



Consideration of breast cancer subtype in targeting the androgen receptor



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ABSTRACT

The androgen receptor (AR) is a drug target in breast cancer, and AR-targeted therapies have induced tumor responses in breast cancer patients. In this review, we summarized the role of AR in breast cancer based on preclinical and clinical data. Response to AR-targeted therapies in unselected breast cancer populations is relatively low. Pre-clinical and clinical data show that AR antagonists might have a role in estrogen receptor (ER)-negative/AR-positive tumors. The prognostic value of AR for patients remains uncertain due to the use of various antibodies and cut-off values for immunohistochemical assessment. To get more insight into the role of AR in breast cancer, we additionally performed a retrospective pooled analysis to determine the prognostic value of the AR using mRNA profiles of 7270 primary breast tumors. Our analysis shows that a higher AR mRNA level is associated with improved disease outcome in patients with ER-positive/human epidermal growth factor receptor 2 (HER2)-negative tumors, but with worse disease outcome in HER2-positive subgroups. In conclusion, next to AR expression, incorporation of additional tumor characteristics will potentially make AR targeting a more valuable therapeutic strategy in breast cancer.

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Abbreviations: AR, androgen receptor; CBR, clinical benefit rate; CI, confidence interval; DFS, disease-free survival; DHT, dihydrotestosterone; ER, estrogen receptor; HR, hazard ratio; HER2, human epidermal growth factor receptor 2; HER3, human epidermal growth factor receptor 3; HR, hormone receptor; IHC, immunohistochemistry; LAR, luminal androgen receptor; LHRH, luteinizing hormone-releasing hormone; NA, not available; OS, overall survival; PFS, progression-free survival; PR, progesterone receptor; TNBC, triple-negative breast cancer; Wnt, Wingless protein.

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1. Introduction

Breast cancer is the most common cancer in women (Stewart & Wild, 2014). Among invasive breast cancers, 75% express the estrogen receptor (ER) and 20–30% overexpress the human epidermal growth factor receptor 2 (HER2). These patients can benefit from therapy that targets ER or HER2, resulting in superior overall survival (OS) in both the curative and non-curative setting (Blamey et al., 2010; Gibson, Dawson, Lawrence, & Bliss, 2007; Swain et al., 2015). However, there is still a need to improve disease outcome, leading to a constant search for new drug targets. In recent studies, the androgen receptor (AR) has shown interesting potential as a drug target in breast cancer.

In prostate cancer, AR is a key driver of proliferation, and AR-targeted drugs are currently part of standard care (Parker, Gillessen, Heidenreich, & Horwich, 2015). Interestingly, AR is considered to be overexpressed in 70–90% of breast cancers, including up to 30% of the triple negative breast cancers (TNBC), and tumor response has been observed following AR-directed therapy (Collins et al., 2011). This makes AR a potentially interesting drug target for many breast cancer patients.

However, AR status is not routinely assessed in breast tumors. Currently, for immunohistochemical analysis, a broad range of cut-off values is used, and AR status is determined by various antibodies. This variance makes it difficult to interpret the role of AR based on expression data obtained with immunohistochemistry (IHC) in breast cancer. Therefore, pooled analyses using gene expression data to determine the association between AR status and disease-free survival (DFS) and OS in breast cancer patients is of interest. To address this, we first performed a literature review to summarize preclinical and clinical data concerning the role of AR in breast cancer, including its role in physiology, and its use in targeted therapy in prostate cancer. In addition, we explored the prognostic value of the AR in breast cancer subgroups using mRNA data of 7270 primary breast cancer samples obtained from the public domain.

2. Search strategy

PubMed was searched for articles published until August 2018 with the terms ‘androgen receptor’, ‘expression’, ‘cancer’, ‘molecular imaging’, and ‘tumor’ in various combinations. Only articles in English were reviewed. The abstracts were screened for relevance. We included *in vitro* studies with breast cancer cell lines and *in vivo* and clinical studies using androgens or AR-targeted drugs. Outside of PubMed, we searched abstracts of annual meetings of the American Society of Clinical Oncology and the San Antonio Breast Cancer Symposium in 2014–2018 with the same terms. Finally, ClinicalTrials.gov was searched for AR-targeted therapy trials in breast cancer patients.

3. Physiological function of AR

AR is expressed in hair follicles, bone, brain, liver, cardiovascular, and breast tissue in both sexes and in males also in testes and prostate tissue (Kimura, Mizokami, Oonuma, Sasano, & Nagura, 1993). AR belongs to the type I nuclear receptors. These receptors are intracellular transcription factors that directly regulate gene expression in response to their ligand. Androgens are ligands that bind to the AR, and are produced in ovaries of women, the prostate and testes of men, and by hair follicles and the zona reticularis of the adrenal glands of both sexes (Burger, 2002; Wilson, 2011; Wilson & French, 1976). After the lipophilic androgens diffuse through the cell membrane into the cytoplasm they bind to intracellular AR. This leads to dissociation of heat shock proteins followed by activation and dimerization of AR. The AR dimer then translocates to the nucleus. Binding of the AR dimer to the androgen response element in the promoter and enhancer regions of target genes leads to upregulation or downregulation of DNA transcription. Depending on tissue type this leads to cell division, differentiation, apoptosis, proliferation, or angiogenesis (Fig. 1).

Female AR knockout mice experience impaired follicular growth and dysfunctional ovulation, illustrating that AR is essential for normal female fertility (Walters, Simanainen, & Handelsman, 2010). In women, low serum androgen levels lead to reduced libido, reduced muscular strength, and vaginal dryness, whereas high levels result in hirsutism, a lower voice, and acne (Bachmann, 2002; van Staa & Sprafka, 2009). Germline AR mutations result in androgen insensitivity syndromes, which cause disorders in secondary sex characteristics such as clitoromegaly, absence of internal genital structures, or presence of testes in phenotypic women (Quigley et al., 1992).

In men, low serum androgen levels are associated with depression and can lead to low libido and erectile dysfunction, whereas high levels have been linked to aggressive behavior (Buvat, Maggi, Guay, & Torres, 2013; Pope Jr, Kouri, & Hudson, 2000). Cardiovascular disease and coagulation abnormalities have also been related to high doses of androgens used in men, but these effects have not been reported in women (Ferenchick, Hirokawa, Mammen, & Schwartz, 1995; Gooren, Wierckx, & Giltay, 2014).

4. Mechanism of AR-targeted therapy in prostate cancer

The AR signaling cascade can be inhibited for therapeutic use in several ways. Firstly, it can be inhibited indirectly by androgen deprivation therapy by lowering circulating androgen levels. This can be done with drugs such as luteinizing hormone-releasing hormone (LHRH)-agonists or CYP17A1 inhibitors like abiraterone acetate, or by orchidectomy (Table 1). In metastatic prostate cancer patients, the addition of abiraterone acetate to prednisone resulted in a median OS of 15.8 months for the combination versus 11.2 months for prednisone alone (Fizazi et al., 2012).

Secondly, the AR can be directly blocked by administering AR antagonists. The first-generation AR antagonists approved by the US Food and Drug Administration and European Medicines Agency are bicalutamide, flutamide, and nilutamide, which inhibit the effects of autocrine testosterone production by the tumor. Unlike these AR antagonists, the second-generation AR antagonist enzalutamide not only competitively binds to the AR ligand-binding domain, but also inhibits nuclear translocation of AR, DNA binding, and coactivator recruitment (Tran et al., 2009).

Thirdly, degradation of AR serves as a novel strategy for interfering the AR signaling. The AR degraders such as ARV-330 are currently in preclinical development (Teply & Antonarakis, 2016).

5. Mechanisms of actions of AR in breast cancer

5.1. Preclinical evidence

In vitro the androgens testosterone and dihydrotestosterone (DHT) mainly reduced proliferation, while AR antagonists stimulated proliferation of ER-positive/AR-positive breast cancer cell lines (Andò et al., 2002; Aspinall, Stamp, Davison, Shenton, & Lennard, 2004; Birrell et al., 1995; Chottanapund et al., 2013; Cops et al., 2008; Macedo et al., 2006; Ortmann et al., 2002; Poulin, Baker, & Labrie, 1988; Reese, Warsaw, Murai, & Siiteri, 1988; Rizza et al., 2014; Szelei, Jimenez, Soto, Luizzi, & Sonnenschein, 1997). However, increased proliferation has been observed at very high androgen concentrations (100 nM–1000 nM), especially in the extensively studied ER-positive/AR-positive MCF-7 cell line (Aspinall et al., 2004; Lin et al., 2009; Lippman, Bolan, & Huff, 1976; Maggiolini, Donzé, Jeannin, Andò, & Picard, 1999; Sonnehansen & Lykkesfeldt, 2005). These proliferative effects of androgen treatment observed at very high concentrations in ER-positive cell lines might be due to conversion of DHT to the estrogen agonist 5 α -androstane-3 β ,17 β -diol (Sikora et al., 2009). In addition, AR agonists and AR antagonists both reduced tumor growth in *in vivo* ER-positive/AR-positive breast cancer models (Bocuzzi et al., 1995; Cochrane et al., 2014; Dauvois, Geng, Lévesque, Mérand, & Labrie, 1991; Spinola,

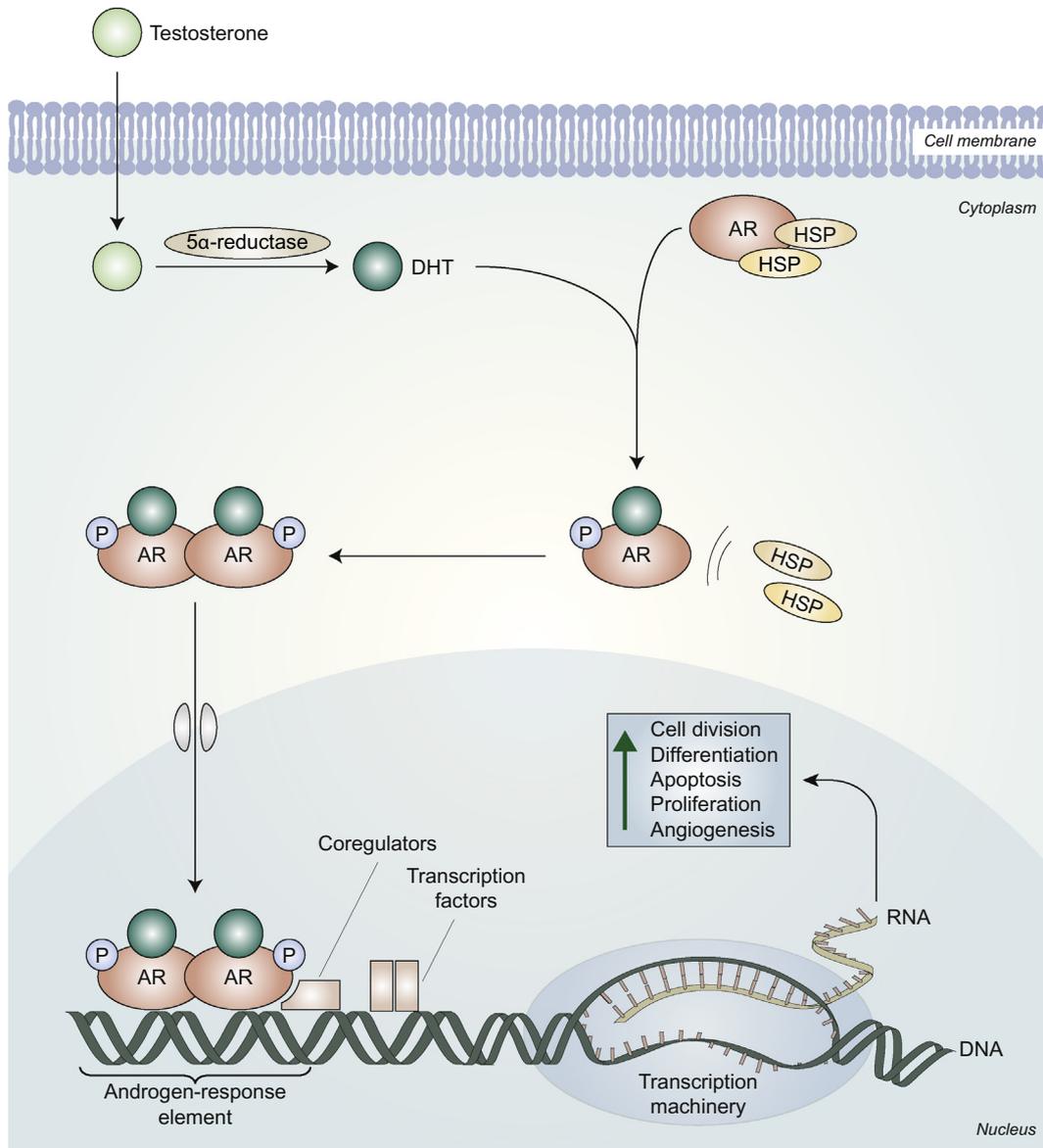


Fig. 1. Effect of androgens on the androgen receptor (AR) in a physiological setting in an androgen-responsive cell. After free testosterone passively diffuses through the plasma membrane, it is converted to dihydrotestosterone (DHT) by 5 α -reductase. In the cell, DHT binds to the AR, which leads to dissociation of heat shock proteins (HSPs), activation by phosphorylation (P), and dimerization of the AR. The AR dimer then translocates to the nucleus, where it binds to the androgen response element in the promoter regions of target genes. The AR dimer-androgen response element complex may act on the transcription machinery itself, or it recruits additional transcription factors or coregulators, ultimately leading to up- or downregulation of DNA transcription. Depending on the tissue this might lead to cell division, differentiation, apoptosis, proliferation or angiogenesis.

Table 1
AR-targeted therapies in use as standard care.

| Class | Subclass | Drugs | Indication | Mechanism of action |
|----------------------|----------------------------------|-----------------------------------------|----------------------------------------------------------------|----------------------------------------------------------------------------------------------------------------------------------|
| Androgen deprivation | LHRH analogues | Leuporelin Goserelin | Prostate cancer, endometriosis | Suppresses luteinizing hormone and follicle stimulating hormone, which stimulate androgen production in the testicles |
| | CYP17A1 inhibitors | Abiraterone acetate | Metastatic castration-resistant prostate cancer | Blocks conversion of precursors pregnenolone and 17 α -hydroxypregnenolone into dehydroepiandrosterone and androstenediol |
| AR blocking | First-generation AR antagonists | Bicalutamide Flutamide Nilutamide | Metastatic prostate cancer | Competes directly with (dihydro-)testosterone for AR binding site |
| | Second-generation AR antagonists | Enzalutamide | Metastatic prostate cancer | Blocks androgen binding to AR, inhibits nuclear translocation, DNA binding, and coactivator recruitment |
| High dose androgens | Androgens | Testosterone propionate | Testosterone deficiency, breast cancer in postmenopausal women | Binds directly to AR |
| Other | | Lixisenatide | Diabetes mellitus type 2 | Glucagon peptide agonist, little AR stimulation |

AR, androgen receptor; LHRH, luteinizing hormone-releasing hormone.

Marchetti, Mérand, Bélanger, & Labrie, 1988; Zava & McGuire, 1977). This phenomenon was also seen with ER-targeted therapy in breast cancer patients. Although anti-estrogen therapy is the cornerstone of endocrine therapy, high dose estrogens have also induced tumor regression (Lewis-Wambi & Jordan, 2009).

In comparison to ER-positive/AR-positive breast cancer cell lines, an opposite effect of androgens and AR antagonists is seen in *in vitro* ER-negative/AR-positive cell lines. In these cell lines, androgens mainly stimulated proliferation, while AR antagonists lowered proliferation (Birrell et al., 1995; Cochrane et al., 2014; Doane et al., 2006; Hall, Birrell, Tilley, & Sutherland, 1994; Lehmann et al., 2011; Naderi & Hughes-Davies, 2008; Ni et al., 2011; Robinson et al., 2011). Also, in *in vivo* ER-negative/AR-positive human breast cancer xenografts AR agonists stimulated tumor growth while AR-antagonists inhibited androgen-mediated growth of ER-negative/AR-positive breast tumors (Lehmann et al., 2011; Ni et al., 2011).

Increased proliferation and cell survival has been associated with the AR-mediated activation of the mitogen-activated protein kinase signaling pathway (Lange, Gioeli, Hammes, & Marker, 2007). Simultaneous stimulation of the epidermal growth factor receptor and AR hyperactivated the mitogen-activated protein kinase pathway. In ER-negative/AR-positive MDA-MB-231 cells this led to reduced proliferation, while stimulation of the epidermal growth factor receptor or AR separately increased proliferation (Garay et al., 2012).

Crosstalk between AR and ER, where signal transduction of the ER can affect the AR and vice versa, appears to increase proliferation. These receptors can co-localize in breast cancer cells, as shown with immunofluorescence and immunoprecipitation (Migliaccio et al., 2005; Peters et al., 2009). Interestingly, blocking the AR in tamoxifen-resistant, ER-positive/AR-positive MCF-7 cells did restore sensitivity to tamoxifen (De Amicis et al., 2010). In addition, an AR:ER ratio ≥ 2 has been linked to an increased risk for failure while on tamoxifen and a worse disease-specific survival in patients with ER-positive breast cancer (Cochrane et al., 2014; Rangel et al., 2018). This suggests that the AR:ER ratio may influence tumor response to ER-targeted therapy.

Crosstalk between AR and HER2 has also been indicated. Testosterone exposure of MDA-MB-453 cells increased *HER2* mRNA levels, and exposure to the human epidermal growth factor receptor 3 (HER3) ligand heregulin increased both *HER2* and *AR* mRNA levels. Moreover, inhibition of HER2 signaling reduced androgen-stimulated cell growth in ER-negative/HER2-positive/AR-positive cell lines (Naderi & Hughes-Davies, 2008; Ni et al., 2011).

Crosstalk between AR and the Wingless proteins (Wnt) signaling pathway has also been observed in ER-negative/AR-positive MDA-MB-453 cells (Ni et al., 2011). Stimulation of AR with DHT directly upregulated *WNT7B* mRNA levels, resulting in β -catenin activation. Nuclear translocation of activated β -catenin stimulates *HER3* transcription.

Table 2

Breast cancer trials with newer AR-targeted drugs or combinations of AR-targeted and standard targeted therapies.

| Treatment | Phase | Subgroup | Results | Adverse events | Reference |
|----------------------------------------------------------------------------------------|-------|------------------------------------------------------------|--------------------------------------------------------------------------------------------------------------------------|-------------------------------------------------------------------------------------------------------------------------------|------------------------------|
| Enzalutamide (AR antagonist) | II | Locally advanced or metastatic AR+/TNBC | AR IHC $\geq 1\%$: 25% CBR at 16 weeks AR IHC $\geq 10\%$: 33% CBR at 16 weeks | Grade 3 fatigue in 3.1% | (Traina et al., 2018) |
| Enobosarm (AR modulator) | II | Metastatic TNBC and ER+ breast cancer | 35% stable disease at 6 months (95% CI 16.6–59.4%) | Grade 3 adverse events in 4% | (Overmoyer et al., 2015) |
| CR1447 (AR modulator) | I | Metastatic AR+/ER+/HER2- breast cancer | Stable disease at 3 months in 2/14 patients | Only grade 1 and 2 | (Zweifel et al., 2017) |
| Orteronel (CYP17A1 inhibitor) | Ib | Metastatic ER+ breast cancer | Stable disease ≥ 6 months in 2/8 patients Serum estrogen and testosterone levels suppressed | Grade 3 hypertension in 2/8 patients | (Rampurwala et al., 2017) |
| Orteronel | II | Metastatic AR+/ER+ breast cancer | Stable disease in 3/29 patients | Grade 3/4 hypertension (7%) and increased lipase (10%) | (Yardley et al., 2016) |
| Seviteronel (CYP17A1 inhibitor) | I | Metastatic ER+ breast cancer and TNBC | 5/19 (26%) CBR at 16 weeks 2/19 (11%) CBR at 24 weeks | Grade 3 dehydration in 1/19 (5%) | (Bardia et al., 2018) |
| Seviteronel | II | Metastatic AR+/ER+ breast cancer and AR+/TNBC | ER+ breast cancer: 18% (2/11) CBR at 24 weeks TNBC: 33% (2/6) CBR at 16 weeks | Only grade 1 and 2 | (Gucalp et al., 2017) |
| Exemestane with or without enzalutamide | II | Metastatic HR+/HER2- breast cancer ^a | Median PFS 4.3 months (95% CI 1.0 – NA) in exemestane arm Median PFS 16.5 months (95% CI 1.9–10.9) in combination arm | Exemestane: 15% discontinuation rate Combination: 16% discontinuation rate | (Krop et al., 2018) |
| Enzalutamide with trastuzumab | II | Locally advanced or metastatic AR+/ER-/HER2+ breast cancer | 27.3% CBR at 24 weeks | Any grade: fatigue (22.7%), nausea (18.2%), diarrhea (13.6%), arthralgia (13.6%) Grade 3/4 adverse events in 5/22 patients | (Krop et al., 2017) |
| Enzalutamide with or without aromatase inhibitor | I/Ib | Metastatic breast cancer | 90% reduction in anastrozole exposure 50% reduction in exemestane exposure No change in fulvestrant exposure | Enzalutamide: grade 3/4 anemia (7%) Combination: grade 3/4 hypertension (7%), fatigue (6%), and neutropenia (4%) | (Schwartzberg et al., 2017) |
| Abiraterone acetate plus prednisone with or without exemestane versus exemestane alone | II | Metastatic ER+ breast cancer | No difference in PFS | Combination: grade 3/4 hypertension (5.8%) and hypertension (5.8%) | (O'Shaughnessy et al., 2016) |

^a Results are only shown for patients who tested positive for a biomarker for response to enzalutamide and had received no prior endocrine therapy. AR, androgen receptor; CBR, clinical benefit rate; CI, confidence interval; ER, estrogen receptor; HER2, human epidermal growth factor receptor 2; HR, hormone receptor; IHC, immunohistochemistry; NA, not available; PFS, progression-free survival; TNBC, triple-negative breast cancer.

HER3 then forms heterodimers with HER2 and activates the mTOR/PI3K/AKT pathway, resulting in cell proliferation (Ni et al., 2011).

In quadruple-negative breast cancer cell lines, comprising TNBC cell lines without AR expression, androgens mostly did not affect proliferation, independent of the concentration (Aspinall et al., 2004; Barton et al., 2015; Birrell et al., 1995; Lippman et al., 1976; Wang et al., 2011).

In conclusion, the effect of AR-targeted therapies differs according to the ER status of breast cancer cells. Whereas androgens mainly inhibit

tumor growth in ER-positive breast cancer cell lines, they stimulate tumor growth in ER-negative cell lines, and anti-androgens were most effective in ER-negative/AR-positive cells. The effects of AR-targeted drugs per breast cancer cell line are described in Supplementary Table 1 (Andò et al., 2002; Aspinall et al., 2004; Barton et al., 2015; Birrell et al., 1995; Chottanapund et al., 2013; Cochrane et al., 2014; Cops et al., 2008; De Amicis et al., 2010; Doane et al., 2006; Garay et al., 2012; Hackenberg et al., 1991; Hall et al., 1994; Lehmann et al.,

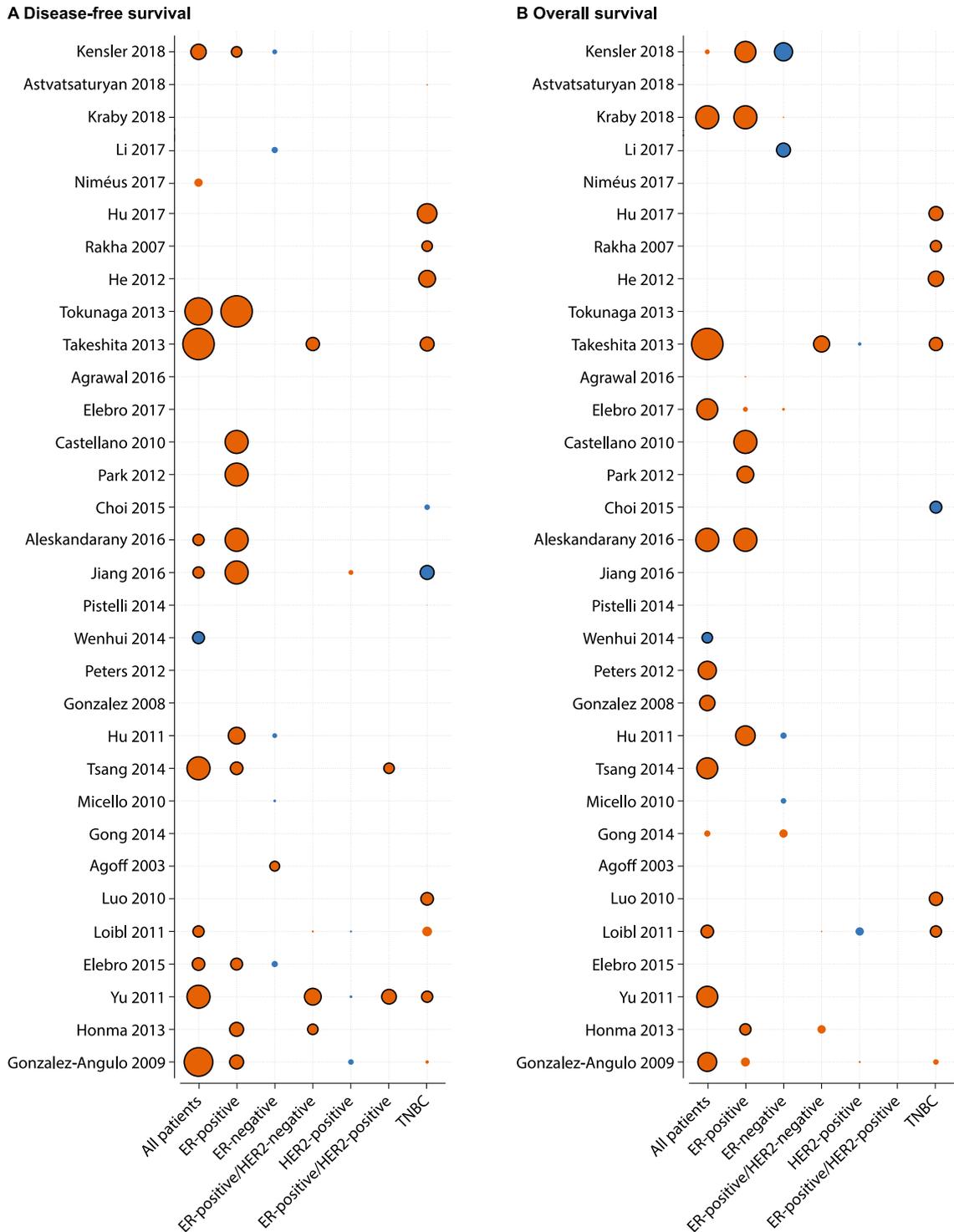


Fig. 2. Overview of studies on the prognostic value of AR expression measured immunohistochemically per breast cancer subgroup. Associations have been studied using log-rank test or univariate Cox regression analysis. An orange bubble indicates an association between androgen receptor (AR) positivity and prolonged disease-free survival (panel A) or overall survival (panel B). A blue bubble indicates an association between AR positivity and shorter survival. The size of the bubble indicates the statistical significance level. Black delineation indicates a P value $\leq .05$. ER, estrogen receptor; HER2, human epidermal growth factor receptor 2; TNBC, triple-negative breast cancer.

2011; Lin et al., 2009; Lippman et al., 1976; Macedo et al., 2006; Maggiolini et al., 1999; Naderi & Hughes-Davies, 2008; Narayanan et al., 2014; Ni et al., 2011; Ortmann et al., 2002; Poulin et al., 1988; Reese et al., 1988; Rizza et al., 2014; Robinson et al., 2011; Sikora et al., 2009; Sonne-Hansen & Lykkesfeldt, 2005; Szelei et al., 1997; Wang et al., 2011).

5.2. Clinical evidence

The ovaries are a main source of androgens. Theoretically, this means that LHRH analogues as well as oophorectomy, which are both used in breast cancer patients with ER-positive tumors, likely result in a reduction of androgen levels. In 13 premenopausal patients with ER-positive breast cancer, androgen serum levels were lower following treatment with the LHRH-analogue goserelin and an aromatase inhibitor (Forward, Cheung, Jackson, & Robertson, 2004). Aromatase inhibitors, also part of standard care for breast cancer patients with ER-positive tumors, inhibit the conversion of androgens into estrogens. To date few data are available with regards to the use of aromatase

inhibitors combined with androgen deprivation therapy in AR-positive breast cancer patients. In a phase II study in 30 women with AR-positive/triple negative metastatic breast cancer, androgen deprivation by abiraterone acetate 1000 mg once daily combined with prednisolone 5 mg twice daily resulted in one complete response and five patients with stable disease (Bonneto et al., 2016).

Until recently, studies exploring the effect of AR-targeted therapy included breast cancer patients regardless of tumor AR expression levels. Non-tissue-selective androgens, such as testosterone propionate and fluoxymesterone, have been used for treatment of metastatic breast cancer since the 1940s (Fels, 1944). High doses of androgens such as fluoxymesterone and testosterone administered to metastatic breast cancer patients showed 19% and 36% tumor response rates, respectively, without selection for AR expression. The treatment coincided with masculinizing side effects such as acne, hirsutism, and lowering of the voice in 15–20% of patients (Adair & Herrmann, 1946; Goldenberg, Waters, Ravdin, Ansfeld, & Segaloff, 1973; Ingle et al., 2006, 1991; Kellokumpu-Lehtinen, Huovinen, & Johansson, 1987). Testosterone propionate administration to patients with ER-positive metastatic breast

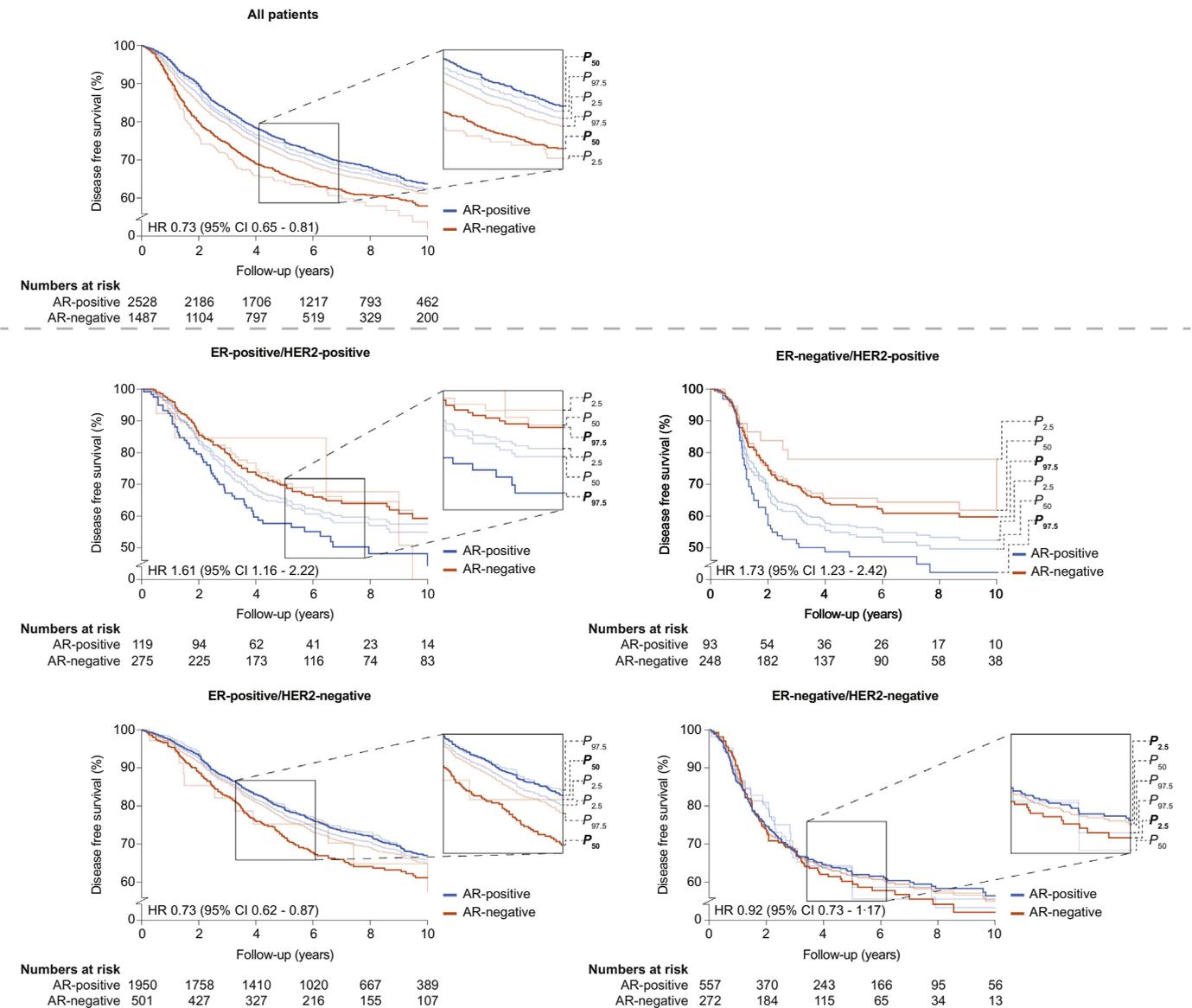


Fig. 3. Disease-free survival curves for different thresholds for AR positivity in breast cancer subgroups. Non-transparent curves show the threshold discriminating best between AR-positive and AR-negative cases in terms of disease-free survival, defined as time of diagnosis to locoregional or distant recurrence, or death. Hazard ratios and corresponding 95% confidence intervals are shown for non-transparent curves. AR, androgen receptor; CI, confidence interval; ER, estrogen receptor; HER2, human epidermal growth factor receptor 2; HR, hazard ratio.

cancer, refractory to ER-targeted therapy, resulted in a complete or partial tumor response in nine out of 53 patients and a median OS of 12 months (Boni et al., 2014). A retrospective analysis evaluated the response to fluoxymesterone in 103 patients with metastatic, ER-positive breast cancer and showed that 33 patients discontinued treatment due to side effects. A clinical benefit, defined as objective tumor response or stable disease ≥ 6 months was seen in 43% of remaining patients (Kono et al., 2016).

Direct blocking of AR in breast cancer patients was first described in 1988. Flutamide, 750 mg orally daily administered, resulted in one partial tumor response and five stable diseases out of 29 patients, but was accompanied by gastrointestinal side effects (Perrault et al., 1988). In postmenopausal women, two out of 14 patients experienced disease stabilization for 20–26 weeks when treated with the AR antagonist nilutamide 100 mg orally per day (Millward, Cantwell, Dowsett, Carmichael, & Harris, 1991). Due to the side effects and modest results observed in clinical trials, the interest for AR-targeted therapy in breast cancer diminished. However, with novel AR-targeted drugs emerging in the prostate cancer setting and the awareness of the high frequency of

AR expression in breast cancer, AR-targeted therapy in breast cancer has regained attention in recent years.

The first study to select patients based on AR expression evaluated the efficacy of the AR blocker bicalutamide 150 mg per day orally in 26 postmenopausal women with ER-negative (IHC positivity $\leq 10\%$ tumor cells), progesterone receptor (PR)-negative, AR-positive (IHC $\geq 10\%$) metastatic breast cancer. A clinical benefit rate was seen in 19% of patients, while the drug was well tolerated (Gucalp et al., 2013). However, most patients in this study were heavily pre-treated, which may explain the low overall response rate. One case study reported a complete response to bicalutamide in a woman with AR-positive metastatic breast cancer (Arce-Salinas, Riesco-Martinez, Hanna, Bedard, & Warner, 2016).

More recently, studies have been performed with newer AR-targeted drugs such as second-generation AR antagonists, AR modulators and novel non-steroidal CYP17A1 inhibitors (Table 2) (Bardia et al., 2018; Gucalp et al., 2017; Krop et al., 2018, 2017; O’Shaughnessy et al., 2016; Overmoyer et al., 2015; Rampurwala et al., 2017; Schwartzberg et al., 2017; Traina et al., 2018; Yardley et al., 2016;

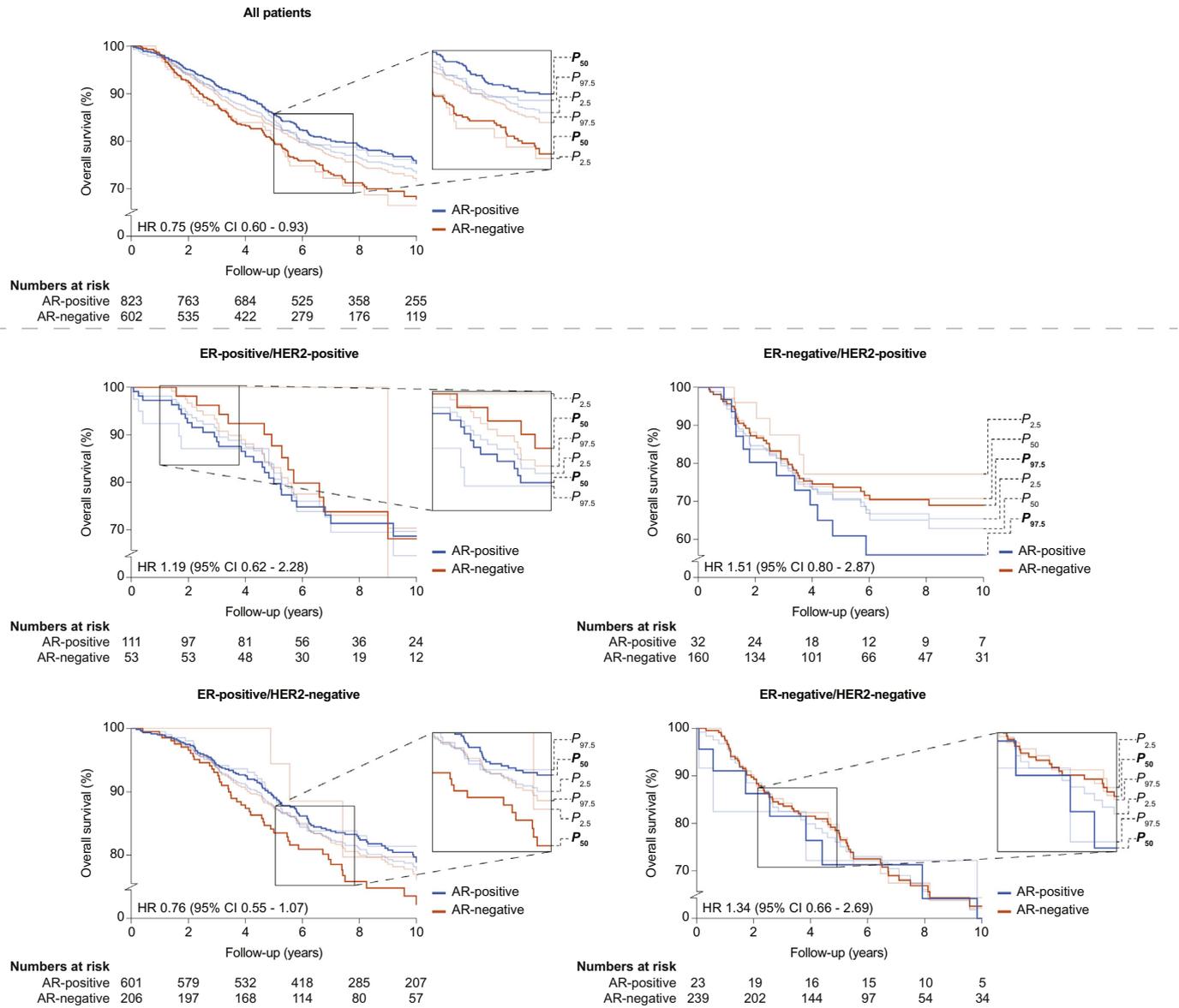


Fig. 4. Overall survival curves for different thresholds for AR positivity in breast cancer subgroups. Non-transparent curves show the threshold discriminating best between AR-positive and AR-negative cases in terms of overall survival, defined as time of diagnosis to death by any cause. Hazard ratios and corresponding 95% confidence intervals are shown for non-transparent curves. AR, androgen receptor; CI, confidence interval; ER, estrogen receptor; HER2, human epidermal growth factor receptor 2; HR, hazard ratio.

Zweifel et al., 2017). A phase II study assessed the efficacy of the second-generation AR antagonist enzalutamide 160 mg per day in 118 patients with locally advanced or metastatic, AR-positive (IHC > 0%), TNBC (ER/PR IHC < 1%). Clinical benefit rates were 25% at 16 weeks and 24% at 24 weeks. In patients whose tumors expressed $\geq 10\%$ nuclear AR ($n = 78$), determined using antibodies optimized for measuring AR expression in breast cancer tissue (Kumar et al., 2017), clinical benefit rates were 33% at 16 weeks and 22% at 24 weeks. Enzalutamide was well tolerated, with fatigue being the only grade 3 side effect occurring in >2% of patients (3.1%) (Traina et al., 2018). Results of the selective AR modulator enobosarm are also of interest: stable disease for >6 months has been reported in up to 35% of heavily pre-treated patients (Overmoyer et al., 2015). Furthermore, combinations of AR-targeted therapy with hormonal or anti-HER2 therapy are currently being investigated. A phase II study evaluated the effect of exemestane with enzalutamide in 247 patients with hormone receptor-positive/HER2-negative, metastatic breast cancer. In the patients that had received no prior endocrine therapy for metastatic breast cancer who tested positive for a gene expression-based biomarker for response to enzalutamide ($n = 50$), exemestane/enzalutamide significantly improved median progression-free survival from 4.3 months (95% confidence interval [CI] 11.0 – NA) to 16.5 months (95% CI 1.9–10.9) compared to exemestane/placebo (Krop et al., 2018). Ongoing trials with AR-targeted therapy in breast cancer are listed in Supplementary Table 2.

6. AR expression measured immunohistochemically in breast cancers

Breast cancer patients with various tumor characteristics have experienced clinical benefit from AR-targeted therapies. However, selecting patients for such therapies has been challenging. Clear guidelines on IHC interpretation of the AR have not been established thus far. Most studies use IHC to determine AR expression and base their cut-off value on 10% tumor cells staining positive. Data concerning the response to AR-targeted therapies in patients with tumors expressing low levels of AR, in the range of 1% to 10% positive cells by IHC, are less frequently described. In the current setting of ER, even patients with low ER expression (1–10%) are eligible for therapy, and guidelines now use the 1% cut-off value (National Comprehensive Cancer Network, 2018).

For AR measurements, different antibodies with varying sensitivity and specificity have been used. Most experience in clinical breast cancer

trials has been obtained with the AR441 mouse monoclonal IgG antibody from DAKO.

Studies on the role of AR in breast cancer have shown that AR positivity in the primary tumor is associated with better OS and DFS (Fig. 2 and Supplementary Table 3) (Agoff, Swanson, Linden, Hawes, & Lawton, 2003; Agrawal et al., 2016; Aleskandarany et al., 2016; Astvatsaturyan, Yue, Walts, & Bose, 2018; Castellano et al., 2010; Choi, Kang, Lee, & Bae, 2015; Elebro, Bendahl, Jernström, & Borgquist, 2017; Elebro et al., 2015; Gong, Wei, Wu, Ueno, & Huo, 2014; Gonzalez-Angulo et al., 2009; Gonzalez et al., 2008; He et al., 2012; Honma et al., 2013; R. Hu et al., 2011; Hu, Chen, Ma, & Jiang, 2017; Jiang et al., 2016; Kensler et al., 2018; Kraby et al., 2018; Li et al., 2017; Loibl et al., 2011; Luo, Shi, Li, & Jiang, 2010; Micello et al., 2010; Niméus, Folkesson, Nodin, Hartman, & Klintman, 2017; Park et al., 2012; Peters et al., 2012; Pistelli et al., 2014; Rakha et al., 2007; Takeshita, Omoto, Yamamoto-Ibusuki, Yamamoto, & Iwase, 2013; Tokunaga et al., 2013; Tsang et al., 2014; Wenhui et al., 2014; Yu et al., 2011). This effect is most profound in patients with ER-positive tumors. In patients with ER-negative breast cancer, the relation between AR expression and disease outcome is less clear, with the exception of TNBC where AR positivity has mainly been associated with improved survival. In patients with HER2-positive tumors, no significant effect of AR expression on DFS or OS has been observed, probably due to limited patient numbers.

Recently, a large study including 4417 women from the Nurses' Health Study cohorts showed that AR-positivity (IHC > 1%) is associated with improved breast cancer-specific survival in patients with ER-positive breast cancer independent of clinicopathological characteristics 7 years after diagnosis (hazard ratio [HR] 0.53, 95% CI 0.41–0.69) (Kensler et al., 2018). In contrast, AR positivity was associated with worse breast cancer-specific survival in patients with ER-negative breast cancer (HR 1.62, 95% CI 1.18–2.22). For patients with HER2-positive breast cancer, AR positivity was not associated with breast cancer-specific survival.

7. Retrospective pooled analysis of AR mRNA expression in breast cancer

Given the limited available data on IHC, retrospective pooled analyses using mRNA expression data is very interesting. Recently a meta-analysis on gene expression data demonstrated that a higher AR mRNA level is associated with favorable clinical outcome in women with early-stage breast cancer (Bozovic-Spasojevic et al., 2017). This analysis was based on intrinsic molecular subtypes, but in current

Table 3
Associations between AR mRNA expression and survival per breast cancer subgroups based on tumor receptor status.

| Subgroup | Univariate | | | | | Multivariate ^a | | | | |
|---------------------------|------------|------------|------|-----------|--------|---------------------------|------------|------|-----------|-------|
| | Total (n) | Events (n) | HR | 95% CI | P | Total (n) | Events (n) | HR | 95% CI | P |
| Disease-free survival | | | | | | | | | | |
| Overall | 4640 | 1335 | 0.87 | 0.82–0.91 | <0.001 | 927 | 314 | 0.96 | 0.85–1.08 | 0.49 |
| ER-positive | 2864 | 874 | 0.88 | 0.82–0.95 | <0.001 | 711 | 236 | 0.88 | 0.76–1.02 | 0.08 |
| HER2-positive | 743 | 303 | 1.12 | 1.00–1.25 | 0.049 | 172 | 72 | 1.30 | 0.98–1.73 | 0.06 |
| ER-positive/HER2-positive | 398 | 155 | 1.13 | 0.94–1.35 | 0.20 | 88 | 37 | 1.03 | 0.66–1.61 | 0.90 |
| ER-positive/HER2-negative | 2466 | 719 | 0.85 | 0.78–0.92 | <0.001 | 623 | 199 | 0.89 | 0.76–1.05 | 0.17 |
| ER-negative/HER2-positive | 345 | 148 | 1.10 | 0.96–1.26 | 0.17 | 84 | 35 | 1.46 | 1.03–2.06 | 0.03 |
| ER-negative/HER2-negative | 837 | 313 | 0.74 | 0.83–1.06 | 0.31 | 132 | 43 | 1.02 | 0.73–1.42 | 0.92 |
| Overall survival | | | | | | | | | | |
| Overall | 1427 | 336 | 0.87 | 0.78–0.96 | 0.008 | 632 | 153 | 1.02 | 0.79–1.23 | 0.80 |
| ER-positive | 972 | 208 | 0.84 | 0.71–0.98 | 0.026 | 472 | 107 | 0.90 | 0.72–1.13 | 0.37 |
| HER2-positive | 357 | 98 | 1.03 | 0.84–1.26 | 0.77 | 119 | 39 | 1.34 | 0.95–1.89 | 0.10 |
| ER-positive/HER2-positive | 165 | 43 | 0.90 | 0.63–1.27 | 0.55 | 55 | 20 | 0.81 | 0.46–1.60 | 0.46 |
| ER-positive/HER2-negative | 807 | 165 | 0.83 | 0.69–0.99 | 0.035 | 417 | 330 | 0.94 | 0.72–1.13 | 0.66 |
| ER-negative/HER2-positive | 192 | 55 | 1.08 | 0.85–1.36 | 0.53 | 64 | 19 | 1.72 | 1.08–2.73 | 0.021 |
| ER-negative/HER2-negative | 263 | 73 | 1.12 | 0.88–1.41 | 0.35 | 96 | 27 | 1.12 | 0.73–1.72 | 0.59 |

Associations were determined using Cox regression analysis. Disease-free survival was defined at time to locoregional or distant recurrence, or death. Overall survival was defined as time to death by any cause. CI, confidence interval; ER, estrogen receptor; HER2, human epidermal growth factor receptor 2; HR, hazard ratio.

^a Adjusted for age, tumor size, grade, lymph node status, ER status, HER2 status and treatment regimen.

Table 4

Associations between AR mRNA expression and survival in breast cancer intrinsic molecular subtypes.

| Subgroup | Univariate | | | | | Multivariate ^a | | | | |
|-----------------------|------------|------------|------|-----------|-------|---------------------------|------------|------|------------|-------|
| | Total (n) | Events (n) | HR | 95% CI | P | Total (n) | Events (n) | HR | 95% CI | P |
| Disease-free survival | | | | | | | | | | |
| Luminal A | 2009 | 548 | 0.97 | 0.87–1.07 | 0.51 | 462 | 126 | 0.98 | 0.79–1.22 | 0.86 |
| Luminal B | 922 | 367 | 0.87 | 0.78–0.98 | 0.018 | 219 | 99 | 0.76 | 0.61–0.94 | 0.014 |
| Normal | 355 | 118 | 0.85 | 0.66–1.09 | 0.20 | 46 | 18 | 1.93 | 0.81–4.59 | 0.14 |
| HER2-enriched | 253 | 111 | 1.04 | 0.88–1.22 | 0.68 | 87 | 38 | 1.31 | 0.89–1.94 | 0.17 |
| Basal | 507 | 191 | 1.05 | 0.81–1.36 | 0.72 | 113 | 33 | 1.04 | 0.57–1.92 | 0.89 |
| Overall survival | | | | | | | | | | |
| Luminal A | 711 | 109 | 0.87 | 0.68–1.10 | 0.24 | 325 | 52 | 1.16 | 0.82–1.64 | 0.41 |
| Luminal B | 327 | 106 | 0.87 | 0.70–1.08 | 0.22 | 125 | 51 | 0.64 | 0.45–0.91 | 0.014 |
| Normal | 100 | 32 | 0.79 | 0.50–1.27 | 0.34 | 25 | 9 | 2.75 | 0.44–17.28 | 0.28 |
| HER2-enriched | 130 | 50 | 1.17 | 0.91–1.49 | 0.22 | 70 | 23 | 1.76 | 1.10–2.82 | 0.019 |
| Basal | 159 | 39 | 1.03 | 0.54–1.97 | 0.94 | 82 | 18 | 0.98 | 0.37–2.65 | 0.98 |

Associations were determined using Cox regression analysis. Disease-free survival was defined as time to locoregional or distant recurrence, or death. Overall survival was defined as time to death by any cause. CI, confidence interval; HER2, human epidermal growth factor receptor 2; HR, hazard ratio.

^a Adjusted for age, tumor size, grade, lymph node status, estrogen receptor status, HER2 status and treatment regimen.

practice immunohistochemically determined receptor statuses are used. Therefore, we used publicly available mRNA profiles to assess associations of predicted AR status and AR mRNA levels with disease outcome in receptor status-based breast cancer subgroups and intrinsic molecular subtypes (Parker et al., 2009).

We analyzed 7270 mRNA expression profiles of primary tumor samples of non-metastatic breast cancer patients, and we assembled a reference group of 172 normal breast tissue samples obtained during reduction mammoplasty. Whenever information on receptor status was missing, we determined these by inference using gene expression data. Detailed analysis methods information has previously been published (Bense et al., 2017). Overall, *ESR1* mRNA and *ERBB2* functional genomic mRNA expression (Fehrmann et al., 2015) clearly discriminated between immunohistochemically determined positive and negative receptor statuses (Supplementary Fig. 1). AR status in the tumor samples was considered positive when the AR mRNA level was above a certain threshold. We explored multiple thresholds by calculating the 2.5th, 25th, 50th, 75th, and 97.5th percentiles of AR mRNA level in normal breast tissue. Differences in survival between predicted AR-positive and AR-negative tumors were determined with Kaplan-Meier curves and log-

rank test. In addition, the association between AR mRNA levels and DFS and OS in the tumors samples was determined with Cox regression.

For the group as a whole, DFS and OS were prolonged in patients with AR-positive tumors in comparison to those with AR-negative tumors (Figs. 3 and 4). The difference in survival was more pronounced when lowering the thresholds defining AR positivity (Supplementary Figs. 2 and 3). Cox regression also showed that a higher AR mRNA level was associated with prolonged DFS and OS in the whole group. However, this association did not remain significant when corrected for relevant clinicopathological parameters (Table 3).

In patients with ER-positive/HER2-negative tumors, AR positivity was also associated with a prolonged DFS, depending on the threshold used (Figs. 3 and 4; Supplementary Figs. 2 and 3). We observed a similar, but less pronounced, trend for prolonged OS with AR positivity. A higher AR mRNA level was associated with prolonged DFS and OS in univariate analyses. This association also did not remain significant when corrected for relevant clinicopathological parameters (Table 3).

For patients with ER-negative/HER2-positive and ER-positive/HER2-positive tumors, AR positivity was associated with a shorter DFS (Figs. 3 and 4). The difference in survival is more pronounced when a higher

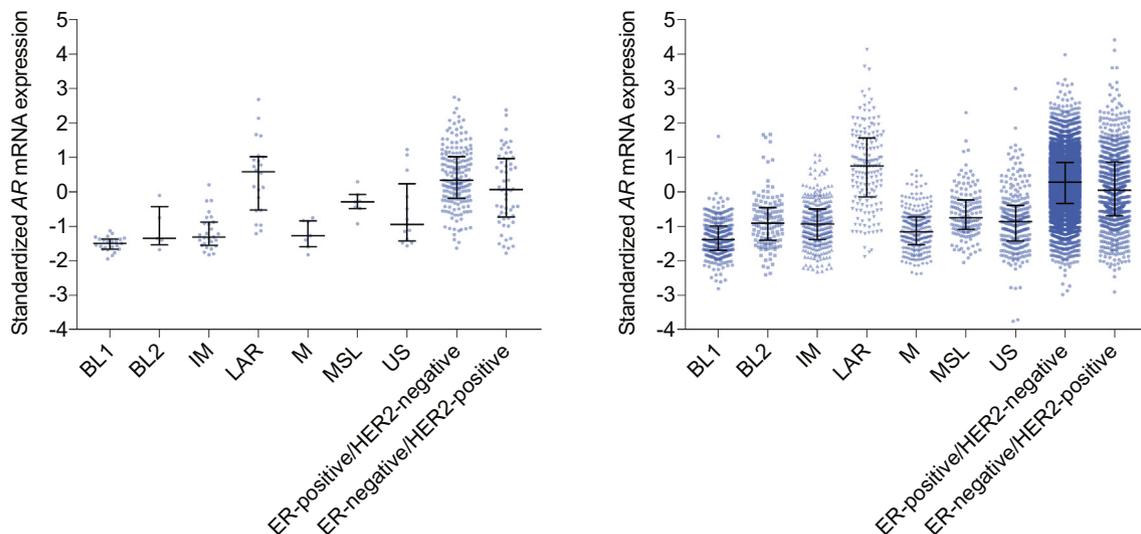


Fig. 5. Scatter dot plot of standardized AR mRNA expression in breast cancer subgroups. AR mRNA expression is shown for triple-negative breast cancer subtypes basal-like 1 (BL1), basal-like 2 (BL2), immunomodulatory (IM), luminal androgen receptor (LAR), mesenchymal (M), mesenchymal stem-like (MSL), and unstable (US), and for ER-positive/HER2-negative and ER-negative/HER2-positive breast cancer subgroups. Whenever information on ER and HER2 status was missing, we determined these by inference using gene expression data. Error bars indicate median mRNA expression and interquartile range. AR, androgen receptor; ER, estrogen receptor; HER2, human epidermal growth factor receptor 2.

threshold is used for defining AR positivity (Supplementary Figs. 2 and 3). In line with this observation, Cox regression showed that a higher AR mRNA level was associated with shorter DFS and OS in patients with ER-negative/HER2-positive breast cancer when corrected for relevant clinicopathological parameters (Table 3).

For the intrinsic molecular subtypes, a higher AR mRNA level was associated with prolonged DFS and OS in the luminal B subtype, independent of other relevant clinicopathological parameters (Table 4). In the HER2-enriched molecular subtype, a higher AR mRNA level was associated with shorter OS independent of clinicopathological parameters.

The results above suggest that the effect of AR status and AR mRNA levels on DFS and OS varies between receptor status-based subgroups as well as between intrinsic molecular subtypes.

We also explored mRNA expression of AR in breast cancer subgroups. Whereas AR expression was comparable in ER-positive/HER2-negative and ER-negative/HER2-positive tumors, it was evidently lower in ER-negative/HER2-negative tumors (Fig. 5). However, in the luminal AR (LAR) TNBC subtype (Lehmann et al., 2011), AR mRNA levels were similar to those found in ER-positive or HER2-positive tumors. *ESR1* and *ERBB2* expression levels in the LAR subtype were similar to other TNBC subtypes (Supplementary Fig. 4). Furthermore, in the ER-negative/HER2-positive subgroup AR mRNA levels positively correlated with *HER2* (R 0.47, 95% CI 0.41–0.52) and *HER3* mRNA levels (R 0.43, 95% CI 0.37–0.48). AR mRNA expression levels did not correlate with *Wnt* or the more downstream *c-Myc* and *β-catenin*.

8. Discussion and future perspectives

This review summarizes information on preclinical and clinical data concerning the role of AR in breast cancer, as well as data on immunohistochemical and mRNA measurement of AR.

For further implementation of AR-directed therapy in breast cancer insight in patient selection criteria seems to be critical. The associations of AR mRNA levels with DFS and OS in the different ER and HER2 status-based subgroups are in agreement with the associations reported in current literature based on IHC data. However, the association between predicted AR positivity as well as a higher AR mRNA level and shorter survival we observed in the ER-negative/HER2-positive subgroup and the HER2-enriched intrinsic molecular subtype is in contrast with another recent mRNA-based analysis (Bozovic-Spasojevic et al., 2017). That analysis showed that a higher AR mRNA level is associated with prolonged survival in the HER2-enriched molecular subtype. The discrepancy indicates that the pooled analyses should be interpreted with some caution as they are based on retrospective, publicly available data that can contain potential confounders. However, based on our analysis, targeting both HER2 and AR might be of interest for patients with ER-negative/HER2-positive/AR-positive tumors. This is supported by a currently ongoing trial in breast cancer patients with HER2-positive/AR-positive tumors assessing the effect of trastuzumab plus enzalutamide (NCT02091960). Preliminary results have shown a 24-week clinical benefit rate of 27.3% in patients who received a median of four prior anti-HER2 therapies (Krop et al., 2017).

We used our retrospective pooled analysis as a hypothesis-generating tool to facilitate insight into the role of AR in the context of different breast tumor characteristics. Here, we aimed at detecting as many potentially relevant observations with reasonable power, which would considerably reduce if we had split our data for validation purposes. As we pursued this hypothesis-generating approach, the results of our pooled analysis require validation in larger and preferably prospective patient cohorts.

The limited amount of data on AR expression in breast cancer suggests that a discrepancy in AR status between primary and distant metastatic breast cancer lesions can exist in up to 33% of patients (D'Amato et al., 2016). Obtaining a biopsy during the course of disease is currently considered the gold standard, but is not always feasible. Furthermore, a

single biopsy from a metastatic lesion is not necessarily representative for the patient's complete AR status.

A different approach to obtain potentially whole body information about tumor hormonal receptor status is via circulating tumor cells or circulating tumor DNA (Bidard et al., 2014; Kasimir-Bauer et al., 2016). Also, whole body *in vivo* expression of AR with intact ligand binding domain is possible by using molecular imaging of the AR with ¹⁸F-fluorodihydrotestosterone positron emission tomography (PET). This tracer showed selective uptake in prostate cancer metastases and could be blocked by flutamide and enzalutamide (Dehdashti et al., 2005; Scher et al., 2012). In metastatic breast cancer patients, ¹⁸F-fluorodihydrotestosterone tumor uptake showed good correlation with IHC staining for AR in representative tumor biopsies ($P = .01$) of 13 patients (Venema et al., 2017).

Although the results of AR-targeted therapies in metastatic breast cancer patients are interesting, all patients eventually showed progression while on treatment. Mechanisms that may be related to resistance to AR-targeted therapies in metastatic prostate cancer include amplification or overexpression of AR, ligand-independent activation, overexpression of coactivators, and the expression of active AR splice variants (Chen et al., 2004; Fujimoto, Mizokami, Harada, & Matsumoto, 2001; Scher et al., 2010; Stanbrough et al., 2006; Teply & Antonarakis, 2016). The most frequently studied AR splice variant in tumors and circulating tumor DNAs in the context of prostate cancer is AR-V7, in which AR is activated without ligand binding; this variant is predictive of resistance to both enzalutamide and abiraterone (Antonarakis et al., 2014). Analysis of different splice variants showed AR-V7 mutations in 53.7% of primary breast cancer samples ($n = 54$) (Hickey et al., 2015). The role of these potential mechanisms for resistance to AR-targeted therapies in breast cancer requires further study.

In summary, increased understanding of the role of AR in breast cancer, and optimal selection for AR-targeted therapies, can potentially improve treatment options for breast cancer patients. With novel (selective) AR antagonists becoming available along with new patient selection methods, AR-targeted therapies deserve further evaluation in clinical breast cancer studies. The response rates to AR-targeted therapies in unselected patient populations are relatively low. Preclinical and clinical data show that AR antagonists could be a potential therapy for patients with ER-negative/AR-positive tumors. In addition, based on our retrospective pooled analysis, patients with HER2-positive/AR-positive tumors might be a preferred subgroup to treat with combined HER2-targeted and AR-targeted treatment. These data indicate that patient selection, using additional tumor characteristics, might increase the role of AR-targeted therapy in patients with breast cancer.

Conflicts of interest statement

EGE de Vries reports consulting/advisory board fees from Synthon, Pfizer and Sanofi, and grants from Novartis, Amgen, Roche/Genentech, Regeneron, Chugai, Synthon, AstraZeneca, Radius Health, CytomX Therapeutics and Nordic Nanovector, all to the hospital and unrelated to the submitted work. TG Steenbruggen reports financial support from Memidis Pharma unrelated to the submitted work. M Brown serves as a scientific advisor to GTx, Inc. and Kronos Bio, and receives sponsored research support from Novartis. The other authors declare no competing interests.

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Appendix A. Supplementary data

Supplementary data to this article can be found online at <https://doi.org/10.1016/j.pharmthera.2019.05.005>.

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