Prognostic value of PTEN loss in men with conservatively managed localised prostate cancer

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Background: The natural history of prostate cancer is highly variable and difficult to predict. We report on the prognostic value of phosphatase and tensin homologue (PTEN) loss in a cohort of 675 men with conservatively managed prostate cancer diagnosed by transurethral resection of the prostate.

Methods: The PTEN status was assayed by immunohistochemistry (PTEN IHC) and fluorescent *in situ* hybridisation (PTEN FISH). The primary end point was death from prostate cancer.

Results: The PTEN IHC loss was observed in 18% cases. This was significantly associated with prostate cancer death in univariate analysis (hazard ratio (HR) = 3.51; 95% CI 2.60–4.73; $P = 3.1 \times 10^{-14}$). It was highly predictive of prostate cancer death in the 50% of patients with a low risk score based on Gleason score, PSA, Ki-67 and extent of disease (HR = 7.4; 95% CI 2.2–24.6; $P = 0.012$)), but had no prognostic value in the higher risk patients. The PTEN FISH loss was only weakly associated with PTEN IHC loss ($\kappa = 0.5$). Both PTEN FISH loss and amplification were univariately predictive of death from prostate cancer, but this was not maintained in the multivariate analyses.

Conclusion: In low-risk patients, PTEN IHC loss adds prognostic value to Gleason score, PSA, Ki-67 and extent of disease.

Differentiation of aggressive from indolent tumours remains a high priority for the appropriate management of prostate cancer and the avoidance of unnecessary treatments and side effects in patients with indolent disease. We have investigated over 40 potentially prognostic biomarkers, including cell surface, cytoplasmic and nuclear markers and chromosomal rearrangements, for their ability to predict prostate cancer-specific survival. Although many were univariately significant, few were significant in multivariate analysis when Gleason score and baseline PSA were also included in the model (Cuzick *et al*, 2006; Attard *et al*, 2008; Berney *et al*, 2009; Foster *et al*, 2009; Kudahetti *et al*, 2009; Rajab *et al*, 2010a, b). Only an RNA-based cell cycle progression score (Cuzick *et al*, 2011, 2012), extent of disease (% chips) (Rajab *et al*, 2010a) and the immunohistochemistry (IHC) proliferation biomarker Ki-67 (Berney *et al*, 2009) provided substantial improvement on assessment of prognosis based on Gleason score and PSA alone.

Phosphatase and tensin homologue (PTEN) 10q23-24 mutations have long been associated with prostate cancer (Gray *et al*,

1998) and PTEN protein and genomic losses have been correlated with poor prognosis (McMenamin et al, 1999; Schmitz et al, 2007; Yoshimoto et al, 2007).

In combination with other genes within the PTEN tumour-suppressor pathway, IHC-detectable PTEN loss was the most significant prognostic protein in breast cancer and correlated significantly with poor patient outcome in prostate cancer and bladder cancer cohorts (Saal et al, 2007). As part of a four gene signature, PTEN expression was shown to be prognostic of PSA biochemical recurrence and lethal metastasis in a radical prostatectomy cohort (Ding et al, 2011).

The prognostic value of PTEN gene status has been suggested using an mRNA microarray signature for a range of pathways in 281 patients (Markert et al, 2011) where a cluster exhibiting a stem cell like signature and also P53 and PTEN loss had a very poor outcome. In molecular subsets based on fluorescent in situ hybridisation (FISH)-detected ERG and ETV1 gene patterns, PTEN was found to be a useful prognostic indicator of prostate cancer-specific

death (Reid et al, 2010) and biochemical recurrence (Krohn et al, 2012).

Less cumbersome and more comprehensive IHC studies have supported the value of PTEN loss in high-risk patients treated by radical prostectomy (Lotan et al, 2011) and have found PTEN to be a weakly independent prognostic factor for progression-free survival in prostate cancer (Antonarakis et al, 2012). In contrast, Bedolla et al (2007) found that, by itself, PTEN was not seen as a good predictor of biochemical recurrence, although it was better in combination with Akt. Combined PTEN IHC and FISH studies have suggested a role for both cytoplasmic and nuclear PTEN loss in progression, but a trend between PTEN loss and prostate cancer death was not significant (McCall et al, 2008). Conflicting results may be partly attributed to differences in methodology (Sangale et al, 2011). In a subset of the current cohort, we previously reported on PTEN loss using FISH to detect gene loss (Reid et al, 2010). In that analysis, PTEN gene loss showed an association with prostate cancer-specific mortality in univariate but not multivariate
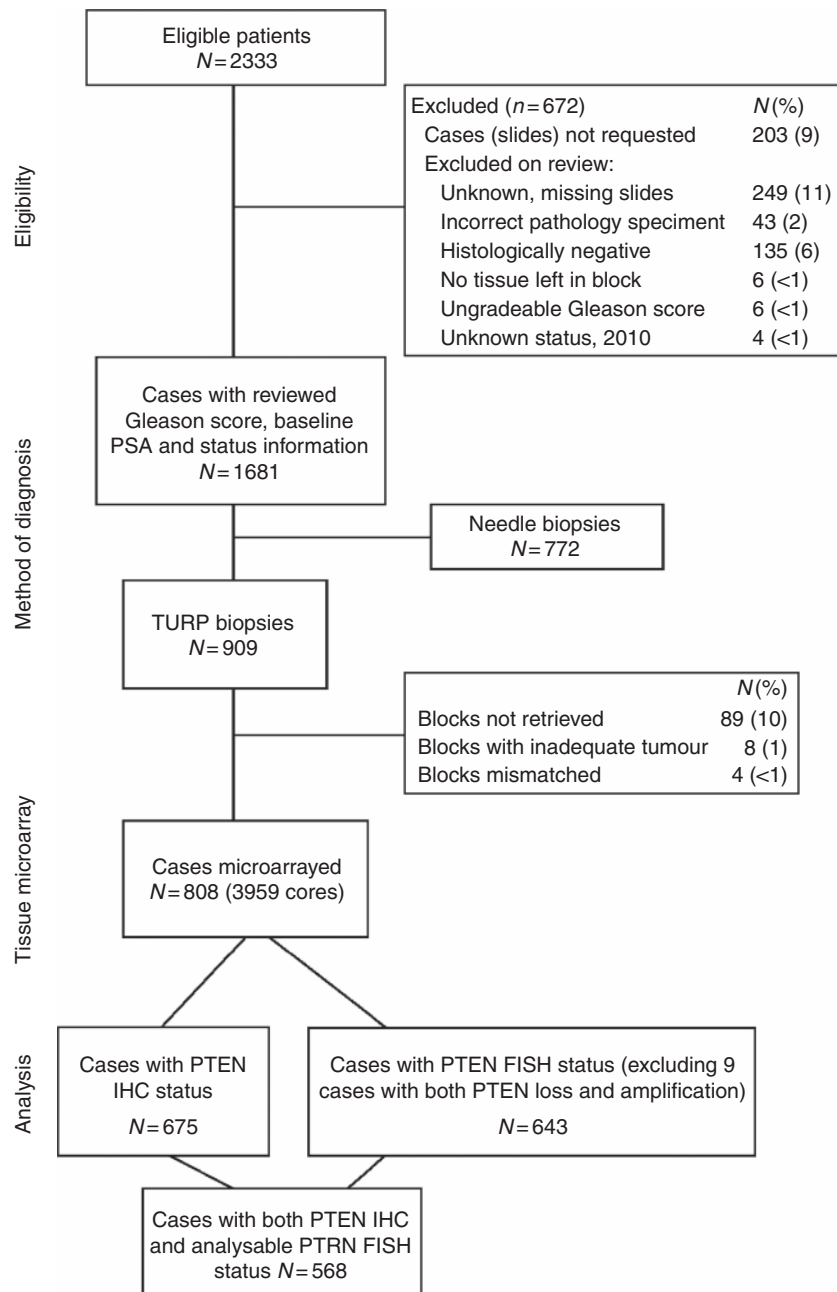

Figure 1. Consort diagram: PTEN cohort derivation.

analyses. An apparent interaction, with *ERG/ETV1* gene rearrangement (ETS), was also observed.

Here we report on the prognostic value of PTEN status for death from prostate cancer evaluated by IHC and FISH in a large cohort of men with conservatively treated prostate cancer with long-term follow-up.

MATERIALS AND METHODS

Patients. Potentially eligible cases of prostate adenocarcinoma diagnosed by TURP were identified from six cancer registries in Great Britain. Case notes from collaborating hospitals were reviewed to assess patient eligibility and confirm they had conservatively managed clinically localised disease, as described previously (Cuzick *et al*, 2006). Briefly, men were included in this study if they had conservatively treated clinically localised prostate cancer diagnosed by TURP between 1990 and 1996 (inclusively), were younger than 76 years at the time of diagnosis and had a baseline PSA measurement. Patients treated with radical prostatectomy or radiation therapy within the first 6 months after diagnosis, or who died or showed evidence of metastatic disease within 6 months of diagnosis or had a baseline PSA > 100 ng ml^{-1} were excluded. Men who had hormone therapy before the diagnostic biopsy were also excluded.

Cases were diagnosed between 1990 and 1996 and the last follow-up took place in December 2009. Deaths were divided into those from prostate cancer and those from other causes, according to the World Health Organisation standardised criteria (WHO, 2010). Approval was obtained from the Northern Multicentre Research Ethics Committee, followed by local ethics committee approval at each collaborating hospital.

The PTEN IHC and FISH assays were conducted on a tissue microarray of up to six 600 μm diameter cancerous cores per biopsy block of formalin-fixed, paraffin-embedded tissue from patients diagnosed by TURP.

IHC assay. The PTEN IHC loss was assayed with rabbit monoclonal antibody 138G6 (Cell Signaling Technology Inc., Danvers, MA, USA). The assay was performed at Myriad Genetics, Inc. (Salt Lake City, UT, USA) and interpreted by a pathologist (ZS). A negative sample was regarded as having no staining in either the cytoplasmic or nuclear cellular compartment; for positive samples, only cytoplasmic staining was scored (Sangale *et al*, 2011). In both the nucleus and the cytoplasm, PTEN staining was seen, but only cytoplasmic staining was used in the scoring. Where multiple cores per individual were available, the average PTEN score was used. The PTEN IHC status was dichotomised as present (positive), or loss (negative), defined as $< 10\%$ tumour cells staining positive.

FISH assay. The PTEN FISH loss was also assayed in the same tissue microarray, using a directly labelled chromosome 10 centromere probe and two overlapping BAC probes to the *PTEN* locus (Reid *et al*, 2010). For each core PTEN FISH status was scored as normal, gene amplification or loss (heterozygous or homozygous). For multiple cores, an individual was assigned the greatest degree of loss seen in any core. Cases showing both loss and amplification were omitted ($N = 9$). For ETS subgroups, cases were defined by previous *ERG/ETV1* FISH analysis as being either normal or rearranged and excluded if no information was available ($N = 1$). Cases with PTEN FISH homozygous loss included all those where both copies of the *PTEN* gene were deleted or one copy was deleted and the second copy dysfunctional because of associated ETS gene rearrangements.

Statistical analysis. The primary end point was time to death from prostate cancer, which was assessed using a proportional hazards model. Observations were censored on the date of last follow-up, or at death from other causes. Covariates evaluated were centrally reviewed Gleason score, baseline PSA value, clinical stage, extent of disease (proportion of positive chips), age at diagnosis and Ki-67 score (% cells positive).

The concentration of PSA was modelled as the natural logarithm of $(1 + PSA \ (ng \ ml^{-1}))$. For simplicity, Gleason scores were grouped into less than 7, equal to 7 and greater than 7. Gleason $3 + 4$ and $4 + 3$ was combined because they previously showed little difference in outcome (Cuzick *et al*, 2006).

All variables were available for 619 men and were combined in a multivariate proportional hazards model to create an overall clinical score that was evaluated as the linear predictor of the multivariate model including Gleason, PSA, Ki-67 and extent of disease.

All P-values were two sided and 95% CIs and P-values, obtained from partial likelihoods of proportional hazards models, were based on χ^2 statistics with 1 degree of freedom (d.f.), unless otherwise indicated. The main assessment was by univariate and multivariate analyses of the prognostic value of PTEN status on death from prostate cancer. For the multivariate Cox proportional hazards models, forward stepwise regression was used. Statistical analyses were done with STATA (version 11.2, StataCorp, 4905 Lakeway Drive, College Station, TX, USA).

RESULTS

PTEN IHC status. The PTEN IHC staining was recorded for 1729 cancer cores from 675 men. The cohort derivation is shown in Figure 1. The PTEN status was scored as negative in 119 (18%) and positive in 556 (82%) cases. Scores were derived from a single core
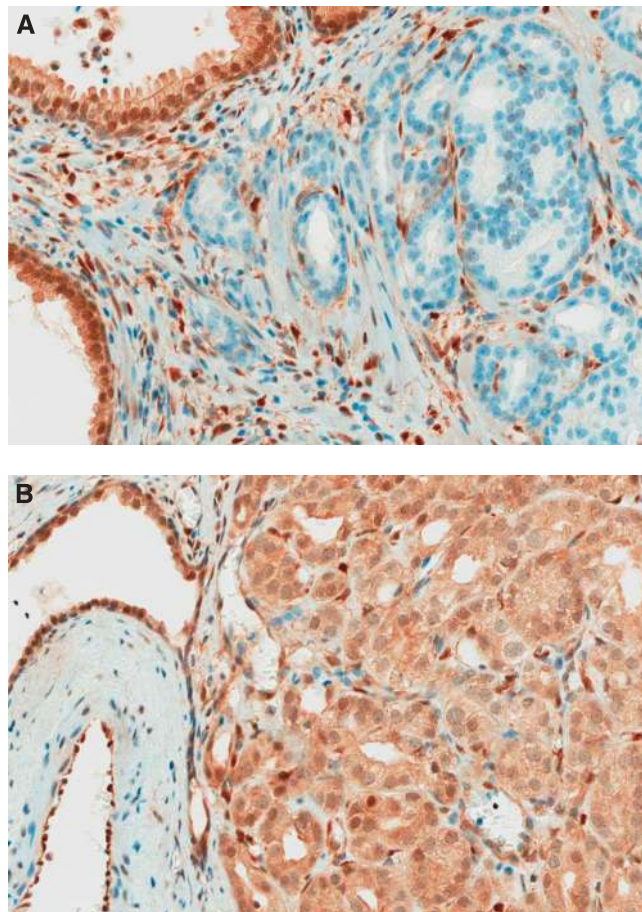

Figure 2. Prostatic adenocarcinoma showing PTEN-deficient (**A**) and PTEN-positive (**B**) staining by immunohistochemistry.

in 132 (20%) cases, from two cores in 238 (35%) men, from three cores in 115 (17%) men, from four cores in 179 (27%) men, from five cores in 6 (1%) men and from six cores in 5 (1%) men. Examples of positively and negatively stained sections are shown in Figure 2. Heterogeneity of staining within cores was seen in <1% of samples. The vast majority (95%) of PTEN IHC-assessed tumours were either negative on all cores ($n = 118$) or positive on all cores ($n = 524$). There were strong correlations ($P < 0.001$) between PTEN IHC and Gleason score, PSA and Ki-67, with PTEN loss more common in cases with Gleason score >7, Ki-67 score >5% or baseline PSA >10 ng ml^{-1} (Supplementary Table 1).

In a univariate analysis PTEN IHC loss was a significant predictor of prostate cancer death in. The hazard ratio (HR) was 3.51 (95% CI 2.60–4.73; χ^2 (1 d.f.) = 57.7; $P = 3.1 \times 10^{-14}$; Table 1). In a multivariate analysis with Gleason score, PSA and PTEN IHC based on the 625 men for whom all covariate scores were available, PTEN IHC added significantly to the overall predictive ability (HR = 1.47; 95% CI 1.07–2.04; $P = 0.02$; Table 1). When Ki-67 and extent of disease, as assessed by percentage of TURP chips containing cancer, were added to the multivariate model, the contribution from PTEN IHC ($P = 0.22$) was markedly reduced. However, the multivariate analysis was strongly influenced by patients with poor risk factors.

There was strong evidence of interaction of PTEN IHC with Gleason score ($P = 0.008$, 1 d.f)., PSA ($P = 0.001$, 1 d.f.), Ki-67 ($P = 0.001$, 1 d.f) and extent of disease ($P = 0.001$, 1 d.f.) in predicting outcome, and PTEN appears to be predictive only in men with low Gleason score, low PSA, low Ki-67 or low extent of disease (Figures 3 and 4).

The best linear predictor using standard factors was given by Gleason, PSA, Ki-67 and extent of disease. A prognostic model was constructed using these factors and a linear predictor was obtained.

This was designated the clinical score and when scaled to vary approximately between 0 and 100 was given by:

Clinical score = 16.2x(Gleason score 7) + 30.2x(Gleason score >7) + 6.6x log(1 + PSA(ng ml^{-1})) + 17.2x(Ki-67 >5%) + 0.3x (extent of disease) + 38.7.

A histogram of the clinical scores is shown in Figure 5 and it was strongly predictive of outcome (Table 1). When stratified by clinical score PTEN IHC was found to strongly predict outcome only in the lowest 50% of this score distribution (Figures 3 and 4) but not for high values. There was a significant interaction of PTEN with this score ($P = 0.047$).

PTEN FISH status. The PTEN FISH staining was recorded for 1672 cancer cores from 652 men. Cases were each assigned a PTEN FISH status of normal, amplification, heterozygous or homozygous loss. Examples of FISH staining for this population have been shown in our previous work (Reid *et al*, 2010). The PTEN FISH was analysed as a categorical variable in these four groups and also in three groups with the few cases of heterozygous loss combined with homozygous loss; normal was used as the reference group. Nine patients with evidence of both heterozygous loss and amplification were excluded. For PTEN FISH analysis in four categories, an additional eight patients with both heterozygous loss and missing ETS status (ambiguous loss status) were excluded. In univariate analysis of PTEN FISH in three categories, PTEN FISH loss was significantly predictive of prostate cancer death (HR = 2.46; 95% CI 1.72–3.51; Wald's test $P = 8 \times 10^{-7}$). Univariate analysis of PTEN FISH with distinct heterozygous loss and homozygous loss indicated the latter group was the most at risk (HR = 2.77; 95% CI 1.89–4.05; Wald's test $P = 1.8 \times 10^{-7}$). However, in a multivariate analysis including Gleason score, PSA,

Table 1. Univariate and multivariate analyses of prostate cancer death: survival results for PTEN IHC loss (PTEN), with Gleason score (G), baseline PSA (P), Ki-67 (K) and extent of disease (C)

| | | Univariate | | Multivariate | | | |
| | | | | PTEN, G, P (N = 675) | | PTEN, G, P, K, C (N = 619) | |
	N[a]	HR (95% CI)	χ^2 (P-value)	HR (95% CI)	χ^2 (P-value)	HR (95% CI)	χ^2 (P-value)
PTEN IHC	(675)						
Normal	556	1[b]	57.68 (3.1×10^{-14})	1[b]	5.37 (0.021)	1[b]	1.52 (0.218)
PTEN loss	119	3.51 (2.60–4.73)		1.47 (1.07–2.04)		1.24 (0.88–1.73)	
Gleason	(675)						
<7	327	1[b]	145.22 (1.9×10^{-33})	1[b]	52.23 (4.9×10^{-13})	1[b]	18.74 (1.5×10^{-5})
7	181	3.51 (2.35–5.26)		1.98 (1.25–3.11)		1.77 (1.09–2.88)	
>7	167	8.45 (5.83–12.26)		4.54 (2.93–7.04)		2.91 (1.76–4.81)	
log(1 + PSA)	(675)	1.93 (1.68–2.20)	98.32 (3.6×10^{-23})	1.51 (1.29–1.76)	28.77 (8.2×10^{-8})	1.30 (1.09–1.54)	8.75 (3.1×10^{-3})
Ki-67	(625)						
≤5%	481	1[b]	80.35 (3.1×10^{-19})			1[b]	15.14 (10^{-6})
>5%	144	4.21 (3.13–5.65)				1.93 (1.39,2.67)	
Extent of disease[c]	(669)	1.25 (1.20–1.30)	128.95 (7.0×10^{-30})			1.10 (1.04–1.16)	12.83 (3.4×10^{-4})
Clinical score	(619)						
<Median	310	1	173.92 (10^{-39})				
50–75th Percentile	154	4.29 (2.77–6.66)					
>75th Percentile	155	11.76 (7.85–17.62)					

Abbreviations: CI = confidence interval; HR = hazard ratio; IHC = immunohistochemistry; PSA = prostate-specific antigen; PTEN = phosphatase and tensin homologue.
[a]Sample size for univariate analysis.
[b]Reference category.
[c]Extent of disease (%) is scaled to be between 0 and 10, so that the hazard ratio corresponds to every 10% increase.
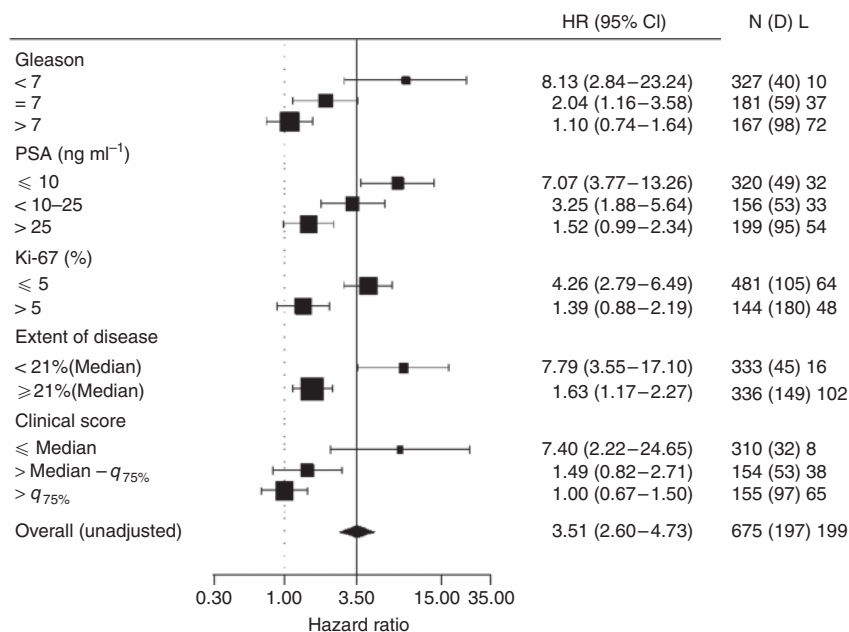
	HR (95% CI)	N (D) L
Gleason		
< 7	8.13 (2.84–23.24)	327 (40) 10
= 7	2.04 (1.16–3.58)	181 (59) 37
> 7	1.10 (0.74–1.64)	167 (98) 72
PSA (ng ml^{-1})		
⩽ 10	7.07 (3.77–13.26)	320 (49) 32
< 10–25	3.25 (1.88–5.64)	156 (53) 33
> 25	1.52 (0.99–2.34)	199 (95) 54
Ki-67 (%)		
⩽ 5	4.26 (2.79–6.49)	481 (105) 64
> 5	1.39 (0.88–2.19)	144 (180) 48
Extent of disease		
< 21%(Median)	7.79 (3.55–17.10)	333 (45) 16
⩾ 21%(Median)	1.63 (1.17–2.27)	336 (149) 102
Clinical score		
⩽ Median	7.40 (2.22–24.65)	310 (32) 8
> Median – $q_{75\%}$	1.49 (0.82–2.71)	154 (53) 38
> $q_{75\%}$	1.00 (0.67–1.50)	155 (97) 65
Overall (unadjusted)	3.51 (2.60–4.73)	675 (197) 199

Figure 3. Univariate hazard ratios (95% CI) for PTEN IHC loss according to Gleason score (<7, =7 and >7), PSA (⩽10, 10–25 and >25 (ng ml^{-1})), Ki-67 (⩽5% and >5%), extent of disease (<21% and ⩾21%) and risk groups based on the clinical score (score<median, median<score<75% quantile, score >75% quantile). N, D and L denote the number at risk, deaths and PTEN loss counts in each subgroup.
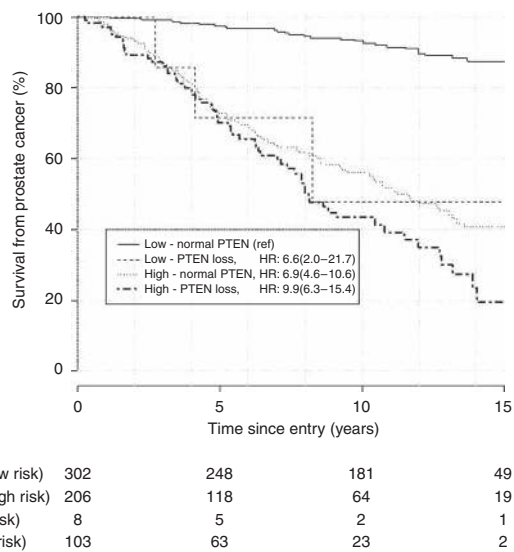

Number at risk:

Normal PTEN (low risk)	302	248	181	49
Normal PTEN (high risk)	206	118	64	19
PTEN loss (low risk)	8	5	2	1
PTEN loss (high risk)	103	63	23	2

Figure 4. Kaplan–Meier survival curves for PTEN IHC loss and normal PTEN divided by the median clinical score (low and high).

Ki-67 and extent of disease (Table 2), the predictive value was lost, as for PTEN IHC. Similarly, PTEN FISH amplification was significantly predictive of prostate cancer death (HR = 1.75; 95% CI 1.23–2.50) in univariate analysis, but not in the multivariate analyses (Table 2). There was no evidence of interaction of PTEN FISH (normal, amplification, loss) with Gleason score (P = 0.99) or our clinical score (P = 0.4). We did not find PTEN FISH (normal, amplification, loss) to be more predictive in the low Gleason score and low clinical score (HR = 1.2; 95% CI 0.4–3.1 for amplification and HR = 1.4; 95% CI 0.3–6.1 for loss).

In contrast to our previous analysis based on a subset of these cases, we found no evidence of an interaction of PTEN FISH (normal, amplification, loss) with ETS (P = 0.2).

PTEN IHC status and PTEN FISH status. Both PTEN IHC and PTEN FISH results were available for 568 cases. We are interested
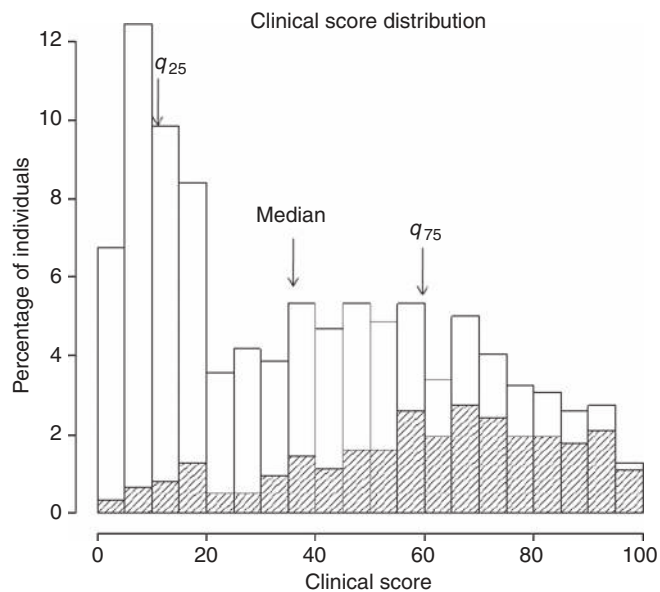

Figure 5. Histogram of the (normalised) clinical score based on the multivariate model using Gleason score, PSA, Ki-67 and extent of disease. The shaded area of each bar denotes the deaths in each clinical score bin. The arrows indicate the median score (36) and interquartile range (13–60).

in comparing PTEN IHC loss with PTEN FISH loss (heterozygous loss combined with homozygous loss). Considering both PTEN FISH amplification and normal as one group, Cohen's κ for PTEN FISH loss and PTEN IHC loss was 0.5 (P<0.001). Excluding those cases classified as PTEN FISH amplification, Cohen's κ for PTEN IHC and PTEN FISH (n = 439) was also 0.5 (P<0.001). PTEN FISH loss was seen in 63% of men (56 out of 89) when PTEN IHC was negative and 11% of men (38 out of 350) when PTEN IHC was positive. In a bivariate analysis (excluding PTEN FISH amplification cases), PTEN IHC loss was a much

Table 2. Univariate and multivariate PTEN FISH analysis hazard ratios (HRs) for normal *vs* amplification (amp) *vs* loss (any, heterozygous (het), homozygous (hom)), with Gleason score (G) and baseline PSA (P) only, and also including Ki-67 (K) and extent of disease (C)

| | | Univariate | | Multivariate | | | |
| | | (*N* = 643) | | PTEN, G, P (*N* = 643) | | PTEN, G, P, K, C (*N* = 575) | |
PTEN FISH	*N*	HR (95% CI)	*P*-value	HR (95% CI)	*P*-value	HR (95% CI)	*P*-value
Normal	399	1[a]		1[a]		1[a]	
Amp	140	1.75 (1.23–2.50)	0.002	1.30 (0.91–1.87)	0.15	1.34 (0.91–1.96)	0.14
Loss (any)	104	2.46 (1.72–3.51)	8.0×10^{-7}	1.20 (0.82–1.73)	0.35	1.19 (0.81–1.76)	0.38
Het	18	1.92 (0.89–4.14)	0.1	0.91 (0.42–1.98)	0.81	0.84 (0.38–1.85)	0.66
Hom	78	2.77 (1.89–4.05)	1.8×10^{-7}	1.33 (0.89–1.98)	0.16	1.34 (0.88–2.03)	0.17

Abbreviations: CI = confidence interval; FISH = fluorescent *in situ* hybridization; PTEN = phosphatase and tensin homologue.
Patients with cores showing both amplification and loss were excluded (*N* = 9). For PTEN FISH analysis in four categories, cases with heterozygous loss and missing ETS status (*N* = 8) were excluded.
[a]Reference category.

stronger predictor of prostate cancer death than PTEN FISH loss ($\chi^2 = 42$ and 0.5, respectively), and PTEN FISH did not add significant prognostic information when PTEN IHC was in the model.

DISCUSSION

Variable results have been seen in different IHC studies of PTEN. The majority of the commercially available PTEN antibodies are directed against the same C-terminal epitope as 138G6, the antibody used in this study (Sangale *et al*, 2011). The antibody 138G6 is directed against the extreme carboxy-terminal sequence of human PTEN protein, and therefore, will only stain full-length protein. Thus, the presence of nonfunctional truncated protein should not confound our results.

A second and perhaps more important reason for the variability between studies of PTEN expression is the differences in antibody specificity. In a previous work (Sangale *et al*, 2011), an evaluation of 11 PTEN antibodies showed that 138G6 was the most specific of the commercially available antibodies. In a panel of control tissues that were molecularly characterised for PTEN status, most of the PTEN antibodies (with the exception of 138G6) were found to have poor sensitivity, specificity or both. This assessment included other antibodies directed at C-terminal peptides and two antibodies directed against the full-length recombinant protein. Their conclusions were confirmed by an independent study (Lotan *et al*, 2011).

In the current analysis, PTEN IHC added prognostic information for prostate survival in univariate analysis, but in multivariate analysis the effect was only apparent when other markers indicated a good prognosis and was not additionally informative when other markers indicated poor prognosis. This is especially relevant for men with Gleason = 6 where additional markers are particularly needed. Here it retained prognostic value in multivariate analysis with a high observed hazard ratio. It was also highly predictive in the low-risk group with PSA $\leqslant 10$ ng ml^{-1}. For men in the lowest 50% of the clinical score, based on Gleason score, PSA, Ki-67 and extent of disease, PTEN IHC was a strong independent predictor with a hazard ratio in excess of seven-fold. Even greater proportions would be scored as low risk in areas where PSA screening is routinely practiced.

The assessment of PTEN using this antibody was very reproducible and very few patients had conflicting results in different cores, suggesting that it is highly reproducible across laboratories. It is also less expensive than molecular markers

(Cuzick *et al*, 2006) and may have a role in triaging low-risk patients.

In multivariate models, PTEN FISH loss was not as strong a predictor as PTEN IHC loss and did not add independent information.

The poor correlation of PTEN FISH status with IHC status contrasts with a previous report for the same antibody (Reid *et al*, 2012). This may be partly explained by the extent of heterogeneity across the cores observed in the current study; staining was consistent across individual cores from the same patient in 95% cases stained by PTEN IHC, but only 75% cases stained by PTEN FISH.

The reason for PTEN loss being important only in low-risk patients is not clear and requires replication in another study. It suggests that PTEN may be involved at an early stage in a pathway associated with disease development, and when this or other pathways are sufficiently dysregulated to lead to cancers with an elevated Gleason Score or a large extent, the effect is overridden. However, we know of no direct mechanistic data to confirm this. The fact that PTEN loss is a much stronger factor in univariate analyses than in multivariate ones also suggests that it is only a part of the carcinogenic process and further changes are needed before aggressive tumours develop.

These findings require replication in an independent data set and also need to be established for patients diagnosed by needle biopsy and managed conservatively. The value of PTEN in predicting recurrence or death from prostate cancer in men who receive radical treatment also needs to be established.

CONFLICT OF INTEREST

Drs Lanchbury, Stone and Sangale are employees of Myriad Genetics. Professor Cuzick is on the speakers bureau for Myriad genetics. The other authors declare no conflict of interest.

DISCLAIMER

The PTEN status was assayed blind to all other data by Myriad Genetics (IHC) and ICR (FISH). Analysis was conducted at QMUL under the direction of Professor Cuzick, following a predefined Statistical Analysis Plan. Interpretation of the data was done jointly by all authors, but the final content of this report was determined by non-corporate authors.

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APPENDIX

Investigators in participating regional cancer registries, research centres and hospital trusts are listed below. Members of the Transatlantic Prostate Group are designated by an asterisk.

Thames Cancer Registry: Henrik Møller*, Shirley Bell (deceased), K Linklater, J Ottey V Fisher; Ashford & St Peter's: M Hall, N Harvey Hills; Barnet & Chase Farm: H Reid; Brighton and Sussex: N Kirkham, P Thomas; Bromley: D Nurse; Dartford & Gravesham: I Dickinson, P Thebe; East & North Hertfordshire: D Hanbury, M Ali-Izzi; Eastbourne: C Moffatt; Epsom & St Helier: M Bailey, L Temple; Essex Rivers Healthcare; W Aung, C Booth; Frimley Park: B Montgomery, P Denham; Greenwich Healthcare: N Cetti, P Pinto; Guy's & St Thomas's: A Chandra, T O'Brien; Hammersmith Hospitals: N Livni; Havering Hospitals: I Saeed; Hillingdon: F Barker, T Beaven; King's Healthcare: G Muir, Z Khan; Kingston: C Jameson; Lewisham; A Giles; Mayday Healthcare; N Arsanious, A Arnaout; The Medway: E Boye; Mid Essex Hospitals: Mid Kent: M Boyle; North West London Hospitals: M. Jarmulowicz; Royal Free Hampstead: RJ Morgan, A Bates; St Bartholomew's and The Royal London Hospitals: F Chinegwundoh*, RTD Oliver*, D Berney*; Wolfson Institute of Preventive Medicine, Queen Mary University of London: J Cuzick*, G Fisher*; Institute of Cancer Research, Sutton: C Cooper*; Royal Surrey County: S De Sanctis; Southend: M Chappell; St George's, London: R Kirby, C Corbishley; St Mary's, London: A Patel, M Walker; West Hertfordshire: J Crisp, W Riddle; Worthing & Southlands Hospitals: J Grant.

Northern & Yorkshire Cancer Registry & Information Service: David Forman*, C Storer, C Bennett, C Spink; Airedale: I Appleyard, JO'Dowd; Hull & East Yorkshire: J Hetherington, A MacDonald; The Leeds Teaching Hospitals: P Whelan, P Quirke and P Harnden.

Oxford Cancer Intelligence Unit: Monica Roche*, Sandra Edwards, S Bose, P Hall; Heatherwood & Wexham Park: M Ali, O Karim; Milton Keynes: E Walker, S Jalloh; Northampton: M Miller, A Molyneux; Oxford Radcliffe: S Brewster, D Davies; Royal Berkshire & Battle: P Malone, C McCormick; Stoke Mandeville: J Greenland and A Padel.

Welsh Cancer Intelligence & Surveillance Unit: John Steward*, Shelagh Reynolds, Lynda Roberts, Judith Adams; Ceredigion and Mid Wales: J Edwards, CGB Simpson; Conwy & Denbighshire: A Dalton, V Srinivasan; NE Wales: A De Bolla, C Burdge; Gwent Healthcare: W Bowsher, M Rashid; Swansea: M Lucas, C O'Brien; Cardiff & Vale: M Varma.

Scottish Cancer Registry: David Brewster*; The Lothian University Hospitals, J Royle, K Grigor; North Glasgow University Hospitals, D Kirk, A Milano and R Reid.

Merseyside & Cheshire Cancer Registry: Lyn Williams*, R Iddenden; Royal Liverpool University Hospital, CS Foster* and P Cornford.

Memorial Sloan Kettering Cancer Center: P Scardino*, P Fearn*, V Reuter*, J Eastham*, M Kattan* and H Lilja*.