Clinical Cancer Research

Combined Androgen and Estrogen Receptor Status in Breast Cancer: Treatment Prediction and Prognosis in a Population-Based Prospective Cohort

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Abstract

Purpose: To evaluate whether tumor androgen receptor (AR) expression was prognostic and/or predictive for endocrine treatment alone or in combination with estrogen receptor (ER). The AR has been hypothesized to have differential prognostic roles in breast cancer depending on tumor ER status, and to influence endocrine treatment response.

Experimental Design: A population-based prospective cohort of 1,026 patients diagnosed with primary invasive breast cancer in Lund, Sweden, between 2002 and 2012 was followed until June 2014. Associations between immunohistochemical AR expression in tumor tissue microarrays, patient and tumor characteristics, and AR genotypes were analyzed. Disease-free survival (DFS) by AR status, and combined ER/AR status was assessed in various treatment groups.

Results: AR expression was assessable in 913 tumors. AR^+ tumors (85.0%) were associated with higher age ($P = 0.036$) and favorable tumor characteristics. The AR^+ status was a prognostic marker for DFS (LogRank $P = 0.025$). There was an interaction between AR and ER expression with respect to prognosis (adjusted $P_{interaction} \leq 0.024$). Tumors with discordant hormone receptor expressions (ER^+AR^- or ER^-AR^+) demonstrated worse prognosis compared with concordant tumor expressions (ER^+AR^+ or ER^-AR^-) in multivariable models [adjusted HRs (95% confidence intervals); $\geq$1.99 (1.28–3.10), $P \leq 0.002$]. ER^+AR^- indicated early treatment failure with aromatase inhibitors (AI) among chemonaïve patients aged 50 or older.

Conclusions: Prediction of breast cancer prognosis and treatment response was improved by combining AR and ER status. AR negativity predicted early treatment failure with AI but not tamoxifen, a finding that warrants confirmation in a randomized setting. Patients may benefit from anti-androgens or selective AR modulators. *Clin Cancer Res; 21(16); 3640–50. ©2015 AACR.*

Introduction

The heterogeneity of breast cancer calls for a deeper understanding of prognostic and predictive markers to improve breast cancer survival. The critical gaps in breast cancer research have recently been portrayed (1), and the androgen receptor (AR) was highlighted as an interesting prognostic and treatment-predictive marker because of its interplay with the estrogen receptor-α (ERα). Based on preclinical findings, Vera-Badillo and colleagues hypothesized AR to be a good prognostic marker in ER^+ tumors, but a poor prognostic marker in ER^- tumors (2). However, this hypothesis was not confirmed in their meta-analysis of clinical studies. Moreover, high AR expression has been proposed as a positive predictive marker for endocrine treatment response; however, endocrine treatment type was not considered (3). Conversely, preclinical data suggest that AR overexpression causes tamoxifen (TAM) and/or aromatase inhibitor (AI) resistance (4, 5). Ongoing clinical trials with anti-androgens (6, 7) further highlight the need for additional studies of AR alone and in combination with ER in breast cancer (8).

In a previous study, we reported a treatment predictive value of AR genotyping for adjuvant TAM, but not for AIs (9). In this study, we hypothesized that the prognostic value of tumor AR expression may depend on ER status and have predictive value for endocrine treatment response. Our primary aim was to analyze AR expression in breast tumors from patients included in the prospective population-based BC Blood Study and to relate AR expression to patient and tumor characteristics. We also aimed to examine if germline AR genotypes were associated with AR tumor expression. Finally, we aimed to elucidate whether AR tumor expression was prognostic and/or predictive for endocrine treatment either alone or in combination with ER.

Materials and Methods

Patients

The BC Blood Study is an ongoing epidemiologic cohort study at the Skåne University Hospital in Lund, Sweden, exploring

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Note: Supplementary data for this article are available at Clinical Cancer Research Online (http://clincancerres.aacrjournals.org/).

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Translational Relevance

In this prospective, population-based study, a differential role of androgen receptor (AR) in estrogen receptor (ER)$^+$ versus ER$^-$ tumors is demonstrated. Further, patients with tumors of discordant receptor status (ER$^+$AR$^-$ or ER$^-$AR$^+$) had worse prognosis in multivariable models compared with patients with tumors of concordant receptor status (ER$^+$AR$^+$ or ER$^-$AR$^-$), suggesting a need for new treatment options for these patients. ER$^+$AR$^-$ indicated early treatment failure with aromatase inhibitors among chemonaïve patients aged 50 or older, a finding of interest to investigate in future randomized trials. Thus, we suggest caution when considering treatment strategies targeting AR, because either inhibitory or stimulatory action might be beneficial depending on the patient's breast cancer ER status. Several clinical trials with either antiandrogens or selective androgen receptor modulators are ongoing. More studies on AR stratified by ER status are needed.

factors, which may be associated with prognosis and treatment response in primary breast cancer. Starting in October 2002, patients with primary breast cancers were invited to participate at the preoperative visit. Patients with a previous cancer during the last 10 years were excluded.

This study included patients enrolled between October 2002 and June 2012. After a written informed consent was signed, a questionnaire regarding lifestyle, reproductive factors, and medications was completed (10). Blood samples were taken and body measurements, including breast volume, were recorded by a research nurse. Breast volume was defined as the total bilateral breast volume among patients without previous breast surgeries and was measured with plastic cups (11, 12). Specific treatment data were collected from patient charts and follow-up questionnaires to enable assessment of adherence to the prescribed therapy (13). Follow-up included additional questionnaires and collection of clinical data after 3 to 6 months, 7 to 9 months, and after 1, 2, 3, 5, 7, 9, and 11 years from inclusion. Information on breast cancer events and date of death was obtained from patient charts, the regional tumor registry, and the population registry. Genotyping of the six haplotypes tagging single nucleotide polymorphisms in the AR (rs1337080, rs17302090, rs6152, rs7061037, rs5031002, and rs5964607) was done as previously described (9).

Between October 2002 and June 2012, a total of 1,116 patients were included. Patients who had received preoperative treatment ($n = 51$) and patients with only ductal carcinoma *in situ* ($n = 39$) were excluded. The total study cohort therefore consisted of 1,026 patients (Fig. 1).

The study was approved by the Lund University Ethics Committee (Dnr LU75-02, LU37-08, LU658-09, LU58-12, and LU379-12).

Histopathologic analyses

Tumor tissue microarrays (TMA) were constructed by sampling cores from representative non-necrotic tumor regions of formalin-fixed paraffin-embedded tissue blocks. Duplicate cores (1.0 mm) from the primary tumors were mounted into recipient blocks. For immunohistochemical analysis, 4-μm sections were automatically pretreated using the PT Link system and stained for the monoclonal antibody of AR (clone AR441, dilution 1:200; Thermo Scientific) in an Autostainer Plus (Dako).

A senior breast pathologist (Anna Ehinger) was consulted to ensure assessability and invasiveness of the TMA sections. Scoring was then performed twice independently (Karin Elebro), and blinded for clinical data. In cases of discrepancies (2%), a third scoring was done to reach consensus (Karin Elebro + Signe Borgquist). Scoring included nuclear staining fractions (0%, 1%–10%, 11%–50%, 51%–75%, 76%–100%) and intensity score (negative, weak, moderate, strong). Tumors were considered AR$^+$, if more than 10% of the nuclei were stained, independent of intensity. In an alternative analysis, a cutoff of >75% of stained nuclei was used to define AR_{75} positive tumors (AR_{75}^+). If the duplicate cores were heterogeneous, the fraction of positively stained nuclei was estimated across both sampled cores.

Tumor characteristics collected from patient charts and pathology reports included invasive tumor size, histologic grade, axillary lymph node involvement, ER, and progesterone receptor (PR) status (positive if greater than 10% nuclei were stained according to standard clinical practice in Sweden; refs. 14, 15). HER2 assessment was routinely analyzed as of November 2005 in patients younger than 70 years. HER2 status determined by FISH (16) was thus included in subgroup analyses of patients included in the study between November 2005 and June 2012 [$n = 738$, of which 50 patients (6.8%) had missing HER2 status; Fig. 1].

Statistical analyses

All analyses were performed using SPSS Statistics 19 (IBM). The anthropometric variables weight (kg), height (m), body mass index (BMI; kg/m^2), and waist-to-hip ratio were used as continuous variables. Breast volume was analyzed as a continuous variable and as a dichotomous variable ($\geq$850 mL; yes/no) as per previous reports from this cohort (11, 12). Reproductive factors such as age at menarche and age at first full-term pregnancy were used as continuous variables (years), whereas parity (yes/no), current smoker prior to surgery (yes/no), and abstainer (yes/no) were used as dichotomous variables. Information on treatment by last follow-up prior to any event was entered as dichotomous variables defined as patients who had received postoperative chemotherapy (yes/no), radiotherapy (yes/no), and endocrine therapy (yes/no), respectively. The endocrine therapy group was stratified according to TAM treatment (yes/no) and AI treatment (yes/no). Trastuzumab treatment (yes/no) was entered as a covariate in subgroup analyses of patients included as of November 2005. Tumor characteristics were categorized as invasive pathologic tumor size (pT; 1–4 or 2+), pathologic axillary lymph node involvement (pN; yes/no) or number of involved lymph nodes (0, 1–3, 4+), and histologic grade (I–III or I–II vs. III).

Associations between patient and tumor characteristics and AR status were assessed. Because some variables were not normally distributed, the Mann–Whitney *U* test was used. Categorized variables were analyzed using χ^2 tests and logistic regression; ORs with 95% confidence intervals (CI) are presented.

Disease-free survival (DFS) in relation to AR status was assessed by the Kaplan–Meier method and the LogRank test. Crude and adjusted Cox proportional hazards regression models provided HRs with 95% CIs. Adjustments were performed using four models. Model 1: age (continuous) and tumor characteristics [pT 2+ yes/no, pN yes/no, histologic grade I–II

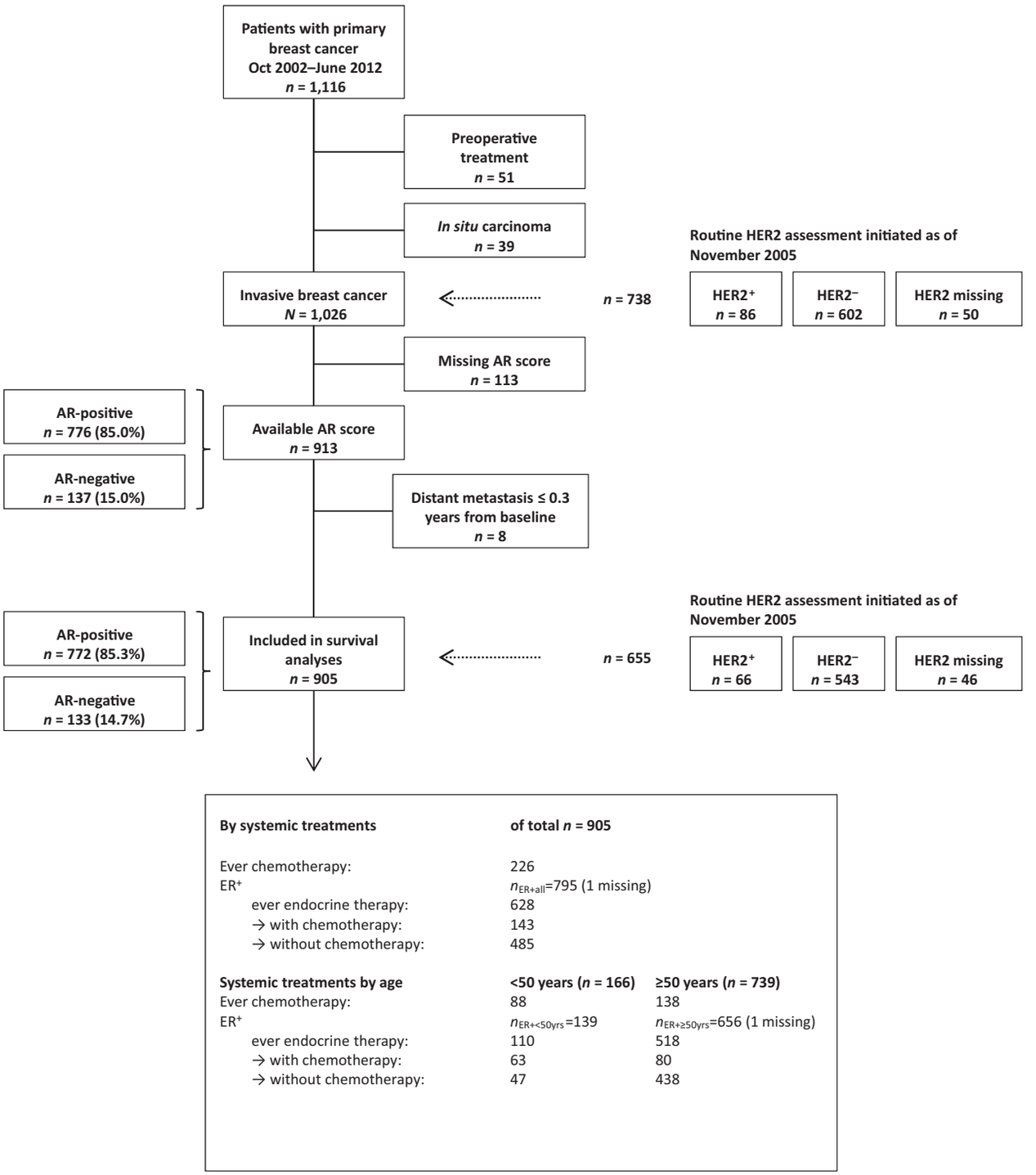

Figure 1.
Flow chart of the study population included in various analyses.

vs. III, ER status (+/−), and AR status (+/−)]. Model 2: age, tumor characteristics, BMI (≥25 kg/m^2 yes/no), and smoking (yes/no). Model 3: age, tumor characteristics, and treatment (chemotherapy yes/no, radiotherapy yes/no, TAM yes/no, AI yes/no). Model 4: model 3 variables with the addition of trastuzumab treatment yes/no and restricted to patients included as of November 2005. An interaction term between ER and AR was calculated and used in the Cox regression analysis, and adjustments using models 1, 2 and 3 were performed. Thereafter, combined ER and AR status (ER$^+$AR$^+$, ER$^-$AR$^-$, ER$^+$AR$^-$,

or ER^-AR^+) were used in the remaining multivariable analyses, using ER^+AR^+ as the reference group.

Survival was calculated from inclusion to a first breast cancer event, death from non-breast cancer related cause or last follow-up by June 30, 2014, whichever came first. Patients with distant metastases detected on the postoperative metastases screen at 0.3 years of inclusion ($n = 8$) or earlier were excluded from the survival analyses. Finally, patients with tumors without available AR status ($n = 113$) were excluded, resulting in 905 patients in the survival analyses (Fig. 1). Breast cancer events were defined as local or regional recurrences, contralateral cancer, or distant metastases.

Prior power calculations assuming 900 patients with an accrual interval of 10 years and additional follow-up time of 0.5 years and a frequency of 15% AR^- tumors showed that the study was able to detect true HRs between 0.731 and 1.403 with 80% power and α of 5% (power and sample size calculation program, PS, version 3.0, developed by Dupont and Plummer; http://biostat.mc.vanderbilt.edu/wiki/Main/).

P values < 0.05 were considered significant. All *P* values were two-tailed. Because this was an exploratory study, nominal *P* values are presented without adjustments for multiple testing. The report follows the REMARK criteria (17).

Results

AR in relation to patient characteristics

Eighty-nine percent of the patients had tumors for which AR status were available ($n = 913$). The majority of tumors were AR^+ ($n = 776$, 85.0%; Fig. 1). Higher age at diagnosis was significantly associated with AR^+ (Table 1). This association remained significant among patients younger than 50 years ($P = 0.008$), indicating that the association was driven by the younger patients (Supplementary Table S1). Smaller breast volumes (<850 mL) were more common among patients with AR^+ tumors (Table 1). This association was more apparent among patients aged less than 50 years, where an association was seen both for the continuous ($P = 0.03$) and the dichotomized variables (OR = 0.45; 95% CI, 0.20–1.03; Supplementary Table S1). No other anthropometric measure was associated with AR status. Reproductive factors, exogenous hormone use, and smoking and alcohol habits were not associated with AR status. Tumor AR expression was not significantly associated with the germline AR diplotypes (data not shown).

AR in relation to tumor characteristics

Tumors ≤20 mm were more likely to be AR^+ compared with larger tumors or tumors with skin or muscle involvement independent of size. No association between axillary lymph node involvement and AR status was observed. Lower histologic grade was significantly associated with AR^+ tumors. Tumor AR was highly coexpressed with ER and PR status. HER2 amplified tumors were evenly distributed between AR^+ and AR^- tumors. However, AR^+ was significantly positively associated with HER2 amplification in the ER^-PR^- tumors (Table 2).

AR and DFS

Patients were followed for up to 11 years (median follow-up for patients still at risk 5.0 years), and 107 events were observed. In general, patients with AR^- tumors had significantly worse prognoses compared with patients with AR^+ tumors (Fig. 2A; Table 3). However, stratification by ER status revealed AR was a positive prognostic marker in patients with ER^+ tumors, but conferred a worse prognosis in patients with ER^- tumors (Fig. 2B–D; Table 3). A significant interaction between AR and ER expressions was seen [$P_{interaction} = 0.010$ univariable and adjusted; $P_{interaction} = 0.019$ (model 1), $P_{interaction} = 0.024$ (model 2), $P_{interaction} = 0.014$ (model 3)] adjusted for age and tumor characteristics, BMI, and smoking, or treatment.

Multivariable analyses for combined ER and AR status showed worse prognosis for the ER^-AR^+ tumors compared with all other combinations (Table 3). The ER^-AR^+ tumors showed significantly worse prognosis compared with ER^+AR^+ tumors. This association remained after adjusting for age and tumor characteristics (model 1), when BMI and smoking were added to the model (model 2), and when adjustments for treatment were added to the model (model 3). When breast size was added to model 2, results remained essentially the same (data not shown). In model 3, which incorporated adjuvant treatment data, double negative

Table 1. Patient characteristics by AR status

	All $N = 1{,}026$	Missing total	Patients with available tumor AR status: AR negative $n = 137$	AR positive $n = 776$	*P* value[a] or OR (95% CI) for AR positive	Missing AR status $n = 113$
	Median (IQR) or %	*n*	Median (IQR) or %	Median (IQR) or %		Median (IQR) or %
Patient characteristics						
Age at diagnosis, years	61.1 (52.1–68.1)	0	59.4 (49.0–68.0)	61.5 (53.1–68.3)	**0.036**	60.0 (47.8–68.1)
Weight, kg	69.0 (62.0–78.0)	26	70.0 (60.9–78.6)	69.0 (62.0–78.5)	0.99	67.8 (61.8–76.0)
Height, m	1.65 (1.62–1.70)	26	1.65 (1.61–1.70)	1.66 (1.62–1.70)	0.39	1.66 (1.62–1.69)
BMI, kg/m^2	25.1 (22.5–28.3)	28	25.1 (22.5–28.9)	25.2 (22.5–28.4)	0.97	24.8 (22.3–27.7)
Waist-to-hip ratio, m/m	0.86 (0.81–0.90)	38	0.86 (0.81–0.91)	0.86 (0.81–0.90)	0.96	0.85 (0.80–0.90)
Total breast volume, mL	1,000 (650–1,500)	160	1,050 (700–1,650)	1,000 (650–1,500)	0.12	1,000 (625–1,300)
≥850 mL, %	57.3	160	65.2	55.9	0.68 (0.45–1.02)	57.0
Age at menarche, years	13 (12–14)	6	13 (12–14)	13 (12–14)	0.24	13 (13–14)
Parous, %	87.9	1	83.9	88.5	1.47 (0.89–2.45)	88.5
Age at first full-term pregnancy, years	25 (22–28)	131	25 (21–29)	24 (22–28)	0.29	25 (22–28)
Ever use of oral contraceptives, %	70.8	1	75.2	69.4	0.75 (0.49–1.14)	75.2
Ever use of HRT, %	43.9	3	37.5	45.9	1.41 (0.97–2.05)	38.1
Current smoker prior to surgery, %	20.5	2	24.8	19.5	0.73 (0.48–1.12)	22.1
Abstainer, %	10.5	7	11.8	10.4	0.87 (0.49–1.54)	9.8

NOTE: Bold letters indicate statistically significant results.

Abbreviations: HRT, hormone replacement therapy; IQR, interquartile range.

[a]Mann–Whitney *U* test.

Table 2. Tumor characteristics by AR status

	All		Patients with available tumor AR status			Missing AR status
			AR negative	AR positive		
	N = 1,026	Missing	*n* = 137	*n* = 776	*P* value or OR (95% CI)	*n* = 113
	n (%)	*n*	*n* (%)	*n* (%)	for AR positive	*n*
Tumor characteristics						
Invasive tumor size		0			0.26[a]	
1 (≤20 mm)	740 (72.1)		89 (65.0)	564 (72.7)	Ref.	87
2 (21–50 mm)	269 (26.2)		46 (33.6)	199 (25.6)		24
3 (≥51 mm)	15 (1.5)		2 (1.5)	11 (1.4)	0.70 (0.47–1.02)	2
4 (skin or muscular involvement independent of size)	2 (0.2)		0	2 (0.3)		0
Axillary lymph node involvement		2			0.43[b]	
0	627 (61.2)		86 (63.2)	466 (60.1)	Ref.	75
1–3	306 (29.9)		35 (25.7)	239 (30.8)	1.14 (0.78–1.66)	32
≥4	91 (8.9)		15 (11.0)	70 (9.0)		6
Histologic grade		1			**<0.0001[b]**	
I	252 (24.6)		16 (11.7)	202 (26.0)	**Ref**.	34
II	511 (49.9)		43 (31.4)	416 (53.6)		52
III	262 (25.6)		78 (56.9)	158 (20.4)	**0.19 (0.13–0.28)**	26
Hormone receptor status						
ER^+	896 (87.5)	2	73 (53.7)	726 (93.6)	**12.53 (8.05–19.50)**	97
PR^+	726 (70.9)	2	55 (40.4)	594 (76.5)	**4.81 (3.29–7.03)**	77
Combined ER and PR status					**<0.0001[a]**	
ER^-PR^-	122 (11.9)		61 (44.9)	46 (5.9)	**Ref**.	15
ER^-PR^+	6 (0.6)	2	2 (1.5)	4 (0.5)	2.65 (0.47–15.11)	0
ER^+PR^-	176 (17.2)		20 (14.7)	136 (17.5)	**9.02 (4.92–16.52)**	20
ER^+PR^+	720 (70.3)		53 (39.0)	590 (76.0)	**14.76 (9.18–23.73)**	77
HER2 amplification, November 2005 onward[c]						
All[c]	86 (12.5)	50[c]	11 (14.1)	57 (10.6)	0.73 (0.36–1.45)	18
ER^-PR^- subgroup[d]	28 (32.9)	0	7 (15.6)	16 (50.0)	**5.43 (1.88–15.72)**	5
Treatment by last follow-up[e]						
Ever chemotherapy	259 (25.2)	0	57 (41.6)	169 (21.8)	**0.39 (0.27–0.57)**	33
ER^+ only						
Ever endocrine therapy	694 (77.5)	0	61 (83.6)	567 (78.1)	0.70 (0.37–1.34)	66
Ever tamoxifen	528 (58.9)	0	55 (75.3)	420 (57.9)	**0.45 (0.26–0.78)**	53
Ever aromatase inhibitor	345 (38.5)	0	22 (30.1)	289 (39.8)	1.53 (0.91–2.58)	34
Ever radiotherapy	641 (62.5)	0	89 (65.0)	489 (63.0)	0.92 (0.63–1.34)	63
Ever trastuzumab[c+f]	66 (8.9)	0[f]	7 (8.2)	46 (8.0)	0.72 (0.36–1.45)	13
November 2005 onward						

NOTE: Bold letters indicate statistically significant results.

Abbreviation: *df*, degree of freedom.

[a]χ^2 3 *df*.

[b]χ^2 2 *df*.

[c]HER2 status routinely analyzed in patients <70 years with invasive tumors as of November 2005. In total, 738 patients were included in the study from November 2005 to June 2012, among which 688 were tested for HER2 status and 50 had missing HER2 status.

[d]Among the 738 patients included as of November 2005, totally 85 patients had ER^-PR^- tumors, all of which were tested for HER2 status.

[e]Patients may have received more than one type of treatment.

[f]Data on trastuzumab treatment were available for all patients as of November 2005. However, 50 patients (6.8%) had missing HER2 status.

(ER^-AR^-) tumors demonstrated a reduced risk compared with the other models. Because tumors with discordant ER and AR status (ER^+AR^- or ER^-AR^+) showed worse prognosis compared with concordant ER and AR status (ER^+AR^+ or ER^-AR^-), a variable for discordant ER and AR status was constructed. Tumors of discordant ER and AR status demonstrated significantly worse prognosis compared with concordant ER and AR status in all multivariable models, including model 4, which was based on the subgroup of patients with data on trastuzumab treatment (Table 3).

AR as a treatment-predictive marker

Tumor AR status did not provide a treatment predictive value for adjuvant chemotherapy alone or in combination with endocrine treatment. This was analyzed among all patients who ever received chemotherapy, and in patients with ER^- tumors who received chemotherapy only, as well as in patients with ER^+ tumors who received chemotherapy followed by endocrine treatment (all Log-Rank *P*s ≥0.17). To explore whether AR status had a treatment predictive value for endocrine treatment, patients with ER^+ tumors who had not received chemotherapy were included in further analyses. Further, patients aged less than 50 years were excluded because AIs are rarely prescribed to premenopausal patients.

Tumor AR status did not provide a prognostic value among patients who never received endocrine treatments (Fig. 3A). However, AR^- was predictive of early failure of sequential treatments with TAM/AI or AI/TAM (Fig. 3B). The association was weaker among patients who had ever received TAM, including those sequentially treated with AIs (Fig. 3C), whereas the predictive value of AR remained strong in the subgroup of patients who had ever received AIs, including those sequentially treated with TAM (Fig. 3D). To further differentiate between TAM and AIs, patients with endocrine monotherapy were analyzed separately. Still, AR^- suggested early failure of AI treatment (Fig. 3F), whereas no predictive value was found for patients who received TAM alone (Fig. 3E).

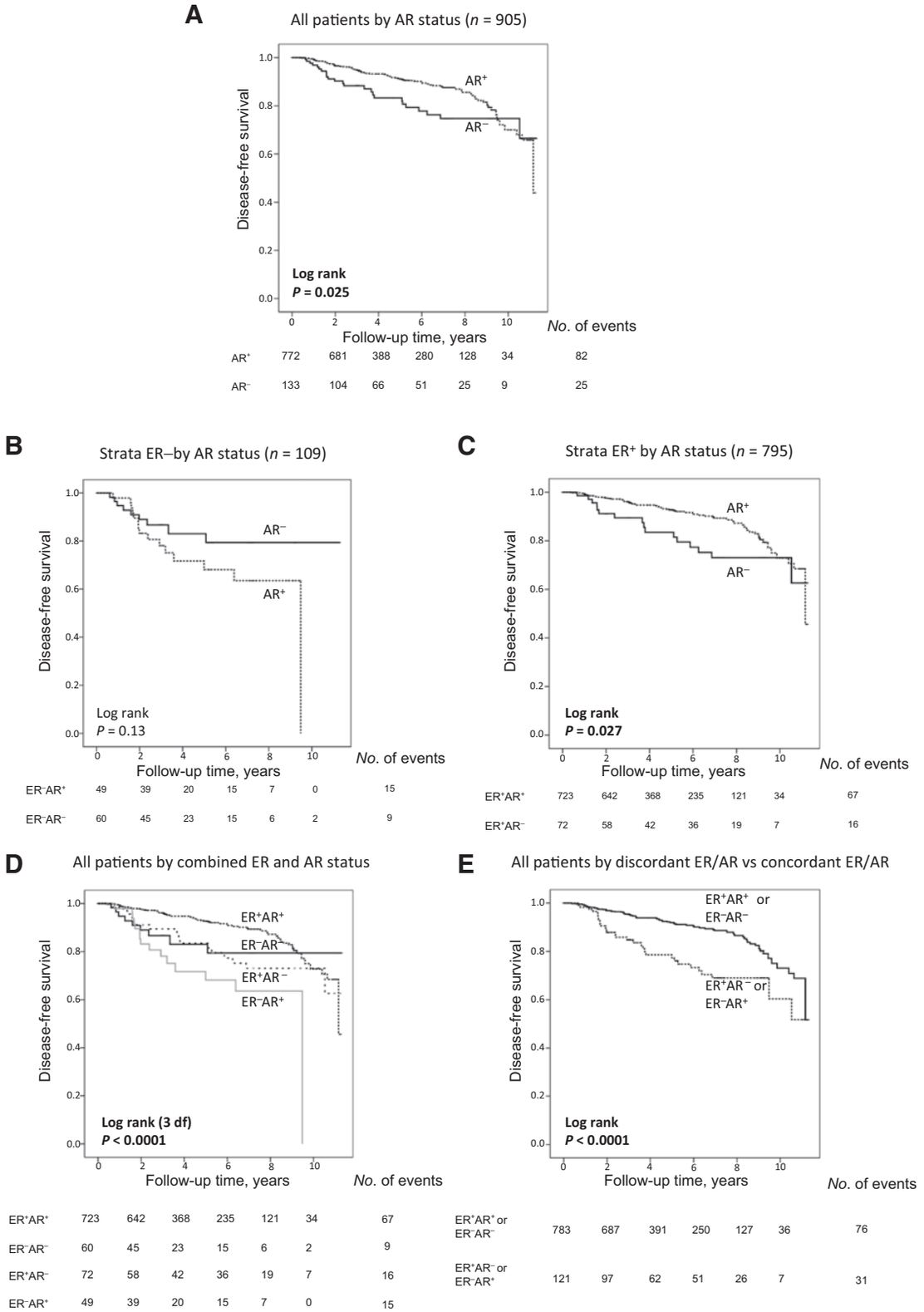

Figure 2.
The prognostic role of AR alone and in combination with ER. Kaplan–Meier estimates of DFS for (A) all patients ($n = 905$) by AR status, (B) ER-negative patients by AR status, (C) ER-positive patients by AR status, (D) combinations of AR and ER status, and (E) discordant versus concordant AR and ER status (ER^+AR^- and ER^-AR^+ vs. ER^+AR^+ and ER^-AR^-). Because this is an ongoing cohort, the number of patients decreased with each follow-up. Bold letters indicate statistically significant results.

Table 3. DFS by AR, ER, and combinations of ER and AR status

Tumor status	Total	Events	Missing	Crude HR		Adjusted HR							
						Model 1		Model 2		Model 3		Model 4[a]	
	n	*n*	*n*	HR (95% CI)	*P*	HR[b] (95% CI)	*P*	HR[b,c] (95% CI)	*P*	HR[b,d] (95% CI)	*P*	HR[b,d,e] (95% CI)	*P*
All	905											655	
AR^-	133	107	0	**1.67 (1.06–2.62)**	**0.026**	1.17 (0.69–1.96)	0.56	1.21 (0.72–2.04)	0.47	1.18 (0.70–2.00)	0.54	1.13 (0.47–2.75)	0.79
ER^-	109	107	1	**2.58 (1.64–4.07)**	**<0.0001**	**2.15 (1.23–3.75)**	**0.007**	**1.94 (1.10–3.42)**	**0.022**	1.68 (0.82–3.43)	0.16	2.14 (0.74–6.18)	0.16
ER^+AR^+	723	67	1	Ref.		Ref.		Ref.		Ref.		Ref.	
ER^-AR^-	60	9		1.98 (0.99–3.98)	0.054	1.73 (0.80–3.75)	0.17	1.65 (0.76–3.59)	0.21	1.28 (0.52–3.16)	0.59	1.90 (0.54–6.62)	0.32
ER^+AR^-	72	16		**1.91 (1.10–3.30)**	**0.021**	**1.77 (1.00–3.13)**	**0.049**	**1.82 (1.03–3.21)**	**0.041**	**1.84 (1.03–3.28)**	**0.038**	2.18 (0.74–6.44)	0.16
ER^-AR^+	49	15		**3.83 (2.18–6.72)**	**<0.0001**	**3.25 (1.79–5.90)**	**0.0001**	**2.91 (1.58–5.36)**	**0.001**	**2.49 (1.20–5.16)**	**0.014**	2.94 (1.00–8.66)	0.051
ER^+AR^- or ER^-AR^+	121	107	1	**2.38 (1.56–3.62)**	**<0.0001**	**2.11 (1.36–3.25)**	**0.001**	**2.05 (1.33–3.18)**	**0.001**	**1.99 (1.28–3.10)**	**0.002**	**2.11 (1.02–4.37)**	**0.044**

NOTE: Bold letters indicate statistically significant results.

[a]Patients included as of November 2005. In total, 655 patients and 46 events. Missing data for 1 patient.

[b]Adjusted for age (continuous), invasive tumor size (≤20 mm vs. ≥21 mm or skin or muscular involvement independent of size), axillary lymph node involvement (yes/no), and tumor grade III (yes/no). Adjusted for ER status (+/−) in AR only analysis, and for AR status (+/−) in ER only analysis. Missing data for 3 patients.

[c]Adjusted for BMI ≥25.0 kg/m^2 (yes/no) and preoperative current smoking (yes/no). Missing data for 31 patients.

[d]Adjusted for treatment; tamoxifen, aromatase inhibitors, chemotherapy, and radiotherapy. Missing data for 3 patients.

[e]Adjusted for trastuzumab treatment.

High AR expression (>75%) as an alternative cutoff for AR positivity

Because AR overexpression may affect endocrine treatment response, analyses were repeated with an alternative cutoff of >75% stained nuclei for AR (AR_{75}^+). This resulted in a near equal distribution of AR_{75}^+ tumors ($n = 463$, 50.7%) and AR_{75} negative (AR_{75}^-) tumors ($n = 450$, 49.3%). However, the associations between tumor AR status and age, invasive tumor size, and breast size were lost. The associations between AR status and grade or ER/PR coexpression were weakened with this cutoff. The association between AR status and HER2 amplification in the ER^-PR^- subgroup was lost. In contrast, a new association between AR_{75} negativity (AR_{75}^-) and HER2 amplification emerged among all tumors unselected for ER status. The prognostic role of AR_{75}^+ in the entire cohort and in the ER^+ subgroup was lost, as well as the interaction between AR_{75}^+ and ER status (data not shown). In terms of treatment response, there was still no association between AR_{75} status and DFS in the group of TAM-treated patients 50 years and older, who had not received chemotherapy. Similarly, the early events seen among the AI-treated patients with AR_{75}^- tumors remained with the new cutoff and became stronger. Among the patients who received AI only, there were six events among 33 patients with AR_{75}^- tumors, and only one event among the 53 patients with AR_{75}^+ tumors [LogRank $P = 0.023$, adjusted HR (model 1) 7.18 (0.84–61.53), $P = 0.072$].

Discussion

In this study, the prognostic value of AR expression was significantly different depending on the ER status of the tumor. Concomitant AR and ER expression was associated with superior prognosis compared with all other AR/ER combinations. In contrast, AR expression among ER^- tumors presented a worse prognosis than ER^-AR^- tumors. Similar findings have been reported by others (18, 19).

No association was seen between tumor AR expression and the AR germline diplotypes, which we previously reported were associated with response to TAM but not AI treatment (9). In contrast, lack of tumor AR expression was predictive of early failure of AI treatment among postmenopausal patients who never received chemotherapy. To our knowledge, this has not been reported previously.

The prognostic role of tumor AR expression in ER^+ breast cancer has consistently been reported to be associated with favorable clinical outcomes (18–24). Results from clinical studies on ER^- breast cancer have, however, been inconsistent, demonstrating positive or negative as well as no associations of AR with clinical outcomes (2, 18–20, 25–27). Many factors may have contributed to the reported inconsistent results; small sample sizes, heterogeneity of study populations, selection of included studies in the meta-analyses and lack of standardized methods and cutoffs for AR assessment but also for ER assessment, and the biologic heterogeneity among ER^- tumors.

The differential role of AR depending on ER status has been suggested to be related to the competitive interaction between AR and ER (20). In the presence of ER, AR interacts with estrogen response elements on ER, blocking downstream estrogen target genes; thus, inhibiting ER-stimulated tumor growth (28, 29). In the absence of ER, AR instead interacts with androgen receptor elements and functions as an oncogene promoting tumor growth (28). Recently, three reviews highlighted this complexity and called

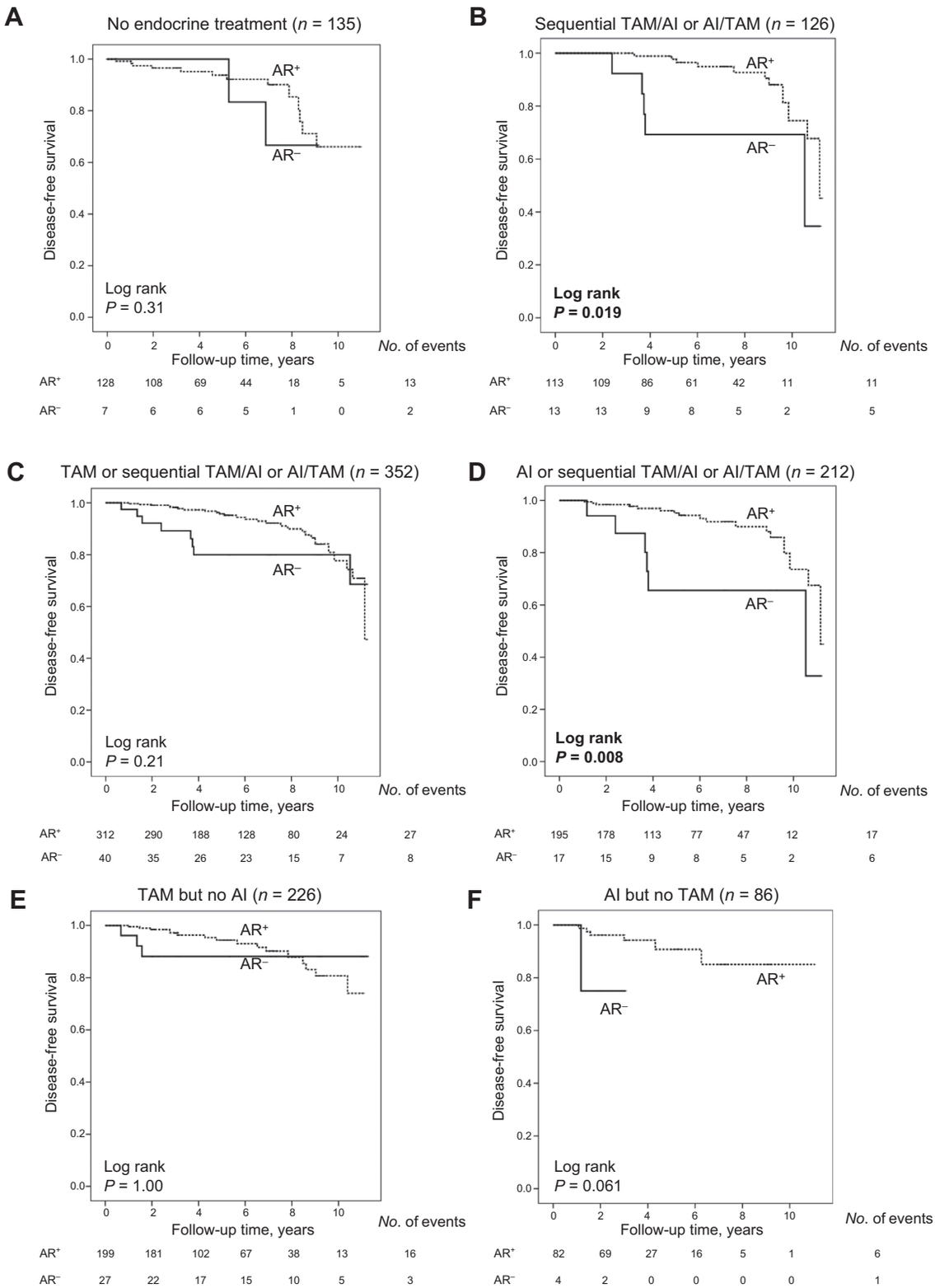

Figure 3.
The predictive role of AR by type of endocrine treatment. Kaplan–Meier estimates of DFS by AR status for patients aged at least 50 years with ER^+ tumors who did not receive chemotherapy, regardless of radiotherapy ($n = 573$). (A), patients who did not receive endocrine therapy ($n = 135$). (B–F), subgroups of endocrine treatments by TAM and/or AIs ($n = 438$). Patients may appear in more than one of these graphs. Because this is an ongoing cohort, the number of patients decreased with each follow-up. Bold letters indicate statistically significant results.

for awareness when implementing AR-targeting agents in clinic practice; certain settings might require anti-androgens, whereas in other settings androgen agonists, such as selective androgen receptor modulators (30), might be warranted (8, 26, 31).

In this study, a significant interaction between AR and ER expression with respect to DFS was found, indicating the importance of stratifying according to ER status in analyses of the prognostic value of AR. In accordance with this finding, results from the Nurses' Health Study also reported that AR positivity confers a negative prognosis for patients with ER^- breast cancer and demonstrated a formal interaction analysis, which to our knowledge has not been performed by other groups. Further, it was shown that discordant ER and AR expression conferred a worse prognosis than concordant ER and AR expression (19), which corresponds to the findings from our multivariable analyses. Herein, the increased risk for early events among the patients with double negative tumors (ER^-AR^-) was no longer evident in the multivariable model adjusted for treatment in all patients, indicating that chemotherapy had the intended effect for this group of patients, and that chemotherapy may be a confounder in this setting. In the model where trastuzumab treatment was added to the multivariable model, ER^-AR^- tumor status was associated with worse prognosis than ER^+AR^+ tumor status but better prognosis than discordant tumors. Because this model only included a subgroup of patients, it is not fully comparable with the other models. However, patients with ER^-AR^+ tumors had the worst outcome compared with all other groups, irrespective of the adjustment model used. The anti-androgen enzalutamide, (ClinicalTrials.gov identifier: NCT01889238; refs. 6, 7, 32) or indirect modulators such as the CYP17 inhibitor abiraterone acetate (ClinicalTrials.gov identifier: NCT00755885) could be novel treatment options in the group of ER^-AR^+ patients. Recently, abiraterone acetate with or without concomitant exemestane was reported not to improve outcome in metastatic breast cancer patients. However, all patients had ER^+ tumors and were unselected for AR status (33).

Previous studies on endocrine treatment have, to the best of our knowledge, not reported the predictive value of AR stratified by type of endocrine treatment. However, this would be of value because TAM and AI inhibit breast cancer growth by different mechanisms (28). The AIs block androgen conversion to estrogens, resulting in very low circulating estradiol levels (34). They might also confer higher androgen levels during treatment (35). Studies have indicated that increased androgens and AR expression following AI treatment may contribute to reduced tumor cell proliferation. This was explained by the growth inhibitory effect of androgens via the AR being revealed in the low estrogen environment (36, 37). Recently, Patani and colleagues compared the transcriptional response with the AI anastrazole with the transcriptional response to the selected ER downregulator, fulvestrant. Compared with anastrazole-mediated estrogen reduction, fulvestrant treatment induced a stronger and more differential transcriptional response, potentially attributable to arrest of estrogen independent ERα activity. The study suggested the involvement of AR associated genes, and it would therefore be of future interest to evaluate AR status in relation to fulvestrant treatment response (38).

Hickey and colleagues have suggested there may be scope for revisiting the combination TAM and androgen treatment in the subgroup of patients not reaching optimal blockage by TAM alone (28). The preclinical findings indicating AR overexpression as a novel mechanism of endocrine resistance (4, 5) could not be confirmed in our study. When analyses were repeated using the high cutoff AR_{75}, the finding of early events among patients with AR_{75}^- tumors remained. Thus, our results indicate that patients with ER^+AR^- do not benefit from AIs and that alternative treatment strategies should be considered for this group of patients. Because the associations between AR_{75} status and conventional tumor characteristics and prognosis were less pronounced, and the interaction between AR and ER status disappeared, the original cutoff (>10%) for AR^+ was assumed to be more biologically relevant and of higher clinical utility to guide clinicians in terms of prognosis and treatment selection. Other groups have previously used the same antibody with a cutoff at 10% or lower (19, 39–42). The AR/ER ratio has been reported to predict endocrine resistance (43). Unfortunately, our data do not allow assessment of the AR/ER ratio. We also did not find associations between AR and chemo/endocrine therapy, as previously shown by others (3, 21).

A strength of this study is that it was a large prospective population-based study. Participation and follow-up rates were high, and included and nonincluded patients were similar with respect to age and tumor characteristics (9, 44). However, the follow-up was relatively short, and the majority of patients had ER^+ tumors, which tend to metastasize late (45). Because the study was population-based, no randomization of treatment was performed. Thus, analyses of the potential treatment predictive value of AR status were restricted to comparisons within treatment groups. Also, patients with more aggressive disease tended to be treated with AIs rather than TAM only. For example, patients aged 50 and older with ER^+ tumors who received AI only were over 30-fold more likely to have node positive disease than those treated with TAM only. Because AR and ER are often coexpressed, the expected number of AR^- tumors in the analyses of endocrine treatment response was low and the subgroup analyses should therefore be interpreted with caution. No predictive value was seen within the chemotherapy group; however, chemotherapy regimens differed somewhat during follow-up. Ki67 analysis was routinely introduced as of March 2009 (9), and thus were not incorporated in the present study, although it would be of interest in future studies. The distribution of patient and tumor characteristics for patients with available AR status was comparable with those without available AR status, suggesting that TMAs were representative.

In conclusion, the prognostic value of AR expression was significantly different depending on the ER status of the tumor. Patients with discordant ER and AR tumor expression had significantly worse prognosis compared with patients with concordant ER and AR tumor expression. Depending on the combined ER/AR status, patients may benefit from new treatment options, such as anti-androgens or selective androgen receptor modulators. Finally, AR negativity was found to be predictive of early failure of AI but not TAM treatment, a finding that warrants confirmation in a randomized setting.

Disclosure of Potential Conflicts of Interest

No potential conflicts of interest were disclosed.

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Development of methodology: H. Jernström
Acquisition of data (provided animals, acquired and managed patients, provided facilities, etc.): K. Elebro, M. Simonsson, A. Markkula, C. Ingvar, H. Jernström

Analysis and interpretation of data (e.g., statistical analysis, biostatistics, computational analysis): K. Elebro, S. Borgquist, C. Ingvar, C. Rose, H. Jernström
Writing, review, and/or revision of the manuscript: K. Elebro, S. Borgquist, M. Simonsson, A. Markkula, K. Jirström, C. Ingvar, C. Rose, H. Jernström
Administrative, technical, or material support (i.e., reporting or organizing data, constructing databases): K. Elebro, A. Markkula, K. Jirström, H. Jernström
Study supervision: C. Ingvar, H. Jernström

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