The Magnitude of Androgen Receptor Positivity in Breast Cancer is Critical for Reliable Prediction of Disease Outcome

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Statement of Translational Relevance

The estrogen receptor alpha (ERα) is routinely assessed in breast cancer to inform clinical management. More recently, the androgen receptor (AR) has been shown to be of potential prognostic value, but the appropriate cut-point for robust prognostication has yet to be established. In this study, an optimal cut-point for AR was determined using ROC analysis with a test cohort and its prognostic capacity validated in an independent cohort. Prognostic capacity was robust with a high (78% positivity) cut-point but not with lower (1% - 10%) positivity cut-points commonly used in previous studies. The 78% cut-point was valid for unselected cases and selected ERα positive cases. Among the latter, an AR:ERα positivity ratio indicative of comparable receptor expression or AR predominance was associated with the best survival outcome. Determination of the AR status in addition to ERα in breast tumors provides important prognostic information providing an optimal cut-point is utilized.
Abstract

**Purpose:** Consensus is lacking regarding the androgen receptor (AR) as a prognostic marker in breast cancer. The objectives of this study were to comprehensively review the literature on AR prognostication and determine optimal criteria for AR as an independent predictor of breast cancer survival.

**Experimental Design:** AR positivity was assessed by immunostaining in two clinically-validated primary breast cancer cohorts (training cohort n=219; validation cohort n=418; 77% and 79% estrogen receptor alpha (ERα) positive, respectively). The optimal AR cut-point was determined by ROC analysis in the training cohort and applied to both cohorts.

**Results:** AR was an independent prognostic marker of breast cancer outcome in 22/46 (48%) of previous studies that performed multivariate analyses. Most studies used cut-points of 1% or 10% nuclear positivity. Herein, neither 1 nor 10% cut-points were robustly prognostic. ROC analysis revealed a higher AR cut-point (78% positivity) provided optimal sensitivity and specificity to predict breast cancer survival in the training (HR 0.41, P=0.015) and validation (HR 0.50, P=0.014) cohorts. Ten-fold cross validation confirmed the robustness of this AR cut-point. Patients with ERα positive tumors and AR positivity ≥78% had the best survival in both cohorts (P<0.0001). Among the combined ERα positive cases, those with comparable or higher levels of AR (AR:ERα positivity ratio >0.87) had the best outcomes (P<0.0001).

**Conclusions:** This study defines an optimal AR cut-point to reliably predict breast cancer survival. Testing this cut-point in prospective cohorts is warranted for implementation of AR as a prognostic factor in the clinical management of breast cancer.
Introduction

Androgen receptor (AR) expression is highly prevalent in primary breast cancers, ranging from 53-99% depending on the characteristics of the cohort analyzed, the assay methodology and the criteria for positivity (1-7). Up to 90% of estrogen receptor alpha (ERα) positive tumors and approximately 20% of ERα negative tumors are also AR positive (1-7). Given the high frequency of AR expression in breast cancer and the availability of old and new generation agents to modulate its activity, there has been a resurgence of interest in targeting the AR signaling pathway to treat women with this disease. However, much controversy exists over how best to target AR in breast cancer as it appears to have pleiotropic roles dependent on disease subtype and stage of progression (reviewed in (8) and (9)). Strong clinical and preclinical evidence supports AR as being growth inhibitory in hormone sensitive, ERα positive breast malignancies, a role that may be sustained from normal breast tissue as AR activity inhibits breast development in men and women (8, 10). Accordingly, non-aromatizable AR agonists such as fluoxymesterone have historically demonstrated an efficacy comparable to that of the selective ERα modulator, tamoxifen, in advanced breast cancer (11, 12). However, use of androgenic agents was discontinued due to virilizing side effects in some women. Development of new selective AR modulators (SARMs) with AR agonist activity in breast tissues may circumvent that problem and one, Enobosarm (GTx024), has shown promise in a phase II trial of women with advanced, hormone sensitive disease (13). This approach is supported by a recent preclinical study in which induction of AR agonist activity using a different SARM inhibited the growth of endocrine-sensitive as well as endocrine-resistant patient-derived breast cancer xenografts (14). However, some pre-clinical studies have suggested that AR antagonism is also a therapeutic option for women with ERα positive disease (15-17), leading to breast cancer trials with new generation AR antagonists such as enzalutamide (e.g. ClinicalTrials.gov Identifier: NCT02953860) currently used to treat prostate cancer. Antagonism of AR activity may have a clinical niche in the treatment of ERα negative, AR positive disease, particularly the triple negative breast cancer (TNBC) subtype, in which AR is
purported to have oncogenic activity (18-24). However, at μM concentrations AR antagonists can have off-target effects in breast cancer cells (25), suggesting that their therapeutic efficacy may not be mediated by inhibition of AR.

Numerous studies have investigated whether AR is a biomarker for predicting survival of breast cancer, but like the potential utility and means of targeting AR, its prognostic value remains controversial. Disparate results among studies may be attributed to heterogeneity of breast cancer cohorts and differences in methodology, including the use of different cut-points for AR positivity. Although several meta-analyses commonly conclude that AR is associated with better outcomes in ERα positive disease (26-29), these analyses used population-level rather than individual patient-level data. Moreover, most studies included in the meta-analyses of AR as a prognostic biomarker used cut-points that are typically those used for ERα and progesterone receptor (PR) as a predictive biomarker (30, 31). To date, no standardized cut-point for AR prognostication has been statistically defined, which most likely has contributed to conflicting results in the literature regarding AR as a prognostic biomarker in breast cancer.

In the current study, we comprehensively reviewed the literature on AR prognostication for breast cancer survival to highlight methodological heterogeneity in previous studies, and utilized two clinically validated breast cancer cohorts from Australia (32) and Canada (33) with long-term follow-up to empirically define optimal criteria for AR to be a robust independent predictor of breast cancer specific survival. Since there has been interest in assessing the clinical relevance of different AR to ERα expression ratios in breast cancer (8, 15, 34, 35), we also evaluated this parameter.

Methods

Study selection for comprehensive review on AR and breast cancer specific outcome

Primary publications that investigated the relationship between AR expression and breast cancer outcome were identified by searching PubMed with the terms ‘breast cancer’ and ‘androgen
receptor’ up to July 2017. Only studies that examined the relationship between AR level and patient outcome were included; there was no restriction based on methodology used to assess AR protein levels. Exclusion criteria for the PubMed search included the following 1) non-English articles, 2) review articles, 3) no information provided for at least one of the following: disease free survival (DFS), relapse-free survival (RFS) or breast cancer specific overall survival (OS) and 4) duplicate publications/cohorts. Our comprehensive review identified a total of 53 articles (Table 1). Articles were divided into 3 cohort subtypes: (1) unselected (i.e. all cases); (2) ERα positive and (3) ERα negative cohorts, including TNBC.

**Patient Cohorts**

Prior approval for this study was obtained from the Human Research Ethics Committee of the University of Adelaide (H-051-2006). Two independent breast cancer cohorts (32, 33) were studied, both represented on tissue microarrays (TMA) consisting of replicate sample cores. For detailed pathological and clinical characteristics see Supplementary Table 1 and for details of immunostaining methods, statistical analyses and cut-point determination see Supplementary Methods.

**Training cohort:** 219 patients with invasive ductal breast carcinoma diagnosed between 1992 and 2002 from St Vincent's Hospital, Sydney, Australia (32). Prior approval for this TMA construction was obtained from the Human Research Ethics Committee of St Vincent’s Hospital, Sydney. Median follow up was 90 months.

**Validation cohort:** 418 patients with invasive breast cancer diagnosed at Vancouver General Hospital, between 1974 and 1995 (33). TMAs were constructed at the Genetic Pathology Evaluation Centre, University of British Columbia, Vancouver, Canada with ethical approval from the Institutional Ethical Review Board. Median follow up was 143 months.

**Immunostaining and AR antisera**

Sections of paraffin-embedded breast cancer tissue (4 μm) from the two TMA cohorts were immunostained with two different AR antibodies, U407 (epitope: amino acids 200-220) (36) for the
training cohort) and AR-N20 (SC-816, epitope: amino acids 1–20; Santa Cruz; Dallas, TX) for the validation cohort. The specificity of both of these AR antibodies has been confirmed previously by western blot and peptide competition experiments (36, 37). Concordance of immunostaining with the two AR antibodies is shown in Supplementary Figure 1. Details of the AR immunohistochemistry methodology and scoring are contained in Supplementary Methods. Ki67 (SP6, Neomarkers, USA; 1:200 dilution) was scored as a visual estimate of continuous percent positivity by a board certified clinical pathologist for both cohorts (38, 39).

ROC Analysis

ROC analysis was used to dichotomize AR and Ki67 positivity for all cases in the individual training and validation cohorts. Additional ROC analyses were performed for all cases and the ERα positive cases in the combined training and validation cohort. There were insufficient cases in the combined cohort to perform ROC analyses for HER2+ or ERα negative sub-groups. The optimal cut-points for predicting breast cancer death were determined using the Youden index (J), which was calculated using the formula $J = \text{max} \left[ \text{sensitivity} + \text{specificity} - 1 \right]$ (40). In addition to ROC analysis, recursive partitioning was applied to the training cohort to select the most appropriate AR cut-point (41). For additional details of the statistical analyses see Supplementary Methods. Statistical power of the ROC-derived AR cut-point for each of the cohorts was determined using Minitab 17 (www.minitab.com, USA).

Ten-fold Cross Validation

Ten-fold cross validation was performed to provide evidence of the accuracy of the classifier model to correctly predict the AR classes generated by ROC analysis (42-44). To this end, we employed supervised learning to build a range of classifiers including Decision Stump, Decision Tree, Decision Tree with information gain and Naïve Bayse from cohort features such as tumor size and overall survival. Ten-fold cross-validation randomly divides the dataset into 10 sub-samples and
uses 9 sub-samples as training data and one sub-sample as test data. This process is repeated 10-
times. Cross-validation is a reliable statistic in supervised learning and class prediction as it avoids
overlapping test sets. The above-mentioned analyses were performed using RapidMiner 6.0
software (RapidMiner, Boston, USA).

Results

A comprehensive analysis of studies investigating AR as a biomarker of breast cancer survival

A comprehensive review of the literature on AR as a prognostic factor in breast cancer was
undertaken. A total of 53 studies that assessed AR status and disease outcome in breast cancer
using either radio-ligand binding assays (n=7), immunostaining (n=45), or reverse phase protein
microarray (n=1) were identified in a PubMed search with the criteria outlined in Methods (Table
1). Of these, 7 did not perform multivariate analyses. AR was predictive of breast cancer outcome
in 22/46 (48%) of all previous studies that performed multivariate analyses. Nearly all previous
studies used cut-points of 1% or 10% nuclear AR positivity. Among the 22 studies involving
unselected breast cancer cohorts, most found that AR is prognostic for OS by univariate analysis,
but only 10/22 (45%) identified AR as an independent prognostic marker of OS by multivariate
analyses (Table 1). Among the 8 studies involving cohorts selected for ERα positive disease, 5/8
(62%) identified AR as an independent predictor of outcome. Findings in cohorts selected for ERα
negative disease were most conflicting, with a significant association between AR expression and
OS reported in only 7/18 (39%) of studies. Of note, 5 of these 7 studies (45-49) reported that AR
expression conferred a survival advantage in tumors that lacked ERα expression, but 2 (50, 51)
reported a survival disadvantage (Table 1). Collectively, the studies published to date highlight the
lack of consensus on whether AR is an independent prognostic factor for breast cancer survival in
unselected or selected cohorts. Importantly, none of the published studies performed ROC analysis
or used an alternative statistical approach to define the optimal cut-point for AR prognostication in
breast cancer. The majority of studies employed an arbitrary cut-point, the most common being 1%
or 10% AR positivity. While these cut-points, commonly used for ERα and PR as a predictive
biomarker (30, 31), may be useful to determine AR status, they are not necessarily optimal for use in prognostication.

**Commonly used cut-points of AR positivity do not reliably predict breast cancer survival**

The majority (>75%) of breast cancers in the training and validation cohorts analyzed in this study were classified as having AR positive nuclear immunostaining irrespective of whether a 1% or 10% criterion was used (Supplementary Table 1). ROC analysis and the generated area under the curve (AUC) for all cases was used to assess how well AR positivity could distinguish between patients that died from breast cancer and those that survived at least 10 years. AR positivity had prognostic capacity in both the training (AUC=0.678, 95% CI; 0.587-0.770, *P*<0.0001, Figure 1A) and validation (AUC=0.588, 95% CI; 0.525-0.651, *P*=0.008, Figure 2A) cohorts. Based on the ROC analysis, a cut-point of 1% AR positivity, used in 14/36 of previous studies (see Table 1), resulted in high sensitivity for predicting breast cancer OS in the training (90.0%) and validation (93.8%) cohorts, but a low specificity in both instances (training: 27.5%; validation: 7.8%). Similarly, the commonly used cut-point of 10% AR positivity (13/36 of studies in Table 1) resulted in high sensitivity (training: 84.7%; validation: 85.8%) but low specificity (training: 35.0%; validation: 18.4%) for predicting OS. Kaplan-Meier and Cox regression analyses indicated that nuclear AR positivity arbitrarily assigned as either ≥1% or ≥10% positivity was significantly associated with OS in the training cohort but not in the validation cohort (Supplementary Figure 2, Table 2, Table 3).

**Defining a cut-point for AR positivity that confers robust prognostication**

As neither a 1% nor a 10% cut-point for AR positivity was prognostic for breast cancer OS in both the training and validation cohorts, the Youden index method, which gives equal weight to sensitivity and specificity, was applied to the ROC analysis to identify an optimal AR cut-point for
all cases in the training cohort. The highest Youden index was obtained using 77.5% AR positivity, which resulted in a sensitivity of 57.1% and a specificity of 75.0% for predicting breast cancer death. This cut-point resulted in a positive predictive value of 90.7%. An alternative method, recursive partitioning, identified 77% as the optimal cut-point for the training cohort to predict OS. The ROC determined cut-point of 77.5% (rounded to 78%) was significantly associated with ERα status and breast cancer subtype in both cohorts (Supplementary Tables 2 and 3, Chi Squared test). The frequency distributions for AR percent positivity and representative images of tumors classified as either high (≥78% positivity) or low (<78%) AR positivity are shown for both the training and validation cohorts in Figure 1B-C and Figure 2B-C, respectively. Most tumors (69.4%) with AR positivity <78% were also ERα negative (i.e. <1% ERα positivity) in the training cohort; 73.6% were ERα negative in the validation cohort. A high proportion of TNBC had low AR positivity in both the training (79%) and validation (69%) cohorts (Supplementary Tables 2 and 3). Ten-fold cross validation was performed using all cases to provide evidence of the accuracy of the classifier model to correctly predict the two AR classes (i.e. <78% and ≥78% positivity) generated by ROC analysis. A high accuracy of 10-fold cross validation (>80%) for most of the classifiers demonstrated the robustness of AR positivity equal to 78% as an optimal cut-point based on cohort features in both the individual and combined training and validation cohorts (Supplementary Table 4). For example, for the combined training and validation cohorts, the accuracy of Decision Tree with information gain criterion in prediction of AR classes <78% and ≥78% was 87.27% and 90.98%, respectively (Supplementary Table 4).

Histological grade III and progesterone receptor (PR) negative tumors were significantly associated with low AR positivity in the training cohort (Supplementary Table 2). The lack of an association between AR and PR in the validation cohort (Supplementary Table 3) is possibly the result of approximately one third of the cases having unknown PR values (150/418; 35.9%), whereas PR was measured in almost all the cases (214/219; 97.7%) in the training cohort (Supplementary Table 1).
Kaplan-Meier survival analyses for all cases demonstrated that an AR positivity ≥78% was significantly associated with OS in both the training (Figure 1D, *P*<0.0001) and the validation (Figure 2D, *P*=0.001) cohorts. Patients with tumor AR positivity ≥78% had approximately a 3-fold reduced risk of cancer-related death in the training cohort (HR=0.32, Cox regression analysis, *P*=0.001, Table 2) and a 2-fold reduced risk in the validation cohort (HR=0.51, Cox regression analysis, *P*=0.001, Table 3). AR immunostaining with a cut-point of 78% was an independent predictor of OS after adjusting for all other variables significant by univariate analysis in both cohorts (Tables 2 and 3) but was not an independent predictor of RFS (Table 2). Neither the 1% nor 10% AR cut-points were significant by univariate analysis in the validation cohort (Table 3).

**AR prognosis with adjuvant therapy**

Treatment information was available for the training cohort but unavailable for the validation cohort. In the training cohort, AR with a 78% cut-point predicted OS (*P*=0.002, log rank statistic=9.63) in patients who received adjuvant chemotherapy (n=82; Supplementary Figure 3A), but not in those who did not receive adjuvant chemotherapy (n=128; *P*=0.067; Supplementary Figure 3B). AR was a significant predictor of OS in all patients irrespective of whether they did (n=110, *P*=0.014, log rank statistic=6.10, Supplementary Figure 3C) or did not (n=100, *P*=0.010, log rank statistic=6.69, Supplementary Figure 3D) receive adjuvant endocrine therapy. Similarly, AR predicted outcome in the ERα positive subgroup irrespective of whether patients received adjuvant endocrine therapy (n=89, *P*=0.01, log rank statistic=6.59) or not (n=67, *P*=0.017, log rank statistic=5.74).

**ERα positive breast cancers with high AR have the best survival outcome**

In both the training and validation cohorts, patients with ERα positive tumors (ERα ≥1%) that contained high levels of AR (i.e. ERα+, AR ≥78%) had significantly increased OS in comparison to those in the other 3 possible ERα and AR classification groups (i.e. ERα positive and AR <78%,...
ERα negative and AR ≥78%, ERα negative and AR <78%; Figure 1E and 2E). Patients with ERα negative disease (ERα <1%) had an increased risk of death regardless of AR status, compared to those with ERα positive AR ≥78% tumors (training cohort: 11.5 and 10.3-fold risk, \( P<0.0001 \), Figure 1F; validation cohort: 1.9 and 2.9-fold risk, \( P=0.006 \) and \( P<0.0001 \), Figure 2F). The training and validation cohorts were combined for greater statistical power to assess the ability of AR to predict OS as a continuous variable or with an AR cut-point of <78% or ≥78% positivity. AR levels predicted OS in ERα positive tumors but not ERα negative tumors (Supplementary Table 5). When assessed by subtype, AR was an independent predictor of OS in Luminal A but not Luminal B cancers (Supplementary Table 5). When ROC analysis was applied to all cases or just the ERα positive cases in the combined training and validation cohorts, the optimal AR cut-point was 77.95%.

The AR to ERα ratio is a determinant of OS in ERα positive disease

To investigate whether the relative levels of AR and ERα influenced OS, the training and validation cohorts were combined for greater statistical power and unbiased tertiles were calculated for the AR to ERα positivity ratio (<0.87, 0.87-1.05 and >1.05). Kaplan-Meier and Cox regression analyses demonstrated that patients with tumors containing comparable levels of AR and ERα (i.e. an AR to ERα positivity ratio approximating 1; range 0.87-1.05), or a predominance of AR (i.e. an AR to ERα positivity ratio >1.05) had the highest 10-year breast cancer survival outcomes (83.3% and 80.5%, respectively; \( P<0.0001 \); Figure 3). In contrast, patients with a predominance of ERα (i.e. an AR to ERα positivity ratio <0.87) had a poorer survival outcome compared to patients with similar AR and ERα immunostaining levels (10-year breast cancer survival 71.6%; Figure 3; \( P<0.0001 \)). Patients with tumors that were either AR or ERα negative or lacked both receptors had a lower 10-year breast cancer specific survival rate of 53-66% (Figure 3; 3-fold increased risk of death, \( P<0.004 \)). ROC analysis showed that the optimal cut-point for the AR to ERα positivity ratio was 0.82. However, in multivariate analyses, neither the AR to ERα ratio tertile groups nor an AR to
ERα ratio cut-point of 0.82 were independent predictors of OS in the combined training and validation cohorts (Supplementary Table 6A and 6B). Consistent with this, the AR to ERα ratio did not differentiate between luminal A and luminal B ERα positive breast cancer subgroups (Supplementary Table 7).

Discussion

The current study demonstrates that clinical assessment of AR in addition to ERα may permit a more precise prediction of breast cancer outcome in women, particularly those with hormone sensitive disease, providing an optimal cut-point for AR is utilized. As highlighted by our comprehensive review of the literature, many published studies have investigated AR as a prognostic factor in breast cancer, but the findings have been inconsistent resulting in doubt about its clinical utility for prognostication. A critical common limitation of previous studies has been the failure to define an optimal AR cut-point for prognosis. Rather, arbitrary cut-points of 1% and 10% positivity, used to determine AR status, were typically applied to dichotomize data for prognostic testing. Herein, we provide the first robust, statistically derived cut-point for future testing of AR as a prognostic factor in prospective cohorts.

ROC analysis using two independent, well characterized breast cancer cohorts with 10-year follow-up demonstrated that a cut-point of 78% AR positivity, which approximated the median value, achieved the best combination of specificity and sensitivity for prediction of breast cancer survival. This cut-point value was reinforced using recursive partitioning of the data. Ten-fold cross validation, a statistic used in supervised learning and class prediction, confirmed the robustness of the AR cut-point defined by ROC analysis. Our finding that the optimal AR cut-point approximated the median percentage positivity determined by immunostaining is consistent with 2 of 3 previous biochemical (radio-ligand binding) studies of unselected breast cancer cohorts that arbitrarily used the median AR protein level to dichotomize the breast cancer survival data (52-54). More recently,
Tokunaga et al (7) employed an arbitrary cut-point of 75% AR positivity, which approximated the median immunostaining, to demonstrate that AR is an independent predictor of outcome in an unselected breast cancer cohort. Hence, our statistically determined AR cut-point is not without precedence in the literature concerning AR and breast cancer prognostication when all cases are considered.

AR was prognostic in this study for all breast cancer cases in both cohorts, which likely reflects its negative correlation with tumor size and proliferative capacity, regardless of ERα status as shown in this study and previously reported (3, 5, 55). A recent meta-analysis in which AR mRNA expression was correlated with various gene signatures in large combined breast cancer cohorts strongly supports this concept (26). Functionally, AR and ERα are hormone-activated nuclear transcription factors that regulate gene expression and are commonly co-expressed in normal and malignant breast epithelial cells (37, 56), indicating the potential for direct cross-talk between these two sex hormone receptor signaling pathways. Indeed, antagonism between AR and ERα signaling is thought to underpin sex-specific breast development (9). In breast cancer, this interaction and its functional consequence is likely to be perturbed by altered receptor levels and a pathological hormone milieu (8, 9, 34).

Our data showing that a high AR cut-point is required to robustly predict survival from ERα positive disease is consistent with the concept that a minimum threshold of AR activity is required to restrain growth of ERα positive breast cancers (34, 37). Indeed, this may explain why AR positivity tends to be higher than ERα positivity in normal and most ERα positive malignant breast tissues, even though the expression of both receptors is increased in the malignant compared to the normal state (8). We have previously shown that exogenous AR dose-dependently inhibited ERα transcriptional activity from an AR to ERα molar ratio of 1:1 to 4:1 (37). This finding is in accordance with another study showing that an AR to ERα ratio approximating 1 was associated with a survival advantage in a tamoxifen-treated cohort of ERα positive patients (15). However, the
same study disagreed with our finding that a predominance of AR over ERα conferred a survival advantage, reporting that an AR to ERα ratio > 2 was associated with treatment failure on tamoxifen (15). In that study, AR positive tumors were defined as AR >0% positivity, which has only been used in 1 other immunohistochemical study (see Table 1), and AR alone did not independently predict outcome. Moreover, the AR=0% cases, which are well established as being associated with a poor prognosis, were included in the AR to ERα ratio <2 group. In our study, in the analysis showing that patients with tumors characterized by a predominance of ERα over AR had a reduced chance of survival, only tumors that were positive for both AR and ERα were included in the analysis. In this scenario, AR may have lost its capacity to antagonize ERα signaling, either due to insufficient expression or activation. Additionally, a recent preclinical study showed that estrogen potentially can activate AR in a non-classical manner to facilitate ERα genomic activity in breast cancer cells (17). Hence, studies examining the prognostic value of AR in ERα positive breast cancers that included tumors with very low levels of AR expression (e.g. cut-points of >0%, >1% or >10%) may have produced disparate results because low AR expression or insufficient activation cannot effectively oppose ERα activity or conditions are such that AR is hijacked into facilitating ERα activity. This presents a therapeutic conundrum as the latter scenario indicates an AR antagonist strategy whereas the former suggests an AR agonist strategy. While both options are supported by recent studies that have employed patient-derived xenograft models (14, 17), the outcome of current clinical trials will provide definitive evidence regarding this controversy.

AR status did not improve the stratification of ERα negative breast cancers in terms of patient outcome, likely due to insufficient power. In a larger study, Hu et al (3) showed that postmenopausal women with ERα negative breast cancers had poor survival irrespective of AR status. Since ERα negative breast cancers are less common than ERα positive cancers and represent a very heterogeneous mix of molecular disease entities, it has been difficult to definitively identify robust prognostic factors. However, meta-analyses of studies examining AR as a prognostic factor
in breast cancer have found that AR is associated with a better outcome in ERα negative disease in general or of its various sub-groupings (27-29). Despite the reported association of AR with a better outcome in this disease context, there has been much enthusiasm for therapeutic targeting of AR, particularly in TNBC, as there are currently no targeted therapies for the treatment of this aggressive disease subtype. The mainstream clinical approach has been AR antagonism based on *in vitro* and *in vivo* pre-clinical studies suggesting that AR has oncogenic activity in some ERα negative breast cancer models (18-24, 57), and two clinical trials of women with advanced TNBC have reported efficacy with this approach (58, 59). However, *in vitro* studies have demonstrated dichotomous proliferative effects of androgens in different AR positive ERα negative breast cancer cell lines (8). For example, while the MDA-MB-453 breast cancer cell line is stimulated by androgen *in vitro* (15, 20-22), other AR positive ERα negative breast cancer cell lines (MFM-223 and CAL-148) are inhibited (25, 60). Androgenic stimulation of tumor growth has also been demonstrated *in vivo* using MDA-MB-453 xenografts (15, 22), but this has not been convincingly demonstrated with other ERα negative models. Rather, these studies have inferred a growth stimulatory effect of AR signaling *in vivo* by showing growth inhibition using an AR antagonist (bicalutamide or enzalutamide) alone (18, 23, 24). The interpretation of these studies is confounded by the potential for off-target effects of AR antagonists at µM doses in ERα negative breast cancer cells (25).

Although the two cohorts in this study have the advantage of long-term clinical follow-up, treatment practices have evolved since the cohorts were assembled. Nevertheless, adjuvant endocrine therapy with tamoxifen or new generation ERα-target therapies remains a critical component of standard-of-care for women with ERα positive disease. A sub-analysis of the training cohort, where treatment information was available, demonstrated that AR, using a 78% cut-point, predicted OS in patients who received tamoxifen as adjuvant endocrine therapy. Considering our findings that AR is a robust independent prognostic factor for breast cancer survival in both unselected and selected ERα positive cases providing an appropriate cut-point was utilized, we
propose that studies to determine the optimal AR cut-point for selection of patients who are likely to respond to hormonal interventions targeting either the AR or ERα are warranted. This is particularly important given the recent interest in targeting AR in breast cancer, with clinical trials initiated to investigate either the stimulation or inhibition of this signaling pathway (ClinicalTrials.gov identifiers NCT00468715, NCT01597193, NCT00755885, NCT01889238, NCT01616758, NCT02463032 and NCT02007512). At present, there is no consensus regarding how best to select breast cancer patients who most likely will benefit from an AR targeted therapeutic intervention, highlighting the need for well-designed and validated prospective studies of AR as a predictive marker as well as a prognostic marker.

In summary, by eliminating the use of arbitrary criteria for AR positivity to predict breast cancer survival, assessment of AR status could become an important clinical tool in the management of this disease. Since pre-clinical studies suggest a role for AR in resistance to tamoxifen (15, 61) and aromatase inhibitors (62, 63), it will be important to test the prognostic power of the AR in the context of the cut-points defined herein using tissues collected from large contemporary, prospective breast cancer cohorts.

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**Competing Interests:** The authors have no conflicts to disclose.
### Tables

#### Table 1. Summary of studies investigating Androgen Receptor as a prognostic factor in breast cancer.

<table>
<thead>
<tr>
<th>Ref</th>
<th>Year</th>
<th>N</th>
<th>Sample</th>
<th>Ab</th>
<th>Cut-point</th>
<th>Univariate</th>
<th>Multivariate</th>
<th>HR (95% CI)</th>
</tr>
</thead>
<tbody>
<tr>
<td>(64)</td>
<td>1979</td>
<td>292</td>
<td>RLB</td>
<td>-</td>
<td>≥10 fmol/mg</td>
<td>NS</td>
<td>ND</td>
<td></td>
</tr>
<tr>
<td>(65)</td>
<td>1984</td>
<td>1181</td>
<td>RLB</td>
<td>-</td>
<td>≥5 fmol/mg</td>
<td>OS (P&lt;0.001)</td>
<td>ND</td>
<td></td>
</tr>
<tr>
<td>(66)</td>
<td>1986</td>
<td>796</td>
<td>RLB</td>
<td>-</td>
<td>≥5 fmol/mg</td>
<td>OS (P&lt;0.05)</td>
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<tr>
<td>(67)</td>
<td>1990</td>
<td>61</td>
<td>RLB</td>
<td>-</td>
<td>≥10 fmol/mg</td>
<td>OS at 36 months (P=0.043)</td>
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<tr>
<td>(54)</td>
<td>1992</td>
<td>224</td>
<td>RLB</td>
<td>-</td>
<td>&gt;50.5 fmol/mg (median)</td>
<td>Yes</td>
<td>MFS (P=0.001)</td>
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<tr>
<td>(52)</td>
<td>1996</td>
<td>269</td>
<td>RLB</td>
<td>-</td>
<td>&gt;43 fmol/mg (median)</td>
<td>NS</td>
<td>ND</td>
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<tr>
<td>(68)</td>
<td>1996</td>
<td>153</td>
<td>Frozen - WS</td>
<td>ARF39.3</td>
<td>≥10%</td>
<td>DFS (P=0.043)</td>
<td>NS</td>
<td></td>
</tr>
<tr>
<td>(69)</td>
<td>2006</td>
<td>232</td>
<td>TMA</td>
<td>AR441</td>
<td>≥10%</td>
<td>DFS (P=0.028)</td>
<td>NS</td>
<td></td>
</tr>
<tr>
<td>(70)</td>
<td>2007</td>
<td>115</td>
<td>FFPE – WS</td>
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<td>≥10%</td>
<td>OS (P=0.03)</td>
<td>NS</td>
<td></td>
</tr>
<tr>
<td>(71)</td>
<td>2007</td>
<td>1087</td>
<td>TMA</td>
<td>AR441</td>
<td>Allred score &gt;3</td>
<td>NS</td>
<td>NS</td>
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<td>(72)</td>
<td>2008</td>
<td>488</td>
<td>FFPE – WS</td>
<td>AR441</td>
<td>NS</td>
<td>RFS (P=0.023)</td>
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<tr>
<td>(73)</td>
<td>2008</td>
<td>111</td>
<td>TMA</td>
<td>AR441</td>
<td>&gt;0</td>
<td>OS (P = 0.01)</td>
<td>OS (P = 0.03)</td>
<td>0.46 (0.23–0.93)</td>
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<tr>
<td>(74)</td>
<td>2008</td>
<td>138</td>
<td>FFPE – WS</td>
<td>AR441</td>
<td>≥15%</td>
<td>OS (P=0.01)</td>
<td>NS</td>
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<tr>
<td>(75)</td>
<td>2008</td>
<td>347</td>
<td>RPA</td>
<td>NR</td>
<td>≥0.085 (median)</td>
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<td>OS (P=0.004)</td>
<td>0.36 (0.22-0.59)</td>
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<tr>
<td>(76)</td>
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<td>673</td>
<td>TMA</td>
<td>ARF39.4.1</td>
<td>Remmele score &gt;3</td>
<td>RFS (P=0.033)</td>
<td>OS (P=0.023)</td>
<td>NS</td>
</tr>
<tr>
<td>(77)</td>
<td>2011</td>
<td>335</td>
<td>FFPE – WS</td>
<td>AR441</td>
<td>Allred score NR</td>
<td>OS (P&lt;0.001)</td>
<td>OS (P&lt;0.001)</td>
<td>0.31 (0.19-0.50)</td>
</tr>
<tr>
<td>(78)</td>
<td>2013</td>
<td>73</td>
<td>TMA</td>
<td>AR441</td>
<td>≥1%</td>
<td>OS (P=0.004)</td>
<td>ND</td>
<td></td>
</tr>
<tr>
<td>(79)</td>
<td>2013</td>
<td>205</td>
<td>NR</td>
<td>AR441</td>
<td>&gt;75%</td>
<td>DFS (P=0.0005)</td>
<td>DFS (P=0.005)</td>
<td>0.46 (0.26-0.79)</td>
</tr>
<tr>
<td>(80)</td>
<td>2014</td>
<td>109</td>
<td>TMA</td>
<td>AR441</td>
<td>≥1%</td>
<td>DFS (P=0.026)</td>
<td>DFS (P=0.031)</td>
<td>0.24 (0.07-0.88)</td>
</tr>
<tr>
<td>(81)</td>
<td>2014</td>
<td>379</td>
<td>NR</td>
<td>AR-318</td>
<td>Histoscore &gt;10</td>
<td>DFS (P&lt;0.0001)</td>
<td>DFS (P&lt;0.0001)</td>
<td>0.24 (0.12-0.50)</td>
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<tr>
<td>(82)</td>
<td>2015</td>
<td>1100</td>
<td>TMA</td>
<td>ARF39.4.1</td>
<td>Histoscore &gt;3</td>
<td>OS (P=0.009)</td>
<td>0.65 (0.47-0.90)</td>
<td></td>
</tr>
<tr>
<td>(83)</td>
<td>2016</td>
<td>1141</td>
<td>TMA</td>
<td>AR-N20</td>
<td>H-score &gt;190</td>
<td>OS (P&lt;0.001)</td>
<td>OS (P=0.033)</td>
<td>0.80 (0.64-0.98)</td>
</tr>
</tbody>
</table>

#### ERα Negative Breast Cancers

<table>
<thead>
<tr>
<th>Ref</th>
<th>Year</th>
<th>N</th>
<th>Sample</th>
<th>Ab</th>
<th>Cut-point</th>
<th>Univariate</th>
<th>Multivariate</th>
<th>HR (95% CI)</th>
</tr>
</thead>
<tbody>
<tr>
<td>(84)</td>
<td>2010</td>
<td>226</td>
<td>ER-PRE-PR-</td>
<td>ARF39.4.1</td>
<td>≥5%</td>
<td>DFS (P=0.049)</td>
<td>NS</td>
<td></td>
</tr>
<tr>
<td>(85)</td>
<td>2007</td>
<td>282</td>
<td>TNBC</td>
<td>ARF39.4.1</td>
<td>Modified H-score &gt;1%</td>
<td>OS (P=0.04)</td>
<td>NS</td>
<td></td>
</tr>
<tr>
<td>(86)</td>
<td>2012</td>
<td>83</td>
<td>FFPE – WS</td>
<td>AR441</td>
<td>≥1%</td>
<td>NS</td>
<td>NS</td>
<td></td>
</tr>
<tr>
<td>(87)</td>
<td>2013</td>
<td>203</td>
<td>FFPE – WS</td>
<td>AR441</td>
<td>H-score &gt;10%</td>
<td>NS</td>
<td>ND</td>
<td></td>
</tr>
<tr>
<td>(88)</td>
<td>2014</td>
<td>699</td>
<td>TNBC</td>
<td>AR27</td>
<td>≥1%</td>
<td>DFS (P=0.05)</td>
<td>ND</td>
<td></td>
</tr>
<tr>
<td>(89)</td>
<td>2014</td>
<td>173</td>
<td>TNBC</td>
<td>ARF39.4.1</td>
<td>≥5%</td>
<td>OS (P=0.032)</td>
<td>NS</td>
<td></td>
</tr>
</tbody>
</table>
Abbreviations: Ab, antibody; AR, androgen receptor; AR+, AR positive; AR-, AR negative; DFS, breast cancer specific relapse or breast cancer death; ER, estrogen receptor; FFPE, formalin fixed paraffin embedded; HR, hazard ratio; IHC, immunohistochemistry; MFS, metastasis free survival; NA, not available; ND, not determined; NR, not reported; NS, not significant; OS, overall survival or breast-cancer specific survival; PR, progesterone receptor; Ref, reference; RFS, relapse free survival; RLB, radio ligand binding assay; RPA, reverse phase protein microarray; TMA, tissue microarray; WS, whole section; a tumor samples from patients who developed metastatic disease; b grade III cancers; c ER cut-point >5%; d ER cut-point ≥10%; e ER cut-point >0%; f ER cut-point not reported; g ER cut-point ≥1%; h ER cut-point ≥10 pmol/mg protein; TNBC, triple negative breast cancer (ER- PR-HER2-); studies in bold were significant by multivariate analysis.
Table 2. Univariate and multivariate analyses for relapse-free survival (RFS) and overall survival (OS) (training cohort)

### Univariate Cox Regression

<table>
<thead>
<tr>
<th>Variable</th>
<th>RFS</th>
<th>95% CI</th>
<th>P value</th>
<th>OS</th>
<th>95% CI</th>
<th>P value</th>
</tr>
</thead>
<tbody>
<tr>
<td>Age(^e) (n=219)</td>
<td>1.01</td>
<td>0.98-1.04</td>
<td>0.460</td>
<td>1.01</td>
<td>1.00-1.02</td>
<td>0.159</td>
</tr>
<tr>
<td>Tumor size(^b) (n=205)</td>
<td>2.18</td>
<td>1.27-3.74</td>
<td>0.005</td>
<td>2.34</td>
<td>1.24-4.40</td>
<td>0.009</td>
</tr>
<tr>
<td>Grade(^b) (n=198)</td>
<td>4.90</td>
<td>1.19-20.17</td>
<td>0.028</td>
<td>3.68</td>
<td>0.89-15.29</td>
<td>0.072</td>
</tr>
<tr>
<td>Lymph status(^d) (n=208)</td>
<td>3.29</td>
<td>1.82-5.94</td>
<td>0.0001</td>
<td>2.42</td>
<td>1.25-4.68</td>
<td>0.008</td>
</tr>
<tr>
<td>ER(\alpha) status(^e) (n=204)</td>
<td>0.37</td>
<td>0.21-0.66</td>
<td>0.0001</td>
<td>0.23</td>
<td>0.12-0.43</td>
<td>&lt;0.0001</td>
</tr>
<tr>
<td>PR status(^d) (n=205)</td>
<td>0.36</td>
<td>0.21-0.61</td>
<td>0.0001</td>
<td>0.22</td>
<td>0.12-0.42</td>
<td>&lt;0.0001</td>
</tr>
<tr>
<td>HER-2/neu status(^d) (n=201)</td>
<td>2.75</td>
<td>1.56-8.44</td>
<td>0.0001</td>
<td>3.06</td>
<td>1.60-5.87</td>
<td>0.001</td>
</tr>
<tr>
<td>Ki67 status(^b) (n=190)</td>
<td>2.73</td>
<td>1.57-4.73</td>
<td>0.0001</td>
<td>3.42</td>
<td>1.77-6.61</td>
<td>&lt;0.0001</td>
</tr>
<tr>
<td>AR status(^d) (n=210)</td>
<td>0.992</td>
<td>0.98-1.00</td>
<td>0.015</td>
<td>0.99</td>
<td>0.98-1.00</td>
<td>0.001</td>
</tr>
<tr>
<td>AR status 1(^e) (n=210)</td>
<td>0.48</td>
<td>0.25-0.92</td>
<td>0.026</td>
<td>0.36</td>
<td>0.18-0.73</td>
<td>0.004</td>
</tr>
<tr>
<td>AR status 10(^e) (n=210)</td>
<td>0.585</td>
<td>0.32-1.06</td>
<td>0.077</td>
<td>0.39</td>
<td>0.20-0.73</td>
<td>0.004</td>
</tr>
<tr>
<td>AR status 78(^d) (n=210)</td>
<td>0.54</td>
<td>0.32-0.92</td>
<td>0.024</td>
<td>0.32</td>
<td>0.16-0.62</td>
<td>0.001</td>
</tr>
</tbody>
</table>

### Multivariate Analysis – AR 1%

<table>
<thead>
<tr>
<th>Variable</th>
<th>RFS (n=196)</th>
<th>95% CI</th>
<th>P value</th>
<th>OS (n=174)</th>
<th>95% CI</th>
<th>P value</th>
</tr>
</thead>
<tbody>
<tr>
<td>Tumor size(^b)</td>
<td>1.58</td>
<td>0.85-2.82</td>
<td>0.150</td>
<td>1.43</td>
<td>0.70-2.92</td>
<td>0.313</td>
</tr>
<tr>
<td>Tumor grade(^e)</td>
<td>1.35</td>
<td>0.31-5.91</td>
<td>0.688</td>
<td>-</td>
<td>-</td>
<td>-</td>
</tr>
<tr>
<td>Lymph node status(^d)</td>
<td>2.57</td>
<td>1.35-4.91</td>
<td>0.004</td>
<td>1.98</td>
<td>1.00-3.91</td>
<td>0.051</td>
</tr>
<tr>
<td>ER(\alpha) status(^e)</td>
<td>0.83</td>
<td>0.29-2.37</td>
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<td>0.47</td>
<td>0.16-1.44</td>
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<tr>
<td>PR status(^d)</td>
<td>0.61</td>
<td>0.22-1.73</td>
<td>0.355</td>
<td>0.57</td>
<td>0.18-1.82</td>
<td>0.527</td>
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<tr>
<td>HER-2/neu status(^d)</td>
<td>1.77</td>
<td>0.89-3.51</td>
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<td>1.83</td>
<td>0.84-3.96</td>
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<tr>
<td>Ki67 status(^b)</td>
<td>1.89</td>
<td>0.92-3.86</td>
<td>0.082</td>
<td>1.73</td>
<td>0.78-3.83</td>
<td>0.178</td>
</tr>
<tr>
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<td>0.36-1.20</td>
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<td>1.002</td>
<td>0.40-2.49</td>
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</table>

### Multivariate Analysis – AR 10%

<table>
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<tr>
<th>Variable</th>
<th>RFS (n=196)</th>
<th>95% CI</th>
<th>P value</th>
<th>OS (n=174)</th>
<th>95% CI</th>
<th>P value</th>
</tr>
</thead>
<tbody>
<tr>
<td>Tumor size(^b)</td>
<td>1.57</td>
<td>0.85-2.92</td>
<td>0.152</td>
<td>1.43</td>
<td>0.72-2.88</td>
<td>0.309</td>
</tr>
<tr>
<td>Tumor grade(^e)</td>
<td>1.36</td>
<td>0.31-5.93</td>
<td>0.684</td>
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<td>-</td>
<td>-</td>
</tr>
<tr>
<td>Lymph node status(^d)</td>
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<td>1.37-4.94</td>
<td>0.004</td>
<td>1.97</td>
<td>0.99-3.91</td>
<td>0.053</td>
</tr>
<tr>
<td>ER(\alpha) status(^e)</td>
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<td>0.28-2.17</td>
<td>0.638</td>
<td>0.48</td>
<td>0.16-1.46</td>
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</tr>
<tr>
<td>PR status(^d)</td>
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<td>0.332</td>
<td>0.58</td>
<td>0.18-1.86</td>
<td>0.358</td>
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<tr>
<td>HER-2/neu status(^d)</td>
<td>1.74</td>
<td>0.86-3.50</td>
<td>0.122</td>
<td>1.85</td>
<td>0.86-3.95</td>
<td>0.114</td>
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<tr>
<td>Ki67 status(^b)</td>
<td>1.88</td>
<td>0.92-3.83</td>
<td>0.085</td>
<td>1.73</td>
<td>0.78-3.83</td>
<td>0.177</td>
</tr>
<tr>
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<td>0.36-1.20</td>
<td>0.129</td>
<td>0.95</td>
<td>0.40-12.22</td>
<td>0.898</td>
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</table>

### Multivariate Analysis – AR 78%

<table>
<thead>
<tr>
<th>Variable</th>
<th>RFS (n=196)</th>
<th>95% CI</th>
<th>P value</th>
<th>OS (n=174)</th>
<th>95% CI</th>
<th>P value</th>
</tr>
</thead>
<tbody>
<tr>
<td>Tumor size(^b)</td>
<td>1.53</td>
<td>0.80-2.76</td>
<td>0.181</td>
<td>1.38</td>
<td>0.74-2.57</td>
<td>0.369</td>
</tr>
<tr>
<td>Tumor grade(^e)</td>
<td>1.25</td>
<td>0.29-5.51</td>
<td>0.788</td>
<td>-</td>
<td>-</td>
<td>-</td>
</tr>
<tr>
<td>Lymph node status(^d)</td>
<td>2.68</td>
<td>1.41-5.08</td>
<td>0.003</td>
<td>1.89</td>
<td>0.95-3.73</td>
<td>0.068</td>
</tr>
<tr>
<td>ER(\alpha) status(^e)</td>
<td>0.88</td>
<td>0.33-2.50</td>
<td>0.797</td>
<td>0.52</td>
<td>0.18-1.52</td>
<td>0.228</td>
</tr>
<tr>
<td>PR status(^d)</td>
<td>0.64</td>
<td>0.22-1.73</td>
<td>0.402</td>
<td>0.69</td>
<td>0.22-2.17</td>
<td>0.527</td>
</tr>
<tr>
<td>HER-2/neu status(^d)</td>
<td>1.80</td>
<td>0.90-3.56</td>
<td>0.085</td>
<td>2.11</td>
<td>1.01-4.38</td>
<td>0.046</td>
</tr>
<tr>
<td>Ki67 status(^b)</td>
<td>1.95</td>
<td>0.96-3.90</td>
<td>0.067</td>
<td>1.77</td>
<td>0.81-3.87</td>
<td>0.151</td>
</tr>
<tr>
<td>AR status 78(^d)</td>
<td>0.62</td>
<td>0.36-1.20</td>
<td>0.129</td>
<td>0.41</td>
<td>0.20-0.84</td>
<td>0.015</td>
</tr>
</tbody>
</table>

\(^a\) Age at diagnosis (continuous variable); \(b\) = tumor size (mm) as a dichotomous variable cut-point \(\leq 20\) vs \(>20\); \(c\) = tumor grade (well or moderate vs poor); \(d\) = lymph node status (negative vs positive); \(e\) = ER\(\alpha\) status (% positive cells) as a dichotomous variable cut-point \(<1\) vs \(\geq 1\) positive cells; \(f\) = PR status (% positive cells) as a dichotomous variable cut-point \(\leq 10\) vs \(>10\) positive cells; \(g\) = HER-2/neu status (negative vs positive); \(h\) = Ki67 status (% positive cells) as a dichotomous variable cut-point \(<7.5\) vs \(\geq 7.5\) positive cells; \(i\) = AR status (% positive cells) as a dichotomous variable cut-point \(<1\) vs \(\geq 1\) positive cells; \(j\) = AR status (% positive cells) as a dichotomous variable cut-point \(<10\) vs \(\geq 10\) positive cells; \(l\) = AR status (% positive cells) as a dichotomous variable cut-point \(<78\) vs \(\geq 78\) positive cells

Author manuscripts have been peer reviewed and accepted for publication but have not yet been edited.
Table 3. Univariate and multivariate analyses for OS (validation cohort)

<table>
<thead>
<tr>
<th>Variable</th>
<th>Univariate Cox Regression</th>
<th>Multivariate Analysis (n=222)</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>HR</td>
<td>95% CI</td>
</tr>
<tr>
<td>Age&lt;sup&gt;a&lt;/sup&gt;</td>
<td>1.01</td>
<td>1.00-1.02</td>
</tr>
<tr>
<td>Tumor size&lt;sup&gt;b&lt;/sup&gt; (n=417)</td>
<td>1.97</td>
<td>1.37-2.83</td>
</tr>
<tr>
<td>Grade&lt;sup&gt;c&lt;/sup&gt; (n=402)</td>
<td>0.89</td>
<td>0.60-1.34</td>
</tr>
<tr>
<td>Lymph status&lt;sup&gt;d&lt;/sup&gt; (n=370)</td>
<td>2.46</td>
<td>1.95-3.92</td>
</tr>
<tr>
<td>ERα status&lt;sup&gt;e&lt;/sup&gt; (n=364)</td>
<td>0.53</td>
<td>0.36-0.77</td>
</tr>
<tr>
<td>PR status&lt;sup&gt;f&lt;/sup&gt; (n=267)</td>
<td>0.63</td>
<td>0.42-0.93</td>
</tr>
<tr>
<td>HER-2/neu status&lt;sup&gt;g&lt;/sup&gt; (n=343)</td>
<td>2.05</td>
<td>1.25-3.35</td>
</tr>
<tr>
<td>Ki67 status&lt;sup&gt;h&lt;/sup&gt; (n=367)</td>
<td>1.64</td>
<td>1.15-2.35</td>
</tr>
<tr>
<td>AR status&lt;sup&gt;i&lt;/sup&gt; (n=376)</td>
<td>0.99</td>
<td>0.99-1.00</td>
</tr>
<tr>
<td>AR status&lt;sup&gt;j&lt;/sup&gt; (n=376)</td>
<td>0.60</td>
<td>0.33-1.10</td>
</tr>
<tr>
<td>AR status&lt;sup&gt;k&lt;/sup&gt; (n=376)</td>
<td>0.79</td>
<td>0.50-1.23</td>
</tr>
<tr>
<td>AR status&lt;sup&gt;l&lt;/sup&gt; (n=376)</td>
<td>0.51</td>
<td>0.34-0.75</td>
</tr>
</tbody>
</table>

<sup>a</sup> = Age at diagnosis (continuous variable)
<sup>b</sup> = Tumor size (mm) as a dichotomous variable cut-point <=20 vs >20
<sup>c</sup> = Tumor grade (well or moderate vs poor)
<sup>d</sup> = Lymph node status (negative vs positive)
<sup>e</sup> = ERα status (% positive cells) as a dichotomous variable cut-point <1 vs ≥1% positive cells
<sup>f</sup> = PR status (% positive cells) as a dichotomous variable cut-point <1 vs ≥1% positive cells
<sup>g</sup> = HER-2/neu status (negative vs positive)
<sup>h</sup> = Ki67 status (% positive cells) as a dichotomous variable cut-point <16 vs ≥16% positive cells
<sup>i</sup> = AR status (% positive cells) as a dichotomous variable cut-point <1 vs ≥1% positive cells
<sup>j</sup> = AR status (% positive cells) as a dichotomous variable cut-point <10 vs ≥10% positive cells
<sup>k</sup> = AR status (% positive cells) as a dichotomous variable cut-point <78 vs ≥78% positive cells
<sup>l</sup> = AR status (% positive cells) as a dichotomous variable cut-point <78 vs ≥78% positive cells
Figure 1. High AR expression is associated with an increased overall survival (OS) in the training cohort. (A) Receiver operating characteristic (ROC) analysis in the training cohort identified an area under the curve of 0.678 (95% CI; 0.587-0.770, \(P<0.0001\)). (B) Frequency distribution for AR as assessed by visual scoring in a cohort of 219 breast cancers. The mean, median and range of AR percent positivity by immunostaining are shown. (C) Left panel: an example of weak nuclear AR immunostaining in approximately 20% of the tumor cells. Right panel: an example of breast cancer with uniform nuclear AR immunoreactivity of moderate intensity in virtually 100% of tumor cells. Arrows denote examples of AR positive tumor cells. (D) Kaplan-Meier analysis showing that high AR (\(\geq 78\%\) nuclear positivity) was significantly associated with increased OS (log rank statistic=12.60, \(P<0.0001\)). (E) Kaplan-Meier analysis showing that breast tumors with high AR that were ER\(\alpha\) positive (AR\(\geq 78\%\) ER\(\alpha\)+) are associated with a significantly increased overall patient survival (log rank statistic=32.34, \(P<0.0001\)) when compared to the remaining tumor groups (AR<78% ER\(\alpha\)+; AR\(\geq 78\%\) ER\(\alpha\)- and AR<78% ER\(\alpha\)-). (F) Cox regression analysis for OS comparing relative risk among AR and ER\(\alpha\) sub-groups in (E); n=204. ER\(\alpha\)+ denotes ER\(\alpha\) \(\geq 1\%\) positive tumor nuclei; ER\(\alpha\)- denotes ER\(\alpha\) negative tumors. AR\(\geq 78\) denotes AR positivity greater than or equal to 78% and AR<78 refers to AR positivity less than 78%. HR, hazard ratio; CI, confidence interval.

Figure 2. AR immunostaining and overall survival (OS) in the validation cohort. (A) Receiver operating characteristic (ROC) analysis in the validation cohort identified an area under the curve of 0.588 (95% CI; 0.525-0.651, \(P=0.008\)). (B) Frequency distribution for AR was assessed by visual scoring in the validation cohort of 418 breast tumors. The mean, median and range of percent positivity by AR immunostaining are shown. (C) Left panel: an example of weak nuclear AR immunostaining in approximately 20% of tumor cells. Right panel: an example of strong AR nuclear immunoreactivity in approximately 80% of tumor cells. Arrows denote examples of AR positive tumor cells. (D) Kaplan-Meier analysis showing that high AR was significantly associated with increased OS (log rank statistic=12.07, \(P=0.001\)). (E) Kaplan-Meier analysis showing that breast tumors with high AR (\(\geq 78\%\) nuclear positivity) that were ER\(\alpha\) positive (AR\(\geq 78\%\) ER\(\alpha\)+) are associated with significantly increased OS (log rank statistic=20.12, \(P<0.0001\)) when compared to the remaining tumor groups (AR<78% ER\(\alpha\)+; AR\(\geq 78\%\) ER\(\alpha\)-).
and AR<78% ERα). (F) Cox regression analysis for OS, comparing relative risk among the AR and ERα sub-groups in (E); n=344. ERα+ denotes ERα ≥1% tumor nuclei; ERα– denotes ERα negative tumors. AR≥78 denotes AR positivity greater than or equal to 78% and AR<78 refers to AR positivity less than 78%. HR, hazard ratio; CI, confidence interval.

**Figure 3. AR:ERα ratio predicts overall survival (OS).** Analysis of the AR:ERα ratio in the combined training and validation cohorts (n=552) with cases separated into tertiles; AR:ERα ratio approximating 1 (i.e. 0.87-1.05), AR:ERα ratio >1.05, AR:ERα ratio <0.87. For the 120 patients with ERα negative tumors, patients were divided into groups according to whether the tumors were AR high (≥ 78% nuclear positivity) or not (< 78% AR nuclear positivity). **Upper panel:** Kaplan-Meier analysis showing the association between the different subgroups and OS (log rank statistic=37.4, P<0.0001). **Lower panel:** Cox regression analysis showing the relative risk of death in the different subgroups relative those with an AR:ERα ratio approximating 1. HR, hazard ratio; CI, confidence interval.
References


Figure 1

A. Sensitivity vs. 1 - Specificity plot.

B. Distribution of AR positive tumor nuclei per case (%).

C. Immunohistochemical staining of AR U407 epitope aa 200-220.

D. Cumulative survival curves for low and high AR.

E. Comparison of AR≥78% ERα+ and AR<78% ERα+.

F. Univariate analysis of AR status on overall survival.

<table>
<thead>
<tr>
<th>Variable</th>
<th>HR</th>
<th>95% CI</th>
<th>P</th>
</tr>
</thead>
<tbody>
<tr>
<td>AR≥78% ERα+</td>
<td>1.0</td>
<td></td>
<td></td>
</tr>
<tr>
<td>AR≥78% ERα+ vs AR≥78% ERα-</td>
<td>11.5</td>
<td>3.63-36.26</td>
<td>&lt;0.0001</td>
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<tr>
<td>AR≥78% ERα+ vs AR&lt;78% ERα-</td>
<td>4.9</td>
<td>1.79-13.59</td>
<td>0.002</td>
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<tr>
<td>AR≥78% ERα+ vs AR&lt;78% ERα-</td>
<td>10.3</td>
<td>3.67-29.05</td>
<td>&lt;0.0001</td>
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</tbody>
</table>
Figure 3

<table>
<thead>
<tr>
<th>Variable</th>
<th>Combined training &amp; validation cohorts OS</th>
</tr>
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<tbody>
<tr>
<td></td>
<td>HR</td>
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<tr>
<td>AR:ERα 0.87-1.05</td>
<td>1.00</td>
</tr>
<tr>
<td>AR:ERα 0.87-1.05 vs AR:ERα &gt;1.05</td>
<td>1.12</td>
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<tr>
<td>AR:ERα 0.87-1.05 vs AR:ERα &lt;0.87</td>
<td>1.69</td>
</tr>
<tr>
<td>AR:ERα 0.87-1.05 vs AR+ERα-</td>
<td>3.24</td>
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<td>AR:ERα 0.87-1.05 vs AR-ERα+</td>
<td>3.00</td>
</tr>
<tr>
<td>AR:ERα 0.87-1.05 vs AR-ERα-</td>
<td>3.22</td>
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Clinical Cancer Research

The Magnitude of Androgen Receptor Positivity in Breast Cancer is Critical for Reliable Prediction of Disease Outcome


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