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Tracking Progesterone Receptor-Mediated Actions in Breast Cancer

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Abstract

Ovarian steroid hormones contribute to breast cancer initiation and progression primarily through the actions of their nuclear transcription factors, the estrogen receptor alpha (ER α) and progesterone receptors (PRs). These receptors are important drivers of the luminal A and B subtypes of breast cancer, where estrogen-blocking drugs have been effective endocrine therapies for patients with these tumors. However, many patients do not respond, or become resistant to treatment. When endocrine therapies fail, the luminal subtypes of breast cancer are more difficult to treat because these subtypes are among the most heterogeneous in terms of mutation diversity and gene expression profiles. Recent evidence suggests that progestin and PR actions may be important drivers of luminal breast cancers. Clinical trial data has demonstrated that hormone replacement therapy with progestins drives invasive breast cancer and results in greater mortality. PR transcriptional activity is dependent upon cross-talk with growth factor signaling pathways that alter PR phosphorylation, acetylation, or SUMOylation as mechanisms for regulating PR target gene selection required for increased cell proliferation and survival. Site-specific PR phosphorylation is the primary driver of gene-selective PR transcriptional activity. However, PR phosphorylation and heightened transcriptional activity is coupled to rapid PR protein degradation; the range of active PR detected in tumors is likely to be dynamic. Thus, PR target gene signatures may provide a more accurate means of tracking PR's contribution to tumor progression rather than standard clinical protein-based (IHC) assays. Further development of antiprogestin therapies should be considered along side antiestrogens and aromatase inhibitors.

Keywords

progesterone receptor (PR); phosphorylation; SUMOylation; gene expression; estrogen receptor (ER); antiprogestins; luminal breast cancer

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Conflict of interest statement

Carol A. Lange is a consultant for Arno Therapeutics, Inc. (Flemington, NJ). Todd P. Knutson declares no conflicts of interest.

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1. Introduction

Breast cancer is the most commonly diagnosed cancer and the second leading cause of cancer-related death in women. In 2013, it is estimated that 232,000 women will be diagnosed with breast cancer and 39,000 women will die from the disease (Siegel, et al., 2013). Clinically, protein expression levels for estrogen receptor alpha (ER α), progesterone receptor (PR), and HER2 are the primary biomarkers used to inform breast cancer treatment strategies. Breast cancers are characterized into three main groups: ER-positive, HER2-amplified, and triple negative tumors, which are negative for ER, PR, and HER2. Up to 70% percent of breast tumors express ER or PR upon biopsy, and these tumors are associated with greater overall survival and decreased metastasis (Bardou, et al., 2003; McGuire, 1978). Tumors that express high levels of HER2, primarily through genomic amplification of the *ERBB2* locus, are associated with worse outcomes (Slamon, et al., 1987).

In addition to testing for ER, PR, and HER2 protein expression levels, many other molecular tests have begun to be used clinically to assess breast tumor aggressiveness, risk of relapse, and optimal treatment strategies. A recent collaborative study characterized untreated primary breast tumors by integrating data from multiple high-throughput genomic technologies including DNA copy number arrays, exome sequencing, mRNA expression, microRNA sequencing, and reverse-phase protein arrays. This comprehensive analysis identified four major subtypes of breast cancer with unique molecular drivers: luminal A, luminal B, HER2-enriched, and basal-like (Table 1) (Cancer Genome Atlas Network, 2012). Luminal A tumors typically expressed high levels of ER and PR, whereas luminal B tumors usually expressed high levels of ER but reduced levels of PR. Nearly 75% of all breast tumors were identified as luminal A or luminal B, and these tumors were the most heterogeneous and had the least prominent molecular drivers. HER2-enriched tumors were generally driven by amplification of the *ERBB2* locus, and basal-like tumors rarely expressed ER, PR, or HER2 and were driven by PI3K pathway mutation. These data indicate that distinct treatment strategies must be developed that target the molecular drivers specific to each breast cancer subtype; however, additional research is needed to characterize the molecular heterogeneity identified among the four breast cancer subtypes, especially the most abundant luminal subtypes.

Antiestrogen therapy targeting ER is the primary treatment strategy for the luminal subtypes of breast cancer. Although this treatment strategy has been very successful, approximately 40% of patients eventually relapse. To improve treatment outcomes, it must be appreciated that breast cancer is a hormonally driven disease that should be analyzed in the context of steroid hormone receptor transcriptional activity in addition to common mutations (Briskin, 2013). Thus, a deeper investigation into the molecular signaling within the luminal subtypes will greatly enhance our understanding of disease biology and improve treatment strategies for patients bearing these tumors. In this review, we discuss how progestins are critical for mammary gland development and increase breast cancer risk in post-menopausal women. Recent advances surrounding PR and its post-translational modifications that mediate breast cancer cell proliferation and survival are presented. The last decade of molecular research has provided a powerful rationale for targeting PR in a subset of breast cancer patients. The potential for clinical antiprogestin therapies is discussed. We propose that PR transcriptional signatures will provide more reliable tumor biomarkers that accurately track activated PR relative to total PR levels as measured by protein-based assays.

2. Steroid hormones influence breast cancer risk

Steroid hormones are associated with many empirically identified breast cancer risk factors. Heritable mutations in the *BRCA1* or *BRCA2* DNA repair genes disproportionally increase a

woman's risk of breast (45–65%) and ovarian (11–39%) cancer compared to other cancers (Antoniou, et al., 2003). The organ-specific cancer penetrance for these mutations has been difficult to understand, but a recent finding that women with *BRCA1/2* mutations had significantly higher levels of estradiol, increased PR expression, and higher circulating progesterone levels during the luteal phase of the menstrual cycle may suggest a possible link to increased cancer risk (Widschwendter, et al., 2013). In a related translational study, nulliparous *BRCA1/p53* deficient mice displayed increased epithelial cell proliferation and differentiation, which normally is only induced during pregnancy, that could be blocked by a PR antagonist (Poole, et al., 2006). These data suggest that rapid cell proliferation in the breast may be synergized through *BRCA1/2* deficiency and increased estrogen and progesterone exposure that may partly explain the organ-specific cancer risk of these mutations.

Lifetime exposure to elevated levels of steroid hormones, including estrogens and progestins, increases the relative risk of breast cancer incidence in pre- and post-menopausal women (Clemons & Goss, 2001; Key, et al., 2002). Multiple epidemiological studies link hormonal contraceptive use in pre-menopausal women with increased breast cancer risk. Progestin-only depot-medroxyprogesterone acetate (DMPA) usage for greater than 12 months was shown to increase breast cancer risk by 2.2 fold (Li, et al., 2012). A pooled analysis found that young women currently using combined oral contraceptives have a 24% increased breast cancer risk than nonusers, but the risk decreases over 10 years of nonuse (Collaborative Group on Hormonal Factors in Breast, 1996). A recent study confirmed the elevated risk for any current oral contraceptive use but revealed that triphasic preparations containing a progestin, levonorgestrel, account for most of the elevated breast cancer risk in pre-menopausal women (3.05 relative risk compared to nonusers) (Hunter, et al., 2010). In a different cohort of pre-menopausal women, current progestin use for greater than 4.5 years was associated with increased breast cancer risk, but this risk also diminished after stopping treatment (Fabre, et al., 2007). Thus, there is broad agreement within the epidemiological literature that women are at greater breast cancer risk while taking progestin-containing hormonal contraception.

Exposure to increased levels of testosterone or androgens also increases breast cancer risk, in part because these molecules are converted to estradiol in post-menopausal women (Key, et al., 2002). In addition, obesity, weight gain after menopause, and alcohol use are associated with elevated breast cancer risk (Chlebowski, et al., 2002; Huang, et al., 1997; Smith-Warner, et al., 1998). Obesity is a strong risk factor for breast cancer incidence, partly because additional adipose can drive higher levels of aromatase enzyme expression, resulting in higher than normal estradiol levels (Siiteri, 1987; Zhao, et al., 1997). Further, anti-inflammatory immune signals that are elevated in excess adipose tissues provide pro-growth paracrine signals to nearby epithelial cells (Brown & Simpson, 2012). Additionally, women who experienced early menarche (before age 11), a late first pregnancy (after age 35), or were never pregnant are at elevated risk for breast cancer (Russo, et al., 2005). In contrast, early pregnancy, multiparous women, and prolonged duration of breast-feeding decrease breast cancer risk (Collaborative Group on Hormonal Factors in Breast Cancer, 2002). The reduced risk associated with multiple pregnancy-lactation cycles is not clearly understood, but may be related to reduced levels of circulating estradiol, progesterone, and prolactin following pregnancy (Faupel-Badger, et al., 2013). Oophorectomy in pre-menopausal women (before age 35) reduces breast cancer risk by 75% (Feinleib, 1968; Trichopoulos, et al., 1972), as estrogen and progestin production occurs in the ovary. Elevated mammographic breast density is associated with a 6-fold higher breast cancer incidence (N. F. Boyd, et al., 2007; Vachon, et al., 2007). Further, increased mammographic breast density is associated with the use of estrogen- and progestin-containing hormone

replacement therapy (HRT) in post-menopausal women (Byrne, et al., 1995; Greendale, et al., 1999; Rutter, et al., 2001).

Epidemiological studies of women taking estrogens as part of post-menopausal HRT suggested that these women have better cardiovascular disease outcomes (Bush, et al., 1987; Grady, et al., 1992; Stampfer & Colditz, 1991) and a higher risk for breast cancer (Collaborative Group on Hormonal Factors in Breast Cancer, 1997; Steinberg, et al., 1991). The Women's Health Initiative (WHI) formally tested these questions in a double-blind, randomized, phase III clinical trial. The results show that HRT with oral continuous combined conjugated equine estrogens (CEE) plus medroxyprogesterone acetate (MPA) significantly increases invasive breast cancer risk, and thus, HRT should be avoided if possible (Chlebowski, et al., 2010; Chlebowski, et al., 2003; Chlebowski, et al., 2009). In addition, data from a separate arm of that clinical trial indicate that post-menopausal women with prior hysterectomy, treated with estrogen-only HRT, have a reduced risk of breast cancer, suggesting that estrogens alone may be protective in this cohort (Anderson, et al., 2012; LaCroix, et al., 2011). The average women's age in the WHI trial was 63 years, suggesting that these findings may not apply to women using HRT shortly after menopause (within 5 years). Post-hoc analysis in these early-menopausal women indicate that estrogens alone do not increase or decrease risk, whereas estrogen plus progestins increase breast cancer risk (Santen, 2013). Furthermore, an observational study by the Million Women Study Collaborators indicates that post-menopausal women on progestin-containing HRT are at higher risk of developing breast cancer than women on estrogens-only HRT or other HRT regimens, such as tibolone (Million Women Study Collaborators, 2003). Additional evidence in non-human primates shows that treatment with estrogen plus MPA upregulates proliferative genes, compared to estrogens alone (Wood, et al., 2013). Thus, these data suggest that elevated progestin levels in post-menopausal women markedly contribute to aggressive tumor development. Conversely, however, a prospective study compared women taking HRT regimens of estrogen plus MPA versus estrogen plus progesterone and found that women in the estrogen plus progesterone cohort did not have increased breast cancer risk (Fournier, et al., 2008; Fournier, et al., 2005). This study did confirm the WHI clinical trial that estrogen plus MPA regimens increase invasive breast cancer risk compared to placebo. MPA may modify breast cancer risk by prolonged activation of PRs, or off-target effects that include modulation of the androgen receptor or glucocorticoid receptor, or some combination of these actions. Randomized trials testing natural progesterone compared to MPA would clarify the effects of progestins relative to progesterone on breast cancer risk. Taken together, these findings argue for a greater understanding of PR signaling in breast cancer.

3. Hormone actions in mammary gland development and breast cancer

Normal mammary gland development has primarily been studied in mouse and rat models (for review, see (Hennighausen & Robinson, 2005)). The mammary gland contains two major tissue compartments: the epithelium includes ductal and alveolar structures, and the stroma is comprised of supporting cells. Alveoli are bud-like structures that form a lumen within the stroma that differentiate into milk-producing secretory luminal epithelial cells during pregnancy. The mammary epithelium is derived from a population of self-renewing mammary stem cells (MSC) that produce committed ductal or alveolar precursor cells (for review, (Smalley & Ashworth, 2003)). These cells are necessary for the hormonally driven massive alveolar expansion that occurs during pregnancy, in preparation for milk production and secretion through ductal epithelial branches. Upon weaning, the breast epithelium undergoes hormone-regulated involution. These multiple cycles of proliferation and apoptosis demonstrate the dynamic nature of these glands and their susceptibility to deregulation and cancer. The stromal compartment contains myoepithelial cells,

extracellular matrix, endothelial cells, fibroblasts, adipose, and a host of immune cells and factors that are important regulators of mammary epithelial cell function. Complex signaling between the epithelium and stroma mediates correct epithelial cell polarity, differentiation, and affect epithelial cell proliferation and invasion during tumorigenesis (for review, see (Polyak & Kalluri, 2010)).

Mammary gland development begins primarily during puberty and is dependent on the actions of the steroid hormones estrogen and progesterone. 17 beta-estradiol is a ligand for ER and is primarily responsible for mediating ductal outgrowth and side branching (C. W. Daniel, et al., 1987), whereas progesterone is necessary for alveolar development (Haslam, 1989; Wang, et al., 1990). During the menstrual cycle in women, pre-ovulatory progesterone levels are low, estrogen levels are elevated and proliferation occurs in the endometrium. During the post-ovulatory phase, high levels of progesterone are sustained and mediate endometrial differentiation and promote proliferation in the breast epithelium. Progesterone-dependent proliferation and development are mediated through PR isoforms (PR-A and PR-B). Isoform specific PR knockouts in mice revealed that PR isoforms have unique transcriptional targets and mediate tissue specific functions (Mulac-Jericevic, et al., 2003; Mulac-Jericevic, et al., 2000). Briefly, PR-A is required for reproductive potential and PR-B is required for mammary gland development and specifically upregulates RANKL, an important paracrine factor that drives alveoli expansion during pregnancy. Prolactin is also an important peptide hormone that mediates mammary gland expansion primarily during pregnancy and lactation. Proliferation in the mammary gland occurs in two waves. First, progesterone stimulates a small population of PR-positive cells to produce and secrete important extracellular growth factors and cytokines (including RANKL and WNTs) that require cyclin D1 (Beleut, et al., 2010). These paracrine factors drive a second larger wave of epithelial cell proliferation in nearby PR-negative cells of the mammary gland. These two waves of proliferation could not be stimulated by estrogen alone; however, RANK or RANKL is required for progesterone-mediated mammary gland expansion and tumor initiation in mouse studies (Beleut, et al., 2010; Robinson, et al., 2000; Schramek, et al., 2010; Tanos, et al., 2013). In human breast microstructures, progesterone-dependent proliferation was shown to be RANKL-mediated and *in vivo*, RANKL was correlated with progesterone levels and expressed in human luminal epithelial cells (Tanos, et al., 2013). RANKL was expressed greater than 2-fold in invasive breast carcinomas compared to normal breast in the TCGA cohort (Cancer Genome Atlas Network, 2012; Rhodes, et al., 2007). In breast cancer cell lines, progestins were unable to induce RANKL expression suggesting this paracrine signaling mechanism requires intact tissue architecture (Tanos, et al., 2013). These data demonstrate that the proliferative progesterone/RANKL signaling axis is conserved from mouse to human and suggests that anti-RANKL drugs, such as denosumab, may be useful for a subset of breast cancer patients (Cummings, et al., 2009; Tanos, et al., 2013).

Estrogen and progesterone also contribute to breast tumor initiation and progression. Although only a few cells are ER/PR-positive in the normal breast epithelium (Aupperlee, et al., 2005), many cells express high levels of ER (75%) and PR (50–70%) in breast tumors (Bardou, et al., 2003; McGuire, 1978). In many cellular contexts, ER and PR have similar expression levels because estrogens stimulate PR expression. Cell proliferation is driven by high levels of PR expression in tumor epithelial cells via both PR-dependent autocrine/paracrine signaling and cell-intrinsic PR-dependent transcriptional action (Haslam & Woodward, 2003; Robinson, et al., 2000; Tanos, et al., 2013). Moreover, elevated levels of estrogen and progesterone, signaling through their cognate receptors, have the potential to initiate DNA mutations in breast tissues through the stimulation of pro-proliferative and pro-survival transcriptional programs, resulting in frequent rounds of cellular replication that cause higher mutational frequencies (Henderson & Feigelson, 2000; Russo, et al., 2001). In

addition, estradiol can be converted into various metabolites, including 4-OH-estradiol and 3,4 estradiol-quinone; these metabolites can covalently bind purine DNA bases, causing depurination and higher rates of error-prone DNA repair (Liehr, 2000; Liehr & Ricci, 1996; Yager & Liehr, 1996).

In summary, mammary gland development, differentiation, and involution are hormonally regulated processes that undergo many cycles of proliferation and apoptosis, making the gland extremely sensitive to changes in steroid hormone receptor action. Changes to hormone receptor action are regulated through post-translational modifications, such as phosphorylation (discussed below). Targeting hormone actions in breast cancer initiation and progression have led to some of the most effective breast cancer prevention and treatment strategies.

4. PR transcriptional activity is controlled by post-translational modifications

Post-translational modifications are chemical alterations of a protein after its translation in which functional moieties are covalently attached to the substrate protein to influence protein-protein interactions and/or enzyme activities related to cellular functions such as DNA repair, replication, transcription, chromosome segregation, genomic stability, and intracellular trafficking (for reviews, see (Gareau & Lima, 2010; Gill, 2003; Hay, 2005; Johnson, 2004)). PR isoforms are post-translationally modified by phosphorylation, ubiquitination, SUMOylation, and acetylation (Abdel-Hafiz, et al., 2002; A. R. Daniel, et al., 2010; Hagan, et al., 2012; Lange, et al., 2000). These modifications are highly dynamic, depend on cellular context, and govern PR transcriptional regulation of selected target genes or gene subsets (further discussed below).

Site-specific PR phosphorylation is an important context-dependent mechanism for regulating PR-target gene selectivity. These experiments have been largely studied in T47D and MCF-7 human breast cancer cell line models that allow for estrogen-independent PR expression and/or independent PR isoform expression (Horwitz, et al., 1982; Sartorius, et al., 1994). PR contains 14 serine residues that are phosphorylated by multiple protein kinases (eg, MAPK, CK2, and CDK2) either basally or in response to ligand binding or treatment of cells with growth factors (reviewed in (Trevino & Weigel, 2013)). Many breast cancers display heightened ERBB/MAPK pathway activation. ERK1/2 phosphorylates PR, and PR also regulates MAPK activation through interaction with signaling pathway components (reviewed in (Hagan, et al., 2012)). As a result, PR phosphorylation is predicted to be commonly deregulated (Knutson, et al., 2012).

PR phosphorylation at Ser294 has been intensively studied. In the presence of ligand, PR-B is rapidly phosphorylated at Ser294, accumulates in the nucleus, and becomes highly transcriptionally active at multiple genes important for cell cycle progression (*CCND1*), proliferation (*MYC*), and survival (*BCL2L1*) (Moore, et al., 2000; Shen, et al., 2001). PR-B Ser294 phosphorylation augments ligand-dependent (regulated) receptor degradation that is functionally coupled to increased transcriptional activity (Shen, et al., 2001); PR is rapidly degraded upon ligand binding via the ubiquitination-proteasome pathway (Lange, et al., 2000). When ligand-induced PR-B is phosphorylated and highly transcriptionally active, its half-life shortens due to rapid turnover; activated PR phospho-proteins are often undetectable by western blotting and proteasome inhibitors block both degradation and PR transcriptional activity (Shen, et al., 2001). These effects result in large part from reciprocal interaction between Ser294 and Lys388, a SUMO-consensus motif in the PR amino-terminus. In the presence of growth factors (EGF) or heightened MAPK activities, PR-B Ser294 phosphorylation negatively regulates PR-B SUMOylation at Lys388, a repressive

modification that also stabilizes PRs in response to progestin (discussed in detail below) (Fig. 1) (A. R. Daniel, et al., 2007). In contrast to PR-B, PR-A is not appreciably phosphorylated at Ser294 and thus heavily SUMOylated at Lys388, transcriptionally repressed, and highly stable relative to PR-B (A. R. Daniel, et al., 2007). Thus, in lieu of the difficulty of detection of activated and dynamic PR-B protein species in the presence of progesterone and/or the influence of local growth factors, it may be feasible to use PR-B target gene expression as a reliable biomarker of transcriptionally hyperactive PR (discussed in detail below).

PR-B phosphorylation at Ser400 is required for ligand-independent PR transcriptional activity during cell cycle progression. In response to progestin or mitogen treatment, CDK2 signaling is activated and the G₁/S-phase transition is initiated. CDK2 has been shown to phosphorylate PR at multiple sites that regulate PR transcriptional activity (Pierson-Mullany & Lange, 2004; Zhang, et al., 1997). PR is basally phosphorylated on Ser400 in resting cells and highly phosphorylated by CDK2 in response to progestins (Zhang, et al., 1997). In addition, treatment with mitogenic growth factors known to activate CDK2 activity, or expression of constitutively active CDK2 *in vitro*, induces ligand-independent PR transcriptional activity. PR Ser400 mutation to alanine (S400A) blocks CDK2-dependent PR transcriptional activity in this context (Pierson-Mullany & Lange, 2004). In T47D breast cancer cells, high expression of the cell cycle inhibitor p27 also blocks CDK2-induced PR Ser400 phosphorylation and ligand-independent PR transcriptional activity, whereas knockdown of p27 restores wild-type PR transcriptional activity (Pierson-Mullany & Lange, 2004). These data suggest that PR Ser400 phosphorylation by activated CDK2 regulates the transcriptional activity of PR during the cell cycle; these events may be particularly favored upon loss of p27 in breast cancer cells.

PR phosphorylation at Ser345 is also required for PR transcriptional regulation of select specificity factor 1 (Sp1)-dependent target genes expressed during cell cycle progression (Faivre, et al., 2008). PR phosphorylation at Ser345 occurs in response to ligand-binding (via rapid activation of MAPK) or during cell cycle progression (via activated CDK2) in the absence of progestin. Phospho-Ser345 PRs upregulate *HSPB8* mRNA levels, a gene whose expression is associated with tamoxifen resistance and poor prognosis in breast cancer cells (Dressing, et al., 2013; Gonzalez-Malerva, et al., 2011). Phosphorylated PR, Sp1, and cyclin D1 physically interact as part of transcription complexes on an enhancer region for *HSPB8*; this complex is required for *HSPB8* expression during the G₂/M phase (Fig. 2) (Dressing, et al., 2013). Thus, cell cycle-induced PR transcriptional regulation requires PR phosphorylation, primarily at Ser345, and its interaction with Sp1/cyclin D1 to regulate a novel subset of PR target genes, including *HSPB8*. These data suggest that targeting the PR/Sp1/cyclin D1 interaction and/or CDK2 activity may be highly beneficial for women with hormone refractory tumors that express high levels of cyclin D1 and *HSPB8*.

PR phosphorylation at Ser345 is also involved in nongenomic, membrane-associated actions, where PR serves as a node within cytoplasmic protein kinase signaling cascades (for reviews, see (Bjornstrom & Sjoberg, 2005; Edwards, 2005; Lange, 2008; Losel & Wehling, 2003)). For example, progestin treatment in breast cancer cells activates the MAPK signaling pathway via rapid (5–10 min) nongenomic mechanisms that require PR/c-Src interaction (Boonyaratankornkit, et al., 2001). Furthermore, progestin treatment drives sustained (18 h) MAPK signaling via phospho-Ser345-dependent PR transcriptional mechanisms, including the upregulation of *WNT1* (Faivre & Lange, 2007). *WNT1* expression stimulates matrix metalloproteinases to release EGFR ligands, driving prolonged EGFR/MAPK signaling (Civenni, et al., 2003). In addition to directly binding DNA (eg, *WNT1* promoter), PR is known to mediate transcription through tethering mechanisms with other transcription factors, such as Sp1 and activator protein 1 (AP-1) (Bamberger, et al.,

1996; Owen, et al., 1998). Rapid progestin-induced PR Ser345 phosphorylation (5–10 min) is dependent on intact c-Src, EGFR, and MAPK signaling pathways, and PR can directly interact with Sp1 at Sp1 DNA-binding motifs to regulate PR-dependent gene activation, including p21 (Fig. 2) (Faivre, et al., 2008). PR with mutations in the poly-proline tract (mPro) or at Ser345 (S345A) does not associate with Sp1 on the p21 promoter and does not activate progestin-dependent p21 transcription (Faivre, et al., 2008) or *HSPB8* transcription during cell cycle progression (discussed above). There are likely multiple additional phospho-Ser345 PR target genes regulated in this manner. Thus, PR genomic (ie, transcriptional) activities are fully integrated with nongenomic activities classically linked to membrane-associated protein kinase signaling that ultimately regulate PR-target gene selectivity.

PR phosphorylation at Ser81 is another example of phosphorylation-dependent PR target gene selectivity. Phosphorylation at this residue is dependent on CK2 (formally casein kinase II) expression and is enhanced by progestin exposure; phosphorylation also occurs during S-phase in the absence of progestins (Hagan, et al., 2011). Mechanistically, PR Ser81 phosphorylation requires DUSP6 (also called MKP3) to bind the PR common docking (CD) domain and scaffold an interaction with CK2 (Hagan, et al., 2013). Phosphorylation at PR Ser81 is important for progesterone-independent breast cancer cell soft agar colony formation and is required for selected (ligand-dependent and -independent) PR target gene regulation (eg, *BIRC3*) (Hagan, et al., 2011). Indeed, only phospho-Ser81 PR is associated with CK2 at a *BIRC3* enhancer region; PR with a mutation at this site (S79/81A) does not regulate *BIRC3* mRNA and is not present at the enhancer (Hagan, et al., 2011). PR Ser81 may be very important for ligand-independent gene transactivation because this site is basally phosphorylated under high kinase activities (ie, during cell cycle G₁/S-phase transition) (Hagan, et al., 2011). In addition, mutation of the PR CD domain attenuates cell cycle progression and dramatically shifts the PR transcriptional program, blocking its ability to regulate JAK/STAT pathway genes, including STAT5 (Hagan, et al., 2013). Thus, CK2-dependent PR Ser81 phosphorylation is likely required for normal mammary gland development and particularly important for directing PR-driven genetic programs (ie, *WNT1* expression) associated with regulation of the mammary stem cell compartment (Hagan, et al., 2013).

Multiple steroid hormone receptors (SR) are also modified by acetylation, including PR (A. R. Daniel, et al., 2010; Faus & Haendler, 2006). The hinge region of PR contains a conserved motif of lysine residues (KxKK) that are rapidly acetylated in response to ligand binding (A. R. Daniel, et al., 2010). Progestin-treated cells expressing lysine mutant PR (alanine substitutions) cannot be acetylated and show defective nuclear retention, delayed global phosphorylation, and reduced Ser400 phosphorylation (A. R. Daniel, et al., 2010). These PR acetylation mutations dramatically influence PR transcriptional selectivity at various promoters and the temporal regulation of PR target genes (A. R. Daniel, et al., 2010). These data suggest that regulatory sites in the PR hinge region, including acetylation motifs and the nuclear localization signal, are necessary for efficient PR nuclear retention and allow for efficient PR phosphorylation in response to ligands (A. R. Daniel, et al., 2010). A functional hinge region is primarily required for effective “early gene” transcription, including *MYC* (A. R. Daniel, et al., 2010), which is induced within minutes of progestin treatment.

Many SRs are covalently modified by SUMO peptides that cause a diverse range of effects on SR function, including altered target gene selectivity, repression or activation of select target genes, and increased protein stability (reviewed in (Knutson & Lange, 2013)). Upon ligand binding, PR becomes SUMOylated at Lys388 (Abdel-Hafiz, et al., 2002). Growth factor pathway activation of MAPK or CDK2 protein kinases drive PR Ser294

phosphorylation, which antagonizes SUMOylation at Lys388, suggesting a mechanism for PR deSUMOylation leading to hypersensitivity to ligand and rapid turnover (A. R. Daniel, et al., 2007). Breast cancer cells stably expressing SUMOylation-deficient PR-B mutant (K388R) have heightened PR transcriptional activity at many cancer-relevant target genes, and have higher rates of proliferation and survival than cells expressing wild-type PR that can be SUMOylated in the presence of progesterone (Knutson, et al., 2012).

5. PR expression, degradation, and activity in breast tumors

Breast tumors of the luminal subtypes contain the greatest molecular heterogeneity in terms of gene expression, mutation diversity, copy number alterations, and patient outcomes (Cancer Genome Atlas Network, 2012). The clinical significance of understanding this molecular heterogeneity lies in distinguishing between the less aggressive luminal A tumors and the more aggressive luminal B tumors. Recent findings suggest that the activity of PR rather than PR protein levels may provide some distinction between these subtypes. In the clinic, biomarker expression levels for ER, PR, and HER2 are routinely measured. A recent analysis showed that approximately 52% of luminal A tumors are ER+, PR+, and HER2-, and 36% of luminal B tumors have this biomarker expression profile. In contrast, luminal B tumors are more likely to lose PR expression and upregulate HER2 expression, with 18% of luminal B tumors and 0% of luminal A tumors being ER+, PR-, and HER2+ (Bastien, et al., 2012). However, it is not clear how to interpret loss of PR protein expression when PR mRNA is either present or simply not measured in the clinical setting. In the TCGA cohort, heterozygous loss of the PGR locus occurs in 40% of luminal tumors. However, this does not entirely explain all PR- IHC profiles, as 25% of luminal tumors are also heterozygous for the *ESR1* locus but these tumors are overwhelmingly ER+ (Cancer Genome Atlas Network, 2012; Cerami, et al., 2012; Gao, et al., 2013). Interestingly, PR and ER copy-number is correlated in individual tumors. In addition, both PR and ER mRNA levels are similar in luminal tumors that are diploid or have lost an allele at these loci, suggesting that a subset of PR- tumors (identified by IHC) may downregulate the protein via other mechanisms.

As discussed above, phospho-PR gene signatures provide an excellent readout of PR transcriptional activity in breast cancer models (Dressing, et al., 2013; Hagan, et al., 2013; Knutson, et al., 2012). PR-target gene sets are clearly implicated in aggressive breast cancer behaviors. Importantly, PR-driven gene signatures reliably predict poor outcome in patients with luminal breast cancer (Knutson, et al., 2012). The involvement of PR activity in luminal B cell proliferation is further bolstered by the WHI clinical trial showing that estrogen plus progestin-containing HRT drives invasive cancer, whereas estrogens alone do not (Chlebowski, et al., 2003; LaCroix, et al., 2011). We suggest that loss of PR protein expression is most often a marker of its rapid (regulated) degradation following growth-factor or progestin-induced phosphorylation and transcriptional activation. Thus for example, when PR-B becomes phosphorylated at Ser294, the receptor is relatively deSUMOylated and becomes highly transcriptionally active at selected target genes important for breast tumor progression (Fig. 1). These events are coupled to receptor loss via rapid ubiquitin-mediated proteasome-dependent turnover (Lange, et al., 2000). Blocking the proteasome blocks PR transcriptional activity and stabilizes PR (Lange, et al., 2000). Thus paradoxically, “activated” PR protein is only weakly detected at the peak of PR-target gene mRNA expression (Knutson, et al., 2012). In the clinic, ER and PR expression are measured by IHC and scored as positive even if a small percentage (greater than 1%–10%) of cells express each receptor (Hammond, et al., 2010). However, dephosphorylated PRs are weakly active (stabilized) proteins and thus predicted to be easily detected in tumor biopsy samples, whereas phosphorylated (active) PRs may appear as PR-low or -null in routine clinical tests. Thus, luminal B tumors that score as PR- may actually contain highly active PRs of

relatively short half-life that function to drive tumor progression in the context of high kinase activities and/or growth factor-rich tumor microenvironments. These phospho-PR species are hypersensitive to low ligand concentrations and clearly regulate gene subsets that confer aggressive breast tumor behavior (A. R. Daniel, et al., 2007; Knutson, et al., 2012).

Notably, clinical IHC measurements for ER, PR, and HER2 cannot accurately identify any of the intrinsic subtypes of breast cancer (ie, luminal A, luminal B, HER2-enriched, and basal-like) (Bastien, et al., 2012). This makes it difficult to distinguish between luminal A and B tumors without using other methods. Even highly sensitive RT-qPCR methods of measuring ER, PR, and HER2 transcript levels in tumor samples cannot identify the intrinsic subtypes (Bastien, et al., 2012). One technique of measuring activated PR is to measure the expression of selected PR target genes that are drivers of cell proliferation. For example, the luminal B subtype contains elevated *MYC*-dependent proliferation (Cancer Genome Atlas Network, 2012). *MYC* is an important PR target gene, suggesting that measuring its expression may better distinguish between luminal subtypes. In addition, PRs in breast cancer cells may also be difficult to detect using clinical IHC and western blotting (discussed above). Not all antibodies detect PR isoforms equally, which may also skew detection levels. Thus, PR-dependent gene signatures may better determine whether PR is activated (ie, phosphorylated and deSUMOylated) in breast tumors. Unique PR-B target gene signatures have been described that can discriminate between wild-type PR and phosphorylated/SUMO-deficient PR expression in T47D cells (Knutson, et al., 2012). These PR gene signatures, derived *in vitro*, accurately predict poor outcome in patients on endocrine therapy (Knutson, et al., 2012). Therefore, elevated mRNA levels of multiple activated PR target genes could potentially identify aggressive tumors, irrespective of PR protein levels.

Growth factor receptors are commonly overexpressed in breast cancer resulting in roughly 50% of tumors exhibiting elevated MAPK activity. Further, approximately 20% of luminal B tumors have heightened HER2 expression and activity (Prat & Perou, 2011), indicating these tumors likely drive MAPK-dependent PR Ser294 phosphorylation and deSUMOylation. EGF and progestins synergize in breast cancer cells, and EGF treatment leads to MAPK phosphorylation and elevated (SUMO-deficient) PR target gene expression; these effects are blocked by MAPK or PR inhibitors (Knutson, et al., 2012). Indeed, PR (and ER) cross-talk with growth factor signaling pathways is extensive, and growth factor pathway activation (ie, HER2, IGF1R, etc.) is a key factor that contributes to endocrine-resistant tumor progression. Over 50% of endocrine-resistant tumors express PR (Encarnacion, et al., 1993). Combination therapies targeting ER and HER2 enhance progression-free survival, suggesting other combinations targeting PR may also be effective if patients are properly selected (ie, the patient's tumor expresses an activated PR gene signature) (Johnston, et al., 2009; Kaufman, et al., 2009).

6. Clinical strategies for hormone-based breast cancer prevention and treatment

Breast cancer prevention strategies focus on blocking estrogen-mediated actions and have been widely employed. Long-term use of tamoxifen dramatically reduced breast cancer risk in high-risk women in the National Surgical Adjuvant Breast and Bowel Project (NSABP) prevention (P-1) clinical trial (Cuzick, et al., 2003; Fisher, et al., 2005; Fisher, et al., 1998). Raloxifene also provided a 76% reduction in all breast cancers in post-menopausal women with osteoporosis (Cummings, et al., 1999). A panel commissioned by the American Society of Clinical Oncologists (ASCO) reviewed the current literature and determined that long-term tamoxifen treatment does indeed prevent breast cancer in women with elevated risk (Chlebowski, et al., 1999). However, continued use of tamoxifen may cause endometrial

cancer (predominantly stage I) and non-fatal pulmonary emboli. No cardiovascular risks or benefits have been observed in patients taking tamoxifen (Reis, et al., 2001; Vogel, et al., 2010). Preventative tamoxifen treatment in women with elevated risk of breast cancer has been associated with lower incidence of disease, but is only associated with a breast cancer survival advantage after 15-years (Fisher, et al., 2005). Thus, whether tamoxifen prevents breast cancers by preventing lesions, reversing the appearance of early lesions, or delaying tumor growth is not clear (Vogel, et al., 2010). Additionally, recent data have shown that long-term use of aromatase inhibitors (eg, exemestane) reduces the incidence of breast cancer in post-menopausal women with few reported side effects (Goss, et al., 2011).

Breast cancer treatment strategies targeting hormone actions are also effective once a tumor has been diagnosed. Estrogens are primarily synthesized in the ovary; thus, oophorectomy is a surgical method of hormone ablation. Surgical oophorectomy was the first effective treatment recognized for breast cancer (Beatson, 1896; S. Boyd, 1900). Although surgical oophorectomy provides a survival advantage (Early Breast Cancer Trialists' Collaborative Group, 1996, 2000), medical oophorectomy with tamoxifen is more often considered for women with ER-positive tumors. Tamoxifen is a widely prescribed breast cancer drug with an impressive long-term benefit in the adjuvant setting (Davies, et al., 2011; Early Breast Cancer Trialists' Collaborative Group, 1998, 2001). However, 40% of patients on tamoxifen relapse during treatment (Campbell, et al., 1981; Ingle, et al., 1991; Jaiyesimi, et al., 1995; Lippman & Allegra, 1980; R. Paridaens, et al., 1980; Ring & Dowsett, 2004; Stewart, et al., 1982). In patients with tamoxifen-resistant tumors, sequential treatment with other hormonal therapies (eg, aromatase inhibitors) can be effective (Ring & Dowsett, 2004). Often patient tumors become resistant to hormonal therapies after 12–18 months due to tumor adaptive mechanisms that include *de novo* production of local estrogen, hypersensitivity of ER to low estrogen levels, estrogenic effects of tamoxifen metabolites, overexpression or activation of growth factor pathway proteins, or downregulation of transcriptional repressors (Ring & Dowsett, 2004; Santen, et al., 1990). There are *in vivo* and *in vitro* data to support the hypothesis that tamoxifen treatment can cause some tumors to become hypersensitive to low levels of estrogen or begin responding to the antagonist (ie, tamoxifen) as an ER agonist, thus stopping tamoxifen treatment may also be effective in resistant tumors (Ring & Dowsett, 2004; Shim, et al., 2000). Tumor regression in these patients may occur if treated with aromatase inhibitors that prevent the production of estrogens or with the pure antiestrogen (ie, devoid of any agonistic activity) fulvestrant (Gottardis, et al., 1989).

Despite the relative success of tamoxifen treatment for ER-positive tumors, recent clinical trials have demonstrated that aromatase inhibitors (ie, anastrozole, letrozole, or exemestane) are better than tamoxifen as first-line adjuvant breast cancer therapies and have fewer side effects in advanced breast cancers (Baum, et al., 2002; Baum, et al., 2003; Bonnetterre, et al., 2000; Buzdar, et al., 2006; Crivellari, et al., 2008; Cuzick, et al., 2010; Duffy, et al., 2006; Howell, et al., 2005; Mouridsen, et al., 2001; Nabholz, et al., 2000; R. J. Paridaens, et al., 2008). The superiority of aromatase inhibition over ER inhibition was primarily tested in the large scale Anastrozole and Tamoxifen Alone or in Combination (ATAC) clinical trial. The reason aromatase inhibitors are more effective than tamoxifen likely depends on their mechanism of action: antiestrogens can only impact ER-mediated transcriptional programs, but do not reduce the absolute levels of free estradiol in breast tissue (Cavalieri, et al., 2000; Santen, 2002). Estradiol levels remain high in breast tissue in post-menopausal women, even though their circulating levels decrease after menopause (Chetrite, et al., 2000; Geisler, et al., 2000; van Landeghem, et al., 1985). In post-menopausal women, estradiol is primarily synthesized in the breast through the conversion of androgens by aromatase enzymes or the conversion of estrone sulfate by sulfatase enzymes (Masamura, et al., 1996; Siiteri, 1987). Free estradiol at high levels may contribute to DNA mutagenesis and/or activate other estrogen-binding proteins, such as GPR30/GPER (reviewed in (Lappano, et al., 2013)).

Conversely, aromatase inhibitors reduce free estradiol levels, thus blocking all estrogen-dependent events, including ER-mediated transcription and estradiol's mutagenic properties. Additionally, treating patients with aromatase inhibitors is clinically advantageous because, unlike the agonistic properties of tamoxifen outside the breast, they do not increase deep vein thrombosis, pulmonary emboli, or adversely affect endometrial tissue, vaginal mucosa, or cholesterol levels in women (Baum, et al., 2003; Buzdar, et al., 2006).

Blocking ER activity or estrogen synthesis are the predominant endocrine therapies available for patients with luminal breast cancer; however, evidence shows that progestins also contribute to invasive breast cancer incidence and mortality (Chlebowski, et al., 2003). These findings in humans are supported by numerous studies conducted in animal models of breast cancer (Balana, et al., 2001; Balana, et al., 1999; Carnevale, et al., 2007; Labriola, et al., 2003; Lanari, et al., 1986; Lydon, et al., 1995; Michna, et al., 1989; Molinolo, et al., 1987; Poole, et al., 2006). Up to 40% of women will relapse on antiestrogen endocrine therapies because of *de novo* (primary) or acquired (secondary) resistance mechanisms. Endocrine resistant cells are often steroid hormone independent and their proliferation utilizes autocrine and paracrine peptide growth factors and downstream protein kinase pathway activation, including IGF1R, HER2, EGFR, MAPK, and AKT signaling. Historically, PR was primarily considered a marker for functional ER activity because PR is an ER target gene, yet recent evidence has shown that PR function and target gene regulation is distinct from ER and is an independent driver of breast cancer cell proliferation and survival (Hilton, et al., 2012). Indeed, PR is a phospho-protein whose regulation of gene expression is highly influenced by protein kinase cross-talk. Thus, in antiestrogen-resistant cells with high kinase activity, PR phosphorylation and transcriptional activation is likely to be enhanced (Knutson, et al., 2012; Shen, et al., 2001; Song, et al., 2002). Therefore, additional investigation is needed to better understand how PR post-translational modifications alter gene regulation in normal breast cells relative to breast cancer cells, including both endocrine-resistant and endocrine-sensitive breast tumor cells, so that the use of combined antiestrogen and antiprogestin targeted therapies may be evaluated in patients with luminal tumors.

7. Development of antiestrogens and antiprogestins to block ER/PR function

Early studies showing that steroid hormone actions drive a substantial portion of breast cancers (Armstrong, et al., 2000; Beatson, 1896; S. Boyd, 1900; Trichopoulos, et al., 1972; Zumoff, 1998) triggered the development of pharmacological strategies to block the hormonal drivers of these tumors. Several ER and PR antagonists have been developed for the prevention and treatment of breast cancers (Cole, et al., 1971; Ward, 1973). Crystal structure studies demonstrate that ER and PR agonists (eg, 17-beta-estradiol and progesterone) bind a hydrophobic pocket and cause a conformational change to the conserved alpha-helix 12, causing it to swing shut and close the ligand-binding pocket (Shiau, et al., 1998; Tanenbaum, et al., 1998). Alpha-helix 12 contains an activation function (AF) domain, and this new structural configuration allows the receptor to interact with coactivator molecules and enhance transcription (Shiau, et al., 1998; Tanenbaum, et al., 1998). Conversely, ER and PR antagonists often contain bulky side chains that block this conformational change in alpha-helix 12, allowing for corepressor interactions and transcriptional repression (Madauss, et al., 2007; Raaijmakers, et al., 2009). Tamoxifen and raloxifene have mixed agonist and antagonist actions (Jordan & Morrow, 1999); drugs of this class are selective estrogen receptor modulators (SERMs) (Jordan & Morrow, 1999). Upon ER binding, SERMs facilitate ER-mediated transcriptional repression or activation, depending on the cellular context. In breast tissue, tamoxifen's effects are antagonistic, but

in the uterus, its effects are agonistic, causing endometrial wall thickening (Jordan & Dowse, 1976; Silfen, et al., 1999). However, the pure ER antagonist fulvestrant causes ER degradation, inhibits ER-mediated transcriptional action, and provides a similar anti-tumor effect as the aromatase inhibitor anastrozole (Howell, et al., 2002; Osborne, et al., 2002). Akin to antiestrogens, the first PR antagonists included several ligands that dramatically inhibited PR transcriptional action. Recent reviews have outlined much of the pre-clinical and clinical results for these compounds in the context of breast cancer treatment (Table 2) (Klijn, et al., 2000; Lanari, et al., 2012). Mifepristone (RU486) is a PR modulator (PRM) that binds in the ligand pocket and completely blocks PR transcriptional activity (depending on cellular context) (Han, et al., 2007). Mifepristone was tested in phase II clinical trials for breast cancer, but it had limited efficacy and substantial toxicity; side effects included lethargy, nausea, and anorexia (Perrault, et al., 1996). Mifepristone also binds the glucocorticoid receptor (GR) with moderate affinity, and this is likely the cause of toxicity in patients (Clark, 2008). Less toxicity was reported for trials with mifepristone in meningioma patients, possibly because of co-administration of glucocorticoids to limit the anti-GR effects of mifepristone (Grunberg, et al., 2006). It would be interesting to know whether mifepristone would have been more effective against tumor progression if patients were selected based on their molecular subtypes (ie, luminal B) or an activated PR gene signature, instead of only PR-positivity. Onapristone (ZK 98299) is another PRM that had significant anti-tumor efficacy in clinical trials (Robertson, et al., 1999). However, the drug caused hepatotoxicity in patients, and the trial was halted (Robertson, et al., 1999). Lonaprisan (ZK 230211) is a third-generation, highly selective PR antagonist that was recently tested in a phase II clinical trial for stage IV metastatic breast cancer patients that were PR+/HER2- and that had progressed on endocrine therapy (Jonat, et al., 2013). In this study, only 14% of patients taking lonaprisan had stable disease >6 months (7% >12 months), and there were no partial or complete responses. In cell culture models, lonaprisan bound PR to induce cell cycle arrest and block proliferation through the induction of p21 (Busia, et al., 2011). This antiprogesterin may have significant clinical impact if administered to a select group of patients with transcriptionally hyperactive PR, independent of clinically determined PR expression status (ie, protein-based PR/HER2 profile). EC304 is a newly synthesized compound that has potent antiprogestational activity. EC304 blocks PR transcriptional activity, breast cancer cell proliferation, and does not contain antiglucocorticoid activity, suggesting this compound may be very useful for cancer patients (Nickisch, et al., 2013). Additional PR antagonists have been investigated to combat breast cancer growth including telapristone (CDB-4124), asoprisnil, and ulipristal acetates with encouraging results (Communal, et al., 2012; Madauss, et al., 2007; Wiehle, et al., 2011). Further development of highly selective PR antagonists, careful selection of patients whose tumors contain activated PRs, and treatment in combination with other endocrine therapies may significantly advance hormone-modulation strategies for breast cancer.

8. Conclusions

Mammary tissue development, differentiation, and remodeling during pregnancy are critically dependent on progesterone and PR actions. Progesterone/PRs also drive increased breast tumor cell proliferation and survival. Exposure to progesterone or synthetic progestins increases invasive breast cancer risk and mortality in post-menopausal women. In breast cancer cells, the proliferative actions of progesterone are directed by PR-B-specific post-translational modifications that readily occur in the context of activated growth factor protein kinase signaling. In the face of heightened signaling pathway activation, protein kinases that input to MAPK-dependent cell cycle progression may diminish or bypass the need for ligand-binding and redirect phospho-PRs to gene subsets important for rapid tumor progression. Context-dependent PR actions clearly impact breast tumors of the luminal subtypes. We conclude that PR levels should not be simply regarded as biomarkers of

functional ER expression. Rather, PR gene signatures should be incorporated into modern diagnostics aimed at more focused molecular targeting of luminal breast cancers. In sum, there is great potential for improved endocrine therapies that include the use of selective antiprogesterins. Additional investigation is warranted to elucidate the contribution of phospho-PRs and the role of PR-isoform specific actions to both early and late stages of breast cancer progression and to establish the biological time frame that is most appropriate for targeting activated PRs (ie, early in endocrine therapy in conjunction with ER-targeted therapies and/or following frank tumor progression/endocrine failure).

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Abbreviations

AF	activation function
AP-1	activator protein 1
CD	common docking
CDK2	cyclin dependent kinase 2
CK2	casein kinase II
DUSP6/MKP3	dual specificity phosphatase 6/ MAPK phosphatase 3
EGF	epidermal growth factor
ER	estrogen receptor alpha
GR	glucocorticoid receptor
HER2	human epidermal growth factor receptor 2
HRT	hormone replacement therapy
IHC	immunohistochemistry
JAK	janus kinase
MAPK	mitogen activated protein kinase
PR	progesterone receptor
PRM	progesterone receptor modulator
RT-qPCR	reverse transcription quantitative polymerase chain reaction
SERM	selective estrogen receptor modulator
Sp1	specificity protein 1
SR	steroid hormone receptor
STAT	signal transducers and activators of transcription
SUMO	small ubiquitin-like modifier

WHI World Health Initiative
WT wild type

References

- Abdel-Hafiz H, Takimoto GS, Tung L, Horwitz KB. The inhibitory function in human progesterone receptor N termini binds SUMO-1 protein to regulate autoinhibition and transrepression. *J Biol Chem*. 2002; 277:33950–33956. [PubMed: 12114521]
- Anderson GL, Chlebowski RT, Aragaki AK, Kuller LH, Manson JE, Gass M, et al. Conjugated equine oestrogen and breast cancer incidence and mortality in postmenopausal women with hysterectomy: extended follow-up of the Women's Health Initiative randomised placebo-controlled trial. *Lancet Oncol*. 2012
- Antoniou A, Pharoah PD, Narod S, Risch HA, Eyfjord JE, Hopper JL, et al. Average risks of breast and ovarian cancer associated with BRCA1 or BRCA2 mutations detected in case Series unselected for family history: a combined analysis of 22 studies. *Am J Hum Genet*. 2003; 72:1117–1130. [PubMed: 12677558]
- Armstrong K, Eisen A, Weber B. Assessing the risk of breast cancer. *N Engl J Med*. 2000; 342:564–571. [PubMed: 10684916]
- Aupperlee MD, Smith KT, Kariagina A, Haslam SZ. Progesterone receptor isoforms A and B: temporal and spatial differences in expression during murine mammary gland development. *Endocrinology*. 2005; 146:3577–3588. [PubMed: 15878961]
- Balana ME, Labriola L, Salatino M, Movsichoff F, Peters G, Charreau EH, et al. Activation of ErbB-2 via a hierarchical interaction between ErbB-2 and type I insulin-like growth factor receptor in mammary tumor cells. *Oncogene*. 2001; 20:34–47. [PubMed: 11244498]
- Balana ME, Lupu R, Labriola L, Charreau EH, Elizalde PV. Interactions between progestins and heregulin (HRG) signaling pathways: HRG acts as mediator of progestins proliferative effects in mouse mammary adenocarcinomas. *Oncogene*. 1999; 18:6370–6379. [PubMed: 10597237]
- Bamberger AM, Bamberger CM, Gellersen B, Schulte HM. Modulation of AP-1 activity by the human progesterone receptor in endometrial adenocarcinoma cells. *Proc Natl Acad Sci U S A*. 1996; 93:6169–6174. [PubMed: 8650238]
- Bardou VJ, Arpino G, Elledge RM, Osborne CK, Clark GM. Progesterone receptor status significantly improves outcome prediction over estrogen receptor status alone for adjuvant endocrine therapy in two large breast cancer databases. *J Clin Oncol*. 2003; 21:1973–1979. [PubMed: 12743151]
- Bastien RR, Rodriguez-Lescure A, Ebbert MT, Prat A, Munarriz B, Rowe L, et al. PAM50 breast cancer subtyping by RT-qPCR and concordance with standard clinical molecular markers. *BMC Med Genomics*. 2012; 5:44. [PubMed: 23035882]
- Baum M, Buzdar AU, Cuzick J, Forbes J, Houghton JH, Klijn JG, et al. Anastrozole alone or in combination with tamoxifen versus tamoxifen alone for adjuvant treatment of postmenopausal women with early breast cancer: first results of the ATAC randomised trial. *Lancet*. 2002; 359:2131–2139. [PubMed: 12090977]
- Baum M, Buzdar A, Cuzick J, Forbes J, Houghton J, Howell A, et al. Anastrozole alone or in combination with tamoxifen versus tamoxifen alone for adjuvant treatment of postmenopausal women with early-stage breast cancer: results of the ATAC (Arimidex, Tamoxifen Alone or in Combination) trial efficacy and safety update analyses. *Cancer*. 2003; 98:1802–1810. [PubMed: 14584060]
- Beatson GT. On the treatment of inoperable cases of carcinoma of the mamma: suggestions for a new method of treatment with illustrative cases. *Lancet*. 1896; 2:104–107.
- Beleut M, Rajaram RD, Caikovski M, Ayyanan A, Germano D, Choi Y, et al. Two distinct mechanisms underlie progesterone-induced proliferation in the mammary gland. *Proc Natl Acad Sci U S A*. 2010; 107:2989–2994. [PubMed: 20133621]
- Bjornstrom L, Sjöberg M. Mechanisms of estrogen receptor signaling: convergence of genomic and nongenomic actions on target genes. *Mol Endocrinol*. 2005; 19:833–842. [PubMed: 15695368]

- Bonnetterre J, Thurlimann B, Robertson JF, Krzakowski M, Mauriac L, Koralewski P, et al. Anastrozole versus tamoxifen as first-line therapy for advanced breast cancer in 668 postmenopausal women: results of the Tamoxifen or Arimidex Randomized Group Efficacy and Tolerability study. *J Clin Oncol*. 2000; 18:3748–3757. [PubMed: 11078487]
- Boonyaratankornkit V, Scott MP, Ribon V, Sherman L, Anderson SM, Maller JL, et al. Progesterone receptor contains a proline-rich motif that directly interacts with SH3 domains and activates c-Src family tyrosine kinases. *Mol Cell*. 2001; 8:269–280. [PubMed: 11545730]
- Boyd NF, Guo H, Martin LJ, Sun L, Stone J, Fishell E, et al. Mammographic density and the risk and detection of breast cancer. *N Engl J Med*. 2007; 356:227–236. [PubMed: 17229950]
- Boyd S. On oophorectomy in cancer of the breast. *Br Med J*. 1900; 2:1161–1167.
- Brisken C. Progesterone signalling in breast cancer: a neglected hormone coming into the limelight. *Nat Rev Cancer*. 2013; 13:385–396. [PubMed: 23702927]
- Brown KA, Simpson ER. Obesity and Breast Cancer: mechanisms and therapeutic implications. *Front Biosci (Elite Ed)*. 2012; 4:2515–2524. [PubMed: 22652657]
- Bush TL, Barrett-Connor E, Cowan LD, Criqui MH, Wallace RB, Suchindran CM, et al. Cardiovascular mortality and noncontraceptive use of estrogen in women: results from the Lipid Research Clinics Program Follow-up Study. *Circulation*. 1987; 75:1102–1109. [PubMed: 3568321]
- Busia L, Faus H, Hoffmann J, Haendler B. The antiprogesterin Lonaprisan inhibits breast cancer cell proliferation by inducing p21 expression. *Mol Cell Endocrinol*. 2011; 333:37–46. [PubMed: 21138753]
- Buzdar A, Howell A, Cuzick J, Wale C, Distler W, Hocht-Boes G, et al. Comprehensive side-effect profile of anastrozole and tamoxifen as adjuvant treatment for early-stage breast cancer: long-term safety analysis of the ATAC trial. *Lancet Oncol*. 2006; 7:633–643. [PubMed: 16887480]
- Byrne C, Schairer C, Wolfe J, Parekh N, Salane M, Brinton LA, et al. Mammographic features and breast cancer risk: effects with time, age, and menopause status. *J Natl Cancer Inst*. 1995; 87:1622–1629. [PubMed: 7563205]
- Campbell FC, Blamey RW, Elston CW, Morris AH, Nicholson RI, Griffiths K, et al. Quantitative oestradiol receptor values in primary breast cancer and response of metastases to endocrine therapy. *Lancet*. 1981; 2:1317–1319. [PubMed: 6118718]
- Cancer Genome Atlas Network. Comprehensive molecular portraits of human breast tumours. *Nature*. 2012; 490:61–70. [PubMed: 23000897]
- Carnevale RP, Proietti CJ, Salatino M, Urtreger A, Peluffo G, Edwards DP, et al. Progesterin effects on breast cancer cell proliferation, proteases activation, and in vivo development of metastatic phenotype all depend on progesterone receptor capacity to activate cytoplasmic signaling pathways. *Mol Endocrinol*. 2007; 21:1335–1358. [PubMed: 17440047]
- Cavalieri E, Frenkel K, Liehr JG, Rogan E, Roy D. Estrogens as endogenous genotoxic agents--DNA adducts and mutations. *J Natl Cancer Inst Monogr*. 2000; 75–93. [PubMed: 10963621]
- Cerami E, Gao J, Dogrusoz U, Gross BE, Sumer SO, Aksoy BA, et al. The cBio cancer genomics portal: an open platform for exploring multidimensional cancer genomics data. *Cancer Discov*. 2012; 2:401–404. [PubMed: 22588877]
- Chetrite GS, Cortes-Prieto J, Philippe JC, Wright F, Pasqualini JR. Comparison of estrogen concentrations, estrone sulfatase and aromatase activities in normal, and in cancerous, human breast tissues. *J Steroid Biochem Mol Biol*. 2000; 72:23–27. [PubMed: 10731634]
- Chlebowski RT, Aiello E, McTiernan A. Weight loss in breast cancer patient management. *J Clin Oncol*. 2002; 20:1128–1143. [PubMed: 11844838]
- Chlebowski RT, Anderson GL, Gass M, Lane DS, Aragaki AK, Kuller LH, et al. Estrogen plus progestin and breast cancer incidence and mortality in postmenopausal women. *JAMA*. 2010; 304:1684–1692. [PubMed: 20959578]
- Chlebowski RT, Collyar DE, Somerfield MR, Pfister DG. American Society of Clinical Oncology technology assessment on breast cancer risk reduction strategies: tamoxifen and raloxifene. *J Clin Oncol*. 1999; 17:1939–1955. [PubMed: 10561236]
- Chlebowski RT, Hendrix SL, Langer RD, Stefanick ML, Gass M, Lane D, et al. Influence of estrogen plus progestin on breast cancer and mammography in healthy postmenopausal women: the

- Women's Health Initiative Randomized Trial. *JAMA*. 2003; 289:3243–3253. [PubMed: 12824205]
- Chlebowski RT, Kuller LH, Prentice RL, Stefanick ML, Manson JE, Gass M, et al. Breast cancer after use of estrogen plus progestin in postmenopausal women. *N Engl J Med*. 2009; 360:573–587. [PubMed: 19196674]
- Chwalisz K, Larsen L, Mattia-Goldberg C, Edmonds A, Elger W, Winkel CA. A randomized, controlled trial of asoprisnil, a novel selective progesterone receptor modulator, in women with uterine leiomyomata. *Fertil Steril*. 2007; 87:1399–1412. [PubMed: 17307170]
- Civenni G, Holbro T, Hynes NE. Wnt1 and Wnt5a induce cyclin D1 expression through ErbB1 transactivation in HC11 mammary epithelial cells. *EMBO Rep*. 2003; 4:166–171. [PubMed: 12612606]
- Clark RD. Glucocorticoid receptor antagonists. *Curr Top Med Chem*. 2008; 8:813–838. [PubMed: 18537690]
- Clemons M, Goss P. Estrogen and the risk of breast cancer. *N Engl J Med*. 2001; 344:276–285. [PubMed: 11172156]
- Cole MP, Jones CT, Todd ID. A new anti-oestrogenic agent in late breast cancer. An early clinical appraisal of ICI46474. *Br J Cancer*. 1971; 25:270–275. [PubMed: 5115829]
- Collaborative Group on Hormonal Factors in Breast C. Breast cancer and hormonal contraceptives: collaborative reanalysis of individual data on 53 297 women with breast cancer and 100 239 women without breast cancer from 54 epidemiological studies. *Lancet*. 1996; 347:1713–1727. [PubMed: 8656904]
- Collaborative Group on Hormonal Factors in Breast Cancer. Breast cancer and hormone replacement therapy: collaborative reanalysis of data from 51 epidemiological studies of 52,705 women with breast cancer and 108,411 women without breast cancer. Collaborative Group on Hormonal Factors in Breast Cancer. *Lancet*. 1997; 350:1047–1059. [PubMed: 10213546]
- Collaborative Group on Hormonal Factors in Breast Cancer. Breast cancer and breastfeeding: collaborative reanalysis of individual data from 47 epidemiological studies in 30 countries, including 50302 women with breast cancer and 96973 women without the disease. *Lancet*. 2002; 360:187–195. [PubMed: 12133652]
- Communal L, Vilasco M, Hugon-Rodin J, Courtin A, Mourra N, Lahlou N, et al. Ulipristal acetate does not impact human normal breast tissue. *Hum Reprod*. 2012
- Crivellari D, Sun Z, Coates AS, Price KN, Thurlimann B, Mouridsen H, et al. Letrozole compared with tamoxifen for elderly patients with endocrine-responsive early breast cancer: the BIG 1-98 trial. *J Clin Oncol*. 2008; 26:1972–1979. [PubMed: 18332471]
- Cummings SR, Eckert S, Krueger KA, Grady D, Powles TJ, Cauley JA, et al. The effect of raloxifene on risk of breast cancer in postmenopausal women: results from the MORE randomized trial. Multiple Outcomes of Raloxifene Evaluation. *JAMA*. 1999; 281:2189–2197. [PubMed: 10376571]
- Cummings SR, San Martin J, McClung MR, Siris ES, Eastell R, Reid IR, et al. Denosumab for prevention of fractures in postmenopausal women with osteoporosis. *N Engl J Med*. 2009; 361:756–765. [PubMed: 19671655]
- Cuzick J, Powles T, Veronesi U, Forbes J, Edwards R, Ashley S, et al. Overview of the main outcomes in breast-cancer prevention trials. *Lancet*. 2003; 361:296–300. [PubMed: 12559863]
- Cuzick J, Sestak I, Baum M, Buzdar A, Howell A, Dowsett M, et al. Effect of anastrozole and tamoxifen as adjuvant treatment for early-stage breast cancer: 10-year analysis of the ATAC trial. *Lancet Oncol*. 2010; 11:1135–1141. [PubMed: 21087898]
- Daniel AR, Faivre EJ, Lange CA. Phosphorylation-dependent antagonism of sumoylation derepresses progesterone receptor action in breast cancer cells. *Mol Endocrinol*. 2007; 21:2890–2906. [PubMed: 17717077]
- Daniel AR, Gaviglio AL, Czaplicki LM, Hillard CJ, Housa D, Lange CA. The progesterone receptor hinge region regulates the kinetics of transcriptional responses through acetylation, phosphorylation, and nuclear retention. *Mol Endocrinol*. 2010; 24:2126–2138. [PubMed: 20861224]

- Daniel CW, Silberstein GB, Strickland P. Direct action of 17 beta-estradiol on mouse mammary ducts analyzed by sustained release implants and steroid autoradiography. *Cancer Res.* 1987; 47:6052–6057. [PubMed: 3664507]
- Davies C, Godwin J, Gray R, Clarke M, Cutter D, Darby S, et al. Relevance of breast cancer hormone receptors and other factors to the efficacy of adjuvant tamoxifen: patient-level meta-analysis of randomised trials. *Lancet.* 2011; 378:771–784. [PubMed: 21802721]
- Dressing GE, Knutson TP, Hagan CR, Schiewer MJ, Daniel AR, Knudsen KE, et al. Progesterone Receptor-Cyclin D1 Complexes Induce Cell Cycle-Dependent Transcriptional Programs. *Mol Endocrinol.* 2013 In review.
- Duffy S, Jackson TL, Lansdown M, Philips K, Wells M, Pollard S, et al. The ATAC ('Arimidex', Tamoxifen, Alone or in Combination) adjuvant breast cancer trial: first results of the endometrial sub-protocol following 2 years of treatment. *Hum Reprod.* 2006; 21:545–553. [PubMed: 16210385]
- Early Breast Cancer Trialists' Collaborative Group. Ovarian ablation in early breast cancer: overview of the randomised trials. *Lancet.* 1996; 348:1189–1196. [PubMed: 8898035]
- Early Breast Cancer Trialists' Collaborative Group. Tamoxifen for early breast cancer: an overview of the randomised trials. *Lancet.* 1998; 351:1451–1467. [PubMed: 9605801]
- Early Breast Cancer Trialists' Collaborative Group. Ovarian ablation for early breast cancer. *Cochrane Database Syst Rev.* 2000:CD000485.
- Early Breast Cancer Trialists' Collaborative Group. Tamoxifen for early breast cancer. *Cochrane Database Syst Rev.* 2001:CD000486. [PubMed: 11279694]
- Edwards DP. Regulation of signal transduction pathways by estrogen and progesterone. *Annu Rev Physiol.* 2005; 67:335–376. [PubMed: 15709962]
- Encarnacion CA, Ciocca DR, McGuire WL, Clark GM, Fuqua SA, Osborne CK. Measurement of steroid hormone receptors in breast cancer patients on tamoxifen. *Breast Cancer Res Treat.* 1993; 26:237–246. [PubMed: 8251648]
- Fabre A, Fournier A, Mesrine S, Desreux J, Gompel A, Boutron-Ruault MC, et al. Oral progestagens before menopause and breast cancer risk. *Br J Cancer.* 2007; 96:841–844. [PubMed: 17299388]
- Faivre EJ, Daniel AR, Hillard CJ, Lange CA. Progesterone receptor rapid signaling mediates serine 345 phosphorylation and tethering to specificity protein 1 transcription factors. *Mol Endocrinol.* 2008; 22:823–837. [PubMed: 18202149]
- Faivre EJ, Lange CA. Progesterone receptors upregulate Wnt-1 to induce epidermal growth factor receptor transactivation and c-Src-dependent sustained activation of Erk1/2 mitogen-activated protein kinase in breast cancer cells. *Mol Cell Biol.* 2007; 27:466–480. [PubMed: 17074804]
- Faupel-Badger JM, Arcaro KF, Balkam JJ, Eliassen AH, Hassiotou F, Lebrilla CB, et al. Postpartum remodeling, lactation, and breast cancer risk: summary of a National Cancer Institute-sponsored workshop. *J Natl Cancer Inst.* 2013; 105:166–174. [PubMed: 23264680]
- Faus H, Haendler B. Post-translational modifications of steroid receptors. *Biomed Pharmacother.* 2006; 60:520–528. [PubMed: 16949786]
- Feinleib M. Breast cancer and artificial menopause: a cohort study. *J Natl Cancer Inst.* 1968; 41:315–329. [PubMed: 5671283]
- Fisher B, Costantino JP, Wickerham DL, Cecchini RS, Cronin WM, Robidoux A, et al. Tamoxifen for the prevention of breast cancer: current status of the National Surgical Adjuvant Breast and Bowel Project P-1 study. *J Natl Cancer Inst.* 2005; 97:1652–1662. [PubMed: 16288118]
- Fisher B, Costantino JP, Wickerham DL, Redmond CK, Kavanah M, Cronin WM, et al. Tamoxifen for prevention of breast cancer: report of the National Surgical Adjuvant Breast and Bowel Project P-1 Study. *J Natl Cancer Inst.* 1998; 90:1371–1388. [PubMed: 9747868]
- Fournier A, Berrino F, Clavel-Chapelon F. Unequal risks for breast cancer associated with different hormone replacement therapies: results from the E3N cohort study. *Breast Cancer Res Treat.* 2008; 107:103–111. [PubMed: 17333341]
- Fournier A, Berrino F, Riboli E, Avenel V, Clavel-Chapelon F. Breast cancer risk in relation to different types of hormone replacement therapy in the E3N-EPIC cohort. *Int J Cancer.* 2005; 114:448–454. [PubMed: 15551359]

- Gao J, Aksoy BA, Dogrusoz U, Dresdner G, Gross B, Sumer SO, et al. Integrative analysis of complex cancer genomics and clinical profiles using the cBioPortal. *Sci Signal*. 2013; 6 pl1.
- Gareau JR, Lima CD. The SUMO pathway: emerging mechanisms that shape specificity, conjugation and recognition. *Nat Rev Mol Cell Biol*. 2010; 11:861–871. [PubMed: 21102611]
- Geisler J, Berntsen H, Lonning PE. A novel HPLC-RIA method for the simultaneous detection of estrone, estradiol and estrone sulphate levels in breast cancer tissue. *J Steroid Biochem Mol Biol*. 2000; 72:259–264. [PubMed: 10822015]
- Gill G. Post-translational modification by the small ubiquitin-related modifier SUMO has big effects on transcription factor activity. *Curr Opin Genet Dev*. 2003; 13:108–113. [PubMed: 12672486]
- Gonzalez-Malerva L, Park J, Zou L, Hu Y, Moradpour Z, Pearlberg J, et al. High-throughput ectopic expression screen for tamoxifen resistance identifies an atypical kinase that blocks autophagy. *Proc Natl Acad Sci U S A*. 2011; 108:2058–2063. [PubMed: 21233418]
- Goss PE, Ingle JN, Ales-Martinez JE, Cheung AM, Chlebowski RT, Wactawski-Wende J, et al. Exemestane for breast-cancer prevention in postmenopausal women. *N Engl J Med*. 2011; 364:2381–2391. [PubMed: 21639806]
- Gottardis MM, Jiang SY, Jeng MH, Jordan VC. Inhibition of tamoxifen-stimulated growth of an MCF-7 tumor variant in athymic mice by novel steroidal antiestrogens. *Cancer Res*. 1989; 49:4090–4093. [PubMed: 2743303]
- Grady D, Rubin SM, Petitti DB, Fox CS, Black D, Ettinger B, et al. Hormone therapy to prevent disease and prolong life in postmenopausal women. *Ann Intern Med*. 1992; 117:1016–1037. [PubMed: 1443971]
- Greendale GA, Reboussin BA, Sie A, Singh HR, Olson LK, Gatewood O, et al. Effects of estrogen and estrogen-progestin on mammographic parenchymal density. Postmenopausal Estrogen/Progestin Interventions (PEPI) Investigators. *Ann Intern Med*. 1999; 130:262–269. [PubMed: 10068383]
- Grunberg SM, Weiss MH, Russell CA, Spitz IM, Ahmadi J, Sadun A, et al. Long-term administration of mifepristone (RU486): clinical tolerance during extended treatment of meningioma. *Cancer Invest*. 2006; 24:727–733. [PubMed: 17162554]
- Hagan CR, Daniel AR, Dressing GE, Lange CA. Role of phosphorylation in progesterone receptor signaling and specificity. *Mol Cell Endocrinol*. 2012; 357:43–49. [PubMed: 21945472]
- Hagan CR, Knutson TP, Lange CA. A Common Docking Domain in Progesterone Receptor-B links DUSP6 and CK2 signaling to proliferative transcriptional programs in breast cancer cells. *Nucleic Acids Res*. 2013
- Hagan CR, Regan TM, Dressing GE, Lange CA. ck2-dependent phosphorylation of progesterone receptors (PR) on Ser81 regulates PR-B isoform-specific target gene expression in breast cancer cells. *Mol Cell Biol*. 2011; 31:2439–2452. [PubMed: 21518957]
- Hammond ME, Hayes DF, Dowsett M, Allred DC, Hagerty KL, Badve S, et al. American Society of Clinical Oncology/College Of American Pathologists guideline recommendations for immunohistochemical testing of estrogen and progesterone receptors in breast cancer. *J Clin Oncol*. 2010; 28:2784–2795. [PubMed: 20404251]
- Han SJ, Tsai SY, Tsai MJ, O'Malley BW. Distinct temporal and spatial activities of RU486 on progesterone receptor function in reproductive organs of ovariectomized mice. *Endocrinology*. 2007; 148:2471–2486. [PubMed: 17303655]
- Haslam SZ. The ontogeny of mouse mammary gland responsiveness to ovarian steroid hormones. *Endocrinology*. 1989; 125:2766–2772. [PubMed: 2477236]
- Haslam SZ, Woodward TL. Host microenvironment in breast cancer development: epithelial-cell-stromal-cell interactions and steroid hormone action in normal and cancerous mammary gland. *Breast Cancer Res*. 2003; 5:208–215. [PubMed: 12817994]
- Hay RT. SUMO: a history of modification. *Mol Cell*. 2005; 18:1–12. [PubMed: 15808504]
- Helle SI, Jonat W, Giurescu M, Ekse D, Holly JM, Lonning PE. Influence of treatment with onapristone on the IGF-system in breast cancer patients. *J Steroid Biochem Mol Biol*. 1998; 66:159–163. [PubMed: 9719450]
- Henderson BE, Feigelson HS. Hormonal carcinogenesis. *Carcinogenesis*. 2000; 21:427–433. [PubMed: 10688862]

- Hennighausen L, Robinson GW. Information networks in the mammary gland. *Nat Rev Mol Cell Biol*. 2005; 6:715–725. [PubMed: 16231422]
- Hilton HN, Graham JD, Kantimm S, Santucci N, Cloosterman D, Huschtscha LI, et al. Progesterone and estrogen receptors segregate into different cell subpopulations in the normal human breast. *Mol Cell Endocrinol*. 2012; 361:191–201. [PubMed: 22580007]
- Horwitz KB, Mockus MB, Lessey BA. Variant T47D human breast cancer cells with high progesterone-receptor levels despite estrogen and antiestrogen resistance. *Cell*. 1982; 28:633–642. [PubMed: 7200400]
- Howell A, Cuzick J, Baum M, Buzdar A, Dowsett M, Forbes JF, et al. Results of the ATAC (Arimidex, Tamoxifen, Alone or in Combination) trial after completion of 5 years' adjuvant treatment for breast cancer. *Lancet*. 2005; 365:60–62. [PubMed: 15639680]
- Howell A, Robertson JF, Quaresma Albano J, Aschermannova A, Mauriac L, Kleeberg UR, et al. Fulvestrant, formerly ICI 182,780, is as effective as anastrozole in postmenopausal women with advanced breast cancer progressing after prior endocrine treatment. *J Clin Oncol*. 2002; 20:3396–3403. [PubMed: 12177099]
- Huang Z, Hankinson SE, Colditz GA, Stampfer MJ, Hunter DJ, Manson JE, et al. Dual effects of weight and weight gain on breast cancer risk. *JAMA*. 1997; 278:1407–1411. [PubMed: 9355998]
- Hunter DJ, Colditz GA, Hankinson SE, Malspeis S, Spiegelman D, Chen W, et al. Oral contraceptive use and breast cancer: a prospective study of young women. *Cancer Epidemiol Biomarkers Prev*. 2010; 19:2496–2502. [PubMed: 20802021]
- Ingle JN, Mailliard JA, Schaid DJ, Krook JE, Gesme DH Jr, Windschitl HE, et al. A double-blind trial of tamoxifen plus prednisolone versus tamoxifen plus placebo in postmenopausal women with metastatic breast cancer. A collaborative trial of the North Central Cancer Treatment Group and Mayo Clinic. *Cancer*. 1991; 68:34–39. [PubMed: 2049750]
- Ioffe OB, Zaino RJ, Mutter GL. Endometrial changes from short-term therapy with CDB-4124, a selective progesterone receptor modulator. *Mod Pathol*. 2009; 22:450–459. [PubMed: 19136935]
- Jaiyesimi IA, Buzdar AU, Decker DA, Hortobagyi GN. Use of tamoxifen for breast cancer: twenty-eight years later. *J Clin Oncol*. 1995; 13:513–529. [PubMed: 7844613]
- Johnson ES. Protein modification by SUMO. *Annu Rev Biochem*. 2004; 73:355–382. [PubMed: 15189146]
- Johnston S, Pippen J Jr, Pivot X, Lichinitser M, Sadeghi S, Dieras V, et al. Lapatinib combined with letrozole versus letrozole and placebo as first-line therapy for postmenopausal hormone receptor-positive metastatic breast cancer. *J Clin Oncol*. 2009; 27:5538–5546. [PubMed: 19786658]
- Jonat W, Bachelot T, Ruhstaller T, Kuss I, Reimann U, Robertson JF. Randomized phase II study of lonaprisan as second-line therapy for progesterone receptor-positive breast cancer. *Ann Oncol*. 2013
- Jordan VC, Dowse LJ. Tamoxifen as an anti-tumour agent: effect on oestrogen binding. *J Endocrinol*. 1976; 68:297–303. [PubMed: 176295]
- Jordan VC, Morrow M. Tamoxifen, raloxifene, and the prevention of breast cancer. *Endocr Rev*. 1999; 20:253–278. [PubMed: 10368771]
- Kaufman B, Mackey JR, Clemens MR, Bapsy PP, Vaid A, Wardley A, et al. Trastuzumab plus anastrozole versus anastrozole alone for the treatment of postmenopausal women with human epidermal growth factor receptor 2-positive, hormone receptor-positive metastatic breast cancer: results from the randomized phase III TAnDEM study. *J Clin Oncol*. 2009; 27:5529–5537. [PubMed: 19786670]
- Key T, Appleby P, Barnes I, Reeves G. Endogenous sex hormones and breast cancer in postmenopausal women: reanalysis of nine prospective studies. *J Natl Cancer Inst*. 2002; 94:606–616. [PubMed: 11959894]
- Khan JA, Tikad A, Fay M, Hamze A, Fagart J, Chabbert-Buffet N, et al. A new strategy for selective targeting of progesterone receptor with passive antagonists. *Mol Endocrinol*. 2013; 27:909–924. [PubMed: 23579486]
- Klijn JG, de Jong FH, Bakker GH, Lamberts SW, Rodenburg CJ, Alexieva-Figusch J. Antiprogestins, a new form of endocrine therapy for human breast cancer. *Cancer Res*. 1989; 49:2851–2856. [PubMed: 2720645]

- Klijn JG, Setyono-Han B, Foekens JA. Progesterone antagonists and progesterone receptor modulators in the treatment of breast cancer. *Steroids*. 2000; 65:825–830. [PubMed: 11108894]
- Knutson TP, Daniel AR, Fan D, Silverstein KA, Covington KR, Fuqua SA, et al. Phosphorylated and sumoylation-deficient progesterone receptors drive proliferative gene signatures during breast cancer progression. *Breast Cancer Res*. 2012; 14:R95. [PubMed: 22697792]
- Knutson TP, Lange CA. Dynamic Regulation of Steroid Hormone Receptor Transcriptional Activity by Reversible SUMOylation. *Vitam Horm*. 2013; 93:227–261. [PubMed: 23810010]
- Labriola L, Salatino M, Proietti CJ, Pecci A, Coso OA, Kornblihtt AR, et al. Heregulin induces transcriptional activation of the progesterone receptor by a mechanism that requires functional ErbB-2 and mitogen-activated protein kinase activation in breast cancer cells. *Mol Cell Biol*. 2003; 23:1095–1111. [PubMed: 12529413]
- LaCroix AZ, Chlebowski RT, Manson JE, Aragaki AK, Johnson KC, Martin L, et al. Health outcomes after stopping conjugated equine estrogens among postmenopausal women with prior hysterectomy: a randomized controlled trial. *JAMA*. 2011; 305:1305–1314. [PubMed: 21467283]
- Lanari C, Molinolo AA, Pasqualini CD. Induction of mammary adenocarcinomas by medroxyprogesterone acetate in BALB/c female mice. *Cancer Lett*. 1986; 33:215–223. [PubMed: 2947675]
- Lanari C, Wargon V, Rojas P, Molinolo AA. Antiprogestins in breast cancer treatment: are we ready? *Endocr Relat Cancer*. 2012; 19:R35–50. [PubMed: 22351709]
- Lange CA. Integration of progesterone receptor action with rapid signaling events in breast cancer models. *The Journal of Steroid Biochemistry and Molecular Biology*. 2008; 108:203–212. [PubMed: 17964138]
- Lange CA, Shen T, Horwitz KB. Phosphorylation of human progesterone receptors at serine-294 by mitogen-activated protein kinase signals their degradation by the 26S proteasome. *Proc Natl Acad Sci U S A*. 2000; 97:1032–1037. [PubMed: 10655479]
- Lappano R, De Marco P, De Francesco EM, Chimento A, Pezzi V, Maggiolini M. Cross-talk between GPER and growth factor signaling. *J Steroid Biochem Mol Biol*. 2013
- Levens ED, Potlog-Nahari C, Armstrong AY, Wesley R, Premkumar A, Blithe DL, et al. CDB-2914 for uterine leiomyomata treatment: a randomized controlled trial. *Obstet Gynecol*. 2008; 111:1129–1136. [PubMed: 18448745]
- Li CI, Beaver EF, Tang MT, Porter PL, Daling JR, Malone KE. Effect of depo-medroxyprogesterone acetate on breast cancer risk among women 20 to 44 years of age. *Cancer Res*. 2012; 72:2028–2035. [PubMed: 22369929]
- Liehr JG. Is estradiol a genotoxic mutagenic carcinogen? *Endocr Rev*. 2000; 21:40–54. [PubMed: 10696569]
- Liehr JG, Ricci MJ. 4-Hydroxylation of estrogens as marker of human mammary tumors. *Proc Natl Acad Sci U S A*. 1996; 93:3294–3296. [PubMed: 8622931]
- Lippman ME, Allegra JC. Quantitative estrogen receptor analyses: the response to endocrine and cytotoxic chemotherapy in human breast cancer and the disease-free interval. *Cancer*. 1980; 46:2829–2834. [PubMed: 7448729]
- Losel R, Wehling M. Nongenomic actions of steroid hormones. *Nat Rev Mol Cell Biol*. 2003; 4:46–56. [PubMed: 12511868]
- Lydon JP, DeMayo FJ, Funk CR, Mani SK, Hughes AR, Montgomery CA Jr, et al. Mice lacking progesterone receptor exhibit pleiotropic reproductive abnormalities. *Genes Dev*. 1995; 9:2266–2278. [PubMed: 7557380]
- Madauss KP, Grygielko ET, Deng SJ, Sulpizio AC, Stanley TB, Wu C, et al. A structural and in vitro characterization of asoprisnil: a selective progesterone receptor modulator. *Mol Endocrinol*. 2007; 21:1066–1081. [PubMed: 17356170]
- Masamura S, Santner SJ, Santen RJ. Evidence of in situ estrogen synthesis in nitrosomethylurea-induced rat mammary tumors via the enzyme estrone sulfatase. *J Steroid Biochem Mol Biol*. 1996; 58:425–429. [PubMed: 8903427]
- McGuire WL. Hormone receptors: their role in predicting prognosis and response to endocrine therapy. *Semin Oncol*. 1978; 5:428–433. [PubMed: 734443]

- Michna H, Schneider MR, Nishino Y, el Etreby MF. Antitumor activity of the antiprogestins ZK 98.299 and RU 38.486 in hormone dependent rat and mouse mammary tumors: mechanistic studies. *Breast Cancer Res Treat.* 1989; 14:275–288. [PubMed: 2514814]
- Million Women Study Collaborators. Breast cancer and hormone-replacement therapy in the Million Women Study. *Lancet.* 2003; 362:419–427. [PubMed: 12927427]
- Molinolo AA, Lanari C, Charreau EH, Sanjuan N, Pasqualini CD. Mouse mammary tumors induced by medroxyprogesterone acetate: immunohistochemistry and hormonal receptors. *J Natl Cancer Inst.* 1987; 79:1341–1350. [PubMed: 2826865]
- Moore MR, Conover JL, Franks KM. Progesterin effects on long-term growth, death, and Bcl-xL in breast cancer cells. *Biochem Biophys Res Commun.* 2000; 277:650–654. [PubMed: 11062008]
- Mouridsen H, Gershanovich M, Sun Y, Perez-Carrion R, Boni C, Monnier A, et al. Superior efficacy of letrozole versus tamoxifen as first-line therapy for postmenopausal women with advanced breast cancer: results of a phase III study of the International Letrozole Breast Cancer Group. *J Clin Oncol.* 2001; 19:2596–2606. [PubMed: 11352951]
- Mulac-Jericevic B, Lydon JP, DeMayo FJ, Conneely OM. Defective mammary gland morphogenesis in mice lacking the progesterone receptor B isoform. *Proc Natl Acad Sci U S A.* 2003; 100:9744–9749. [PubMed: 12897242]
- Mulac-Jericevic B, Mullinax RA, DeMayo FJ, Lydon JP, Conneely OM. Subgroup of reproductive functions of progesterone mediated by progesterone receptor-B isoform. *Science.* 2000; 289:1751–1754. [PubMed: 10976068]
- Nabholtz JM, Buzdar A, Pollak M, Harwin W, Burton G, Mangalik A, et al. Anastrozole is superior to tamoxifen as first-line therapy for advanced breast cancer in postmenopausal women: results of a North American multicenter randomized trial. Arimidex Study Group. *J Clin Oncol.* 2000; 18:3758–3767. [PubMed: 11078488]
- Nickisch K, Nair HB, Kesavaram N, Das B, Garfield R, Shi SQ, et al. Synthesis and antiprogesterone properties of novel 17-fluorinated steroids. *Steroids.* 2013; 78:909–919. [PubMed: 23607964]
- Osborne CK, Pippen J, Jones SE, Parker LM, Ellis M, Come S, et al. Double-blind, randomized trial comparing the efficacy and tolerability of fulvestrant versus anastrozole in postmenopausal women with advanced breast cancer progressing on prior endocrine therapy: results of a North American trial. *J Clin Oncol.* 2002; 20:3386–3395. [PubMed: 12177098]
- Owen GI, Richer JK, Tung L, Takimoto G, Horwitz KB. Progesterone regulates transcription of the p21(WAF1) cyclin- dependent kinase inhibitor gene through Sp1 and CBP/p300. *J Biol Chem.* 1998; 273:10696–10701. [PubMed: 9553133]
- Paridaens R, Sylvester RJ, Ferrazzi E, Legros N, Leclercq G, Heuson JC. Clinical significance of the quantitative assessment of estrogen receptors in advanced breast cancer. *Cancer.* 1980; 46:2889–2895. [PubMed: 7448734]
- Paridaens RJ, Dirix LY, Beex LV, Nooij M, Cameron DA, Cufer T, et al. Phase III study comparing exemestane with tamoxifen as first-line hormonal treatment of metastatic breast cancer in postmenopausal women: the European Organisation for Research and Treatment of Cancer Breast Cancer Cooperative Group. *J Clin Oncol.* 2008; 26:4883–4890. [PubMed: 18794551]
- Perrault D, Eisenhauer EA, Pritchard KI, Panasci L, Norris B, Vandenberg T, et al. Phase II study of the progesterone antagonist mifepristone in patients with untreated metastatic breast carcinoma: a National Cancer Institute of Canada Clinical Trials Group study. *J Clin Oncol.* 1996; 14:2709–2712. [PubMed: 8874331]
- Pierson-Mullany LK, Lange CA. Phosphorylation of progesterone receptor serine 400 mediates ligand-independent transcriptional activity in response to activation of cyclin-dependent protein kinase 2. *Mol Cell Biol.* 2004; 24:10542–10557. [PubMed: 15572662]
- Polyak K, Kalluri R. The role of the microenvironment in mammary gland development and cancer. *Cold Spring Harb Perspect Biol.* 2010; 2:a003244. [PubMed: 20591988]
- Poole AJ, Li Y, Kim Y, Lin SCJ, Lee WH, Lee EYHP. Prevention of Bcr1-mediated mammary tumorigenesis in mice by a progesterone antagonist. *Science.* 2006; 314:1467–1470. [PubMed: 17138902]
- Prat A, Perou CM. Deconstructing the molecular portraits of breast cancer. *Mol Oncol.* 2011; 5:5–23. [PubMed: 21147047]

- Raaijmakers HC, Versteegh JE, Uitdehaag JC. The X-ray structure of RU486 bound to the progesterone receptor in a destabilized agonistic conformation. *J Biol Chem.* 2009; 284:19572–19579. [PubMed: 19372222]
- Reis SE, Costantino JP, Wickerham DL, Tan-Chiu E, Wang J, Kavanah M. Cardiovascular effects of tamoxifen in women with and without heart disease: breast cancer prevention trial. National Surgical Adjuvant Breast and Bowel Project Breast Cancer Prevention Trial Investigators. *J Natl Cancer Inst.* 2001; 93:16–21. [PubMed: 11136837]
- Rhodes DR, Kalyana-Sundaram S, Mahavisno V, Varambally R, Yu J, Briggs BB, et al. OncoPrint 3.0: genes, pathways, and networks in a collection of 18,000 cancer gene expression profiles. *Neoplasia.* 2007; 9:166–180. [PubMed: 17356713]
- Ring A, Dowsett M. Mechanisms of tamoxifen resistance. *Endocr Relat Cancer.* 2004; 11:643–658. [PubMed: 15613444]
- Robertson JF, Willsher PC, Winterbottom L, Blamey RW, Thorpe S. Onapristone, a progesterone receptor antagonist, as first-line therapy in primary breast cancer. *Eur J Cancer.* 1999; 35:214–218. [PubMed: 10448262]
- Robinson GW, Hennighausen L, Johnson PF. Side-branching in the mammary gland: the progesterone-Wnt connection. *Genes Dev.* 2000; 14:889–894. [PubMed: 10783160]
- Romieu G, Maudelonde T, Ulmann A, Pujol H, Grenier J, Cavalie G, et al. The antiprogesterin RU486 in advanced breast cancer: preliminary clinical trial. *Bull Cancer.* 1987; 74:455–461. [PubMed: 3311238]
- Russo J, Hu YF, Tahin Q, Mihaila D, Slater C, Lareef MH, et al. Carcinogenicity of estrogens in human breast epithelial cells. *APMIS.* 2001; 109:39–52. [PubMed: 11297193]
- Russo J, Moral R, Balogh GA, Mailo D, Russo IH. The protective role of pregnancy in breast cancer. *Breast Cancer Res.* 2005; 7:131–142. [PubMed: 15987443]
- Rutter CM, Mandelson MT, Laya MB, Seger DJ, Taplin S. Changes in breast density associated with initiation, discontinuation, and continuing use of hormone replacement therapy. *JAMA.* 2001; 285:171–176. [PubMed: 11176809]
- Santen RJ. To block estrogen's synthesis or action: that is the question. *J Clin Endocrinol Metab.* 2002; 87:3007–3012. [PubMed: 12107191]
- Santen RJ. Menopausal hormone therapy and breast cancer. *J Steroid Biochem Mol Biol.* 2013
- Santen RJ, Manni A, Harvey H, Redmond C. Endocrine treatment of breast cancer in women. *Endocr Rev.* 1990; 11:221–265. [PubMed: 2194783]
- Sartorius CA, Groshong SD, Miller LA, Powell RL, Tung L, Takimoto GS, et al. New T47D breast cancer cell lines for the independent study of progesterone B- and A-receptors: only antiprogesterin-occupied B-receptors are switched to transcriptional agonists by cAMP. *Cancer Res.* 1994; 54:3868–3877. [PubMed: 8033109]
- Schramek D, Leibbrandt A, Sigl V, Kenner L, Pospisilik JA, Lee HJ, et al. Osteoclast differentiation factor RANKL controls development of progestin-driven mammary cancer. *Nature.* 2010; 468:98–102. [PubMed: 20881962]
- Shen T, Horwitz KB, Lange CA. Transcriptional hyperactivity of human progesterone receptors is coupled to their ligand-dependent down-regulation by mitogen-activated protein kinase-dependent phosphorylation of serine 294. *Mol Cell Biol.* 2001; 21:6122–6131. [PubMed: 11509655]
- Shiau AK, Barstad D, Loria PM, Cheng L, Kushner PJ, Agard DA, et al. The structural basis of estrogen receptor/coactivator recognition and the antagonism of this interaction by tamoxifen. *Cell.* 1998; 95:927–937. [PubMed: 9875847]
- Shim WS, Conaway M, Masamura S, Yue W, Wang JP, Kmar R, et al. Estradiol hypersensitivity and mitogen-activated protein kinase expression in long-term estrogen deprived human breast cancer cells in vivo. *Endocrinology.* 2000; 141:396–405. [PubMed: 10614662]
- Siegel R, Naishadham D, Jemal A. Cancer statistics, 2013. *CA Cancer J Clin.* 2013; 63:11–30. [PubMed: 23335087]
- Siiteri PK. Adipose tissue as a source of hormones. *Am J Clin Nutr.* 1987; 45:277–282. [PubMed: 3541569]

- Silfen SL, Ciaccia AV, Bryant HU. Selective estrogen receptor modulators: tissue selectivity and differential uterine effects. *Climacteric*. 1999; 2:268–283. [PubMed: 11910661]
- Slamon DJ, Clark GM, Wong SG, Levin WJ, Ullrich A, McGuire WL. Human breast cancer: correlation of relapse and survival with amplification of the HER-2/neu oncogene. *Science*. 1987; 235:177–182. [PubMed: 3798106]
- Smalley M, Ashworth A. Stem cells and breast cancer: A field in transit. *Nat Rev Cancer*. 2003; 3:832–844. [PubMed: 14668814]
- Smith-Warner SA, Spiegelman D, Yaun SS, van den Brandt PA, Folsom AR, Goldbohm RA, et al. Alcohol and breast cancer in women: a pooled analysis of cohort studies. *JAMA*. 1998; 279:535–540. [PubMed: 9480365]
- Song RX, Santen RJ, Kumar R, Adam L, Jeng MH, Masamura S, et al. Adaptive mechanisms induced by long-term estrogen deprivation in breast cancer cells. *Mol Cell Endocrinol*. 2002; 193:29–42. [PubMed: 12160999]
- Stampfer MJ, Colditz GA. Estrogen replacement therapy and coronary heart disease: a quantitative assessment of the epidemiologic evidence. *Prev Med*. 1991; 20:47–63. [PubMed: 1826173]
- Steinberg KK, Thacker SB, Smith SJ, Stroup DF, Zack MM, Flanders WD, et al. A meta-analysis of the effect of estrogen replacement therapy on the risk of breast cancer. *JAMA*. 1991; 265:1985–1990. [PubMed: 1826136]
- Stewart J, King R, Hayward J, Rubens R. Estrogen and progesterone receptors: correlation of response rates, site and timing of receptor analysis. *Breast Cancer Res Treat*. 1982; 2:243–250. [PubMed: 7150779]
- Tanenbaum DM, Wang Y, Williams SP, Sigler PB. Crystallographic comparison of the estrogen and progesterone receptor's ligand binding domains. *Proc Natl Acad Sci U S A*. 1998; 95:5998–6003. [PubMed: 9600906]
- Tanos T, Sfllomos G, Echeverria PC, Ayyanan A, Gutierrez M, Delaloye JF, et al. Progesterone/RANKL is a major regulatory axis in the human breast. *Sci Transl Med*. 2013; 5:182ra155.
- Trevino LS, Weigel NL. Phosphorylation: a fundamental regulator of steroid receptor action. *Trends Endocrinol Metab*. 2013
- Trichopoulos D, MacMahon B, Cole P. Menopause and breast cancer risk. *J Natl Cancer Inst*. 1972; 48:605–613. [PubMed: 5058966]
- Vachon CM, van Gils CH, Sellers TA, Ghosh K, Pruthi S, Brandt KR, et al. Mammographic density, breast cancer risk and risk prediction. *Breast Cancer Res*. 2007; 9:217. [PubMed: 18190724]
- van Landeghem AA, Poortman J, Nabuurs M, Thijssen JH. Endogenous concentration and subcellular distribution of androgens in normal and malignant human breast tissue. *Cancer Res*. 1985; 45:2907–2912. [PubMed: 3157451]
- Vogel VG, Costantino JP, Wickerham DL, Cronin WM, Cecchini RS, Atkins JN, et al. Update of the National Surgical Adjuvant Breast and Bowel Project Study of Tamoxifen and Raloxifene (STAR) P-2 Trial: Preventing breast cancer. *Cancer Prev Res (Phila)*. 2010; 3:696–706. [PubMed: 20404000]
- Wang S, Counterman LJ, Haslam SZ. Progesterone action in normal mouse mammary gland. *Endocrinology*. 1990; 127:2183–2189. [PubMed: 2226309]
- Ward HW. Anti-oestrogen therapy for breast cancer: a trial of tamoxifen at two dose levels. *Br Med J*. 1973; 1:13–14. [PubMed: 4567104]
- Widschwendter M, Rosenthal AN, Philpott S, Rizzuto I, Fraser L, Hayward J, et al. The sex hormone system in carriers of BRCA1/2 mutations: a case-control study. *Lancet Oncol*. 2013; 14:1226–1232. [PubMed: 24140203]
- Wiehle R, Lantvit D, Yamada T, Christov K. CDB-4124, a progesterone receptor modulator, inhibits mammary carcinogenesis by suppressing cell proliferation and inducing apoptosis. *Cancer Prev Res (Phila)*. 2011; 4:414–424. [PubMed: 21119048]
- Wood CE, Branstetter D, Jacob AP, Cline JM, Register TC, Rohrbach K, et al. Progestin effects on cell proliferation pathways in the postmenopausal mammary gland. *Breast Cancer Res*. 2013; 15:R62. [PubMed: 23938070]
- Yager JD, Liehr JG. Molecular mechanisms of estrogen carcinogenesis. *Annu Rev Pharmacol Toxicol*. 1996; 36:203–232. [PubMed: 8725388]

- Yudt MR, Russo LA, Berrodin TJ, Jelinsky SA, Ellis D, Cohen JC, et al. Discovery of a novel mechanism of steroid receptor antagonism: WAY-255348 modulates progesterone receptor cellular localization and promoter interactions. *Biochem Pharmacol.* 2011; 82:1709–1719. [PubMed: 21854761]
- Zhang Y, Beck CA, Poletti A, Clement JPt, Prendergast P, Yip TT, et al. Phosphorylation of human progesterone receptor by cyclin-dependent kinase 2 on three sites that are authentic basal phosphorylation sites in vivo. *Mol Endocrinol.* 1997; 11:823–832. [PubMed: 9171245]
- Zhao Y, Agarwal VR, Mendelson CR, Simpson ER. Transcriptional regulation of CYP19 gene (aromatase) expression in adipose stromal cells in primary culture. *J Steroid Biochem Mol Biol.* 1997; 61:203–210. [PubMed: 9365191]
- Zumoff B. Does postmenopausal estrogen administration increase the risk of breast cancer? Contributions of animal, biochemical, and clinical investigative studies to a resolution of the controversy. *Proc Soc Exp Biol Med.* 1998; 217:30–37. [PubMed: 9421204]

Figure 1. Kinase dependent PR Ser294 phosphorylation antagonizes PR Lys388 SUMOylation and mediates rapid protein turnover

Progestins diffuse through the plasma membrane and bind PR causing rapid SUMOylation on Lys388 on a subset of receptors resulting in transcriptional repression at many cancer relevant genes. Persistent MAPK (or CDK2) pathway activation (e.g. EGF treatment) results in efficient PR Ser294 phosphorylation, inhibition of PR SUMOylation, and transcriptional activation. Phosphorylated PR is highly ubiquitinated and rapidly degraded by the 26S proteasome; whereas, SUMOylated PR is highly stable with a longer half-life.

Figure 2. Cell cycle- or MAPK-dependent PR Ser345 phosphorylation controls transcriptional selectivity

PR Ser345 phosphorylation by CDK2 during cell cycle progression mediates ligand-independent expression of *HSPB8* through direct interaction with cyclin D1 and Sp1. In addition, rapid ligand-dependent PR Ser345 phosphorylation, which requires c-Src and MAPK signaling, drives *p21* transcription via Sp1 tethering mechanisms.

Table 1

Molecular subtypes of breast cancer

Molecular Subtype	Clinically reported status (% within subtype)	Common Mutations (% within subtype)	Common copy number amplifications	Common copy number deletions	Average Mutations per Mb
Luminal A	ER+ (96), ER- (3) PR+ (90), PR- (8) HER2+ (6), HER2- (90)	<i>PIK3CA</i> (45), <i>TP53</i> (12), <i>GATA3</i> (14), <i>MAP3K1</i> (13)			0.84
Luminal B	ER+ (99), ER- (1) PR+ (77), PR- (23) HER2+ (16), HER2- (80)	<i>PIK3CA</i> (29), <i>TP53</i> (29), <i>GATA3</i> (15), <i>MLL3</i> (6)		<i>TP53</i> , <i>MAP2K4</i> , <i>CDKN2A</i>	1.38
HER2- enriched	ER+ (53), ER- (43) PR+ (36), PR- (64) HER2+ (67), HER2- (28)	<i>PIK3CA</i> (39), <i>TP53</i> (72)	<i>ERBB2</i>	<i>TP53</i> , <i>MAP2K4</i>	2.05
Basal-like	ER+ (14), ER- (84) PR+ (9), PR- (88) HER2+ (2), HER2- (95)	<i>TP53</i> (80), <i>PIK3CA</i> (9)	<i>PIK3CA</i>	<i>PTEN</i>	1.68
All Tumors	ER+ (76), ER- (22) PR+ (65), PR- (33) HER2+ (15), HER2- (82)	<i>TP53</i> (37), <i>PIK3CA</i> (36), <i>GATA3</i> (11), <i>MAP3K1</i> (8)			

Data derived from a comprehensive breast cancer study (Cancer Genome Atlas Network, 2012).

Table 2**Antiprogestins in preclinical and clinical development**

Antiprogesterin	Clinical Development	Disease or condition	References
Mifepristone (RU486)	Phase 2	Breast cancer	(Romieu, et al., 1987), (Perrault, et al., 1996), (Klijn, et al., 1989)
Onapristone (ZK98299)	Phase 2	Breast cancer	(Robertson, et al., 1999), (Helle, et al., 1998)
Lonaprisan (ZK230211, BAY86-5044)	Phase 2	Breast cancer	(Jonat, et al., 2013)
APR19	Preclinical	Breast cancer	(Khan, et al., 2013)
EC304	Preclinical	Breast cancer	(Nickisch, et al., 2013)
WAY-255348	Preclinical	Breast cancer	(Yudt, et al., 2011)
ORG31710	Preclinical	Breast cancer	(Klijn, et al., 2000)
Asoprisnil (J867)	Phase 2	Uterine leiomyomata	(Chwalisz, et al., 2007)
Telapristone (Proellex, CDB- 4124)	Phase 2	Endometriosis, Uterine leiomyomata	(Ioffe, et al., 2009)
CDB-2914 (Ulipristal acetates)	Phase 2	Uterine leiomyomata	(Levens, et al., 2008)