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Prognostic value of microsatellite instability (MSI) and PTEN expression in women with endometrial cancer: Results from studies of the NCIC Clinical Trials Group (NCIC CTG)

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ABSTRACT

Aim: The impact of PTEN status and microsatellite instability (MSI) on the prognosis of women with endometrial cancer is controversial. The aim of this study was to investigate MSI and PTEN expression in two patient populations using data from NCIC CTG studies.

Methods: Archival paraffin embedded tumour from women with endometrial cancer enrolled in NCIC CTG studies: EN5 (stage I/II) and IND 126, 148 and 160 (advanced/recurrent disease) were examined for MSI using BAT25/26 and for PTEN expression using immunohistochemistry. PTEN and MSI status were correlated with clinicopathologic variables and survival using data from NCIC CTG trial databases.

Results: PTEN and MSI results were available from 128 and 163 patients, respectively. MSI+ tumours were more common in women enrolled in EN5 compared to the IND studies ($p = 0.01$). PTEN negative tumours were associated with improved survival in both univariate (hazard ratio (HR) 0.55, 95% confidence interval (CI) 0.32–0.94; $p = 0.03$) and multivariate (adjusted HR 0.54, 95% CI 0.30–0.96; $p = 0.03$) analyses in women enrolled in IND studies. Microsatellite stable tumours were associated with an improved prognosis in univariate (HR 0.18, 95% CI 0.06–0.51; $p < 0.0001$) and multivariate (adjusted HR 0.16, 95% CI 0.05–0.5; $p < 0.0001$) analyses in women enrolled in EN5. There was no significant correlation between MSI and PTEN status.

Conclusions: PTEN negative tumours in women with advanced disease are associated with improved survival. MSI+ tumours are more common in early stage disease and in this group of women are associated with a worse prognosis.

1. Introduction

Endometrial cancer is the fourth most common female malignancy in North America and its incidence is rising.[1] Whilst most patients with early stage disease have a good prognosis there are a group of women who will recur. The prognosis for patients with recurrent or advanced disease at presentation, incurable by surgery or radiotherapy, is poor.[2] Several

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molecular alterations have been reported to be associated with endometrial cancer. Microsatellite instability (MSI) and mutations in PTEN are among the most common and are reported to occur predominantly in endometrioid endometrial cancer.[3]

Loss of DNA mismatch repair, either as a result of germ line mutations or epigenetic silencing, leads to an increase in frameshift mutations producing genomic instability at repeat sequences in DNA, manifest as MSI. This process potentially effects many cancer-related genes.[4,5] Colorectal tumours exhibiting MSI are associated with various clinicopathologic characteristics and a good prognosis.[6] Furthermore, in colorectal cancer, the presence of MSI may predict response to cytotoxic chemotherapy.[7] Prognostic and predictive roles for defective DNA mismatch repair and the presence of MSI are postulated for other tumour types including endometrial cancers.[8–10] MSI occurs in 20–45% of endometrial tumours, the majority being sporadic. The prognostic value of MSI in endometrial cancer is controversial with conflicting results arising from different investigators data are scarce in the recurrent/ metastatic patient population.[9–17]

Loss of PTEN function occurs in 26–80% of endometrial carcinomas and leads to deregulated phosphoinositol 3 kinase/ Akt/mTOR signalling. This leads to effects on angiogenesis, protein translation and cell cycle progression which may provide neoplastic cells with a selective survival advantage.[3] The prognostic value of PTEN mutations and loss of PTEN expression is controversial with some reporting a worse prognosis for PTEN negative tumours and others no association.[18–21] PTEN mutations are reported to be associated with MSI.[3] One study suggesting that tumours which display both present at a more advanced stage and have a worse clinical outcome.[22]

NCIC Clinical Trials Group has initiated and/or participated in a number of studies for women with endometrial cancer. Patients with stage I/II disease were enrolled into EN5 and randomized to either observation or radiation therapy.[23] IND 126, 148 and 160 were single arm, phase II studies for women with recurrent or metastatic endometrial cancer developed through the NCIC CTG Investigational New Drug Program (IND).[24–26]

The aim of this study was to investigate the relationship between PTEN and MSI status and clinicopathologic variables including overall survival in two groups of patients: those with early, FIGO stage I/II, endometrial cancer who participated in EN5 and in those women participating in IND 126, 148 and 160 for metastatic and advanced disease using data from the trial databases of NCIC CTG.

2. Materials and methods

Diagnostic archival paraffin embedded tumour samples from patients participating in closed NCIC CTG studies were obtained and examined for MSI and PTEN expression. Samples were obtained from patients with stage I/II endometrial cancer participating in EN5[23] and from patients entered into phase II IND studies with metastatic/advanced disease: IND 126 (letrozole), IND 148 (erlotinib) and IND 160 (temsirolomus).[24–26] All patients entered in these studies except IND 160 had not received chemotherapy. All these studies had full ethics board approval at each of the participating sites and patients signed written informed consent before study entry which included use of tissue samples.

2.1. *Analysis of MSI*

A pathologist identified the tumour area from a section of archival paraffin embedded tumour samples from each subject. The identified area was macrodissected from one 10 μm section of tissue and DNA was isolated using Qiagen columns. DNA was quantitated using spectrophotometry and PCR was performed for each DNA sample using BAT25 and BAT26 markers. Presence or absence of microsatellite instability was documented for each locus.

2.2. *Analysis of PTEN status*

Formalin fixed paraffin embedded tissue sections from archival surgical blocks were dewaxed in xylene and rehydrated through graded alcohol to water. Endogenous peroxidase was blocked in 3% hydrogen peroxide. Heat induced epitope retrieval was carried out in 10 mM Citrate buffer, pH 6.0 in a Milestone T/T Mega microwave oven. After blocking for endogenous biotin (Vector Laboratories) sections were incubated in primary antibody (anti-PTEN, rabbit monoclonal from Cell Signaling Technology, 1:200 dilution) for 16 h at room temperature in a moist chamber. Following washing in PBS, secondary incubations were carried out in biotin conjugated anti-rabbit IgG (Vector Laboratories) and streptavidin-HRP (ID Labs Inc.) for 30 min each. Immuno reactivities were revealed by incubation in NovaRed substrate (Vector Laboratories) for 5 min. Slides were counterstained in Mayer's haematoxylin and mounted with Permount®.

Tumour cells were scored for PTEN immunoreactivity based on predominant staining intensity and percentage of tumour cells showing staining. tumours were scored as PTEN negative when there was complete absence of nuclear or cytoplasmic staining in the tumour cells, and positive staining in stroma, lymphocytes or normal endometrial cells present in the same section.

2.3. *Statistical analysis*

Fisher's exact test was used to study the correlation between PTEN or MSI status and other patient characteristics, which included stage of disease, age, performance status, tumour grade, and histology type. The correlation of PTEN or MSI status and other patient characteristics with survival, defined as from time to randomization (for EN5 patients) or registration (for IND patients) to the date of death from any cause or last follow-up, was tested by using Cox models with each of them as single covariate in univariate analysis and with all of them as covariates in multivariate analysis. For EN5 patients only, the correlation with disease-free survival (DFS), defined as the time from disease diagnosis to first disease recurrence or death, was also analysed using the same approach. Patients alive without recurrence were censored at the time of last fellow-up in this analysis. All p-values were two-sided.

3. Results

One hundred eighty-eight tumour samples were obtained from patients enrolled in EN5 (97 patients), IND 126 (12), IND 148 (32) and IND 160 (47). PTEN immunohistochemistry results were available from 128 patients (58 from EN5 and 70 from IND studies). One hundred and sixty three patient samples (83 from EN5 and 80 from IND studies) were examined successfully for microsatellite instability. Overall, 71 (55%) of the tumour samples examined had lost PTEN expression and 32 (19.3%) were MSI+. Characteristics of patients with available PTEN or MSI results are presented by PTEN or MSI status in Table 1. The MSI+ phenotype was significantly more common in the patients from EN5 (stage I/II disease) compared to those in the IND studies ($p = 0.01$). Microsatellite instability ($p = 0.02$) but not PTEN status ($p = 0.19$) was significantly associated with endometrioid histology. There was no significant correlation between PTEN and MSI statuses ($p = 0.28$).

3.1. Prognostic significance of PTEN status

Kaplan–Meier curves for survival are shown for all patients with PTEN status data patients enrolled in EN5 (Fig. 1A) and the IND studies (Fig. 1B) by PTEN status.

In univariate analysis, including all patients with PTEN results, there was a significantly improved survival for patients with PTEN –ve tumours (hazard ratio (HR) 0.57, 95% CI 0.35–0.94; $p = 0.02$), early stage disease (EN5 versus IND patients HR 0.06, 95% CI 0.03–0.14; $p < 0.0001$), grade 1 tumour (HR 0.48, 95% CI 0.27–0.84; $p = 0.01$), endometrioid histological type (HR 0.25, 95% CI 0.14–0.45; $p < 0.0001$), and an ECOG performance status (PFS) of 0 (HR 0.58, 95% CI 0.36–0.94; $p = 0.03$). The association between PTEN status and survival was still significant after adjusting for other potential prognostic factors including age, ECOG PFS, stage, histology type and tumour grade (adjusted HR 0.52, 95% CI 0.31–0.87; $p = 0.01$). Early stage disease was the only other independent predictor of survival identified from multivariate analysis (adjusted HR 0.07, 95% CI 0.03–0.15; $p < 0.0001$).

When only EN5 patients were included in the analysis, there was no significant association between PTEN status and disease-free or overall survival found in either uni or multivariate analysis (Tables 2A and 2B). There was a trend in improved survival for patients with ECOG PFS 0 in univariate analysis (HR 0.33, 95% CI 0.10–1.09; $p = 0.06$).

For patients enrolled in IND studies, there was a statistically significant improvement in survival for patients with PTEN –ve tumours (HR 0.55, 95% CI 0.32–0.94; $p = 0.03$). The

Table 1 – Patient characteristics.

Characteristics	PTEN –ve	PTEN +ve	MSS	MSI+	*p*-Value
Stage of disease					
I/II (EN5)	33 (56.9%)	25 (43.1%)	61 (72.6%)	23 (27.4%)	PTEN, $p = 0.73$
Metastatic (IND)	38 (53.5%)	33 (46.5%)	73 (89.0%)	9 (11.0%)	MSI, $p = 0.01$
Age, years					
$\leqslant 65$	31 (57.4%)	23 (42.6%)	62 (83.8%	12 (16.2%)	PTEN, $p = 0.58$
>65	40 (54.1%)	34 (46.0%)	69 (77.5%)	20 (22.5%)	MSI, $p = 0.48$
Missing	0	1	3	0	
Mean, years (SD)	65.4 (9.9)	66.8 (9.3)	65.5 (9.3)	66.1 (8.8)	
Performance status					
0	38 (54.3%)	32 (45.7%)	71 (78.0%)	20 (22.0%)	PTEN, $p = 0.60$
1	29 (59.2%)	20 (40.8%)	51 (81%)	12 (19.1%)	MSI, $p = 0.45$
2	4 (44.4%)	5 (55.6%)	9 (100%)	0	
Missing	0	1	3	0	
Tumour grade					
1	28 (60.9%)	18 (39.1%)	46 (83.6%)	9 (16.4%)	PTEN, $p = 0.56$
2	25 (56.8%)	19 (43.2%)	43 (78.2%)	12 (21.8%)	MSI, $p = 0.50$
3	16 (45.7%)	19 (54.3%)	37 (77.1%)	11 (22.9%)	
Missing	2 (50%)	2 (50%)	8 (100%)	0 (0%)	
Histology					
Endometrioid	62 (57.9%)	45 (42.1%)	100 (76.3%)	31 (23.7%)	PTEN, $p = 0.19$
Others	9 (42.9%)	12 (57.1%)	31 (96.9%)	1 (3.1%)	MSI, $p = 0.02$
Missing	0 (0%)	1 (100%)	3 (100%)	0 (0%)	
Prior chemotherapy					
Yes	1 (1%)	1 (2%)	11 (8%)	2 (6%)	PTEN, $p = 1.00$
No	70 (99%)	56 (98%)	120 (92%)	30 (94%)	MSI, $p = 1.00$
Prior hormone therapy					
Yes	11 (15%)	9 (16%)	22 (17%)	3 (9%)	PTEN, $p = 1.00$
No	60 (85%)	48 (84%)	109 (83%)	29 (91%)	MSI, $p = 0.42$
Prior radiotherapy					
Yes	25 (35%)	18 (32%)	48 (37%)	6 (19%)	PTEN, $p = 0.71$
No	46 (65%)	35 (68%)	83 (63%)	26 (81%)	MSI, $p = 0.06$

No patients included in this analysis had received chemotherapy.

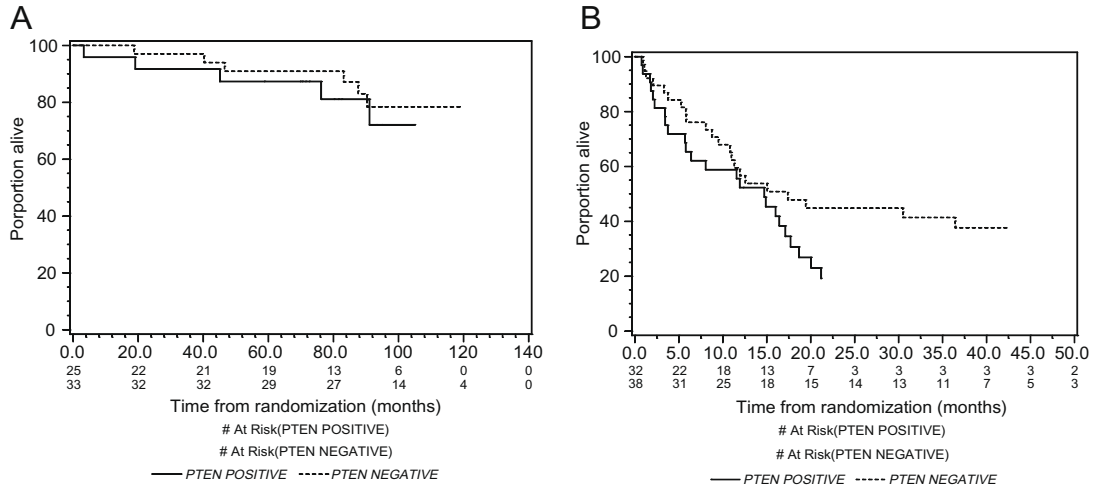

Fig. 1 – Kaplan–Meier curves for overall survival for (A) patients enrolled in EN5 and (B) patients enrolled in IND studies by PTEN status.

improvement was still significant in multivariate analysis adjusting for other prognostic factors (adjusted HR 0.54, 95% CI 0.30–0.96; $p = 0.03$; Table 2C). No other factor was found to be significantly associated with improved survival in either univariate analysis or multivariate analysis.

All Survival analyses were also performed using 'date of diagnosis' as the start time point to calculate overall survival. This did not change the significance of the results.

3.2. *Prognostic significance of MSI status*

Kaplan–Meier curves for survival are shown for all patients enrolled in EN5 (Fig. 2A) and the IND studies (Fig. 2B) by MSI status.

When all patients with MSI status results were included in the analysis, no statistically significant difference was found in survival between patients with MSS and MSI+ tumours in either univariate (HR 1.34, 95% CI 0.72–2.49; $p = 0.35$) or multivariate analyses (HR 0.67, 95% CI 0.34–1.33; $p = 0.25$). Early stage disease (EN5 versus IND patients HR 0.06, 95% CI 0.03–0.12; $p < 0.0001$), grade 1 tumour (HR 0.48, 95% CI 0.28–0.82; $p = 0.01$), endometrioid histological type (HR 0.33, 95% CI 0.20–0.54; $p < 0.0001$), and an ECOG PFS of 0 (HR 0.48, 95% CI 0.30–0.75; $p < 0.0001$) were found to be significantly associated with improved survival in univariate analysis but only early stage disease (adjusted HR 0.07, 95% CI 0.03–0.14; $p < 0.0001$) and an ECOG PFS of 0 (adjusted HR 0.54, 95% CI 0.34–0.88; $p = 0.01$) were independent predictors of improved survival in multivariate analysis.

Table 2A – Analysis of disease-free survival for patients in EN5 with available PTEN results.

Prognostic factors	Univariate analysis			Multivariate analysis	
	N	Hazard ratio[a] (95% CI)	Log-rank *p*-value	Hazard ratio[a] (95% CI)	*p*-Value from Cox regression
PTEN			0.50		0.51
Negative	32	0.72		0.73	
Positive	25	(0.28, 1.88)		(0.28, 1.89)	
Age			0.56		0.69
⩽65 years	24	0.75		0.79	
>65 years	33	(0.28, 2.02)		(0.24, 2.56)	
ECOG performance status			0.25		0.46
0	33	0.58		0.66	
1–2	24	(0.22, 1.50)		(0.22, 1.97)	
Histology			NA		NA
Endometrioid	57	NA		NA	
Others	0				
Grade			0.47		0.51
1	29	0.70		0.70	
>1	28	(0.27, 1.84)		(0.24, 2.02)	

NA = not available.
[a] Hazard ratio of first category over second category.

Table 2B – Analysis of survival for patients in EN5 with available PTEN results.

Prognostic factors		Univariate analysis		Multivariate analysis	
	N	Hazard ratio[a] (95% CI)	Log-rank p-value	Hazard ratio[a] (95% CI)	p-Value from Cox regression
PTEN			0.35		0.37
Negative	33	0.58		0.59	
Positive	25	(0.19, 1.82)		(0.19, 1.88)	
Age			0.17		0.29
⩽65 years	24	0.41		0.44	
>65 years	34	(0.11, 1.53)		(0.09, 2.00)	
ECOG performance status			0.06		0.29
0	34	0.33		0.47	
1–2	24	(0.10, 1.09)		(0.12, 1.87)	
Histology			NA		NA
Endometrioid	58	NA		NA	
Others	0				
Grade			0.19		0.17
1	30	0.47		0.40	
>1	28	(0.15, 1.51)		(0.11, 1.48)	

NA = not available.
[a] Hazard ratio of first category over second category.

Table 2C – Analysis of survival for patients in IND studies with available PTEN results.

Prognostic factors		Univariate analysis		Multivariate analysis	
	N	Hazard ratio[a] (95% CI)	Log-rank p-value	Hazard ratio[a] (95% CI)	p-Value from Cox regression
PTEN			0.03		0.03
Negative	38	0.55		0.54	
Positive	32	(0.32, 0.94)		(0.30, 0.96)	
Age			0.22		0.40
⩽65 years	30	0.71		0.77	
>65 years	40	(0.41, 1.23)		(0.43, 1.40)	
ECOG performance status			0.17		0.31
0	36	0.69		0.74	
1–2	34	(0.40, 1.18)		(0.42, 1.31)	
Histology			0.15		0.65
Endometrioid	49	0.65		0.86	
Others	21	(0.36, 1.18)		(0.44, 1.67)	
Grade			0.79		0.82
1	16	0.92		0.93	
>1	51	(0.48, 1.75)		(0.48, 1.78)	

[a] Hazard ratio of first category over second category.

When only EN5 patients were included, patients with MSS tumours had a significantly improved disease free and overall survival compared to patients with MSI+ tumours in both univariate (HR 0.24, 95% CI 0.11–0.55; $p = 0.0002$ for disease-free survival and HR 0.18, 95% CI 0.06–0.51; $p < 0.0001$ for overall survival) and multivariate (adjusted HR 0.20, 95% CI 0.09–0.49; $p = 0.0003$ for disease-free survival and adjusted HR 0.16, 95% CI 0.05–0.50; $p < 0.0001$ for overall survival) analyses (Tables 3A and 3B).

For patients enrolled in IND studies there was no significant difference in survival between patients with MSS and MSI+ tumours in uni or multivariate analyses (Table 3C). Endometrioid histological type was associated with improved survival in univariate analysis (HR 0.58, 95% CI 0.34–0.99; $p = 0.04$).

If only the patients with endometrioid histology are included in the analyses patients with MSS tumours enrolled in EN5 continue to have a significantly improved survival (adjusted HR 0.17, 95% CI 0.05–0.54; $p < 0.0001$). MSI status is not associated with survival for women with endometrioid histology enrolled in the IND studies (adjusted HR 1.58, 95% CI 0.53–4.33; $p = 0.42$) or in the overall MSI dataset ($p = 0.2$).

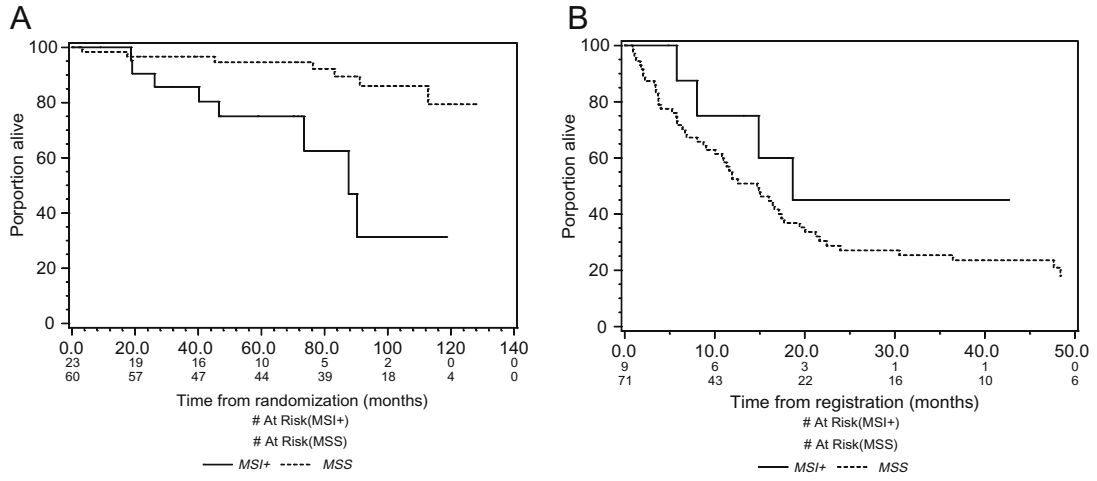

Fig. 2 – Kaplan–Meier curves for overall survival for (A) patients enrolled in EN5 and (B) patients enrolled in IND studies by MSI status.

Table 3A – Analysis of disease-free survival for patients in EN5 with available MSI results.

Prognostic factors	Univariate analysis			Multivariate analysis	
	N	Hazard ratio[a] (95% CI)	Log-rank *p*-value	Hazard ratio[a] (95% CI)	*p*-Value from Cox regression
Data set: all randomized patients					
MSI			0.0002		0.0003
MSS	60	0.24		0.20	
High	23	(0.11, 0.55)		(0.09, 0.49)	
Age			0.20		0.35
⩽65 years	38	0.58		0.65	
>65 years	45	(0.25, 1.35)		(0.26, 1.61)	
ECOG performance status			0.17		0.17
0	54	0.57		0.55	
1–2	29	(0.26, 1.28)		(0.24,1.30)	
Histology			NA		NA
Endometrioid	83	NA		NA	
Others	0				
Grade			0.99		0.61
1	38	1.00		1.25	
>1	45	(0.44, 2.23)		(0.53, 2.99)	

NA = not available.
[a] Hazard ratio of first category over second category.

Survival analyses were also performed using 'date of diagnosis' to define overall survival. There was no change of conclusion from these analyses.

4. Discussion

We are increasingly moving beyond risk stratification based on clinicopathologic variables for patients diagnosed with malignancy. Molecular characteristics with prognostic value, not only identify patient groups at risk, but potentially lead to the development of new therapeutic strategies and novel targeted agents for these patient populations. MSI and loss of PTEN expression have previously been identified as potential prognostic markers. Previous studies however have produced conflicting results.[9–21] This probably reflects the differing study designs, use of retrospective data (or institutional databases) and inclusion of mixed patient populations, in terms of histology and stage. This is the first study, to our knowledge, that has linked trial based clinicopathologic data to patient tumour samples. A classic approach to determining potential prognostic factors is to look at expression variations between early (and mostly good prognosis) versus recurrent (by definition bad prognosis) disease. We have therefore investigated two distinct patient populations: those with early, stage I/II, disease and those, who have already demonstrated they have poor prognostic disease, with recurrent or

Table 3B – Analysis of survival for patients in EN5 with available MSI results.

Prognostic factors		Univariate analysis		Multivariate analysis	
	N	Hazard ratio[a] (95% CI)	Log-rank *p*-value	Hazard ratio[a] (95% CI)	*p*-Value from Cox regression
Data set: all randomized patients					
MSI			<0.0001		<0.0001
MSS	60	0.18		0.16	
High	23	(0.06, 0.51)		(0.05, 0.50)	
Age			0.33		0.28
⩽65 years	37	0.59		0.53	
>65 years	46	(0.20, 1.72)		(0.17, 1.67)	
ECOG performance status			0.08		0.12
0	53	0.41		0.42	
1–2	30	(0.14, 1.15)		(0.14, 1.26)	
Histology			0.33		0.99
Endometrioid	78	NA		NA	
Others	5				
Grade			0.24		0.60
1	39	0.54		0.73	
>1	44	(0.19, 1.55)		(0.23, 2.33)	

NA = not available.
[a] Hazard ratio of first category over second category.

Table 3C – Analysis of survival for patients in IND studies with available MSI results.

Prognostic factors		Univariate analysis		Multivariate analysis	
	N	Hazard ratio[a] (95% CI)	Log-rank *p*-value	Hazard ratio[a] (95% CI)	*p*-Value from Cox regression
Data set: all randomized patients					
MSI			0.23		0.41
MSS	71	1.85		1.58	
High	9	(0.67, 5.12)		(0.54, 4.65)	
Age			0.84		0.99
⩽65 years	37	0.95		1.00	
>65 years	43	(0.57, 1.58)		(0.57, 1.76)	
ECOG performance status			0.05		0.09
0	38	0.60		0.63	
1–2	42	(0.36, 1.01)		(0.37, 1.07)	
Histology			0.04		0.23
Endometrioid	53	0.58		0.69	
Others	27	(0.34, 0.99)		(0.38, 1.27)	
Grade			0.82		0.90
1	16	0.93		0.96	
>1	59	(0.49, 1.75)		(0.50, 1.80)	

[a] Hazard ratio of first category over second category.

metastatic disease. This latter group in particular has been under represented in the existing studies. In order to place this in the context of earlier mixed population studies we also included some analyses using the whole data set.

We analysed MSI using two National Cancer Institute workshop recommended, consensus loci (BAT25 and BAT26) in paraffin embedded tumour samples.[27,28] Previous studies have used up to five markers (included those reported here) mostly in fresh frozen tissue and have compared tumour with normal tissue from the same patient.[9,10] However, routine MSI testing using archival paraffin blocks is considered a standard technique in colorectal studies. Moreover, the use of the two monomorphic mononucleotide markers, BAT25/26 has been shown to accurately identify high frequency microsatellite instability tumours.[29,30] The overall incidence of MSI+ tumours (19.3%) was similar to rates reported in other

studies.[9–17] Women with stage I/II disease, however, were more likely (27.4%) than those with recurrent or metastatic disease (11.0%) to have MSI+ tumours at the outset of their disease ($p = 0.01$). This differs from data reported in earlier studies including non-endometrioid histology suggesting that the MSI+ phenotype is more common in later stage disease.[11] This may reflect, however, the two patient populations that we have chosen to compare. In the study by Black et al. for example, they compared stage IA with stage IB–V tumours, with only 21% of women having stage III/IV disease.[9] Others have shown a trend towards MSI+ phenotype being more common in earlier stage disease when only patients with endometrioid histology are included.[10] Our study confirms the association between MSI and the endometrioid histological subtype. Of the patient samples examined for MSI 94% from EN5 and 66% from IND studies had endometrioid histology. If only those patients with endometrioid histology are included the difference between the incidence of MSI+ tumours (28% EN% versus 17% IND) did not reach statistical significance.

Overall, there was no difference in survival between women with MSS and MSI+ tumours. This conflicts with data presented by Black et al. which suggested an improved survival for woman with MSI+ tumours.[9] Other studies, including only endometrioid tumours, have reported no association with survival.[10] In our study, however, in women with stage I/II disease, the MSI+ phenotype was strongly associated with a worse overall survival in both uni and multivariate analyses (Table 3A, Fig. 2B), These results are consistent with an earlier report of worse disease free survival associated with MSI+ tumours in stage I/II disease in a mixed histology population.[15] The association with survival was maintained when the analyses were repeated including only patients from EN5 with endometrioid tumours. There was no association with survival seen within the advanced group of patients. Whether other prognostic factors impact more on survival than MSI status remains unclear. The advanced patients included a higher proportion of aggressive histological subtypes, however, no relationship was observed between MSI and survival when the analyses were repeated including only IND patients with endometrioid histology. Whilst the patients recruited onto the IND studies had not received prior chemotherapy the impact of chemotherapy post study cannot be discounted. Study therapy may also have implications for the prognosis of patients. Although there are theoretical reasons, at least, why MSI+ tumours might be less likely to respond to letrozole or an MTOR inhibitor.[22,31] The results were unchanged when date of diagnosis rather than date of study enrollment was used in the analyses.

The proportion (55%) of patients whose tumours lack expression of PTEN reported here is similar to that reported in the literature.[3] In this study there was no difference in loss of PTEN expression between the early and advanced patient groups and we did not observe an association between PTEN –ve tumours and endometrioid histology.[3] In early stage disease loss of PTEN expression was not associated with overall survival. This confirms the results reported by Steinbakk et al. in their population-based analysis of stage I/II EEC.[21] Elsewhere, PTEN expressing tumours are reported to have an improved prognosis in stage I disease.[18] Overall, and in patients with advanced/recurrent disease, we observed a significantly improved survival for women with PTEN –ve tumours in both uni and multianalyses (Table 2B, Fig. 1). One study reports an improved survival for patients with PTEN expressing tumours who received chemotherapy, however, this improvement was not seen in patients who just received radiotherapy.[20] Once again the effect of post study chemotherapy cannot be discounted. Furthermore, we cannot exclude an effect of the experimental therapy on prognosis particularly given that over half the recurrent patient samples came from IND 160, a study investigating the efficacy of the MTOR inhibitor, temsirolomus. However, data has previously been presented suggesting that PTEN status does not correlate with response in this study.[26] The results were unchanged when date of diagnosis rather than date of study enrollment was used in the analyses. Whether immunohistochemistry is the best way to look at inactivation of PTEN is debatable. Many studies have undertaken mutational analysis of the PTEN gene and controversy does exist over the use of immunohistochemistry and choice of antibodies.[3] Furthermore there is some evidence to suggest that even a hemizygous inactivation leading to a protein deficient state may be functionally significant when combined with abnormalities of other genes which converge downstream.[32] Further research is required in this important area of endometrial tumour biology.

tumours exhibiting MSI have been reported to be associated with a higher rates of PTEN mutation by some authors but not by others.[17,32] PTEN contains 2 mononucleotide microsatellite regions where MSI related PTEN mutations have frequently been found.[32] In a previous study, including patients with non-endometrioid histology with MSI+ tumours PTEN mutations were more common in late stage disease. The presence of mutated PTEN and the MSI+ phenotype was associated with a worse prognosis than patients with PTEN mutations alone.[22] A further study reported a trend towards a higher frequency of MSI in tumours exhibiting loss of PTEN expression assessed by immunohistochemistry.[17] In our study there was no significant association between PTEN expression and MSI status. Thirteen patients had MSI+/PTEN– tumours (4 in EN5 and 9 in IND studies) compared to 8 MSI+/PTEN+ tumours (3 in EN5 and 5 in IND studies). No survival effect was observed (data not shown) although numbers were small.

Microsatellite instability and PTEN are among the most common molecular changes seen in endometrial cancer. Linked to trial quality databases we have shown that the MSI+ phenotype is associated with a worse prognosis in women with stage I/II disease an effect which was maintained when only women with endometrioid histology were included. In women with advanced or metastatic tumours loss of PTEN expression is associated with improved survival. The interplay of molecular pathways is complex, by identifying patient populations at risk we can develop more targeted therapeutic strategies to improve the outcome for women with endometrial cancer.

Conflict of interest statement

None declared.

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