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The role of the AR/ER ratio in ER-positive breast cancer patients

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Abstract

The significance of androgen receptor (AR) in breast cancer (BC) management is not fully defined, and it is still ambiguous how the level of AR expression influences oestrogen receptor-positive (ER+) tumours. The aim of the present study was to analyse the prognostic impact of AR/ER ratio, evaluated by immunohistochemistry (IHC), correlating this value with clinical, pathological and molecular characteristics. We retrospectively selected a cohort of 402 ER+BC patients. On each tumour, IHC analyses for AR, ER, PgR, HER2 and Ki67 were performed and AR+ cases were used to calculate the AR/ER value. A cut-off of ≥2 was selected using receiver-operating characteristic (ROC) curve analyses. RNA from 19 cases with AR/ER≥2 was extracted and used for Prosigna-PAM50 assays. Tumours with AR/ER≥2 (6%) showed more frequent metastatic lymph nodes, larger size, higher histological grade and lower PgR levels than cases with AR/ER<2. Multivariate analysis confirmed that patients with AR/ER≥2 had worse disease-free interval (DFI) and disease-specific survival (DSS) (hazard ratios (HR) = 4.96 for DFI and HR = 8.69 for DSS, both $P \leq 0.004$). According to the Prosigna-PAM50 assay, 63% (12/19) of these cases resulted in intermediate or high risk of recurrence categories. Additionally, although all samples were positive for ER assessed by IHC, the molecular test assigned 47.4% (9/19) of BCs to intrinsic non-luminal subtypes. In conclusion, the AR/ER ratio ≥2 identifies a subgroup of patients with aggressive biological features and may represent an additional independent marker of worse BC prognosis. Moreover, the Prosigna-PAM50 results indicate that a significant number of cases with AR/ER≥2 could be non-luminal tumours.

Key Words
- breast cancer
- androgen receptor
- oestrogen receptor
- subtypes
- prosigna

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Introduction

Oestrogen receptor (ER) and progesterone receptor (PgR) are expressed in most breast cancers (BCs) (~75%) and both have wide prognostic and predictive utility (Early Breast Cancer Trialists' Collaborative Group 2011). In contrast, the clinical and biological significance of androgen receptor (AR) expression in BC is not fully defined. AR positivity has been detected in up to 61% of primary and metastatic BC lesions (Park *et al.* 2010, Hu *et al.* 2011, Yu *et al.* 2011) and approximately 75% of ER-positive (ER+) BCs are also AR positive (AR+). Several studies have shown that AR expression in luminal tumours (ER+) is associated with lower tumour

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grade, smaller tumour size, lower proliferative index (Ki67 level) and more importantly, AR expression in ER+ tumours is an independent prognostic factor of a good outcome (Castellano *et al.* 2010, Niemeier *et al.* 2010, Aleskandarany *et al.* 2016, Bozovic-Spasojevic *et al.* 2016). On the other hand, up to 31% of ER-negative (ER–) BCs are reported to be AR+ (Niemeier *et al.* 2010, Park *et al.* 2010), but the prognostic impact of AR expression in this subset of BCs is not clear (Luo *et al.* 2010, Hu *et al.* 2011, Park *et al.* 2011, Pistelli *et al.* 2014, Vera-Badillo *et al.* 2014, Hilborn *et al.* 2016, Jiang *et al.* 2016, Asano *et al.* 2017).

While the interaction between the signalling pathways of ER and AR (Peters *et al.* 2009) is well known, it is still ambiguous how the level of AR expression influences ER+ tumours. *In vitro* studies have shown that AR signalling inhibits oestrogen-induced proliferation of ER+ MCF7 BC cells (Ando *et al.* 2002, Greeve *et al.* 2004, Macedo *et al.* 2006, Cops *et al.* 2008). This inhibitory effect seems to be mediated by several mechanisms, but the most important is the ability of AR to compete with ER for binding of oestrogen response elements (EREs), preventing ER-dependent gene transcription (Need *et al.* 2012). In line with this observation, some studies have reported that increasing AR expression results in a greater androgen-dependent inhibition of ER function (Buchanan *et al.* 2005, Peters *et al.* 2009). However, other studies performed on ER+ MCF7 BC cells described an increase in proliferation when the AR signalling pathway is stimulated (Birrell *et al.* 1995, Lin *et al.* 2009). Moreover, Cochrane and coworkers (Cochrane *et al.* 2014) recently reported that high AR levels and low ER levels (higher AR/ER ratio) could be associated with a worse prognosis and tamoxifen (TAM) resistance.

Considering these data, the aim of the present study was to analyse the prognostic impact of AR expression with respect to ER (AR/ER ratio) in a large case series of ER+/HER2-negative (HER2–) BC patients. We evaluated if the AR/ER ratio may identify a subset of tumours with different clinical and pathological characteristics. In addition, in the subgroup of BCs with high AR/ER ratio values, we performed Prosigna-PAM50 assays to assess the molecular subtypes of these BCs.

Patients and methods

Study design and population

We collected a cohort of 402 ER+/HER2– primary invasive BC patients with available follow-up (Fig. 1), who underwent surgery from January 1998 to December 2012 at the Breast Unit of the Città della Salute e della Scienza of Torino, University Hospital of Torino in Turin, Italy. In the diagnostic setting, the cut-off value considered for ER and PgR positivity was ≥1%, as suggested by the St Gallen and ASCO/CAP Guideline Recommendations (Hammond *et al.* 2010, Coates *et al.* 2015), and the same cut-off was adopted for AR positivity (Castellano *et al.* 2010). For all cases, the following clinico-pathological data were obtained from the clinical charts and pathological reports: age, type of surgery (conservative surgery vs radical mastectomy), tumour size (<15 mm vs ≥15 mm), histological type, tumour grade and nodal involvement. Ethical approval for this study was obtained from the Comittee for human Biospecimen Utilization (Department of Medical Sciences – ChBU). The project provided an informed consent, obtained from the patients at the time of surgery due to the retrospective approach of the study, which did not impact on their treatment. The procedure for collecting the consent was approved by the Committee for human Biospecimen Utilization (Department of Medical Sciences – ChBU). All the cases were anonymously recorded, and data were accessed anonymously.

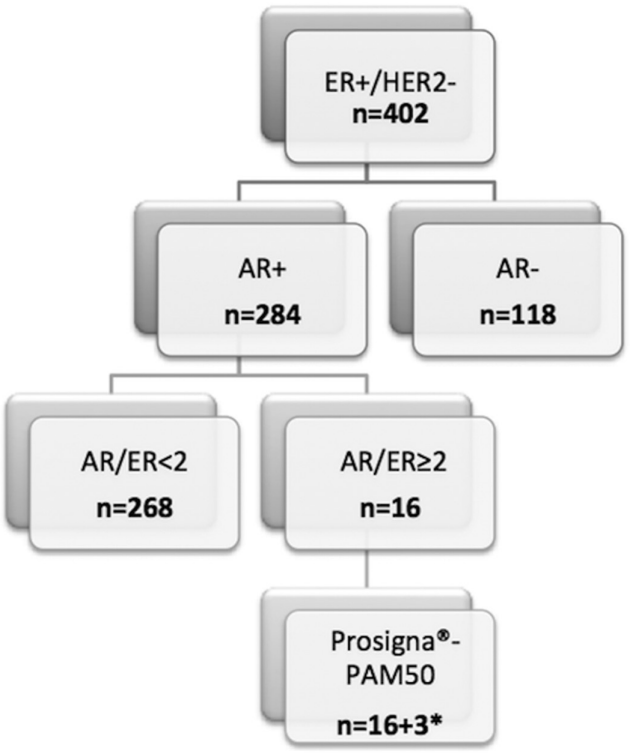

Figure 1
Study flowchart. ER – estrogen receptor; HER2 – human epidermal growth factor receptor 2; AR –androgen receptor. *Three additional cases (without follow-up) with a ratio of AR/ER≥2 were included for the Prosigna-PAM50 assay.

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Immunohistochemistry (IHC)

For each case, representative blocks were selected and multicore tissue microarrays (TMAs) were prepared, as previously described (Sapino *et al.* 2006). IHC was performed using an automated slide processing platform (Ventana BenchMark AutoStainer, Ventana Medical Systems, Tucson, AZ, USA) with the following primary antibodies: prediluted anti-ER rabbit monoclonal antibody (SP1, Ventana Medical Systems); prediluted anti-PgR rabbit monoclonal antibody (1E2, Ventana Medical Systems); anti-AR mouse monoclonal antibody (AR441, diluted 1:50, Dako) and anti-Ki67 mouse monoclonal antibody (MIB1, diluted 1:50, Dako). Measurement of HER2 expression was performed by an anti-HER2 polyclonal antibody (A0485, diluted 1:800, Dako). IHC equivocal cases (score 2+) were assessed for HER2 status by fluorescence *in situ* hybridization (FISH) (Marchio *et al.* 2009). Positive and negative controls (omission of the primary antibody and IgG-matched serum) were included for each immunohistochemical run. All cases were confirmed as ER+ and HER2−.

For statistical analyses and according to the St Gallen Consensus Recommendations (Coates *et al.* 2015), we adopted a cut-off of 20% for dichotomizing tumours as having low and high levels of PgR and Ki67. In addition, this cut-off agrees with the median Ki67 value of our laboratory, previously established to differentiate tumours with a higher proliferative index (Coates *et al.* 2015, Bustreo *et al.* 2016).

AR/ER ratio calculation

AR and ER nuclear staining percentages were compared. Post estimation ROC curve after logistic regression was used to establish the optimal AR/ER ratio cut-off value, which allowed us to subdivide the patients into those with good and worse prognosis as described below.

Statistical analyses

Pearson's chi-square test and Student's *t*-test were preliminarily performed to compare categorical and continuous variables, respectively, and to evaluate the potential differences in the variable distribution among the groups. The disease-free interval (DFI) was calculated from the date of surgical excision of the primary tumour to the date of the first relapse or last check-up. Disease-specific survival (DSS) was calculated from the surgical excision date of the primary tumour to the date of BC death or last check-up. Survival distribution curves were plotted using the Kaplan–Meier method and the statistical comparisons were performed using the log-rank test. Cox regression analyses were carried out on the DFI and DSS to calculate the crude and adjusted HR and 95% CIs for the different study group. The cases lost to follow-up and cases with non-breast cancer-related deaths were censored at the last follow-up. Models were created to evaluate the prognostic role of different variables. The proportional hazard assumption was assessed with the Schoenfeld residuals. This did not give reasons to suspect a violation of this assumption. The nature of the variables

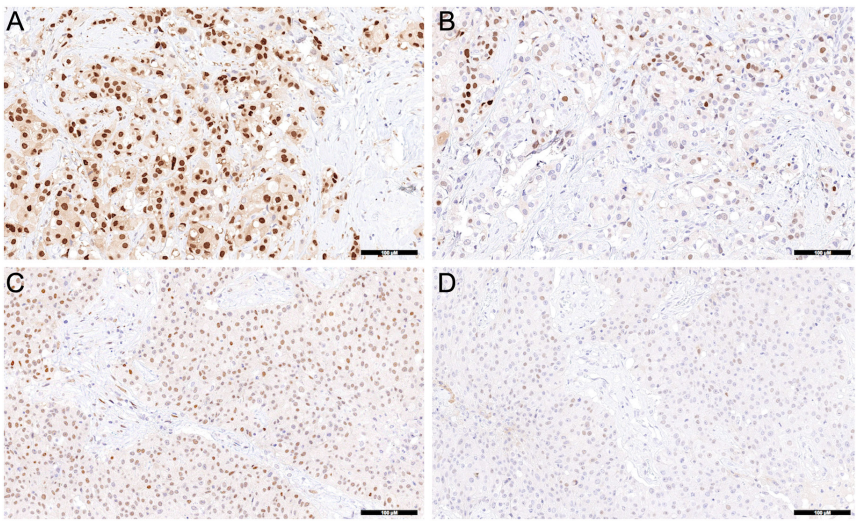

Figure 2
Immunohistochemical staining. Representative IHC for the androgen receptor (A and C) and estrogen receptor (B and D) in two BC cases with high AR levels with respect to ER (AR/ER≥2). Although ER is expressed at the IHC level in these cases, the molecular test classified them as non-luminal subtypes.

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Table 1 Clinical and pathological characteristics of ER+/AR+BC patients.

Clinical-pathological features	Total (%)	AR/ER<2 (%)	AR/ER≥2 (%)	*P* value (Fisher test)
Number of patients	284 (100)	268 (94.3)	16 (5.7)	–
Median age (interval)	62 (31–88)	62 (31–87)	65 (47–88)	0.309*
ER% median (interval)	90 (2–100)	95 (30–100)	18 (2–45)	<0.001*
AR% median (interval)	50 (5–99)	40 (5–99)	80 (25–99)	0.01*
Grade				
1	104 (36.7)	103 (38.4)	1 (6.3)	<0.001
2	128 (45)	122 (45.5)	6 (37.5)	
3	52 (18.3)	43 (16.1)	9 (56.2)	
Tumour size				
<15 mm	149 (52.5)	146 (54.5)	3 (18.7)	0.004
≥15 mm	135 (47.5)	122 (45.5)	13 (81.3)	
Metastatic lymph nodes				
0	191 (67.2)	183 (68.3)	8 (50)	<0.001
1–3	62 (21.8)	61 (22.8)	1 (6.3)	
4–9	22 (7.8)	18 (6.8)	4 (25)	
>9	9 (3.2)	6 (2.1)	3 (18.7)	
Ki67				
<20%	168 (59.2)	162 (60.4)	6 (37.5)	0.075
≥20%	116 (40.8)	106 (39.6)	10 (62.5)	
PgR				
<20%	42 (14.8)	33 (12.3)	9 (56.3)	0.001
≥20%	242 (85.2)	235 (87.7)	7 (43.7)	
Relapse				
No	233 (82)	225 (84)	8 (50)	0.001
Local	5 (1.8)	5 (1.9)	0	
Distant	46 (16.2)	38 (14.1)	8 (50)	
Surgery				
Quadrantectomy	188 (66.2)	181 (67.5)	7 (43.7)	0.036
Mastectomy	96 (33.8)	87 (32.5)	9 (56.3)	
Therapy†				
HT	198 (69.7)	190 (70.9)	8 (50)	0.073
CT	85 (29.4)	77 (28.7)	8 (50)	

Patients were grouped according to AR/ER ratio cut-off ≥2.
**P* value from Student's *t*-test; †1 patient refused therapy.
CT, patients who received hormonal therapy plus chemotherapy; HT, patients who received hormonal therapy.

Table 2 Univariate analysis in the group of ER+/AR+ BC patients.

Clinical-pathological features	DFI		DSS	
	HR (95% CI)	*P*	HR (95% CI)	*P*
Age	0.99 (0.96–1.02)	0.694	0.97 0.93–1.01	0.264
Grade	3.02 (1.96–4.66)	<0.001	5.26 (2.37–11.7)	<0.001
Tumor size ≥15 mm	6.97 (3.22–15.06)	<0.001	11.9 (2.74–52)	<0.001
Metastatic lymph nodes				
0	1			
1–3	2.92 (1.37–6.23)	<0.005	2.85 (0.71–11.4)	0.138
4–9	5.58 (2.41–12.9)	<0.001	12.2 (3.44–43.3)	<0.001
>9	15.5 (6.25–38.6)	<0.001	23.17 (5.74–93.3)	<0.001
Ki67≥20%	7.66 (3.55–16.52)	<0.001	12.25 (2.81–53.3)	<0.001
PgR≥20%	0.65 (0.34–1.25)	0.201	0.77 (0.27–2.16)	0.061
ER% expression	0.98 (0.97–0.99)	0.027	0.98 (0.96–0.99)	0.016
AR% expression	1.00 (0.99–1.01)	0.541	0.99 (0.98–1.02)	0.994
AR/ER≥2	7.55 (3.31–17.2)	<0.001	10.84 (3.52–33.3)	<0.001
HT vs CT	3.77 (2.02–7.03)	<0.001	3.85 (1.42–10.42)	0.008

CT, patients who received hormonal therapy plus chemotherapy; DFI, disease-free interval; DSS, disease-specific survival; HR, hazard ratio; HT, patients who received hormonal therapy.

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(continue/categorical) included in the models was evaluated considering literature reports and the results of the log-likelihood ratio test. For model selection, the Akaike information criterion (AIC) test was used. All statistical tests were two-sided. *P*-values <0.05 were considered significant. Statistical analyses were performed using STATA/SE12.0 Statistical Software (STATA, College Station, TX, USA).

Prosigna multigene prognostic assay

Sixteen ER+/HER2− BC cases with an AR/ER ratio ≥2 with long follow-up and 3 additional cases collected during the routine diagnostic assessment of ER and AR were selected for Prosigna-PAM50 analysis (NanoString Technologies, Seattle, WA, USA). Briefly, tissue obtained after macrodissection of formalin-fixed paraffin-embedded (FFPE) tumours were processed with a Roche FFPET RNA Isolation Kit (Roche). The isolated RNA was hybridized to 58 gene-specific probe pairs, plus 6 positive and 8 negative controls (Capture and Reporter Probes – Prosigna CodeSet. NanoString Technologies), overnight at 65°C in a single hybridization reaction. The removal of excess probes, followed by binding of the probe–target complexes on the surface of a specific nCounter cartridge, was performed on the nCounter Prep Station (NanoString Technologies). Finally, the nCounter cartridge with immobilized probe/target complexes was read in the nCounter Digital Analyzer (NanoString Technologies). The conversion of gene expression measurements into intrinsic molecular subtypes, risk of recurrence (ROR) scores and risk categories used a fully prespecified algorithm has been previously described (Parker *et al.* 2009, Dowsett *et al.* 2013).

Results

Patients and tumour characteristics

Clinical and pathological features of the 402 ER+/HER2− tumours according to the AR status are shown in Supplementary Table 1 (see section on supplementary data given at the end of this article). The median time of follow-up was 8 years. The majority of cases, 70.6% (284/402), were AR+. The distribution plots of IHC ER and AR nuclear staining percentages are presented in Supplementary Fig. 1. According to our previous reports (Castellano *et al.* 2010, 2013), we confirmed that AR expression (≥1% nuclear staining) was significantly correlated with a longer DSS (*P*=0,0008; Supplementary Fig. 2) of ER+ BC patients.

AR/ER ratio and correlation with histological and immunohistochemical features

The median AR/ER ratio was 0.51. Two was the optimal AR/ER ratio that differentiated the cohort by prognosis (AR/ER≥2: AUC=0.74; *P*=0.002) (Supplementary Fig. 3). The characteristics of the 284 ER+/HER2−/AR+ BC cases stratified by an AR/ER ratio cut-off are reported in Table 1. Of the 284 AR+/ER+ cases, 268 (94%) had an AR/ER ratio <2 and 16 (6%) an AR/ER ratio ≥2 (Fig. 1 and Fig. 2). In the descriptive analysis, patients with a higher AR/ER ratio carried larger tumours with a higher histological grade and lower PgR levels, and they frequently had more metastatic lymph nodes and had a higher number of relapse events (*P*≤0.004) (Table 1).

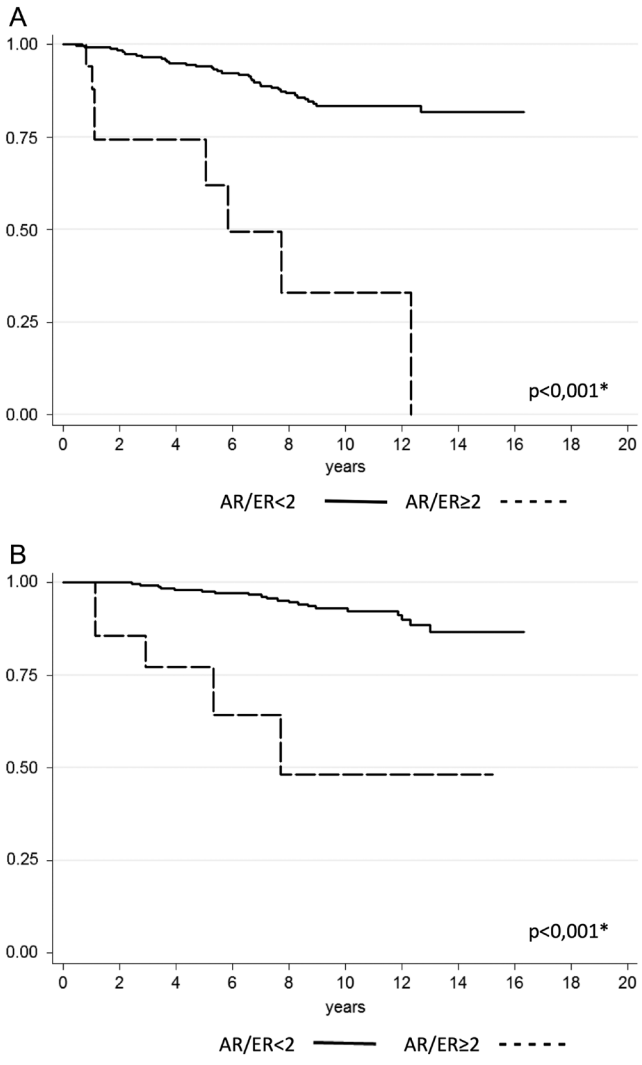

Figure 3
Survival curves for AR/ER<2 vs AR/ER≥2. (A) Disease-free interval (DFI). (B) Disease-specific survival (DSS). *Log-rank test for equality of survivor functions.

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Table 3 Multivariate analysis in the group of ER+/AR+ BC patients.

Clinical-pathological features	DFI*		DSS*	
	HR (95% CI)	*P*	HR (95% CI)	*P*
Age	1.01 (0.98–1.04)	0.474	0.98 (0.94–1.03)	0.602
Tumor size ≥15 mm	4.16 (1.88–9.18)	<0.001	8.87 (1.71–46)	0.009
Metastatic lymph nodes				
0	1			
1–3	1.41 (0.58–3.40)	0.441	1.28 (0.23–7.1)	0.778
4–9	1.59 (0.63–3.99)	0.321	3.45 (0.81–14.7)7)	0.095
>9	4.42 (1.66–11.79)	0.003	5.71 (1.17–27.7)	0.031
Ki67≥20%	3.98 (1.78–8.86)	<0.001	5.26 (1.12–24.6)	0.035
AR/ER≥2	4.96 (1.95–12.68)	<0.001	8.69 (2.02–37.44)	0.004
HT vs CT	1.64 (0.72–7.03)	0.234	1.02 (0.25–4.18)	0.974

*Test of proportional-hazards assumption global test DFI $P=0.3188$, DSS $P=0.3871$.
CT, patients who received hormonal therapy plus chemotherapy; DFI, disease-free interval; DSS, disease-specific survival; HR, hazard ratio; HT, patients who received hormonal therapy.

AR/ER ratio and impact on prognosis

As shown in Table 2, univariate analysis confirmed that an AR/ER ratio ≥2 was one of the most significant markers of poor survival (HR=7.55 for DFI, and HR=10.84 for DSS, both $P<0.001$), together with tumour grade, tumour size ≥15 mm, nodal involvement ≥4 and high Ki67 index. Moreover, the Kaplan–Meier curves and the log-rank test showed significant differences in the survival times between the two groups (DFI and DSS $P<0.001$) (Fig. 3A and B). In the analysis, we also included ER and AR expression as continuous variables to compare the weight on the prognosis of different levels of the receptor expression with the AR/ER ratio. While the percentage of AR expression did not show any impact on prognosis, the levels of ER were correlated with prognosis although at a lower significance compared to the AR/ER ratio (Table 2). Multivariate analysis confirmed an independent effect on the prognosis of the AR/ER ratio. According to this model, patients with an AR/ER≥2 were five times more likely to relapse (HR=4.96, $P<0.001$ for DFI) and eight times more likely to die of BC (HR=8.69, $P=0.004$ for DSS) compared with patients with a ratio <2. Tumour size ≥15 mm, lymph nodes >9 and a high Ki67 index had an unfavourable effect on DFI and DSS (Table 3). The proportionality assumption was satisfied both for the DFI ($P=0.1227$) and DSS ($P=0.3517$).

To exclude the possibility that prognostic information from the AR/ER ratio was only a consequence of the low ER levels, we additionally tested a cut-off point for ER nuclear staining at 10%. As expected, patients with lower ER levels (<10%) were associated with worse DFI and DSS (Supplementary Fig. 4). However, according to the AIC

Table 4 Characteristics of BC cases evaluated with Prosigna – PAM50 assay.

Clinical and molecular features	AR/ER≥2 *n* (%)
Grade	
1	1 (5.3)
2	8 (42.1)
3	10 (52.6)
Tumor size	
<15 mm	5 (26.3)
≥15 mm	14 (73.7)
Metastatic lymph nodes	
0	11 (57.9)
1–3	1 (5.3)
4–9	4 (21)
>9	3 (15.8)
Ki67	
<20%	8 (42.1)
≥20%	11 (57.9)
PgR	
<20	12 (63.2)
≥20	7 (36.8)
IHC-based subtype	
Luminal A	3 (15.8)
Luminal B	16 (84.2)
Prosigna-PAM50 molecular subtype	
Luminal A	9 (47.4)
Luminal B	1 (5.3)
HER2-Enriched	2 (10.5)
Basal-like	7 (36.8)
Prosigna-PAM50 risk category	
Low	7 (36.8)
Intermediate	4 (21.1)
High	8 (42.1)
Prosigna-PAM50 risk category – PDR†	
Low	7 (4.57)*
Intermediate	4 (10.25)*
High	8 (34.87)*

†Probability of distant recurrence; *Mean percentage of PDR at 10 years for each risk category.

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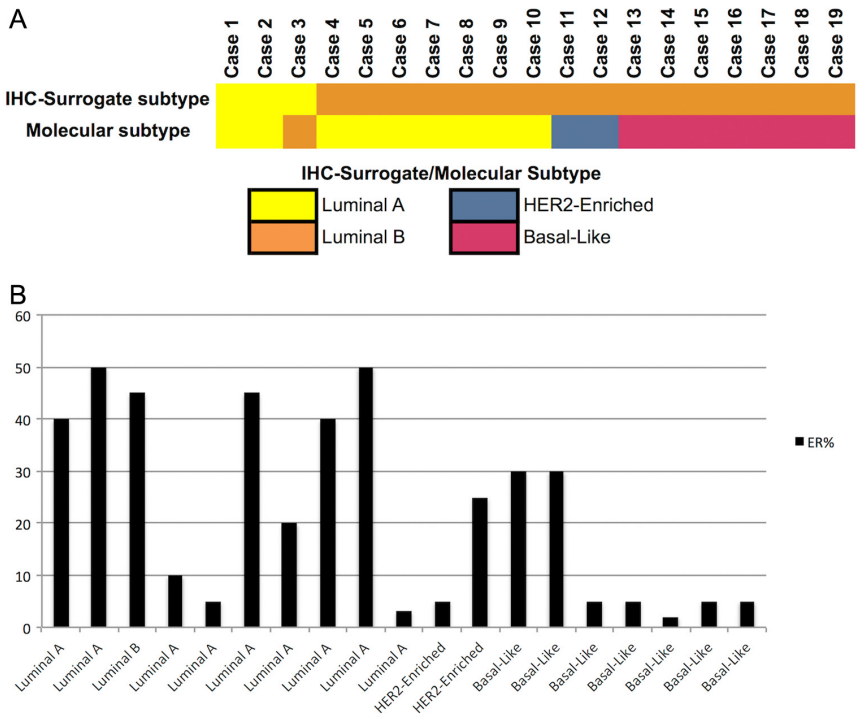

Figure 4
IHC-based vs intrinsic molecular subtypes. (A) Following guidelines recommendations, all BC with an AR/ER ratio ≥2 (19 cases) were classified as luminal by IHC. However, the Prosigna-PAM50 assay changed the classification of 17 cases (89.5%), almost half of them to the non-luminal subtypes. (B) Correlation of the ER percentage (ER%) nuclear staining (IHC) and intrinsic molecular subtypes in BC with an AR/ER ratio ≥2.

test, which was used for model selection, the AR/ER ratio model received the lowest AIC score (DFI AIC=378.8, DSS AIC=139.7), indicating that this model is more effective at providing prognostic information than the model with an ER cut-off at 10% (DFI AIC=384.6, DSS AIC=145.9). Furthermore, although patients with lower ER levels were more likely to have AR/ER≥2, 56.2% of tumours (9/16 cases) with a high AR/ER ratio had a high ER level (≥10%).

AR/ER≥2 and association with intrinsic molecular subtypes

A Prosigna-PAM50 assay was performed on the 19 cases with a ratio AR/ER≥2 to evaluate their ROR and molecular subtype. Twelve out of the 19 cases (63.2%) resulted in intermediate or high-risk categories (high probability of distant recurrence at 10 years) (Table 4).

We then compared the IHC-based subtypes (Hammond *et al.* 2010, Coates *et al.* 2015) with the intrinsic molecular subtype obtained by the Prosigna-PAM50 assay and the percentage of ER expression. Three cases were classified as IHC luminal A (15.8%) and 16 cases as luminal B (84.2%). The concordance between the IHC subtypes and intrinsic molecular subtypes was very low (k=0.0583) since only 2 cases (10.5%) maintained the same subtype (luminal A) using the Prosigna-PAM50 assay. Molecular tests classified 47.4% of samples as luminal A, 5.3% as luminal B, 10.5% as HER2-enriched and 36.8% as a basal-like subtype (Fig. 4A and Table 4). Thus, gene expression analysis showed that 47.4% of BCs with an AR/ER ratio ≥2 were assigned to non-luminal subtypes (Fig. 2, Fig. 4A and Table 4). The correlation with the percentage of ER expression showed that 6 of the cases that switched from luminal to non-luminal had an ER<10%, although two cases classified as luminal A by Prosigna-PAM50 assay had an ER<10% (2% and 5%, respectively) (Fig. 4B).

Discussion

In our study, we demonstrated that within ER+ BCs, the AR/ER ratio may represent an additional independent prognostic marker. Specifically, we showed that BCs with an AR/ER ratio ≥2 had a worse DFF and DSS. This particular subset of tumours is rare within ER+ BCs and, from the molecular point of view, they do not always fit with the luminal subtype.

The prognostic role of AR in ER+ BC has been extensively studied. Several authors have reported that AR expression in luminal cancers is associated with a better outcome compared to AR negative BCs (Park *et al.* 2011, Castellano *et al.* 2013, Kim *et al.* 2015, Bozovic-Spasojevic *et al.* 2016). However, some reports suggest that AR could be related to BC progression (Grogg *et al.* 2015), as it is detected in a significantly higher percentage of ductal carcinomas '*in situ*' (DCIS) that are adjacent to invasive carcinomas than in pure DCIS (Yu *et al.* 2011).

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Moreover, although the expression of ER and PgR decrease during BC progression (from DCIS to invasive and from G1 to G3), AR expression is highly conserved during BC progression, as it is detected in a high percentage of metastatic tumours (Cimino-Mathews *et al.* 2012, Grogg *et al.* 2015). In addition, Gonzalez and coworkers (Gonzalez *et al.* 2008) found that AR+ tumours are frequently positive for matrix metalloproteinases (MMPs), which have been involved in breast tumour dissemination. Finally, a recent study indicated that AR expression can induce the epithelial-to-mesenchymal transition in ER+ BC cells, conferring them with metastatic potential (Feng *et al.* 2016).

Panet-Raymond and coworkers (Panet-Raymond *et al.* 2000) reported that co-expression of both ER and AR reduces the trans-activation function of AR, and Takagi and coworkers (Takagi *et al.* 2010) suggested that AR signalling is suppressed in BC by high ER signalling activity.

All these results indicate that the interaction between the ER and AR levels may influence the AR activity. In line with this hypothesis, we found that BCs with a high AR/ER ratio are associated with aggressive biological features and worse prognosis.

To the best of our knowledge, only Cochrane and coworkers reported an association between AR/ER ratio and outcome (Cochrane *et al.* 2014). They showed that AR/ER ratio ≥2 is a good predictor of DFI and DSS, in a cohort of ER+ BC patients. AR/ER optimal ratio (AR/ER≥2) was further defined and confirmed to predict DFI and DSS in our cohort (ER+/HER2− BC patients) by univariate Cox (HR) analysis. However, they reported a higher percentage of cases with an AR/ER ratio ≥2 than in our series (11.4% vs 6% respectively), which is probably related to differences in case selection since we excluded tumours with HER2 positivity.

The molecular analysis with Prosigna-PAM50 confirmed that most cases with AR/ER≥2 had a high-to-intermediate ROR. In addition, Prosigna-PAM50 assay assigned 47.4% of our IHC luminal cases to the non-luminal intrinsic molecular subtypes. These results could suggest that tumours with a high AR/ER ratio could be resistant to hormone therapy. In fact, *in vitro* studies have demonstrated that hormone therapy-resistant tumours express higher levels of AR and lower ER levels than hormone therapy-sensitive tumours (De Amicis *et al.* 2010, Fujii *et al.* 2014, Rechoum *et al.* 2014, Ciupek *et al.* 2015). To confirm these experimental data, Cochrane and coworkers demonstrated that AR/ER≥2 was associated with an increased risk of tamoxifen therapy failure in BC patients (Cochrane *et al.* 2014). Taken together, these data may suggest that BCs with an AR/ER≥2 could represent tumours that are changing or evolving from ER dependence (luminal subtype) to AR dependence, with the progressive loss of ER expression (non-luminal subtype).

Our study has some limitations due to its retrospective design. We included in the analysis patients with different treatment (hormone therapy and chemotherapy), and we do not have validation setting of patients to confirm our data. To address these limitations and validate our data, future studies need to include larger cohort of patients, who possibly underwent the same therapeutic approach.

In conclusion, our results suggest that tumours with AR/ER≥2 should be carefully evaluated and reinforce the idea of targeting AR for BC treatment.

Supplementary data

This is linked to the online version of the paper at https://doi.org/10.1530/ERC-17-0417.

Declaration of interest

The authors declare that there is no conflict of interest that could be perceived as prejudicing the impartiality of the research reported.

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Author contribution statement

Study conception and design: N R, M R L, L A, A S and I C.; Acquisition of data: N R, J M, M P M, L B, P C, S O A, I C.; Analysis and interpretation of data: N R, L A, S O A, M P M, L B, P C, A S; Drafting of manuscript: N R, M R L, J M and I C.; Critical revision: N R, M R L, L A, S O A, J M, M P M, L B, P C, A S and I C.; Final approval of the version to be submitted: N R, M R L, L A, S O A, J M, M P M, L B, P C, A S and I C.

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References

Aleskandarany MA, Abduljabbar R, Ashankyty I, Elmouna A, Jerjees D, Ali S, Buluwela L, Diez-Rodriguez M, Caldas C, Green AR, *et al.* 2016 Prognostic significance of androgen receptor expression in invasive breast cancer: transcriptomic and protein expression analysis. *Breast Cancer Research and Treatment* **159** 215–227. (https://doi.org/10.1007/s10549-016-3934-5)

Ando S, De Amicis F, Rago V, Carpino A, Maggiolini M, Panno ML & Lanzino M 2002 Breast cancer: from estrogen to androgen receptor. *Molecular and Cellular Endocrinology* **193** 121–128. (https://doi.org/10.1016/S0303-7207(02)00105-3)

Asano Y, Kashiwagi S, Goto W, Tanaka S, Morisaki T, Takashima T, Noda S, Onoda N, Ohsawa M, Hirakawa K, *et al*. 2017 Expression and clinical significance of androgen receptor in triple-negative breast cancer. *Cancers* **9** 4. (https://doi.org/10.3390/cancers9010004)

Birrell SN, Bentel JM, Hickey TE, Ricciardelli C, Weger MA, Horsfall DJ & Tilley WD 1995 Androgens induce divergent proliferative responses in human breast cancer cell lines. *Journal of Steroid Biochemistry and Molecular Biology* **52** 459–467. (https://doi.org/10.1016/0960-0760(95)00005-K)

Bozovic-Spasojevic I, Zardavas D, Brohee S, Ameye L, Fumagalli D, Ades F, de Azambuja E, Bareche Y, Piccart M, Paesmans M, *et al*. 2016 The prognostic role of androgen receptor in patients with early-stage breast cancer: a meta-analysis of clinical and gene expression data. *Clinical Cancer Research* **23** 2707–2712. (https://doi.org/10.1158/1078-0432.CCR-16-0979)

Buchanan G, Birrell SN, Peters AA, Bianco-Miotto T, Ramsay K, Cops EJ, Yang M, Harris JM, Simila HA, Moore NL, *et al*. 2005 Decreased androgen receptor levels and receptor function in breast cancer contribute to the failure of response to medroxyprogesterone acetate. *Cancer Research* **65** 8487–8496. (https://doi.org/10.1158/0008-5472.CAN-04-3077)

Bustreo S, Osella-Abate S, Cassoni P, Donadio M, Airoldi M, Pedani F, Papotti M, Sapino A & Castellano I 2016 Optimal Ki67 cut-off for luminal breast cancer prognostic evaluation: a large case series study with a long-term follow-up. *Breast Cancer Research and Treatment* **157** 363–371. (https://doi.org/10.1007/s10549-016-3817-9)

Castellano I, Allia E, Accortanzo V, Vandone AM, Chiusa L, Arisio R, Durando A, Donadio M, Bussolati G, Coates AS, *et al*. 2010 Androgen receptor expression is a significant prognostic factor in estrogen receptor positive breast cancers. *Breast Cancer Research and Treatment* **124** 607–617. (https://doi.org/10.1007/s10549-010-0761-y)

Castellano I, Chiusa L, Vandone AM, Beatrice S, Goia M, Donadio M, Arisio R, Muscara F, Durando A, Viale G, *et al*. 2013 A simple and reproducible prognostic index in luminal ER-positive breast cancers. *Annals of Oncology* **24** 2292–2297. (https://doi.org/10.1093/annonc/mdt183)

Cimino-Mathews A, Hicks JL, Illei PB, Halushka MK, Fetting JH, De Marzo AM, Park BH & Argani P 2012 Androgen receptor expression is usually maintained in initial surgically resected breast cancer metastases but is often lost in end-stage metastases found at autopsy. *Human Pathology* **43** 1003–1011. (https://doi.org/10.1016/j.humpath.2011.08.007)

Ciupek A, Rechoum Y, Gu G, Gelsomino L, Beyer AR, Brusco L, Covington KR, Tsimelzon A & Fuqua SA 2015 Androgen receptor promotes tamoxifen agonist activity by activation of EGFR in ERalpha-positive breast cancer. *Breast Cancer Research and Treatment* **154** 225–237. (https://doi.org/10.1007/s10549-015-3609-7)

Coates AS, Winer EP, Goldhirsch A, Gelber RD, Gnant M, Piccart-Gebhart M, Thurlimann B, Senn HJ & Panel M 2015 Tailoring therapies – improving the management of early breast cancer: St Gallen International Expert Consensus on the primary therapy of early breast cancer 2015. *Annals of Oncology* **26** 1533–1546. (https://doi.org/10.1093/annonc/mdv221)

Cochrane DR, Bernales S, Jacobsen BM, Cittelly DM, Howe EN, D'Amato NC, Spoelstra NS, Edgerton SM, Jean A, Guerrero J, *et al*. 2014 Role of the androgen receptor in breast cancer and preclinical analysis of enzalutamide. *Breast Cancer Research* **16** R7. (https://doi.org/10.1186/bcr3599)

Cops EJ, Bianco-Miotto T, Moore NL, Clarke CL, Birrell SN, Butler LM & Tilley WD 2008 Antiproliferative actions of the synthetic androgen, mibolerone, in breast cancer cells are mediated by both androgen and progesterone receptors. *Journal of Steroid Biochemistry and Molecular Biology* **110** 236–243. (https://doi.org/10.1016/j.jsbmb.2007.10.014)

De Amicis F, Thirugnansampanthan J, Cui Y, Selever J, Beyer A, Parra I, Weigel NL, Herynk MH, Tsimelzon A, Lewis MT, *et al*. 2010 Androgen receptor overexpression induces tamoxifen resistance in human breast cancer cells. *Breast Cancer Research and Treatment* **121** 1–11. (https://doi.org/10.1007/s10549-009-0436-8)

Dowsett M, Sestak I, Lopez-Knowles E, Sidhu K, Dunbier AK, Cowens JW, Ferree S, Storhoff J, Schaper C & Cuzick J 2013 Comparison of PAM50 risk of recurrence score with oncotype DX and IHC4 for predicting risk of distant recurrence after endocrine therapy. *Journal of Clinical Oncology* **31** 2783–2790. (https://doi.org/10.1200/JCO.2012.46.1558)

Early Breast Cancer Trialists' Collaborative Group, Davies C, Godwin J, Gray R, Clarke M, Cutter D, Darby S, McGale P, Pan HC, Taylor C, *et al*. 2011 Relevance of breast cancer hormone receptors and other factors to the efficacy of adjuvant tamoxifen: patient-level meta-analysis of randomised trials. *Lancet* **378** 771–784. (https://doi.org/10.1016/S0140-6736(11)60993-8)

Feng J, Li L, Zhang N, Liu J, Zhang L, Gao H, Wang G, Li Y, Zhang Y, Li X, *et al*. 2016 Androgen and AR contribute to breast cancer development and metastasis: an insight of mechanisms. *Oncogene* **36** 2775–2790. (https://doi.org/10.1038/onc.2016.432)

Fujii R, Hanamura T, Suzuki T, Gohno T, Shibahara Y, Niwa T, Yamaguchi Y, Ohnuki K, Kakugawa Y, Hirakawa H, *et al*. 2014 Increased androgen receptor activity and cell proliferation in aromatase inhibitor-resistant breast carcinoma. *Journal of Steroid Biochemistry and Molecular Biology* **144** 513–522. (https://doi.org/10.1016/j.jsbmb.2014.08.019)

Gonzalez LO, Corte MD, Vazquez J, Junquera S, Sanchez R, Alvarez AC, Rodriguez JC, Lamelas ML & Vizoso FJ 2008 Androgen receptor expresion in breast cancer: relationship with clinicopathological characteristics of the tumors, prognosis, and expression of metalloproteases and their inhibitors. *BMC Cancer* **8** 149. (https://doi.org/10.1186/1471-2407-8-149)

Greeve MA, Allan RK, Harvey JM & Bentel JM 2004 Inhibition of MCF-7 breast cancer cell proliferation by 5alpha-dihydrotestosterone; a role for p21(Cip1/Waf1). *Journal of Molecular Endocrinology* **32** 793–810. (https://doi.org/10.1677/jme.0.0320793)

Grogg A, Trippel M, Pfaltz K, Ladrach C, Droeser RA, Cihoric N, Salhia B, Zweifel M & Tapia C 2015 Androgen receptor status is highly conserved during tumor progression of breast cancer. *BMC Cancer* **15** 872. (https://doi.org/10.1186/s12885-015-1897-2)

Hammond ME, Hayes DF, Dowsett M, Allred DC, Hagerty KL, Badve S, Fitzgibbons PL, Francis G, Goldstein NS, Hayes M, *et al*. 2010 American Society of Clinical Oncology/College Of American Pathologists guideline recommendations for immunohistochemical testing of estrogen and progesterone receptors in breast cancer. *Journal of Clinical Oncology* **28** 2784–2795. (https://doi.org/10.1200/JCO.2009.25.6529)

Hilborn E, Gacic J, Fornander T, Nordenskjold B, Stal O & Jansson A 2016 Androgen receptor expression predicts beneficial tamoxifen response in oestrogen receptor-alpha-negative breast cancer. *British Journal of Cancer* **114** 248–255. (https://doi.org/10.1038/bjc.2015.464)

Hu R, Dawood S, Holmes MD, Collins LC, Schnitt SJ, Cole K, Marotti JD, Hankinson SE, Colditz GA & Tamimi RM 2011 Androgen receptor expression and breast cancer survival in postmenopausal women. *Clinical Cancer Research* **17** 1867–1874. (https://doi.org/10.1158/1078-0432.CCR-10-2021)

Jiang HS, Kuang XY, Sun WL, Xu Y, Zheng YZ, Liu YR, Lang GT, Qiao F, Hu X & Shao ZM 2016 Androgen receptor expression predicts different clinical outcomes for breast cancer patients stratified by hormone receptor status. *Oncotarget* **7** 41285–41293. (https://doi.org/10.18632/oncotarget.9778)

Kim Y, Jae E & Yoon M 2015 Influence of androgen receptor expression on the survival outcomes in breast cancer: a meta-analysis. *Journal of*

Breast Cancer **18** 134–142. (https://doi.org/10.4048/jbc.2015.18.2.134)

Lin HY, Sun M, Lin C, Tang HY, London D, Shih A, Davis FB & Davis PJ 2009 Androgen-induced human breast cancer cell proliferation is mediated by discrete mechanisms in estrogen receptor-alpha-positive and -negative breast cancer cells. *Journal of Steroid Biochemistry and Molecular Biology* **113** 182–188. (https://doi.org/10.1016/j.jsbmb.2008.12.010)

Luo X, Shi YX, Li ZM & Jiang WQ 2010 Expression and clinical significance of androgen receptor in triple negative breast cancer. *Chinese Journal of Cancer* **29** 585–590. (https://doi.org/10.5732/cjc.009.10673)

Macedo LF, Guo Z, Tilghman SL, Sabnis GJ, Qiu Y & Brodie A 2006 Role of androgens on MCF-7 breast cancer cell growth and on the inhibitory effect of letrozole. *Cancer Research* **66** 7775–7782. (https://doi.org/10.1158/0008-5472.CAN-05-3984)

Marchio C, Lambros MB, Gugliotta P, Di Cantogno LV, Botta C, Pasini B, Tan DS, Mackay A, Fenwick K, Tamber N, *et al*. 2009 Does chromosome 17 centromere copy number predict polysomy in breast cancer? A fluorescence in situ hybridization and microarray-based CGH analysis. *Journal of Pathology* **219** 16–24. (https://doi.org/10.1002/path.2574)

Need EF, Selth LA, Harris TJ, Birrell SN, Tilley WD & Buchanan G 2012 Research resource: interplay between the genomic and transcriptional networks of androgen receptor and estrogen receptor alpha in luminal breast cancer cells. *Molecular Endocrinology* **26** 1941–1952. (https://doi.org/10.1210/me.2011-1314)

Niemeier LA, Dabbs DJ, Beriwal S, Striebel JM & Bhargava R 2010 Androgen receptor in breast cancer: expression in estrogen receptor-positive tumors and in estrogen receptor-negative tumors with apocrine differentiation. *Modern Pathology* **23** 205–212. (https://doi.org/10.1038/modpathol.2009.159)

Panet-Raymond V, Gottlieb B, Beitel LK, Pinsky L & Trifiro MA 2000 Interactions between androgen and estrogen receptors and the effects on their transactivational properties. *Molecular and Cellular Endocrinology* **167** 139–150. (https://doi.org/10.1016/S0303-7207(00)00279-3)

Park S, Koo J, Park HS, Kim JH, Choi SY, Lee JH, Park BW & Lee KS 2010 Expression of androgen receptors in primary breast cancer. *Annals of Oncology* **21** 488–492. (https://doi.org/10.1093/annonc/mdp510)

Park S, Koo JS, Kim MS, Park HS, Lee JS, Lee JS, Kim SI, Park BW & Lee KS 2011 Androgen receptor expression is significantly associated with better outcomes in estrogen receptor-positive breast cancers. *Annals of Oncology* **22** 1755–1762. (https://doi.org/10.1093/annonc/mdq678)

Parker JS, Mullins M, Cheang MC, Leung S, Voduc D, Vickery T, Davies S, Fauron C, He X, Hu Z, *et al*. 2009 Supervised risk predictor of breast cancer based on intrinsic subtypes. *Journal of Clinical Oncology* **27** 1160–1167. (https://doi.org/10.1200/JCO.2008.18.1370)

Peters AA, Buchanan G, Ricciardelli C, Bianco-Miotto T, Centenera MM, Harris JM, Jindal S, Segara D, Jia L, Moore NL, *et al*. 2009 Androgen receptor inhibits estrogen receptor-alpha activity and is prognostic in breast cancer. *Cancer Research* **69** 6131–6140. (https://doi.org/10.1158/0008-5472.CAN-09-0452)

Pistelli M, Caramanti M, Biscotti T, Santinelli A, Pagliacci A, De Lisa M, Ballatore Z, Ridolfi F, Maccaroni E, Bracci R, *et al*. 2014 Androgen receptor expression in early triple-negative breast cancer: clinical significance and prognostic associations. *Cancers* **6** 1351–1362. (https://doi.org/10.3390/cancers6031351)

Rechoum Y, Rovito D, Iacopetta D, Barone I, Ando S, Weigel NL, O'Malley BW, Brown PH & Fuqua SA 2014 AR collaborates with ERalpha in aromatase inhibitor-resistant breast cancer. *Breast Cancer Research and Treatment* **147** 473–485. (https://doi.org/10.1007/s10549-014-3082-8)

Sapino A, Marchio C, Senetta R, Castellano I, Macri L, Cassoni P, Ghisolfi G, Cerrato M, D'Ambrosio E & Bussolati G 2006 Routine assessment of prognostic factors in breast cancer using a multicore tissue microarray procedure. *Virchows Archiv* **449** 288–296. (https://doi.org/10.1007/s00428-006-0233-2)

Takagi K, Miki Y, Nagasaki S, Hirakawa H, Onodera Y, Akahira J, Ishida T, Watanabe M, Kimijima I, Hayashi S, *et al*. 2010 Increased intratumoral androgens in human breast carcinoma following aromatase inhibitor exemestane treatment. *Endocrine-Related Cancer* **17** 415–430. (https://doi.org/10.1677/ERC-09-0257)

Vera-Badillo FE, Templeton AJ, de Gouveia P, Diaz-Padilla I, Bedard PL, Al-Mubarak M, Seruga B, Tannock IF, Ocana A & Amir E 2014 Androgen receptor expression and outcomes in early breast cancer: a systematic review and meta-analysis. *Journal of the National Cancer Institute* **106** djt319. (https://doi.org/10.1093/jnci/djt319)

Yu Q, Niu Y, Liu N, Zhang JZ, Liu TJ, Zhang RJ, Wang SL, Ding XM & Xiao XQ 2011 Expression of androgen receptor in breast cancer and its significance as a prognostic factor. *Annals of Oncology* **22** 1288–1294. (https://doi.org/10.1093/annonc/mdq586)

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