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# Progesterone receptor expression is an independent prognostic variable in early breast cancer: a population-based study

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**Background:** Progesterone receptor (PR) expression assessment in early invasive breast cancer remains controversial. This study sought to re-evaluate PR expression as a potential therapeutic guide in early breast cancer; particularly in oestrogen receptor (ER)-positive, lymph node (LN)-negative disease.

**Methods:** A population cohort of 1074 patients presenting to a single Cancer Centre over 4 years (2000–2004) underwent surgery for primary invasive breast cancer with curative intent. Prospective data collection included patient demographics, pathology, ER and PR expression, HER2 status, adjuvant chemotherapy and endocrine therapy. Progesterone receptor expression was compared with (all causes) overall survival (OS), breast cancer-specific survival (BCSS) and disease-free survival (DFS).

**Results:** Overall survival was 71.0% and BCSS was 83.0% at median follow-up of 8.34 years. Absent PR expression was significantly associated with poorer prognosis for OS, BCSS and DFS ( $P < 0.0001$ , log-rank), even within the ER-positive, LN-negative group (hazard ratio for BCSS 3.17, 95% CI 1.43–7.01) and was not influenced by endocrine therapy. Cox's regression analysis demonstrated that PR expression was an independent prognostic variable.

**Conclusion:** Absence of PR expression is a powerful, independent prognostic variable in operable, primary breast cancer even in ER-positive, LN-negative patients receiving endocrine therapy. Absence of PR expression should be re-evaluated as a biomarker for poor prognosis in ER-positive breast cancer and such patients considered for additional systemic therapy.

Endocrine therapy is well established for early and advanced breast cancer with treatment decisions currently based on the semi-quantitative, immunohistochemical assessment of oestrogen receptor (ER) expression on histological material (SIGN, 2007; National Institute for Health and Excellence, 2009; Hammond *et al*, 2010). In contrast, progesterone receptor (PR) estimation is recommended in some (SIGN, 2007; Hammond *et al*, 2010), but not all (National Institute for Health and Excellence, 2009) national guidelines.

The role of PR measurement in the management of breast cancer remains controversial. Some commentators suggest that it could be dispensed with (Olivetto *et al*, 2004) as ER-negative,

PR-positive patients who may respond to endocrine therapy are very rare, limiting its usefulness. However, others consider that, as a prognostic indicator, PR is still worth assessing (MacGrogan *et al*, 2005).

The potential utility of PR expression as a prognostic marker has been appreciated since 1975 when it was first suggested that PR expression (by ligand binding assay) could predict outcome and response to ER-directed therapy in advanced disease (Horwitz and McGuire, 1975). This was later confirmed in a prospective study (Ravdin *et al*, 1992) and in 2004 an immunohistochemical estimation of PR expression was validated and shown to be superior to ligand binding in predicting outcome (Mohsin *et al*, 2004).

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Progesterone receptor expression predicts response to tamoxifen in premenopausal women and response increases with higher levels of PR expression (Stendahl *et al*, 2006). In postmenopausal women, the ATAC study (Arimidex, tamoxifen alone, or in combination), using the locally derived hormone receptor expression data, suggested a superior response to aromatase inhibitor (AI) over tamoxifen in patients with ER-positive, PR-negative cancers (Dowsett *et al*, 2005); a finding not confirmed by other groups studying other AIs (Breast International Group (BIG) 1-98 Collaborative Group *et al*, 2005; Viale *et al*, 2007). Subsequent, central ER and PR testing (in the ATAC study patients) also failed to confirm that AIs were superior in ER-positive, PR-negative disease (Dowsett *et al*, 2008) demonstrating the importance of consistent and quality assured testing. This was further reinforced by the central review of tumour sections in the BIG1-98 and ATAC clinical trials which showed significant discordance between local and central hormone receptor assessment (Viale *et al*, 2007; Dowsett *et al*, 2008).

Potential biasing factors in other studies of PR expression in early breast cancer include: PR analysis at multiple, peripheral facilities using different methodologies and patient inclusion/exclusion criteria (as used in clinical trials). In order to avoid these confounding factors, this study used the preoperative, diagnostic core biopsies (ensuring consistent, rapid fixation and processing) within a single, quality assured and accredited diagnostic pathology service in line with current ASCO/CAP guidelines (Hammond *et al*, 2010). Furthermore, this study represents a 'real-world' breast cancer population in that every patient presenting to a single regional cancer centre with operable breast cancer over a period of 4 years was included, making it ideal for evaluating PR immunohistochemistry (IHC) for prognosis and treatment planning.

## PATIENTS AND METHODS

**Study population.** The patient population comprised a consecutive series of all new patients presenting with operable, invasive breast cancer (symptomatic and screen detected) to a single Regional Cancer Centre between 1 July 2000 and 30 June 2004. The Caldicott Guardian granted permission for the use of patient data. The Cancer Centre treats all breast cancer patients from a defined geographic area with all patient records maintained within one institution. All patients undergoing curative surgery were discussed at a multidisciplinary meeting postoperatively and appropriate adjuvant therapy (including chemotherapy) was prescribed as per national guidelines (SIGN, 2007).

**Pathology.** All pathology was reviewed by a single specialist breast pathologist (CAP) working within an accredited Pathology Laboratory. Data were recorded as per National Guidelines concerning tumour grade, tumour size and LN status (BSP, 2005). The Nottingham Prognostic Index (NPI) was derived from these data (Haybittle *et al*, 1982). In the 65 patients (6.1%) who had multiple tumours and underwent surgery, the cancer with the highest grade was used as the index lesion for the survival analysis.

**Hormone receptor expression analysis.** Oestrogen receptor and PR analysis were carried out as described (Purdie *et al*, 2010a). Diagnostic core biopsies were immunostained using primary antibodies for ER (clone 6F11, 1:200) and PR (clone 16, 1:800); both Novocastra Laboratories Ltd, Newcastle Upon Tyne, UK. The stained slides were scored using the 'Quickscore' method (Detre *et al*, 1995). Cancers scoring 0–3 were regarded as negative whereas cases scoring 4–18 (maximum) were regarded as positive. Adjuvant endocrine therapy decisions were made on the basis of this assessment. Subsequently, all cancers were further assessed using the ASCO/CAP guidelines (Hammond *et al*, 2010) for ER and PR

expression (1% cutoff) and also using the 'Allred' method with cases scoring >2 defined as positive (Harvey *et al*, 1999).

**HER2 assessment.** HER2 assessment by IHC and fluorescent *in situ* hybridization (FISH) was carried out as described (Purdie *et al*, 2010a, 2010b) in laboratories accredited by CPA UK Ltd and quality assured by the UK National External Quality Assurance Scheme. All cancers underwent HER2 IHC using the CB11 monoclonal antibody on the diagnostic core biopsy and scored using published criteria (Ellis *et al*, 2000; Walker *et al*, 2008). All cancers scoring 'equivocal (2+)' on IHC were subjected to HER2 FISH analysis carried out using the PathVysion HER2 DNA probe kit (Vysis, Abbott Laboratories, Abbott Park, IL, USA) and assessed by standard criteria (Purdie *et al*, 2010b). The ratio of orange HER2 signals to green alphasatellite CEP17 signals was calculated and amplification defined as a ratio of  $\geq 2.00$ . Cases scoring IHC-negative (0), IHC-negative (1+) or IHC-equivocal (2+) but FISH-negative were classified as HER2-negative and those scoring IHC equivocal (2+) and FISH-positive or IHC-positive (3+) were regarded as HER2-positive.

**Follow-up.** Follow-up data were obtained from the Cancer Centre breast oncology database, backed up, where necessary, by contact with the patient's general medical practitioner or the registrar of deaths. Complete follow-up data were obtained for 1072 of the 1074 patients (99.8%) who underwent surgery. For patients who died, the date and cause of death was recorded; all deaths not attributable to breast cancer were censored at the date of death. The primary outcome in this analysis was time to breast cancer death; time to death by any cause and time to recurrence (first episode, local and/or distant) were also analysed. Accordingly, the primary end points were overall survival (OS), breast cancer-specific survival (BCSS) and disease-free survival (DFS).

**Statistical analysis.** Statistical analyses were carried out using SPSS version 20 (IBM, NY, USA). All associations for  $2 \times 2$  tables were carried out using a two-sided Fisher's exact test. For  $3 \times 2$  tables a chi-squared ( $\chi^2$ ) test with Yates' correction was employed. Survival analysis was carried out by Kaplan–Meier survival curves analysed by the log-rank test and multivariate analysis was carried out using the Cox regression. Proportional hazards assumptions of the Cox regression analysis were verified using the 'survival' package in R using the *cox.zph* procedure (Grambsch and Therneau, 1994).

## RESULTS

**Study population.** A total of 1283 patients presented with invasive breast cancer (Figure 1). Of these, 209 (16.8%) did not undergo surgery because of advanced disease or significant comorbidities. The remaining 1074 (84%) patients had surgical management with curative intent; 328 of whom (30.5%) were screen detected. Surgery was followed by adjuvant therapy determined by multidisciplinary team discussion with patient choice informed by appropriate guidelines (SIGN, 2007). Chemotherapy was administered to 307 patients (28.6%) including 53 who received neo-adjuvant chemotherapy. Endocrine therapy was prescribed to 865 patients (80.5%). Of these, 488 received tamoxifen (Tam) only, 35 an AI only, 261 Tam and AI, 47 were part of the BIG 1-98 study (Breast International Group (BIG) 1-98 Collaborative Group *et al*, 2005) receiving Tam or letrozole and 34 were treated by ovarian suppression.

**Pathology.** The distribution of tumour grade (15.8% grade 1, 42.1% grade 2 and 42.1% grade 3) and nodal status (39.6% node positive) are what would be expected for a population which was largely symptomatic in presentation (Table 1) (Rakha *et al*, 2008). Oestrogen receptor-positivity was present in 80.2% of cancers and

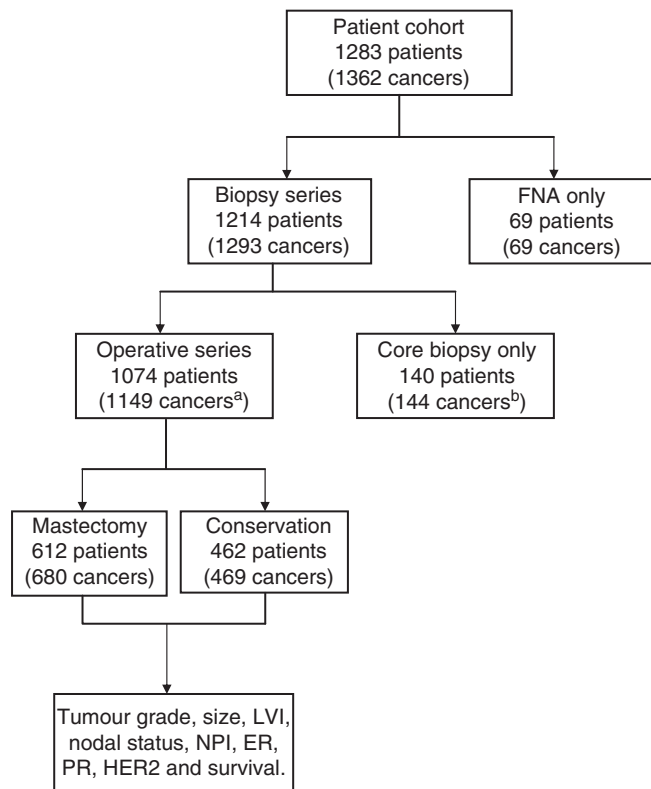


Figure 1. CONSORT diagram of patient and tumour groups. <sup>a</sup>Fifty-five patients each had two tumours, 10 patients each had three tumours. <sup>b</sup>Four patients in this group each had two tumours. Abbreviations: ER = oestrogen receptor expression; HER2 = human epidermal growth factor receptor 2 status; LVI = lymphovascular invasion; NPI = Nottingham Prognostic Index; PR = progesterone receptor expression.

67.2% were PR-positive. HER2-positivity was detected in 13.9% (Purdie *et al*, 2010a). Survival analysis confirmed that tumour grade, tumour size, lymphovascular invasion, LN status and NPI score were all predictive of prognosis in the manner that would be expected (data not shown).

**PR expression.** PR expression was strongly associated with age, tumour grade, ER expression, NPI group, negative HER2 status and not receiving chemotherapy. There was no significant association between PR expression and nodal status, tumour size, lymphovascular invasion or type of surgery (Table 1). Oestrogen receptor expression was strongly associated with PR expression and only three cases were ER-negative and PR-positive (0.3%). The same associations were detected when the Allred score or the ASCO/CAP cutoff (1%) for ER and PR expression were used (data not shown).

**HER2 status.** Cancers that were ER-positive, PR-negative were significantly more likely to be HER2-positive (18%) than those that were ER-positive, PR-positive (8%,  $P < 0.001$ ) (Table 2).

**Survival.** Follow-up data for OS, BCSS and DFS were obtained from 1072 patients (99.8%). Overall survival was 71.0% and BCSS 83.0% at median follow-up of 8.34 years. For all variables where there was a significant association with BCSS, there was a similar, significant association with DFS and hence DFS data are not shown.

Separating the entire cohort of patients into four groups on the basis of ER, PR and HER2 status demonstrated a highly significant difference in BCSS in these groups (Figure 2A). When PR was

Table 1. PR characteristics in 1074 cancers

	PR negative N (%)	PR positive N (%)	Total N (%)	P*
Symptomatic	256 (72.7)	490 (67.9)	746 (69.5)	0.126
Screening	96 (27.3)	232 (32.1)	328 (30.5)	
<b>Total</b>	352 (100.0)	722 (100.0)	1074 (100.0)	
Age 20–39	24 (6.8)	27 (3.7)	51 (4.7)	0.0381
Age 40+	328 (93.2)	695 (96.3)	1023 (95.3)	
<b>Total</b>	352 (100.0)	722 (100.0)	1074 (100.0)	
Grade 1	21 (6.0)	149 (20.6)	170 (15.8)	<0.0001
Grade 2	96 (27.2)	356 (49.3)	452 (42.1)	
Grade 3	235 (66.8)	217 (30.1)	452 (42.1)	
<b>Total</b>	352 (100.0)	722 (100.0)	1074 (100.0)	
Node negative	212 (60.6)	431 (60.3)	643 (60.4)	1.00
Node positive	138 (39.4)	284 (39.7)	422 (39.6)	
<b>Total</b>	350 (100.0)	715 (100.0)	1065 (100.0) <sup>a</sup>	
Node negative	212 (60.6)	431 (60.3)	643 (60.4)	0.3396
Node positive (1–3)	91 (26.0)	207 (30.0)	298 (28.0)	
Node positive (4+)	47 (13.4)	77 (10.7)	124 (11.6)	
<b>Total</b>	350 (100.0)	715 (100.0)	1065 (100.0) <sup>a</sup>	
Size 1–20 mm	166 (47.6)	368 (51.0)	534 (49.9)	0.4892
Size 21–50 mm	157 (45.0)	297 (41.1)	454 (42.4)	
Size >50 mm	26 (7.4)	57 (7.9)	83 (7.7)	
<b>Total</b>	349 (100.0)	722 (100.0)	1071 (100.0) <sup>b</sup>	
GPG (2.00–3.40) <sup>c</sup>	70 (20.1)	286 (40.0)	356 (33.5)	<0.0001
MPG (3.41–5.40) <sup>c</sup>	182 (52.3)	283 (39.6)	465 (43.7)	
PPG (>5.40) <sup>c</sup>	96 (27.6)	146 (20.4)	242 (22.8)	
<b>Total</b>	348 (100.0)	715 (100.0)	1063 (100.0) <sup>d</sup>	
LVI absent	249 (70.7)	535 (74.1)	784 (73.0)	0.2753
LVI present	103 (29.3)	187 (25.9)	290 (27.0)	
<b>Total</b>	352 (100.0)	722 (100.0)	1074 (100.0)	
ER positive	142 (40.3)	719 (99.6)	861 (80.2)	<0.0001
ER negative	210 (59.7)	3 (0.3)	213 (19.8)	
<b>Total</b>	352 (100.0)	722 (100.0)	1074 (100.0)	
HER2 positive	90 (25.6)	59 (8.2)	149 (13.9)	<0.0001
HER2 negative	262 (74.4)	662 (91.8)	924 (86.1)	
<b>Total</b>	352 (100.0)	721 (100.0)	1073 (100.0) <sup>e</sup>	
Chemotherapy	153 (43.5)	154 (21.3)	307 (28.6)	<0.0001
No chemotherapy	199 (56.5)	568 (78.7)	767 (71.4)	
<b>Total</b>	352 (100.0)	722 (100.0)	1074 (100.0)	
Mastectomy	207 (58.8)	405 (56.1)	612 (57.0)	0.4386
Conservation	145 (41.2)	317 (43.9)	462 (43.0)	
<b>Total</b>	352 (100.0)	722 (100.0)	1074 (100.0)	

Abbreviations: GPG = good prognostic group; LVI = lymphovascular invasion; MPG = moderate prognostic group, PPG = poor prognostic group. PR characteristics in 1074 cancers. \* $\chi^2$  test with Yates' correction for 3 × 2 tables, two-sided Fisher's exact test for 2 × 2 tables.

<sup>a</sup>Nine patients had no axillary nodes as a result of previous axillary surgery. <sup>b</sup>Three cancers showed a pathological complete response (pCR) to neo-adjuvant chemotherapy. <sup>c</sup>Nottingham Prognostic Index, <sup>d</sup>Nine patients had no nodes and two had a pCR to neo-adjuvant chemotherapy. <sup>e</sup>One cancer had insufficient tissue for HER2 FISH analysis.

examined as the sole variable for all patients, absence of PR expression was strongly associated with a worse BCSS (HR 3.24, 95% CI 2.42–4.34, Figure 2B).

Absence of PR expression was associated with poorer prognosis in ER-positive patients treated with any endocrine therapy (Figure 2C). Furthermore, the association of PR-negative tumours with poor prognosis was also seen in both LN-positive ( $P < 0.0001$ ) and LN-negative ( $P < 0.0001$ ) groups (Figure 3A and B) as well as in those patients who did ( $P < 0.0001$ ) or did not ( $P < 0.0001$ ) receive chemotherapy (Figure 3C and D). These findings are summarised in Table 3.

**Cox regression analysis.** Cox regression analysis was carried out using PR expression as well as the standard pathological and

biomarker variables employed by NPI and Adjuvant! Online in addition to HER2 (Table 4). Progesterone receptor was an independent prognostic variable for both BCSS and DFS in this analysis, and, despite the very close association with ER, PR was a more powerful predictor of BCSS and DFS than ER. Conventional clinical and pathological factors were prognostic in keeping with the literature (Blamey *et al*, 1979; Haybittle *et al*, 1982; Mook *et al*, 2009).

**DISCUSSION**

This study is the largest, population-based analysis of PR expression in breast cancer carried out using the current standard of care methodology (Hammond *et al*, 2010). Although retrospective, it does have the advantage of comprising an unselected, operable breast cancer population without any exclusions or

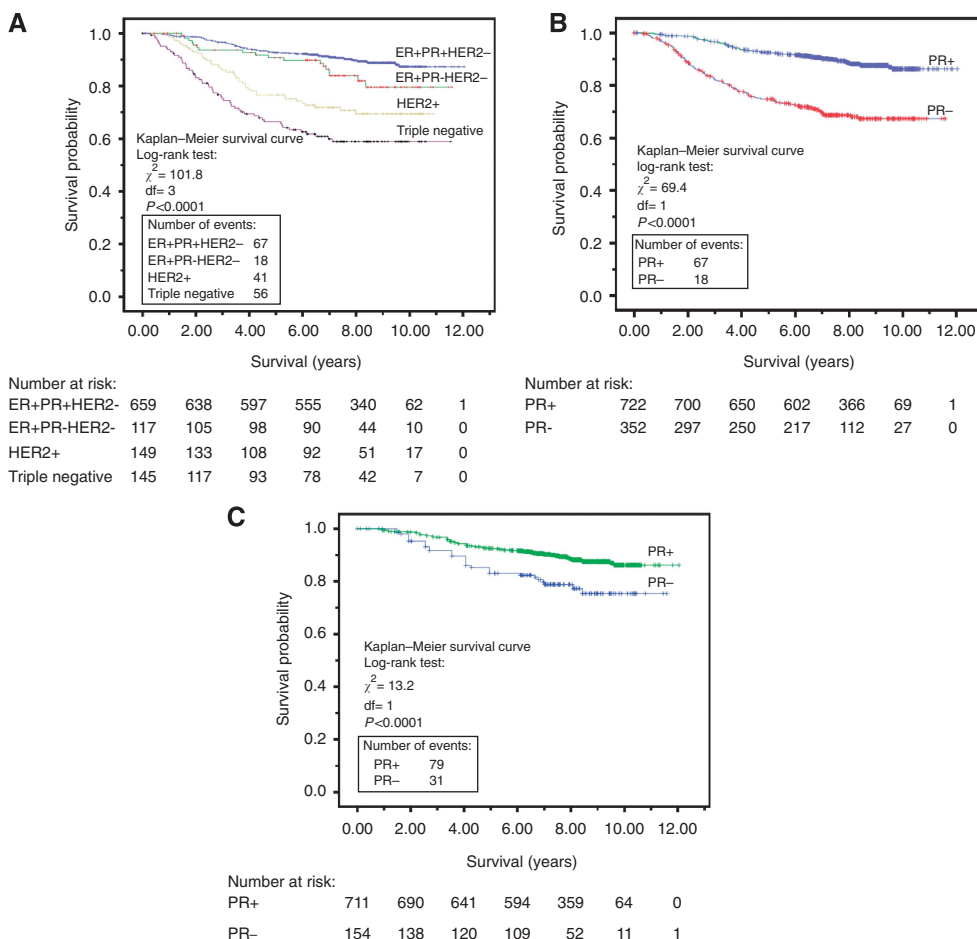
selection bias. Furthermore, the pathology and biomarker analyses were carried out prospectively in a single, accredited laboratory and represent a ‘real-world’ assessment of the value of PR expression in clinical practice (Hammond *et al*, 2010).

Breast cancer gene expression profiling has yielded significant advances in recent years (Sørlie *et al*, 2001; West *et al*, 2001; van de Vijver *et al*, 2002; van t Veer *et al*, 2002) indicating that ER-positive disease can be divided into luminal A and B sub-types which differ on the basis of the greater expression of proliferation-associated genes in luminal B cancers. Supporting evidence comes from an association between Ki67 proliferation index and gene expression profiling which, in at least one study, allowed the identification of a poorer prognosis (luminal B) subgroup of ER-positive patients (Cheang *et al*, 2009). However, the method only correctly assigned the tumours to luminal A or B in 75% of patients reflecting the need for further analysis using validated methods before widespread adoption of Ki67 (Yerushalmi *et al*, 2010).

It has been suggested that ER-positive, PR-negative cancers belong to the luminal B group (Creighton *et al*, 2009), implying an overlap between measures of proliferation and absence of PR expression in ER-positive cancers. Much interest has centred on the assessment of cell proliferation by counting cells immunostained for the proliferation marker Ki67 (recently reviewed (Dowsett *et al*, 2011)). Many studies, using this technique, have shown that Ki67 predicts outcome. Our finding of a significant association of PR-negative subtype with tumour grade (of which mitotic count is a key part (BSP, 2005)) would be consistent with this. Unfortunately, the methodology for Ki67 assessment varies from one study to another with cutoffs ranging from 0 to 30%. As a result, there is, as yet, no accepted diagnostic

**Table 2.** Association of HER2 status with PR expression in ER-positive cancers

	PR-negative N (%)	PR-positive N (%)	Total N (%)	P-value
HER2-negative	117 (82.4)	659 (91.8)	776 (90.2)	
HER2-positive	25 (17.6)	59 (8.2)	84 (9.8)	<0.001
Total	142 (100.0)	718 (100.0)	860 (100.0)	



**Figure 2.** Kaplan-Meier plot of BCSS for all patients by: (A) ER, PR and HER2 status, (B) PR status only and (C) PR status in patients who received any endocrine therapy (all were ER-positive).

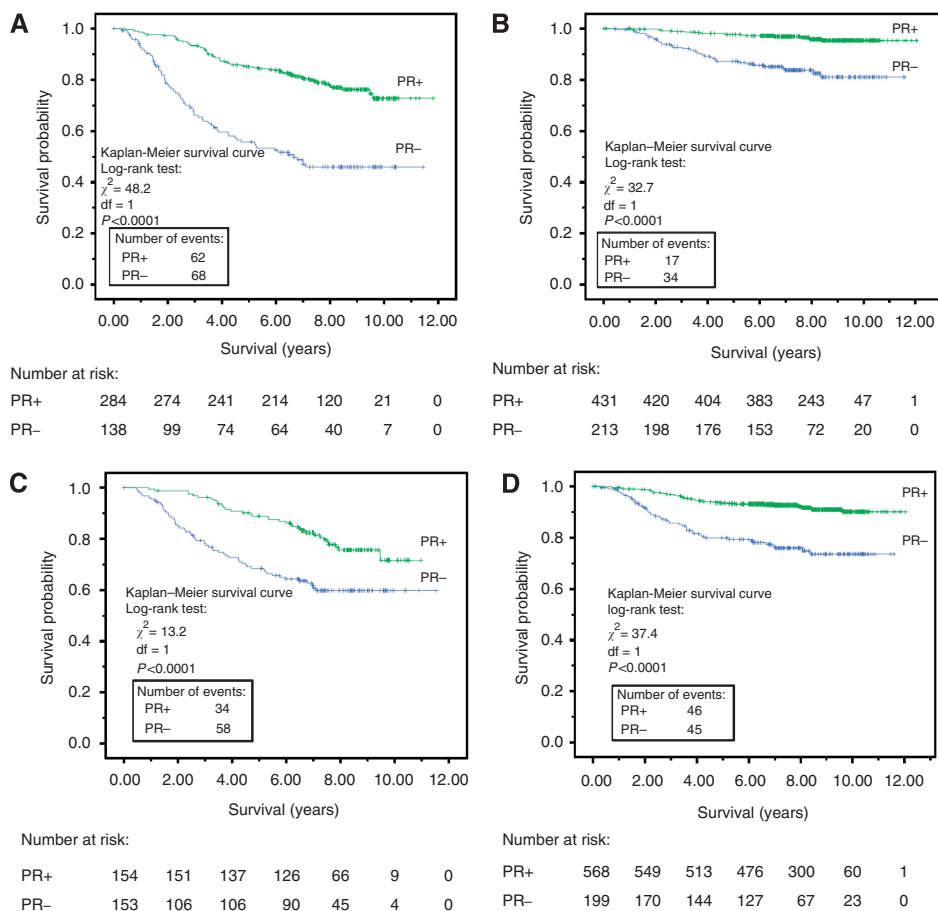


Figure 3. Kaplan-Meier plot of BCSS by PR status in: (A) LN-positive patients, (B) LN-negative patients, (C) patients who received chemotherapy and (D) patients who did not receive chemotherapy.

standard for the measurement of Ki67 proliferation fraction and current guidelines suggest that it cannot be generally applied to diagnostic, clinical situations (Dowsett *et al*, 2011). Progesterone receptor expression, conversely, is internationally used (SIGN, 2007; Hammond *et al*, 2010), is subject to rigorous quality assurance and has well-defined parameters for assessment as a result of the work carried out to ensure the validity of ER assessment in guiding endocrine therapy. As such, PR expression, rather than Ki67, could be a useful marker to identify a group of ER-positive patients (particularly those who are LN-negative) who have a significantly worse prognosis and might benefit from more aggressive adjuvant therapy.

Intratumoral heterogeneity of PR expression is a significant feature and has been identified in 28.9% of tumours (Torhorst *et al*, 2001). Tissue micro-arrays (TMAs) using a single 0.6 mm diameter core per tumour showed only 88% concordance with a full section of resected tumour (Torhorst *et al*, 2001). A single 0.6 mm core in a TMA has a surface area of 1.13 mm<sup>2</sup> for assessment whereas we examined at least two 14-gauge cores giving an area of 68.4 mm<sup>2</sup> for examination (more than 60 times that of a TMA core). Even using a larger (1.0 mm) TMA core gave a concordance of only 67–90% (Kyndi *et al*, 2008). Furthermore, many studies use cell counts of 50–100 cells in order to classify a tumour (Kyndi *et al*, 2008; Faratian *et al*, 2009); this may not be suitable for PR due to heterogeneity of expression. TMA material may also suffer from inconsistent and sometimes poor fixation and tissue processing; parameters known to adversely affect immunohistochemical scoring (Mann *et al*, 2005; Hammond *et al*, 2010). Thus, studies using a single TMA core per tumour to examine PR expression need to be viewed with some caution, as there is a significant risk of false-negative scoring.

Although other studies have examined PR expression in larger cohorts of breast cancer patients, these have either employed TMAs (Liu *et al*, 2010) or (now obsolete) biochemical assays (Bardou *et al*, 2003). Our study identified PR expression in 67.2% whereas studies using the TMA methodology identified PR expression in only 51.2% (Liu *et al*, 2010). Current ASCO/CAP guidelines recommend that the diagnostic assessment of ER and PR be carried out on core biopsy material (Hammond *et al*, 2010); the technique used throughout this study.

ER-negative, PR-positive cancers are exceptionally rare (0.3%). This suggests that the assessment of PR expression in ER-negative tumours to identify those that might still benefit from endocrine therapy may not be justified (Olivotto *et al*, 2004). Progesterone receptor expression correlates with ER expression, negative HER2 status, tumour grade and age at presentation but not with LN status, tumour size or lymphovascular invasion. These data are all in keeping with previous studies (Liu *et al*, 2010) and indicate that PR-expressing tumours are more common in post-menopausal women with low-grade, ER-positive breast cancers who, generally, have a good prognosis.

Using ER, PR and HER2 can stratify patients into four distinct prognostic groups (Figure 2A). The best prognosis cancers are ER-positive, PR-positive and HER2-negative, whereas the worst prognosis belongs to the triple negative cancers. This stratification of prognosis, using already available biomarkers, persists despite the use of guideline-based adjuvant therapies (but predates the use of trastuzumab in this population) and provides very powerful information to guide treatment planning.

Of note, many of the PR-negative patients recur and/or die from breast cancer in the first 5 years following diagnosis, at a time when

Table 3. Summary of BCSS and DFS analysis (Cox)

Subset	PR	Survival (%)	$\chi^2$	P-value	Hazard ratio (95% CI)
<b>BCSS</b>					
All cases	- +	70.7 89.8	62.026	<0.0001	3.239 (2.418–4.340)
ER +	- +	83.1 89.0	5.885	0.015	1.761 (1.115–2.782)
ER + LN +	- +	68.2 78.1	3.987	0.046	1.806 (1.011–3.266)
ER + LN -	- +	89.8 96.3	8.130	0.004	3.170 (1.434–7.006)
ER + Chemo +	- +	62.9 78.4	5.924	0.015	2.226 (1.169–4.239)
ER + Chemo -	- +	89.7 91.9	1.064	0.302	1.414 (0.732–2.731)
<b>DFS</b>					
All cases	- +	64.5 83.7	60.42	<0.001	2.719 (2.113–3.498)
ER +	- +	76.1 83.7	7.749	0.005	1.722 (1.174–2.524)
ER + LN +	- +	56.8 70.7	5.739	0.017	1.841 (1.117–3.034)
ER + LN -	- +	84.7 92.3	8.145	0.004	2.444 (1.323–4.515)
ER + Chemo +	- +	51.4 71.9	8.520	0.004	2.319 (1.318–4.078)
ER + Chemo -	- +	84.1 86.9	1.406	0.236	1.376 (0.812–2.333)

Abbreviations: BCSS = breast cancer-specific survival; DFS = disease-free survival; ER = oestrogen receptor; LN = lymph node; PR = progesterone receptor.

Table 4. Cox analysis for BCSS and DFS analysis

	B	SE	Wald	P-value	Hazard ratio (95% CI)
<b>BCSS</b>					
LN status	1.177	0.176	44.662	0.000	3.245 (2.298–4.582)
Size	0.720	0.125	33.366	0.000	2.055 (1.609–2.624)
PR-negative	0.753	0.236	10.154	0.001	2.124 (1.336–3.376)
Grade	0.413	0.155	7.092	0.008	1.511 (1.115–2.047)
ER-negative	0.575	0.246	5.460	0.019	1.777 (1.097–2.879)
HER2-positive	0.132	0.187	0.496	0.481	1.141 (0.791–1.646)
<b>DFS</b>					
LN status	0.935	0.146	40.741	0.000	2.547 (1.911–3.393)
PR-negative	0.663	0.197	11.372	0.001	1.941 (1.320–2.854)
Size	0.582	0.108	28.818	0.000	1.790 (1.447–2.214)
Grade	0.390	0.127	9.389	0.002	1.477 (1.151–1.895)
ER-negative	0.375	0.211	3.150	0.076	1.455 (0.962–2.200)
HER2-positive	0.084	0.167	0.255	0.614	1.088 (0.785–1.508)

Abbreviations: BCSS = breast cancer-specific survival; DFS = disease-free survival; ER = oestrogen receptor; HER2 = human epidermal growth factor receptor 2; LN = lymph node; PR = progesterone receptor.

additional adjuvant therapy might be most effective. Unfortunately, anthracycline-based regimes employed within the study period do not appear to have restored the BCSS or DFS for

PR-negative to that of PR-positive (Figure 3C and D). However, PR expression was not used for decision-making regarding the use of chemotherapy during the study. The prognostic significance of PR expression is present within all breast cancer subsets and, furthermore, the deleterious influence of the absence of PR expression (in ER-positive disease) is still evident despite endocrine therapy (Viale *et al*, 2007; Dowsett *et al*, 2008).

Multivariate analysis demonstrates that PR expression is an independent prognostic variable more powerful than ER for predicting BCSS and DFS. For example, in this series, in the ER-positive subgroup, PR expression is second only to LN status in predicting BCSS (all patients in this subgroup received endocrine therapy). Progesterone receptor is, therefore, a powerful, independent predictor of outcome with a hazard ratio (HR) of 3.24 for BCSS and 2.72 for DFS in all patients and 1.76 for BCSS and 1.72 for DFS in the endocrine-treated ER-positive subgroup. Oestrogen receptor-positive, LN-negative patients fall into a good prognostic group and may receive endocrine therapy as their only adjuvant, systemic therapy. However, survival analysis of this group again shows that absence of PR expression predicts significantly poorer outcome with a HR of 3.17 for BCSS and 2.44 for DFS.

The expression of PR is directly related to oestrogen binding to ERs and the function of PR is dependent on the normal structure and function of ER (Horwitz and McGuire, 1978; Yu *et al*, 1981). Thus, simplistically, absence of PR expression may simply be a marker of loss of normal ER pathway function that would account for the relative unresponsiveness to endocrine therapy that we have demonstrated in this study. A number of possible mechanisms for the development of ER-positive, PR-negative breast cancers have been postulated including downregulation of PR by crosstalk with the epidermal growth factor receptor pathways (recently reviewed (Thakkar and Mehta, 2011)). Our finding that ER-positive, PR-negative breast cancer is more likely than ER-positive, PR-positive to be HER2-positive (18% vs 8%,  $P < 0.001$ ) is consistent with these data (Dowsett *et al*, 2001; Arpino *et al*, 2005).

On the basis of an assessment at the time of primary diagnosis, ER-positive, PR-negative breast cancers have a poorer prognosis than ER-positive, PR-positive cancers. We have previously shown that when ER-positive breast cancer recurs, it frequently shows a change from PR-positive to PR-negative (26%) suggesting that loss of PR expression and the development of hormone therapy unresponsiveness occur with disease progression (Thompson *et al*, 2010).

The prognostic effect of PR-negativity in the ER-positive, HER2-negative group becomes most pronounced beyond 6 years of follow-up where the survival curves diverge (Figure 2A). This corresponds to the period beyond standard endocrine therapy of 5 years raising the possibility that extended adjuvant endocrine therapy could improve the outcome of these patients. This question might be answered by subgroup analysis in clinical trials of extended adjuvant endocrine therapy such as MA17 (Jin *et al*, 2012). This finding also emphasises the importance of extended follow-up to identify markers of late relapse, which will become increasingly important as the survival of breast cancer patients improves with better management.

**CONCLUSION**

Absence of PR expression in primary breast cancer is strongly and independently associated with worse prognosis and this effect is seen in all subgroups including the ER-positive LN-negative group that usually has a particularly good prognosis. Assessment of PR expression by IHC in breast cancer is already subject to well-established and rigorous QA measures. Thus, PR expression could be used to identify patients in, otherwise good prognostic groups, who might benefit from additional adjuvant chemotherapy, extended endocrine therapy and/or treatments targeting growth factor receptor pathways.

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