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Androgen Receptor Levels and Association with PIK3CA Mutations and Prognosis in Breast Cancer

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Abstract

Purpose: To examine the androgen receptor (AR) levels in breast cancer and to assess the impact of AR expression on patient outcomes.

Experimental Design: Reverse-phase protein arrays were used to measure AR levels and a mass spectroscopy–based approach was used to detect *PIK3CA* mutations. Means and SDs were generated for AR levels. Linear regression models were used to determine if AR levels differed by tumor subtype and *PIK3CA* mutation status. Two-sample *t* tests were used to identify pair-wise differences. Survival probabilities were estimated with the use of the Kaplan-Meier product and log-rank test.

Results: The median age was 59 years (23-89 years). Significant differences in AR levels existed among different breast tumor subtypes (highest in estrogen receptor–positive and/or progesterone receptor–positive tumors) as well as by *PIK3CA* mutation status ($P < 0.0001$ for both). AR levels were significantly higher in breast tumors with kinase domain PIK3CA mutations versus tumors that are wild type or with PIK3CA helical mutations ($P = 0.017$ and $P < 0.0001$, respectively). In 347 patients, dichotomized AR level by the median was a significant prognostic factor of recurrence-free survival ($P = 0.0002$) and overall survival ($P = 0.004$). High AR levels were associated with a significantly improved recurrence-free survival in 207 patients with early-stage estrogen/progesterone receptor–positive tumors after adjuvant hormonal therapy. A trend ($P = 0.07$) was found toward higher AR expression in *PIK3CA* mutant versus *PIK3CA* wild-type triple-negative breast tumors.

Conclusions: AR levels may represent a prognostic marker in breast cancers and may provide a valuable tool for selecting treatment. There was an association of *PIK3CA* mutation (kinase domain) with increased AR levels.

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The androgen receptor (AR), a member of the steroid receptor subfamily, is expressed in >70% of breast cancers (1–4) and has been implicated in breast cancer pathogenesis (1–5). There is evidence that the androgen signaling pathway may play a critical role in breast carcinogenesis through the activation of a number of estrogen-responsive genes as observed in other tumors (2, 6–14). Other pathology studies have shown that the direct AR-mediated action of androgens could be the major mechanism used by androgens to influence the growth of breast carcinomas independent of estrogen and progesterone receptors (3, 15). Preclinical studies have shown that androgen action in breast cancer cell lines is cell-type specific and results in either stimulation or inhibition of proliferation (16). Further, androgen-induced regulation of proliferation has been reported to occur through both AR-mediated and AR-independent mechanisms, the latter possibly a result of active metabolites of dihydrotestosterone with estrogenic-like actions (17). A recent genome-wide expression analysis of 99 primary breast cancer samples and 8 breast cancer cell lines identified a subset of estrogen receptor (ER)-negative/progesterone receptor (PR)-negative tumors with

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Translational Relevance

The androgen receptor (AR), a member of the steroid receptor subfamily, is expressed in ›70% of breast cancers and has been implicated in breast cancer pathogenesis. We show an association of *PIK3CA* mutation (kinase domain) with increased AR levels and that AR levels may represent a prognostic marker in breast cancers. AR expression and PI3K pathway aberrations, including *PIK3CA* mutations, are common in breast cancer and show significant concordance, possibly pointing to an important interaction between these two signaling pathways in breast carcinogenesis. Patients with *PIK3CA* mutant breast tumors may benefit from androgen blockade alone or from androgen blockade added to other (e.g., cytotoxic or PI3K-targeted) therapies.

paradoxical expression of genes known to be either direct targets of ER, responsive to estrogen, or typically expressed in ER-positive breast cancer (14). These tumors were distinct from the luminal A and basal subtypes identified by Perou et al. and were determined to express AR at relatively high levels (18).

The phosphatidylinositol 3-kinase (PI3K) signaling pathway is crucial to many aspects of cell growth and survival. It is targeted by genomic aberrations, including mutation, amplification, and rearrangement, more frequently than any other pathway in human cancers, with the possible exception of the p53 and retinoblastoma pathways. In addition, the PI3K pathway is stimulated in cancer as a physiologic consequence of many growth factors and regulators (19). Abnormalities resulting in the activation of the PI3K pathway are common in breast cancer and play an important role in neoplastic transformation. The most common genetic aberrations in breast cancer are activating somatic missense mutations in the gene encoding the p110α (*PIK3CA*) subunit of PI3K (20, 21). Our group (22) and others have shown a significant positive correlation between nuclear steroid receptor positivity and the presence of PIK3CA mutations. For example, 85% of *PIK3CA*-mutated breast cancers have been reported to be ER and/or PR positive (14). Further, a significant association between AR positivity and the presence of *PIK3CA* mutations has been shown in a distinct molecular subset of ER/PR-negative but AR-positive breast tumors possessing a hormonally regulated molecular phenotype (14, 23).

The objectives of the current study were to examine AR levels overall by tumor subtype and by *PIK3CA* domain mutation status (helical versus kinase domain, mutant versus wild-type tumors) in a large series of breast cancers and to assess the impact of AR expression on relapse-free survival (RFS) and overall survival (OS) in all breast cancers and within each breast tumor subtype defined with the use of clinical criteria.

Patients and Methods

Human tumor samples. Three hundred and forty-seven primary breast tumors were obtained from the breast tissue frozen tumor bank at M.D. Anderson Cancer Center. All specimens were collected under Institutional Review Board–approved protocols. These breast tumors were subdivided into three clinically relevant categories defined by immunohistochemistry for ER and PR status, and by immunohistochemistry, fluorescence in situ hybridization, and/or reverse-phase protein lysate array (22) for human epidermal growth factor receptor 2 (HER2) status. Thus, the 347 tumors included 97 triple-negative (TN), 207 ER-positive and/or PR-positive and HER2-negative, and 43 HER2-positive breast cancers.

Reverse-phase protein lysate microarray. Protein was extracted from the human tumors and reverse-phase protein lysate microarray was done in our laboratory as described previously (24–27). Briefly, lysis buffer was used to lyse frozen tumors by homogenization. Tumor lysates were normalized to 1 μg/μL concentration with the use of bicinchoninic acid assay and were boiled with 1% SDS, and the supernatants were manually diluted in six or eight 2-fold serial dilutions with lysis buffer. An Aushon Biosystems 2470 arrayer created 1,056 sample arrays on nitrocellulose-coated FAST slides (Schleicher & Schuell BioScience, Inc.) from the serial dilutions. A slide was then probed with a validated primary AR antibody (Epitomics), and the signal was amplified with a DakoCytomation catalyzed system. A secondary antibody was used as a starting point for amplification. The slides were scanned, analyzed, and quantitated with the use of the Microvigene software (VigeneTech Inc.) to generate serial dilution signal intensity curves for each sample with the logistic fit model: $\ln(y) = a + (b - a)/(1 + \exp\{c^*[d - \ln(x)]\})$. A representative natural logarithmic value of each sample curve on the slide (curve average) was then used as a relative quantification of the amount of each protein in

Table 1. Patient characteristics

Characteristic		Overall	Low AR (<-0.0852)	High AR (≥ -0.0852)	*P* value
Age (y)	Median range	59 (23-89)	55 (23-84)	65 (30-89)	—
	I	69 (20%)	28 (16%)	42 (24%)	0.09
	II	212 (61%)	100 (58%)	109 (63%)	0.38
Stage	III	66 (19%)	45 (26%)	23 (13%)	0.002
	1	61 (17%)	14 (8%)	45 (26%)	<0.001
	2	105 (30%)	29 (16%)	78 (45%)	<0.001
Tumor grade	3	181 (52%)	130 (75%)	51 (29%)	<0.001
	HER2 positive	43 (12%)	26 (15%)	17 (10%)	0.14
	ER and/or PR positive	207 (60%)	66 (38%)	141 (81%)	<0.001
Breast cancer subtype	Triple receptor negative	97 (28%)	81 (47%)	16 (9%)	<0.001
	Helical	23 (7%)	11 (6%)	12 (7%)	1.0
PIK3CA	Kinase	55 (16%)	19 (11%)	36 (21%)	0.01
mutation	Wild type	269 (78%)	143 (83%)	126 (72%)	0.03

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Table 2. AR expression by tumor subtype and by *PIK3CA* mutations

AR expression by tumor subtype

	Mean AR	SD AR	N	F-test *P* value	Versus HER2+ *P* value	Versus ER+ and/or PR+ *P* value
HER2+	0.04743	1.109	43	<0.0001	—	—
ER+ and/or PR+	0.24175	0.739	207		0.155	—
TN	-0.81954	0.82	97		<0.0001	<0.0001

AR expression by *PIK3CA* mutation type

	Mean AR	SD AR	N	F-test *P* value	Versus helical *P* value	Versus kinase *P* value
Helical	-0.111627	0.7998	23	<0.0001	—	—
Kinase	0.43001	0.9954	55		0.017	—
Wild type	0.1803036	0.902	269		0.729	<0.0001

each sample. The level of AR in each sample was expressed as a log-mean centered value after correction for protein loading with the use of the average expression levels of >50 proteins as previously described (22, 24–27).

Mass spectroscopy–based approach evaluating single-nucleotide polymorphisms. DNA was extracted from frozen tumors with the use of a QIAamp Micro Kit (Qiagen Inc.) according to the manufacturer's instructions. A mass spectroscopy–based approach evaluating single-nucleotide polymorphisms was used to detect known mutations in *PIK3CA* (PIK3CA_A1046V, PIK3CA_C420R, PIK3CA_E110K, PIK3CA_E418K, PIK3CA_E453K, PIK3CA_E542K, PIK3CA_E545K, PIK3CA_F909L, PIK3CA_G1049R, PIK3CA_G451L456_V, PIK3CA_H1047L, PIK3CA_H1047R, PIK3CA_H1047Y, PIK3CA_H701P, PIK3CA_K111N, PIK3CA_M1043V, PIK3CA_N345K, PIK3CA_P539R, PIK3CA_Q060K, PIK3CA_Q546E, PIK3CA_R088Q, PIK3CA_S405F, and PIK3CA_T1025S; refs. 22, 28, 29). PCR and extension primers for *PIK3CA* were designed with the Sequenom, Inc. Assay Design. PCR-amplified DNA was cleaned with the use of EXO-SAP (Sequenom) primer extended by iPLEX chemistry, desalted with Clean Resin (Sequenom), and spotted onto Spectrochip matrix chips with the use of a nano-dispenser (Samsung). The chips were run in duplicate on a Sequenom matrix-assisted laser desorption ionization–time of flight MassARRAY system. Sequenom Typer Software and visual inspection were used to interpret mass spectra. Reactions in which >15% of the resultant mass ran in the mutant site in both reactions were scored as positive.

Statistical methods. Baseline patient characteristics were calculated overall and by AR expression group with medians and ranges for age, frequency, and percentages for all other characteristics. Means and SDs were generated for AR expression overall by tumor subtype and by *PIK3CA* domain mutation. Linear regression models were used to determine if the mean AR expression was different by tumor subtype and *PIK3CA* mutation status. Two-sample *t* tests were then used to identify pair-wise differences. A frequency table was created to examine the distribution of *PIK3CA* mutations among the various tumor subtypes.

To examine survival, the time to death or censoring was computed in years since diagnosis for each patient, and RFS time to event was computed as years to first relapse or death after diagnosis for each patient. OS time was censored at the date of the last follow-up if death was not observed. RFS time was censored at the date of the last follow-up if no relapses were observed and death was not observed. RFS and OS probabilities were estimated nonparametrically with the use of the Kaplan-Meier product limit method. Log-rank tests were used to evaluate the equality of survival functions of AR expression, dichotomized with the median into low and high expression, overall and within each breast tumor subtype. Cox proportional hazards models were built; *t* tests were done to examine if mean AR expression was

Table 3. Survival estimates by AR level for all patients and by tumor type

RFS

		No. of relapses	No.	5-y survival	95% CI upper	95% CI lower	Log-rank *P* value
Overall	AR < -0.085	71	173	0.5238	0.4154	0.6213	0.0002
	AR ≥ -0.085	53	174	0.7564	0.6647	0.8263	
HER2+	AR < -0.085	14	26	0.2122	0.0446	0.4595	0.1893
	AR ≥ -0.085	10	17	0.1674	0.0119	0.4866	
ER+ and/or PR+	AR < -0.085	30	66	0.6109	0.4575	0.7330	0.0144
	AR ≥ -0.085	39	141	0.8266	0.7329	0.8899	
TN	AR < -0.085	27	81	0.5286	0.3343	0.6901	0.3422
	AR ≥ -0.085	4	16	0.7467	0.3943	0.9123	

OS

		No. of Deaths	No.	5-y survival	95% CI upper	95% CI lower	Log-rank *P* value
Overall	AR < -0.085	50	162	0.6407	0.5301	0.7318	0.0037
	AR ≥ -0.085	39	162	0.7859	0.6886	0.8559	
HER2+	AR < -0.085	10	18	0.3399	0.1187	0.5789	0.4447
	AR ≥ -0.085	6	14	0.4923	0.1627	0.7586	
ER+ and/or PR+	AR < -0.085	25	63	0.7385	0.5807	0.8444	0.0600
	AR ≥ -0.085	32	132	0.8176	0.7158	0.8857	
TN	AR < -0.085	15	81	0.7255	0.5663	0.8344	0.1650
	AR ≥ -0.085	1	16	0.9167	0.5390	0.9878	

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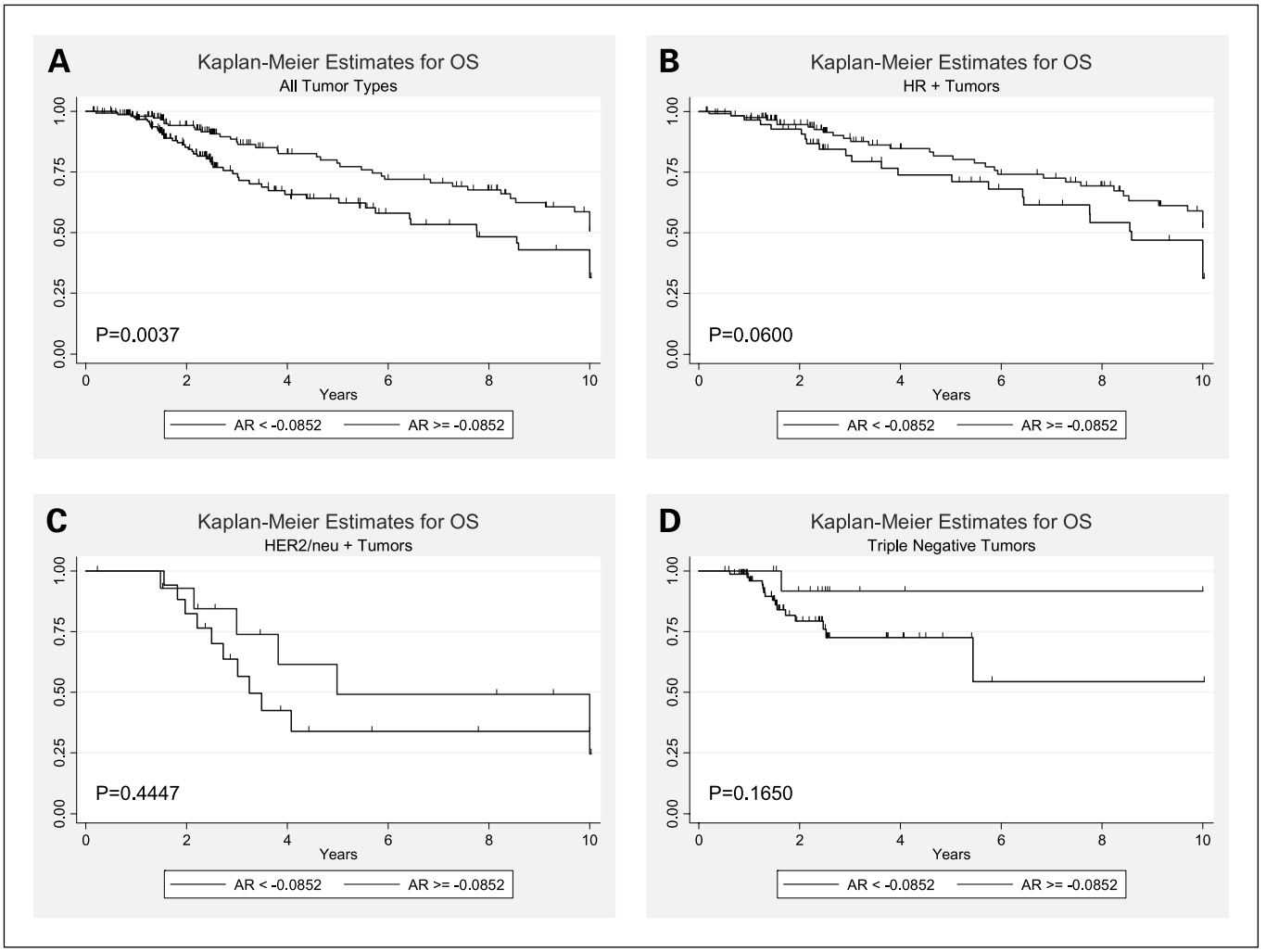

Fig. 1. Kaplan-Meier estimates of the RFS of patients by AR levels. *A,* all patients, *B,* patients with ER-positive and/or PR-positive tumors. *C,* patients with HER2-positive tumors. *D,* patients with TN tumors.

significantly different between *PIK3CA* wild-type and *PIK3CA* mutant tumors. Similarly, Fisher's exact tests were used to examine if there was a relationship between *PIK3CA* mutation type and AR expression level. All statistical analyses were done with the use of Stata 10 (Stata Corp.).

Results

Patient and breast tumor characteristics by AR expression level. Patients were diagnosed between 1989 and 2006. Patient characteristics are summarized in Table 1. The median age of the patients was 59 years (23-89 years). Sixty percent (n = 207) of patients had ER-positive and/or PR-positive breast cancer, 28% (n = 97) had TN tumors, and 12% (n = 43) had HER2-positive tumors. Patients with tumors expressing high [i.e., above median expression on reverse-phase protein lysate microarray (-0.0852)] AR levels tended to be older and have stage III breast cancer less frequently (P = 0.002), and their tumors were of lower nuclear grade (P < 0.001) and express ER and PR (P < 0.001). There were significant differences in mean AR levels between breast tumor subtypes (F-test, P < 0.0001 for both; Table 2). The mean AR levels were highest in ER-positive and/or PR-positive tumors followed by HER2-positive tumors whereas TN breast cancers had the lowest AR levels. Pair-wise, all tumor subtype comparisons were significantly different (Table 2).

PIK3CA mutation status and AR expression. A mass spectroscopy–based approach with the use of methods designed to detect single-nucleotide polymorphisms was used to detect mutations in *PIK3CA* in the 347 breast cancers. This approach is more sensitive than conventional Sanger sequencing, having the potential to detect mutations that are present in only a subset of tumor cells or in tumors with high levels of normal cell contamination, which is commonly the case in breast cancer (28, 29). *PIK3CA* mutations were detected in 78 of 347 breast cancers (22.5%), in 57 of 207 (27.5%) ER-positive and/or PR-positive tumors, in 12 of 43 (27.9%) HER2-positive tumors, and in 9 of 97 (9.3%) TN tumors (P = 0.04). Of the 23 mutation sites in *PIK3CA* that were assessed, 55 mutations were detected in exon 20 that encode the catalytic domain of PI3K (51 PIK3CA_H1047R, 2 PIK3CA_H1047L, 1 PIK3CA_H1047Y, and 1 PIK3CA_G1049R), and 23 mutations were detected in exon 9 that encode the PI3K helical domain (20 PIK3CA_E545K and 3 PIK3CA_E542K). There were significant differences in AR expression levels by *PIK3CA* mutation status

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(F-test, $P < 0.0001$; Table 2). In *PIK3CA* wild-type tumors and breast tumors with helical domain *PIK3CA* mutations, mean AR levels were significantly lower than in breast tumors with kinase domain *PIK3CA* mutations ($P = 0.017$ and $P < 0.0001$, respectively). AR expression did not differ significantly between *PIK3CA* wild-type tumors and tumors possessing helical domain *PIK3CA* mutations ($P = 0.729$; Table 2).

We also looked at the association between AR and *PIK3CA* kinase mutations by ER and/or PR group. For the ER-positive and/or PR-positive group, *PIK3CA* kinase mutations were present in 18.1% (low AR levels) versus 19.8% (high AR levels), $P = 0.776$. For the ER/PR-negative group, *PIK3CA* kinase mutations were present in 6.5% (low AR levels) versus 24.2% (high AR levels), $P = 0.004$.

Patient outcomes. At a median follow-up of 50.4 months, (range, 9.6-110.4 months), there have been 124 recurrences and 89 deaths. Dichotomized AR levels by the median (-0.0852) was a significant prognostic factor of OS ($P = 0.004$) and RFS ($P = 0.0002$). Five-year survival estimates are summarized in Table 3. The estimated 5-year OS rate was 79% [95% confidence interval (95% CI), 69%-86%] among patients with high AR levels and 64% (95% CI, 53%-73%) among patients with low AR levels (Fig. 1A). The estimated 5-year RFS rate was 76% (95% CI, 66%-83%) among patients with high AR levels and 52% (95% CI, 41%-62%) among patients with low AR levels (Fig. 2A). AR was not a significant predictor of OS or RFS times within HER2-positive or TN breast tumors (Table 3). However, high AR expression was associated with significantly better RFS in patients with hormone receptor–positive breast cancer ($P = 0.016$), with a trend toward improved OS times in these patients ($P = 0.06$). Note that most patients with early stage ER-positive and/or PR-positive breast cancer were treated with adjuvant tamoxifen alone. Figures 1 and 2 show the Kaplan-Meier survival curves for RFS and OS for all patients and by breast tumor subtype.

When examining RFS and OS, neither *PIK3CA* mutation status nor a combination of *PIK3CA* mutation status and AR expression (high levels of AR and the presence of a *PIK3CA* mutation versus other tumors) proved to be significant predictors of patient outcomes across all patients or within each specific breast tumor subtype (data not shown).

Table 4 shows the results of the multivariable models for OS and RFS. After adjustment for ER and/or PR status, HER2 status, *PIK3CA* mutation status, and therapy (tamoxifen alone versus

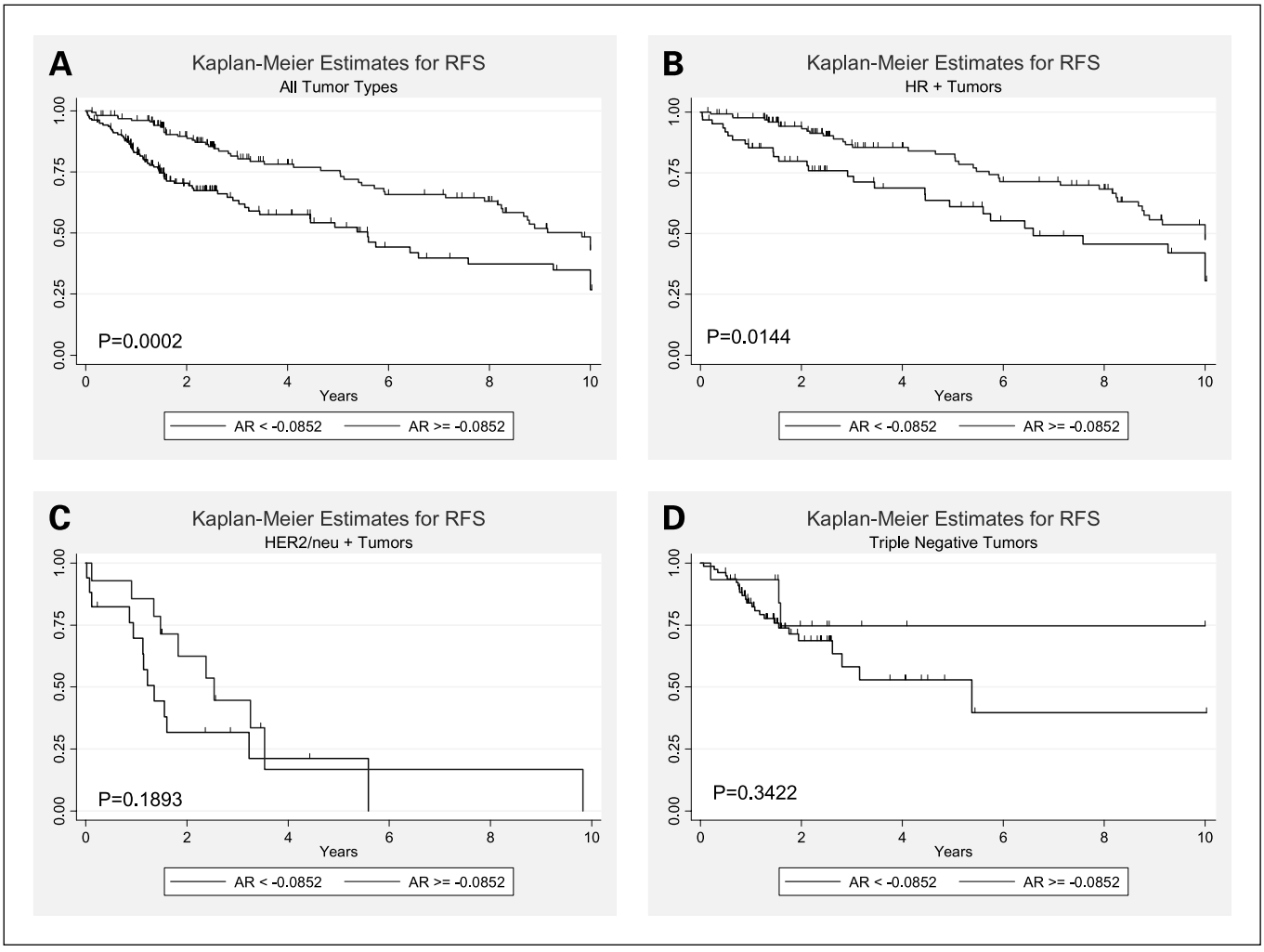

Fig. 2. Kaplan-Meier estimates of the OS of patients by AR levels. *A,* all patients, *B,* patients with ER-positive and/or PR-positive tumors. *C,* patients with HER2-positive tumors. *D,* patients with TN tumors.

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Table 4. Multivariate model of RFS and OS

Model	OS				RFS			
	HR	Lower CI	Upper CI	*P* value	HR	Lower CI	Upper CI	*P* value
AR (high versus low)	0.57	0.36	0.89	0.013	0.53	0.36	0.80	0.002
HER2 (positive versus negative)	1.80	0.88	3.71	0.108	3.21	1.81	5.71	<0.0001
ER and/or PR (positive versus negative)	1.07	0.43	2.64	0.885	0.83	0.38	1.79	0.626
PIK3CA (kinase mutation versus other)	1.02	0.58	1.77	0.950	1.21	0.74	1.98	0.437
Tamoxifen alone versus any chemotherapy	0.84	0.36	1.93	0.676	0.80	0.38	1.66	0.544

Abbreviation: HR, hazards ratio.

chemotherapy use), patients with breast cancer with high AR levels had a significantly deceased risk of both recurrence (hazards ratio, 0.53; 95% CI, 0.36-0.80; $P = 0.002$) and death (hazards ratio, 0.57; 95% CI, 0.36-0.89; $P = 0.013$) compared with patients with breast cancer with low AR levels.

AR levels in TN breast tumors. When looking at AR levels, specifically within the TN tumor subtype, a trend was seen toward higher AR expression in *PIK3CA* mutant (mean AR, -0.1578; SD, 1.0556) versus *PIK3CA* wild-type (mean AR, -0.8878; SD, 0.7587) tumors ($P = 0.07$). Of note, of the nine *PIK3CA* mutations in TN tumors, eight were within the kinase domain of *PIK3CA*. Further, we looked at the proportion of *PIK3CA* kinase mutations by AR level in the TN group: 3 of 16 (18.8%) TN tumors with high AR levels had a *PIK3CA* kinase mutation and 5 of 76 (6.2%) TN tumors with low AR levels had a *PIK3CA* kinase mutation ($P = 0.095$).

Discussion

AR expression varies significantly between different breast tumor subtypes and may be a significant predictor of breast cancer patient outcomes. Mean AR levels were highest in ER-positive and/or PR-positive followed by HER2-positive tumors. TN breast cancers showed the lowest AR levels. Patients with high AR levels had a more favorable prognosis as shown by longer RFS and OS times; this finding was confirmed by multivariate analysis. However, within each breast cancer subtype, these differences were not statistically significant with the exception that ER-positive and/or PR-positive breast cancer patients whose tumors possess high AR levels show significantly improved RFS times. Because most patients with early-stage ER-positive and/or PR-positive breast cancer in our study were treated with adjuvant tamoxifen, AR, like PR, may be a predictive factor for benefit from adjuvant hormonal therapy.

AR is expressed in >70% of breast cancers (1–4) and has been directly implicated in breast cancer pathogenesis (1–5). Androgen signaling may also activate estrogen-responsive genes (3, 6-17). ER-negative and/or PR-negative breast cancers represent ~30% of all breast cancers and are known to have a more aggressive clinical course, partly because these tumors are more likely to be poorly differentiated and of higher histologic grade (30, 31). Moinfar et al. (12) studied the frequency of AR expression with the use of immunohistochemistry in 200 cases of breast carcinoma and found 60% of all invasive carcinomas and 46% of ER-negative invasive carcinomas to be AR positive. Further, among poorly differentiated invasive carcinomas, 39% were ER and PR negative but AR positive. Because preclinical work suggests that ER/PR-negative AR-positive breast cancer cells may respond to antiandrogen therapy (32, 33), the latter therapy is now being explored in this patient population. We used a different method, reverse-phase protein lysate microarray, to measure AR. Although the limitations of this technology may bring in the issue of tissue heterogeneity and stromal contamination avoided by immunohistochemistry, reverse-phase protein lysate microarray provides absolute quantification rather than simply assigning samples as "positive" or "negative". However, we did explore whether AR expression was dichotomously expressed in TN breast tumors and found that 81 of 97 (83.5%) TN tumors had low AR levels and 16 of 97 (16.5%) tumors had high AR levels.

In targeting AR with androgen-based hormonal therapy in ER-negative and/or PR-negative breast cancer, it may be necessary to take into consideration potential interactions between AR and other tumor growth–related factors. We and others have previously shown that PI3K pathway aberrations are common in breast cancer, pointing to a critical role for this signaling pathway in breast carcinogenesis (22, 30). In a retrospective analysis of the incidence of *PIK3CA* mutation status in 98 invasive breast cancers at Memorial Sloan-Kettering Cancer Center, a significant positive correlation between nuclear steroid receptor status and *PIK3CA* mutation status was identified, with 85% of *PIK3CA*-mutated breast cancers being ER, PR and/or AR positive (14, 23). We detected a similar distribution of *PIK3CA* mutations within breast cancer subtypes defined by hormone receptor (ER/PR) status along with a relatively high frequency of mutations in HER2-positive breast tumors. In addition, we found that breast cancers with kinase domain *PIK3CA* mutations expressed significantly higher levels of AR than breast cancers with helical domain *PIK3CA* mutations or with a wild-type *PIK3CA* gene in the ER/PR-negative tumors ($P = 0.004$). Further, in the TN breast tumors within our series, there was a trend toward *PIK3CA* mutant tumors showing significantly higher AR expression than *PIK3CA* wild-type tumors. This trend maintained when looking particularly at kinase mutations. Consistent with this, in the retrospective study from Memorial Sloan-Kettering, investigators showed a significant association between AR positivity and the presence of *PIK3CA* mutations to be particularly striking in a distinct molecular subset of ER/PR-negative but AR-positive tumors (14, 23). Indeed, 80% of the tumors within this subset possessed *PIK3CA* mutations, with the kinase domain *PIK3CA* mutation being over-represented in comparison with the helical domain mutation.

Preclinical and clinical evidence suggests that androgens and the androgen signaling pathway may play a critical role in breast

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carcinogenesis in some cases. High AR expression is likely to be associated with a good prognosis in women with hormone receptor–positive breast cancer after adjuvant hormonal therapy. AR expression and PI3K pathway aberrations, including *PIK3CA* mutations, are common in breast cancer and show significant concordance, possibly pointing to an important interaction between these two signaling pathways in breast carcinogenesis. Patients with *PIK3CA* mutant TN breast tumors may benefit from androgen blockade alone or from androgen blockade added to other (e.g., cytotoxic or PI3K-targeted) therapies (34).

Disclosure of Potential Conflicts of Interest

No potential conflicts of interest were disclosed.

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