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Androgen Receptor Expression and Breast Cancer Survival: Results From the Nurses' Health Studies

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Abstract

Background: Hormone receptor signaling is critical in the progression of breast cancers, although the role of the androgen receptor (AR) remains unclear, particularly for estrogen receptor (ER)–negative tumors. This study assessed AR protein expression as a prognostic marker for breast cancer mortality.

Methods: This study included 4147 pre- and postmenopausal women with invasive breast cancer from the Nurses' Health Study (diagnosed 1976–2008) and Nurses' Health Study II (1989–2008) cohorts. AR protein expression was evaluated by immunohistochemistry and scored through pathologist review and as a digitally quantified continuous measure. Hazard ratios (HR) and 95% confidence intervals (CI) of breast cancer mortality were estimated from Cox proportional hazards models, adjusting for patient, tumor, and treatment covariates.

Results: Over a median 16.5 years of follow-up, there were 806 deaths due to breast cancer. In the 7 years following diagnosis, AR expression was associated with a 27% reduction in breast cancer mortality overall (multivariable HR = 0.73, 95% CI = 0.58 to 0.91) a 47% reduction for ER+ cancers (HR = 0.53, 95% CI = 0.41 to 0.69), and a 62% increase for ER– cancers (HR = 1.62, 95% CI = 1.18 to 2.22) (*P* heterogeneity < .001). A log-linear association was observed between AR expression and breast cancer mortality among ER– cancers (HR = 1.14, 95% CI = 1.02 to 1.26 per each 10% increase in AR), although no log-linear association was observed among ER+ cancers.

Conclusions: AR expression was associated with improved prognosis in ER+ tumors and worse prognosis in ER– tumors in the first 5–10 years postdiagnosis. These findings support the continued evaluation of AR-targeted therapies for AR+/ER– breast cancers.

Sex-steroid hormone receptor signaling is critical in the development and progression of breast cancers (1,2). The estrogen receptor (ER) is of particular importance, as many breast cancer risk factors are believed to act, at least partially, through their effects on the estrogenic milieu in the breast (3). Moreover, targeting ER signaling through use of selective estrogen receptor modulators and aromatase inhibitors is a cornerstone of current breast cancer therapeutics. Less is known about the androgen receptor (AR), which is an emerging potential prognostic biomarker and therapeutic target for breast cancer (4). Androgen

therapy was commonly used in the mid-20th century (5,6), before falling out of favor due to frequent side effects and the development of selective estrogen receptor modulators. A resurgence of interest in targeting AR to treat ER– cancers has led to several recent and ongoing trials of AR-targeted therapies (7,8).

AR is expressed in 60%–80% of breast cancers, with more frequent coexpression in ER+ cancers, and expression in about 40% of ER– cancers (9–13). The effects of AR signaling may differ for ER+ and ER– breast cancers (14). Androgen treatments

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stimulate cell growth in ER-/AR+ cell line models, and this growth can be inhibited by cotreatment with AR antagonists (15). In contrast, androgen treatment reduces ER signaling in an ER+/AR+ cell line (16). A potential prognostic benefit of AR expression in ER+ tumors has been consistently observed in epidemiologic studies (17–19), with a recent meta-analysis (20) finding AR expression to be associated with statistically significantly improved disease-free survival (HR=0.40, 95% confidence interval [CI] = 0.31 to 0.52). The studies that have evaluated AR expression in ER- tumors have yielded inconsistent results, observing harmful (21,22), protective (23,24), and null (10,17,19,25,26) associations. In a prior analysis of 1467 women in the Nurses' Health Study (NHS) (27), we observed statistically significantly lower breast cancer mortality (HR=0.68, 95% CI = 0.47 to 0.99) with AR expression among women with ER+ cancers, and statistically nonsignificantly high breast cancer mortality (HR=1.59, 95% CI = 0.94 to 2.68) among women with ER- cancers.

This analysis builds on prior work in the NHS (27) through the addition of the Nurses' Health Study II (NHSII) cohort, the inclusion of premenopausal cases from NHS, and longer follow-up to further evaluate AR as a biomarker of breast cancer prognosis. In particular, this study aims to clarify the association for ER- cancers. To that end, this analysis nearly triples the population and the number of breast cancer deaths, in total and for ER- cancers, of the prior analysis. We hypothesized that AR expression would be associated with improved survival for ER+ cancers, and worse survival for ER- tumors. Finally, we evaluated a potential dose-response relationship between AR expression and breast cancer survival, conditional on tumor ER expression.

Methods

Nurses' Health Study Cohorts

The NHS began in 1976, when 121 700 US registered nurses 30–55 years of age completed a baseline questionnaire. The NHSII is composed of 116 429 US registered nurses who returned a baseline questionnaire in 1989 at 25–42 years of age. Members of both cohorts complete biennial questionnaires that provide detailed information on demographic, lifestyle, and medical history covariates. Cohort procedures have been described in detail elsewhere (28). All study protocols were approved by the institutional review board of Brigham and Women's Hospital and informed consent was obtained from all study participants.

Endpoints

Deaths in the cohorts are reported by next of kin or postal authorities, and the names of nonresponders are cross-referenced with the National Death Index (29). The cause of death is then ascertained from the death certificate or medical records. Breast cancer-specific survival (BCS) was defined as the time (in months) elapsed between breast cancer diagnosis and death from breast cancer or end of follow-up. Women who died of other causes were censored at the time of death. Secondary endpoints (overall survival [OS], recurrence-free survival [RFS]) are described in the [Supplementary Methods](#) (available online). All endpoints were ascertained through December 2015.

Immunohistochemistry Assays

Study investigators attempt to obtain formalin-fixed paraffin-embedded tumor tissue blocks and hematoxylin and eosin stained slides from the diagnosing hospital. Breast pathologists then select three representative areas of tumor, which are cored (0.6 mm), and assembled into tissue microarrays (TMAs) for immunohistochemistry assays. TMA sections were stained for AR (Dako AR441 antibody), ER- α (Dako 1D5 antibody), and HER2 (Dako A0485 antibody). Three cores per individual were scored through manual pathologist review and categorized as negative (0%), low positive (1%–10%), or high positive (>10%), and expression level was evaluated as the maximum across cores. Images of representative cores are shown in [Figure 1](#). Tumors with at least 1% expression were considered AR+ or ER+ in primary analyses. TMAs stained for AR were also scored using Definiens Tissue Studio image analysis software (Munich, Germany) to yield a continuous quantitative estimate of the percentage of AR+ epithelial nuclei (AR positivity score). Four TMAs had poor staining quality and were excluded from the Definiens analysis. Average batch recalibration was used to account for staining batch variability adjusting for cohort, year of diagnosis, and age at diagnosis (30). The Spearman correlation between the three-tier manual scoring and positivity score was 0.42 for AR ([Supplementary Table 1](#), available online).

Study Population

A total of 4200 nurses with stage I–III breast cancer diagnosed from cohort baseline (1976 NHS; 1989 NHSII) through 2008 and measured AR and ER protein expression by manual pathologist review were identified for this analysis ([Figure 2](#)). Three women were excluded for implausible survival times (<0 months) and 50 women were excluded because they died from breast cancer within 12 months of diagnosis and were assumed to have metastatic breast cancer, leaving a total of 4147 women in the final study population. Of these women, 3505 also had AR expression measured by Definiens Tissue Studio.

Statistical Analyses

Survival curves stratified by joint AR and ER expression were constructed using the Kaplan-Meier estimator and compared with the log-rank test. Cox proportional hazards models were used to evaluate the association between AR expression and survival endpoints. Models were stratified by cohort and adjusted for age at diagnosis (continuous), year of diagnosis (before 1990, 1990–1994, 1995–1999, 2000 and later), tumor stage (I, II, III), tumor grade (1, 2, 3, unknown), ER expression (positive $\geq 1\%$, negative <1%), type of surgery (lumpectomy, mastectomy, unknown), self-reported receipt of hormone therapy (yes, no, unknown), chemotherapy (yes, no, unknown), and radiation therapy (yes, no, unknown). Participant body mass index and menopausal status at diagnosis were considered as potential covariates, but were not statistically significant predictors of survival and not included in the final models. The proportional hazards assumption was tested using a likelihood ratio test for the inclusion of a product term between AR expression and log survival time (31). As the proportional hazards assumption was violated, piecewise Cox models were fit before and after 7 years postdiagnosis—the median event time for the primary outcome BCS. Alternative cut points of 5 and 10 years postdiagnosis were evaluated in sensitivity analyses. Effect modification between

Figure 1. Androgen receptor (AR) immunoreactivity in three tissue microarray cores. Cores were graded as negative (0%; A, D), low positive (1%–10%; B, E) and high positive (>10%; C, F). Images in the top row are at magnification $\times 15$. The bottom row contains the identical image as shown in the top row at magnification $\times 40$ to demonstrate AR localization.

AR expression and patient, tumor, and treatment covariates was evaluated using a likelihood ratio test for an interaction term between AR expression and the covariate of interest. We also evaluated possible nonlinearity for the association between the AR positivity score and survival nonparametrically with restricted cubic splines (32). Tests for nonlinearity were statistically nonsignificant using the likelihood ratio test, and we therefore evaluated quantitative AR expression as a linear predictor of the log HR. A test for a nonlinearity for age at diagnosis was likewise null, and this covariate was modeled as linear predictor. All statistical tests were two-sided and used a 0.05 level of statistical significance. Statistical analyses were performed using SAS version 9.4 (SAS Institute, Cary, NC).

Results

Age-standardized participant, tumor, and treatment characteristics stratified by tumor AR expression ($\geq 1\%$ positive) are shown in Table 1. AR was expressed in 76.6% of breast cancers in total, 85.9% of ER+ cancers, and 40.4% of ER– cancers. AR was also expressed in 31.7% of triple negative (ER–/PR–/HER2–) breast cancers. Women with AR+ cancers tended to be older, have lower BMI, and have lower stage and histologic-grade cancers. Given the association with age, we also conducted secondary analyses stratified by menopausal status. Women with AR+ tumors were also more likely to receive hormone therapy, and less likely to receive chemotherapy.

The 4147 breast cancer patients were followed for a median 16.5 years (interquartile range = 11.4–21.9). Over this period, there were 806 deaths due to breast cancer, 1120 breast cancer recurrences, and 1864 total deaths. In the Kaplan-Meier analysis, AR expression was associated with improved BCS by the log-rank test ($P = .001$). Ten-year BCS was 88.1% (95% CI = 86.9% to 89.2%) for AR+ cancers and 82.2% (95% CI = 79.7% to 84.5%) for AR– cancers. Moreover, statistically significant heterogeneity in BCS was observed when stratifying by AR and ER expression ($P < .001$) (Figure 3). AR expression was associated with improved BCS among ER+ tumors ($P < .001$) and worse BCS among ER– tumors ($P = .02$). Similar findings were observed for the endpoints of RFS (Supplementary Figure 1, available online) and OS (Supplementary Figure 2, available online). AR was associated with improved BCS among ER+/HER2– tumors ($P = .001$), worse BCS among ER–/HER2– tumors ($P = .01$), and not associated with BCS among ER+/HER2+ tumors ($P = .45$) or ER–/HER2+ tumors ($P = .96$) (Supplementary Figure 3, available online). These trends were similar for pre- and postmenopausal patients (Supplementary Figure 4, available online).

In Cox proportional hazards models adjusted for patient, tumor, and treatment covariates, AR expression was associated with a 27% reduction in breast cancer-specific mortality (BCM) (HR = 0.73, 95% CI = 0.58 to 0.91) in the 7 years following diagnosis (Table 2). However, beyond 7 years postdiagnosis, AR expression was associated with a 48% increase in BCM (HR = 1.48, 95% CI = 1.14 to 1.92). The association between AR and survival differed by tumor ER expression (P heterogeneity $< .001$). In the

Figure 2. Flowchart for identification of analytic population in the Nurses' Health Study (NHS) and Nurses' Health Study II (NHSII). AR = androgen receptor; ER = estrogen receptor; TMA = tissue microarray.

first 7 years postdiagnosis AR expression was associated with 47% lower BCM ($HR=0.53$, 95% CI = 0.41 to 0.69) for ER+ breast cancers, and with 62% higher BCM ($HR=1.62$, 95% CI = 1.18 to 2.22) for ER- breast cancers. The inverse association observed for ER+ tumors appeared to be limited to ER+/HER2- tumors, whereas the positive association observed for ER- tumors was present for both ER-/HER2+ and ER-/HER2- tumors. These associations attenuated beyond 7 years postdiagnosis. Comparable associations and interactions were observed when evaluating RFS and OS (Supplementary Tables 2 and 3, available online).

In the subset of 3505 patients with continuous measures of tumor AR expression, each 10% increase in the AR positivity score was not associated with BCM over 7 years ($HR=1.03$, 95% CI = 0.96 to 1.09) (Figure 4). Among ER+ cancers, each 10%

increase in AR positivity score was associated with a statistically nonsignificant 3% reduction in BCM ($HR=0.97$, 95% CI = 0.89 to 1.05). In contrast, among ER- cancers, there was 14% higher BCM ($HR=1.14$, 95% CI = 1.02 to 1.26) per 10% increase in AR positivity. Findings were similar for RFS and OS, although the association among ER- tumors for OS did not achieve statistical significance.

Sensitivity analyses evaluating an alternative cut point of 10% expression to define AR positivity (Supplementary Table 4, available online) and alternative cut points of 5 years and 10 years for use in piecewise Cox models (Supplementary Table 5, available online) yielded similar associations with BCM to those found in primary analyses. Finally, in exploratory analyses, AR expression was not found to modify the associations between treatment and BCM (Supplementary Table 6,

Table 1. Age-standardized characteristics of study population by tumor androgen receptor (AR) expression*

Characteristic	AR+† (n = 3178)	AR-† (n = 969)	P‡
Mean age at cancer diagnosis, years§ (SD)	57.1 (10.1)	55.5 (10.0)	<.001
Year of diagnosis, %			.002
Before 1990	28.0	27.8	
1990–1994	24.1	18.5	
1995–1999	27.9	32.8	
2000 or later	20.0	20.9	
Tumor ER expression, %			<.001
ER+	89.2	48.6	
ER–	10.8	51.4	
Tumor HER2 expression, %			.07
HER2+	20.4	23.6	
HER2–	78.0	74.9	
Missing	1.6	1.5	
Tumor grade, %			<.001
Grade 1	23.4	7.7	
Grade 2	53.9	39.8	
Grade 3	17.1	48.5	
Unknown	5.6	4.1	
Tumor stage, %			<.001
Stage I	55.4	47.3	
Stage II	31.9	36.4	
Stage III	12.6	16.3	
Type of surgery received, %			.11
Lumpectomy	36.4	33.0	
Mastectomy	51.9	53.1	
Unknown surgery	11.7	13.9	
Receipt of chemotherapy, %			<.001
Received chemotherapy	41.2	57.7	
No chemotherapy	39.2	23.9	
Missing	19.6	18.5	
Receipt of radiation therapy, %			.14
Received radiation therapy	48.0	49.0	
No radiation therapy	33.5	30.8	
Missing	18.6	20.2	
Receipt of hormonal therapy, %			<.001
Received hormonal therapy	66.6	51.1	
No hormonal therapy	16.3	27.1	
Missing	17.1	21.8	
Menopausal status at diagnosis, %			.03
Premenopausal	31.5	30.1	
Postmenopausal	61.0	61.7	
Unknown	7.5	8.3	
Mean BMI at diagnosis, kg/m ² (SD)	25.8 (5.1)	26.3 (5.0)	.02

*Values are means (SD) or percentages and are standardized to the age distribution of the study population. Values of polytomous variables may not sum to 100% due to rounding. BMI = body mass index; ER = estrogen receptor.

†AR+: ≥1% of nuclei staining positive; AR–: <1% of nuclei staining positive.

‡P values are from Pearson χ^2 tests for categorical variables and t tests for continuous variables, excluding individuals with missing data. All statistical tests were two-sided.

§Value is not age-adjusted.

available online). The associations between AR expression and BCM differed by tumor grade, but this heterogeneity was driven by the differential prevalence of ER expression in the high- and low-grade cancers (Supplementary Table 7, available online). There was no additional heterogeneity by other patient and tumor characteristics.

Discussion

In two large prospective cohorts, we found that AR expression was associated with improved BCS in the first 5–10 years following diagnosis, after adjustment for patient, tumor, and treatment covariates. This association differed by tumor ER expression, with AR expression associated with improved survival for women with ER+ cancers (specifically ER+/HER2–) in the first 5–10 years postdiagnosis, but worse survival beyond 10 years. Women with AR+/ER– tumors experienced worse survival outcomes in earlier follow-up postdiagnosis compared to women with AR–/ER– tumors, although this association attenuated with longer follow-up. Moreover, a log-linear dose-response relationship was observed between AR expression and BCM among women with ER– tumors, although the results for the continuous AR positivity score were null for ER+ cancers.

Our findings are consistent with prior epidemiologic studies that found AR to be a favorable prognostic marker for ER+ cancers in early follow-up. A recent meta-analysis (20) of nine studies with a total of 2843 patients found statistically significant improved disease-free survival (DFS) (HR = 0.53, 95% CI = 0.44 to 0.63) comparing women with AR+/ER+ tumors to women with AR–/ER+ tumors in the univariate analysis, replicating the findings of previous meta-analyses (33–35). It is postulated that AR may antagonize ER signaling through binding at certain estrogen response elements, thereby reducing transcription of ER target genes that stimulate tumor growth (16,36). Alternatively, AR may occupy its own unique response elements, from which it could directly or indirectly exert inhibitory influences on the transcription of ER target genes (37). Interestingly, the direction of the association changed in later follow-up. Prior studies have found that AR expression is conserved in recurrent ER+ breast cancers as compared to the primary tumor, whereas ER expression diminishes (38,39). Thus, this change in association may reflect a differential biologic effect of AR, wherein AR signaling potentially increases the rate of late recurrence among women with ER+ breast cancer (40), or a selection bias due to depletion of susceptibles among women with AR–/ER+ cancers (41).

Epidemiologic studies have provided inconsistent evidence regarding the prognostic value of AR in ER– breast cancers. Although we observed moderately worse survival outcomes with AR expression in this subgroup in the NHS and NHSII, the most recent meta-analysis (20) found AR to be a statistically significant predictor of improved survival for triple negative breast cancers (TNBC) for both DFS (HR = 0.64, 95% CI = 0.51 to 0.81, n = 1373) and OS (HR = 0.64, 95% CI = 0.49 to 0.88, n = 1285). There was no association among women with ER– but PR+ or HER2+ cancers for DFS (HR = 0.33, 95% CI = 0.04 to 2.44, n = 383) or OS (HR = 1.32, 95% CI = 0.98 to 1.80, n = 687). Another recent meta-analysis (34) found that AR expression predicted improved DFS for ER– breast cancer (HR = 0.42, 95% CI = 0.26 to 0.67), but did not predict OS. Two additional meta-analyses yielded null results in this subgroup (33,35). The epidemiologic evidence largely does not support experimental evidence from in vitro models. Androgen treatment promotes cell proliferation in AR+/ER–/HER2+ cell lines, and these growth signals can be blocked by cotreatment with AR antagonists (15,42). Our analysis suggests that AR may have value as a prognostic biomarker in ER– tumors independent of other patient, tumor, and treatment characteristics, raising its potential as a possible therapeutic target.

The potential of AR as a therapeutic target is particularly relevant for TNBC, within which luminal AR has emerged as a subtype of interest (43). Luminal AR tumors are characterized by

Figure 3. Kaplan-Meier survival curves of breast cancer-specific survival by cross-classified AR and ER tumor expression ($\geq 1\%$ positive, $< 1\%$ positive by manual read). P values from log-rank test were $P = .001$ comparing AR+ to AR- tumors, $P < .001$ comparing AR+/ER+ to AR-/ER+ tumors, and $P = .02$ comparing AR+/ER- to AR-/ER- tumors. All statistical tests were two-sided. AR = androgen receptor; ER = estrogen receptor.

Table 2. Multivariable analysis of breast cancer-specific survival by tumor androgen receptor (AR) expression*

Breast cancer type †	No. of individuals	0–7 y after diagnosis HR (95% CI)	>7 y after diagnosis HR (95% CI)	Total follow-up period HR (95% CI)
All tumors	4147	406 events	400 events	806 events
Model 1		0.64 (0.52 to 0.80)	1.34 (1.03 to 1.73)	0.89 (0.75 to 1.07)
Model 2		0.72 (0.58 to 0.91)	1.50 (1.15 to 1.95)	1.00 (0.83 to 1.20)
Model 3		0.73 (0.58 to 0.91)	1.48 (1.14 to 1.92)	1.00 (0.83 to 1.19)
ER+ tumors	3303	248 events	341 events	589 events
Model 1		0.44 (0.34 to 0.56)	1.14 (0.86 to 1.52)	0.69 (0.56 to 0.85)
Model 2		0.50 (0.39 to 0.65)	1.29 (0.97 to 1.73)	0.78 (0.63 to 0.97)
Model 3		0.53 (0.41 to 0.69)‡	1.38 (1.03 to 1.84)	0.83 (0.67 to 1.03)
ER- tumors	844	158 events	59 events	217 events
Model 1		1.55 (1.14 to 2.12)	0.93 (0.59 to 1.46)	1.33 (1.02 to 1.73)
Model 2		1.72 (1.25 to 2.36)	1.03 (0.65 to 1.63)	1.48 (1.12 to 1.94)
Model 3		1.62 (1.18 to 2.22)‡	0.91 (0.57 to 1.43)	1.34 (1.01 to 1.77)
ER+/HER2+ tumors§	625	59 events	32 events	91 events
Model 1		0.75 (0.44 to 1.28)	1.02 (0.56 to 1.87)	0.83 (0.51 to 1.37)
Model 2		0.92 (0.53 to 1.57)	1.17 (0.64 to 2.15)	0.99 (0.60 to 1.63)
Model 3		0.82 (0.48 to 1.41)	1.01 (0.55 to 1.86)	0.88 (0.54 to 1.45)
ER+/HER2- tumors§	2624	187 events	301 events	488 events
Model 1		0.38 (0.29 to 0.51)	1.20 (0.88 to 1.64)	0.67 (0.53 to 0.85)
Model 2		0.43 (0.32 to 0.57)	1.36 (0.99 to 1.87)	0.75 (0.59 to 0.95)
Model 3		0.48 (0.36 to 0.64)	1.50 (1.09 to 2.06)	0.82 (0.65 to 1.05)
ER-/HER2+ tumors§	254	56 events	14 events	70 events
Model 1		1.32 (0.80 to 2.18)	0.43 (0.18 to 1.06)	1.01 (0.63 to 1.62)
Model 2		1.64 (0.99 to 2.72)	0.50 (0.20 to 1.23)	1.21 (0.75 to 1.96)
Model 3		1.43 (0.86 to 2.36)	0.41 (0.16 to 1.00)	1.01 (0.63 to 1.64)
ER-/HER2- tumors§	581	101 events	44 events	145 events
Model 1		1.47 (0.99 to 2.17)	1.33 (0.80 to 2.21)	1.42 (1.02 to 1.98)
Model 2		1.63 (1.10 to 2.43)	1.56 (0.94 to 2.61)	1.64 (1.17 to 2.30)
Model 3		1.59 (1.07 to 2.37)	1.41 (0.84 to 2.36)	1.54 (1.10 to 2.15)

*Hazard ratios (HR) and 95% confidence intervals (95% CI) compare AR+ to AR- tumors over total follow-up period, within 7 years of diagnosis, and after 7 years post-diagnosis. AR expression was defined as $\geq 1\%$ positive, $< 1\%$ positive by manual read.

†Model 1: adjusted for age at diagnosis, ER expression (all tumor analysis), and stratified by cohort; Model 2: covariates from Model 1 + date of diagnosis, tumor stage, tumor grade, and HER2 expression; Model 3: covariates from Model 2 + type of surgery, radiation therapy, chemotherapy, and hormonal therapy.

‡P heterogeneity for AR-by-ER interaction is $< .001$.

§Excluding 63 individuals with unknown HER2 expression.

Figure 4. Hazard ratio and 95% confidence intervals of breast cancer-specific, recurrence-free, and overall survival within 7 years of diagnosis per 10% increase in AR positivity score. Hazard ratios are stratified by cohort, and adjusted for age at diagnosis, date of diagnosis, tumor stage, tumor grade, HER2 expression, and ER status (all tumor analyses only). AR positivity score was estimated by Definiens Tissue Studio for 3505 breast cancer cases (2803 ER+, 702 ER–). P values for heterogeneity of AR positivity score-by-ER interaction are .02 for breast cancer-specific survival, .003 for recurrence free survival, and .02 for overall survival. All statistical tests were two-sided. AR = androgen receptor; CI = confidence interval; ER = estrogen receptor; HR = hazard ratio.

high mRNA levels of AR and AR targets, and frequent mutations in the *PIK3CA* gene. Moreover, they demonstrate at least some sensitivity to treatment with AR antagonists. As such, there are several recent or ongoing clinical trials of AR-targeted agents for treatment of early and advanced stage AR+ TNBC (4,7,8). These agents include AR antagonists, selective AR modulators, and inhibitors of 17- α -hydroxylase/17, 20-lyase (CYP17) enzymatic activity. In a single-arm phase II trial among 26 patients with AR+/ER–/PR– metastatic breast cancer, bicalutamide treatment (an AR antagonist) yielded a clinical benefit rate of 19% (95% CI = 7% to 39%) and was well tolerated (44). Enzalutamide treatment led to a clinical benefit rate of 25% (95% CI = 17% to 33%) at 16 weeks in a phase II trial among 118 patients with AR+ TNBC (45). A clinical benefit of enzalutamide was also observed in a prior phase II trial, particularly for tumors with high levels of an androgen-driven gene signature (46). In a phase II trial, abiraterone acetate (a CYP17 inhibitor) plus prednisone led to a 20% clinical benefit rate (95% CI = 8% to 39%) at 6 months for AR+ TNBC (47). These trials have applied variable cutoffs (1%, 10%) for AR expression in patient eligibility. Prior epidemiologic studies have suggested that higher expression levels may improve the prognostic values of AR (48,49). As we observed a log-linear dose-response between AR expression and BCM for ER– breast cancer, it may be that women with higher AR expression would stand to benefit more from AR-targeted therapies, although this should be evaluated further in trial settings.

This analysis represents the single largest study of AR as a marker of breast cancer prognosis to date. This provides particular insight for the association among ER– breast cancers, where there have been a few studies that were limited by small sample sizes (most <250 individuals). Increased duration of follow-up also allowed for evaluation of long-term survival outcomes.

Moreover, to our knowledge, we are the first to evaluate the relationship between a continuous measure of AR protein expression and BCM. This study also benefits from the use of centralized staining and scoring of immunohistochemistry markers.

Possible limitations of this study include the modest concordance observed between manual pathologist scoring and the AR positivity score estimated by Definiens Tissue Studio. The AR positivity score tended to estimate higher AR expression relative to manual scoring. This measurement error is likely nondifferential with respect to participant outcomes, potentially attenuating observed associations. Standard methods of AR assessment have yet to emerge, as a variety of antibodies and cut points have been used previously (20,49). Additionally, we were unable to account for detailed treatment covariates in the analysis. Treatment information was extracted through medical record review or reported by participants in supplemental questionnaires, resulting in broad categorizations of treatment. Treatment information was also unavailable for about 20% of women in this analysis, potentially leading to residual confounding by these covariates. However, after adjusting for ER and HER2 expression, the associations between AR and treatment variables would be relatively weak, as AR has not been commonly used in clinical practice. We were also unable to obtain tumor tissue from all breast cancer cases that occur in the cohorts. However, the demographic and tumor characteristics are similar for the women for whom we are able and unable to obtain tissue (50).

AR expression was associated with improved survival for ER+ breast cancers and worse survival for ER– cancers in the first 5–10 years postdiagnosis. These findings suggest that AR may have value as a therapeutic target for the treatment of AR+/ER– breast cancers.

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