Seminars in Cancer Biology xxx (xxxx) xxx–xxx

Contents lists available at ScienceDirect

Seminars in Cancer Biology

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

Review

Androgen receptor in breast cancer: A wolf in sheep's clothing? A lesson from prostate cancer.

Samanta Salvi[a], Massimiliano Bonafè[a,b], Sara Bravaccini[a,*]

[a] Istituto Scientifico Romagnolo per lo Studio e la Cura dei Tumori (IRST) IRCCS, Meldola, Italy
[b] Department of Experimental, Diagnostic and Specialty Medicine, Alma Mater Studiorum, University of Bologna, Bologna, Italy

ARTICLE INFO

Keywords:
Androgen receptor
In situ breast cancer
Invasive breast cancer
Prognostic and predictive biomarker
Therapeutic target

ABSTRACT

The possibility that a receptor for androgen is expressed in Breast Cancer (BC) is fascinating given that the tumor is predominantly estrogen-dependent.

The androgen receptor (AR) is emerging as a new marker and a potential new therapeutic target in the treatment of BC patients. The recent availability of selective AR inhibitors (*e.g.* bicalutamide, enzalutamide, apalutamide) approved for the treatment of prostate cancer has opened up the possibility to use them in BC patients whose tumors express AR. However, AR appears to have various functions according to the BC subtype, *e.g.* ER-positive or triple negative BC and the patient prognosis is different on the basis of the presence or absence of estrogen and progesterone receptors.

Moreover, a different AR expression was seen according to the various ethnicities. Of note, in population at low economical income, the availability of anti-AR compounds at low cost could open the possibility to treat AR-positive triple negative BC that are highly present in these populations.

Up to now, AR detection is not routinely performed in BC. The standardization of AR detection methods could render AR an easily detectable marker in primary BC and metastatic samples. Nevertheless, the overall concordance of 60% of AR expression in primary tumor and metastasis implies that a clinician who need the AR value to give anti-AR therapy should have the data on both the tumor materials.

Following the comprehensive studies on prostate cancer the possibility to test AR on liquid biopsies suggest the use of this biomarker for a real-time disease monitoring.

Finally, considering the possibility to treat patients with immune checkpoint inhibitors there is the need to know the relation between microenvironment and AR in BC.

1. Why androgen receptor?

The possibility that a receptor for androgen is expressed in Breast Cancer (BC) is fascinating given that the tumor is predominantly estrogen-dependent. However, the heterogeneity of the disease could explain why not all BC expressing hormones respond to hormonal treatments [1].

The androgen receptor (AR) is emerging as a new marker and a potential new therapeutic target in the treatment of BC patients. Circulating androgens are detected at physiological conditions in females, and their levels are different during life. However, the role of genomic or expression alterations of AR in relation to BC is not well known [1].

Researchers are currently trying to understand whether AR interferes with estrogen receptor (ER) and/or progesterone receptor (PgR) activities. AR is already a therapeutic target, and the recent availability of selective AR inhibitors (*e.g.* bicalutamide, enzalutamide, apalutamide) approved for the treatment of prostate cancer has opened up the possibility to use them in BC patients whose tumors express AR. However, AR appears to have different functions according to the BC subtype, *e.g.* ER-positive or triple negative BC.

This paper aimed to provide an overview of the role of AR detected by liquid biopsies and on tissues in relation to various BC subtypes.

2. AR structure and functions

AR gene is located on the chromosome Xq11-12. The receptor has three domains: an amino-terminal domain (NTD, residues 1–555), containing activation functional domains; a DNA binding domain (DBD, residues 555–623); and a carboxyl-terminal domain (CTD, residues

* Corresponding author at: IRST Srl IRCCS, Via Piero Maroncelli 40, 47014 Meldola FC Italy.
E-mail address: sara.bravaccini@irst.emr.it (S. Bravaccini).

https://doi.org/10.1016/j.semcancer.2019.04.002
Received 8 March 2019; Received in revised form 12 April 2019; Accepted 15 April 2019
1044-579X/

665–919) which including the ligand-binding domain (LBD). Moreover, a nuclear localization signal (NLS), which is the responsible for AR nuclear import, and a hinge region are located between DBD and CTD. AR protein is located in cytoplasm, in the absence of ligand, associated with heat-shock and other chaperone proteins. The binding of AR with androgens lead to a conformational change and exposure of NLS. The translocation of androgen/AR complex to the nucleus causes its dimerization and the binding to AREs, within classical target genes, to modulate gene transcription.

Moreover, AR could be activated also in ligand-independent manner by different growth factors, by phosphorylation or other modifications, or following interaction with co-activators [1].

3. A lesson from prostate cancer

Prostate cancer (PCa) is dependent on AR activation for growth and development; for this reason, androgen deprivation therapy (ADT) is the gold standard treatment in advanced PCa. AR upregulation is the most common event involved in the progression from hormone sensitive to castration-resistant prostate cancer (CRPC). In PCa setting several mechanisms responsible for AR transcriptional re-activation have been demonstrated, including mutation, amplification, or rearrangement of the *AR* gene, and elevated expression of truncated AR variants [2–5].

Various AR signaling-directed therapies, such as abiraterone, enzalutamide and more recently apalutamide have been developed. Abiraterone is a selective inhibitor of the enzyme cytochrome P450 involved in androgens biosynthesis, reducing the circulating testosterone levels in PCa [6]. Enzalutamide is a new anti-androgen with greater affinity for AR than abiraterone [7]. On February 14, 2018, the Food and Drug Administration approved apalutamide for patients with non-metastatic castration-resistant prostate cancer (NM-CRPC) but up to now none demonstrated its role on AR-positive BC. The availability of anti-AR compounds open the possibility to treat also AR-positive BC patients.

4. "The issue" of AR detection

4.1. Tissues approaches

AR is localized to the cytoplasm in the absence of androgen. AR translocates to the nucleus, upon ligand binding, where it can modulate transcription of AR-responsive genes. The withdrawal of androgen results in the export of unliganded AR from the nucleus to the cytoplasm, where it is transcriptionally inactive. AR is expressed in the nucleus of the cells but can be present also at cytoplasm.

The tissue approaches permit to detect the AR status at cellular level (nuclear and/or cytoplasmic) distinguishing epithelial cells from inflammatory cells and surrounding stroma.

Among the different methods to test AR both in primary tumor and in metastasis, immunohistochemistry (IHC) is the cheapest method and can be performed routinely in all laboratories (Fig. 1). Different way to classify AR-positive cases have been used as well as different cut off such as 1%, 10%, 50% and staining intensity 0, 1, 2, 3 + . In addition some authors have use H score (the product of percentage and staining intensity) to define AR positivity. Other methods to detect AR on tissue are the measure i) of the gene copy number (GCN) by Fluorescence *in situ* hybridization (FISH), ii) the mutational status of the AR gene by NGS and digital PCR approaches and iii) the *in situ* evaluation of mRNA by RNA scope (ACD Technology).

4.2. AR in ductal carcinoma in situ of the breast

AR is expressed in normal breast tissue, and expression decreases with advancement to Ductal Carcinoma *in situ* of the Breast (DCIS) and invasive cancer. AR has recently been shown to play an oncogenic or oncosuppressive role in cancer. Despite some studies in invasive BC have reported that AR expression is related to better survival when it is co-expressed with ER and PgR, its prognostic role in *in situ* BC has been never investigated.

For the first time retrospective analyses of DCIS relapsed and non-relapsed patients treated with quadrantectomy alone and/or quadrantectomy plus radiotherapy were recently performed [8–10]. AR and AR/ER in DCIS patients showed to have an unfavorable prognostic role independently of the treatment [8–10]. In particular, Tumedei and colleagues showed that the AR/ER ratio value in relapsed patients was statistically different from that of not relapsed patients ($p = 0.011$) and, at a cut off of 1.13, showed a sensitivity of 75% and a specificity of 94% for predicting relapse as *in situ* or invasive carcinoma. The ratio AR/PgR at a cut off of 1.00 has a sensitivity of 75% and specificity of 53%, while at a cut off of 3.00 has a sensitivity of 50% and a specificity of 84%. Moreover, while the single variables showed an Area Under the Curve (AUC) values from 52% to 77%, the ratio of AR/ER reached a very high AUC (92%). AR and ER play an important role in discriminating tumors which will relapse or not and can give important information in planning therapy. The hormonal variables together with AR, seem to be important prognostic tools able to increase the accuracy in terms of relapse prediction up to 92% for *in situ* tumors. As in clinical practice DCIS patients are treated almost exclusively with surgery and radiotherapy, the predictive role of specific markers, especially AR, on the clinical outcome in this population was investigated by the same group. The unfavorable prognostic role of AR and AR/ER was seen also in this subset of patients [9,10].

AR expression was seen in all grades of DCIS. Of the 72 positive AR cases, 21 (29%) were ER negative, corresponding to 10% (21/221) of all patients [11]. The majority of the AR-positive cases were high grade, and the most common histological subtype in this subset was a solid growth pattern with apocrine features. Early data from clinical trials evaluating AR antagonists in invasive/metastatic triple-negative BC suggest that some patients may benefit from androgen blockade.

Given that up to now the literature furnish data on the unfavorable prognostic role of AR, the role of AR as therapeutic target in DCIS has to

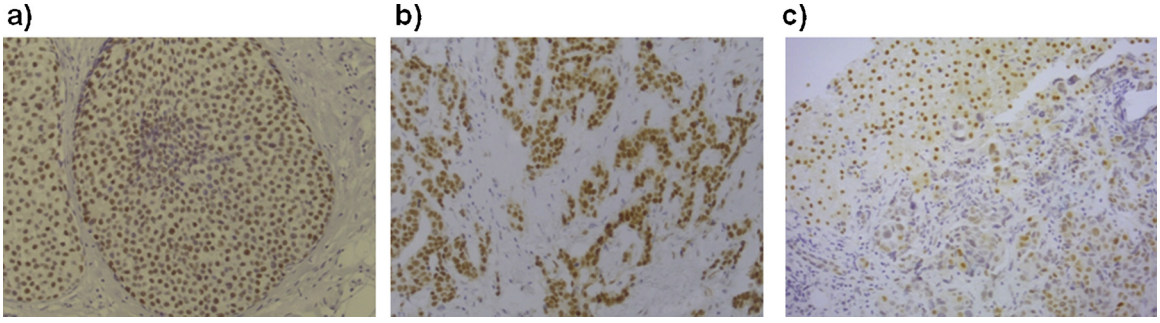

Fig. 1. AR expression by immunohistochemistry (20X magnification) on (a) ductal carcinoma *in situ* of the breast, (b) invasive primary tumor, and (c) metastatic BC sample.

be explored yet.

Moreover, Oshilaja and colleagues recently reported the usefulness of IHC testing and potential clinical trials of AR antagonists for chemoprevention in patients with AR-positive and ER-negative DCIS [11].

4.3. AR in invasive BC

Luminal BC have been reported to be positive for AR expression with higher level in Luminal A tumor and lower in Luminal B tumors respect to Her2 enriched and Triple Negative BC (TNBC) [12–15]. These findings are controversial because some of them described a role of AR status in predicting response rate and overall survival (OS) under hormonal treatment and at the same time they reported no association between AR expression and disease free survival in ER-positive tumors. In the same works ER status maintained the principal role as independent prognostic marker for disease free survival (DFS) [16–18]. However, for Cochrane and colleagues it seems to be an independent prognostic marker if hormone receptor are expressed while for Vera Badillo and colleagues its prognostic role seems to be independent from the expression of the hormonal receptors [19,20]. Thus, AR could be a wolf or a lamb on the bases of the BC subset in which it is evaluated. Kraby and colleagues demonstrated that AR was an independent predictor of good prognosis in BC, particularly in grade 3 and Luminal A tumors.

4.4. AR expression in TNBC

It appears that in ER-negative BC cells, AR acts in a more homogeneous way as compared to ER-positive BC cells. In these tumors the receptor clearly promotes cell proliferation and spreading by acting at different levels. This evidence depicts AR as a therapeutic target potentially very exploitable for TNBC and provides new opportunities for the treatment of this subtype of BC.

The role of AR as a prognostic/predictive biomarker in this subset of patients is controversial, but increasing evidence suggests that AR positive TNBC may respond to therapeutic agents targeting AR [21].

AR-positive TNBC was seen to be more common in older patients and in whom had a higher propensity for lymph node metastases (LNM). AR-positive TNBC may represent a BC subtype with unique features that may be amenable to treatment with alternative targeted therapies.

4.5. AR concordance between primary tumors and metastasis

Only few studies have been performed with the attempt to evaluate AR expression in primary tumor and metastasis.

Kutasovic and colleagues in a study of molecular profiling on BC metastasis to gynaecological organs identified novel AR mutations in the metastatic specimens [22].

Bronte and colleagues highlighted an overall concordance of AR detection between primary tumor and metastasis greater than 60%. This implies that a clinician who need the AR value to give anti AR therapy should have the data on both the tumor materials available given that AR status in primary tumor could be different respect to that of metastasis [15]. As mentioned in Kraby et al. paper, discordant AR expression data between primary tumor and LNM were observed in 21.4% of cases and most often there was a switch from AR-negative primary tumor to AR-positive axillary LNM [23].

5. AR predictive role

Patients with ER and AR-positive tumor have a better outcome than those with ER-positive and AR-negative disease [24]. This has been attributed to the competition between AR and ER at the level of estrogen response elements (EREs) and consequent impairment of ER-dependent gene transcription [25]. In fact, some studies highlighted that in ER-positive BC, AR could compete with ER-dependent transcription for the binding to the same sites or facilitating the ER binding to the DNA. In parallel, also in ER- and PgR-positive BC cells AR seems to compete [26]. Instead, in PgR-negative BC cells, AR increases the ER gene transcription providing a protumorigenic role [27]. The AR/ER ratio has been reported to impact prognosis and response to anti-estrogen endocrine therapy. For Cochrane and colleagues an high AR/ER ratio seems to be important to predict failure from Tamoxifen [20]. Bronte and colleagues assessed whether AR in primary tumors and/or matched metastases is a predictor of efficacy of first-line ET in advanced BC. AR status did not affect time to progression (TTP) significantly, whereas PgR and Ki67 status did. AR/PgR ≥ 0.96 was associated with a significantly shorter TTP (HR = 1.65, 95% CI 1.05–2.61, p = 0.028) [28]. AR status in primary tumors or metastases was not associated with progressive disease (PD) as best response. In contrast, Ki67 $\geq$ 20% and PgR $<$ 10% showed a statistically significant association with PD as best response. AR expression does not appear to be useful to predict the efficacy of ET in advanced BC, whereas Ki67 and PgR exert a greater impact on its efficacy [28]. AR can also predict response to AR inhibitors [29].

6. Liquid biopsy approaches

The need of biomarkers assessment by using noninvasive methods lead researchers to study and develop new approaches for AR testing on liquid biopsy. Circulating androgen can be detected with different concentrations in pre- and postmenopausal status. In particular, androgens levels decreased in menopause, even if is less drastic than the decrease in circulating levels of estrogen and progesterone [30].

The correlation between high androgens serum concentrations and BC risk is still controversial.

Several studies have been focused on the evaluation of AR aberrations on serum/plasma or urine in PCa setting, highlighted the correlation between copy number changes, mutations and splice variants identification with diagnosis, prognosis, tumor evolution monitoring and outcome prediction [2,5,6,31]. Regarding BC, few studies were conducted with the main aim to evaluate AR on liquid biopsy. Of note, as well as in PCa, BC circulating tumor cells (CTCs) were evaluated for the expression of the AR active splice variant of AR, called AR-v7, which lacks the ligand-binding domain. In BC, AR-v7 expression seems to be related to an increased number of bone metastasis [32]. Given the evidences on PCa, the detection of AR-v7 in CTCs could be is a potential predictive marker for abiraterone and enzalutamide efficacy also in BC setting [2]. Recently, in metastatic BC, AR mRNA expression was evaluated in CTCs finding 31% AR-positive samples. Moreover, 58% of matched CTC and primary tumor samples of different BC subtypes showed a discordance of AR status, concluding that the determination of AR expression in CTCs could help to select metastatic BC patients for AR inhibitors [33].

7. Anti-androgen therapies

Natural and synthetic steroidal androgens [34–37] have been used for therapeutic purpose. Steroidal androgens, however, induce many side effects [38]. The use of first and second generation AR-directed antagonists (bicalutamide and enzalutamide), is the most used therapy for advanced BC (Tamoxifen-resistent BCs and TNBCs) [39–41]. Both the antagonists have been used in clinical trials with positive results [42].

The most recent studies were conducted by using *in vitro* and *in vivo* experiments with the principal aim to test the dose, efficacy, safety, tolerability of different new potential anti-AR therapies alone and the combination with other drugs. In a phase 1 study of seviteronel, a selective CYP17 lyase and AR inhibitor, *in vitro* and *in vivo* anti-tumor activity was tested. In particular, the safety, tolerability, pharmacokinetics (PK), and activity of once-daily seviteronel were evaluated in

women with estrogen receptor-positive or TNBC, showing to be well tolerated [43]. Abiraterone acetate and seviteronel, CYP17A1 inhibitors, reduce the androgen production and the androgen levels and they are now being tested in phase 2 clinical trials (available from: https://clinicaltrials.gov/ct2/show/study/NCT02580448) [44], alone or in combination with AR-directed antagonists (available from: https://clinicaltrials.gov/ct2/show/NCT02605486). Preclinical and clinical findings, however, have indicated that AR stimulates the growth of TNBC or Her2 positive BC in combination with other effectors. Optimal results might be obtained by approaches in which AR antagonists are used in combination with inhibitors of these pathways [45–48].

The use of selective AR modulators (SARMs, *i.e.*, enobosarm GTx-024) represent the therapy of ER-positive advanced BCs. These compounds activate AR with scant side effects. Enobosarm, for instance, is giving favorable results (Overmoyer B) and is still investigated in a phase II clinical trials in patients with ER positive BC (available from: https://clinicaltrials.gov/ct2/show/NCT01889238).

Giovannelli P. and colleagues showed that in TNBC-derived cell lines (MDA-MB231 and MDA-MB453), expressing AR, S1 peptide could be a promising therapeutic option. In fact, it mimics AR proline-rich motif responsible for the interaction of AR with SH3-Src leading to the inhibition of motility and invasiveness of TNBC cells [49]. These *in vivo* findings suggest also that S1 peptide blocking should be considered as anti-AR strategy.

Other studies suggest to use combined therapeutic approaches to improve the treatment efficacy. For example, cell lines studies showed that AR enriched TNBC cell lines carry PI3KCA mutations acquire sensitivity to PI3K/mTOR inhibition, promoting the cancer cell growth [50,51].

The emerging researches have proved that poly (ADP-ribose) polymerase (PARP) inhibitor is effective in BRCA1-deficient BCs. Some authors demonstrated that combination of AR antagonist (bicalutamide) and PARP inhibitor (ABT-888) could inhibit cell viability and induce cell apoptosis significantly whatever *in vitro* or *in vivo* setting in AR-positive TNBC. Previous studies have proved that both BRCA1 and PARP1 have close connections with AR in prostate cancer. Jiayan Luo and colleagues analyzed the correlation among AR, PARP1 and BRCA1 in TNBC for the first time [52]. After BRCA1 overexpression, the expression of AR and PARP1 were decreased in mRNA and protein levels. Additionally, AR positively regulated PARP1 while PARP1 also upregulated AR expression *in vitro*. They confirmed that BRCA1 expression was negatively correlated with AR and PARP1 in TNBC patients using a tissue microarray with TNBC patient samples. These findings highlighted that the combination of bicalutamide and PARP inhibitor may be a potential strategy for TNBC patients and merits further evaluation. These results were recently confirmed by *in vivo* and *in vitro* experiments performed by Sang M. and colleagues on sporadic TNBC [53].

8. AR in BC in male

BC in male is a rare tumor with biological differences between female BC. Male BC is exclusively hormone receptor positive, also for AR. Male BC showed a prevalence of BRCA2 germline mutations. Di Oto and colleagues showed that X chromosome gain is related to increased AR expression in male BC [54]. X chromosome gain was observed in 74.7% of invasive duct carcinoma, in 20.6% of *in situ* duct carcinoma, and in 14.6% of gynecomastia when associated with cancer, while all cases of tumor-free gynecomastia showed wildtype X chromosome composition. AR IHC expression was observed in 100% of male BC tested. AR gene methylation status revealed low level or absence of methylation. These data suggest that X chromosome can play a role in the neoplastic transformation of male breast epithelium. X chromosome gain is paralleled by AR gene polysomy. Polysomic AR genes showed low methylation levels and high AR protein expression on IHC [54].

9. AR expression in the different ethnicities

Information on BC biomarkers is poor in the majority of low-resource countries, such as Sub-Saharan Africa. A different biology in terms of biomarker expression was previously seen in between Caucasian and Tanzanian BC patients [55].

For the first time a comparison of AR expression in Tanzanian and Caucasian BC patients was done demonstrating that AR expression in Tanzanian BC patients was lower than the Caucasian population in terms of percentage, H score, and staining intensity [56]. These findings were in agreement with Thike and colleagues that reported that the lower AR expression reflects the higher aggressiveness of tumors, but their study was performed in a different ethnicity, such as Asian population [57]. The lower AR expression in African respect to Caucasian patients might be a consequence of a major tumor aggressiveness (low hormonal receptor expression and highly proliferating tumors) and probably of a different carcinogenesis [56]. AR loss could represent an unfavorable prognostic marker in the African population.

The use of expensive drugs, such as monoclonal antibodies, is prohibitive for African patients. Given the high proportion of AR-positive TNBC, AR could represent a therapeutic target. The availability of cheaper drugs such as anti-AR compounds could open the possibility for the treatment also in this population at low economical income.

Davis and colleagues demonstrated in African American women that AR-negative triple negative or "quadruple negative" women have an enriched basal and immune signature suggesting that AR could be used as a prognostic marker for BC, particularly in this BC subtype [58].

AR expression was evaluated among internationally diverse patient populations by Jiagge and colleagues [59]. AR expression was higher in White American patients and decrease in African American, Ethiopian and Ghanaian patients albeit the difference was not statistically significant.

In a clinicopathological study from Jordan on the expression of AR in invasive ductal breast carcinomas a significant relationship of AR expression with ER status was found. AR expression was significantly associated with smaller tumor size. Although AR status was not independently associated with survival, their data suggest AR is a good prognostic factor [60].

10. Conclusions and future perspectives

PCa studies suggested AR as prominent prognostic and predictive marker. Given that the prognostic and predictive role of AR in BC is matter of debate, AR detection is not routinely performed. The standardization of IHC methods could render AR an easily detectable marker in primary BC and metastatic samples. The differences of AR expression between primary and metastatic tumor suggest that AR has to be detected in all biological material available for the patient considering also the different role of this biomarker in these two subsets of disease.

The need to compare AR status on different populations given the possibility to treat patients at low economical income with anti-AR compounds considering the low cost and the relatively high incidence of AR-positive TNBC, for example in Tanzanian population.

PCa evidences suggest that AR evaluation on liquid biopsy can be used to monitor the tumor evolution and find therapy for the patients for whom the biopsy of metastasis is not available. However, in this field, additional studies are needed to verify the usefulness of AR noninvasive analysis in BC and to define which type of AR alterations are more clinically relevant.

In order to improve the efficacy of the treatment, the evaluation of combined therapeutic approaches, such as anti-AR with PARP, mTOR, Her2 and immune checkpoint inhibitors, have to be better explored.

Conflict of interest statement

The authors declare that there are no conflicts of interest.

Funding

None.

References

[1] P. Giovannelli, M. Di Donato, G. Galasso, E. Di Zazzo, A. Bilancio, A. Migliaccio, The androgen receptor in breast cancer, Front. Endocrinol. (Lausanne) 9 (2018) 492.
[2] E.S. Antonarakis, C. Lu, H. Wang, B. Luber, M. Nakazawa, J.C. Roeser, et al., AR-V7 and resistance to enzalutamide and abiraterone in prostate cancer, N. Engl. J. Med. 371 (2014) 1028–1038.
[3] X. Zhang, S.Z. Hong, E.J. Lin, D.Y. Wang, Z.J. Li, L.I. Chen, Amplification and protein expression of androgen receptor gene in prostate cancer cells: fluorescence in situ hybridization analysis, Oncol. Lett. 9 (2015) 2617–2622.
[4] C. Henzler, Y. Li, R. Yang, T. McBride, Y. Ho, C. Sprenger, et al., Truncation and constitutive activation of the androgen receptor by diverse genomic rearrangements in prostate cancer, Nat. Commun. 7 (2016) 13668.
[5] B. De Laere, P.J. van Dam, T. Whitington, M. Mayrhofer, E.H. Diaz, G. Van den Eynden, et al., Comprehensive profiling of the androgen receptor in liquid biopsies from castration-resistant prostate cancer reveals novel intra-AR structural variation and splice variant expression patterns, Eur. Urol. 72 (2017) 192–200.
[6] J.S. de Bono, C.J. Logothetis, A. Molina, K. Fizazi, S. North, L. Chu, et al., Abiraterone and increased survival in metastatic prostate cancer, N. Engl. J. Med. 364 (2011) 1995–2005.
[7] H.I. Scher, K. Fizazi, F. Saad, M.E. Taplin, C.N. Sternberg, K. Miller, et al., Increased survival with enzalutamide in prostate cancer after chemotherapy, N. Engl. J. Med. 367 (2012) 1187–1197.
[8] M.M. Tumedei, R. Silvestrini, S. Ravaioli, I. Massa, R. Maltoni, A. Rocca, et al., Role of androgen and estrogen receptors as prognostic and potential predictive markers of ductal carcinoma in situ of the breast, Int. J. Biol. Markers 30 (2015) e425–8.
[9] S. Ravaioli, M.M. Tumedei, F. Foca, R. Maltoni, A. Rocca, I. Massa, et al., Androgen and oestrogen receptors as potential prognostic markers for patients with ductal carcinoma in situ treated with surgery and radiotherapy, Int. J. Exp. Pathol. 98 (2017) 289–295.
[10] S. Ravaioli, M. Puccetti, M.M. Tumedei, R. Silvestrini, L. Bedei, S. Bravaccini, Are androgen and estrogen receptors in DCIS patients prognostic indicators of relapse independently of treatment? Appl. Immunohistochem. Mol. Morphol. (2017).
[11] O. Oshilaja, L. Nomani, B.C. Calhoun, A.J. Montero, C.D. Sturgis, Androgen receptors in resected ductal carcinoma in situ of breast: novel insights with possible implications for testing and targeted endocrine chemoprevention trials, Appl. Immunohistochem. Mol. Morphol. (2018).
[12] Y.C. Wong, B. Xie, The role of androgens in mammary carcinogenesis, Ital. J. Anat. Embryol. 106 (2001) 111–125.
[13] B. Xie, S.W. Tsao, Y.C. Wong, Sex hormone-induced mammary carcinogenesis in female noble rats: the role of androgens, Carcinogenesis 20 (1999) 1597–1606.
[14] J.J. Park, R.A. Irvine, G. Buchanan, S.S. Koh, J.M. Park, W.D. Tilley, et al., Breast cancer susceptibility gene 1 (BRCAI) is a coactivator of the androgen receptor, Cancer Res. 60 (2000) 5946–5949.
[15] G. Bronte, S. Bravaccini, S. Ravaioli, M. Puccetti, E. Scarpi, D. Andreis, et al., Androgen receptor expression in breast cancer: what differences between primary tumor and metastases? Transl. Oncol. 11 (2018) 950–956.
[16] R.M. Bryan, R.J. Mercer, R.C. Bennett, G.C. Rennie, T.H. Lie, F.J. Morgan, Androgen receptors in breast cancer, Cancer 54 (1984) 2436–2440.
[17] V. Kuenen-Boumeester, T.H. Van der Kwast, C.C. Claassen, M.P. Look, G.S. Liem, J.G. Klijn, et al., The clinical significance of androgen receptors in breast cancer and their relation to histological and cell biological parameters, Eur. J. Cancer 32A (1996) 1560–1565.
[18] J.A. Soreide, O.A. Lea, J.E. Varhaug, A. Skarstein, S. Kvinnsland, Androgen receptors in operable breast cancer: relation to other steroid hormone receptors, correlations to prognostic factors and predictive value for effect of adjuvant tamoxifen treatment, Eur. J. Surg. Oncol. 18 (1992) 112–118.
[19] F.E. Vera-Badillo, A.J. Templeton, P. de Gouveia, I. Diaz-Padilla, P.L. Bedard, M. Al-Mubarak, et al., Androgen receptor expression and outcomes in early breast cancer: a systematic review and meta-analysis, J. Natl. Cancer Inst. 106 (2014) djt319.
[20] D.R. Cochrane, S. Bernales, B.M. Jacobsen, D.M. Cittelly, E.N. Howe, N.C. D'Amato, et al., Role of the androgen receptor in breast cancer and preclinical analysis of enzalutamide, Breast Cancer Res. 16 (2014) R7.
[21] K. Astvatsaturyan, Y. Yue, A.E. Walts, S. Bose, Androgen receptor positive triple negative breast cancer: clinicopathologic, prognostic, and predictive features, PLoS One 13 (2018) e0197827.
[22] J.R. Kutasovic, A.E. McCart Reed, R. Males, S. Sim, J.M. Saunus, A. Dalley, et al., Breast cancer metastasis to gynaecological organs: a clinico-pathological and molecular profiling study, J. Pathol. Clin. Res. 5 (2019) 25–39.
[23] M.R. Kraby, M. Valla, S. Opdahl, O.A. Haugen, J.E. Sawicka, M.J. Engstrom, et al., The prognostic value of androgen receptors in breast cancer subtypes, Breast Cancer Res. Treat. 172 (2018) 283–296.
[24] I. Bozovic-Spasojevic, D. Zardavas, S. Brohee, L. Ameye, D. Fumagalli, F. Ades, et al., The prognostic role of androgen receptor in patients with early-stage breast cancer: a meta-analysis of clinical and gene expression data, Clin. Cancer Res. 23 (2017) 2702–2712.
[25] E.F. Need, L.A. Selth, T.J. Harris, S.N. Birrell, W.D. Tilley, G. Buchanan, Research resource: interplay between the genomic and transcriptional networks of androgen receptor and estrogen receptor alpha in luminal breast cancer cells, Mol. Endocrinol. 26 (2012) 1941–1952.
[26] A.A. Peters, G. Buchanan, C. Ricciardelli, T. Bianco-Miotto, M.M. Centenera, J.M. Harris, et al., Androgen receptor inhibits estrogen receptor-alpha activity and is prognostic in breast cancer, Cancer Res. 69 (2009) 6131–6140.
[27] M.V. Karamouzis, K.A. Papavassiliou, C. Adamopoulos, A.G. Papavassiliou, Targeting androgen/estrogen receptors crosstalk in cancer, Trends Cancer 2 (2016) 35–48.
[28] G. Bronte, A. Rocca, S. Ravaioli, M. Puccetti, M.M. Tumedei, E. Scarpi, et al., Androgen receptor in advanced breast cancer: is it useful to predict the efficacy of anti-estrogen therapy? BMC Cancer 18 (348) (2018) 018-4239-3.
[29] T.A. Traina, K. Miller, D.A. Yardley, J. Eakle, L.S. Schwartzberg, J. O'Shaughnessy, et al., Enzalutamide for the treatment of androgen receptor-expressing triple-negative breast cancer, J. Clin. Oncol. 36 (2018) 884–890.
[30] J. Kotsopoulos, S.A. Narod, Androgens and breast cancer, Steroids 77 (2012) 1–9.
[31] V. Conteduca, D. Wetterskog, M.T.A. Sharabiani, E. Grande, M.P. Fernandez-Perez, A. Jayaram, S. Salvi, Androgen receptor gene status in plasma DNA associates with worse outcome on enzalutamide or abiraterone for castration-resistant prostate cancer: a multi-institution correlative biomarker study, Ann. Oncol. 28 (7) (2017) 1508–1516.
[32] N. Aceto, A. Bardia, B.S. Wittner, M.C. Donaldson, R. O'Keefe, A. Engstrom, et al., AR expression in breast cancer CTCs associates with bone metastases, Mol. Cancer Res. 16 (2018) 720–727.
[33] I.E. de Kruijff, A.M. Sieuwerts, W. Onstenk, A. Jager, P. Hamberg, F.E. de Jongh, et al., Androgen receptor expression in circulating tumor cells of patients with metastatic breast cancer, Int. J. Cancer (2019) [Epub ahead of print].
[34] A.A. Loeser, Stilboestrol: therapeutic trials, Br. Med. J. 1 (1939) 13.
[35] J.B. Herrmann, F.E. Adair, The effect of testosterone propionate on carcinoma of the female breast with soft tissue metastases, J. Clin. Endocrinol. Metab. 6 (1946) 769–775.
[36] F.M. Muggia, P.A. Cassieth, M. Ochoa Jr, F.A. Flatow, A. Gellhorn, G.A. Hyman, Treatment of breast cancer with medroxyprogesterone acetate, Ann. Intern. Med. 68 (1968) 328–337.
[37] M. Kono, T. Fujii, G.R. Lyons, L. Huo, R. Bassett, Y. Gong, et al., Impact of androgen receptor expression in fluoxymesterone-treated estrogen receptor-positive metastatic breast cancer refractory to contemporary hormonal therapy, Breast Cancer Res. Treat. 160 (2016) 101–109.
[38] C.C. Coss, A. Jones, J.T. Dalton, Selective androgen receptor modulators as improved androgen therapy for advanced breast cancer, Steroids 90 (2014) 94–100.
[39] D.M. Harvell, N.S. Spoelstra, M. Singh, J.L. McManaman, C. Finlayson, T. Phang, et al., Molecular signatures of neoadjuvant endocrine therapy for breast cancer: characteristics of response or intrinsic resistance, Breast Cancer Res. Treat. 112 (2008) 475–488.
[40] F. De Amicis, J. Thirugnansampanthan, Y. Cui, J. Selever, A. Beyer, I. Parra, et al., Androgen receptor overexpression induces tamoxifen resistance in human breast cancer cells, Breast Cancer Res. Treat. 121 (2010) 1–11.
[41] R. Huang, J. Han, X. Liang, S. Sun, Y. Jiang, B. Xia, et al., Androgen receptor expression and bicalutamide antagonize androgen receptor inhibit beta-catenin transcription complex in estrogen receptor-negative breast cancer, Cell. Physiol. Biochem. 43 (2017) 2212–2225.
[42] C. Arce-Salinas, M.C. Riesco-Martinez, W. Hanna, P. Bedard, E. Warner, Complete response of metastatic androgen receptor-positive breast cancer to bicalutamide: case report and review of the literature, J. Clin. Oncol. 34 (2016) e21–4.
[43] A. Bardia, A. Gucalp, N. DaCosta, N. Gabrail, M. Danso, H. Ali, 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 Res. Treat. 171 (1) (2018) 111–120.
[44] H. Bonnefoi, T. Grellety, O. Tredan, M. Saghatchian, F. Dalenc, A. Mailliez, et al., 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), Ann. Oncol. 27 (2016) 812–818.
[45] G. Bianchini, J.M. Balko, I.A. Mayer, M.E. Sanders, L. Gianni, Triple-negative breast cancer: challenges and opportunities of a heterogeneous disease, Nat. Rev. Clin. Oncol. 13 (2016) 674–690.
[46] D.W. Fry, P.J. Harvey, P.R. Keller, W.L. Elliott, M. Meade, E. Trachet, et al., Specific inhibition of cyclin-dependent kinase 4/6 by PD 0332991 and associated antitumor activity in human tumor xenografts, Mol. Cancer Ther. 3 (2004) 1427–1438.
[47] B.D. Lehmann, J.A. Bauer, J.M. Schafer, C.S. Pendleton, L. Tang, K.C. Johnson, et al., PIK3CA mutations in androgen receptor-positive triple negative breast cancer confer sensitivity to the combination of PI3K and androgen receptor inhibitors, Breast Cancer Res. 16 (2014) 406 014-0406-x.
[48] A.J. Robles, S. Cai, R.H. Cichewicz, S.L. Mooberry, Selective activity of deguelin identifies therapeutic targets for androgen receptor-positive breast cancer, Breast Cancer Res. Treat. 157 (2016) 475–488.
[49] P. Giovannelli, M. Di Donato, F. Auricchio, G. Castoria, A. Migliaccio, Androgens induce invasiveness of triple negative breast cancer cells through AR/Src/PI3-K complex assembly, Sci. Rep. 9 (1) (2019) 4490.
[50] V.N. Barton, N.C. D'Amato, M.A. Gordon, J.L. Christenson, A. Elias, J.K. Richer, Androgen receptor biology in triple negative breast cancer: a case for classification as AR+ or quadruple negative disease, Horm. Cancer 6 (2015) 206–213.
[51] B. Rahim, R.A.R. O'Regan, Signaling in breast cancer, Cancers (Basel) 9 (10) (2017) 3390/cancers9030021.
[52] J. Luo, J. Jin, F. Yang, Z. Sun, W. Zhang, Y. Shi, et al., The correlation between

PARP1 and BRCA1 in AR positive triple-negative breast cancer, Int. J. Biol. Sci. 12 (2016) 1500–1510.
[53] M. Sang, L. Meng, C. Ma, S. Liu, M. Sang, S. Chang, et al., Effect of AR antagonist combined with PARP1 inhibitor on sporadic triple-negative breast cancer bearing AR expression and methylation-mediated BRCA1 dysfunction, Biomed. Pharmacother. 111 (2019) 169–177.
[54] E. Di Oto, G.B. Biserni, Z. Varga, L. Morandi, M.C. Cucchi, R. Masetti, et al., X chromosome gain is related to increased androgen receptor expression in male breast cancer, Virchows Arch. 473 (2018) 155–163.
[55] D. Amadori, P. Serra, S. Bravaccini, A. Farolfi, M. Puccetti, E. Carretta, et al., Differences in biological features of breast cancer between Caucasian (Italian) and African (Tanzanian) populations, Breast Cancer Res. Treat. 145 (2014) 177–183.
[56] S. Bravaccini, S. Ravaioli, D. Amadori, E. Scarpi, M. Puccetti, A. Rocca, et al., Are there differences in androgen receptor expression in invasive breast cancer in African (Tanzanian) population in comparison with the Caucasian (Italian) population? Front. Endocrinol. (Lausanne) 9 (2018) 137.
[57] A.A. Thike, L. Yong-Zheng Chong, P.Y. Cheok, H.H. Li, G. Wai-Cheong Yip, B. Huat Bay, et al., Loss of androgen receptor expression predicts early recurrence in triple-negative and basal-like breast cancer, Mod. Pathol. 27 (2014) 352–360.
[58] M. Davis, S. Tripathi, R. Hughley, Q. He, S. Bae, B. Karanam, et al., AR negative triple negative or "quadruple negative" breast cancers in African American women have an enriched basal and immune signature, PLoS One 13 (2018) e0196909.
[59] E. Jiagge, A.S. Jibril, M. Davis, C. Murga-Zamalloa, C.G. Kleer, K. Gyan, et al., Androgen receptor and ALDH1 expression among internationally diverse patient populations, J. Glob. Oncol. 4 (2018) 1–8.
[60] F.N. Obeidat, M. Ahram, A. Al-Khader, S.A. Mbaideen, H. Hassan, B. Altarawneh, et al., Expression of androgen receptor in invasive ductal breast carcinomas: a clinicopathological study from Jordan, Ann. Saudi Med. 38 (2018) 326–335.