Pushing estrogen receptor around in breast cancer

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Abstract

The estrogen receptor-α (herein called ER) is a nuclear sex steroid receptor (SSR) that is expressed in approximately 75% of breast cancers. Therapies that modulate ER action have substantially improved the survival of patients with ER-positive breast cancer, but resistance to treatment still remains a major clinical problem. Treating resistant breast cancer requires co-targeting of ER and alternate signalling pathways that contribute to resistance to improve the efficacy and benefit of currently available treatments. Emerging data have shown that other SSRs may regulate the sites at which ER binds to DNA in ways that can powerfully suppress the oncogenic activity of ER in breast cancer. This includes the progesterone receptor (PR) that was recently shown to reprogram the ER DNA binding landscape towards genes associated with a favourable outcome. Another attractive candidate is the androgen receptor (AR), which is expressed in the majority of breast cancers and inhibits growth of the normal breast and ER-positive tumours when activated by ligand. These findings have led to the initiation of breast cancer clinical trials evaluating therapies that selectively harness the ability of SSRs to 'push' ER towards anti-tumorigenic activity. Our review will focus on the established and emerging clinical evidence for activating PR or AR in ER-positive breast cancer to inhibit the tumour growth-promoting functions of ER.

Key Words
- estrogen receptor
- endocrine therapy
- endocrine resistance
- breast cancer
- progesterone receptor
- androgen receptor

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Introduction

There are three major sex steroid hormones – estrogen, progesterone and androgen – and each affect the signalling activity of its cognate sex steroid receptor (SSR), that is the estrogen receptor-α (ER), progesterone receptor (PR) and androgen receptor (AR), respectively. In women, sex steroid hormones are produced by the ovaries (and adrenal glands in the case of androgens) and through peripheral conversion of circulating precursors (Simpson 2003, Nicolas Diaz-Chico *et al*. 2007, McNamara *et al*. 2014, McNamara & Sasano 2015). SSRs are structurally related and evolutionarily conserved, have similar consensus DNA-binding motifs and use common co-factors for activity (Germain *et al*. 2006).

ER is expressed in approximately 75% of all breast cancers. When present, ER drives neoplasia and is a *bona fide* therapeutic target. The underlying aim of current endocrine therapy is to either reduce ER activity or reduce receptor levels within breast cancer cells.

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Despite the success of ER-directed treatments, a significant proportion of patients with ER-positive breast cancer relapse from their cancer due to inherent or acquired resistance to endocrine therapy. It has been recently shown that endocrine resistance may be the result of genetic and epigenetic factors (Ellis *et al*. 2012, Fuqua *et al*. 2014, Jeselsohn *et al*. 2015, Stone *et al*. 2015). Gain-of-function mutations in *ESR1*, the gene encoding the ER, are a relatively rare event in primary breast cancer (Cancer Genome Atlas Network 2012), but can be detected using next-generation sequencing in approximately 20% of patients with metastatic ER-positive disease who had received prior endocrine therapies (Robinson *et al*. 2013, Toy *et al*. 2013, Fuqua *et al*. 2014, Jeselsohn *et al*. 2015). A higher prevalence (up to 55%) of *ESR1* mutations has been reported in circulating free DNA (cfDNA) in metastatic ER-positive breast cancers with prior AI therapy when using digital droplet PCR to increase mutation detection sensitivity (Chandarlapaty *et al*. 2016, Fribbens *et al*. 2016, Spoerke *et al*. 2016). These mutations cluster in the ligand-binding domain of the ER and lead to ligand-independent ER activity that promotes tumour growth, partial resistance to endocrine therapy and potentially enhanced metastatic capacity. It has also been shown that DNA hypermethylation of estrogen-responsive elements can result in reduced ER binding and decreased gene expression of key regulators of ER activity and resistance to ER-directed therapies (Stone *et al*. 2015).

The clinical challenge of disease recurrence after ER-directed therapy (endocrine therapy) has led to increased attention being focussed on combining endocrine therapy with novel agents that target resistance mechanisms. Although many of these novel therapies have increased the time to progression, they are not curative and the development of resistance ultimately limits their use. Therefore, further scientific and clinical research into new endocrine therapies as well as strategies to enhance the effectiveness of currently available endocrine agents are essential to improve outcomes in both early and metastatic disease. In this regard, the high level of co-expression of PR and AR in ER-positive breast cancer makes these sex steroid receptors attractive targets for broad-based therapeutic intervention. The purpose of this review is to examine the emerging evidence for inter-connected roles of ER, PR and AR, and discuss how modulating PR and AR may be an effective therapeutic strategy for sex hormone receptor-positive breast cancer.

ER-directed therapies and targeted combination therapies

Current ER-directed strategies involve disrupting the process of estrogen production or modulating either the function or level of ER in breast cancer cells. In pre-menopausal women, the majority of circulating estrogen comes from the ovarian follicles, stimulated by luteinizing hormone (LH) and follicle-stimulating hormone (FSH) secreted by the anterior pituitary gland. Pituitary production of LH and FSH is controlled by secretion of GnRH (also known as luteinising-hormone-releasing hormone) from the hypothalamus. In the postmenopausal setting, estrogen production is dependent on peripheral aromatisation of circulating pro-androgens, predominantly in the liver, adrenal glands and adipose tissue. Whatever the source, estrogen exerts its effect via binding to ER, which in turn binds DNA to directly regulate the transcription of target genes. Endocrine therapy is aimed at modulating and disrupting these processes either by blocking pituitary production of LH/FSH (GnRH analogues), blocking ER (tamoxifen), degrading ER (fulvestrant) or by inhibiting the peripheral production of estrogen (aromatase inhibitors).

All women diagnosed with ER-positive BC should be considered for endocrine therapy. The introduction and widespread use of adjuvant tamoxifen and subsequently aromatase inhibitors (AIs) in the postmenopausal population has resulted in significant improvements in the overall survival of women with ER-positive early BC (Dowsett *et al*. 2010, Early Breast Cancer Trialists' Collaborative Group 2011). Therefore, ER-directed therapies represent a cornerstone strategy in the management of ER-positive breast cancers. In spite of this, approximately 30% of patients will experience relapse due to inherent or acquired-resistance to the above-mentioned therapies. In the metastatic setting, the introduction of sequential lines of different endocrine therapies involving aromatase inhibitors and fulvestrant has led to stepwise improvements in disease control and outcomes for women with metastatic ER-positive disease (Lonning 2000, Mehta *et al*. 2012, Robertson *et al*. 2012).

Alternative target therapies have recently been used in combination with ER-directed therapies to improve survival outcomes, representing a major advance in the treatment options for patients with metastatic ER-positive breast cancer. These include drugs that target the phosphatidylinositol-4,5-bisphosphate 3-kinase (PI3K) cell signalling pathway. One such drug, which is FDA approved for use in advanced breast cancer,

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is everolimus, an inhibitor of mammalian target of rapamycin (mTOR), which is downstream of PI3K. The combination of everolimus with ER-directed therapies has approximately doubled the duration of progression-free survival compared with ER-directed therapies alone (Bachelot *et al*. 2012, Baselga *et al*. 2012, Piccart *et al*. 2014). Conversely, the addition of a pan-PI3K inhibitor to fulvestrant has demonstrated either no improvement in clinical outcome (Krop *et al*. 2016) or a very modest overall effect with those patients with PIK3CA mutations detectable in cell-free DNA deriving the greatest benefit (Baselga *et al*. 2016).

Another class of drugs that has demonstrated clinical efficacy in the treatment of metastatic breast cancer is the inhibitors of cyclin-dependent kinases 4 and 6 (CDK 4/6), which regulate cell cycle progression. Cyclin D-CDK4/6-INK4-Rb pathway activation is a feature of endocrine-resistance breast cancer (Thangavel *et al*. 2011). *CCND1* gene amplification and overexpression of the cyclin D protein are found in a significant proportion of ER-positive breast cancer (Cancer Genome Atlas Network 2012). Co-administration of palbociclib, a CDK 4/6 inhibitor, with ER-directed therapies approximately doubles progression-free survival compared with ER-directed therapy alone both in patients receiving first-line metastatic therapy, and patients with metastatic breast cancer that progressed on previous endocrine therapy (Finn *et al*. 2015, 2016, Turner *et al*. 2015). The results of similarly designed large phase III trials of other CDK 4/6 inhibitors, including ribociclib (Clinicaltrials.gov identifiers NCT01958021 and NCT02278120) and abemaciclib (Clinicaltrials.gov identifiers NCT02107703 and NCT02246621) in combination with endocrine therapy would be reported in the near future.

PR-directed strategies for treating breast cancer

PR is expressed in a large proportion of ER-positive breast cancer (Nadji *et al*. 2005), and elevated PR levels have been shown to correlate with increased probability of response to endocrine therapy, longer time to treatment failure, and longer overall survival, independent of ER expression (Ravdin *et al*. 1992, Bardou *et al*. 2003, Purdie *et al*. 2014, Koornstra *et al*. 2015). Multivariate analyses have demonstrated that PR expression is prognostic compared with biomarkers such as ER and HER2, but less so in node-negative breast cancer (Fisher *et al*. 1988, Cuzick *et al*. 2011). However, PR expression does not influence the relative benefit of adjuvant AI therapy over tamoxifen in ER-positive breast cancers (Viale *et al*. 2007, Early Breast Cancer Trialists' Collaborative Group 2015). ER drives the expression of PR, and this serves as a well-established biomarker of ER functionality. The functional role of PR in breast cancer on the other hand, has not been as extensively investigated as ER.

The use of progesterone for the treatment of breast cancer was limited by the need for intramuscular injection leading to the development of synthetic orally available progestogens (Stoll 1967). Subsequent clinical trials of the synthetic progestogens megestrol acetate (megace) and medroxyprogesterone acetate (MPA) demonstrated consistent benefit in women with advanced ER-positive breast cancer (Table 1). In the context of failure of prior endocrine therapy, median durations of response of up to 10 months have been reported (Brufman *et al*. 1994, Birrell *et al*. 1995*b*, Abrams *et al*. 1999, Bines *et al*. 2014). Progestins have similar efficacy to tamoxifen, oophorectomy and aminoglutethimide (an AI and inhibitor of cholesterol conversion to steroids) in comparative trials in the metastatic setting (Ingle *et al*. 1982, Ettinger *et al*. 1986, van Veelen *et al*. 1986, Canney *et al*. 1988, Muss *et al*. 1988, 1994, Lundgren *et al*. 1989, Martoni *et al*. 1991). An adjuvant study in high-risk breast cancer demonstrated no difference in outcomes between tamoxifen for 1 year, tamoxifen for 2 years or tamoxifen for 6 months followed by megestrol acetate for 6 months (Andersen *et al*. 2008). Moreover, a study of a single injection of 500 mg of hydroxyprogesterone before surgery in postmenopausal women demonstrated an improvement in outcomes overall, with a significant improvement in patients with lymph node metastases (Badwe *et al*. 2011).

Interestingly, AR was shown to be a positive biomarker in predicting response to MPA, suggesting that its action in breast cancer may be mediated in part by AR, or indeed that AR may be a key determinant of endocrine responsiveness generally (Birrell *et al*. 1995*b*). In spite of the above-mentioned findings and more recent data showing that progesterone inhibits estrogen-stimulated growth in patient-derived xenograft models of ER- and PR-positive breast cancer (Kabos *et al*. 2012), progestogens have now been supplanted by newer therapies targeting ER alone or in combination with other emerging non-ER-directed therapies in the treatment of advanced breast cancer.

Another PR targeting strategy that has been evaluated in breast cancer is the use of PR antagonists such as mifepristone (RU486) and onapristone. Trials with these agents have been limited to small sample sizes, and the response rates have been modest

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Table 1 Clinical trials using progesterone receptor agonists and antagonists in the treatment of advanced breast cancer.

Treatment	No. of patients	Median duration of response	Objective response and clinical benefit rate	Reference
Progesterone receptor antagonists				
Mifepristone, *2nd/3rd line*	22	–	CBR: 54%, 18% after 3 months	Romieu *et al.* (1987)
Mifepristone, *1st line*	28	–	ORR: 10.7%	Perrault *et al.* (1996)
Lonaprisan, *2nd line*	68	–	CBR: 21% (25 mg); 7% (100 mg)	Jonat *et al.* (2013)
Progestins				
MPA	52	–	ORR: 43%. CBR 63%	Pannuti *et al.* (1978)
Megace, *1st/2nd line*	172	HD, 8 months; LD, 3.2 months (P=0.019)	ORR: HD, 27%; LD, 10% (P=0.005)	Muss *et al.* (1990)
Progestin treatment after specific previous treatment failure				
Megace after TAM/AG, *2nd line*	73	9–10 months	ORR: 4% CBR: 52%	Brufman *et al.* (1994)
MPA after TAM, *2nd line*	83	9.7 months	ORR: 38.6%	Birrell *et al.* (1995*b*)
Megace after NSAI, *2nd line*	48	10 months	ORR: 0% CBR: 39.6%	Bines *et al.* (2014)
Progestins in comparative studies				
Megace vs TAM, *1st line*	55	MA, 65 days; TAM, 58 days	CBR: MA, 14%; TAM, 26%: (NS)	Ingle *et al.* (1982)
Megace vs TAM, *1st line*	190	–	CBR: MA, 35%; TAM, 42%	Ettinger *et al.* (1986)
MPA vs TAM, *1st line*	129	MPA, 17 months; TAM, 23 months (NS)	CBR: MPA, 44%; TAM, 35%: (NS)	van Veelen *et al.* (1986)
MPA vs AG, *2nd line*	218	MPA, 42 weeks; AG, 44 weeks (NS)	ORR: MPA, 31%; AG, 27% (NS) CBR: MPA, 54%; AG, 51% (NS)	Canney *et al.* (1988)
Megace vs TAM, *1st/2nd line*	136	MA, 7.7 months; TAM, 7.7 months (NS)	ORR: MA, 28%; TAM, 31%	Muss *et al.* (1988)
Megace vs AG, *2nd line*	150	MA, 13 months; AG, 13.1 months	ORR: MA, 31%; AG, 34%	Lundgren *et al.* (1989)
MPA vs oophorectomy, *2nd line*	40	MPA, 9 months; OPX, 7 months	ORR: MPA, 55%; OPX, 33% (P=0.17)	Martoni *et al.* (1991)
MPA vs TAM, *1st line*	166	MPA, 6.3 months; TAM, 5.5 months (P=0.48)	ORR: MPA, 34%; TAM, 17% (P=0.01)	Muss *et al.* (1994)

MPA, medroxyprogesterone acetate; Megace, megestrol acetate; TAM, tamoxifen; AG, aminoglutethimidine; LD/HD, low/high Dose; OPX, oophorectomy; CBR, clinical benefit rate; ORR, objective response rate; NS, not significant.

Many clinical trials have been conducted that have studied the effects of progestins alone and in combination with other therapies in the treatment of advanced breast cancer. Table 1 presents a sample of studies accruing more than 20 patients. In general, progestins show comparable efficacy to other endocrine therapies in 1st and 2nd line treatment of advanced breast cancer.

(Romieu *et al.* 1987, Klijn *et al.* 1989, Perrault *et al.* 1996). Onapristone was associated with hepatic toxicity, and the study was prematurely terminated (Robertson *et al.* 1999). Hence, this class of therapy has not progressed to routine clinical use.

Role of AR in breast cancer

Overall, AR is the most prevalent SSR in all stages of breast cancer, occurring in up to 90% of primary tumours and 75% of metastatic breast cancer (Lea *et al.* 1989, Moinfar *et al.* 2003, Park *et al.* 2010, Cimino-Mathews *et al.* 2012, Honma *et al.* 2012, Ren *et al.* 2013). The frequency of AR expression varies between breast cancer subtypes, with ER-positive cancers more likely to be AR-positive compared with ER-negative cancers (Gonzalez-Angulo *et al.* 2009, Peters *et al.* 2009, Micello *et al.* 2010, Niemeier *et al.* 2010, Park *et al.* 2010, Collins *et al.* 2011, Hu *et al.* 2011, Loibl *et al.* 2011, Yu *et al.* 2011). Some studies have reported AR expression based on luminal sub-types with luminal A cancers expressing AR more frequently than luminal B (Collins *et al.* 2011, Yu *et al.* 2011). AR and ER co-localize at select genomic loci within the nuclei of breast cancer cells (Peters *et al.* 2009) and AR expression in ER-positive cancers has been associated with favourable clinicopathological characteristics such

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as older age at diagnosis, lower tumour grade, lower Ki-67 positivity, smaller tumours and less necrosis (Castellano *et al*. 2010, Hu *et al*. 2011, Witzel *et al*. 2013), as well as being associated with response to endocrine therapy and chemotherapy (Gonzalez-Angulo *et al*. 2009, Peters *et al*. 2009, Niemeier *et al*. 2010, Hu *et al*. 2011, Honma *et al*. 2012, Witzel *et al*. 2013). However, the prognosis of women with AR-positive breast cancer is exquisitely context dependent. When breast cancer is classified into molecular subgroups, AR expression is a consistent independent predictor of breast cancer survival only in the context of ER-positive disease (Luminal A and B subtypes) (Poulin *et al*. 1989, Birrell *et al*. 1998, Panet-Raymond *et al*. 2000, Peters *et al*. 2009, Castellano *et al*. 2010, Park *et al*. 2010, Hu *et al*. 2011). This reflects the anti-proliferative, anti-estrogenic influence of AR signalling in nearly all pre-clinical models of ER-positive breast cancer (Lanzino *et al*. 2005) and the powerful ability of androgens to suppress ER-mediated growth of the normal mammary epithelium (Peters *et al*. 2011).

The proposed use of androgens as a possible treatment for breast cancer dates as far back as 1939 (Ulrich 1939). Data regarding the inhibitory effect of androgens in pre-clinical models of breast cancer are supported by the clinical efficacy of androgen therapies such as methyl-testosterone and fluoxymesterone in the treatment of metastatic breast cancer, where disease regression was reported in up to 30% of cases (Adair & Herrmann 1946, Kennedy 1958, Goldenberg & Hayes 1961, Goldenberg 1964, Manni *et al*. 1981).

Role of AR in ER-negative breast cancer

AR action critically depends on context, in particular whether ER is expressed or not. AR is an inconsistent biomarker of survival in the ER-negative context (Agoff *et al*. 2003, Doane *et al*. 2006, Peters *et al*. 2009, Hu *et al*. 2011) and AR exhibits a plasticity of action in models of ER-negative breast cancer in which both oncogenic and tumour suppressive effects have been reported, even in the same model (Hickey *et al*. 2012, Lim *et al*. 2014, Chia *et al*. 2015).

In a small proportion of ER-negative breast cancers known as molecular apocrine or luminal AR subtype, AR has been shown to genomically mimic the oncogenic actions of ER (Robinson *et al*. 2011). In breast cancer cell line models of molecular apocrine breast cancer, AR activates oncogenic Wnt and HER2 signalling via transcriptional induction of key proteins within those pathways (Ni *et al*. 2011). AR-associated oncogenic activity is dependent upon overexpression of the pioneer factor FOXA1 and altered interaction with other nuclear proteins. It has also been shown that different AR ligands exert opposing growth responses in the same ER-negative breast cancer cell line (Moore *et al*. 2012), potentially via ligand-specific differential recruitment of nuclear co-factors. It is important to note that the aforementioned studies rely heavily on a single cell line model of molecular apocrine breast cancer, namely MDA-MB-453, which has a mutation in the ligand-binding domain of AR that compromises receptor stability and potentially the response to androgenic ligands (Moore *et al*. 2012). This highlights a need to develop better models of apocrine breast cancer to fully understand the basis of AR-driven breast cancer growth in an ER-negative context. Currently there are several clinical trials investigating the efficacy of AR-directed therapies in cohorts of triple-negative breast cancer (TNBC, cancers that do not express ER, PR or HER2) (Gucalp *et al*. 2013, Traina *et al*. 2015). For the purposes of this review, we will concentrate on the role of AR and AR-directed therapy in ER-positive breast cancer.

AR in ER-positive breast cancer

In ER-positive breast cancer, the outcome of cross-talk between AR and ER appears to depend on their expression ratio. Although clinical and pre-clinical evidence overwhelmingly supports a tumour suppressive role for AR signalling in treatment-naïve ER-positive breast cancer, the MCF7 breast cancer cell line stands out as an exception, exhibiting AR-mediated proliferative effects dependent upon culture conditions. MCF7 cells typically overexpress ER and have low levels of AR, although this may depend on cell culture conditions resulting in fluctuations in AR activity. It has been shown that the AR antagonist enzalutamide inhibits estrogen-stimulated growth of MCF7 cells grown as tumour xenografts (Cochrane *et al*. 2014), in part by restricting AR nuclear uptake (D'Amato *et al*. 2016), whereas others have shown androgen-induced inhibition of ER-positive breast cancer cell proliferation (Birrell *et al*. 1995*a*). When ectopically overexpressed in MCF7 cells, AR exerts a robust anti-proliferative effect, in part due to altered interactions of ER and AR with a common transcriptional co-factor, ARA70 (Lanzino *et al*. 2005, Peters *et al*. 2009). In other ER-positive breast cancer cell lines (e.g. ZR75-1, T47D) that express AR and ER in more equal proportions, AR activation consistently inhibits proliferation (reviewed in Hickey *et al*. 2012). This also occurs in vivo, whereby androgen treatment delayed the onset of DMBA-induced rat mammary carcinomas

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(Zava & McGuire 1977), whereas genetic ablation of AR hastened the onset of HER2- or DMBA-induced mouse mammary tumours in mice (Simanainen *et al*. 2012, Hodgson *et al*. 2013). Collectively, these data support the notion that the AR:ER ratio critically reflects the proliferative outcome of sex steroid hormone crosstalk in luminal breast cancers (Birrell *et al*. 2007).

In the setting of tamoxifen-resistant ER-positive breast cancer, there are conflicting studies with one study reporting that high AR conferred a survival advantage in a consecutive series of over 900 breast cancers (Castellano *et al*. 2010), and another in a cohort of 192 cases reporting the opposite (Cochrane *et al*. 2014). However, the latter study compared the relative outcome of a tamoxifen-resistant group dichotomised by level of AR expression rather than comparing tamoxifen-sensitive to resistant disease. In that study, a high AR:ER ratio was associated with resistance to tamoxifen, but a key determinant of the increase in the AR:ER ratio was a low ER, with 45% of cases studied having an ER positivity of <20%. AR has been shown to facilitate estrogen-independent ER activity in MCF7 cells that are resistant to anastrozole (Rechoum *et al*. 2014). A comparison of 21 patient-matched cases of primary and recurrent breast cancer (Fujii *et al*. 2014) showed a significant decrease in ER levels with no change in AR levels after the development of resistance to AI therapy, consistent with the ER and AR staining patterns in the aforementioned study by Cochrane and coworkers. AI-resistant derivatives of the ER-positive T47D breast cancer cell line have similarly been shown to maintain AR expression, but completely lose ER expression (Fujii *et al*. 2014). Collectively, these data suggest that alteration in the AR:ER expression ratio is a common feature of resistance to ER-directed therapy, but that this may be determined more by ER loss than AR gain. Additional, larger studies in well-annotated populations are required to define the precise role of alterations in the AR:ER ratio with progression from endocrine-sensitive to resistant disease.

Two studies that compared tamoxifen to tamoxifen in combination with fluoxymesterone demonstrated improved clinical benefit with the combination in unselected breast cancer patients (Tormey *et al*. 1983, Ingle *et al*. 1991). In an exploratory analysis, patients aged older than 65 years with an ER level of >10 fmol had a significant improved survival from 7 to 18 months with the combination (Ingle *et al*. 1991). These studies provide clinical evidence that dual targeting of ER and AR may be of clinical benefit. Collectively, the pre-clinical and clinical evidence provide compelling support for a role of ligand-activated AR as a tumour suppressor in ER-positive breast cancer (Table 2).

Despite the therapeutic benefits seen with androgens, they fell from use as a class of agents due to virilising side effects, concerns regarding aromatization to estrogen, and the emergence of tamoxifen (Cole *et al*. 1971) and AIs (Coombes *et al*. 1984) as effective therapies in ER-positive breast cancer. Their initial use predated knowledge regarding AR expression and its potential role in ER-positive breast cancer. In light of recent new understanding of the interplay between SSRs in breast cancer, there has been a resurgence in interest in initiating innovative clinical trials to identify endocrine-based therapeutic strategies that enhance or are complementary to ER-directed interventions (Lonning 2009) (Table 3). These strategies are largely focused on targeting the AR signaling axis with antagonists of AR or inhibitors of androgen biosynthesis. It is intriguing that two opposing treatment strategies have been used in targeting AR in ER-positive breast cancer, namely an agonistic and an antagonistic strategy. The preclinical data supporting

Table 2 Studies of androgens and SARMs in metastatic breast cancer.

Treatment	No. of patients	Median duration of response	Objective response and clinical benefit rate	Reference
Androgen receptor agonists				
Fluoxymesterone	29	5.3 months	CBR: 48%	Kennedy (1957)
Fluoxymesterone vs TAM, *1st line*	79	–	CBR: TAM, 30%; FLU, 19%	Westerberg (1980)
Fluoxymesterone + TAM vs TAM, *1st line*	238	FLU + TAM, 11.6 months; TAM, 6.5 months (P=0.03)	ORR: FLU + TAM, 54%; TAM, 42% (P=0.07)	Ingle *et al*. (1991)
Selective androgen receptor modulators				
Enobosarm, *2nd line*	17	–	CBR: 35%	Overmoyer *et al*. (2015)

FLU, fluoxymesterone; CBR, clinical benefit rate; ORR, objective response rate.
Modulation of AR has not been studied as extensively as PR in the context of breast cancer. As with Table 1, a representative sample of studies accruing more than 20 participants is presented.

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Table 3 Current clinical trials investigating AR directed therapy strategies in breast cancer.

Treatment	Other treatments	Phase	Cancer subtype	Clinicaltrials.gov identifier
Androgen receptor antagonists				
Enzalutamide	Anastrozole, exemestane, fulvestrant	I	Any/AR+	NCT01597193
Enzalutamide	Exemestane	II	ER+ and/or PR+, HER2–	NCT02007512
Enzalutamide	Trastuzumab	II	AR+/HER2+	NCT02091960
Enzalutamide	Exemestane	Window	ER+	NCT02676986
AZD5312	–	II	AR+ solid tumours	NCT02144051
Selective androgen receptor modulators				
Enobosarm	–	II	ER+, AR+	NCT02463032
Enobosarm	–	Window	ER+, AR+	EMERALD
CR1447	–	I/II	P I: ER+/HER2–P II: ER+/HER2– or TNBC/AR+	NCT02067741
Androgen biosynthesis inhibitors				
VT-464	–	I/II	P I: TNBC or ER+/HER2–P II: TNBC/AR+ or ER+/HER2–	NCT02580448
Irosustat	Aromatase inhibitor (continued beyond progression)	II	ER+	NCT01785992
Irosustat	–	Window	ER+	NCT01662726
Orteronel	–	I	ER+	NCT01808040
Orteronel	–	II	TNBC/AR+; ER+ and/or PR+/AR+	NCT01990209
Androgens				
DHEA	Aromatase inhibitor	II	ER–/PR–/AR+ or ER or PR+/AR+	NCT02000375 (terminated)

AR, androgen receptor; DHEA, dehydroepiandrosterone; ER, estrogen receptor; PR, progesterone receptor.
Recruitment of advanced/metastatic breast cancer patients unless otherwise stated.
The majority of current clinical effort in this area is focussed on interventions affecting AR activity.

either of these two strategies in the context of ER-positive breast cancer is limited, but this has not deterred clinical trials to be conducted with both classes of drug, primarily in patients with endocrine-resistant metastatic breast cancer as summarized below.

AR antagonists and androgen biosynthesis inhibitors in ER-positive breast cancer

Enzalutamide is currently being evaluated in clinical trials of ER-positive breast cancer (Table 3). It acts by competitively inhibiting androgen binding, subsequent AR nuclear translocation and interaction with chromatin, and has been shown to bind to AR with greater relative affinity compared with other AR antagonists (Tran *et al*. 2009). In a phase I study, the pharmacokinetics and tolerability of enzalutamide in combination with AIs were similar to that reported in the initial trials of men with prostate cancer (Traina *et al*. 2014). As enzalutamide is an inducer of CYP3A4, it resulted in decreased circulating levels of AI and a corresponding increase in circulating estradiol levels in the study. Current clinical trials of enzalutamide in ER-positive breast cancer are summarized in Table 3. Finally, a phase I study (Clinicaltrials.gov identifier NCT02144051) to investigate the safety and pharmacokinetics of an antisense oligonucleotide AZD5312 (ISIS-ARRx), which targets AR mRNA, has just been completed in patients with advanced solid tumours, including breast cancer (Clinicaltrials.gov identifier NCT02144051).

Abiraterone acetate inhibits the hydroxylase CYP17, an enzyme involved in the biosynthesis of several steroidal hormones and hormone precursors, including ultimately androgens and estrogens. The FDA has approved its use in castrate-resistant prostate cancer. A randomized phase II study in postmenopausal patients with ER-positive metastatic breast cancer (O'Shaughnessy *et al*. 2016) reported no significant difference in progression-free survival with abiraterone compared with exemestane (3.7 vs 3.7 months; HR=1.1; P=0.437) or for the combination of abiraterone plus exemestane compared with exemestane alone (4.5 vs 3.7 months; HR=0.96; P=0.794). The reason for the lack of efficacy seen with abiraterone may be related to a reduction in testosterone or an increase in progesterone. Another CYP17 inhibitor, orteronel, has demonstrated promising results in pre-clinical studies (Kaku *et al*. 2011, Yamaoka *et al*. 2012, 2013).

In an alternative approach, irosustat (STX64), a first-generation irreversible inhibitor of steroid sulfatase (STS), has been shown to prevent the formation of androgenic steroids with estrogenic properties such as androstenedione 5α-androstane-3β,17β-diol, which have

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been implicated in endocrine resistance (Sikora *et al*. 2009, 2012, Palmieri *et al*. 2011, O'Hara *et al*. 2012). Two phase I studies have demonstrated irosustat to be well tolerated and provided evidence of clinical activity (Stanway *et al*. 2006, Coombes *et al*. 2013).

Selective AR modulators (SARMs)

The rationale for using a SARM in the context of ER- and AR-positive breast cancer is based on preclinical studies that consistently demonstrate an anti-proliferative effect of AR agonists in this breast cancer subtype (reviewed in Hickey *et al*. 2012). Enobosarm (GTx-024) is a non-steroidal SARM, which binds and activates AR with an affinity, potency and efficacy similar to dihydrotestosterone (DHT) (Kim *et al*. 2005, Narayanan *et al*. 2008). Enobosarm has the advantage of having selective anabolic activity and lacking cutaneous androgenic activity. Additionally, enobosarm is not converted to estrogenic metabolites (Chen *et al*. 2005, Mohler *et al*. 2009, Coss *et al*. 2014).

A proof-of-concept phase II study in post-menopausal women with ER-positive metastatic breast cancer (Clinicaltrials.gov identifier NCT01616758) who had a mean of three prior lines of ER-directed therapies, demonstrated that enobosarm at a dose of 9 mg daily has clinical activity (Overmoyer *et al*. 2015). Of the AR-positive patients, 35% derived clinical benefit, with the six-month Kaplan–Meier estimate of progression free survival being 40.1% (95% CI: 18.1–62.1%). Enobosarm has been shown to be safe and well tolerated in the context of a number of randomised phase II clinical studies (Dalton *et al*. 2011, Dobs *et al*. 2013). In the phase II metastatic breast cancer study, 95% of adverse events recorded were grade 1/2, and included pain, fatigue, nausea, hot flashes/night sweats, arthralgia and anxiety (Overmoyer *et al*. 2015). A follow on phase II trial is currently recruiting patients with ER-positive metastatic breast cancer (Clinicaltrials.gov identifier NCT02463032). A randomised phase II pre-surgical window of opportunity study is also being undertaken to evaluate the effect of 2 weeks of enobosarm in untreated ER and AR-positive early breast cancer (EMERALD study, Cancer Research UK Grant number A20712). The primary endpoint in this study is change in Ki67 between baseline and end of treatment.

Another SARM currently being investigated is 4-hydroxytestosterone (CR1447), which strongly binds to AR and has aromatase-inhibiting activity (Ghosh *et al*. 2009). Pre-clinical studies demonstrated that 4-hydroxytestosterone has anti-proliferative activity *in vitro* in both ER-positive and TNBC cell lines and is dependent on the presence of the AR. Animal data have shown selective anabolic effects. In a phase I study, in patients with advanced breast cancer where 4-hydroxytestosterone was transdermally administered to avoid first-pass metabolism, it was well tolerated up to a dose of 400 mg per day, with no dose-limiting toxicities noted (Schoenfeld *et al*. 2015). A phase II trial with 4-hydroxytestosterone at 400 mg in metastatic, endocrine responsive/HER2-negative and AR-positive TNBC is currently underway (Clinicaltrials.gov identifier NCT02067741).

Contemporary strategies for improving the treatment and outcomes for ER-positive breast cancer

Biological insights acquired from new transcription factor mapping techniques such as Chromatin Immunoprecipitation followed by Sequencing (ChIP-Seq) and Rapid Immunoprecipitation Mass spectrometry of Endogenous protein (RIME) (Hurtado *et al*. 2011, Ross-Innes *et al*. 2012, Mohammed *et al*. 2013, 2015) have highlighted the roles played by PR and AR in the regulation of ER signalling: opening new paths to tackling the most pressing needs in breast cancer therapy. It is becoming increasingly clear that substantial cross-talk occurs between SSRs, whereby the activation of one has a significant impact on the others. The mechanisms underlying receptor cross-talk have yet to be fully elucidated, but include competition for co-factors or consensus DNA-binding sites (Peters *et al*. 2009, Lim *et al*. 2012). More recently, it was demonstrated that activated PR reprograms ER chromatin binding, with many new ER-DNA interaction sites being detected within a short time after treatment with progesterone (Mohammed *et al*. 2015). This reprogramming of ER to novel cis-regulatory elements resulted in changes in gene expression profiles associated with cell cycle arrest, suggesting that activated PR was able to redirect ER chromatin binding and inhibit cell growth (Fig. 1). In support of this, progesterone inhibited estradiol-induced breast cancer cell proliferation as measured by Ki67 in patient-derived samples of primary breast cancer cultured *ex-vivo*. Moreover, treatment of MCF7 and T47D breast cancer xenografts with progesterone inhibited tumour growth, consistent with what has been reported in a patient-derived xenograft model of ER-positive and PR-positive breast cancer (Kabos *et al*. 2012). When combined with tamoxifen, the combination had greater

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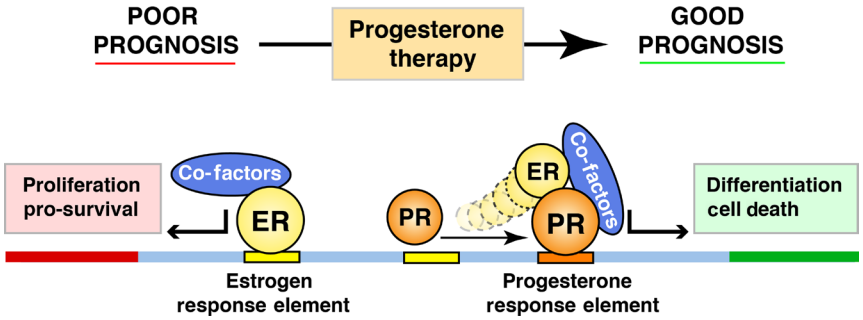

Figure 1
Progesterone receptor (PR) reprograms estrogen receptor (ER) binding towards genes associated with good prognosis. In the absence of progesterone or the PR (left), ER binds to estrogen response elements and activates the expression of genes involved in cell proliferation and survival. In the presence of PR and progesterone (right), PR binds to and physically displaces the ER by binding to progesterone response elements at genes associated with cell differentiation and death. Therefore, progesterone is able to exert influence on the ER genomic binding landscape and expected to promote favourable outcomes in a subset of patients with ER- and PR-positive breast cancer.

efficacy than either drug alone. Importantly, increased expression of a gene signature derived from progesterone-stimulated ER chromatin binding (comprising 38 genes) conferred a good prognosis in the Metabric cohort of breast cancer patients ($n = 1957$) (Curtis *et al*. 2012). This paper was seminal in that it challenged the previous common understanding of PR as a passive downstream marker of ER activity and identified a key role for PR in the regulation of ER function in breast cancer. These exciting new findings have the potential to open up a novel strategy to treat ER-positive breast cancer, specifically by modulating SSR crosstalk to reprogram ER signalling. Important considerations in translating these preclinical findings into clinical trials include knowing the SSRs that are co-expressed with ER, the type of SSR-directed drug used (i.e. antagonist or modulators, synthetic or natural), and whether the approach should be evaluated in endocrine-resistant or treatment-naive settings.

Although the traditional pathway for the majority of new therapies to be evaluated clinically begins in the metastatic context, pre-surgical clinical trials represent another validated strategy to evaluate novel therapies, particularly in ER-positive breast cancer, and can help to characterise the optimal target population (Dowsett *et al*. 2007, 2011, Goetz & Suman 2016). The POETIC (perioperative aromatase inhibitor therapy followed by standard adjuvant therapy) (Clinicaltrials.gov identifier NCT02338310) and the ALTERNATE (fulvestrant and/or anastrozole therapy in postmenopausal patients with stage II-III breast cancer undergoing surgery) (Clinicaltrials.gov identifier NCT01953588) trials are prospectively testing whether post-treatment Ki-67 levels can predict relapse-free survival. These trials will add to our understanding of whether the Ki-67 response is a valid biomarker strategy to identify patients with endocrine-sensitive disease. A valid criticism of Ki-67 as a predictive biomarker is the inter-laboratory and inter-observer variability, necessitating rigorous guidelines and quality assurance for clinical utility of this biomarker (Polley *et al*. 2013). A trial of a single depot injection of progesterone before surgery for ER-positive breast cancers in 976 patients demonstrated a significant improvement in survival outcomes in patients with higher risk node positive disease (Badwe *et al*. 2011). Window of opportunity studies to assess the effect of antiestrogen therapies, alone and in combination with micronized progesterone (prometrium) or a progestin (megestrol acetate) in patients with newly diagnosed ER and PR-positive breast cancer are currently being developed in Australia and UK. In the context of these trials, it is worth noting that although synthetic progestins have been associated with increased risk of developing breast cancer in the context of menopausal hormone therapy, in contrast, other studies have shown that hormone replacement therapies using native progesterone have resulted in no change or a slight decrease in breast cancer incidence (de Lignieres *et al*. 2002, Fournier *et al*. 2005, Espie *et al*. 2007, Fournier *et al*. 2008, Schneider *et al*. 2009).

Concluding statements

Emergent technologies are unravelling the extent and functional significance of SSR crosstalk in breast cancer, and ushering a new wave of clinical trials to understand how the potential breast cancer-suppressive effects of PR and AR can be harnessed in ER-positive breast cancer. Traditional approaches of studying the effects of individual hormones in isolation do not accurately reflect the interplay between different SSRs in breast cancer. Given that SSRs are structurally related, have similar consensus DNA-binding motifs and commonly use the same co-factors for activity, it is not surprising that there is substantial cross-talk between these receptors in breast

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cancer. Activation or inhibition of parallel hormonal pathways may therefore impact on the key receptor responsible for driving tumour growth.

The development of new generation AR antagonists in the context of prostate cancer has fast-tracked clinical trials focussed on inhibiting AR action in breast cancer, particularly in ER-negative breast cancer. Although it is still unclear if this is the correct strategy in ER-positive disease, where interaction of AR and ER signaling is complex and a critical consideration, clinical trials with AR antagonists have commenced in this setting. The emergence of SARMs, which act in opposing ways to AR antagonists, as a potential therapeutic strategy, highlights the lack of certainty regarding the best strategy and context to target AR. It is therefore critical that the SSR crosstalk be fully elucidated mechanistically in preclinical models that recapitulate the *in vivo* context to better inform the design of future clinical trials. The recent demonstration that progesterone stimulation of breast cancer cells *in vitro* and *in vivo* reprogrammed ER chromatin binding has identified a key role for PR in the regulation of ER–DNA interaction that has prognostic implications on patients with ER- and PR-positive breast cancer. This has led to a rethink of the role of PR, previously regarded solely as a downstream effector of ER, and a renewed interest in the development of PR-modulating strategies clinically. A key issue moving forward is if ER-directed therapies are required as a backbone for treatments that target AR and PR, as is the case as in the case of combination strategies with mTOR and CDK 4/6 inhibitors. Of note, combination endocrine therapy has had mixed outcome. In the adjuvant setting, the combination of tamoxifen and anastrozole did not improve outcomes compared with tamoxifen alone (ATAC Group 2008). By contrast, in the metastatic setting, evidence does exist for combining anastrozole and fulvestrant (Mehta *et al*. 2012). Therefore, when considering potential combinatorial strategies, a rational approach based on either scientific data such the pre-clinical evidence for combining progesterone plus tamoxifen or the historical clinical data such as for fluoxymesterone and tamoxifen (Tormey *et al*. 1983, Ingle *et al*. 1991). Ultimately, any combination will need to be tested clinically and ideally in a window study as this is the most efficient mechanism for testing novel combination and will also allow the integration of targeted therapy.

The convergence of the necessary tools to study SSR crosstalk and next generation SSR modulators makes this an opportune time for new therapeutic strategies for pushing ER around in breast cancer.

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Author contribution statement

All authors contributed to the writing of the review.

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