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Androgen receptor expression predicts beneficial tamoxifen response in oestrogen receptor-α-negative breast cancer

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Background: Although the androgen receptor (AR) is frequently expressed in breast cancer, its relevance in the disease is not fully understood. In addition, the relevance of AR in determining tamoxifen treatment efficiency requires evaluation.

Purpose: To investigate the tamoxifen predictive relevance of the AR protein expression in breast cancer.

Methods Patients were randomised to tamoxifen 40 mg daily for 2 or 5 years or to no endocrine treatment. Mean follow-up was 15 years. Hazard ratios were calculated with recurrence-free survival as end point.

Results: In patients with oestrogen receptor (ER)-negative tumours, expression of AR predicted decreased recurrence rate with tamoxifen (hazard ratio (HR) = 0.34; 95% confidence interval (CI) = 0.14–0.81; $P = 0.015$), whereas the opposite was seen in the AR− group (HR = 2.92; 95% CI = 1.16–7.31; $P = 0.022$). Interaction test was significant $P < 0.001$. Patients with triple-negative and AR+ tumours benefitted from tamoxifen treatment (HR = 0.12; 95% CI = 0.014–0.95 $P = 0.044$), whereas patients with AR− tumours had worse outcome when treated with tamoxifen (HR = 3.98; 95% CI = 1.32–12.03; $P = 0.014$). Interaction test was significant $P = 0.003$. Patients with ER+ tumours showed benefit from tamoxifen treatment regardless of AR expression.

Conclusions: AR can predict tamoxifen treatment benefit in patients with ER− tumours and triple-negative breast cancer.

Breast cancer is a heterogeneous disease (Perou *et al*, 2000; Carey *et al*, 2006). Current clinical subgrouping is based on protein expression of oestrogen receptor alpha (ER), progesterone receptor (PgR) and human epidermal growth factor receptor 2 (HER2) into three groups: Luminal (ER+, PgR+/−, HER+/−), HER2 amplified (ER−, PgR−, HER2+) and triple-negative breast cancer (TNBC: ER−, PgR−, HER2−). The ER+ breast cancers constitute 70–80% of all cases (Niemeier *et al*, 2010; Qi *et al*, 2012). Endocrine treatment is the primary treatment for these cases and improves patient outcome (Palmieri *et al*, 2014). ER− disease is heterogeneous and has poorer outcome (Prat *et al*, 2015). TNBC cases are difficult to treat and are associated with increased risk of recurrence and poor prognosis compared with other subtypes. The androgen receptor (AR) is frequently expressed in normal breast epithelium and in malignant breast tumours (up to 80%; Moinfar *et al*, 2003; Park *et al*, 2010), its expression differs in breast cancer subtypes, with 84–95% in luminal, 50–63% in HER2 amplified and 10–53% in TNBC (Chia *et al*, 2015). Despite the high prevalence, the role of AR in breast cancer is not fully understood. In breast cancer *in vitro* models, androgens induce either growth inhibition or increased proliferation (Birrell *et al*, 1995). This varying response is not clearly elucidated, but seems to be related to the expression of ER, PgR and HER2 (Cops *et al*, 2008; Peters *et al*, 2009; Ni *et al*, 2011). In ER+ breast cancer cell lines, AR is reported to inhibit proliferation in a manner depending on the ER/AR ratio, with a higher AR to ER ratio indicating a stronger inhibition of proliferation. This signalling is reported to be mediated through the oestrogen response element

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(Peters *et al*, 2009). Several studies have reported an improved patient outcome associated with increased AR expression in ER+ breast cancer (Castellano *et al*, 2010; Park *et al*, 2011; Park *et al*, 2012; Qu *et al*, 2013; Vera-Badillo *et al*, 2013). In the ER−, AR+ subgroup, AR has been reported to predict improved patient outcome (Agoff *et al*, 2003); however, AR has also been associated with worse outcome (Hu *et al*, 2011). The molecular apocrine microarray profile (ER−, AR+) described by Farmer *et al* (2005) is associated with worse outcome in the material where it was first tested, but has also been associated with favourable outcomes (Lakis *et al*, 2014). In ER− and HER2+ cell lines, AR activated the Wnt and HER2 pathways, and induced proliferation (Ni *et al*, 2011). In addition, AR and HER2 are positively correlated in several breast cancer cohorts (Micello *et al*, 2010; Niemeier *et al*, 2010; Park *et al*, 2011). TNBC is a diverse group, which is difficult to treat with high risk of recurrence and poor prognosis compared with other subtypes. However, the TNBC AR+ group has been shown to respond to AR antagonists, and in addition a portion of these express the luminal AR gene expression profile, which resembles that of ER+ breast cancer (Lehmann *et al*, 2011; Chia *et al*, 2015). Further, several reports on TNBC indicate a positive correlation between AR expression and better clinical outcome (Rakha *et al*, 2007; He *et al*, 2012; Tang *et al*, 2012; Thike *et al*, 2013).

Tamoxifen is a selective ER modulator (SERM) used to treat ER+ breast cancer resulting in improved outcome. Patients with ER− breast cancer generally do not respond to this therapy, however, there is a fraction that does respond (McGuire, 1975; E.B.C.T.C.G, 1992, 1998). The relevance of AR in determining tamoxifen treatment efficiency is not fully elucidated, with opposing findings complicating the clinical relevance. In one study by Park *et al* (2012), AR status was shown to be a positive factor in determining treatment response to tamoxifen in patients with ER+ breast cancer, on the other hand, using an *in vitro* model, (De Amicis *et al* (2010) showed that increased AR to ER ratio was an indicator of tamoxifen resistance.

The aim of this study was to investigate the prognostic (defined as outcome irrespective of treatment status) and tamoxifen predictive (defined as outcome influenced by treatment) relevance of AR protein expression in breast cancer and its subgroups. This was done using a retrospective cohort of lymph node-negative postmenopausal breast cancer patients with a long follow-up period that were randomised to no endocrine treatment or tamoxifen treatment, independently of ER expression.

PATIENTS AND METHODS

The present study was designed and presented with regard to the reporting recommendations for tumour marker prognostic studies (REMARK) guidelines (McShane *et al*, 2006).

Patient material. This retrospective cohort study was conducted using tumours from patients participating in a randomised tamoxifen trial conducted during 1976–1990 in Stockholm, Sweden. Results and details of the 'Stockholm Trial' were previously described (Rutqvist *et al*, 2007). All patients were post-menopausal with tumours $\leqslant$30 mm and were negative for axillary lymph node involvement (N_0). The patients received either breast-conserving surgery followed by radiation treatment with a dose of 50 Gy with 2 Gy per fraction 5 days weekly or modified radical mastectomy. After surgery, patients were randomised to tamoxifen 40 mg daily or to no endocrine treatment. After 2 years of tamoxifen treatment, most disease-free patients were randomised to tamoxifen for an additional 3 years or no further therapy. Tumour material from 912 women was available for the current investigation. The mean follow-up period for all patients was 15 years, for patients evaluated for AR the follow-up was 14 years and the mean follow-up until a recurrence occurred was 6 years. A retrospective study of biomarkers was approved by the Research Ethics Committee at the Karolinska Institute (dnr 97–451, with amendments). To conduct tissue microarray analysis, a pathologist selected representative parts of the tumours. Three tissue cores per patient with a diameter of 0.8 mm were chosen and transferred to paraffin blocks using a manual arrayer (Beecher Instruments, Sun Prairie, WI). From these blocks, sections were cut and placed on slides, forming the basis of the tissue microarray. ER and PgR status were determined with cutoff levels at 1% and 10% of positively stained tumour cell nuclei, respectively. For ER, the original cytosol measurements were used in the case of missing immunohistochemical data, with a cutoff of 0.05 fmol μg^{-1} DNA (71 (9%) of ER cases; Rutqvist *et al*, 2007). HER2 expression scored 0–3+ was previously described (Jansson *et al*, 2009), and for all analysis in this paper, the clinically used 3+ expression was considered HER2+. Grade was scored previously according to the Nottingham grade system (Jerevall *et al*, 2011).

Determination of AR expression through immunohistochemistry. Deparaffinisation, rehydration and antigen retrieval were accomplished using the Pre-Treatment Module for Tissue Specimens (DAKO, Glostrup, Denmark) with Buffer Envision FLEX (Target Retrieval Solution; DAKO) for high pH, and treated according to the manufacturer's instructions. Endogenous peroxidases were blocked with 3% H_2O_2 + MeOH for 5 min, washed in PBS and treated with Protein Block (DAKO) for 10 min. The primary antibody, monoclonal mouse anti-human AR (DAKO, clone AR441), was diluted 1 : 400 and applied to the tissue sections and incubated overnight at 4 °C. The slides were washed and a secondary antibody, DAKO Envision + System – HRP K4000 Anti-Mouse (DAKO), was applied to the slides and incubated for 30 min at room temperature. The slides were stained with 3.3-diaminobenzidin hydrochloride/H_2O_2 and incubated for 8 min. After washing, the tissue sections were counterstained with Mayer's Haematoxylin (Sigma-Aldrich, St Louis, MO, USA), dehydrated and mounted with Pertex (Histolab, Göteborg, Sweden).

Sample scoring was done without knowledge of clinical or pathological data for patients. The tumour cell nuclei were scored and the occurrence of positive nuclei was divided into three groups, 0% (−); 1–10% (+) and >10% (++). Two investigators (JG and AJ) evaluated all slides independently with a concordance rate of 97%. In the remaining 3% of cases, both evaluators re-evaluated the sample jointly to reach a consensus. Intra-patient heterogeneity was present in ~1% of cases, and in these cores the choice was made to only evaluate the core with the highest percentage of stained cells in these cases. Representative slides were photographed using an Axio cam ICc5 digital camera (Zeiss, Oberkochen, Germany) using the AxioVision software (Zeiss). Validation of the antibody is described in the Supplementary Materials.

Statistical analysis. The relationships between grouped variables were analysed using Spearman's rank order correlation. To compensate for multiple testing, $P<0.01$ was set as significant. The survival curves were produced according to the life table method described by Kaplan and Meier, and differences between groups were evaluated with Gehan's generalised Wilcoxon test. Patients with missing data were excluded. Univariate and multivariate analyses were conducted using Cox proportional hazards regression and $P<0.05$ was considered significant. The chosen end point was recurrence, defined as regional relapse or distant metastasis. Breast cancer-specific survival was chosen as a secondary end point. The statistical package Statistica 12.0 (StatSoft Scandinavia, Uppsala, Sweden) was used for all calculations with the exception of the comparison of the TMA and the

original cohort, where STATA 13.1 (StataCorp, Stocholm, Sweden) was used.

RESULTS

AR expression in breast cancer. Using immunohistochemistry, the expression of AR was analysed in tumours from 912 patients. A flow-chart of patients included in the initial tamoxifen trial and further included in the current analysis is shown in (Supplementary Figure 1). The patient selection available as TMA resembles the original patient cohort in terms of recurrence rate comparing the two treatment arms (hazard ratio (HR) = 0.623 95% confidence interval (CI) = 0.486–0.799; $P<0.001$ compared with HR = 0.678 95% CI = 0.571–0.805; $P<0.001$) for the TMA and original cohort, respectively. Similar results were also acquired when selecting for only ER+ cases (HR = 0.581; CI = 0.436–0.773; $P<0.001$ and HR = 0.612; CI = 0.498–0.752; $P<0.001$) for the TMA and original cohort, respectively (Supplementary Figure 2). The patient and tumour characteristics were also similar (supplementary Table 1). The specificity of the anti-AR antibody was determined using western blot, where a single band at 110 kDa was detected, which corresponds to the size of AR in western blot (Supplementary Figure 3). Tissue microarrays from 769 (84.3%) patients were successfully scored, of these, 372 (48.4%) patients did not receive any endocrine treatment and 397 (51.6%) patients received tamoxifen treatment. There were 136 (17.7%) cases with 0% AR expression (−), 33 (4.3%) cases showed AR expression in 1–10% of the tumour cells (+), and 601 (78%) cases showed AR expression in >10% of the tumour cells (++; Table 1). Representative images of immunohistochemical staining of AR protein expression can be seen in (Supplementary Figure 4). There was a significant association of AR with ER ($P<0.0001$) and PgR expression ($P<0.0001$). There was an inverse correlation between AR expression and grade ($P<0.0001$), mitotic index ($P<0.0001$) and tumour size ($P=0.0005$; Table 1). AR positivity varied based on hormone receptor and HER2 expression; 532 of 590 (90.2%), 84 of 160 (52.5%), 38 of 46 (82.6) and 28 of 87 (32.2) were AR+ in ER+, ER−, HER2 amplified and TNBC tumours, respectively (Supplementary Table 2). Supplementary Table 2 shows AR frequency based on clinical subgrouping. In the group of ER− tumours, AR expression was strongly correlated to high expression of HER2 ($P<0.0001$). When analysing the prognostic and treatment predictive value of AR, ⩾1% was considered positive.

Table 1. Expression of androgen receptor in relation to clinicopathological characteristics

		AR, *n* (%)			
	n	−	+	++	***P*-values**
	770	136 (17.7)	33 (4.3)	601 (78.0)	
Tumour size					
⩽20 mm	586	87 (14.9)	25 (4.3)	474 (80.9)	**$P=0.0005$**
>20 mm	166	44 (26.5)	8 (4.8)	114 (68.7)	
ER status 1%[a]					
ER−	160	76 (47,5)	12 (7.5%)	72 (45)	**$P<0.0001$**
ER+	590	58 (9.8)	20 (3.4)	512 (86,8)	
PgR status 10%					
PR−	326	95 (29.1)	21 (6.4)	210 (64.4)	**$P<0.0001$**
PR+	358	29 (8.1)	7 (2.0)	322 (90.0)	
HER2 status					
0–2+	629	115 (18.3)	27 (4.3)	487 (77.4)	$P=0.38$
3+	82	12 (14.6)	3 (3.7)	67 (81.7)	
Grade					
1	123	12 (9.8)	7 (5.7)	104 (84.6)	**$P<0.0001$**
2	381	48 (12.6)	12 (3.2)	321 (84.3)	
3	157	51 (32.5)	8 (5.1)	98 (64.4)	
Mitosis					
1	432	46 (10.7)	18 (4.2)	368 (85.2)	**$P=0.0001$**
2	103	20 (19.4)	3 (2.9)	80 (77.7)	
3	126	45 (35.7)	6 (4.8)	75 (59.5)	
Tamoxifen					
No tamoxifen	373	64 (17.1)	19 (5.1)	290 (77.8)	$P=0.54$
Tamoxifen	397	72 (18.1)	14 (3.5)	311 (78.3)	

Abbreviations: AR = androgen receptor, ER = oestrogen receptor, PgR = progesterone receptor, HER2 = human epithelial growth factor receptor 2.
[a]0.05 fmol μg^{-1} DNA when no immunohistochemical data was available. Bold entries indicate $P<0.05$.

AR and recurrence-free survival

AR predicts benefit from tamoxifen treatment in patients with ER− tumours. For patients with ER− tumours and no tamoxifen treatment, AR was a negative prognostic factor (HR = 2.64; 95% CI = 1.04–6.66; $P=0.040$; Figure 1 and Supplementary Table 3). In univariate analysis of ER− disease, AR+ cases were associated with a beneficial tamoxifen response (HR = 0.34; 95% CI = 0.14–0.81; $P=0.015$), the opposite was observed in patients with AR− tumours (HR = 2.92 95% CI 1.16–7.31; $P=0.022$), test for interaction $P<0.001$ (Figure 2 and Table 2). Furthermore, a multivariate interaction test between tamoxifen and AR adjusting for tumour size and grade was significant ($P=0.002$). AR expression was not a prognostic factor in patients with ER− and HER2+ tumours (HR = 1.32; 95% CI = 0.16–10.59; $P=0.795$) and AR could not predict tamoxifen treatment outcome in this group (Supplementary Table 3 and Table 2).

AR is a prognostic marker in patients with TNBC. In the subgroup of tamoxifen untreated patients with TNBC, high AR expression was associated with poor prognosis (HR = 3.80; 95% CI = 1.11–12.99; $P=0.033$; Figure 3 and Supplementary Table 3). Tamoxifen treatment benefit was seen in TNBC patients with tumours positive for AR (HR = 0.12; 95% CI = 0.014–0.95 $P=0.044$), whereas those with tumours without AR expression had increased recurrence rate if treated with tamoxifen (HR = 3.98; 95% CI = 1.32–12.03; $P=0.014$). The interaction test between tamoxifen and AR was significant ($P=0.003$; Figure 4 and Table 2).

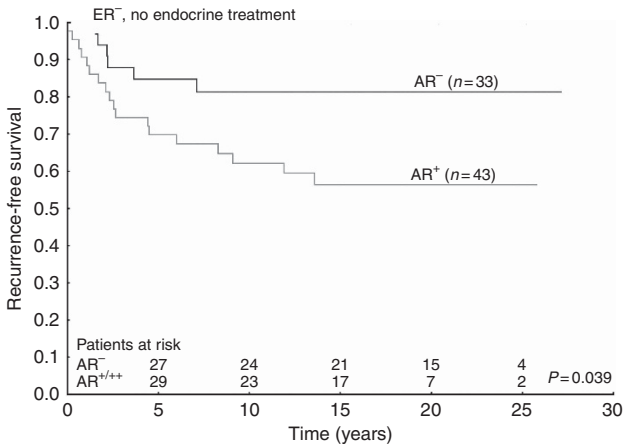

Figure 1. Survival curves with recurrence-free survival as end point for patients who did not receive endocrine treatment, grouped according to nuclear androgen receptor expression. All patient tumours were oestrogen receptor negative.

AR expression in patients with ER+ tumours with or without tamoxifen treatment. In patients with ER+ tumours without tamoxifen treatment, no significant difference was found when grouped by AR status ($P>0.05$; Supplementary Figure 5 and Supplementary Table 3). Patients with ER+ tumours showed benefit from tamoxifen regardless of AR expression (Supplementary Figure 6 and Table 2).

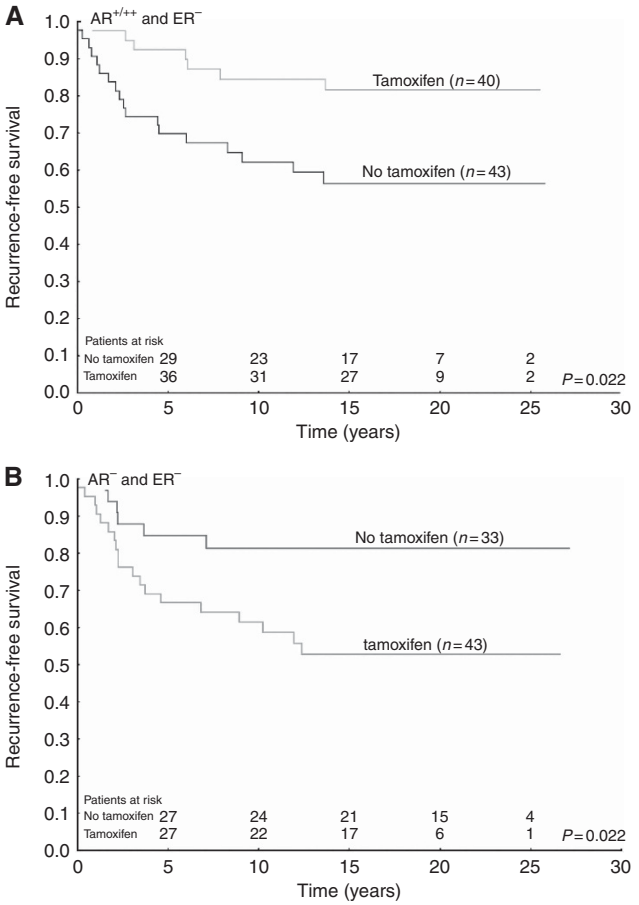

Figure 2. Survival curves with recurrence-free survival as end point, grouped according to treatment status. All patient tumours were oestrogen receptor negative. (**A**) Patients with ⩾1% androgen receptor expression; (**B**) Patients with no androgen receptor expression.

AR expression and breast cancer-specific survival. In terms of prognosis, AR had no significant impact on breast cancer-specific survival in either of the tested subgroups; however, there was a trend for ER− and TNBC patients to have worse outcome when AR+ (Supplementary Table 3). When treated with tamoxifen, patients with ER− and AR− tumours had increased risk (HR = 3.04; 95% CI = 1.00–9.25; $P=0.049$), as did TNBC AR− patients (HR = 3.97; 95% CI = 1.12–14.10; $P=0.033$). The test for interaction between tamoxifen and AR was significant for both the ER− and TNBC groups ($P=0.004$ and 0.009, respectively; Table 2). There was a benefit for ER+ and AR+ patients (HR = 0.43; 95% CI = 0.27–0.70; $P=0.001$); however, there was a similar trend towards benefit in the AR− group, and the interaction test was not significant (Table 2).

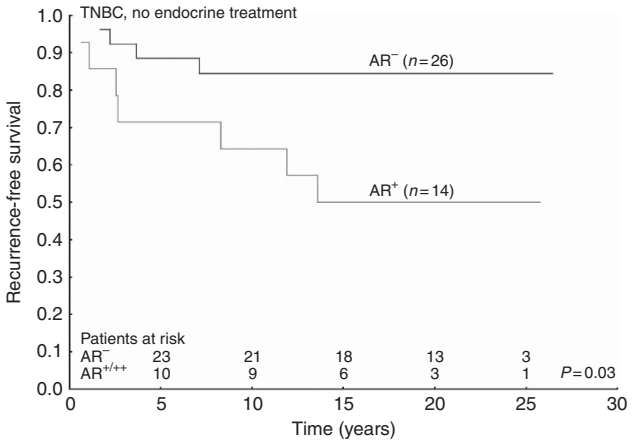

Figure 3. Survival curves with recurrence-free survival as end point for patients who did not receive endocrine treatment, grouped according to nuclear androgen receptor expression. All patient tumours were triple negative (oestrogen receptor, progesterone receptor and epidermal growth factor receptor 2 negative).

Table 2. Risk based on tamoxifen treatment status

	Recurrence-free survival				Breast cancer-specific survival		
	HR (95% CI)	***P***	**Pi**		**HR (95% CI)**	***P***	**Pi**
ER−			**<0.001**	**ER−**			**0.004**
AR− (*n*=76)	2.92 (1.16–7.31)	**0.022**		AR− (*n*=76)	3.04 (1.00–9.25)	**0.049**	
AR+ (*n*=83)	0.34 (0.14–0.81)	**0.015**		AR+ (*n*=83)	0.48 (0.18–1.28)	0.144	
ER− HER2+			0.946	**ER− HER2+**			0.794
AR− (*n*=8)	0.39 (0.02–6.62)	0.513		AR− (*n*=8)	0.39 (0.022–6.62)	0.513	
AR+ (*n*=38)	0.39 (0.12–1.32)	0.131		AR+ (*n*=38)	0.43 (0.11–1.71)	0.231	
TNBC			**0.003**	**TNBC**			**0.009**
AR− (*n*=58)	4.14 (1.38–12.41)	**0.011**		AR− (*n*=59)	3.97 (1.12–14.10)	**0.033**	
AR+ (*n*=28)	0.12 (0.01–0.95)	**0.044**		AR+ (*n*=28)	0.18 (0.02–1.53)	0.115	
ER+			0.531	**ER+**			0.935
AR− (*n*=58)	0.30 (0.10–0.96)	**0.042**		AR− (*n*=58)	0.16 (0.019–1.33)	0.089	
AR+ (*n*=532)	0.47 (0.33–0.67)	**<0.001**		AR+ (*n*=532)	0.43 (0.27–0.70)	**0.001**	

Abbreviations: HR = hazard ratio; CI = confidence interval; ER = oestrogen receptor; AR = androgen receptor; HER2 = human epithelial growth factor receptor 2; TNBC = triple-negative breast cancer (ER, progesterone and HER2 negative), Pi = *P*-interaction. Bold entries indicate $P<0.05$.

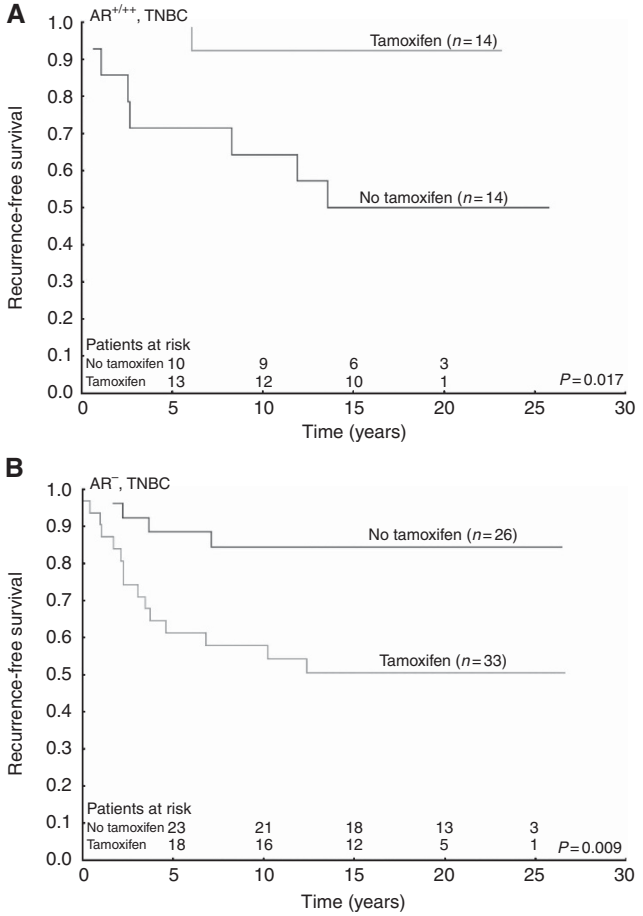

Figure 4. Survival curves with recurrence-free survival as end point, grouped according to treatment status. All patient tumours were triple negative. (**A**) Patients with ⩾1% androgen receptor expression; (**B**) patients with no androgen receptor expression.

DISCUSSION

Here, we present the findings of a large retrospective cohort of nodal negative, low-risk breast cancer patients who were randomised to endocrine or no endocrine treatment independently of ER status. We demonstrate a negative relationship of AR expression with tumour size, grade and mitotic index, and a positive correlation with ER and PR, and in ER− tumours, with HER2, all of which is consistent with similar studies (Gonzalez-Angulo *et al*, 2009; Mrklic *et al*, 2013; Shibahara *et al*, 2013; Elebro *et al*, 2014). In this study, AR expression was detected in 82% of the tumours, previous studies observed that the frequency of AR+ breast cancer in 58.8–90.5% of cases (Niemeier *et al*, 2010; Hu *et al*, 2011; Park *et al*, 2011; Elebro *et al*, 2014). The different percentages of AR+ tumours may depend on use of different antibodies, use of paraffin embedded or frozen sections and varying cutoff values.

We gained similar results regardless of whether we used ⩾1% or ⩾10% cutoff for AR when conducting survival analysis. However, there was slightly better significance when ⩾1% nuclei stained was used. As 1% cutoff is the current standard for ER, and a large number of studies regarding the role of AR also use this cutoff, it was chosen for all survival analyses in the current study (Rakha *et al*, 2007; Castellano *et al*, 2010; Hu *et al*, 2011; Thike *et al*, 2013; Choi *et al*, 2015).

ER− breast cancers are difficult to treat, and have poorer outcome than ER+ disease (Prat *et al*, 2015). Here we show that the ER− AR+ cases have worsened outcome. A similar trend was shown previously in the Nurses' Health study for breast cancer-specific survival (Hu *et al*, 2011). Adverse outcome was also seen using the molecular apocrine profile on the Van 't Veer and Sorlie data sets (Farmer *et al*, 2005). In addition, a recent publication showed a connection between AR negativity and increased expression of metabolic proteins, which correlated with worse outcome (Noh *et al*, 2014). Peters *et al* (2009) and Tokunaga *et al* (2013) failed to show any significance of AR in terms of prognosis when using 75% cutoff for AR. Improved survival was seen in a small ($n=69$) cohort of primarily high-grade and metastatic ER− cases (Agoff *et al*, 2003). Two recent meta-analyses have shown that AR positivity is a positive prognostic factor in ER− and ER+ breast cancer (Qu *et al*, 2013; Vera-Badillo *et al*, 2013). However, the selection appears somewhat biased as no studies were included that indicate AR as a negative factor. In addition, many of the patients in both meta-analyses were of Asian descent, which could indicate that the role of AR may differ in different populations. We show that the ER− and AR+ subgroup of patients benefitted from tamoxifen in terms of recurrence, with a similar trend in terms of breast cancer-specific survival, whereas patients who were both ER− and AR− fared worse on adjuvant tamoxifen treatment in terms of both recurrence and breast cancer-specific survival. One explanation for this could be the ability of tamoxifen to bind directly to AR (Fang *et al*, 2003). In prostate cancer, tamoxifen was shown to inhibit AR activity and cell replication (Mangerini *et al*, 2012; Piccolella *et al*, 2013) and the selective ER downregulator fulvestrant effectively downregulated AR and induced growth inhibition in several human prostate cancer cells (Bhattacharyya *et al*, 2006). These results suggest an anti-androgenic effect of anti-oestrogens. Our finding may have clinical implications and we suggest that tamoxifen and possibly other anti-oestrogens should be evaluated in breast cancer patients with ER− but AR+ tumours.

To evaluate the impact of HER2 expression on the role of AR in ER− patients, we examined the ER− and HER2+ subgroup. We found no significant change in outcome based on AR expression, which could be attributed to the small sample size ($n=21$). Lin Fde *et al* (2012) reported increased grade in the AR and ER− HER2+ cohort.

TNBC cases are heterogeneous and have poor prognosis. There is a strong need to find better therapeutic targets for these patients. Our results indicate that high AR expression was associated with worse outcome, which is supported by previous findings (Choi *et al*, 2015; Luo *et al*, 2010). However, others report no role of AR expression (McGhan *et al*, 2013), or improved outcome in AR+ TNBC (Rakha *et al*, 2007; He *et al*, 2012; Tang *et al*, 2012; Thike *et al*, 2013). There is no clear indicator as to why these studies have opposing results, the number of patients with high grade, metastatic and nodal involvement varied somewhat between studies, as did AR cutoff value and the fraction of AR+ patients, which ranged from 13 to 25.8%. We did notice a trend towards lower grade and less metastasis and nodal involvement in the studies where AR was an indicator of poor prognosis. In AR+ TNBC cases, tamoxifen treatment provided significantly improved outcome. Of note is the adverse outcome in the AR− cases, who fared worse on tamoxifen treatment in both outcomes studied. In the current cohort, 28 TNBC patients were AR+, and a larger patient cohort is needed to strengthen these results.

No prognostic impact of AR expression in ER+ patients was observed, despite this being reported previously by several groups for ER+ patients (Castellano *et al*, 2010; Park *et al*, 2011; Park *et al*, 2012; Qu *et al*, 2013). It is worth noting several differences between this and many previously published studies in terms of prognosis, where this study presents findings from patients with nodal negative disease who did not receive endocrine treatment. Many of the authors reporting that AR expression correlated to

positive outcome analysed more heterogeneous patient groups in terms of stage, age and endocrine treatment status (Peters *et al*, 2009; Niemeier *et al*, 2010; Hu *et al*, 2011; Park *et al*, 2011; Elebro *et al*, 2014).

The beneficial effects of tamoxifen treatment in ER+ patients remained regardless of AR status. And although an improvement in breast cancer-specific survival was seen in the current cohort, the AR− group had a similar trend, and the interaction test was not significant. In a previous study, AR status predicted good response to tamoxifen in ER+ patients (Park *et al*, 2012). However, these findings are from a cohort with different AR cutoffs and higher grade in the patient population as compared with ours, complicating comparison of results. De Amicis *et al* (2010) demonstrated that AR may constitute a possible mechanism for tamoxifen resistance, however, these findings are based on *in vitro* analysis and a small ($n=9$) patient sample and no such association could be detected in the present study.

Although the ability to target AR using SERMs opens for further treatment options, the clinical benefit of anti-androgen treatment in AR+ patients was shown 40 years ago in metastatic breast cancer upon the administration of dihydrotestosterone and fluoxymesterone (Goldenberg, 1964; Goldenberg *et al*, 1975; Manni *et al*, 1981). Despite the advantages, these treatments fell out of use because of virilising side effects, and the advent of SERMs. Recently, the ER− and AR+ group has been shown to benefit from modern androgen therapy (Doane *et al*, 2006). Further, Lehmann *et al* (2011) showed that TNBC and AR+ cells responded well to the AR antagonist bicalutamide. A clinical trial examining the clinical outcome of bicalutamide in stage IV patients with AR+ and ER and PgR− disease (NCT00468715) was completed recently, the results indicating that the treatment was well-tolerated and yielded clinical benefit (Gucalp *et al*, 2013). Two phase 2 clinical trials (NCT02353988 and NCT02348281) are evaluating the benefit of bicalutamide in TNBC, the results of these studies are eagerly awaited. In addition, the first preview of the results of clinical trial NCT01889238 studying the effect of Enzalutamide in TNBC was presented recently, indicating clinical benefit Traina *et al* (2015). In addition to ER− cohorts, Overmoyer (2014) show that targeting the AR utilising the selective AR modulator Enobosarm in ER+ breast cancer was well-tolerated and has significant clinical benefit. These new treatment options provide an important opportunity in the treatment of AR+ patients.

Known limitations of this study are that the use of retrospective materials could infer a potential bias on patient selection for the current study. Furthermore, although no known bias exists for the patients who were included in this study for which no AR staining could be made, it is not possible to determine if these patients would alter the results. In addition, the tamoxifen administration performed in this study follows an older clinical approach of 40 mg daily, compared with 20 mg daily, which is the current standard, this could reduce the external validity of these findings. Another possible result of the higher dose of tamoxifen could be increased AR antagonism, compared with 20 mg (Mangerini *et al*, 2012; Piccolella *et al*, 2013). In this study, $\geqslant 1\%$ ER was designated as positive, which is the clinical practice in several countries, however, similar results were obtained when $\geqslant 10\%$ ER was set as the limit for ER positivity.

CONCLUSION

We show that AR status might be used to identify a subgroup of patients with ER− tumours benefitting from adjuvant tamoxifen treatment. We interpret this to mean that patients with ER− tumours may have their tumours tested for AR and could be candidates for tamoxifen therapy. We also identified a subgroup of patients with TNBC who had AR+ tumours that may be treated with tamoxifen to improve outcome. These hypotheses generating observations need confirmation by further studies with larger number of ER− and TNBC patients in prospective cohorts.

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CONFLICT OF INTEREST

The authors declare no conflict of interest.

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