European Journal of Cancer (2013) 49, 3431–3441

Available at www.sciencedirect.com

SciVerse ScienceDirect

journal homepage: www.ejcancer.com

Hormone receptor loss in endometrial carcinoma curettage predicts lymph node metastasis and poor outcome in prospective multicentre trial

Jone Trovik [a,b,*], Elisabeth Wik [a,b], Henrica M.J. Werner [a,b], Camilla Krakstad [b], Harald Helland [a], Ingrid Vandenput [c], Tormund S. Njolstad [b], Ingunn M. Stefansson [d,e], Janusz Marcickiewicz [f,g], Solveig Tingulstad [h], Anne C. Staff [i], MoMaTEC study group [1], Frederic Amant [c], Lars A. Akslen [d,e], Helga B. Salvesen [a,b]

[a] Department of Obstetrics and Gynecology, Haukeland University Hospital, Bergen, Norway
[b] Department of Clinical Medicine, University of Bergen, Bergen, Norway
[c] Department of Gynecologic Oncology, UZGasthuisberg, KULeuven, Leuven, Belgium
[d] The Gade Institute, Section for Pathology, University of Bergen, Bergen, Norway
[e] Department of Pathology, Haukeland University Hospital, Bergen, Norway
[f] Department of Gynecology, Sahlgrenska Academy, University of Gothenburg, Gothenburg, Sweden
[g] Department of Obstetrics and Gynecology, Halland's Hospital Varberg, Varberg, Sweden
[h] Department of Gynecology, St. Olav's Hospital, Trondheim, Norway
[i] Department of Gynecology, Oslo University Hospital, Ulleval, Oslo, Norway

Available online 8 August 2013

KEYWORDS
Endometrial cancer
Biomarker
Curettage
Hormone receptors
Lymph node metastases
Prognosis

Abstract ***Background:*** Preoperative histologic examination of tumour tissue is essential when deciding if endometrial cancer surgery should include lymph node sampling. We wanted to investigate if biomarkers could improve prediction of lymph node metastasis and outcome.

Patients and methods: Curettage specimens from 832 endometrial carcinoma patients prospectively recruited from 10 centres in the MoMaTEC trial (Molecular Markers in Treatment of Endometrial Cancer) were investigated for hormone receptor and p53 status.

Results: Eighteen per cent of tumours were double negative for oestrogen- and progesterone receptors (ER/PR loss), 24% overexpressed p53. Pathologic expression of all markers correlated with nodal metastases, high FIGO (Federation International of Gynecology and

* *Corresponding author*: Address: Department of Gynecology and Obstetrics, Haukeland University Hospital, Jonas Liesv 72, 5021 Bergen, Norway. Tel.: +47 55974200; fax: +47 55974968.
E-mail addresses: jone.trovik@helse-bergen.no, jone.trovik@med.uib.no (J. Trovik).
[1] See acknowledgments.

http://dx.doi.org/10.1016/j.ejca.2013.06.016

Obstetrics) stage, non-endometrioid histology, high grade and poor prognosis (all $P < 0.001$). ER/PR loss independently predicted lymph node metastasis (odds ratios (OR) 2.0, 95% confidence interval (CI) 1.1–3.7) adjusted for preoperative curettage histology and predicted poor disease-specific survival adjusted for age, FIGO stage, histologic type, grade and myometrial infiltration (hazard ratio (HR) 2.3, 95% CI 1.4–3.9). For lymph node negative endometrioid tumours, ER/PR loss influenced survival independent of grade.

Conclusion: Double negative hormone receptor status in endometrial cancer curettage independently predicts lymph node metastasis and poor prognosis in a prospective multicentre setting. Implementing hormone receptor status to improve risk-stratification for selecting patients unlikely to benefit from lymphadenectomy seems justified.

1. Introduction

Endometrial cancer is the most common gynaecologic malignancy in industrialised countries. Fifteen to twenty per cent of patients with presumed localised disease at primary treatment recur.[1,2] Of all patients dying from this disease, one third was initially classified as low risk for recurrence.[3] Contrasting breast cancer,[4,5] improved knowledge of molecular alterations relevant for prognostication and targeting therapies in endometrial cancer[6,7] has not been systematically incorporated to tailor therapy.[8]

Metastatic lymph nodes detected as part of staging during primary surgery, identifies patients with poor prognosis.[1,9] Routine lymph node sampling has not confirmed to contribute any survival benefit in randomised studies,[10,11] but is associated with increased complication rates.[11]

Preoperative endometrial biopsy by pipelle or curettage is the cornerstone in diagnostics of endometrial cancer and the first step of treatment algorithm planning for primary surgical treatment.[12] Still, final risk stratification of early stage disease has, until recently,[13] been based on assessing histologic subtype, grade and depth of myometrial infiltration in hysterectomy specimens.[9,12,14] Several retrospective studies support that status for oestrogen receptor (ER), progesterone receptor (PR) and the tumour suppressor p53 in primary tumours are independent prognostic markers.[8] This knowledge has not been systematically studied for implementation of individualised surgical therapy in endometrial cancer.[10,11] Instead, the treatment algorithm has moved towards more aggressive surgery including pelvic and para-aortic lymphadenectomy,[15,16] despite lack of established criteria and measures for reproducibility, sensitivity and negative predictive value for the procedure.[17] Systematic clinical implementation studies of biomarkers potential useful in surgically staged endometrial cancer patients have been called for.[8]

On this background, we have investigated if assessment of ER, PR and p53 in endometrial biopsies, could improve preoperative identification of patients with lymph node metastasis and poor prognosis in the prospective international multicentre trial MoMaTEC (Molecular Markers in Treatment of Endometrial Cancer).[18]

2. Materials and methods

In total, 1192 consenting endometrial carcinoma patients, have been prospectively recruited from 10 centres for collection of curettage specimens and clinical information between May 2001 through 2010 as previously reported and summarised in Fig. 1.[18] Distribution of clinicopathologic data is listed in Table 1. Histologic diagnosis from the routine pathology report and local tumour boards from each centre were utilised. Preoperative curettage histology reports, available for 1166 patients, were classified as low- versus high-risk; the latter including endometrioid grade 3, serous, clear cell, carcinosarcoma and undifferentiated subtypes. The 853 cases preoperatively classified as low-risk included 795 endometrioid grade 1 or 2 tumours and 58 hyperplasias with or without atypia or other, benign diagnoses later confirmed as endometrial carcinoma in hysterectomy specimens. Grading was performed both on the curettage and hysterectomy specimen according to World Health Organization (WHO) classification, based on

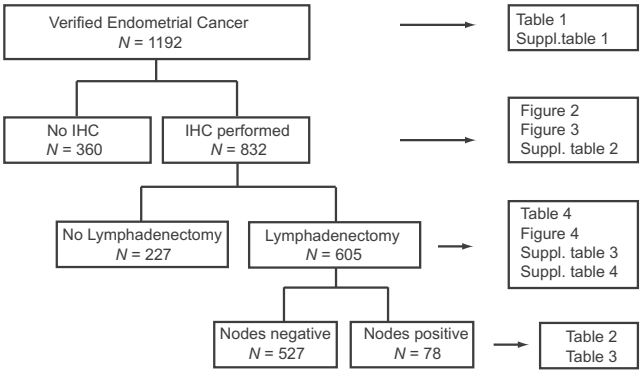

Fig. 1. Overview of patients and data available from the prospective international multicentre Molecular Markers in Treatment in Endometrial Cancer (MoMaTEC) trial with corresponding tables and figures. IHC = immunohistochemistry, N = number of cases.

Table 1
Characteristics at primary treatment of 1192 endometrial cancer patients included in the MoMaTEC* trial.

Characteristics	*N*	%
Mean age (years)	66	
Range	28–94	
Menopausal status		
Pre-/perimenopausal	120	10
Postmenopausal	1072	90
FIGO 2009 classification stage		
I	919	77
II	84	7
III	133	11
IV	56	5
Histological subtype[a]		
Endometrioid	954	80
Adenosquamous	13	1
Clear cell	46	4
Serous	107	9
Carcinosarcoma	54	5
Undifferentiated	18	2
Histological differentiation[a,b,c]		
Grade 1	458	39
Grade 2	343	29
Grade 3	143	32
Primary surgery		
Hysterectomy and oophorectomy	1147	96
Diagnostic curettage only	38	3
Palliative surgery	7	1
Lymph node sampling		
Performed	856	72
Not performed	336	28
Additional treatment[d]		
None	801	70
Radiation	134	12
Chemotherapy	151	13
Chemo radiation	56	5
Hormonal treatment	5	1
Status at last follow-up[e]		
Alive without disease	889	75
Alive with recurrent disease	89	8
Dead without disease	54	5
Dead with but not due to disease	13	1
Dead due to endometrial cancer	144	12

* MoMaTEC: Molecular Markers for Treatment of Endometrial Cancer http://www.clinicaltrials.gov/ct2/show/NCT00598845.
[a] Based on evaluation of hysterectomy specimen.
[b] Missing data in 10 cases.
[c] Data pertaining to endometrioid subtype only.
[d] One thousand one hundred and forty-seven patients subjected to hysterectomy and oophorectomy included.
[e] Data available for 1189 patients, *N* = number of patients.

percentage of solid growth and nuclear atypia. Non-endometrioid tumours were all considered as high grade.[19]

Pelvic lymph node sampling up to the aorta bifurcation was performed as part of surgical staging in 72% of the patients ($n = 856$). Para-aortic sampling was done if suspicious nodes were encountered during the operation. Median number of nodes removed was 14 (range 1–72), 10 or more lymph nodes were harvested in 625 (73%), and 105 (12%) had metastatic lymph nodes. The responsible surgeon, blinded for the biomarker study results, decided the extent of sampling, balancing preoperatively known histologic risk factors and the patient's co-morbidity. The patient group without lymph node sampling was significantly older with more superficial myometrial infiltration; otherwise not different from the sampled group (Supplementary Table 1).

Additional systemic treatment was given to 346 patients (30%), including nearly all patients with FIGO (Federation International of Gynecology and Obstetrics) stages III and IV and half of patients with less advanced stages with endometrioid grade 3 or non-endometrioid subtypes.

Follow-up information regarding recurrence and survival was retrieved as previously reported.[13] Date of last follow-up was December 10th 2012 with mean and median follow-up time for survivors 39 and 38 months (range 0–96). One hundred and forty-four patients died from endometrial carcinoma.

Curettage samples were sufficient for biomarker analyses in 832 women (70%). These were more often endometrioid compared to non-endometrioid (84% versus 76%, $P = 0.001$) and differentiated (grade 1–2, 71% versus grade 3, 64%, $P = 0.017$) as compared to tumours with insufficient tissue available. Otherwise patient and tumour characteristics (Supplementary Table 2) as well as disease-specific survival (log-rank test $P = 0.476$) were similar.

2.1. Immunohistochemistry and tissue microarray (TMA)

TMAs consisting of triplets from each patient's curettage sample were prepared as described and validated earlier.[20,21] Microwave antigen retrieval (750 W for 10 and 350 W for 15 min) in Tris–EDTA buffer pH 9 before using DAKO Autostainer (No 3400-9567), peroxidase blocking (Dako S-2032) for 5 min. and incubating with: Oestrogen Receptor α (ER) (Dako M7047) diluted 1:50, Progesterone Receptor (PR) (Dako M3569) diluted 1:150 both for 30 min, and tumour protein 53 (p53) (Dako M7001) diluted 1:1000 for 60 min. The EnVision+Mouse HRP labelled polymer secondary antibody with DAB+ (K4006) was used. Slides were counterstained with Dako Automation Haematoxylin.[20]

2.2. Evaluation of staining

Blinded for patient characteristics and outcome, slides were evaluated by two authors (J.T. and H.B.S.) using a standard light microscope. Nuclear staining was scored using a semi-quantitative staining index (range 0–9) as product of staining intensity (score 0–3) and tumour area staining positive (0 = no staining,

$1 \leqslant 10\%$, $2 = 10$–50% and $3 > 50\%$), described earlier.[20,22,23] In line with the former study,[20] lower quartile of the dataset was applied as cut-off corresponding to staining index $\leqslant 3$ for ER and 0 for PR. Pathologic expression of p53 (high) was defined as upper quartile (staining index $\geqslant 4$) in line with previous reports.[23,24] Inter-observer reproducibility was evaluated re-scoring random slides blinded for previous scoring, for 97, 104 and 76 patients respectively, yielding Kappa values of 0.91 for ER, 0.88 for PR and 0.86 for p53 stainings.

2.3. Statistical analyses

Assessing the immuno-markers' predictive value for lymph node metastasis was the primary objective, and the prognostic impact the secondary objective of the study. Disease-specific survival was defined as time from surgery to death from endometrial carcinoma. Living patients were censored at last follow-up. Recurrence-free survival was defined as time from surgery to relapse for patients considered cured by primary treatment. Statistical analyses were performed with IBM SPSS 20 program (Statistical Product and Service Solutions version 20.0, IBM, New York) using Pearson's chi-square test exploring associations between categorical variables and binary logistic regression to estimate odds ratios (OR) for lymph node metastasis. Analysis of recurrence-free survival and disease-specific survival was performed by Kaplan–Meyer method and compared using Mantel–Cox (log-rank) test and Cox' proportional hazard method, adjusting for multiple baseline characteristics found significant in the univariate model as previously reported.[23] We tested for potential interactions between variables and examined that hazard functions were proportional over time by log–log plots. All statistical tests were two-sided and considered significant if $P < 0.05$. Power calculation was done as described in earlier publication regarding the MoMaTEC Trial.[25]

2.4. Approvals

Norwegian Data Inspectorate (961478-2), Norwegian Social Science Data Services (15501) and the local Institutional Review Board (REKIII No. 052.01) approved the MoMaTEC study registered at Clinical Trials (http://www.clinicaltrials.gov/ct2/show/NCT00598845)[18] and prepared in accordance with STROBE[26] and REMARK recommendations.[27]

3. Results

3.1. Metastatic lymph nodes associate with pathologic expression of curettage biomarkers

Presence of metastatic lymph nodes was significantly associated with histologic features known to correlate

Table 2
Lymph node status in 605 endometrial cancer patients in the Molecular Markers in Treatment in Endometrial Cancer (MoMaTEC) trial subjected to lymphadenectomy in relation to clinicopathological variables and expression of biomarkers evaluated by Pearson's chi-square test.

Variables	LNneg[a] *N* (%)	LNpos[b] *N* (%)	*P*
Age			0.583
<66 years	281 (88)	39 (12)	
⩾66 years	246 (86)	39 (14)	
Histological type			<0.001
Endometrioid	450 (90)	51 (10)	
Non-endometrioid	77 (74)	27 (26)	
Histological differentiation[c]			<0.001
Grade 1–2	387 (92)	36 (9)	
Grade 3	138 (76)	42 (24)	
Myometrial infiltration[d]			<0.001
<50%	351 (98)	7 (2)	
⩾50%	145 (77)	46 (23)	
Oestrogen Receptor (ER)/Progesterone Receptor (PR)			<0.001
Normal	443 (90)	49 (10)	
Loss[e]	84 (74)	29 (26)	
p53			0.001
Normal	408 (90)	47 (10)	
Pathological	119 (79)	31 (21)	

[a] Lymph node negative (LNneg): 527 patients (87%).
[b] Lymph node positive (LNpos): 78 women (13%) with histologically confirmed metastasis to ⩾1 lymph nodes.
[c] Data available for $N = 603$.
[d] Data available for $N = 549$.
[e] Loss of both ER and PR expression, N = number of patients.

with aggressive disease; non-endometrioid subtype, grade 3 and deep myometrial infiltration (Table 2). All three investigated biomarkers showed mainly nuclear staining (Fig. 2). Loss of oestrogen receptor (ER-) staining (as opposed to positive staining in normal endometrium) was seen in 200 of 832 evaluable patient samples (24%), progesterone receptor loss (PR-) in 197 (24%) and loss of both receptors (ER-/PR-) in 151 (18%). Pathologic (high) expression of p53 was demonstrated in 197 patients (24%). Pathologic expression of all three markers was significantly associated with high age at diagnosis, advanced FIGO stage, lymph node metastasis, non-endometrioid histology and grade 3. Double negative ER/PR correlated with deep myometrial infiltration (Supplementary Table 3). Evaluating complete negative p53 (staining index = 0) as a separate category, complete loss of p53 expression turned out as an intermediate group, between low (Index 1–3) or high (Index $\geqslant$4) demonstrated in Supplementary Table 3. Percentage of lymph node metastasis was not significantly different between negative (8.5%) and low (10.6%, $P = 0.616$).

The hormone receptor stainings in curettage specimens were compared to stainings from $n = 364$ corresponding hysterectomy TMA specimens. The correlations were highly significant, all $P < 0.001$ (chi-square test), ER 84% accuracy and PR 88%. ER/PR had 89% accuracy (Supplementary Table 3).

3.2. Loss of ER and PR expression independently predicts lymph node metastasis

High-risk histology in curettage, loss of ER/PR and pathologic expression of p53, all predicted presence of metastatic lymph nodes in a univariate model (Table 3). 26% of patients (29 of 113) with combined loss of ER/PR expression had lymph node metastasis. Double loss of hormone receptors was stronger than p53 in predicting metastatic nodes with OR 3.12 (95% CI 1.87–5.22, $P < 0.001$). When adjusting for preoperative histology, p53 lost its predictive value, in contrast to ER-/PR- status being an independent predictor for lymph node metastasis with adjusted OR 2.04 (95% CI 1.12–3.70, $P = 0.02$, Table 3). Using the three category p53 variable (negative, low, high) in the logistic regression regarding lymph node metastasis did not alter the conclusion; p53 was not a significant factor while ERPR loss still had independent OR 2.1 with $P = 0.018$. For

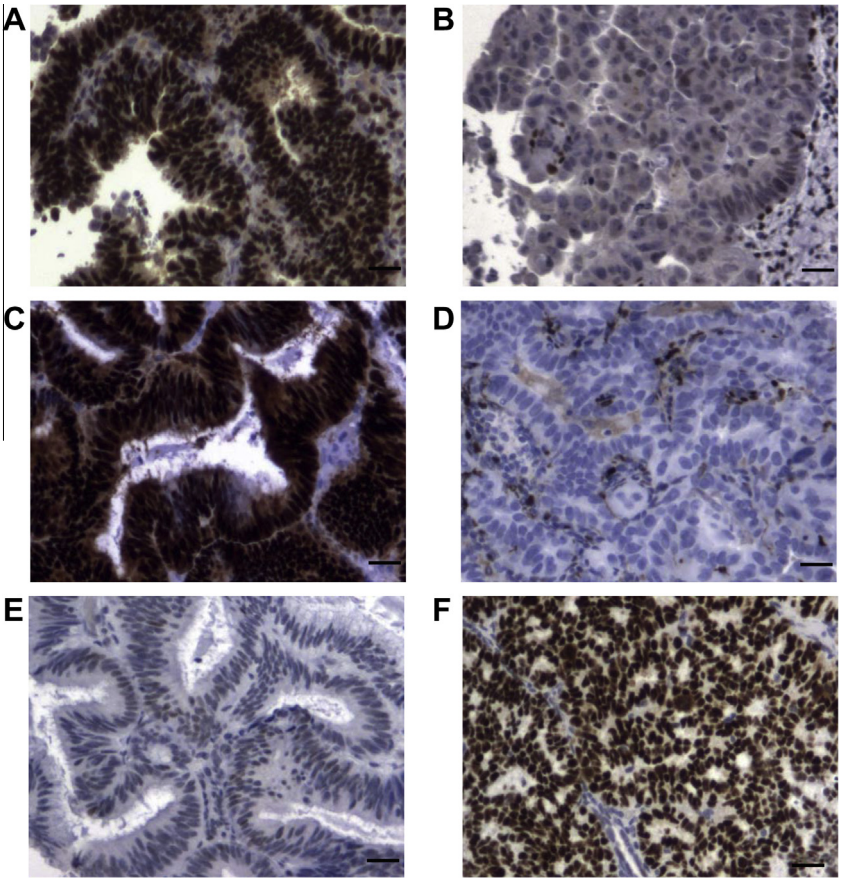

Fig. 2. Representative photomicrographs of immunohistochemical stainings for estimation of Oestrogen Receptor α (ER), Progesterone Receptor (PR) and p53; ER normal (A), ER loss (B), PR normal (C), PR loss (D), p53 normal (E), and p53 pathologic (F), each bar represent 20 μm. Final histology of tumour A and C–F were endometrioid, B was serous.

Table 3
Prediction of lymph node metastasis based on conventional curettage histology and new biomarkers for 605 lymph node sampled endometrial cancer patients in the Molecular Markers in Treatment in Endometrial Cancer (MoMaTEC) trial.

Variable	N[a]	Univariate odds ratios (OR)	95% confidence interval (CI)	P	Multivariate OR	95% CI	P	Sensitivity	Specificity	Positive predictive value	Negative predictive value
All patients											
Curettage histology				<0.001			0.016	0.43	0.80	0.24	0.91
Low-risk[b]	465	1			1						
High-risk[c]	140	3.07	1.87–5.04		2.07	1.14–3.75					
Oestrogen Receptor (ER)/Progesterone Receptor (PR) expression				<0.001			0.020	0.37	0.84	0.26	0.90
Normal	492	1			1						
Loss[d]	113	3.12	1.87–5.22		2.04	1.12–3.70					
p53 expression				0.001			0.438	0.40	0.77	0.21	0.90
Normal	455	1			1						
Pathological	150	2.26	1.38–3.72		1.26	0.70–2.27					
Low-risk patients By curettage histology[b]											
ER/PR expression				0.014			0.015	0.20	0.91	0.20	0.92
Normal	420	1	–		1	–					
Loss[d]	45	2.75	1.23–6.17		2.89	1.23–6.78					
P53 expression				0.766			0.721	0.16	0.86	0.10	0.91
Normal	398	1	–		1						
Pathological	67	1.14	0.49–2.67		0.85	0.34–2.11					
CurRisk & ER/PR expression											
Low-risk[b] & ER/PR normal	420	1									
High-risk &/or ER/PR loss[e]	185	3.33	2.05–5.42	<0.001				0.56	0.73	0.23	0.92

[a] $N = 605$ patients with data available for all variables included in uni- and multivariate logistic regression analysis.
[b] CurLow-risk: Benign, hyperplasia, endometrioid grade 1–2, $N = 465$.
[c] CurHigh-risk: Serous, clear cell, carcinosarcoma, undifferentiated carcinomas, endometrioid grade 3.
[d] Patients with double loss of ER/PR expression.
[e] Fifty-seven patients with curettage histology low risk had ER/PR loss and their final histology were Endometrioid ($N = 47$), adenosquamous ($N = 1$), clear cell ($N = 4$), serous ($N = 4$) undifferentiated ($N = 1$).

patients presumed as low-risk based on preoperative histology, loss of ER/PR expression predicted lymph node metastasis with adjusted OR 2.89 (95% CI 1.23–6.78, $P = 0.015$). Combining hormone receptor status and preoperative histology, prediction of metastatic nodes improved: The frequency of metastatic nodes was 8% for low-risk (ER/PR normal and low risk histology), and 23% for patients with either ER/PR loss or high-risk curettage histology with OR 3.33 (95% CI 2.05–5.42), $P < 0.001$, Table 3). Thus selecting for lymph node sampling if either preoperative histology is high risk or hormone receptor staining is negative will increase the sensitivity of detecting metastatic nodes to 0.56 (from 0.43 curettage or 0.37 ER/PR loss if used separately, Table 3).

Number nodes sampled correlated with detection of metastatic lymph nodes with an OR 1.04 (95% CI 1.02–1.06) tested as logistic regression. However when adding number of lymph nodes as a factor in the multivariate logistic regression model the OR of ER/PR status is still a significant independent predictor of metastatic lymph nodes with OR 2.2, $P = 0.011$. Interestingly, OR for curettage risk group based on histology was weakened when adjusted for number of nodes in the model with OR 1.8, 95% CI 0.97–3.3.

In selecting for lymph node sampling or not the histology or grade was not significantly different (Supplementary Table 2) but when sampling was performed patients with high risk histology had significantly more lymph nodes sampled ($P = 0.004$ chi-square test, using

median 14 as cut-off). Still we found no survival difference for patients subjected to lymphadenectomy when comparing patients with more than 10 nodes removed to those with less than 10 nodes removed (log-rank test $P = 0.876$).

3.3. Loss of hormone receptors independently predicts poor outcome

Pathologic p53 expression and loss of ER/PR expression, significantly predicted poor disease-specific survival (Supplementary Table 4, Fig. 3B and C), along with high age, non-endometrioid histology, high grade, high FIGO stage, deep myometrial infiltration and metastatic lymph nodes. There was 14% discrepancy between preoperative and hysterectomy based histologic risk group classification, with 79 changing to high-risk versus 35 changing to low-risk based on hysterectomy evaluation. Preoperative high risk histology identified patients with poor prognosis (Fig. 3A). A combination of preoperative histology and ER/PR status further refined the identification of poor survivors (Fig. 3D).

Double negative ER/PR showed independent prognostic impact in Cox survival analysis, adjusted for age, FIGO stage, myometrial infiltration, histologic subtype and grade assessed in hysterectomy specimens, with hazard ratio (HR) 2.28 (95% CI 1.35–3.86, $P = 0.002$, Table 4). p53 lost its independent prognostic impact in this multivariate model. Incorporating p53 as three categories (negative, low, high) in the Cox multivariate model did not alter the conclusion: p53 was not statistical independently significant while ERPR negativity retained HR 2.19 (95% CI 1.29–3.74, $P = 0.004$).

Interestingly, ER/PR status improved prediction of survival also within prognostic subgroups defined by lymphadenectomy (Fig. 4). In stratified multivariate analysis among patients without lymph node sampling, double negative ER/PR status predicted poor outcome with HR 4.15 (95% CI 1.60–10.77, $P = 0.003$) adjusted for age, FIGO stage, myometrial infiltration, histologic subtype and grade. Also for the lymph node negative endometrioid subgroup, ER/PR negative status influenced survival independently of tumour grade with HR 5.36 (95% CI 1.32–21.73, $P = 0.019$). For endometrioid grade 1–2 patients, a subgroup considered low risk for recurrence; double negative ER/PR was an independent and significant predictor of poor recurrence-free survival with HR 2.80 (95% CI 1.50–5.36, $P = 0.002$) adjusted for FIGO stage and age.

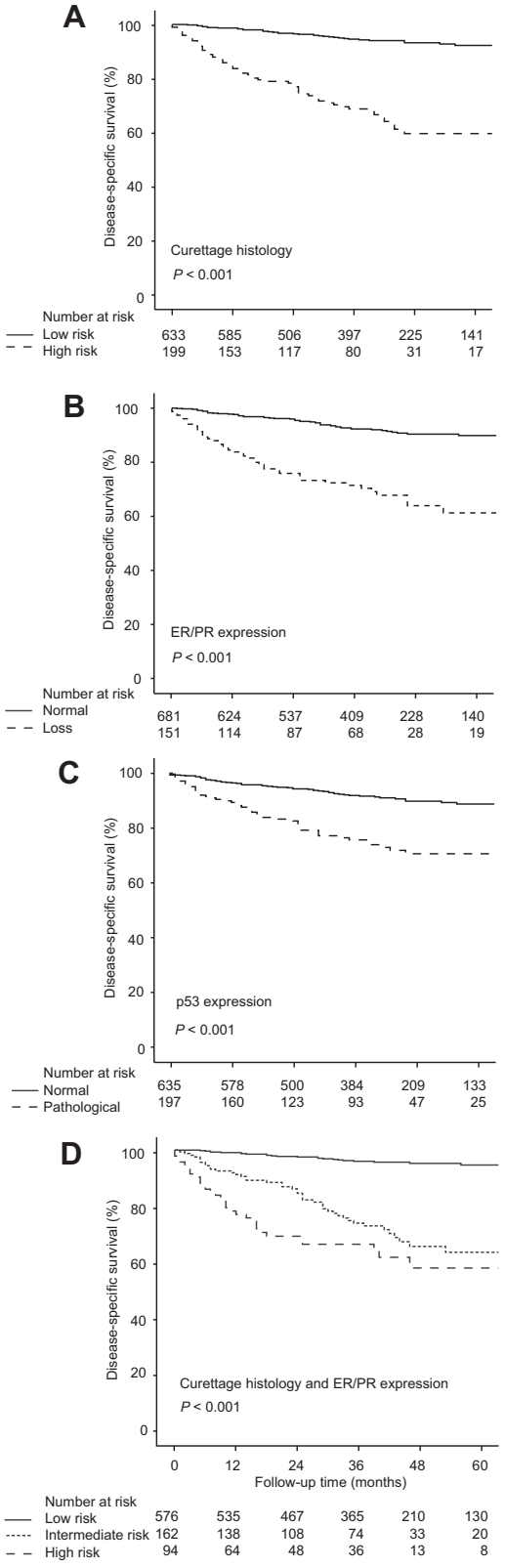

Fig. 3. Disease-specific univariate survival for endometrial carcinoma patients according to preoperative low risk (endometrioid grade 1–2) versus high risk histology (endometrioid grade 3/non-endometrioid subtypes) (A), Oestrogen Receptor (ER)/Progesterone Receptor (PR) expression (B), p53 expression (C), and combination of preoperative histology and ER/PR expression; Low risk: Curettage histology and ER/PR both low risk, Intermediate risk: Curettage histology or ER/PR high risk, High risk: Curettage histology and ER/PR both high risk (D).

4. Discussion

We report for the first time in a large prospective multicentre setting, that hormone receptor loss in preoperative endometrial carcinoma biopsies independently

Table 4
Multivariate survival analysis of endometrial cancer patients in the Molecular Markers in Treatment in Endometrial Cancer (MoMaTEC) trial according to Cox' proportional hazards regression model.

Variable	N[a]	Unadjusted hazard ratio (HR)	95% confidence interval (CI)	P	Adjusted HR	95% CI	P
Age	734	1.06	1.03–1.08	<0.001	1·04	1.02–1.07	0.001
FIGO stage				<0.001			<0.001
I–II	661	1	–		1	–	
III–IV	73	10.44	6.54–16.65		5.13	3.07–8.57	
Myometrial infiltration				<0.001			<0.001
<50%	485	1	–		1	–	
>50%	249	4.61	2.81–7.57		3.11	1.81–5.36	
Histological type[b]				<0.001			0.002
Endometrioid	635	1			1	–	
Non-endometrioid	99	5.77	3.61–9.23		3.42	1.67–7.01	
Histological grade[b]				<0.001			0.646
Grade 1–2	546	1	–		1	–	
Grade 3	188	4.66	2.92–7.45		1.19	0.57–2.46	
Oestrogen Receptor (ER)/Progesterone Receptor (PR)				<0.001			0.002
Normal	614	1	–		1	–	
Loss[c]	120	4.89	3.07–7.78		2.28	1.35–3.86	
p53				<0.001			0.531
Normal	573	1	–		1	–	
Pathological	161	3.54	2.23–5.62		0.81	0.42–1.56	

[a] $N = 734$ for cases with data available for all variables included in uni- and multivariate analyses.
[b] Based on evaluation of hysterectomy specimen.
[c] Patients with double loss of ER/PR expression.

and in addition to preoperative histology, predicts lymph node metastasis.

Lymph node sampling is widely advocated as a compulsory part of complete surgical staging, in particular for intermediate- and high-risk endometrial cancer.[12,16] No randomised trials have demonstrated any survival benefit from lymphadenectomy,[10,11,28] a procedure associated with prolonged operation time and increased complication rate[11] in an obese and co-morbid patient population.[12] Due to the relatively low frequency of lymph node metastasis and recurrence in low-risk groups, adequately powered randomised surgical trials have been difficult to conduct. Improved tools identifying patients with high risk for lymph node metastasis would reduce the required sample size in a randomised clinical trial of lymphadenectomy, while avoiding potential unnecessary side effects from sampling low-risk patients. Standard preoperative histology carefully assessed to identify patients with high and low risk for aggressive disease should always be the first step in a treatment algorithm. But by applying a combination of preoperative histology and ER/PR status in curettage, we were able to define 69% of the patients as low-risk with only 8% risk of lymph node metastasis but 95% 5-year disease specific survival.

Non-endometrioid subtypes, endometrioid grade 3 and deep myometrial infiltration in the excised uterus have consistently demonstrated to predict lymph node metastasis,[1,29] in line with our findings. Several methods evaluating myometrial infiltration pre- or perioperatively exist, with variation in reported accuracy for detection of deep infiltration; ranging from 67% to 84% for vaginal ultrasound,[30,31] 47% to 100% for MRI,[32] 87% for gross intra-operative inspection[30] and perioperative frozen section evaluation.[33]

The 14% discrepancy of preoperative versus final histologic subtype and grade in our study, when dichotomised as high- versus low-risk, is well in line with others ranging from 15% to 32%.[33–35] Assessment of histologic type and grade in preoperative specimens is considered less reliable compared to hysterectomy evaluation, and 31% verified as high-risk patients based on hysterectomy histopathology was missed preoperatively, illustrating the clinical need for improved tools identifying high-risk cases. Tumour tissue in curettage specimens/preoperative office biopsies are normally more scant than tumour tissue available for evaluation from the removed uterus. Still the sample size available is unlikely to be the whole explanation for this discrepancy as we found and report in Supplementary Table 2, that the percentage of discordant histologic diagnose (10% change between endometrioid or non-endometrioid histologic type, 14% change between low or high-risk histology (incorporating grade)) was not significantly different whether tissue were successfully retrieved for TMA for further immunohistochemistry analysis ($n = 832$) or unsuccessfully retrieved for TMA preparation ($n = 360$), the latter considered a surrogate

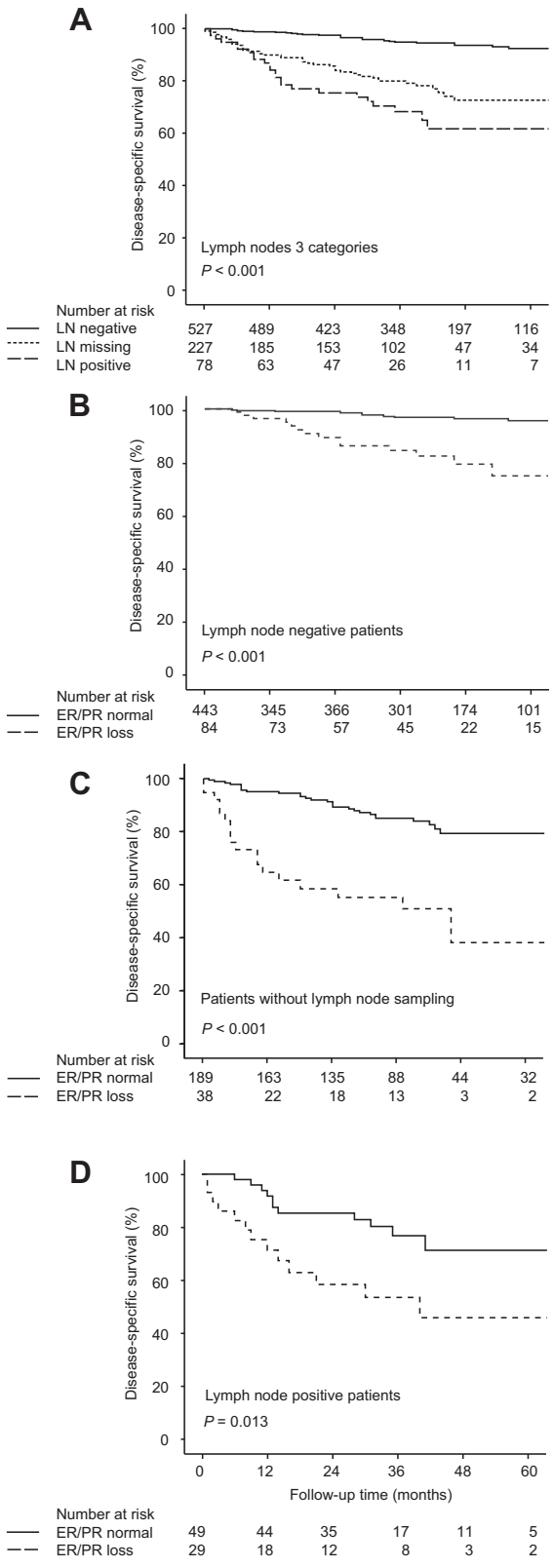

Fig. 4. Disease-specific univariate survival for endometrial carcinoma patients according to lymph node status (A), Oestrogen Receptor (ER)/Progesterone Receptor (PR) expression stratified for lymph node status; lymph nodes negative (LNneg) patients (B), not lymph node sampled (LNmissing) patients (C), positive lymph node (LNpos) patients (D).

marker for more scant tissue availability in preoperatively collected tissue.

We have previously reported[36] that discordant histology in preoperative and post-operative assessments represent a group of patients with a distinct survival/prognosis, significantly different from and intermediate between those with concordant low risk curettage and hysterectomy histology and those with concordant high risk features. This may be related to tumour heterogeneity. Curettage specimens, investigated in this study, probably reflect mostly the part of tumour protruding in the uterine cavity. Deeper parts of the tumour may have different histologic and molecular findings that will not necessarily be reflected in curettage specimens. Although tumour heterogeneity has not been systematically studied, data regarding correlation between discordant and concordant histology assessments and the immunohistochemical biomarkers (Supplementary Table 3) demonstrate that pathologic biomarker expressions are significantly higher in the group with discordant histology. This also suggests that biomarkers may aid in identifying this group of patients with poorer prognosis.

Receptor status and p53 expression have been evaluated as significant predictors of endometrial cancer survival in several retrospective studies.[37] A case–control study investigating curettings from 76 patients found p53 as independent predictor of metastatic nodes.[38] ER or PR status, not determined in their study, was a stronger predictor of lymph node metastasis and prognosis in our larger prospective study. Immunohistochemical expression of ER, PR and p53 has been evaluated in retrospective series to correlate well between curettage and final hysterectomy specimen,[20,24] supporting preoperative staining as representative for status in tumour. Also in our series the hormone receptor status correlated well between curettage and hysterectomy specimens. The correlation for hormone receptor status in full sections versus TMA has earlier been studied comprehensively for breast cancer specimens, demonstrating a good concordance of 97% for ER staining and 98% for PR staining.[39]

Of particular clinical relevance is our finding that double negative ER/PR significantly adds predictive and prognostic information for patients in the lowest risk group; with endometrioid grade 1 or 2 tumours subjected to lymphadenectomy, and also for patients without lymph node sampling, thus providing important information when addressing need for adjuvant therapy.

When evaluating cut-points, using lower quartiles, this corresponded to staining index 0–3 for ER and 0 for PR. The same cut-offs have been found in earlier data sets published from our group.[20,24] In breast cancer where receptor negativity has been incorporated in treatment algorithms for several years there are large studies using 10% of cells staining positive as upper lim-

its for receptor negativity, which would correspond with staining index 3 (strong staining in less than 10%).[40] Thus further studies for standardization of assessments and cut points for new endometrial cancer biomarkers for implementation in the clinic will be important.

Contrasting the treatment algorithms for breast cancer,[4] and despite several studies demonstrating a strong link between hormone receptor status and clinical phenotype in endometrial cancer,[41] biomarkers including ER and PR receptor status is still not routinely used to tailor endometrial cancer treatment. Even in five out of six randomised controlled trials regarding hormonal treatment, this information is not incorporated in the patient stratification.[42] Our prospective multicentre study demonstrates ER/PR status as an independent factor predicting lymph node metastasis as well as survival. Such classification has recently also been demonstrated of relevance for targeting systemic therapies in a metastatic setting.[41]

This study was not designed to evaluate the role of lymphadenectomy in the treatment of endometrial cancer but offers an alternative approach with "molecular staging" of patients as a supplement to the well-established surgical staging for risk stratification. Based on our presented data we suggest that hormone receptor status in the future is integrated in randomised clinical trials of surgical and systemic therapies. ER/PR status in preoperative biopsies in addition to histopathologic classification improves the identification of low- and high-risk patients. A better preoperative differentiation of patients may also allow the allocation of those with low-risk and localised tumours for treatment at local hospitals while the more aggressive cancers can be handled adequately at more specialised centres.[43]

Funding

This work was supported by Helse Vest Research Fund; Norwegian Research Council; the University of Bergen Meltzer Foundation; and the Norwegian Cancer Society (The Harald Andersen's legacy). Frederic Amant is senior researcher for the Research Fund-Flanders (F.W.O.). The funding sponsors had no role in the study design, collection, analysis or interpretation of data, nor in writing the report.

Notes

Institutions participating in the MoMaTEC trial: *Norwegian centers:* Haukeland University Hospital, St. Olav's Hospital, Oslo University Hospital, Ulleval, Akershus University Hospital, Haugesund Hospital, Hospital of Vestfold, Førde Hospital, Ålesund Hospital; *International centers:* University Hospital Gasthuisberg Leuven, Belgium and Sahlgrenska Academy, Sweden.

Conflict of interest statement

None declared.

Acknowledgements

We thank Bendik Nordanger, Gerd Lillian Hallseth, Britt Edvardsen, Mari Kyllesø Halle, Pål Christian Njølstad and Erlend Njølstad for technical assistance, and biostatistician Geir Egil Eide for power calculation. We also thank participants responsible for recruiting patients at the other MoMaTEC (Molecular Markers in Treatment of Endometrial Cancer) sentres: Marie E. Engh, Klaus Oddenes, Jan A. Rokne, Jostein Tjugum and Margaret S. Lode.

Appendix A. Supplementary data

Supplementary data associated with this article can be found, in the online version, at http://dx.doi.org/10.1016/j.ejca.2013.06.016.

References

1. Morrow CP, Bundy BN, Kurman RJ, et al. Relationship between surgical–pathological risk factors and outcome in clinical stage I and II carcinoma of the endometrium: a Gynecologic Oncology Group study. *Gynecol Oncol* 1991;**40**(1):55–65.
2. Abeler VM, Kjorstad KE. Endometrial adenocarcinoma in Norway. A study of a total population. *Cancer* 1991;**67**(12):3093–103.
3. Bray F. *Cancer in Norway 2008 report*. Oslo: Cancer Registry of Norway; 2009.
4. EBCTCG EBCTCG. Effects of chemotherapy and hormonal therapy for early breast cancer on recurrence and 15-year survival: an overview of the randomised trials. *Lancet* 2005;**365**(9472):1687–717.
5. Chang HR. Trastuzumab-based neoadjuvant therapy in patients with HER2-positive breast cancer. *Cancer* 2010;**116**(12):2856–67.
6. Dedes KJ, Wetterskog D, Ashworth A, Kaye SB, Reis-Filho JS. Emerging therapeutic targets in endometrial cancer. *Nat Rev Clin Oncol* 2011;**8**(5):261–71.
7. Salvesen HB, Carter SL, Mannelqvist M, et al. Integrated genomic profiling of endometrial carcinoma associates aggressive tumors with indicators of PI3 kinase activation. *Proc Natl Acad Sci U S A* 2009;**106**(12):4834–9.
8. Salvesen HB, Haldorsen IS, Trovik J. Markers for individualised therapy in endometrial carcinoma. *Lancet Oncol* 2012;**13**(8):e353–61.
9. Mariani A, Dowdy SC, Keeney GL, Long HJ, Lesnick TG, Podratz KC. High-risk endometrial cancer subgroups: candidates for target-based adjuvant therapy. *Gynecol Oncol* 2004;**95**(1):120–6.
10. Kitchener H, Swart A, Qian Q, Amos C, Parmar M. Efficacy of systematic pelvic lymphadenectomy in endometrial cancer (MRC ASTEC trial): a randomised study. *Lancet* 2009;**373**(9658):125–36.
11. Panici Benedetti P, Basile S, Maneschi F, et al. Systematic pelvic lymphadenectomy vs no lymphadenectomy in early-stage endometrial carcinoma: randomized clinical trial. *J Natl Cancer Inst* 2008;**100**(23):1707–16.
12. Amant F, Moerman P, Neven P, Timmerman D, Van Limbergen E, Vergote I. Endometrial cancer. *Lancet* 2005;**366**(9484):491–505.

13. Werner HM, Trovik J, Marcickiewicz J, et al. A discordant histological risk classification in preoperative and operative biopsy in endometrial cancer is reflected in metastatic risk and prognosis. *Eur J Cancer* 2013;**49**(3):625–32.
14. Bokhman JV. Two pathogenetic types of endometrial carcinoma. *Gynecol Oncol* 1983;**15**(1):10–7.
15. Todo Y, Kato H, Kaneuchi M, Watari H, Takeda M, Sakuragi N. Survival effect of para-aortic lymphadenectomy in endometrial cancer (SEPAL study): a retrospective cohort analysis. *Lancet* 2010;**375**(9721):1165–72.
16. Pecorelli S. Revised FIGO staging for carcinoma of the vulva, cervix, and endometrium. *Int J Gynaecol Obstet* 2009;**105**(2):103–4.
17. Creasman WT. The current status of lymphadenectomy in the management of endometrial cancer. *Womens Health (Lond Engl)* 2011;**7**(1):33–5.
18. ClinicalTrials.gov. *Molecular Markers in Treatment in Endometrial Cancer, NCT00598845*. Bethesda: U.S. National Institutes of Health.
19. Tavasolli FA, Devillee P. (Eds.). World Health Organization Classification of Tumours. Pathology and Genetics of Tumours of the Breast and Female Genital Organs. IARC Press: Lyon, France; 2003. p. 217–232.
20. Engelsen IB, Stefansson IM, Akslen LA, Salvesen HB. GATA3 expression in estrogen receptor alpha-negative endometrial carcinomas identifies aggressive tumors with high proliferation and poor patient survival. *Am J Obstet Gynecol* 2008;**199**(5), 543.e1–7.
21. Kononen J, Bubendorf L, Kallioniemi A, et al. Tissue microarrays for high-throughput molecular profiling of tumor specimens. *Nat Med* 1998;**4**(7):844–7.
22. Aas T, Borresen AL, Geisler S, et al. Specific P53 mutations are associated with de novo resistance to doxorubicin in breast cancer patients. *Nat Med* 1996;**2**(7):811–4.
23. Salvesen HB, Iversen OE, Akslen LA. Prognostic significance of angiogenesis and Ki-67, p53, and p21 expression: a population-based endometrial carcinoma study. *J Clin Oncol* 1999;**17**(5):1382–90.
24. Engelsen IB, Stefansson I, Akslen LA, Salvesen HB. Pathologic expression of p53 or p16 in preoperative curettage specimens identifies high-risk endometrial carcinomas. *Am J Obstet Gynecol* 2006;**195**(4):979–86.
25. Trovik J, Wik E, Stefansson IM, et al. Stathmin overexpression identifies high-risk patients and lymph node metastasis in endometrial cancer. *Clin Cancer Res* 2011;**17**(10):3368–77.
26. von Elm E, Altman DG, Egger M, Pocock SJ, Gotzsche PC, Vandenbroucke JP. The Strengthening the Reporting of Observational Studies in Epidemiology (STROBE) statement: guidelines for reporting observational studies. *J Clin Epidemiol* 2008;**61**(4):344–9.
27. McShane LM, Altman DG, Sauerbrei W, Taube SE, Gion M, Clark GM. REporting recommendations for tumor MARKer prognostic studies (REMARK). *Nat Clin Pract Oncol* 2005;**2**(8):416–22.
28. May K, Bryant A, Dickinson HO, Kehoe S, Morrison J. Lymphadenectomy for the management of endometrial cancer. *Cochrane Database Syst Rev* 2010(1):CD007585.
29. Greven KM, Lanciano RM, Corn B, Case D, Randall ME. Pathologic stage III endometrial carcinoma. Prognostic factors and patterns of recurrence. *Cancer* 1993;**71**(11):3697–702.
30. Marcickiewicz J, Sundfeldt K. Accuracy of intraoperative gross visual assessment of myometrial invasion in endometrial cancer. *Acta Obstet Gynecol Scand* 2011;**90**(8):846–51.
31. Savelli L, Ceccarini M, Ludovisi M, et al. Preoperative local staging of endometrial cancer: transvaginal sonography vs. magnetic resonance imaging. *Ultrasound Obstet Gynecol* 2008;**31**(5):560–6.
32. Haldorsen IS, Salvesen HB. Staging of endometrial carcinomas with MRI using traditional and novel MRI techniques. *Clin Radiol* 2012;**67**(1):2–12.
33. Wang X, Zhang H, Di W, Li W. Clinical factors affecting the diagnostic accuracy of assessing dilation and curettage vs frozen section specimens for histologic grade and depth of myometrial invasion in endometrial carcinoma. *Am J Obstet Gynecol* 2009;**201**(2):194.e1–194.e10.
34. Lampe B, Kurzl R, Hantschmann P. Reliability of tumor typing of endometrial carcinoma in prehysterectomy curettage. *Int J Gynecol Pathol* 1995;**14**(1):2–6.
35. Frumovitz M, Singh DK, Meyer L, et al. Predictors of final histology in patients with endometrial cancer. *Gynecol Oncol* 2004;**95**(3):463–8.
36. Werner HM, Trovik J, Marcickiewicz J, et al. A discordant histological risk classification in preoperative and operative biopsy in endometrial cancer is reflected in metastatic risk and prognosis. *Eur J Cancer* 2013;**49**(3):625–32.
37. Engelsen IB, Akslen LA, Salvesen HB. Biologic markers in endometrial cancer treatment. *APMIS* 2009;**117**(10):693–707.
38. Mariani A, Sebo TJ, Katzmann JA, et al. Endometrial cancer: can nodal status be predicted with curettage? *Gynecol Oncol* 2005;**96**(3):594–600.
39. Zhang D, Salto-Tellez M, Putti TC, Do E, Koay ES. Reliability of tissue microarrays in detecting protein expression and gene amplification in breast cancer. *Mod Pathol* 2003;**16**(1):79–84.
40. Haffty BG, Yang Q, Reiss M, et al. Locoregional relapse and distant metastasis in conservatively managed triple negative early-stage breast cancer. *J Clin Oncol* 2006;**24**(36):5652–7.
41. Wik E, Raeder MB, Krakstad C, et al. Lack of Estrogen receptor alpha is associated with epithelial-mesenchymal transition and PI3Kinase alterations in endometrial carcinoma. *Clin Cancer Res* 2013;**19**(5):1094–105.
42. Kokka F, Brockbank E, Oram D, Gallagher C, Bryant A. Hormonal therapy in advanced or recurrent endometrial cancer. *Cochrane Database Syst Rev* 2010(12):CD007926.
43. Chan JK, Sherman AE, Kapp DS, et al. Influence of gynecologic oncologists on the survival of patients with endometrial cancer. *J Clin Oncol* 2011;**29**(7):832–8.