Consideration of breast cancer subtype in targeting the androgen receptor

Venema, Clasina M; Bense, Rico D; Steenbruggen, Tessa G; Nienhuis, Hilde H; Qiu, Si-Qi; van Kruchten, Michel; Brown, Myles; Tamimi, Rulla M; Hospers, Geke A P; Schröder, Carolina P

Published in:
Pharmacology & Therapeutics

DOI:
10.1016/j.pharmthera.2019.05.005

Publication date:
2019

Pharmacology & Therapeutics 200 (2019) 135–147

Contents lists available at ScienceDirect

Pharmacology & Therapeutics

journal homepage: www.elsevier.com/locate/pharmthera

Consideration of breast cancer subtype in targeting the androgen receptor

Clasina M. Venema [a], Rico D. Bense [a], Tessa G. Steenbruggen [a], Hilde H. Nienhuis [a], Si-Qi Qiu [a,b], Michel van Kruchten [a], Myles Brown [c], Rulla M. Tamimi [d,e], Geke A.P. Hospers [a], Carolina P. Schröder [a], Rudolf S.N. Fehrmann [a], Elisabeth G.E. de Vries [a,*]

[a] Department of Medical Oncology, University of Groningen, University Medical Center Groningen, Groningen, The Netherlands
[b] The Breast Center, Cancer Hospital of Shantou University Medical College, Shantou, China
[c] Department of Medical Oncology, Dana-Farber Cancer Institute, Harvard Medical School, Boston, United States
[d] Channing Division of Network Medicine, Department of Medicine, Brigham and Women's Hospital, Harvard Medical School, Boston, MA, USA
[e] Department of Epidemiology, Harvard T.H. Chan School of Public Health, Boston, MA, USA

ARTICLE INFO

Available online 8 May 2019

Keywords:
Breast cancer
Androgen receptor
AR antagonist
Estrogen receptor
Human epidermal growth factor receptor 2
Triple-negative breast cancer

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. Preclinical 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.

Contents

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.

* Corresponding author at: Department of Medical Oncology, University Medical Center Groningen, University of Groningen, PO Box 30.001, 9700 RB Groningen, The Netherlands.
E-mail address: e.g.e.de.vries@umcg.nl (E.G.E. de Vries).

https://doi.org/10.1016/j.pharmthera.2019.05.005

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, Warshaw, 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; Sonne-Hansen & 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 5α-androstane-3β,17β-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 (Boccuzzi et al., 1995; Cochrane et al., 2014; Dauvois, Geng, Lévesque, Mérand, & Labrie, 1991; Spinola,

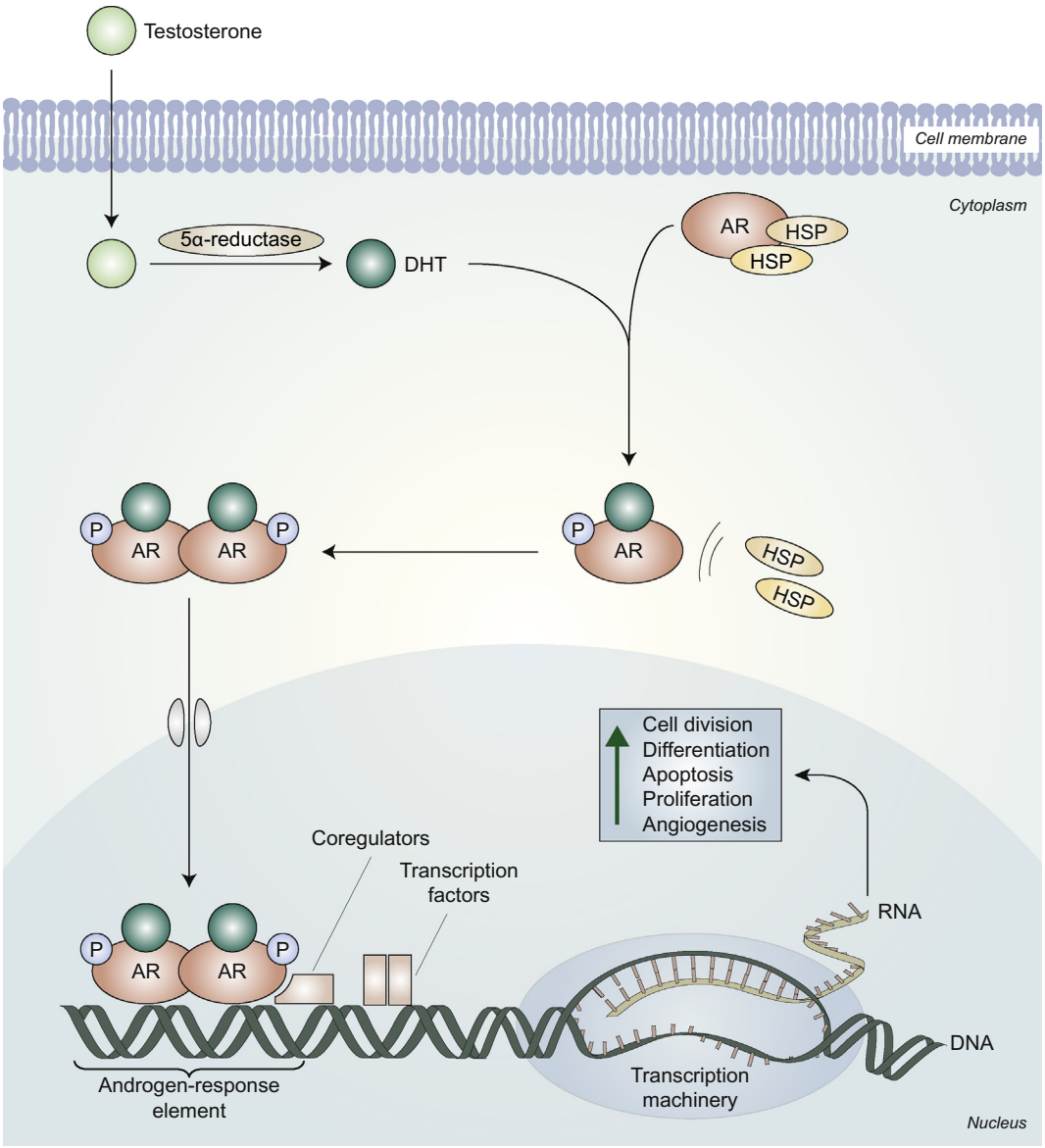

Fig. 1. Effect of androgens on the androgen receptor (AR) in a physiological setting in an androgen-responsive cell. After free testosterone passively diffuses through the plasma membrane, it is converted to dihydrotestosterone (DHT) by 5α-reductase. In the cell, DHT binds to the AR, which leads to dissociation 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 promotor 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	Leuprorelin 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 17α-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 ≥ 1%: 25% CBR at 16 weeks AR IHC ≥ 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 11.0 – 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.,

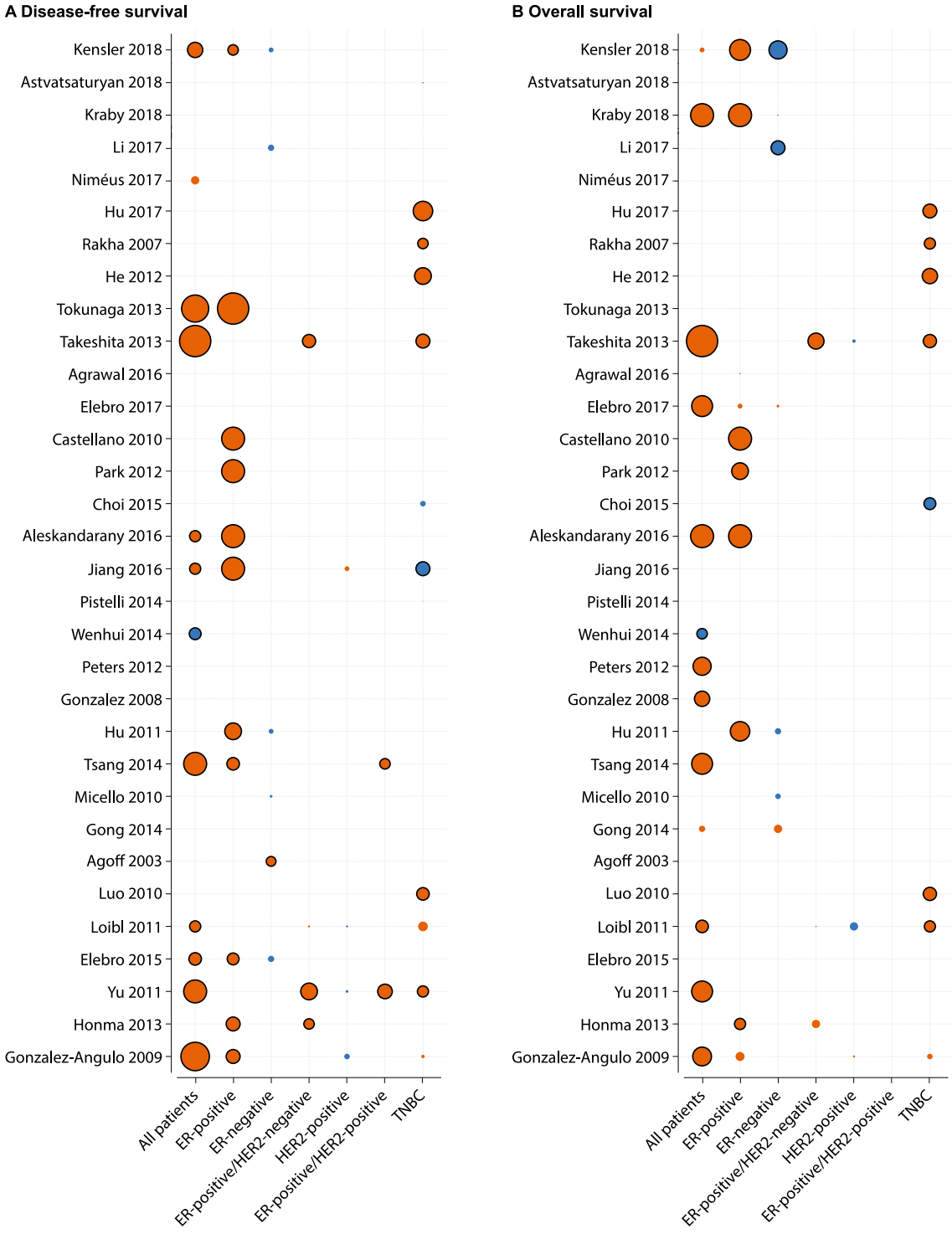

Fig. 2. Overview of studies on the prognostic value of AR expression measured immunohistochemically per breast cancer subgroup. Associations have been studied using log-rank test or univariate Cox regression analysis. An orange bubble indicates an association between androgen receptor (AR) positivity 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 ≤ .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 (Bonnefoi 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, Ansfield, & Segaloff, 1973; Ingle et al., 2006, 1991; Kellokumpu-Lehtinen, Huovinen, & Johansson, 1987). Testosterone propionate administration to patients with ER-positive metastatic breast

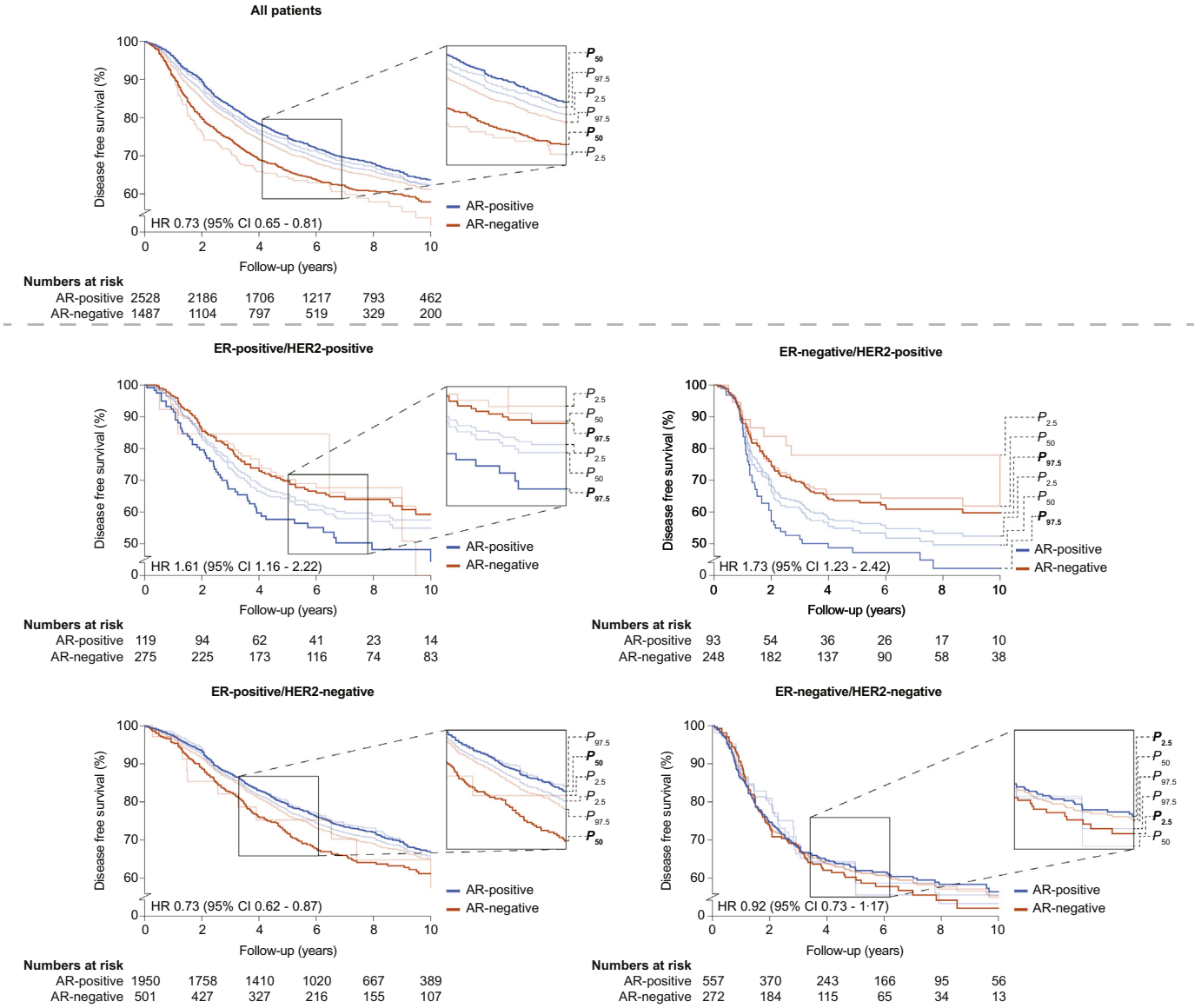

Fig. 3. Disease-free survival curves for different thresholds for AR positivity in breast cancer subgroups. Non-transparent curves show the threshold discriminating best between AR-positive and AR-negative cases in terms of disease-free survival, defined as time of diagnosis to locoregional or distant 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 ≤10% tumor cells), progesterone receptor (PR)-negative, AR-positive (IHC ≥ 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;

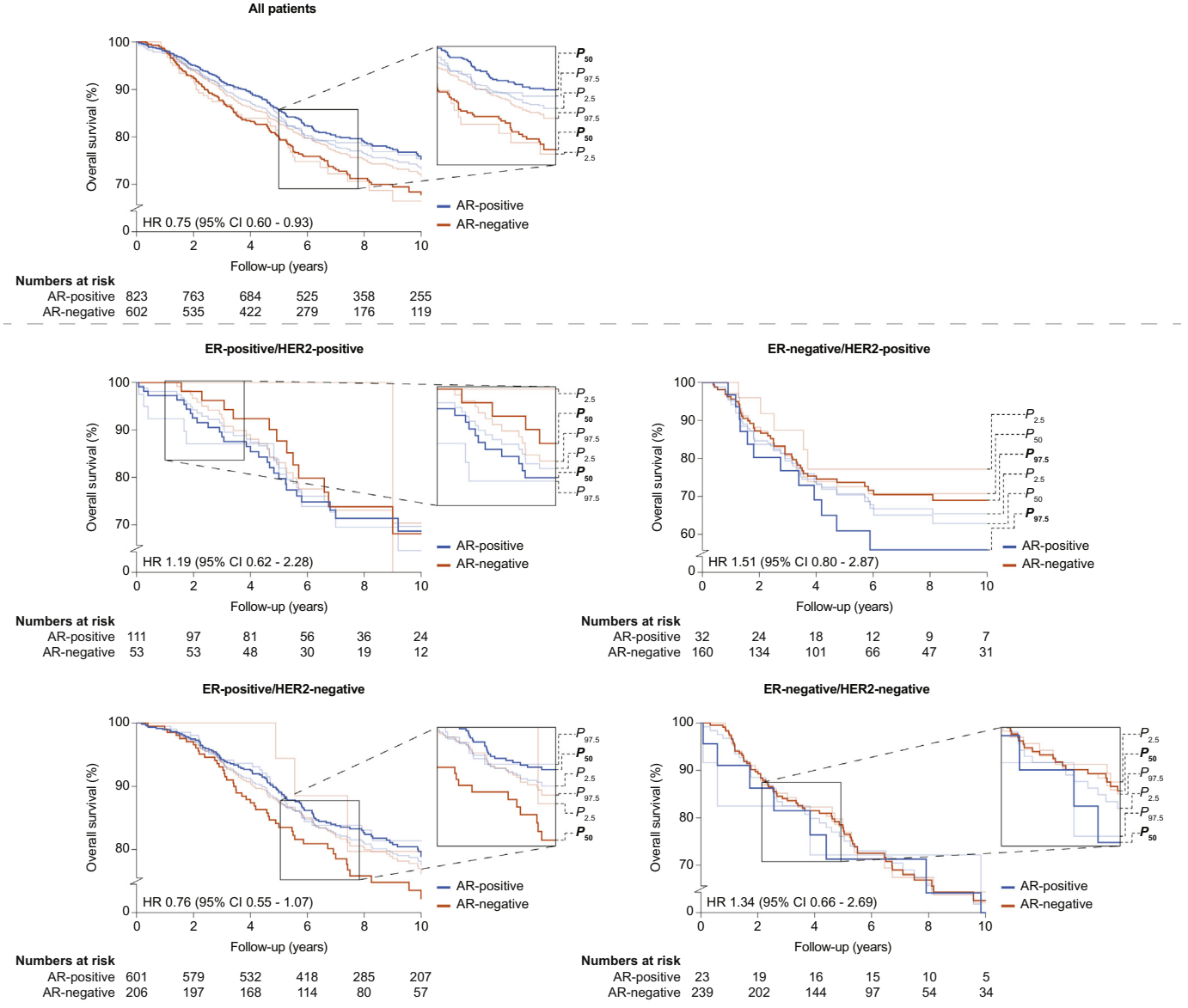

Fig. 4. Overall survival curves for different thresholds for AR positivity in breast cancer subgroups. Non-transparent curves show the threshold discriminating best between AR-positive and AR-negative cases in terms of overall survival, defined as time of diagnosis to death by any cause. Hazard ratios 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 ≥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 at 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

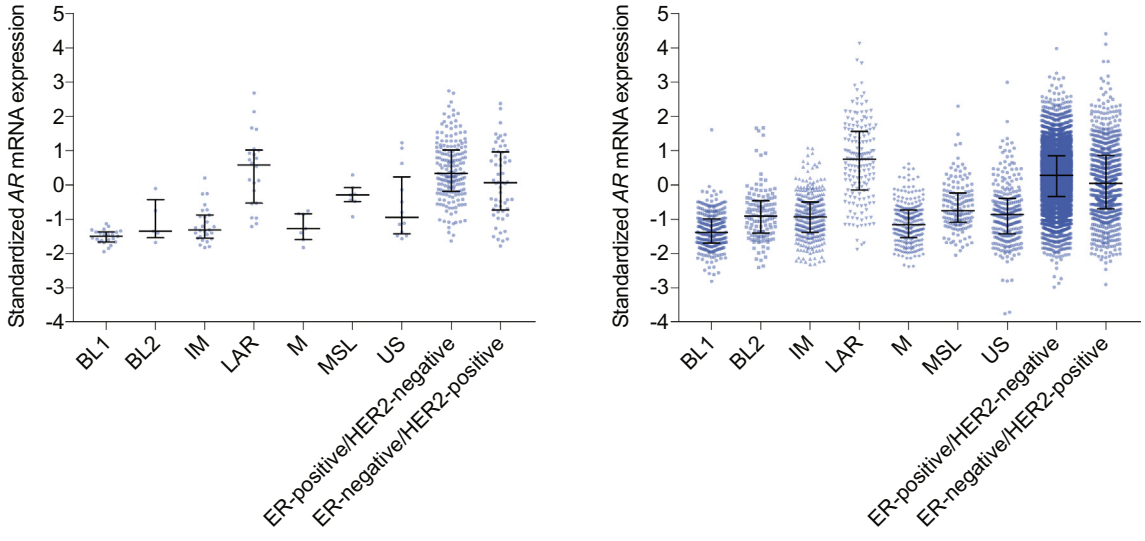

Fig. 5. Scatter dot plot of standardized *AR* mRNA expression in breast cancer subgroups. *AR* mRNA expression is shown for triple-negative breast cancer subtypes basal-like 1 (BL1), basal-like 2 (BL2), immunomodulatory (IM), luminal androgen receptor (LAR), mesenchymal (M), mesenchymal stem-like (MSL), 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 ^{18}F-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, ^{18}F-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.

Acknowledgments

This research was supported by NWO-VENI grant (916-16025), the Bas Mulder award of Alpe d'HuZes/Dutch Cancer Society (RUG 2013-5960), Ubbo Emmius Fund grant (510215), Van der Meer-Boerema Foundation, Anna Dorothea den Hingst Foundation, a Mandema Stipendium, and a grant from the Breast Cancer Research Foundation.

Appendix A. Supplementary data

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

References

Adair, F. E., & Herrmann, J. B. (1946). The use of testosterone propionate in the treatment of advanced carcinoma of the breast. *Annals of Surgery 123*, 1023–1035.

Agoff, S. N., Swanson, P. E., Linden, H., Hawes, S. E., & Lawton, T. J. (2003). Androgen receptor expression in estrogen receptor–negative breast cancer. Immunohistochemical, clinical, and prognostic associations. *American Journal of Clinical Pathology 120*, 725–731. https://doi.org/10.1309/42F00D0DJD0J5EDT.

Agrawal, A., Ziolkowski, P., Grzebieniak, Z., Jelen, M., Bobinski, P., & Agrawal, S. (2016). Expression of androgen receptor in estrogen receptor-positive breast cancer. *Applied Immunohistochemistry & Molecular Morphology 24*, 550–555. https://doi.org/10.1097/PAI.0000000000000234.

Aleskandarany, M. A., Abduljabbar, R., Ashankyty, I., Elmouna, A., Jerjees, D., Ali, S., ... Rakha, E. A. (2016). Prognostic significance of androgen receptor expression in invasive breast cancer: Transcriptomic and protein expression analysis. *Breast Cancer Research and Treatment 159*, 215–227. https://doi.org/10.1007/s10549-016-3934-5.

Andò, S., De Amicis, F., Rago, V., Carpino, A., Maggiolini, M., Panno, M. L., & Lanzino, M. (2002). Breast cancer: From estrogen to androgen receptor. *Molecular and Cellular Endocrinology 193*, 121–128. https://doi.org/10.1016/S0303-7207(02)00105-3.

Antonarakis, E. S., Lu, C., Wang, H., Luber, B., Nakazawa, M., Roeser, J. C., ... Luo, J. (2014). AR-V7 and resistance to enzalutamide and abiraterone in prostate cancer. *New England Journal of Medicine 371*, 1028–1038. https://doi.org/10.1056/NEJMoa1315815.

Arce-Salinas, C., Riesco-Martinez, M. C., Hanna, W., Bedard, P., & Warner, E. (2016). Complete response of metastatic androgen receptor-positive breast cancer to bicalutamide: Case report and review of the literature. *Journal of Clinical Oncology 34*, e21–e24. https://doi.org/10.1200/JCO.2013.49.8899.

Aspinall, S. R., Stamp, S., Davison, A., Shenton, B. K., & Lennard, T. W. J. (2004). The proliferative effects of 5-androstene-3β,17β-diol and 5α-dihydrotestosterone on cell cycle analysis and cell proliferation in MCF7, T47D and MDAMB231 breast cancer cell lines. *Journal of Steroid Biochemistry and Molecular Biology 88*, 37–51. https://doi.org/10.1016/j.jsbmb.2003.10.011.

Astvatsaturyan, K., Yue, Y., Walts, A. E., & Bose, S. (2018). Androgen receptor positive triple negative breast cancer: Clinicopathologic, prognostic, and predictive features. *PLoS One 13*, e0197827. https://doi.org/10.1371/journal.pone.0197827.

Bachmann, G. A. (2002). The hypoandrogenic woman: Pathophysiologic overview. *Fertility and Sterility 77*, S72–S76. https://doi.org/10.1016/S0015-0282(02)03003-0.

Bardia, A., Gucalp, A., DaCosta, N., Gabrail, N., Danso, M., Ali, H., ... Traina, T. A. (2018). Phase 1 study of seviteronel, a selective CYP17 lyase and androgen receptor inhibitor, in women with estrogen receptor-positive or triple-negative breast cancer. *Breast Cancer Research and Treatment 171*, 111–120. https://doi.org/10.1007/s10549-018-4813-z.

Barton, V. N., D'Amato, N. C., Gordon, M. A., Lind, H. T., Spoelstra, N. S., Babbs, B. L., ... Richer, J. K. (2015). Multiple molecular subtypes of triple-negative breast cancer critically rely on androgen receptor and respond to enzalutamide in vivo. *Molecular Cancer Therapeutics 14*, 769–778. https://doi.org/10.1158/1535-7163.MCT-14-0926.

Bense, R.D., Sotiriou, C., Piccart-Gebhart, M.J., Haanen, J.B.A.G., van Vugt, M.A.T.M., de Vries, E.G.E., … Fehrmann, R.S.N. (2017). Relevance of tumor-infiltrating immune cell composition and functionality for disease outcome in breast cancer. Journal of the National Cancer Institute, 109, (djw192). doi:https://doi.org/10.1093/jnci/djw192.

Bidard, F. C., Peeters, D. J., Fehm, T., Nolé, F., Gisbert-Criado, R., Mavroudis, D., ... Michiels, S. (2014). Clinical validity of circulating tumour cells in patients with metastatic breast cancer: A pooled analysis of individual patient data. *The Lancet Oncology 15*, 406–414. https://doi.org/10.1016/S1470-2045(14)70069-5.

Birrell, S. N., Bentel, J. M., Hickey, T. E., Ricciardelli, C., Weger, M. A., Horsfall, D. J., & Tilley, W. D. (1995). Androgens induce divergent proliferative responses in human breast cancer cell lines. *The Journal of Steroid Biochemistry and Molecular Biology 52*, 459–467. https://doi.org/10.1016/0960-0760(95)00005-K.

Blamey, R. W., Hornmark-Stenstam, B., Ball, G., Blichert-Toft, M., Cataliotti, L., Fourquet, A., ... Ellis, I. (2010). ONCOPOOL - a European database for 16,944 cases of breast cancer. *European Journal of Cancer 46*, 56–71. https://doi.org/10.1016/j.ejca.2009.09.009.

Boccuzzi, G., Tamagno, E., Brignardello, E., Di Monaco, M., Aragno, M., & Danni, O. (1995). Growth inhibition of DMBA-induced rat mammary carcinomas by the antiandrogen flutamide. *Journal of Cancer Research and Clinical Oncology 121*, 150–154. https://doi.org/10.1007/BF01198096.

Boni, C., Pagano, M., Panebianco, M., Bologna, A., Sierra, N. M., Gnoni, R., ... Bisagni, G. (2014). Therapeutic activity of testoterone in metastatic breast cancer. *Anticancer Research 34*, 1287–1290.

Bonnefoi, H., Grellety, T., Tredan, O., Saghatchian, M., Dalenc, F., Mailliez, A., ... Gonçalves, A. (2016). A phase II trial of abiraterone acetate plus prednisone in patients with triple-negative androgen receptor positive locally advanced or metastatic breast cancer (UCBG 12-1). *Annals of Oncology 27*, 812–818. https://doi.org/10.1093/annonc/mdw067.

Bozovic-Spasojevic, I., Zardavas, D., Brohée, S., Ameye, L., Fumagalli, D., Ades, F., ... Sotiriou, C. (2017). The prognostic role of androgen receptor in patients with early-stage breast cancer: A meta-analysis of clinical and gene expression data. *Clinical Cancer Research 23*, 2702–2712. https://doi.org/10.1158/1078-0432.CCR-16-0979.

Burger, H. G. (2002). Androgen production in women. *Fertility and Sterility 77*, S3–S5.

Buvat, J., Maggi, M., Guay, A., & Torres, L. O. (2013). Testosterone deficiency in men: Systematic review and standard operating procedures for diagnosis and treatment. *Journal of Sexual Medicine 10*, 245–284. https://doi.org/10.1016/S0015-0282(02)02985-0.

Castellano, I., Allia, E., Accortanzo, V., Vandone, A. M., Chiusa, L., Arisio, R., ... Sapino, A. (2010). Androgen receptor expression is a significant prognostic factor in estrogen receptor positive breast cancers. *Breast Cancer Research and Treatment 124*, 607–617. https://doi.org/10.1007/s10549-010-0761-y.

Chen, C. D., Welsbie, D. S., Tran, C., Baek, S. H., Chen, R., Vessella, R., ... Sawyers, C. L. (2004). Molecular determinants of resistance to antiandrogen therapy. *Nature Medicine 10*, 33–39. https://doi.org/10.1038/nm972.

Choi, J. E., Kang, S. H., Lee, S. J., & Bae, Y. K. (2015). Androgen receptor expression predicts decreased survival in early stage triple-negative breast cancer. *Annals of Surgical Oncology 22*, 82–89. https://doi.org/10.1245/s10434-014-3984-z.

Chottanapund, S., Van Duursen, M. B. M., Navasumrit, P., Hunsonti, P., Timtavorn, S., Ruchirawat, M., & Van Den Berg, M. (2013). Effect of androgens on different breast cancer cells co-cultured with or without breast adipose fibroblasts. *Journal of Steroid Biochemistry and Molecular Biology 138*, 54–62. https://doi.org/10.1016/j.jsbmb.2013.03.007.

Cochrane, D. R., Bernales, S., Jacobsen, B. M., Cittelly, D. M., Howe, E. N., D'Amato, N. C., ... Richer, J. K. (2014). Role of the androgen receptor in breast cancer and preclinical analysis of enzalutamide. *Breast Cancer Research 16*, R7. https://doi.org/10.1186/bcr3599.

Collins, L. C., Cole, K. S., Marotti, J. D., Hu, R., Schnitt, S. J., & Tamimi, R. M. (2011). Androgen receptor expression in breast cancer in relation to molecular phenotype: Results from the Nurses' health study. *Modern Pathology 24*, 924–931. https://doi.org/10.1038/modpathol.2011.54.

Cops, E. J., Bianco-Miotto, T., Moore, N. L., Clarke, C. L., Birrell, S. N., Butler, L. M., & Tilley, W. D. (2008). Antiproliferative actions of the synthetic androgen, mibolerone, in breast cancer cells are mediated by both androgen and progesterone receptors. *Journal of Steroid Biochemistry and Molecular Biology 110*, 236–243. https://doi.org/10.1016/j.jsbmb.2007.10.014.

D'Amato, N. C., Gordon, M. A., Babbs, B., Spoelstra, N. S., Carson Butterfield, K. T., Torkko, K. C., ... Richer, J. K. (2016). Cooperative dynamics of AR and ER activity in breast cancer. *Molecular Cancer Research 14*, 1054–1067. https://doi.org/10.1158/1541-7786.MCR-16-0167.

Dauvois, S., Geng, C., Lévesque, C., Mérand, Y., & Labrie, F. (1991). Additive inhibitory effects of an androgen and the antiestrogen EM-170 on estradiol-stimulated growth of human ZR-75-1 breast tumors in athymic mice. *Cancer Research 51*, 3131–3135.

De Amicis, F., Thirugnansampanthan, J., Cui, Y., Selever, J., Beyer, A., Parra, I., ... Fuqua, S. A. W. (2010). Androgen receptor overexpression induces tamoxifen resistance in human breast cancer cells. *Breast Cancer Research and Treatment 121*, 1–11. https://doi.org/10.1007/s10549-009-0436-8.

Dehdashti, F., Picus, J., Michalski, J. M., Dence, C. S., Siegel, B. A., Katzenellenbogen, J. A., & Welch, M. J. (2005). Positron tomographic assessment of androgen receptors in prostatic carcinoma. *European Journal of Nuclear Medicine and Molecular Imaging 32*, 344–350. https://doi.org/10.1007/s00259-005-1764-5.

Doane, A. S., Danso, M., Lal, P., Donaton, M., Zhang, L., Hudis, C., & Gerald, W. L. (2006). An estrogen receptor-negative breast cancer subset characterized by a hormonally regulated transcriptional program and response to androgen. *Oncogene 25*, 3994–4008. https://doi.org/10.1038/sj.onc.1209415.

Elebro, K., Bendahl, P., Jernström, H., & Borgquist, S. (2017). Androgen receptor expression and breast cancer mortality in a population-based prospective cohort. *Breast Cancer Research and Treatment 165*, 645–657. https://doi.org/10.1007/s10549-017-4343-0.

Elebro, K., Borgquist, S., Simonsson, M., Markkula, A., Jirström, K., Ingvar, C., ... Jernström, H. (2015). Combined androgen and estrogen receptor status in breast cancer: Treatment prediction and prognosis in a population-based prospective cohort. *Clinical Cancer Research 21*, 3640–3650. https://doi.org/10.1158/1078-0432.CCR-14-2564.

Fehrmann, R. S. N., Karjalainen, J. M., Krajewska, M., Westra, H., Maloney, D., Simeonov, A., ... Franke, L. (2015). Gene expression analysis identifies global gene dosage sensitivity in cancer. *Nature Genetics 47*, 115–125. https://doi.org/10.1038/ng.3173.

Fels, E. (1944). Treatment of breast cancer with testosterone propionate. *Journal of Clinical Endocrinology 4*, 121–125. https://doi.org/10.1210/jcem-4-3-121.

Ferenchick, G. S., Hirokawa, S., Mammen, E. F., & Schwartz, K. A. (1995). Anabolic-androgenic steroid abuse in weight lifters: Evidence for activation of the hemostatic system. *American Journal of Hematology 49*, 282–288. https://doi.org/10.1002/ajh.2830490405.

Fizazi, K., Scher, H. I., Molina, A., Logothetis, C. J., Chi, K. N., Jones, R. J., ... de Bono, J. S. (2012). Abiraterone acetate for treatment of metastatic castration-resistant prostate cancer: Final overall survival analysis of the COU-AA-301 randomised, double-blind, placebo-controlled phase 3 study. *The Lancet Oncology 13*, 983–992. https://doi.org/10.1016/S1470-2045(12)70379-0.

Forward, D. P., Cheung, K. L., Jackson, L., & Robertson, J. F. R. (2004). Clinical and endocrine data for goserelin plus anastrozole as second-line endocrine therapy for premenopausal advanced breast cancer. *British Journal of Cancer 90*, 590–594. https://doi.org/10.1038/sj.bjc.6601557.

Fujimoto, N., Mizokami, A., Harada, S., & Matsumoto, T. (2001). Different expression of androgen receptor coactivators in human prostate. *Urology 58*, 289–294.

Garay, J. P., Karakas, B., Abukhdeir, A. M., Cosgrove, D. P., Gustin, J. P., Higgins, M. J., ... Park, B. H. (2012). The growth response to androgen receptor signaling in ERα-negative human breast cells is dependent on p21 and mediated by MAPK activation. *Breast Cancer Research 14*, R27. https://doi.org/10.1016/S0090-4295(01)01117-7.

Gibson, L. J., Dawson, C., Lawrence, D. H., & Bliss, J. M. (2007). Aromatase inhibitors for treatment of advanced breast cancer in postmenopausal women. *Cochrane Database of Systematic Reviews CD003370*. https://doi.org/10.1002/14651858.CD003370.pub2.

Goldenberg, I. S., Waters, N., Ravdin, R. S., Ansfield, F. J., & Segaloff, A. (1973). Androgenic therapy for advanced breast cancer in women. A report of the cooperative breast

cancer group. *JAMA 223*, 1267–1268. https://doi.org/10.1001/jama.1973.03220110045012.
Gong, Y., Wei, W., Wu, Y., Ueno, N. T., & Huo, L. (2014). Expression of androgen receptor in inflammatory breast cancer and its clinical relevance. *Cancer 120*, 1775–1779. https://doi.org/10.1002/cncr.28667.
Gonzalez, L. O., Corte, M. D., Vazquez, J., Junquera, S., Sanchez, R., Alvarez, A. C., ... Vizoso, F. J. (2008). Androgen receptor expresion in breast cancer: Relationship with clinicopathological characteristics of the tumors, prognosis, and expression of metalloproteases and their inhibitors. *BMC Cancer 8*, 149. https://doi.org/10.1186/1471-2407-8-149.
Gonzalez-Angulo, A. M., Stemke-Hale, K., Palla, S. L., Carey, M., Agarwal, R., Meric-Berstam, F., ... Hennessy, B. T. (2009). Androgen receptor levels and association with PIK3CA mutations and prognosis in breast cancer. *Clinical Cancer Research 15*, 2472–2478. https://doi.org/10.1158/1078-0432.CCR-08-1763.
Gooren, L. J., Wierckx, K., & Giltay, E. J. (2014). Cardiovascular disease in transsexual persons treated with cross-sex hormones: Reversal of the traditional sex difference in cardiovascular disease pattern. *European Journal of Endocrinology 170*, 809–819. https://doi.org/10.1530/EJE-14-0011.
Gucalp, A., Danso, M. A., Elias, A. D., Bardia, A., Ali, H. Y., Potter, D., ... Traina, T. A. (2017). Phase (Ph) 2 stage 1 clinical activity of seviteronel, a selective CYP17-lyase and androgen receptor (AR) inhibitor, in women with advanced AR+ triple-negative breast cancer (TNBC) or estrogen receptor (ER)+ BC: CLARITY-01. *Journal of Clinical Oncology 35*(suppl). https://doi.org/10.1200/JCO.2017.35.15_suppl.1102 (abstr 1102).
Gucalp, A., Tolaney, S., Isakoff, S. J., Ingle, J. N., Liu, M. C., Carey, L. A., ... Traina, T. A. (2013). Phase II trial of bicalutamide in patients with androgen receptor-positive, estrogen receptor-negative metastatic breast cancer. *Clinical Cancer Research 19*, 5505–5512. https://doi.org/10.1158/1078-0432.CCR-12-3327.
Hackenberg, R., Lüttchens, S., Hofmann, J., Kunzmann, R., Hölzel, F., & Schulz, K. D. (1991). Androgen sensitivity of the new human breast cancer cell line MFM-223. *Cancer Research 51*, 5722–5727.
Hall, R. E., Birrell, S. N., Tilley, W. D., & Sutherland, R. L. (1994). MDA-MB-453, an androgen-responsive human breast carcinoma cell line with high level androgen receptor expression. *European Journal of Cancer 30*, 484–490. https://doi.org/10.1016/0959-8049(94)90424-3.
He, J., Peng, R., Yuan, Z., Wang, S., Peng, J., Lin, G., ... Qin, T. (2012). Prognostic value of androgen receptor expression in operable triple-negative breast cancer: A retrospective analysis based on a tissue microarray. *Medical Oncology 29*, 406–410. https://doi.org/10.1007/s12032-011-9832-0.
Hickey, T. E., Irvine, C. M., Dvinge, H., Tarulli, G. A., Hanson, A. R., Ryan, N. K., ... Selth, L. A. (2015). Expression of androgen receptor splice variants in clinical breast cancers. *Oncotarget 6*, 44728–44744. https://doi.org/10.18632/oncotarget.6296.
Honma, N., Horii, R., Iwase, T., Saji, S., Younes, M., Ito, Y., & Akiyama, F. (2013). Clinical importance of androgen receptor in breast cancer patients treated with adjuvant tamoxifen monotherapy. *Breast Cancer 20*, 323–330. https://doi.org/10.1007/s12282-012-0337-2.
Hu, X. Q., Chen, W. L., Ma, H. G., & Jiang, K. (2017). Androgen receptor expression identifies patient with favorable outcome in operable triple negative breast cancer. *Oncotarget 8*, 56364–56374. https://doi.org/10.18632/oncotarget.16913.
Hu, R., Dawood, S., Holmes, M. D., Collins, L. C., Schnitt, S. J., Cole, K., ... Tamimi, R. M. (2011). Androgen receptor expression and breast cancer survival in postmenopausal women. *Clinical Cancer Research 17*, 1867–1874. https://doi.org/10.1158/1078-0432.CCR-10-2021.
Ingle, J. N., Suman, V. J., Mailliard, J. A., Kugler, J. W., Krook, J. E., Michalak, J. C., ... Perez, E. A. (2006). Randomized trial of tamoxifen alone or combined with fluoxymesterone as adjuvant therapy in postmenopausal women with resected estrogen receptor positive breast cancer. North central cancer treatment group trial 89-30-52. *Breast Cancer Research and Treatment 98*, 217–222. https://doi.org/10.1007/s10549-005-9152-1.
Ingle, J. N., Twito, D. I., Schaid, D. J., Cullinan, S. A., Krook, J. E., Mailliard, J. A., ... Pfeifle, D. M. (1991). Combination hormonal therapy with tamoxifen plus fluoxymesterone versus tamoxifen alone in postmenopausal women with metastatic breast cancer. An updated analysis. *Cancer 67*, 886–891. https://doi.org/10.1002/1097-0142(19910215)67:4<886::AID-CNCR2820670405>3.0.CO;2-O.
Jiang, H. S., Kuang, X. Y., Sun, W. L., Xu, Y., Zheng, Y. Z., Liu, Y. R., ... Shao, Z. M. (2016). Androgen receptor expression predicts different clinical outcomes for breast cancer patients stratified by hormone receptor status. *Oncotarget 7*, 41285–41293. https://doi.org/10.18632/oncotarget.9778.
Kasimir-Bauer, S., Bittner, A. K., König, L., Reiter, K., Keller, T., Kimmig, R., & Hoffmann, O. (2016). Does primary neoadjuvant systemic therapy eradicate minimal residual disease? Analysis of disseminated and circulating tumor cells before and after therapy. *Breast Cancer Research 18*, 20. https://doi.org/10.1186/s13058-016-0679-3.
Kellokumpu-Lehtinen, P., Huovinen, R., & Johansson, R. (1987). Hormonal treatment of advanced breast cancer. A randomized trial of tamoxifen versus nandrolone decanoate. *Cancer 60*, 2376–2381. https://doi.org/10.1002/1097-0142(19871115)60:10<2376::AID-CNCR2820601005>3.0.CO;2-N.
Kensler, K. H., Poole, E. M., Heng, Y. J., Collins, L. C., Glass, B., Beck, A. H., ... Tamimi, R. M. (2018). Androgen receptor expression and breast cancer rurvival: Results from the Nurses' health studies. *Journal of the National Cancer Institute 111*, djy173. https://doi.org/10.1093/jnci/djy173.
Kimura, N., Mizokami, A., Oonuma, T., Sasano, H., & Nagura, H. (1993). Immunocytochemical localization of androgen receptor with polyclonal antibody in paraffin-embedded human tissues. *Journal of Histochemistry and Cytochemistry 41*, 671–678. https://doi.org/10.1177/41.5.8468448.
Kono, M., Fujii, T., Lyons, G. R., Huo, L., Bassett, R., Gong, Y., ... Ueno, N. T. (2016). Impact of androgen receptor expression in fluoxymesterone-treated estrogen receptor-positive metastatic breast cancer refractory to contemporary hormonal therapy. *Breast Cancer Research and Treatment 160*, 101–109. https://doi.org/10.1007/s10549-016-3986-6.
Pope, H. G., Jr., Kouri, E. M., & Hudson, J. I. (2000). Effects of supraphysiologic doses of testosterone on mood and aggression in normal men: A randomized controlled trial. *Archives of General Psychiatry 57*, 133–140. https://doi.org/10.1001/archpsyc.57.2.133.
Kraby, M. R., Valla, M., Opdahl, S., Haugen, O. A., Sawicka, J. E., Engstrøm, M. J., & Bofin, A. M. (2018). The prognostic value of androgen receptors in breast cancer subtypes. *Breast Cancer Research and Treatment 172*, 283–296. https://doi.org/10.1007/s10549-018-4904-x.
Krop, I., Abramson, V., Colleoni, M., Traina, T., Holmes, F., Estevez, L., ... Yardley, D. A. (2018). Results from a randomized placebo-controlled phase 2 trial evaluating exemestane ± enzalutamide in patients with hormone receptor-positive breast cancer. *Cancer Research 78*(4 suppl) (abstr GS4–07).
Krop, I., Cortes, J., Miller, K., Huizing, M. T., Provencher, L., Gianni, L., ... Wardley, A. (2017). A single-arm phase 2 study to assess clinical activity, efficacy and safety of enzalutamide with trastuzumab in HER2+ AR+ metastatic or locally advanced breast cancer. *Cancer Research 77*(4 suppl) (abstr P4-22-08).
Kumar, V., Yu, J., Phan, V., Tudor, I. C., Peterson, A., & Uppal, H. (2017). Androgen receptor immunohistochemistry as a companion diagnostic approach to predict clinical response to enzalutamide in triple-negative breast cancer. *JCO Precision Oncology 1*, 1–19. https://doi.org/10.1200/PO.17.00075.
Lange, C. A., Gioeli, D., Hammes, S. R., & Marker, P. C. (2007). Integration of rapid signaling events with steroid hormone receptor action in breast and prostate cancer. *Annual Review of Physiology 69*, 171–199. https://doi.org/10.1146/annurev.physiol.69.031905.160319.
Lehmann, B. D., Bauer, J. A., Chen, X., Sanders, M. E., Chakravarthy, A. B., Shyr, Y., & Pietenpol, J. A. (2011). Identification of human triple-negative breast cancer subtypes and preclinical models for selection of targeted therapies. *Journal of Clinical Investigation 121*, 2750–2767. https://doi.org/10.1172/JCI45014DS1.
Lewis-Wambi, J. S., & Jordan, V. C. (2009). Estrogen regulation of apoptosis: How can one hormone stimulate and inhibit? *Breast Cancer Research 11*, 206. https://doi.org/10.1186/bcr2255.
Li, C., Cao, L., Xu, C., Liu, F., Xiang, G., Liu, X., ... Niu, Y. (2017). The immunohistochemical expression and potential prognostic value of HDAC6, AR in invasive breast cancer. *Human Pathology 75*, 16–25. https://doi.org/10.1016/j.humpath.2017.11.010.
Lin, H. Y., Sun, M., Lin, C., Tang, H. Y., London, D., Shih, A., ... Davis, P. J. (2009). Androgen-induced human breast cancer cell proliferation is mediated by discrete mechanisms in estrogen receptor-α-positive and -negative breast cancer cells. *Journal of Steroid Biochemistry and Molecular Biology 113*, 182–188. https://doi.org/10.1016/j.jsbmb.2008.12.010.
Lippman, M., Bolan, G., & Huff, K. (1976). The effects of androgens and antiandrogens on hormone-responsive human breast cancer in long-term tissue culture. *Cancer Research 36*, 4610–4618.
Loibl, S., Müller, B. M., Von Minckwitz, G., Schwabe, M., Roller, M., Darb-Esfahani, S., ... Denkert, C. (2011). Androgen receptor expression in primary breast cancer and its predictive and prognostic value in patients treated with neoadjuvant chemotherapy. *Breast Cancer Research and Treatment 130*, 477–487. https://doi.org/10.1007/s10549-011-1715-8.
Luo, X., Shi, Y. X., Li, Z. M., & Jiang, W. Q. (2010). Expression and clinical significance of androgen receptor in triple negative breast cancer. *Chinese Journal of Cancer 29*, 585–590.
Macedo, L. F., Guo, Z., Tilghman, S. L., Sabnis, G. J., Qiu, Y., & Brodie, A. (2006). Role of androgens on MCF-7 breast cancer cell growth and on the inhibitory effect of letrozole. *Cancer Research 66*, 7775–7782. https://doi.org/10.1158/0008-5472.CAN-05-3984.
Maggiolini, M., Donzé, O., Jeannin, E., Andò, S., & Picard, D. (1999). Adrenal androgens stimulate the proliferation of breast cancer cells as direct activators of estrogen receptor α. *Cancer Research 59*, 4864–4869.
Micello, D., Marando, A., Sahnane, N., Riva, C., Capella, C., & Sessa, F. (2010). Androgen receptor is frequently expressed in HER2-positive, ER/PR-negative breast cancers. *Virchows Archiv 457*, 467–476. https://doi.org/10.1007/s00428-010-0964-y.
Migliaccio, A., Di Domenico, M., Castoria, G., Nanayakkara, M., Lombardi, M., De Falco, A., ... Auricchio, F. (2005). Steroid receptor regulation of epidermal growth factor signaling through Src in breast and prostate cancer cells: Steroid antagonist action. *Cancer Research 65*, 10585–10593. https://doi.org/10.1158/0008-5472.CAN-05-0912.
Millward, M. J., Cantwell, B. M. J., Dowsett, M., Carmichael, J., & Harris, A. L. (1991). Phase II clinical and endocrine study of anandron (RU-23908) in advanced post-menopausal breast cancer. *British Journal of Cancer 63*, 763–764. https://doi.org/10.1038/bjc.1991.170.
Naderi, A., & Hughes-Davies, L. (2008). A functionally significant cross-talk between androgen receptor and ErbB2 pathways in estrogen receptor negative breast cancer. *Neoplasia 10*, 542–548. https://doi.org/10.1593/neo.08274.
Narayanan, R., Ahn, S., Cheney, M. D., Yepuru, M., Miller, D. D., Steiner, M. S., & Dalton, J. T. (2014). Selective androgen receptor modulators (SARMs) negatively regulate triple-negative breast cancer growth and epithelial:Mesenchymal stem cell signaling. *PLoS One 9*, e103202. https://doi.org/10.1371/journal.pone.0103202.
National Comprehensive Cancer Network. (2018). NCCN clinical practice guidelines in oncology (NCCN guidelines); breast cancer version 4.2017. Retrieved from https://www.nccn.org/professionals/physician_gls/f_guidelines.asp#breast (accessed March 4, 2018).
Ni, M., Chen, Y., Lim, E., Wimberly, H., Bailey, S. T., Imai, Y., ... Brown, M. (2011). Targeting androgen receptor in estrogen receptor-negative breast cancer. *Cancer Cell 20*, 119–131. https://doi.org/10.1016/j.ccr.2011.05.026.
Niméus, E., Folkesson, E., Nodin, B., Hartman, L., & Klintman, M. (2017). Androgen receptor in stage I-II primary breast cancer - prognostic value and distribution in subgroups. *Anticancer Research 37*, 6845–6853.

O'Shaughnessy, J., Campone, M., Brain, E., Neven, P., Hayes, D., Bondarenko, I., ... Johnston, S. (2016). Abiraterone acetate, exemestane or the combination in postmenopausal patients with estrogen receptorpositive metastatic breast cancer. *Annals of Oncology 27*, 106–113. https://doi.org/10.1093/annonc/mdv487.

Ortmann, J., Prifti, S., Bohlmann, M. K., Rehberger-Schneider, S., Strowitzki, T., & Rabe, T. (2002). Testosterone and 5α-dihydrotestosterone inhibit in vitro growth of human breast cancer cell lines. *Gynecological Endorcinology 16*, 113–120. https://doi.org/10.1080/gye.16.2.113.120.

Overmoyer, B., Sanz-Altimira, P., Partridge, A. H., Extermann, M., Liu, J., Winer, E., ... Johnston, M. A. (2015). Enobosarm for the treatment of metastatic, estrogen and androgen receptor positive, breast cancer. Final results of the primary endpoint and current progression free survival. *Cancer Research 75*(9 suppl) (abstr P1–13-04).

Park, S., Park, H. S., Koo, J. S., Yang, W. I., Kim, S. I., & Park, B. W. (2012). Higher expression of androgen receptor is a significant predictor for better endocrine-responsiveness in estrogen receptor-positive breast cancers. *Breast Cancer Research and Treatment 133*, 311–320. https://doi.org/10.1007/s10549-011-1950-z.

Parker, C., Gillessen, S., Heidenreich, A., & Horwich, A. (2015). Cancer of the prostate: ESMO clinical practice guidelines for diagnosis, treatment and follow-up. *Annals of Oncology 26*, v69–v77.

Parker, J. S., Mullins, M., Cheang, M. C. U., Leung, S., Voduc, D., Vickery, T., ... Bernard, P. S. (2009). Supervised risk predictor of breast cancer based on intrinsic subtypes. *Journal of Clinical Oncology 27*, 1160–1167.

Perrault, D. J., Logan, D. M., Stewart, D. J., Bramwell, V. H., Paterson, A. H., & Eisenhauer, E. A. (1988). Phase II study of flutamide in patients with metastatic breast cancer. A National Cancer Institute of Canada clinical trials group study. *Investigational New Drugs 6*, 207–210.

Peters, A. A., Buchanan, G., Ricciardelli, C., Bianco-Miotto, T., Centenera, M. M., Harris, J. M., ... Tilley, W. D. (2009). Androgen receptor inhibits estrogen receptor-α activity and is prognostic in breast cancer. *Cancer Research 69*, 6131–6140. https://doi.org/10.1158/0008-5472.CAN-09-0452.

Peters, K. M., Edwards, S. L., Nair, S. S., French, J. D., Bailey, P. J., Salkield, K., ... Brown, M. A. (2012). Androgen receptor expression predicts breast cancer survival: The role of genetic and epigenetic events. *BMC Cancer 12*, 132.

Pistelli, M., Caramanti, M., Biscotti, T., Santinelli, A., Pagliacci, A., De Lisa, M., ... Cascinu, S. (2014). Androgen receptor expression in early triple-negative breast cancer: Clinical significance and prognostic associations. *Cancers 6*, 1351–1362. https://doi.org/10.3390/cancers6031351.

Poulin, R., Baker, D., & Labrie, F. (1988). Androgens inhibit basal and estrogen-induced cell proliferation in the ZR-75-1 human breast cancer cell line. *Breast Cancer Research and Treatment 12*, 213–225. https://doi.org/10.1007/BF01805942.

Quigley, C. A., Friedman, K. J., Johnson, A., Lafreniere, R. G., Silverman, L. M., Lubahn, D. B., ... French, F. S. (1992). Complete deletion of the androgen receptor gene: Definition of the null phenotype of the androgen insensitivity syndrome and determination of carrier status. *Endocrinology and Metabolism 74*, 927–933.

Rakha, E. A., El-Sayed, M. E., Green, A. R., Lee, A. H. S., Robertson, J. F., & Ellis, I. O. (2007). Prognostic markers in triple-negative breast cancer. *Cancer 109*, 25–32. https://doi.org/10.1002/cncr.22381.

Rampurwala, M., Wisinski, K. B., Burkard, M. E., Ehsani, S., O'Regan, R. M., Carmichael, L., ... Tevaarwerk, A. J. (2017). Phase 1b study of orteronel in postmenopausal women with hormone-receptor positive (HR+) metastatic breast cancer. *Investigational New Drugs 35*, 87–94. https://doi.org/10.1007/s10637-016-0403-2.

Rangel, N., Rondon-Lagos, M., Annaratone, L., Osella-Abate, S., Metovic, J., Mano, M. P., ... Castellano, I. (2018). The role of the AR/ER ratio in ER-positive breast cancer patients. *Endocrine-Related Cancer 25*, 163–172. https://doi.org/10.1530/ERC-17-0417.

Reese, C. C., Warshaw, M. L., Murai, J. T., & Siiteri, P. K. (1988). Alternative models for estrogen and androgen regulation of human breast cancer cell (T47D) growth. *Annals of the New York Academy of Sciences 538*, 112–121. https://doi.org/10.1111/j.1749-6632.1988.tb48856.x.

Rizza, P., Barone, I., Zito, D., Giordano, F., Lanzino, M., De Amicis, F., ... Andò, S. (2014). Estrogen receptor β as a novel target of androgen receptor action in breast cancer cell lines. *Breast Cancer Research 16*, R21. https://doi.org/10.1186/bcr3619.

Robinson, J. L. L., MacArthur, S., Ross-Innes, C. S., Tilley, W. D., Neal, D. E., Mills, I. G., & Carroll, J. S. (2011). Androgen receptor driven transcription in molecular apocrine breast cancer is mediated by FoxA1. *The EMBO Journal 30*, 3019–3027. https://doi.org/10.1038/emboj.2011.216.

Scher, H. I., Beer, T. M., Higano, C. S., Anand, A., Taplin, M. E., Efstathiou, E., ... Sawyers, C. L. (2010). Antitumour activity of MDV3100 in castration-resistant prostate cancer: A phase 1-2 study. *The Lancet 375*, 1437–1446. https://doi.org/10.1016/S0140-6736(10)60172-9.

Scher, H. I., Fizazi, K., Saad, F., Taplin, M. E., Sternberg, C. N., Miller, K., ... de Bono, J. S. (2012). Increased survival with enzalutamide in prostate cancer after chemotherapy. *New England Journal of Medicine 367*, 1187–1197. https://doi.org/10.1056/NEJMoa1207506.

Schwartzberg, L. S., Yardley, D. A., Elias, A. D., Patel, M., Lorusso, P., Burris, H. A., ... Traina, T. A. (2017). A phase I/Ib study of enzalutamide alone and in combination with endocrine therapies in women with advanced breast cancer. *Clinical Cancer Research 23*, 4046–4054. https://doi.org/10.1158/1078-0432.CCR-16-2339.

Sikora, M. J., Cordero, K. E., Larios, J. M., Johnson, M. D., Lippman, M. E., & Rae, J. M. (2009). The androgen metabolite 5α-androstane-3β,17β-diol (3βAdiol) induces breast cancer growth via estrogen receptor: Implications for aromatase inhibitor resistance. *Breast Cancer Research and Treatment 115*, 289–296. https://doi.org/10.1007/s10549-008-0080-8.

Sonne-Hansen, K., & Lykkesfeldt, A. E. (2005). Endogenous aromatization of testosterone results in growth stimulation of the human MCF-7 breast cancer cell line. *Journal of Steroid Biochemistry and Molecular Biology 93*, 25–34. https://doi.org/10.1016/j.jsbmb.2004.11.005.

Spinola, P. G., Marchetti, B., Mérand, Y., Bélanger, A., & Labrie, F. (1988). Effects of the aromatase inhibitor 4-hydroxyandrostenedione and the antiandrogen flutamide on growth and steroid levels in DMBA-induced rat mammary tumors. *Breast Cancer Research and Treatment 12*, 287–296. https://doi.org/10.1007/BF01811241.

van Staa, T. P., & Sprafka, J. M. (2009). Study of adverse outcomes in women using testosterone therapy. *Maturitas 62*, 76–80. https://doi.org/10.1016/j.maturitas.2008.11.001.

Stanbrough, M., Bubley, G. J., Ross, K., Golub, T. R., Rubin, M. A., Penning, T. M., ... Balk, S. P. (2006). Increased expression of genes converting adrenal androgens to testosterone in androgen-independent prostate cancer. *Cancer Research 66*, 2815–2825. https://doi.org/10.1158/0008-5472.CAN-05-4000.

Stewart, B., & Wild, C. (Eds.). (2014). *World cancer report 2014*. Lyon: International Agency for Research on Cancer/World Health Organization.

Swain, S. M., Baselga, J., Kim, S. B., Ro, J., Semiglazov, V., Campone, M., ... Cortés, J. (2015). Pertuzumab, trastuzumab, and docetaxel in HER2-positive metastatic breast cancer. *New England Journal of Medicine 372*, 724–734. https://doi.org/10.1046/NEJMoa413513.

Szelei, J., Jimenez, J., Soto, A. M., Luizzi, M. F., & Sonnenschein, C. (1997). Androgen-induced inhibition of proliferation in human breast cancer MCF7 cells transfected with androgen receptor. *Endocrinology 138*, 1406–1412. https://doi.org/10.1210/en.138.4.1406.

Takeshita, T., Omoto, Y., Yamamoto-Ibusuki, M., Yamamoto, Y., & Iwase, H. (2013). Clinical significance of androgen receptor and its phosphorylated form in breast cancer. *Endocrine-Related Cancer 20*, L15–L21. https://doi.org/10.1530/ERC-13-0317.

Teply, B. A., & Antonarakis, E. S. (2016). Novel mechanism-based therapeutics for androgen axis blockade in castration-resistant prostate cancer. *Current Opinion in Endocrinology, Diabetes and Obesity 23*, 279–290. https://doi.org/10.1097/MED.0000000000000254.

Tokunaga, E., Hisamatsu, Y., Taketani, K., Yamashita, N., Akiyoshi, S., Okada, S., ... Maehara, Y. (2013). Differential impact of the expression of the androgen receptor by age in estrogen receptor-positive breast cancer. *Cancer Medicine 2*, 763–773. https://doi.org/10.1002/cam4.138.

Traina, T. A., Miller, K., Yardley, D. A., Eakle, J., Schwartzberg, L. S., O'Shaughnessy, J., ... Cortes, J. (2018). Enzalutamide for the treatment of androgen receptor-expressing triple-negative breast cancer. *Journal of Clinical Oncology 36*, 884–890. https://doi.org/10.1200/JCO.2016.71.3495.

Tran, C., Ouk, S., Clegg, N. J., Chen, Y., Watson, P. A., Arora, V., ... Sawyers, C. L. (2009). Development of a second-generation antiandrogen for treatment of advanced prostate cancer. *Science 324*, 787–790. https://doi.org/10.1038/ncomms5988.

Tsang, J. Y. S., Ni, Y. B., Chan, S. K., Shao, M. M., Law, B. K. B., Tan, P. H., & Tse, G. M. (2014). Androgen receptor expression shows distinctive significance in ER positive and negative breast cancers. *Annals of Surgical Oncology 21*, 2218–2228. https://doi.org/10.1245/s10434-014-3629-2.

Venema, C. M., Mammatas, L. H., Schröder, C. P., van Kruchten, M., Apollonio, G., Glaudemans, A. W. J. M., ... Hospers, G. A. (2017). Androgen and estrogen receptor imaging in metastatic breast cancer patients as a surrogate for tissue biopsies. *Journal of Nuclear Medicine 58*, 1906–1912. https://doi.org/10.2967/jnumed.117.193649.

Walters, K. A., Simanainen, U., & Handelsman, D. J. (2010). Molecular insights into androgen actions in male and female reproductive function from androgen receptor knockout models. *Human Reproduction Update 16*, 543–558. https://doi.org/10.1093/humupd/dmq003.

Wang, Y., Romigh, T., He, X., Tan, M. H., Orloff, M. S., Silverman, R. H., ... Eng, C. (2011). Differential regulation of PTEN expression by androgen receptor in prostate and breast cancers. *Oncogene 30*, 4327–4338. https://doi.org/10.1038/onc.2011.144.

Wenhui, Z., Shuo, L., Dabei, T., Ying, P., Zhipeng, W., Lei, Z., ... Qingyuan, Z. (2014). Androgen receptor expression in male breast cancer predicts inferior outcome and poor response to tamoxifen treatment. *European Journal of Endocrinology 171*, 527–533. https://doi.org/10.1530/EJE-14-0278.

Wilson, E. M. (2011). Analysis of interdomain interactions of the androgen receptor. *Methods in Molecular Biology 776*, 113–129. https://doi.org/10.1007/978-1-61779-243-4.

Wilson, E. M., & French, F. S. (1976). Binding properties of androgen receptors. Evidence for identical receptors in rat testis, epididymis, and prostate. *The Journal of Biological Chemistry 251*, 5620–5629.

Yardley, D. A., Peacock, N., Young, R. R., Silber, A., Chung, G., Webb, C. D., ... Burris, H. (2016). A phase 2 study evaluating orteronel, an inhibitor of androgen biosynthesis, in patients with androgen receptor (AR)-expressing metastatic breast cancer: Interim analysis. *Cancer Research 76*(4 suppl) (abstr P5-14-04).

Yu, Q., Niu, Y., Liu, N., Zhang, J. Z., Liu, T. J., Zhang, R. J., ... Xiao, X. Q. (2011). Expression of androgen receptor in breast cancer and its significance as a prognostic factor. *Annals of Oncology 22*, 1288–1294. https://doi.org/10.1093/annonc/mdq586.

Zava, D. T., & McGuire, W. L. (1977). Estrogen receptors in androgen-induced breast tumor regression. *Cancer Research 37*, 1608–1610.

Zweifel, M., Thürlimann, B., Riniker, S., Weder, P., von Moos, R., Pagani, O., ... Sessa, C. (2017). Phase I trial of the androgen receptor modulator CR1447 in breast cancer patients. *Endocrine Connections 6*, 549–556. https://doi.org/10.1530/EC-17-0174.