The Prognostic Role of Androgen Receptor in Patients with Early-Stage Breast Cancer: A Meta-analysis of Clinical and Gene Expression Data

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Abstract

Purpose: Androgen receptor (AR) expression has been observed in about 70% of patients with breast cancer, but its prognostic role remains uncertain.

Experimental Design: To assess the prognostic role of AR expression in early-stage breast cancer, we performed a meta-analysis of studies that evaluated the impact of AR at the protein and gene expression level on disease-free survival (DFS) and/or overall survival (OS). Eligible studies were identified by systematic review of electronic databases using the MeSH-terms "breast neoplasm" and "androgen receptor" and were selected after a qualitative assessment based on the REMARK criteria. A pooled gene expression analysis of 35 publicly available microarray data sets was also performed from patients with early-stage breast cancer with available gene expression and clinical outcome data.

Results: Twenty-two of 33 eligible studies for the clinical meta-analysis, including 10,004 patients, were considered as evaluable for the current study after the qualitative assessment. AR positivity defined by IHC was associated with improved DFS in all patients with breast cancer [multivariate (M) analysis, HR 0.46; 95% confidence interval (CI) 0.37–0.58, P < 0.001] and better OS [M-HR 0.53; 95% CI, 0.38–0.73, P < 0.001]. Thirty-five datasets including 7,220 patients were eligible for the pooled gene expression analysis. High AR mRNA levels were found to confer positive prognosis overall in terms of DFS (HR 0.82; 95% CI 0.72–0.92; P = 0.0007) and OS (HR 0.84; 95% CI, 0.75–0.94; P = 0.02) only in univariate analysis.

Conclusion: Our analysis, conducted among more than 17,000 women with early-stage breast cancer included in clinical and gene expression analysis, demonstrates that AR positivity is associated with favorable clinical outcome. Clin Cancer Res; 1–11. ©2016 AACR.
antagonism between AR and ER mediated by competition for the
DNA-binding sites in the cells and a possible adoption of AR in
ER-like oncogenic role in the absence of ER (2, 5).
AR expression by IHC has been observed in up to 90% of
primary breast cancer, and in up to 75% of breast cancer metas-
tases, depending on the method, patient’s population and cutoffs
used. These studies indicate that the frequency of AR positivity
differs between different breast cancer subtypes; the highest
positivity observed in ER-positive tumors in up to 80%–90%
(6–10) and the lowest in triple-negative (TN) tumors, in up to
30% (6, 7, 11). Of note, a phase II trial assessing enzalutamide
in metastatic TNBC, reported AR positivity (defined as ≥1%) in 79%
of 404 cases analyzed (12). Retrospective clinical studies showed
that AR by IHC adds prognostic information beyond the estab-
lished clinicopathologic parameters in all patient groups and in
patients with early-stage ER-positive breast cancer (9, 13, 14).
Tumors coexpressing AR and ER are smaller, have lower Nott-
ingham grade and low proliferative index (14), lymph node involve-
ment is less frequent, and they are more likely to be found in
postmenopausal women (4). However, the clinical significance of
AR in patients with ER-negative breast cancer is less clear.
To address the prognostic role of AR status in early-stage breast
cancer, we conducted a meta-analysis of published clinical studies
to evaluate the impact of AR protein expression defined by IHC on
disease-free survival (DFS) and overall survival (OS). Further-
more, we conducted a pooled gene expression analysis of publicly
available microarray datasets to assess the prognostic significance
of mRNA AR status in early-stage breast cancer and explore
potential associations of AR mRNA expression with the expres-
sion of other individual genes and gene signatures (GS) of interest.

Methods
Systematic review of published clinical studies
Meta-analysis of clinical studies that evaluated the impact of AR
protein expression on DFS and OS in patients with early-stage
breast cancer was performed within all breast cancer subtypes
together and, whenever possible, within different breast cancer
subtypes, defined as follows: (i) ER-positive (ER+); (ii) ER-neg-
ative (ER−); (iii) HER2+ and (iv) TN (ER−/HER2−). Patients were
considered to have AR-positive tumors according to the cut-off
points defined by the authors in each of the eligible studies.

Identification and selection of studies
The eligible studies were identified by an electronic search on
PubMed/Medline, Cochrane Review, and Google Scholar using the
MeSH terms “breast neoplasm” and “androgen receptor.” The
literature search was conducted independently by two investiga-
tors (I. Bozovic-Spasojevic and D. Zardavas) up to June 2015.
Identified studies were eligible for this meta-analysis if they met
the following inclusion criteria: studies conducted in patients with
early-stage breast cancer that assessed AR expression in primary
breast cancer, studies that compared clinical outcomes (DFS
and/or OS) in association with AR status, studies published in extenso
and reported in the English language.
Of note, no minimum threshold for either number of patients
or duration of follow-up period was mandated. Cross-referencing
from relevant studies was performed to confirm retrieval of all
potentially eligible studies. To avoid duplication of data, when the
same patient population was reported in several publications,
only study with the highest number of events was included in the
current analysis. In terms of study eligibility, final decisions were
taken upon consensus between two investigators (I. Bozovic-
Sapsojevic and D. Zardavas).

Data extraction
Data, such as authors, publication date, number of patients,
subgroup analysis, method of AR assessment, and AR threshold
were extracted by two investigators (I. Bozovic-Sapsojevic and
D. Zardavas) using a predesigned abstraction form. Survival data
within all patients and whenever possible within different breast
cancer subtypes [including HR and P value] were extracted inde-
pendently by two statisticians (L. Ameye and M. Paesmans).

Quality of the eligible studies
The study methodology of each eligible study was independ-
ently scored by three reviewers [two medical doctors (I. Bozovic-
Sapsojevic and D. Zardavas) and one statistician (M. Paesmans)],
according to REMARK (Reporting Recommendations for Tumor
Marker Prognostic Studies; ref. 15). Each of the 20 criteria listed in
REMARK was scored for each eligible study, using an ordinal scale
with possible values 0, 1, and 2, making a maximal score of 40.
The overall score evaluated several scopes of the methodology
including the scientific rational and design, the description of the
methods used to identify AR expression, data generation, analysis
of the study data, and discussion of their relevance. The attributed
value per item was 2 points if clearly defined in the article, 1 point
if description incomplete or unclear, and 0 point if not defined,
inadequate, or not applicable. The scores for each individual study
were compared and consensus for each item was reached among
the three investigators. We did not redefine a threshold for the
REMARK score of each study as inclusion criterion in the meta-
analysis. However, scoring was performed as quality assessment
of the clinical studies data included in this meta-analysis and used
for sensitivity analyses (Supplementary Table S1).

The studies being eligible for the systematic review are called
“eligible,” and those providing data for the clinical meta-analysis

Translational Relevance
Androgen receptor (AR) recently regained interest as a
possible therapeutic option in breast cancer treatment;
however, little is known about the clinical significance of
AR in breast carcinogenesis. To our knowledge, we report the
largest combined clinical and gene expression meta-analy-
sis, assessing the prognostic significance of AR expression in
early-stage breast cancer indicating that AR expression at
both protein and mRNA level serves as a positive prognos-
ticator for women with early-stage breast cancer. In addi-
tion, we explore connection among AR and pathologic
complete response after neoadjuvant chemotherapy in the
gene expression publicly available datasets, and also cor-
relation of AR and genes and gene signatures of interest.
Additional information about distinct prognostic relevance
of AR expression in different breast cancer subtypes was
provided; however, further studies are warranted to confirm
these findings. It is apparent that AR has many effects on the
biology of breast cancer and deserves more clinical and
translational research attention.
with at least retrievable HR in one of the defined endpoints are called "evaluable." We excluded studies with insufficient or with unreliable data to estimate clinical outcome. In terms of defining the evaluable studies, final decisions were taken upon consensus between the two physicians (I. Bozovic-Spasojevic and D. Zardavas) and the two statisticians (L. Ameye and M. Paesmans).

Pooled gene expression analysis
Thirty-five datasets of gene expression profiling analysis of more than 7,220 primary breast cancer were retrieved from public databases or authors’ websites, 32 previously described in the manuscript of Haibe-Kains and colleagues, with three additional sets: TCGA (TCGA Data Portal; ref. 16), PNC (GSE20713; ref. 17), and METABRIC (European Genome-Phenome Archive under accession number EGAS0000000083; ref. 18).

To ensure comparability of expression values across multiple datasets, we performed a 0.95-quantile normalization of all genes and GS of interest.

AR expression levels were calculated as continuous and categorical variable where classified as low (corresponding to the lower tertile across all available expression values), medium (intermediate tertile), and high (upper tertile).

Table 1. Eligible clinical studies for clinical meta-analysis and their characteristics

<table>
<thead>
<tr>
<th>First author</th>
<th>Type of study</th>
<th>N AR (total)</th>
<th>Method of AR assessment</th>
<th>Antibody used</th>
<th>AR threshold for positivity (chosen by)</th>
<th>RS</th>
<th>ER threshold for positivity</th>
</tr>
</thead>
<tbody>
<tr>
<td>Collett K. (46), 1996</td>
<td>Retrospective analysis</td>
<td>137/269</td>
<td>Dextran-coated charcoal technique</td>
<td>10nM 5H-labeled methyltrienolone R-1881</td>
<td>&gt;5 fmol/mg Median value</td>
<td>12</td>
<td>&gt;15 fmol/mg</td>
</tr>
<tr>
<td>Agoff A. (6), 2003</td>
<td>Retrospective analysis of random cases</td>
<td>51/88</td>
<td>IHC</td>
<td>F39.4.1</td>
<td>&gt;5% arbitrary</td>
<td>13</td>
<td>&gt;5% of cells</td>
</tr>
<tr>
<td>Rahka EA. (41), 2006</td>
<td>Retrospective analysis of consecutive cases</td>
<td>36/282</td>
<td>IHC on TMA</td>
<td>F39.4.1</td>
<td>&gt;1% arbitrary/literature based</td>
<td>16</td>
<td>&gt;1% of cells</td>
</tr>
<tr>
<td>Soiland H. (42), 2008</td>
<td>Retrospective analysis</td>
<td>WS-ARn: 103</td>
<td>IHC on TMA and Aromatase and TMA plus charc-AR</td>
<td>AR441; R1881</td>
<td>charc-AR&gt;30 pmol/g,</td>
<td>25</td>
<td>&gt;10% of cells</td>
</tr>
<tr>
<td>Gonzalez-Angulo AM. (8), 2009</td>
<td>Retrospective analysis of selected cases/frozen</td>
<td>174/347</td>
<td>RPPL Microarray</td>
<td>Primary AR antibody - Epitomics AR -- U4077 antiserum</td>
<td>&gt;0.0852 dichotomized by mean</td>
<td>15</td>
<td>Not reported</td>
</tr>
<tr>
<td>Peters A. (40), 2010</td>
<td>Retrospective analysis</td>
<td>116/215</td>
<td>IHC on TMA</td>
<td>AR441</td>
<td>Median % of positive cells 75% (range 0-96%)</td>
<td>6</td>
<td>&gt;10% of cells</td>
</tr>
<tr>
<td>Castellano L. (9), 2010</td>
<td>Retrospective analysis of consecutive cases</td>
<td>172/375</td>
<td>IHC on TMA</td>
<td>AR441</td>
<td>≥1% literature based</td>
<td>26</td>
<td>&gt;1% of cells</td>
</tr>
<tr>
<td>Luo X. (39), 2010</td>
<td>Retrospective analysis</td>
<td>148/269</td>
<td>IHC</td>
<td>ZYMED PV-6000-G Kit AR antibody not specified</td>
<td>≥2 (intensity %) arbitrary</td>
<td>13</td>
<td>Not reported</td>
</tr>
<tr>
<td>Micello D. (7), 2010</td>
<td>Retrospective analysis of consecutive cases</td>
<td>128/226</td>
<td>IHC</td>
<td>AR27</td>
<td>&gt;10% literature based</td>
<td>16</td>
<td>&gt;10% of cells</td>
</tr>
<tr>
<td>Loibl S. (44), 2010</td>
<td>Prospective study</td>
<td>358/673</td>
<td>IHC on TMA</td>
<td>F39.4.1</td>
<td>&gt;3 (x intensity)</td>
<td>25</td>
<td>&gt;10% of cells</td>
</tr>
<tr>
<td>Park S. (11), 2011</td>
<td>Prospectively collected tissues</td>
<td>541/931</td>
<td>IHC on TMA</td>
<td>AR 441</td>
<td>≥10% arbitrary</td>
<td>26</td>
<td>&gt;10% of cells</td>
</tr>
<tr>
<td>Yu Q. (43), 2011</td>
<td>Retrospective analysis of consecutive cases</td>
<td>237/327</td>
<td>IHC</td>
<td>AR441</td>
<td>≥1 Combined score (intensity x %)</td>
<td>18</td>
<td>&gt;1% of cells</td>
</tr>
<tr>
<td>Peters KM. (54), 2012</td>
<td>Retrospective analysis</td>
<td>41/73</td>
<td>IHC on TMA</td>
<td>Anti-AR (Biocare Medical)</td>
<td>≥1% not reported</td>
<td>11</td>
<td>Not reported</td>
</tr>
<tr>
<td>Hu R. (53), 2012</td>
<td>Prospective study</td>
<td>1154/1467</td>
<td>IHC on TMA</td>
<td>AR441</td>
<td>≥1% (1%−10%) + &gt;10% arbitrary</td>
<td>24</td>
<td>Not reported</td>
</tr>
<tr>
<td>He J. (47), 2012</td>
<td>Retrospective analysis</td>
<td>73/287</td>
<td>IHC on TMA</td>
<td>AR 441 Dako</td>
<td>≥5% nuclear staining arbitrarily</td>
<td>11</td>
<td>Not reported</td>
</tr>
<tr>
<td>Nonna N. (48), 2013</td>
<td>Retrospective analysis</td>
<td>212/403</td>
<td>IHC on FFPE</td>
<td>Anti-AR Ab clone AR27; Novocastro Laboratories</td>
<td>≤10% nuclear staining literature based</td>
<td>13</td>
<td>&gt;10% of cells</td>
</tr>
<tr>
<td>Witzel I. (52), 2013</td>
<td>Retrospective analysis of consecutive cases plus population-based cohort</td>
<td>Cohort 1 126/165 Cohort 2 133/200 Cohort 3 167/223</td>
<td>AR mRNA</td>
<td>Affymetrix HG-U 133A GeneChip System</td>
<td>Expression value of AR mRNA higher than 75%</td>
<td>18</td>
<td>Not reported</td>
</tr>
<tr>
<td>Tokunaga E. (50), 2013</td>
<td>Retrospective analysis of consecutive pts</td>
<td>555/250</td>
<td>IHC</td>
<td>AR441; Dako</td>
<td>75% nuclear staining literature based</td>
<td>9</td>
<td>&gt;1% of cells</td>
</tr>
<tr>
<td>Takeshita T. (65), 2013</td>
<td>Retrospective analysis</td>
<td>295/379</td>
<td>IHC</td>
<td>AR7B Leica Biosystems</td>
<td>Histo score &gt;10 literature based</td>
<td>11</td>
<td>&gt;1% of cells</td>
</tr>
<tr>
<td>Thike AA. (49), 2013</td>
<td>Retrospective cohort analysis</td>
<td>267/699</td>
<td>IHC on TMA</td>
<td>AR 27 NCL-AR-318</td>
<td>≥1% nuclear staining literature based</td>
<td>11</td>
<td>&gt;1% of cells</td>
</tr>
<tr>
<td>Tsang J. (51), 2014</td>
<td>Retrospective analysis of consecutive histologic files of 3 institutions</td>
<td>549/1144</td>
<td>IHC on TMA</td>
<td>AR 441 Dako</td>
<td>≥1% nuclear staining literature based</td>
<td>12</td>
<td>&gt;1% of cells</td>
</tr>
<tr>
<td>Pistelli M. (45), 2014</td>
<td>Retrospective analysis</td>
<td>15/81</td>
<td>IHC</td>
<td>F39.4.1 Biogenex</td>
<td>≥10% nuclear staining arbitrarily</td>
<td>18</td>
<td>&gt;10% of cells</td>
</tr>
</tbody>
</table>

Abbreviations: AR, androgen receptor; ARc, cytoplasmic androgen receptor; ARn, nuclear androgen receptor; charc-AR, charcoal androgen receptor; RPPL, reverse-phase protein lysate microarray; RS, REMARK score (15)—reporting recommendations for tumor marker prognostic studies; please refer to Supplementary Table S1 for details; TMA, tissue microarray; WS, whole section.
Breast cancer molecular subtypes were defined on the basis of the PAM50 classifier as luminal A, luminal B, HER2-enriched, and basal-like. As no clear consensus has ever been established on the existence of this PAM50 group, the normal like subgroup of tumor was not specifically considered in the subgroup analyses. Moreover, as not every study had complete information about ER and HER2 status, ESR1 and ERBB2 status were derived from the bimodal distribution of these two genes expression.

We chose to evaluate potential associations between AR gene expression levels and individual genes and GS that can be classified as follows: estrogen GS (ESR1), proliferation-based prognostic GS [AURKA (19), CIN70 (20), GEN70 (21), GCI (22)], immune-related individual genes and GS [CTLA4 gene, IDO1 gene, IFNG gene, IGKC gene, CD3D gene, CD80 gene, CD8A gene, FOXP3 gene, Immune1 GS (23), Immune2 GS (23), PD1 gene, PDL1 gene, stroma-related genes, and GS]; CXCL13 gene, CXCL9 gene, RANKL gene, Stromal GS (24, 25), Stromal GS (24)], as well as other individual genes and GS related to further oncogenic signaling pathways [RAS GS (26), SRC GS (26), MYC GS (26), E2F3 GS (26), BetaCatatin GS (26), BRCAl and BRCA2 genes, AKT/mTOR GS (27), HER2 gene, IGF1 GS (28), MAPK GS (29), PI3KCA GS (30), PTEN GS (31), VEGFA gene, WNT7B gene].

Furthermore, we assessed the possible impact of AR expression on pathologic complete response (pCR), using neoadjuvant datasets previously published in Ignatiadis and colleagues (32), with availability of gene expression profiling data, and pCR status.

Statistical analysis

Clinical meta-analysis. We used as a measure of the prognostic effect of AR protein expression the HR for the comparison of the DFS or OS distributions using AR-negative patients as a reference. For each study and each considered subtype (when applicable and possible), we extracted the individual HR estimates and their variances if reported. When only the HR estimates were reported, we calculated their variances using the confidence interval for the HR estimate or the log-rank statistic value and the number of events. If none of these methods was applicable, we read the survival curves and calculated back the HRs with their variances. Whenever available, we used both the individual HRs from the univariate and the multivariate analyses in separate analyses. We report combined HRs with 95% confidence intervals (CIs) using fixed-effect models or random-effects models depending on the detected heterogeneity between the individual HRs estimates; if heterogeneity was detected, random effect was reported. Heterogeneity of HRs between the different studies was assessed using a χ² test for heterogeneity.

Pooled gene expression mRNA analysis. We calculated potential associations between AR mRNA levels with clinicopathologic variables among all patients with breast cancer and within breast cancer subtypes using t tests. The clinicopathologic variables that were assessed were the following: age (both as continuous and categorical variable ≤ and > 50 years); tumor size (both as continuous and categorical variable following the UICC-TNM classification); grade (grade 1, 2, and 3); nodal status (positive vs negative); ER status (positive vs negative); and HER2 status (positive vs negative). Furthermore, we assessed potential associations of AR mRNA expression with the expression levels of the previously mentioned individual genes and GS using a Wilcoxon test.

For the survival analysis, the two endpoints were disease-free survival (DFS) and overall survival (OS). Survival plots according to the groups were drawn using the Kaplan–Meier method, and the differences were evaluated with a log-rank test. The median follow-up was calculated with the reversed Kaplan–Meier method and data were censored after 10 years of follow-up.

To compute HR and 95% CI in a univariate analysis we used a Cox linear regression model (adjusting only for the dataset). Multivariate analysis was computed by the linear Cox regression model, adjusted for the ESR1 and ERBB2 gene expression, patient age, tumor grade, tumor size, and the lymph node involvement. In the 8 no-adjuvant datasets, we looked for the association of AR mRNA expression levels among breast cancer subtypes and percentage of pCR by using a logistic regression model (uni- and multivariate adjusted for dataset, tumor grade, and treatment). pCR was defined as loss of the invasive component of the primary tumor in one study (33) and no residual invasive cancer in the breast and axillary lymph nodes in other seven studies (34–38).

Results

Clinical meta-analysis.

We retrieved a total of 913 references. After applying secondary exclusion criteria (Supplementary Fig. S1A), a total of 33 studies were eligible for the analysis and were scored on the basis of REMARK criteria (15). We then excluded studies where none of the HR of interest was available or retrievable with its variance or where the number of observed events was < 15. (Supplementary Fig. S1A; Supplementary Table S1).

Out of the 33 eligible studies, 22 studies were found to be evaluable, including 10,004 patients, with 5,860 cases of AR positivity (Table 1). Twenty studies (6–9, 11, 39–52) were evaluable for the DFS and sixteen (7–9, 11, 39–42, 44–49, 53, 54) for the OS (Supplementary Tables S2A and S2B); among studies only two (44, 53) were prospective, the rest was retrospective.

In the multivariate analysis, we assessed the possible impact of AR expression on pathologic complete response (pCR), using neoadjuvant datasets previously published in Ignatiadis and colleagues (32), with availability of gene expression profiling data, and pCR status.

Table 2A. Univariate and multivariate analyses of AR prognostic role in all patients with breast cancer

<table>
<thead>
<tr>
<th></th>
<th>DFS (21 studies, N = 8036) Random-effects Heterogeneity, P = 0.01</th>
<th>OS (18 studies, N = 8301) Random-effects Heterogeneity, P = 0.005</th>
</tr>
</thead>
<tbody>
<tr>
<td>Univariate analysis</td>
<td>HR (95% CI)</td>
<td>HR (95% CI)</td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>DFS</td>
<td>0.61 (0.52–0.72)</td>
<td>0.62 (0.51–0.75)</td>
</tr>
<tr>
<td>OS</td>
<td>0.46 (0.37–0.58)</td>
<td>0.53 (0.38–0.73)</td>
</tr>
<tr>
<td>Multivariate analysis</td>
<td></td>
<td></td>
</tr>
<tr>
<td>DFS</td>
<td></td>
<td></td>
</tr>
<tr>
<td>OS</td>
<td></td>
<td></td>
</tr>
</tbody>
</table>

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basis of available information, confirming good prognostication conferred by AR positivity in regard to DFS (HR 0.46; 95% CI, 0.37–0.58; P < 0.001) and OS (HR 0.53; 95% CI, 0.38–0.73; P < 0.001; Table 2A; Fig. 1B).

Subgroup analysis was performed on the basis of available data, whenever feasible for the following breast cancer subtypes: ER−, ER+; HER2−ER+ (representative of the HER2-enriched) and TN (representative the basal-like subtype) (Table 2B). This analysis revealed that AR positivity resulted in significantly improved DFS and OS in ER+ patients, both in univariate (HR 0.53; 95% CI, 0.44–0.63, P < 0.001 and HR = 0.59; 95% CI, 0.49–0.72, P < 0.001, respectively) and multivariate analysis (HR 0.40; 95% CI, 0.31–0.52, P < 0.001 and HR = 0.37; 95% CI 0.16–0.85, P = 0.02, respectively). Similar positive association was seen in TNBC patients (HR 0.64; 95% CI 0.51–0.81, P < 0.001 and HR 0.64 95% CI, 0.49–0.88, P < 0.001, respectively), in univariate analysis. In HER2+ breast cancer, AR positivity was not significantly associated with DFS (HR 1.20; 95% CI, 0.86–1.69, P = 0.28) while overall survival was worse (HR 1.50; 95% CI, 1.01–2.22 P = 0.04). No significant association was found either for DFS and OS in ER+ breast cancer subgroup (HR 0.33; 95% CI, 0.04–2.44, P = 0.28 and HR 1.32; 95% CI, 0.98–1.80, P = 0.08 respectively).

Pooled gene expression mRNA analysis

Datasets. A total of 35 datasets with data from 7,737 patients, and median follow-up time of 10.17 years were available (Supplementary Fig. S1B; Supplementary Table S3), including eight studies with 1,005 patients in the neoadjuvant setting (Supplementary Fig. S1B; Supplementary Table S3), including eight studies with 1,005 patients in the neoadjuvant setting (Supplementary Fig. S1B; Supplementary Table S3), including eight studies with 1,005 patients in the neoadjuvant setting (Supplementary Table S3), including eight studies with 1,005 patients in the neoadjuvant setting (Supplementary Table S3), including eight studies with 1,005 patients in the neoadjuvant setting (Supplementary Table S3), including eight studies with 1,005 patients in the neoadjuvant setting (Supplementary Table S3), including eight studies with 1,005 patients in the neoadjuvant setting (Supplementary Table S3), including eight studies with 1,005 patients in the neoadjuvant setting (Supplementary Table S3), including eight studies with 1,005 patients in the neoadjuvant setting (Supplementary Table S3). Clinicopathologic characteristics of these patients according to the datasets can be found in Supplementary Table S3; ref. 32). Clinicopathologic characteristics of these patients according to the datasets can be found in Supplementary Table S3; ref. 32). Clinicopathologic characteristics of these patients according to the datasets can be found in Supplementary Table S3; ref. 32). Clinicopathologic characteristics of these patients according to the datasets can be found in Supplementary Table S3; ref. 32). Clinicopathologic characteristics of these patients according to the datasets can be found in Supplementary Table S3; ref. 32). Clinicopathologic characteristics of these patients according to the datasets can be found in Supplementary Table S3; ref. 32). Clinicopathologic characteristics of these patients according to the datasets can be found in Supplementary Table S3; ref. 32).

Associations between AR mRNA levels with clinicopathologic variables among all patients with breast cancer. Associations between AR mRNA expression and clinicopathologic variables were evaluated among all patients: a significant inverse correlation was observed between AR mRNA expression and tumor size (P = 0.03). Women older than 50 years had higher AR levels (P < 0.001). In addition, AR expression was positively correlated with ESR2 expression (P < 0.001; Table 3; Supplementary Fig. S2A). We also evaluated AR expression among different breast cancer subtypes defined by PAM50 classifier (Table 3, Supplementary Fig. S2B). High AR expression was seen in luminal A tumors with tumors less than 2 cm. High AR expression was found in luminal B tumors in women older than 50 years and with lymph node-negative disease (P = 0.05); AR mRNA levels were positively correlated with ESR1 (P < 0.001).

Survival analysis according to AR mRNA expression levels. To address the impact of AR mRNA expression levels on prognosis, we compared the risk of relapse among three subgroups of patients corresponding to low, intermediate, and high AR mRNA levels (level 1, 2, and 3, respectively) among all patients, and within different breast cancer subtypes (see Methods). Overall, high AR mRNA levels were found to confer better prognosis in terms of DFS (HR 0.82; 95% CI, 0.72–0.92; P = 0.0007) and OS (HR 0.84; 95% CI, 0.75–0.94; P = 0.02) in univariate analysis, but did not remain significant in the multivariate analysis (DFS: HR 0.97; 95% CI 0.84–1.13; P = 0.72 and OS HR 0.98; 95% CI 0.85–1.12, P = 0.72; Table 4).

In contrast, higher AR mRNA expression levels were found to be associated with improved OS in both, uni- and multivariate analyses for all women with HER2-enriched breast cancer (HR 0.75; 95% CI, 0.56–1.00, P = 0.05 and HR 0.72; 95% CI,
Table 2B. Univariate analysis of AR prognostic role in breast cancer (BC) subtypes and multivariate analysis in ER+ subtype

<table>
<thead>
<tr>
<th>Univariate analysis</th>
<th>HR (95% CI)</th>
<th>P</th>
<th>Multivariate analysis</th>
<th>HR (95% CI)</th>
<th>P</th>
</tr>
</thead>
<tbody>
<tr>
<td>ER+</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>DFS (9 studies, N = 2,843) fixed effects, Heterogeneity P = 0.53</td>
<td>0.53 (0.44–0.63)</td>
<td>&lt;0.001</td>
<td>DFS (5 studies, N = 1,571) fixed effects, Heterogeneity P = 0.65</td>
<td>0.40 (0.31–0.52)</td>
<td>&lt;0.001</td>
</tr>
<tr>
<td>OS (6 studies, N = 3,383) fixed effects, Heterogeneity P = 0.26</td>
<td>0.59 (0.49–0.72)</td>
<td>&lt;0.001</td>
<td>OS (3 studies, N = 2,180) random effects, Heterogeneity P = 0.003</td>
<td>0.37 (0.16–0.85)</td>
<td>0.02</td>
</tr>
<tr>
<td>ER-</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>DFS (3 studies, N = 383) random effects, Heterogeneity P = 0.05</td>
<td>0.33 (0.04-2.44)</td>
<td>0.28</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>OS (4 studies, N = 687) fixed effects, Heterogeneity P = 0.11</td>
<td>1.32 (0.98-1.80)</td>
<td>0.08</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>HER2*/ER-</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>DFS (4 studies, N = 410) fixed effects, Heterogeneity P = 0.99</td>
<td>1.20 (0.86-1.69)</td>
<td>0.28</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>OS (3 studies, N = 358) fixed effects, Heterogeneity P = 0.50</td>
<td>1.50 (1.01-2.22)</td>
<td>0.04</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>TN</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>DFS (9 studies, N = 1,373) fixed effects, Heterogeneity P = 0.16</td>
<td>0.64 (0.51-0.81)</td>
<td>&lt;0.001</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>OS (5 studies, N = 1,285) fixed effects, Heterogeneity P = 0.07</td>
<td>0.64 (0.49-0.88)</td>
<td>&lt;0.001</td>
<td></td>
<td></td>
<td></td>
</tr>
</tbody>
</table>

0.53–0.97, P = 0.03 respectively). Similar results were found in HER2-enriched subgroup treated with hormone therapy only (multivariate HR 0.50; 95% CI, 0.26–0.97, P = 0.04). We did not find any other significant association with clinical outcome in the luminal A, luminal B, and basal-like breast cancer subtypes (Supplementary Table S5).

Associations of AR mRNA expression levels with individual genes and gene signatures. We next looked for associations between AR mRNA expression and expression of individual genes and GS of potential clinical relevance. Among all breast cancer subtypes, the following significant associations were found: negative correlation with proliferation-based GS such AURKA GS (19), CIN70 (20), CCG (22), and GENE70 (21; all P < 0.001): negative correlation with immune-related genes and GS such as Immune 1 (23) and Immune 2 (24) GS (P = 0.004 and P < 0.001, respectively), as well as CTLA4, IDO1, IFNγ, IKK, PD1, CXCL9, CXCL13, and PD-L1 genes (all P < 0.001); positive correlation with stroma1 GS (ref. 25; P < 0.001) but not with stroma2 GS (ref. 24; P = 0.13); and finally, positive correlations with several oncogenic pathways including WNT7B, ESR1, HER3, Pik3ca, BRCA1 genes (all P < 0.001), and negative correlations with IGF1 (28), BetaCatenin, PTEN, MAPK, MYC, RAS, SRC GS (26) and BRCA2, VEGFA, CD80, CD8A, CD3D, and S duel genes (all P < 0.001; Supplementary Table S6A). Associations between AR mRNA and expression levels of individual genes and GS among different breast cancer subtypes are presented in Supplementary Table S6B in supplementary file.

Response to neoadjuvant CT according to AR mRNA levels. We further evaluated whether AR mRNA expression was associated with response to neoadjuvant CT (anthracyclines ± taxanes). Out of 1,005 patients from eight datasets (33–38, 39), 8, 39, 44, 52), low grade (8, 39, 44, 52), negative lymph node status, ER and/or PR positivity (44) and older age (8). These associations were confirmed by our AR mRNA pooled analysis, which showed that higher AR mRNA expression levels serve as positive prognosticator among all women with early-stage breast cancer.

In addition, we assessed the prognostic relevance of AR positivity among different breast cancer subtypes, defined differently in the clinical and transcriptomic meta-analysis (IHC and PAM50 based respectively). In the former, a strong better prognostication was conferred by AR positivity among patients with ER+ breast cancer both in univariate and multivariate analysis, which is in line with previously published studies (8, 9, 11, 41, 42, 53).

Discussion

To our knowledge, we report the largest combined clinical and gene expression meta-analysis, assessing the prognostic significance of AR expression in early-stage breast cancer indicating that AR expression at both protein and mRNA level serves as a positive prognosticator for women with early-stage breast cancer.

Our results show that AR protein expression confers a DFS and OS advantage among all patients with breast cancer. A possible explanation for this is the documented association of AR expression with favorable prognostic factors such as small tumor size (39), low grade (8, 39, 44, 52), negative lymph node status, ER and/or PR positivity (44) and older age (8). These associations were confirmed by our AR mRNA pooled analysis, which showed that higher AR mRNA expression levels serve as positive prognosticator among all women with early-stage breast cancer.

In addition, we assessed the prognostic relevance of AR positivity among different breast cancer subtypes, defined differently in the clinical and transcriptomic meta-analysis (IHC and PAM50 based respectively). In the former, a strong better prognostication was conferred by AR positivity among patients with ER+ breast cancer both in univariate and multivariate analysis, which is in line with previously published studies (8, 9, 11, 41, 42, 53).

Preclinical evidence suggesting an AR-ER cross-talk also supports these findings, indicating that AR can antagonize ER signaling depending on the relative levels of these two steroid receptors (2). Interesting results have been reported by Cochrane and colleagues, assessing the potential prognostic/predictive relevance of AR:ER ratio at the protein level assessed through IHC in the setting of ER-positive breast cancer (55). The study was fueled by previous observations that in ER-positive breast cancer responding to endocrine treatment, AR downregulation at the protein and mRNA level is observed, whereas no such effect is seen in non-responsive tumors (56, 57). In a cohort of 192 patients with early-stage ER-positive breast cancer receiving adjuvant tamoxifen treatment and another one of a randomized phase II trial assessing exemestane with or without tamoxifen, high AR:ER ratio was found to predict resistance to endocrine therapy in a statistically significant manner. These findings need further confirmatory
Table 3. Correlation of AR mRNA with tumors' and patients' characteristics in all and in breast cancer subtypes.

<table>
<thead>
<tr>
<th>Subtype</th>
<th>Cor</th>
<th>Wilcox P</th>
<th>Kruskal–Wallis test</th>
<th>DDRT-PCR P</th>
<th>DDRT-PCR cor</th>
</tr>
</thead>
<tbody>
<tr>
<td>ALL</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Age cat</td>
<td>0.60</td>
<td>&lt;0.001</td>
<td>NA</td>
<td>NA</td>
<td>NA</td>
</tr>
<tr>
<td>Tumor size</td>
<td>0.33</td>
<td>&lt;0.001</td>
<td>NA</td>
<td>NA</td>
<td>NA</td>
</tr>
<tr>
<td>Grade</td>
<td>0.26</td>
<td>&lt;0.001</td>
<td>NA</td>
<td>NA</td>
<td>NA</td>
</tr>
<tr>
<td>ESR1</td>
<td>0.85</td>
<td>&lt;0.001</td>
<td>NA</td>
<td>NA</td>
<td>NA</td>
</tr>
<tr>
<td>ERBB2</td>
<td>0.85</td>
<td>&lt;0.001</td>
<td>NA</td>
<td>NA</td>
<td>NA</td>
</tr>
</tbody>
</table>

Abbreviations: Age cat, age category; tumor size cat, tumor size category; cor, correlation; ESR1, estrogen gene level; ERBB2, erb-b2 avian erythroblastic leukemia viral oncogene homolog 2; DDRT-PCR, digital differential display reverse transcription-PCR; ESR1, estrogen receptor alpha (ERα); ERBB2, erb-b2 avian erythroblastic leukemia viral oncogene homolog 2; Wnt, wingless/integrated; Ki67, Ki-67.}

According to the recently proposed "androgen excess" theory, increased androgen activity is one of the most important hormonal abnormalities seen in the patients with both ER$^+$ and ER$^-$ breast cancer (38). This theory suggests that androgens could stimulate ER$^+$ tumors by increased conversion into estrogens within the tumor tissue; concentration of estradiol within tumor tissues about 20 times greater than in the circulation has been reported, an observation that could be explained by the local hormone production as androgen precursors are highly present within the tumor. According to this theory, ER$^+$ tumors could also be stimulated via androgen activity, and depending on testosterone serum levels and AR positivity, either through EGFR or direct stimulation via androgen pathway (38).

Another notable finding of our clinical meta-analysis is the worse prognostication conferred by AR positivity for women with HER2$^+$ ER$^+$ (Her2-enriched) disease, albeit the analysis included three studies with 358 patients (7, 11, 44). Similar findings have been reported, with AR positivity associated with worse clinical outcome among HER2$^+$ (7) as well as patients with HER2$^+$ ER$^+$ (44) breast cancer. The biologic basis for the interaction between AR and HER2 in breast cancer cell lines was recently studied through an analysis of the androgen regulated gene expression in HER2$^+$ ER$^+$ breast cancer, indicating that AR signaling results in ligand-dependent Wnt- and HER2-signaling pathways activation through direct transcriptional induction of WNT17B and HER3, the latter being a dimerization partner to HER2. These findings suggest that anti-androgens merit clinical evaluation as anticancer agents in the setting of HER2$^+$ ER$^+$ breast cancer (59). There is an ongoing phase II, single-arm clinical trial assessing the combination of trastuzumab plus enzalutamide in the setting of HER2$^+$/AR-positive metastatic pretreated breast cancer (NCT02091960, https://clinicaltrials.gov/ct2/show/NCT02091960, accessed on July 15, 2016). However, contrasting to our clinical meta-analysis our gene expression analysis find significantly better OS for AR mRNA expression within all HER2-enriched breast cancers and within HER2-enriched subgroup treated only with hormone therapy. Caution is warranted when we compare data from IHC subtyping with data from gene expression profiling as these two approaches may not produce identical calls for the same tumors and published data suggested modest overlap between AR IHC and transcriptional profiles (2). It must be noted, however, that there is no subset of patients included in the present analysis, for whom information concerning the AR status at both the mRNA and protein level, as assessed through IHC, is available.

We also investigated the prognostic value of AR in the more aggressive TN phenotype, with the clinical meta-analysis indicating that it serves as positive prognosticator in the univariate analysis, confirming previously published results (6, 8, 39, 41, 44). This might be partly explained by the negative correlation of Ki67 as marker of decreased tumor cell proliferation and AR expression, which was seen in TNBC (60). However, other studies did not show any effect of AR on the TNBC outcome (11, 40, 53). In the gene-expression profiling analysis, AR mRNA levels were not prognostic in basal-like breast cancer. Recent findings identified a luminal androgen subtype within basal-like breast cancer confirming the extensive heterogeneity of basal-like breast cancer (60), and the role of AR within this breast cancer subtype requires further assessment.
The different prognostic relevance of AR positivity found among the different breast cancer subtypes indicates that the broader molecular profile constituting the tumor influences the functional output of AR signaling and its relevance for the clinical outcome of patients. Thus, studies conducted among homogeneous breast cancer populations in terms of subtypes are needed to better delineate the prognostic relevance of AR positivity in early-stage breast cancer. Ideally, analyses should be performed within the context of prospective randomized clinical trials, where the abovementioned conditions are fulfilled, along with homogeneous treatment approaches, along with high-level clinical annotation in terms of clinical outcomes. It is important to have a clear definition of what is considered as AR positivity in such studies, as mentioned before, different cutoffs were used in the studies we included in this meta-analysis.

We also investigated the potential impact of AR expression in pCR rates among all and different breast cancer subtypes. Only one study, using prospectively collected samples from the GeparTrio trial, in which AR expression was evaluated by IHC on a TMA of 673 core biopsies, evaluated the predictive value of AR for pCR after neoadjuvant chemotherapy in patients with primary breast cancer (44). This study found that AR+ tumors had lower rates of pCR in comparison with AR− tumors, 12.8% versus 25.4%, respectively P < 0.0001, but AR+ tumors had significantly better DFS and OS including those not reaching pCR (44). Moreover, AR, ER, and HER2 were independent predictors for pCR in a multivariate model (44). When pCR was analyzed across different IHC-assessed breast cancer subtypes, it was found for all subgroups that AR+ tumors had the lower rate of pCR, although the differences were not significant. Hormone receptor positivity is known to be one of the strongest negative predictive biomarkers for pCR after neoadjuvant chemotherapy (61). Indeed, we did confirm that AR positivity was associated with lower pCR rate, but only in univariate analysis.

Finally, we looked for correlations among AR mRNA and other genes and GS of interest; it must be noted that none of these genes or GS is validated and they are therefore not routinely assessed in clinical practice. As expected and in accordance with clinical

**Table 4.** Disease free survival (DFS) and overall survival (OS) analysis according to AR mRNA expression levels in all patients with breast cancer

<table>
<thead>
<tr>
<th>ALL patients (no. of patients)</th>
<th>Treatment</th>
<th>HR Uni (95% CI) DFS</th>
<th>P</th>
<th>HR Multi (95% CI) DFS</th>
<th>P</th>
</tr>
</thead>
<tbody>
<tr>
<td>3,190</td>
<td>treated_and_not_treated</td>
<td>0.82 (0.72–0.92)</td>
<td>0.0007</td>
<td>0.97 (0.84–1.13)</td>
<td>0.72</td>
</tr>
<tr>
<td>1,219</td>
<td>untreated</td>
<td>0.89 (0.72–1.09)</td>
<td>0.26</td>
<td>0.90 (0.69–1.19)</td>
<td>0.49</td>
</tr>
<tr>
<td>1,268</td>
<td>HT_only</td>
<td>0.91 (0.71–1.18)</td>
<td>0.12</td>
<td>0.99 (0.76–1.32)</td>
<td>0.99</td>
</tr>
<tr>
<td>463</td>
<td>CT_only</td>
<td>0.98 (0.76–1.28)</td>
<td>0.91</td>
<td>0.87 (0.61–1.23)</td>
<td>0.43</td>
</tr>
<tr>
<td>240</td>
<td>HT_and_CT</td>
<td>0.82 (0.52–1.29)</td>
<td>0.40</td>
<td>1.05 (0.65–1.77)</td>
<td>0.87</td>
</tr>
</tbody>
</table>

| 2,671                         | treated_and_not_treated | 0.84 (0.75–0.94)     | 0.002  | 0.98 (0.85–1.12)       | 0.72  |
| 914                           | untreated           | 0.87 (0.70–1.08)     | 0.22   | 0.86 (0.66–1.21)       | 0.26  |
| 1,143                         | HT_only             | 1.05 (0.86–1.29)     | 0.62   | 0.99 (0.79–1.23)       | 0.93  |
| 574                           | CT_only             | 1.02 (0.79–1.32)     | 0.85   | 0.92 (0.67–1.27)       | 0.63  |
| 240                           | HT_and_CT           | 0.89 (0.60–1.30)     | 0.62   | 1.01 (0.66–1.57)       | 0.93  |

Abbreviations: CT, chemotherapy; HT, hormonotherapy.
In conclusion, our study shows that AR expression at both protein and mRNA levels serves as positive prognosticator among more than 17,000 women with early-stage breast cancer. Additional information about distinct prognostic relevance of AR expression in different breast cancer subtypes was provided; however, further studies are warranted to confirm these findings. It is apparent that androgens and AR have many effects on the biology of breast cancer and deserve more clinical and translational research attention. Currently, AR is being actively investigated as therapeutic target in breast cancer.

Disclosure of Potential Conflicts of Interest
No potential conflicts of interest were disclosed.

Authors’ Contributions
Conception and design: I. Bozovic-Spasojevic, D. Zardavas, E. De Azambuja, M. Piccart, M. Paesmans, C. Sotiriou
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Acquisition of data (provided animals, acquired and managed patients, provided facilities, etc.): I. Bozovic-Spasojevic, D. Zardavas, L. Ameye, D. Fumagalli, M. Paesmans
Analysis and interpretation of data (e.g., statistical analysis, biostatistics, computational analysis): I. Bozovic-Spasojevic, D. Zardavas, S. Brohé, L. Ameye, D. Fumagalli, F. Ades, E. De Azambuja, M. Paesmans, C. Sotiriou
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Administrative, technical, or material support (i.e., reporting or organizing data, constructing databases): I. Bozovic-Spasojevic, D. Fumagalli, E. De Azambuja, M. Paesmans, C. Sotiriou
Study supervision: I. Bozovic-Spasojevic, E. De Azambuja, M. Paesmans, C. Sotiriou

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Received April 28, 2016; revised October 3, 2016; accepted October 27, 2016; published OnlineFirst October 27, 2016.

References


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Clin Cancer Res  Published OnlineFirst November 9, 2016.

Updated version
Access the most recent version of this article at:
doi:10.1158/1078-0432.CCR-16-0979

Supplementary Material
Access the most recent supplemental material at:
http://clincancerres.aacrjournals.org/content/suppl/2016/11/09/1078-0432.CCR-16-0979.DC1

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