recurrence was lower for MELF invasion positive as compared to MELF invasion negative cases (4.9% vs. 12.7%, $p = 0.026$). However, MELF invasion had no independent impact on risk of recurrence (HR 0.65, $p = 0.30$) after correction for clinicopathological and molecular factors.

Conclusions. MELF invasion has no independent impact on risk of recurrence in early-stage EC, and is frequently observed in low-grade NSMP tumors. Routine assessment of MELF invasion has no clinical implications and is not recommended.

* Corresponding author at: Leiden University Medical Center, Albinusdreef 2, 2333 ZA Leiden, the Netherlands.
E-mail address: T.Bosse@lumc.nl (T. Bosse).

https://doi.org/10.1016/j.ygyno.2022.06.027

1. Introduction

A specific type of invasion pattern is occasionally observed at the invasive front of endometrial cancer (EC) specimens; a pattern of elongated, dilated or fragmented glands, and typically lined by an attenuated epithelium, known by its acronym MELF (microcystic, elongated and fragmented) pattern of myometrial invasion [1]. Based on its histological aspects, it was initially suspected that MELF invasion could be a result of a tumor degenerative process [1]. However, subsequent studies noted morphological and immunohistochemical similarities between MELF invasion and a specific tumor-stroma interaction. This led to the suggestion that this process could be similar to epithelium-mesenchymal transition (EMT). Subsequently alterations in cell-cycle proteins, hormone receptors, cell-adhesion elements and molecular alterations, such as *KRAS,* have been described in the context of MELF invasion [2–7]. Since EMT has been associated with poor prognostic parameters in multiple cancers, several studies have been conducted to determine whether MELF invasion could be a phenotype of EMT and as such prognostic in EC [8,9]. Based on current literature, MELF invasion positive tumors are mostly low-grade with deep myometrial invasion, and are associated with adverse histological findings, such as lymph-vascular space invasion (LVSI) and lymph node metastasis [10–17]. Although the majority of studies did not demonstrate a statistically significant negative effect of MELF invasion on clinical outcomes [7,10–15,18], a few did [16,17,19]. Potential explanations for these differences in outcomes are the wide variety of study populations, the small number of cases, and study methods used (mostly cohort studies or case-control designs). Additionally, only limited data is available on whether a quantitative scoring method for MELF invasion is useful and/or prognostic [6,11,15]. Due to conflicting data, and lack of large prospective studies, the clinical significance of MELF invasion has remained unclear. As a result, there is no consensus on the clinical implications when MELF invasion is reported.

Since the publication of the landmark paper of The Cancer Genome Atlas (TCGA) on EC, multiple studies have underlined the importance of differentiating between the four molecular subclasses; *POLE*-ultra mutated EC (*POLE*mut), mismatch repair deficient EC (MMRd), p53-abberant EC (p53abn) or no-specific-molecular-profile EC (NSMP) [20–22]. Only very limited data has been published on MELF invasion in the context of the molecular EC subgroups [6,19].

The aim of our analysis was to determine the relation of MELF invasion with clinicopathological and molecular characteristics, and to define its prognostic relevance in early-stage EC.

2. Methods

2.1. Study population

Trial data from the post-operative radiation therapy in endometrial carcinoma (PORTEC)-1 and − 2 trials were used. PORTEC-1 involved 714 patients with FIGO 2009 stage I EC, grade 1 or 2 with deep myometrial invasion, or grade 2 or 3 with superficial invasion [23]. In the PORTEC-2 trial a total of 427 patients with high-intermediate risk features, FIGO 2009 stage I, age > 60 years, grade 1–2 with deep invasion or cervical glandular involvement or grade 3 with superficial invasion were included [24]. In both trials, all patients underwent total hysterectomy and bilateral salpingo-oophorectomy without standard lymphadenectomy. In PORTEC-1, patients were randomly allocated to pelvic external beam radiation therapy versus no additional treatment, whereas in the PORTEC-2 patients received external beam radiotherapy or vaginal brachytherapy [23,24].

2.2. Molecular and histopathological data

A comprehensive description of the histopathological, immunohistochemical (IHC) and molecular analyses of the PORTEC-1 and -2 tumor samples have been published [21,25,26]. In short, TCGA molecular subgroups were assigned using a surrogate marker approach. IHC was used to assess for mismatch protein repair deficiency and p53 expression. Sanger and/or Next Generation Sequencing (NGS) were performed to identify cases with a pathogenic mutation in the exonuclease domain of DNA polymerase epsilon (*POLE*), and were classified as *POLE*mut EC [21,27]. In case of multiple-classifying alterations, assignment was done according to the WHO 2020 criteria [28,29]. Presence and quantification of LVSI, other molecular alterations in genes, such as *CTNNB1* and *KRAS*, and additional IHC analyses for estrogen receptor (ER) and L1CAM, both deemed positive if >10% (over)expression, were determined as previously described [21,25,26].

2.3. MELF invasion definition and scoring system

MELF invasion was evaluated according to the definition of Murray et al. [1] According to this definition MELF invasion is characterized by loss of the conventional glandular architecture. Attenuated neoplastic cells with a squamous or vacuolated appearance are lined by flattened, endothelial-like, cells with eosinophilic cytoplasm. These microcysts can appear as compressed elongated structures, which can appear disrupted or fragmented. Notably, these detached glands lie in an edematous or fibromyoxoid background.

Assessment of MELF invasion in this study was done by a stepwise approach. During all screening steps reviewers scored independently, and were blinded for clinical outcomes, clinicopathological and molecular reports. During each step, if there was no consensus at initial review, the case would be discussed at a multiheaded microscope with the expert observers until consensus was reached. First, a senior pathology resident (KTSA) and researcher (ASVMH) were trained to recognize MELF invasion by two expert gynecologic pathologists (TB and VTHBMS). After this training, a single representative hematoxylin and eosin (H&E) stained whole tumor slide of each PORTEC-1 and -2 case was screened for the presence or absence of MELF invasion. During this initial screening process MELF invasion negative cases were logged. During the second phase, all identified cases that potentially displayed MELF invasion were evaluated for presence of MELF invasion by two expert gynecologic pathologists (TB and VTHBMS). Finally, the extent of MELF invasion was quantified in all MELF invasion positive cases by scoring the presence of either one, or more than one focus (TB and VTHBMS). An arbitrary cut-off for focal MELF invasion was set at the presence of one MELF gland, whereas more than one MELF gland was considered multifocal (Fig. 1).

2.4. Analysis in TCGA PanCancer Atlas

Exploratory analyses were performed in an external and independent dataset. Digitalized H&E slides from the TCGA, PanCancer Atlas were downloaded (publicly available at NCI-GDC data Portal (http://portal.gdc.cancer.gov; accessed on 30 October 2020). Clinicopathologic and molecular data of the TCGA, PanCancer Atlas, were obtained via the cBio Cancer Genomics Portal (http://cbioportal.org; accessed on 17 January 2022). The digitalized H&E images were scored by two reviewers (ASVMH and TB), while blinded for clinical outcomes, clinicopathological and molecular reports. Consensus was reached if both observers agreed. Discordant cases were discussed until consensus was reached.

2.5. Statistical analyses

Patient and tumor characteristics of the PORTEC-1 and -2 patients such as histological type, grade and stage, presence and extent of LVSI, molecular subgroups, L1CAM and ER overexpression, and *CTNNB1* and *KRAS* mutational status, were compared between MELF invasion positive and negative cases. Differences in patient and tumor characteristics were analyzed with chi-square or Fisher's exact test for categorical

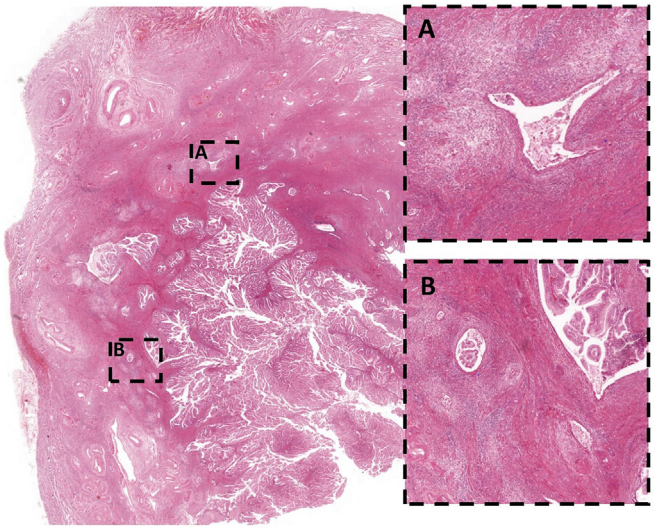

Fig. 1. Representative example of a multifocal MELF invasion positive case.
MELF invasion can be identified at low-power magnification by its distinct fibromyoxoid stroma. A: at higher magnification a gland shows the typical microcystic and elongated appearance. B: at higher magnification, a gland shows it typical microcystic appearance, which is lined by flattened, endothelial-like cells.

variables and Mann-Whitney *U* test for non-normally distributed continuous variables. Time-to-event analyses were done using the Kaplan-Meier method, log-rank tests and Cox' proportional hazards models and were calculated from the date of randomization as starting point. Events were defined as EC recurrences, with censoring at last follow-up in case of no recurrence or death due to other causes. Statistical analyses were performed with SPSS (Statistical Package of Social Science) version 25 (IBM, Armonk, NY, USA). All tests were two-sided, and results were considered statistically significant at *p*-values <0.05.

3. Results

3.1. Clinicopathological characteristics

Of the 1141 randomized PORTEC-1 and -2 EC cases, a total of 979 cases (85.8%) had a single representative H&E-stained histological slide available for review of the MELF invasion (Fig. 2). Table 1 shows the clinicopathologic characteristics of the cases included in our study. In 598 of 979 cases no MELF invasion was observed. Of the remaining cases which were suspected for MELF invasion only 128 cases were finally classified as MELF invasion positive (13.1%, 95% CI 11.0%–15.3%). Only 57 cases showed one focus of MELF invasion (44.5%), whereas in 71 cases multifocal MELF invasion was observed (55.5%). The MELF invasion positive tumors were associated with grade 1–2, deep myometrial invasion and substantial LVSI. All but one of the MELF invasion positive EC showed ER positivity. There was no statistically significant association between MELF invasion and L1CAM using the predefined threshold of ≥10% L1CAM overexpression. However, we observed that at the site of the MELF foci, which usually only make up about 1–5% of the tumor, L1CAM was locally upregulated, while often ER expression was lost (Supplementary S1). MELF invasion was observed in all molecular classes, and mostly in *CTNNB1* wild type tumors. MELF invasion was significantly more often found within the NSMP subgroup (85.6% of all MELF invasion positive tumors), of which over 92.0% were *CTNNB1* wild type, while 23.5% showed mutations in *KRAS*. In the MELF invasion negative group only 15% of cases harbored such a mutation. In other subgroups no differences were seen in *CTNNB1* and *KRAS* mutational status.

No notable differences in clinicopathological and molecular characteristics were observed when comparing patients with no, focal, and multifocal MELF invasion (Supplementary S3).

3.2. Prognostic value of MELF invasion

Median follow-up time in the combined PORTEC-1 and -2 database was 11.3 years (95% CI 11.1–11.5 years). During the entire follow-up,

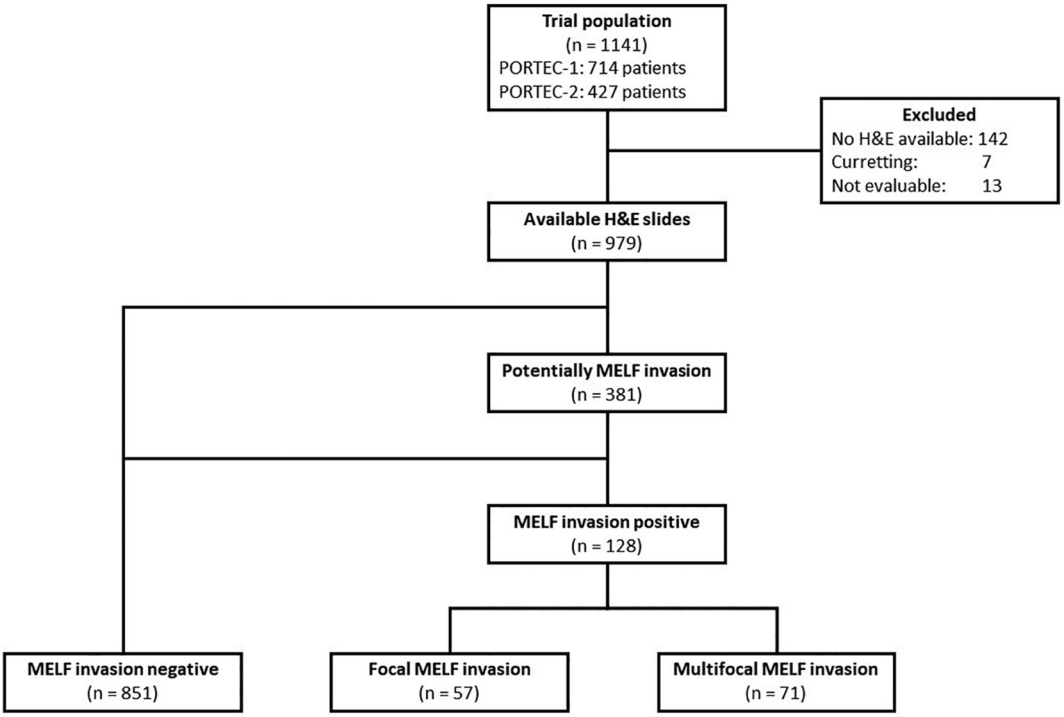

Fig. 2. Flowchart of cohort selection.
H&E: hematoxylin and eosin-stained whole tumor slides; MELF: microcystic elongated fragmented pattern of invasion.

Table 1
Clinicopathological characteristics of endometrial cancer with MELF invasion.

	Total ($n = 979$)	MELF invasion negative ($n = 851$)	MELF invasion positive ($n = 128$)	p-value
Age				0.88
Mean (range)	68 (41–90)	68 (41–90)	69 (50–82)	
Histotype[#]				0.76
Endometrioid	955 (97.5%)	829 (97.4%)	126 (98.4%)	
Non-endometrioid	24 (2.5%)	22 (2.6%)	2 (1.6%)	
Grade				<0.0001
1–2	841 (85.9%)	716 (84.1%)	125 (97.7%)	
3	138 (14.1%)	135 (15.9%)	3 (2.3%)	
Stage				<0.0001
IA	303 (30.9%)	296 (34.8%)	7 (5.5%)	
≥IB[*]	676 (69.1%)	555 (65.2%)	121 (94.5%)	
LVSI				0.049
None or focal	872 (95.5%)	761 (96.1%)	111 (91.7%)	
Substantial	41 (4.5%)	31 (3.9%)	10 (8.3%)	
TCGA				
*POLE*mut	62 (7.3%)	60 (8.1%)	2 (1.8%)	
MMRd	236 (27.6%)	223 (30.1%)	13 (11.7%)	
p53 abnormal	72 (3.2%)	71 (9.6%)	1 (0.9%)	
NSMP	484 (56.7%)	389 (52.3%)	95 (85.6%)	
L1CAM overexpression				0.001
≤10% (negative)	786 (92.9%)	673 (91.9%)	113 (99.1%)	
>10% (positive)	60 (7.1%)	59 (8.1%)	1 (0.9%)	
ER expression				0.050
≤10% (negative)	45 (5.6%)	44 (6.3%)	1 (0.9%)	
>10% (positive)	761 (94.4%)	649 (93.7%)	112 (99.1%)	
CTNNB1				<0.0001
Wild type	730 (80.4%)	620 (78.6%)	110 (92.4%)	
Mutant	178 (19.6%)	169 (21.4%)	9 (7.6%)	
KRAS				0.023
Wild type	762 (83.9%)	671 (85.0%)	91 (76.5%)	
Mutant	146 (16.1%)	118 (15.0%)	28 (23.5%)	
Received treatment				0.42
No treatment	307 (31.4%)	278 (32.6%)	29 (22.7%)	
Vaginal Brachytherapy	197 (20.1%)	164 (19.3%)	33 (25.8%)	
External beam radiotherapy	475 (48.5%)	409 (48.1%)	66 (51.5%)	

ER, estrogen receptor; LVSI, lymph-vascular space invasion; L1CAM, L1-cell adhesion molecule; MELF, microcystic elongated fragmented pattern of invasion; MMRd, mismatch repair deficiency; NSMP, no-specific-molecular-profile; TCGA, The Cancer Genome Atlas.

[*] PORTEC 1–2 inclusion criteria did not allow FIGO stages higher than IB (according to the 2009 definition), nonetheless 2 cases were classified as stage II, 2 as IIIA and 1 as IIIB at central revision. These 5 cases were all MELF invasion negative.

[#] PORTEC 1–2 inclusion criteria did not allow non-endometrioid endometrial cancer, however central revision after randomization reclassified 24 cases as non-endometrioid endometrial cancer.

a total of 133 recurrences occurred, of which 124 in MELF invasion negative (14.6%) and 9 in MELF invasion positive cases (7.0%). When differentiating between no, focal or multifocal MELF invasion, we observed a small non-significant prognostic effect of MELF invasion, Supplementary S2. Five-year risk of recurrence was highest in the MELF invasion negative group, followed by the groups with multifocal focal and focal MELF invasion (12.7% vs 5.9% vs 3.6% respectively, $p = 0.074$). When only distinguishing MELF invasion negative from MELF invasion positive cases, a significantly lower 5-year risk of recurrence was observed for MELF invasion positive tumors, compared to MELF invasion negative cases (4.9% vs. 12.7%, $p = 0.026$, Fig. 3). Of the 9 MELF invasion positive cases who had a recurrence, none were *POLE*mut EC. Seven of the 9 recurrences had occurred in the NSMP group whereas only one recurrence occurred in p53abn and MMRd EC.

To evaluate the independency of the prognostic value of MELF invasion, a multivariable analysis was performed with clinicopathologic (grade, stage, LVSI), immunohistochemical and molecular factors (molecular classes, L1CAM, ER and *CTNNB1*), which is shown in Table 2. After correction for these factors, the presence of MELF invasion was no longer associated with EC recurrence (HR 0.65, 95% CI 0.29 to 1.48, $p = 0.30$), in contrast to grade (HR 2.26, 95% CI 1.30 to 3.93, $p = 0.004$), stage (HR 1.97, 95% CI 1.16 to 3.93, $p = 0.012$) and substantial LVSI (HR 3.92, 95% CI 2.09 to 7.37, $p < 0.0001$).

3.3. MELF invasion in the NSMP EC subclass

We found that all NSMP EC with MELF invasion ($n = 95$) were low-grade, ER positive tumors, and all but one had deep myometrial invasion, see Table 3. Also, more substantial LVSI was observed (1.9% vs. 9.8%, $p = 0.001$) The majority (92.2%) of MELF invasion positive NSMP cases were *CTNNB1* wild type, in comparison to 67.8% in the MELF invasion negative subgroup. NSMP MELF invasion positive cases showed slightly more often *KRAS* aberrations, yet this difference did not reach statistical significance.

A total of 53 recurrences were registered among patients with NSMP EC, of which only 7 had occurred in MELF invasion positive cases. As a result, the 5-year risk of recurrence for NSMP EC with MELF invasion positivity was only 4.3% compared to 9.5% in NSMP EC without MELF invasion; this difference was not statistically significant ($p = 0.23$, see Fig. 3). The prognostic value of MELF invasion within NSMP remained non-significant after correction for the main prognostic factors (HR 0.96, 95% CI 0.40 to 2.31, $p = 0.94$), see Supplementary S4.

3.4. TCGA explorative analyses

Analyses in the TCGA Uterine Corpus Endometrial Carcinoma cohort, identified 8 MELF invasion positive cases (2.7%). Similar results were

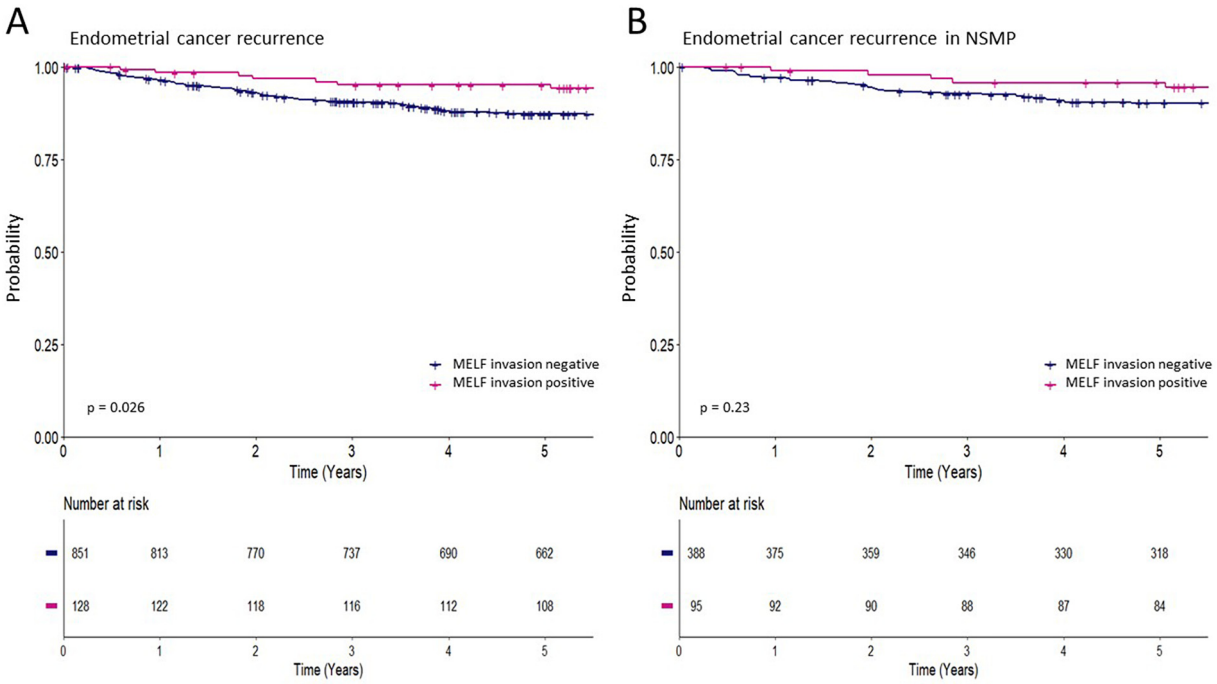

Fig. 3. Endometrial cancer recurrence in MELF invasion negative and MELF invasion positive cases in (A) all cases and in (B) NSMP cases.

Table 2
Multivariable analysis of risk factors for endometrial cancer recurrence.

Parameter	Total number	HR	95% CI	p-value
Grade				
1–2	646			
3	96	2.26	1.30 to 3.93	0.004
Stage				
IA	206			
≥IB	536	1.97	1.16 to 3.93	0.012
LVSI				
None or focal	710			
Substantial	32	3.92	2.09 to 7.37	<0.0001
TCGA				
MMRd	207			
*POLE*mut	48	0.49	0.15 to 1.64	0.25
p53 abnormal	61	2.42	1.23 to 4.76	0.010
NSMP	426	0.71	0.42 to 1.19	0.19
L1CAM overexpression				
≤10% (negative)	671			
>10% (positive)	51	2.6	1.36 to 4.97	0.07
ER expression				
≤10% (negative)	33			
>10% (positive)	691	0.94	0.44 to 2.03	0.09
CTNNB1				
Wild type	601			
Mutation	141	1.65	0.93 to 2.93	0.09
MELF invasion				
Negative	642			
Positive	100	0.65	0.29 to 1.48	0.30

CI, confidence interval; ER, estrogen receptor; HR, hazard ratio; LVSI, lymph-vascular space invasion; L1CAM, L1-cell adhesion molecule; MELF, microcystic elongated fragmented pattern of invasion; MMRd, mismatch repair deficiency; NSMP, no-specific-molecular-profile; TCGA, The Cancer Genome Atlas.

observed; MELF invasion positive cases were predominantly of endometrioid histology, low-grade, and consistent in *CTNNB1* and *KRAS* mutational status.

4. Discussion

In this study, we investigated MELF invasion in a pooled analysis of 979 early-stage EC of the PORTEC-1 and -2 trials. MELF invasion was found in 13.1% of early-stage low-grade, mainly endometrioid EC. Semi-quantification of MELF invasion in focal and multifocal had no added value. The previously reported associations of MELF invasion with deep myometrial invasion and LVSI were confirmed [7,10,15]. At univariate analysis, MELF invasion was associated with a lower risk of recurrence. However, in multivariable analysis MELF invasion was not an independent prognostic factor, and this lower risk of recurrence with MELF invasion stems by its association with low-grade histology and other favorable factors. This was in line with previous findings [7,15,18].

Previous studies on MELF invasion have given a wide range of estimates on its incidence, 5.8% to 48% [7,10–18]. Our analyses, which are based on a large number of patients, high quality data of two randomized controlled trials and a robust MELF invasion assessment, give an estimation of the incidence of MELF invasion in early-stage EC: 13.1% (95% CI, 11.0–15.3%). Yet, only 2.7% of the EC in the external TCGA validation dataset had MELF invasion. This could be explained by the strong correlation of MELF invasion with low-grade tumors (97.7% in our dataset), which were less common in the TCGA validation set (40.8%). Additionally, the PORTEC-1 and -2 inclusion criteria were restricted to endometrioid EC, while in the TCGA 24.6% of ECs were non-endometrioid. Lastly, the TCGA dataset also contained images with a lack of sufficient myometrium wall, due to which correct MELF invasion assessment could not be done in these particular cases.

Another reason for the varying reported incidence of MELF invasion could be the confusion with the presence of LVSI. It is well accepted that MELF invasion is not only associated with LVSI, but that MELF invasion can also mimic LVSI. Due to the flattening of the epithelium of the MELF glands, it may be seen as vascular endothelium and lead to the perception that instead of a microcyst, LVSI is observed [30,31]. It is important to point out, that in contrast to MELF invasion, substantial LVSI is a known risk factor for especially lymph node metastasis and a higher

Table 3
Clinicopathological characteristics of NSMP endometrial cancer with MELF invasion.

	Total ($n = 483$)	No MELF invasion ($n = 388$)	MELF invasion ($n = 95$)	p-value
Age				0.19
Mean (range)	68 (41–90)	68 (41–90)	70 (50–82)	
Histotype				1.00
Endometrioid	476 (98.5%)	382 (98.5%)	94 (98.9%)	
Non-endometrioid	7 (1.5%)	6 (1.5%)	1 (1.1%)	
Grade				<0.0001
1–2	446 (92.3%)	351 (90.5%)	95 (100%)	
3	37 (7.7%)	37 (9.5%)	0 (0%)	
Stage				<0.0001
IA	115 (23.8%)	114 (29.4%)	1 (1.1%)	
≥IB	368 (76.2%)	274 (70.6%)	94 (98.9%)	
LVSI				0.001
None or focal	444 (96.5%)	361 (98.1%)	83 (90.2%)	
Substantial	16 (3.5%)	7 (1.9%)	9 (9.8%)	
L1CAM overexpression				0.14
≤10% (negative)	442 (96.1%)	350 (95.4%)	92 (98.9%)	
>10% (positive)	18 (3.9%)	17 (4.6%)	1 (1.1%)	
ER expression				0.14
≤10% (negative)	12 (6.7%)	12 (3.4%)	0 (0%)	
>10% (positive)	438 (97.3%)	345 (96.6%)	93 (100%)	
CTNNB1				<0.0001
Wild type	338 (72.5%)	255 (67.8%)	83 (92.2%)	
Mutant	128 (27.5%)	121 (32.2%)	7 (7.8%)	
KRAS				0.09
Wild type	382 (82.0%)	314 (83.5%)	68 (75.6%)	
Mutant	84 (18.0%)	62 (16.5%)	22 (24.4%)	

ER, estrogen receptor; LVSI, lymph-vascular space invasion; L1CAM, L1-cell adhesion molecule; MELF, microcystic elongated fragmented pattern of invasion; NSMP, no-specific-molecular-profile.

risk for recurrence [31,32]. Distinguishing LVSI from MELF invasion is essential, as presence of substantial LVSI changes recommended adjuvant treatment strategy in early-stage EC [33]. Mistaking LVSI for MELF invasion could be a reason why some studies observed a negative prognostic value of MELF invasion. Although a higher incidence of lymph node metastases has been observed in (low-grade) MELF invasion positive EC cases, no negative prognostic effect of MELF invasion has been demonstrated [7,11–14,34].

This paper is one of the first to report on MELF invasion in the context of the molecular EC subgroups. A recent paper suggested that MELF invasion may be associated with a negative prognostic impact in *POLE*mut EC patients [19]. This finding could have clinical consequences as *POLE*mut EC patients have an excellent prognosis and treatment de-escalation is currently recommended [33]. However, there are two major concerns about the validity of this finding by He et al. [19] First, the *POLE*mut status in this study was not appropriately assessed, as a number of EC of other molecular subgroups were classified as *POLE*mut EC based on variants of unknown significance (VUS) [19,27]. Second, according to the authors, MELF invasion in *POLE*mut EC was associated with a 15.1-fold increase in tumor recurrence (95% CI 1.57 to 145.3). The extremely wide range of the confidence interval indicates overfitting of the model, which is not surprising considering the incorporation of 7 predictors in a model using a dataset containing only 4 events. In our dataset, we had almost twice as many cases with pathogenic *POLE* mutations available ($n = 62$). Among those, we observed only two MELF invasion positive cases and neither had a disease recurrence. It is therefore unlikely that MELF invasion truly is a negative prognostic factor in *POLE*mut EC. We acknowledge that defining prognostic markers in *POLE*mut EC is difficult because of its rarity and the low number of events, and recommend cautious interpretation and external validation to prevent misinformation.

Significantly more MELF invasion positive cases were found within the NSMP subgroup (85.6%), and particularly NSMP EC without mutations in *CTNNB1* (92.2% vs 7.8%, $p < 0.0001$). This association of MELF invasion with *CTNNB1* wild type has been previously reported, and the absence of MELF invasion was positioned as one the most sensitive predictors of *CTNNB1* mutations [35]. Mutually exclusivity of *CTNNB1* and *KRAS* mutations have been suggested [20]. In colorectal cancer, RAS signaling has been associated with tumor budding, which is suspected to be the morphological initiation of EMT and could be comparable to MELF invasion in EC, since both histological entities have parallel EMT-related findings [36]. A non-statically significant association between *KRAS* aberrations and MELF invasion has been previously reported [6]. In our study *KRAS* mutations were indeed more frequently observed in MELF invasion positive cases, although there was no mutual exclusivity. It is conceivable that MELF invasion is prone to arise in the context of a NSMP EC with activated RAS-pathway.

In early descriptions of MELF invasion it was thought to be a tumor degenerative process resulting from tumor-stroma interaction at the invasive front of EC [1,10]. However, this concept has evolved and MELF invasion is now thought to represent a morphological substrate of locally activated EMT pathways rather than degeneration. This is supported by immunophenotypic studies, showing local upregulation of EMT markers such as L1CAM and downregulation of ER and E-cadherin [2–7]. As EMT-activation is highly associated with aggressive tumor behavior in many tumor types, MELF invasion subsequently has also been proposed as a marker for poor clinical outcome [37]. The lack of an association between MELF invasion and tumoral L1CAM overexpression may be viewed as counterintuitive, however this is explained by the fact that MELF invasion is a localized, instead of a diffuse, process. In support of this, also other studies did not observe a significant association between EMT and tumoral L1CAM overexpression [18,38]. MELF invasion may still represent a very localized form of EMT, however our study shows this does not impact prognosis, as also found by others [7,10–15,18]. Recently MELF invasion has also been studied in ovarian endometrioid and endocervical carcinomas; also without independent association with survival [39,40]. Viewed from this lack of prognostic relevance, MELF foci may be indeed better be seen as a local degenerative process rather than EMT.

Strengths of this study are the use of the largest dataset to date from 2 randomized trials. The prospective, uniform, high quality data with complete long-term follow-up is unique in the MELF invasion literature [23,24]. All included cases have been reviewed by expert gynecologic pathologists, and determination of the molecular profiles has been

performed according to the WHO2020 guideline [21]. A limitation of our study is that only a single representative H&E-stained histological slide per case could be used for assessment of MELF invasion. The true incidence of MELF may be slightly higher since not all H&E slides were available for MELF assessment within our study. Nevertheless, our observed incidence is in line with other reported incidences on MELF [10,12,14,18]. Secondly, our reported incidence and relevance of MELF invasion may be limited to early-stage endometrioid type EC, without known lymph nodes.

In conclusion, this study demonstrated that MELF invasion is not a prognostic marker for recurrence in early-stage endometrioid EC. It is more frequently observed in low-grade NSMP tumors, without *CTNNB1* mutations, that regularly harbor *KRAS* mutations. Also within the NSMP subgroup, MELF invasion is not a candidate for further prognostic refinement. Routine assessment of MELF invasion has no clinical implications and is not recommended.

Supplementary data to this article can be found online at https://doi.org/10.1016/j.ygyno.2022.06.027.

Credit authorship contribution statement

A.S.V.M. van den Heerik: Conceptualization, Methodology, Formal analysis, Investigation, Writing – original draft. **K.T.S. Aiyer:** Methodology, Investigation, Writing – review & editing. **E. Stelloo:** Writing – review & editing. **I.M. Jürgenliemk-Schulz:** Writing – review & editing. **L.C.H.W. Lutgens:** Writing – review & editing. **J.J. Jobsen:** Writing – review & editing. **J.W.M. Mens:** Writing – review & editing. **E.M. van der Steen-Banasik:** Writing – review & editing. **C.L. Creutzberg:** Conceptualization, Methodology, Writing – review & editing. **V.T.H.B.M. Smit:** Conceptualization, Methodology, Investigation, Writing – review & editing. **N. Horeweg:** Conceptualization, Methodology, Formal analysis, Writing – review & editing. **T. Bosse:** Conceptualization, Methodology, Investigation, Writing – review & editing.

Declaration of Competing Interest

ASVMvdH, NH and CLC report a grant from the Dutch Cancer Society (unrelated). NH reports grant from Varian (unrelated), CLC reports grants from Varian (unrelated) and Elekta (non-financial, unrelated), IDMC membership Merck (unrelated) and chair Endometrial committee CGIG.

References

[1] S.K. Murray, R.H. Young, R.E. Scully, Unusual epithelial and stromal changes in myoinvasive endometrioid adenocarcinoma: a study of their frequency, associated diagnostic problems, and prognostic significance, Int. J. Gynecol. Pathol. 22 (4) (2003) 324–333.

[2] C.J. Stewart, M.L. Crook, Y.C. Leung, M. Platten, Expression of cell cycle regulatory proteins in endometrial adenocarcinoma: variations in conventional tumor areas and in microcystic, elongated and fragmented glands, Mod. Pathol. 22 (5) (2009) 725–733.

[3] C.J. Stewart, L. Little, Immunophenotypic features of MELF pattern invasion in endometrial adenocarcinoma: evidence for epithelial-mesenchymal transition, Histopathology. 55 (1) (2009) 91–101.

[4] C.J. Stewart, M.L. Crook, J. Lacey, K. Louwen, Cytokeratin 19 expression in normal endometrium and in low-grade endometrioid adenocarcinoma of the endometrium, Int. J. Gynecol. Pathol. 30 (5) (2011) 484–491.

[5] C.J. Stewart, W.G. McCluggage, Epithelial-mesenchymal transition in carcinomas of the female genital tract, Histopathology. 62 (1) (2013) 31–43.

[6] C.J. Stewart, B. Amanuel, F. Grieu, A. Carrello, B. Iacopetta, KRAS mutation and microsatellite instability in endometrial adenocarcinomas showing MELF-type myometrial invasion, J. Clin. Pathol. 63 (7) (2010) 604–608.

[7] A. Kihara, H. Yoshida, R. Watanabe, K. Takahashi, T. Kato, Y. Ino, et al., Clinicopathologic association and prognostic value of microcystic, elongated, and fragmented (MELF) pattern in endometrial Endometrioid carcinoma, Am. J. Surg. Pathol. 41 (7) (2017) 896–905.

[8] J.P. Thiery, H. Acloque, R.Y. Huang, M.A. Nieto, Epithelial-mesenchymal transitions in development and disease, Cell. 139 (5) (2009) 871–890.

[9] E. Colas, N. Pedrola, L. Devis, T. Ertekin, I. Campoy, E. Martínez, et al., The EMT signaling pathways in endometrial carcinoma, Clin. Transl. Oncol. 14 (10) (2012) 715–720.

[10] C.J. Stewart, B.A. Brennan, Y.C. Leung, L. Little, MELF pattern invasion in endometrial carcinoma: association with low grade, myoinvasive endometrioid tumours, focal mucinous differentiation and vascular invasion, Pathology. 41 (5) (2009) 454–459.

[11] J.D. Hertel, P.C. Huettner, J.D. Pfeifer, Lymphovascular space invasion in microcystic elongated and fragmented (MELF)-pattern well-differentiated endometrioid adenocarcinoma is associated with a higher rate of lymph node metastasis, Int. J. Gynecol. Pathol. 33 (2) (2014) 127–134.

[12] A.S. Joehlin-Price, K.E. McHugh, J.A. Stephens, Z. Li, F.J. Backes, D.E. Cohn, et al., The microcystic, elongated, and fragmented (MELF) pattern of invasion: a single institution report of 464 consecutive FIGO grade 1 endometrial endometrioid adenocarcinomas, Am. J. Surg. Pathol. 41 (1) (2017) 49–55.

[13] E. Euscher, P. Fox, R. Bassett, H. Al-Ghawi, R. Ali-Fehmi, D. Barbuto, et al., The pattern of myometrial invasion as a predictor of lymph node metastasis or extrauterine disease in low-grade endometrial carcinoma, Am. J. Surg. Pathol. 37 (11) (2013) 1728–1736.

[14] K. Pavlakis, I. Messini, T. Vrekoussis, T. Panoskaltsis, D. Chrysanthakis, P. Yiannou, et al., MELF invasion in endometrial cancer as a risk factor for lymph node metastasis, Histopathology. 58 (6) (2011) 966–973.

[15] I. Espinosa, N. Serrat, G.F. Zannoni, R. Rovira, E. D'Angelo, J. Prat, Endometrioid endometrial carcinomas with microcystic, elongated, and fragmented (MELF) type of myoinvasion: role of immunohistochemistry in the detection of occult lymph node metastases and their clinical significance, Hum. Pathol. 70 (2017) 6–13.

[16] D.A. Zinovkin, M.Z.I. Pranjol, D.R. Petrenyov, E.A. Nadyrov, O.G. Savchenko, The potential roles of MELF-pattern, microvessel density, and VEGF expression in survival of patients with Endometrioid endometrial carcinoma: a Morphometrical and Immunohistochemical analysis of 100 cases, J. Pathol. Transl. Med. 51 (5) (2017) 456–462.

[17] M. Sanci, K. Güngördük, V. Gülseren, T. Karadeniz, M. Kocaer, O. Gungorduk, et al., MELF pattern for predicting lymph node involvement and survival in grade I-II endometrioid-type endometrial cancer, Int. J. Gynecol. Pathol. 37 (1) (2018) 17–21.

[18] S. Kommoss, A.D. Hartkopf, B. Krämer, A.K. Bunz, F. Grevenkamp, F. Kommoss, et al., Disseminated tumor cells are not associated with established risk factors, L1CAM immunoreactivity and outcome in endometrial carcinoma, J. Cancer Res. Clin. Oncol. 143 (11) (2017) 2183–2188.

[19] D. He, H. Wang, Y. Dong, Y. Zhang, J. Zhao, C. Lv, et al., POLE mutation combined with microcystic, elongated and fragmented (MELF) pattern invasion in endometrial carcinomas might be associated with poor survival in Chinese women, Gynecol. Oncol. 159 (1) (2020) 36–42.

[20] C. Kandoth, N. Schultz, A.D. Cherniack, R. Akbani, Y. Liu, H. Shen, et al., Integrated genomic characterization of endometrial carcinoma, Nature. 497 (7447) (2013) 67–73.

[21] E. Stelloo, R.A. Nout, E.M. Osse, I.J. Jürgenliemk-Schulz, J.J. Jobsen, L.C. Lutgens, et al., Improved risk assessment by integrating molecular and Clinicopathological factors in early-stage endometrial Cancer-combined analysis of the PORTEC cohorts, Clin. Cancer Res. 22 (16) (2016) 4215–4224.

[22] A. León-Castillo, S.M. de Boer, M.E. Powell, L.R. Mileshkin, H.J. Mackay, A. Leary, et al., Molecular classification of the PORTEC-3 trial for high-risk endometrial Cancer: impact on prognosis and benefit from adjuvant therapy, J. Clin. Oncol. 38 (29) (2020) 3388–3397.

[23] C.L. Creutzberg, W.L. van Putten, P.C. Koper, M.L. Lybeert, J.J. Jobsen, C.C. Warlam-Rodenhuis, et al., Surgery and postoperative radiotherapy versus surgery alone for patients with stage-1 endometrial carcinoma: multicentre randomised trial. PORTEC Study Group. Post Operative Radiation Therapy in Endometrial Carcinoma, Lancet (London, England) 355 (9213) (2000) 1404–1411.

[24] R.A. Nout, V.T. Smit, H. Putter, I.M. Jurgenliemk-Schulz, J.J. Jobsen, L.C. Lutgens, et al., Vaginal brachytherapy versus pelvic external beam radiotherapy for patients with endometrial cancer of high-intermediate risk (PORTEC-2): an open-label, non-inferiority, randomised trial, Lancet (London, England) 375 (9717) (2010) 816–823.

[25] T. Bosse, R.A. Nout, E. Stelloo, E. Dreef, H.W. Nijman, I.M. Jurgenliemk-Schulz, et al., L1 cell adhesion molecule is a strong predictor for distant recurrence and overall survival in early stage endometrial cancer: pooled PORTEC trial results, Europ. J. Cancer (Oxf. Engl. 1990) 50 (15) (2014) 2602–2610.

[26] E.E.M. Peters, A. Leon-Castillo, V. Smit, M. Boennelycke, E. Hogdall, C. Hogdall, et al., Defining substantial Lymphovascular space invasion in endometrial Cancer, Int. J. Gynecol. Pathol. May (1;41(3)) (2021) 220–226.

[27] A. Leon-Castillo, H. Britton, M.K. McConechy, J.N. McAlpine, R. Nout, S. Kommoss, et al., Interpretation of somatic POLE mutations in endometrial carcinoma, J. Pathol. 250 (3) (2020) 323–335.

[28] L. Vermij, V. Smit, R. Nout, T. Bosse, Incorporation of molecular characteristics into endometrial cancer management, Histopathology. 76 (1) (2020) 52–63.

[29] Board WCoTE, WHO Classification of Female Genital Tumours, International Agency for Research on Cancer, Lyon, 2020.

[30] A.J. Cole, C.M. Quick, Patterns of myoinvasion in endometrial adenocarcinoma: recognition and implications, Adv. Anat. Pathol. 20 (3) (2013) 141–147.

[31] T. Bosse, E.E. Peters, C.L. Creutzberg, I.M. Jurgenliemk-Schulz, J.J. Jobsen, J.W. Mens, et al., Substantial lymph-vascular space invasion (LVSI) is a significant risk factor for recurrence in endometrial cancer–A pooled analysis of PORTEC 1 and 2 trials, Europ. J. Cancer (Oxf. Engl. 1990) 51 (13) (2015) 1742–1750.

[32] K. Stålberg, M. Bjurberg, C. Borgfeldt, J. Carlson, P. Dahm-Kähler, A. Flöter-Rådestad, et al., Lymphovascular space invasion as a predictive factor for lymph node metastases and survival in endometrioid endometrial cancer - a Swedish Gynecologic Cancer Group (SweGCG) study, Acta Oncol. (Stockholm, Sweden) 58 (11) (2019) 1628–1633.

[33] N. Concin, X. Matias-Guiu, I. Vergote, D. Cibula, M.R. Mirza, S. Marnitz, et al., ESGO/ESTRO/ESP guidelines for the management of patients with endometrial carcinoma, Int. J. Gynecol. Cancer 31 (1) (2021) 12–39.

[34] G. Han, D. Lim, M.M. Leitao Jr., N.R. Abu-Rustum, R.A. Soslow, Histological features associated with occult lymph node metastasis in FIGO clinical stage I, grade I endometrioid carcinoma, Histopathology. 64 (3) (2014) 389–398.
[35] I. Ruz-Caracuel, Á. López-Janeiro, V. Heredia-Soto, J.L. Ramón-Patino, L. Yébenes, A. Berjón, et al., Clinicopathological features and prognostic significance of CTNNB1 mutation in low-grade, early-stage endometrial endometrioid carcinoma, Virchows Arch. 479 (6) (2021) 1167–1176.
[36] V. Maffeis, L. Nicolè, R. Cappellesso, RAS, cellular plasticity, and tumor budding in colorectal Cancer, Front. Oncol. 9 (2019) 1255.
[37] A. Dongre, R.A. Weinberg, New insights into the mechanisms of epithelial-mesenchymal transition and implications for cancer, Nat. Rev. Mol. Cell Biol. 20 (2) (2019) 69–84.
[38] D. de Freitas, F.N. Aguiar, C. Anton, C.E. Bacchi, J.P. Carvalho, F.M. Carvalho, L1 cell adhesion molecule (L1CAM) expression in endometrioid endometrial carcinomas: a possible pre-operative surrogate of lymph vascular space invasion, PLoS One 13 (12) (2018), e0209294.
[39] A. Goldberg, L. Hand, D. DeCotiis, N. Rosenblum, J. Chan, Microcystic, elongated, and fragmented pattern invasion in ovarian Endometrioid carcinoma: Immunohistochemical profile and prognostic implications, Int. J. Gynecol. Pathol. 37 (1) (2018) 44–51.
[40] S.E. Segura, L. Hoang, M. Boros, C. Terinte, A. Pesci, S. Aviel-Ronen, et al., Clinicopathologic association and prognostic value of MELF pattern in invasive Endocervical adenocarcinoma (ECA) as classified by IECC, Int. J. Gynecol. Pathol. 39 (5) (2020) 436–442.