REVIEW

Hormone therapy and breast cancer: emerging steroid receptor mechanisms

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Abstract
Although hormone therapy is widely used by millions of women to relieve symptoms of menopause, it has been associated with several side effects such as coronary heart disease, stroke and increased invasive breast cancer risk. These side effects have caused many women to seek alternatives to conventional hormone therapy, including the controversial custom-compounded bioidentical hormone therapy suggested to not increase breast cancer risk. Historically, estrogens and the estrogen receptor were considered the principal factors promoting breast cancer development and progression; however, a role for other members of the steroid receptor family in breast cancer pathogenesis is now evident, with emerging studies revealing an interplay between some steroid receptors. In this review, we discuss examples of hormone therapy used for the relief of menopausal symptoms, highlighting the distinction between conventional hormone therapy and custom-compounded bioidentical hormone therapy. Moreover, we highlight the fact that not all hormones have been evaluated for an association with increased breast cancer risk. We also summarize the current knowledge regarding the role of steroid receptors in mediating the carcinogenic effects of hormones used in menopausal hormone therapy, with special emphasis on the influence of the interplay or crosstalk between steroid receptors. Unraveling the intertwined nature of steroid hormone receptor signaling pathways in breast cancer biology is of utmost importance, considering that breast cancer is the most prevalent cancer among women worldwide. Moreover, understanding these mechanisms may reveal novel prevention or treatment options and lead to the development of new hormone therapies that do not cause increased breast cancer risk.

Introduction
Menopause is characterized by the natural, age-related decrease in endogenous estrogen production in women, often leading to a variety of symptoms such as hot flashes, mood swings and night sweats (Johnson 1998, Greendale et al. 1999, Marsden 2003). Conventional United States Food and Drug Administration (FDA)-approved hormone therapy (HT) has been used for decades to alleviate these symptoms and is typically administered as estrogen alone to hysterectomized women or an estrogen-progestin combination to women with a uterus (Johnson 1998, Greendale et al. 1999, Marsden 2003, Cuzick 2008). While the estrogen component alleviates the symptoms of menopause by compensating for reduced endogenous estrogen production, the progestin constituent
counteracts the proliferative effects of estrogens on the uterine epithelium (Johnson 1998). Despite the fact that HT is effective in relieving menopausal symptoms, some HT regimens have been associated with several severe side effects including coronary heart disease, stroke and increased invasive breast cancer risk (Writing Group for the Women’s Health Initiative Investigators 2002, Million Women Study Collaborators 2003, Krieger et al. 2005, Vickers et al. 2007, Cuzick 2008, Marjoribanks et al. 2017). Considering that breast cancer is the most prevalent cancer among women in developed counties (Ferlay et al. 2015, Torre et al. 2015, Siegel et al. 2016), the association between HT and increased breast cancer risk is of significant concern.

The increased breast cancer risk linked to conventional HT has caused many women and medical professionals to seek various safer HT options, including the use of ‘natural’ alternatives such as custom-compounded bioidentical HT (bHT) (Curcio et al. 2006, Gass et al. 2015). Notably, some bioidentical hormones such as bioidentical estradiol (bE$_2$) or bioidentical progesterone (bP$_4$) are available in FDA-approved standard-dose prescription medications (Sood et al. 2011, 2014). However, unlike these FDA-approved HT products containing bioidentical hormones, custom-compounded bHT formulations are administered in personalized doses and are typically composed of a mixture of up to six hormones (Pinkerton 2012, 2014). Uncertainty remains regarding the efficacy and safety of custom-compounded bHT, especially pertaining to these multiple-hormone combination therapies (Pinkerton 2012, 2014). Although proponents of bHT claim that there is in fact evidence to support the efficacy and safety of custom-compounded bHT in terms of breast cancer risk (Boothby et al. 2004, Bosarge & Freeman 2009), these claims are unsubstantiated due to the lack of large-scale, double-blinded clinical trials investigating custom-compounded multiple-hormone bHT regimens at various doses.

Estrogen only and estrogen-progestin combination conventional HT have both been implicated in increased breast cancer risk; however, evidence suggests that estrogen-progestin combination therapies are associated with a greater increased risk than estrogen-alone therapies (Writing Group for the Women’s Health Initiative Investigators 2002, Million Women Study Collaborators 2003, Fournier et al. 2005, 2008a, Marjoribanks et al. 2017). Estrogens predominantly mediate their effects by binding to the estrogen receptor (ER), while progestins are synthetic progestogens (progesterone receptor (PR) ligands) that were designed to mimic the activity of the natural progestogen, progesterone (P$_4$), by binding to the PR. However, it is known that some progestins can bind to the glucocorticoid receptor (GR), mineralocorticoid receptor (MR), androgen receptor (AR) (Koubovec et al. 2005, Africander et al. 2013, 2014, Louw-du Toit et al. 2017, reviewed in Schindler et al. 2003, Africander et al. 2011, Hapgood et al. 2014) and/or the ER (Larrea et al. 2001, Escande et al. 2006, Louw-du Toit et al. 2017, reviewed in Africander et al. 2011). Whether progestins bind to the ER is contradictory. Some studies suggest that medroxyprogesterone acetate (MPA) and norethisterone (NET) can bind to the ER and elicit estrogenic activity, others suggest that they do not (Larrea et al. 2001, Pasapera et al. 2002, Escande et al. 2006, Lemus et al. 2009, Louw-du Toit et al. 2017). Interestingly, we recently showed that NET-acetate (NET-A), levonorgestrel (LNG) and gestodene (GES) can bind to ER-A, but not ER-B, while P$_4$, MPA, nestorone (NES), nomegestrol acetate (NoMAC) and drospirenone (DRSP) do not bind to either ER subtype (Louw-du Toit et al. 2017). Furthermore, some studies suggest that it is the progestin metabolites rather than the parent progestin itself that bind to the ER. Although a number of studies have investigated effects of progestins via steroid receptors other than the PR (Stanczyk et al. 2013, Hapgood et al. 2014), these studies seldom directly compare different progestins in parallel and often use cell lines that endogenously express many steroid receptors to which progestins can bind, which may result in inaccurate results in terms of binding affinities, as well as potencies and efficacies for gene expression. It is thus essential that the pharmacological properties of the progestins for each individual steroid receptor are determined in parallel in a model system expressing only the receptor of interest, as has been done for MPA and NET via the human GR, MR and AR (Koubovec et al. 2005, Africander et al. 2013, 2014).

The role of the ER, which exists as two subtypes transcribed from two distinct genes (Kuiper et al. 1996, Kuiper & Gustafsson 1997, Enemark & Gustafsson 1999), ER-A and ER-B (Ascenzi et al. 2006), has been extensively studied in breast cancer cell biology. Traditionally, estrogens and ER-A were thought to be the main etiological factors contributing to breast cancer pathogenesis, while the PR was considered only as an indicator of a functional ER in breast cancer tumors, implying that the cancer should be sensitive to endocrine-targeting therapies (Horwitz & McGuire 1975, Carroll et al. 2016, Lim et al. 2016). However, recent studies have highlighted novel roles for the PR in breast cancer cell biology (Daniel et al. 2011, Giulianelli et al. 2012, Mohammed et al. 2015, Singhal et al. 2016). Two main PR isoforms have been
identified, PR-A and PR-B (Kastner et al. 1990), which have been shown to elicit differential effects (Conneely et al. 2000, 2003, Jacobsen et al. 2002, Richer et al. 2002, Mulac-Jericevic et al. 2003, Faire & Lange 2007, Lanari et al. 2012, Brisken 2013). A recent study has shown that unliganded PR-B enhances the effects of ER agonists on ER-A-mediated breast cancer cell proliferation and gene expression by forming a complex with ER-A (Daniel et al. 2015). Moreover, it has been shown that when PR-B is activated by P4 or the synthetic PR agonist, promegestone (RS020), it is recruited to the ER-A complex and redirects the complex to different target genes such that the new gene expression profile is associated with a good clinical outcome (Mohammed et al. 2015). ER-A has also been shown to be required for PR-mediated increased cell proliferation and the expression of PR-regulated genes induced by the progestin MPA (Giulianelli et al. 2012). Interestingly, the presence and critical roles of other steroid receptors in breast cancer cell biology have been highlighted in recent studies. For example, the AR is expressed in 90% of breast cancer tumors (reviewed in Hickey et al. 2012), and its expression is associated with either a good or poor prognosis depending on the absence or presence of the ER (McNamara et al. 2014). Similarly, GR expression has been associated with a good outcome in ER-A-positive cancers, but is associated with a poor outcome in ER-A-negative cancers (Pan et al. 2011, Leehy et al. 2016).

Steroid receptor signaling pathways have often been studied in isolation; however, it is becoming increasingly clear that these pathways are intertwined. The ability of some steroid hormones, such as progestins, to activate multiple steroid receptors, coupled with the complexity of steroid receptor crosstalk, highlights the intricacies of the mechanisms through which hormones used in HT may increase breast cancer risk and promote breast cancer pathogenesis. In order to elucidate the involvement of steroid receptor crosstalk in the mechanism behind HT and increased breast cancer risk, additional comparative studies of hormones used in HT are need at the cellular level. The aim of this review is to highlight differences between conventional HT and custom-compounded bHT and to discuss known mechanisms behind conventional HT-induced increased breast cancer risk with an emphasis on the role of steroid receptor crosstalk.

**Menopause and hormone therapy**

Menopausal transition typically occurs in women between the ages of 40 and 60 years and is characterized by the natural age-related loss of ovarian follicular reserve using numerous chemical reactions (Hudson 1996) and are thus semi-synthetic (Taylor 2005, Cirigliano 2007, Chervenak 2009, Bhavnani & Stanczyk 2012).
Class C steroids differ from class B steroids in that they are synthesized from non-steroidal, rather than steroidal, precursors in a process called total synthesis (Bhavnani & Stanczyk 2012). The shortcoming of class B and C steroids is that various isomers are produced during the synthesis process, with only one of these isomers structurally identical to the endogenous human hormone (Bhavnani & Stanczyk 2012, Gass et al. 2015). For example, during total E₁₁ synthesis, eight racemates (differentiated by the left- and right-handed enantiomers of a chiral molecule) are produced, resulting in 16 isomers of which only one is structurally and biochemically identical to endogenous human E₃₁ (Bhavnani & Stanczyk 2012). The remaining isomers have different structures and varying degrees of estrogenicity, while some are even completely inactive (Bhavnani & Stanczyk 2012). Lastly, class D steroids are manmade steroidal compounds synthesized either from the same steroidal plant precursors as class B hormones by semi-synthesis or from non-steroidal starting material by total synthesis (Bhavnani & Stanczyk 2012). Examples of class D steroids include estrogens such as estropipate, esterified estrogens and ethinylestradiol (EE), as well as progestins such as MPA, NET-A, LNG and norgestimate (NGM).

Premarin is an example of a HT containing natural CEEs and has been effective in relieving menopausal symptoms from as early as 1942 (Cuzick 2008). Various other estrogens have subsequently become available for use in conventional HT and include synthetic, rather than natural CEEs, bE₂, as well as the less commonly used esterified estrogens, estropipate and synthetic E₂ derivatives including EE, E₂ valerate, E₂ cypionate and E₂ acetate (Table 2). Although estrogen-only HT effectively relieves menopausal symptoms, studies in the 1960s reported increased incidence of endometrial cancer in Premarin users (Smith et al. 1975, Weiss et al. 1976, Cuzick 2008). This necessitated the addition of a progestin to CEE regimens for women with a uterus, to prevent estrogen-induced endometrial hyperplasia (Cuzick 2008). Progestins are used to mimic the activity of P₄, as they have a longer half-life and a higher bioavailability (Bhavnani & Stanczyk 2012, Stanczyk et al. 2013). Products such as Provera (MPA only) and Prempro (CEE-MPA) (Table 3) thus became commercially available as early as 1965 (Nachtigall et al. 1979). Although Provera is produced as a progestin-only HT, it is administered in combination with an estrogen-only HT (Stefanick 2005). Various generations of progestins have subsequently been developed, derived from P₄, T or the MR antagonist spironolactone, where each new generation is designed to have a greater affinity for the PR and elicit biological effects more similar to P₄ than progestins from the earlier generations (Sitruk-Ware 2004, Sitruk-Ware & Nath 2010, Africander et al. 2011). Note that P₄ derivatives can be either 17-hydroxy-P₄ derivatives or 19-Nor-P₄ derivatives (Fig. 1).

Progestins currently used in FDA-approved HT include the first-generation progestins MPA and NET-A, second-generation progestin LNG, third-generation progestin NGM and the fourth-generation progestin, DRSP (Fig. 1). Interestingly, not all progestins are used clinically in the USA. For example, although NoMAC is used in HT in Europe (Fournier et al. 2005), it is not used in the USA. However, NoMAC and other progestins such as NES and GES (Fig. 1) are currently being investigated in clinical trials for use in contraception in the USA (Sitruk-Ware & Nath 2010, Bahamondes & Bahamondes 2014). The continual evolution of HT is thought to be aimed at designing estrogens and progestogens that effectively manage menopausal symptoms without eliciting unwanted side effects. More recent advances in HT evolution saw the introduction of bioidentical hormones. FDA-approved bE₂-only products (Files et al. 2011, The North American Menopause Society (NAMS) 2012 Hormone Therapy Position Statement Advisory Panel 2012) are available in standardized doses in products such as Alora, Vivelle-Dot, Divigel, Elestrin, Estrogel and Estrace (Table 2), or in combination with progestins such as NET-A (Activella, Mimvey, Combipatch), DRSP (Angelil), NGM (Prefest) or LNG (Climara Pro) (Table 3). Interestingly, although FDA-approved bP₄ is available as Prometrium in the form of a cream or as a pill that is administered together with an estrogen-only HT, there are no standardized

### Table 1  Serum estradiol (E₂), estriol (E₃), estrone (E₁), progesterone (P₄) and testosterone levels in pre- and post-menopausal women.a

<table>
<thead>
<tr>
<th></th>
<th>Pre-menopausal (pg/mL)</th>
<th>Postmenopausal (pg/mL)</th>
</tr>
</thead>
<tbody>
<tr>
<td>E₂</td>
<td>7–400</td>
<td>1–20</td>
</tr>
<tr>
<td>E₃</td>
<td>8–2408</td>
<td>&lt;10</td>
</tr>
<tr>
<td>E₁</td>
<td>12–144</td>
<td>7–44</td>
</tr>
<tr>
<td>P₄</td>
<td>566–15,700</td>
<td>39–700</td>
</tr>
<tr>
<td>Testosterone</td>
<td>217–2200</td>
<td>461–1050</td>
</tr>
</tbody>
</table>

Table 2  FDA-approved estrogen-only HT products.a

<table>
<thead>
<tr>
<th>Composition</th>
<th>Products</th>
<th>Route of administration</th>
<th>Administered doses (min–max) (mg)</th>
<th>Serum E₂ (pg/mL)</th>
<th>Serum E₁ (pg/mL)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Natural CEE</td>
<td>Premarin</td>
<td>Pill</td>
<td>0.3–1.25</td>
<td>NA</td>
<td>87–4500</td>
</tr>
<tr>
<td></td>
<td></td>
<td>Vaginal cream</td>
<td>0.625*</td>
<td>NA</td>
<td>42–600</td>
</tr>
<tr>
<td>Synthetic CEE</td>
<td>Canestrin, Enjuvia</td>
<td>Pill</td>
<td>0.3–1.25</td>
<td>NA</td>
<td>20–85</td>
</tr>
<tr>
<td>bE₂</td>
<td>Alora, Climara, Esclim, Estraderm, Estradot, Menostar, Minivelle, Vivellet-Dot</td>
<td>Patch</td>
<td>0.025–0.1</td>
<td>6–174</td>
<td>9–65</td>
</tr>
<tr>
<td></td>
<td>Divigel, Elestrin, Estrogel</td>
<td>Topical gel</td>
<td>0.025–2.0*</td>
<td>9–67</td>
<td>33–66</td>
</tr>
<tr>
<td></td>
<td>Estrace, Gynodiol</td>
<td>Pill</td>
<td>0.5–2.0</td>
<td>203–355</td>
<td>ND</td>
</tr>
<tr>
<td></td>
<td>Estrace</td>
<td>Vaginal cream</td>
<td>0.1*</td>
<td>ND</td>
<td>ND</td>
</tr>
<tr>
<td></td>
<td>Estrasorb</td>
<td>Topical cream</td>
<td>2.5*</td>
<td>ND</td>
<td>ND</td>
</tr>
<tr>
<td></td>
<td>Estring</td>
<td>Vaginal insert</td>
<td>2.0</td>
<td>8–63</td>
<td>44–66</td>
</tr>
<tr>
<td></td>
<td>Evamist</td>
<td>Topical spray</td>
<td>1.53*</td>
<td>11–57</td>
<td>NA</td>
</tr>
<tr>
<td></td>
<td>Vagifem</td>
<td>Vaginal tablet</td>
<td>0.01–0.025</td>
<td>6–21</td>
<td>17–28</td>
</tr>
<tr>
<td>E₂ derivatives</td>
<td>Delestrogen</td>
<td>IM injection</td>
<td>10–40</td>
<td>ND</td>
<td>ND</td>
</tr>
<tr>
<td>E₂ valerate</td>
<td>Depo-Estradiol</td>
<td>IM injection</td>
<td>1.0–5.0</td>
<td>ND</td>
<td>ND</td>
</tr>
<tr>
<td>E₂ cypionate</td>
<td>Femring</td>
<td>Vaginal ring</td>
<td>0.05–0.10</td>
<td>41–76</td>
<td>36–46</td>
</tr>
<tr>
<td>E₂ acetate</td>
<td>Femtrace</td>
<td>Pill</td>
<td>0.45–1.8</td>
<td>57–177</td>
<td>155–680</td>
</tr>
<tr>
<td>Esterified</td>
<td>Menest</td>
<td>Pill</td>
<td>0.3–2.5</td>
<td>ND</td>
<td>ND</td>
</tr>
<tr>
<td>estrogen</td>
<td>Estragyn</td>
<td>Vaginal cream</td>
<td>1.0*</td>
<td>ND</td>
<td>ND</td>
</tr>
<tr>
<td>Estropipate</td>
<td>Ogen</td>
<td>Pill</td>
<td>0.75–3.0</td>
<td>ND</td>
<td>ND</td>
</tr>
<tr>
<td></td>
<td></td>
<td>Vaginal cream</td>
<td>1.5*</td>
<td>ND</td>
<td>ND</td>
</tr>
</tbody>
</table>

bE₂, bioidentical estradiol; CEE, conjugated equine estrogens; E₁, estrone; E₂, estradiol; IM, intramuscular injection; NA, not applicable as these HT products do not contain the relevant hormone; ND, serum concentrations have not been reported.

FDA-approved bE₂-bP₄ combination formulations available. Notably, bP₄ is administered in a micronized form, referring to the fact that the particle size has been decreased to generate finer powders that are more readily absorbed, and thus, have an increased bioavailability to compensate for the short half-life of the natural hormone (Chaumeil 1998, Boothby et al. 2004). However, both oral and transdermal micronized bP₄ formulations are dissolved in peanut oil and consequently cannot be used by women with nut allergies (Sood et al. 2013, Xie et al. 2014, Mirkin et al. 2015, Stuenkel et al. 2015, Santoro et al. 2016). Another alternative HT regimen involves the use of selective estrogen receptor modulators (SERMs). SERMs elicit tissue-selective estrogenic activity by acting as ER agonists in bone tissue, increasing bone mineral density and bone strength, but as ER antagonists in the breast and endometrium to prevent breast and endometrial cancer (Komm & Chines 2012). FDA approval was recently granted to Duavee, a CEE-SERM (bazedoxifene) combination to be used for the relief of menopausal symptoms as well as to prevent postmenopausal osteoporosis (Kharode et al. 2008, Pinkerton et al. 2009, Bachmann et al. 2010, Kagan et al. 2010, Komm & Chines 2012).

Estrogen only and progestogen-containing FDA-approved HT products are outlined in Tables 2 and 3, respectively. These tables indicate the routes of administration and dose range of the various HTs and, where available, the resulting serum concentrations of E₂, E₁, EE, the progestogens or bazedoxifene. Although these conventional HT regimens are still widely used and have proved efficient at relieving menopausal symptoms (The North American Menopause Society (NAMS)
Custom-compounded bioidentical hormone therapy

Custom-compounded bHT refers to the personalized bHT regimens containing class B steroids that are prepared by compounding pharmacies and can include any number of bioidentical hormones including bioidentical estrone (bE1), estriol (bE3), testosterone (bT), dehydroepiandrosterone (bDHEA), bE2 and/or bP4 (Chervenak 2009, Bhavnani & Stanczyk 2012). Unlike FDA-approved HT, which is available in standardized doses, a customized dose of bHT is prescribed based on a saliva test that estimates serum hormone levels (Boothby et al. 2004, Santoro et al. 2016). However, this method contradicts a global consensus that the lowest possible dose of HT that effectively relieves menopausal symptoms should be prescribed (Bosarge & Freeman 2009, The North American Menopause Society (NAMS) 2012 Hormone Therapy Position Statement Advisory Panel 2012, de Villiers et al. 2013). Moreover, numerous studies have shown a poor correlation between hormone levels found in saliva and serum, due to saliva hormone levels fluctuating based on time of day, diet and other variables (Boothby et al. 2004, Ciriglano 2007, Fugh-berman & Bythrow 2007, Bosarge & Freeman 2009, Chervenak 2009, Santoro et al. 2016).

The safety and efficacy of custom-compounded bHT is controversial and proponents of bioidentical hormones claim that these hormones are natural and identical in structure to endogenous human hormones; hence, they are safer than conventional HT products (Chervenak 2009). This is despite the fact that the proposed ‘natural’ hormones used in custom-compounded bHT are in fact semi-synthetic and synthesized in a similar manner to the bioidentical hormones used in FDA-approved HT (Ciriglano 2007, Fugh-berman & Bythrow 2007, Bosarge & Freeman 2009, Chervenak 2009). However, because of this ‘natural’ classification, custom-compounding pharmacies may legally dispense products containing bioidentical hormones without obtaining FDA approval for each product (Fugh-berman & Bythrow 2007, Chervenak 2009). This means that personalized hormone preparations are dispensed without the rigorous quality control checks that FDA-approved drugs are subjected to. Furthermore, unlike 2012 Hormone Therapy Position Statement Advisory Panel 2017), the reported side effects associated with conventional HT have caused so much alarm that many women have sought alternate menopausal relief in the form of custom-compounded bHT (Bhavnani & Stanczyk 2012).

### Table 3: FDA-approved progestogen or SERM-containing HT products.

<table>
<thead>
<tr>
<th>Composition</th>
<th>Route of administration</th>
<th>Administered doses (mg)</th>
<th>Serum progestogen (µg/mL)</th>
<th>Serum E2 (pg/mL)</th>
<th>Serum E1 (pg/mL)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Estrone- and progesterone-only products</td>
<td>Pill (2.5–10 mg)</td>
<td>0.45–2.0</td>
<td>&lt;30</td>
<td>&lt;260</td>
<td></td>
</tr>
<tr>
<td>Estrone- and progesterone combinations</td>
<td>Patch</td>
<td>0.3–0.625</td>
<td>1.0–1.5</td>
<td>&lt;260</td>
<td></td>
</tr>
<tr>
<td>Estrone, estrogenic, and progestogen combinations</td>
<td>Patches</td>
<td>0.002–0.01</td>
<td>0.05–0.1</td>
<td>&lt;260</td>
<td></td>
</tr>
<tr>
<td>Estradiol alone</td>
<td>Patches</td>
<td>0.025–0.01</td>
<td>0.05–0.1</td>
<td>&lt;260</td>
<td></td>
</tr>
<tr>
<td>Estradiol</td>
<td>Patch</td>
<td>0.02–0.05</td>
<td>0.05–0.1</td>
<td>&lt;260</td>
<td></td>
</tr>
</tbody>
</table>

### Notes:
- bP4, bioidentical progesterone; CEE, conjugated equine estrogens; DRSP, drospirenone; E1, estrone; E2, estradiol; E3, estriol; EE, equine estrogens; MPA, medroxyprogesterone acetate; NA, not applicable as these HT products do not contain the relevant hormone; ND, serum concentrations have not been reported; NET-A, norethisterone acetate; NGM, norgestimate; SERM, selective estrogen receptor modulator.
It has been suggested that this may be due to insufficient bP₄ absorption and thus low bioavailability (Cooper et al. 1998, Burry et al. 1999, Wren et al. 1999, Carey et al. 2000, O’leary et al. 2000, Lewis et al. 2002). However, the Postmenopausal Estrogen and Progestin Intervention (PEPI) trial revealed that oral micronized bP₄ effectively relieves vasomotor symptoms (The Writing Group for the PEPI Trial 1995, Barrett-Connor et al. 1997, Greendale 1998), suggesting that oral micronized bP₄ may be more effective at relieving vasomotor symptoms than localized micronized bP₄ creams.

Custom-compounded bHT often contains bE₂ in combination with bE₃ and/or bE₄ (Taylor 2005, Bosarge & Freeman 2009, Chervenak 2009, Pinkerton 2014). Biest or triest combination regimens can be obtained from compounding pharmacies, where a biest is composed of bE₂ and bE₃ in a 20:80 ratio and a triest is composed of bE₂, bE₃ and bE₄ in a 10:80:10 ratio (Boothby et al. 2004, Curcio et al. 2006, Fugh-berman & Bythrow 2007, Sites 2008). Proponents of bHT claim that bE₃ and bE₄ are weaker, safer estrogens than bE₂ (Sites 2008, Chervenak 2009, Holtorf 2009) and that bE₃ antagonizes the potent estrogenic activity of bE₂ (Melamed et al. 1997, Boothby et al. 2004). However, we have recently shown that both bE₃ and bE₄ are not necessarily weaker estrogens than bE₂ in terms of transactivation and transrepression of gene expression, or their effects on breast cancer cell proliferation and anchorage-independent growth (Perkins et al. 2017). Moreover, we showed that E₃ did not antagonize E₂-induced gene expression, proliferation or anchorage-independent growth of the MCF-7 BUs breast cancer cell line (Perkins et al. 2017). To the best of our knowledge, however, no other detailed comparisons of the agonist and antagonist properties of these hormones for transactivation and transrepression via the ER subtypes have been reported. In fact, the incorporation of E₃ into bHT products appears to be based on murine work conducted by Lemon, more than 30 years ago (Lemon 1975, 1987), showing that E₃ was more protective against carcinogen-induced neoplasms than E₂ or E₄ (reviewed in (Taylor 2005)). However, these claims have not been validated in human models (Melamed et al. 1997) and large-scale, double-blinded, placebo-controlled clinical trials evaluating the safety and efficacy of E₃ or bE₄ are lacking. However, some preliminary small-scale trials have suggested that E₃ sometimes relieves vasomotor symptoms but does not protect against bone loss (Yang et al. 1995, Takahashi et al. 2000), while others have provided evidence that E₃ can protect against bone loss (Cheng et al. 1993, Minaguchi et al. 1996, Iito et al. 1997, 2000).

Conventional HT products, custom-compounded bHT products lack black-box warnings of the potential adverse effects of HT (Fugh-berman & Bythrow 2007, Bhavnani & Stanczyk 2012, Pinkerton 2014). A major concern raised by randomized FDA checks is the fact that custom-compounded preparations frequently result in accidental under- or overdosing, possibly due to variations in purity and/or human error associated with personalized combination constitution (Cirigliano 2007, Eden et al. 2007, Fugh-berman & Bythrow 2007, Bhavnani & Stanczyk 2012, Sood et al. 2013, Pinkerton 2014). Moreover, compounded bHT patches have been shown to yield lower serum estrogen levels than bioequivalent standard-dose E₂ patches, emphasizing that the pharmacodynamics of compounded bHT requires further research (Sood et al. 2013).

Kamenov et al. 2000, Hayashi et al. 2002), highlighting the uncertainties regarding E₃ use. Interestingly, although there are no FDA-approved E₃-containing HT products (Boothby et al. 2004), bE₃ is used in regulated HT products in parts of Europe and Asia (Cirigliano 2007, Lommen & Mead 2013), where it is, usually referred to as E₃ rather than bE₃ (Wright 2005, Conaway 2011).

Androgens such as bT and bDHEA, are also often used in personalized bHT formulations in combination with estrogens and/or progestogens to relieve the symptoms of menopause (Reed-Kane 2001, Boothby et al. 2004, Eden et al. 2007, Sites 2008). However, observational studies have reported adverse effects of androgen-containing HT such as endometrial cancer, hair loss, acne, hirsutism and deepening of the voice (Greendale et al. 1999, Eden et al. 2007). In fact, cases of endometrial cancer have been reported in users of oral bHT products containing combinations of bE₂, bP₄, bT and bDHEA or bE₂, bE₃, bP₄, bT and bDHEA (Eden et al. 2007). Interestingly, various androgens, including the testosterone precursor and methyltestosterone, are approved for HT use in Europe (Davis 2015), while there is no FDA-approved androgen-containing female HT (Pinkerton 2014, Gass et al. 2015). Moreover, although there is a lack of clinical trials examining the effectiveness and possible side effects of androgen use in HT, bT and bDHEA are distributed by compounding pharmacies in both the USA (Boothby et al. 2004, Cirigliano 2007, GuidoZZi et al. 2014) and South Africa (Golding 2009). In terms of breast cancer risk, the inclusion of bT is especially concerning as testosterone can be aromatized to E₂ within breast tissue (Hickey et al. 2012) and endogenous testosterone levels are only marginally decreased after menopause (Table 1). This suggests that the incorporation of bT into an estrogen containing bHT may thus result in greater estrogen exposure than intended, which may increase risk of breast cancer development.

Overall, the lack of large-scale clinical trials investigating the safety and efficacy of custom-compounded bHT such as biest and triest regimens (Cirigliano 2007, Bosarge & Freeman 2009), together with the absence of blackbox warnings, lack of thorough regulatory bodies and uncertainties regarding salivary testing, has resulted in a consensus between several organizations including the North American Menopause Society (NAMS), The International Menopause Society (IMS), The Endocrine Society and The European Menopause and Andropause Society (EMAS), recommending against the use of custom-compounded bHT (de Villiers et al. 2013).

Hormone therapy and breast cancer risk

Numerous clinical trials and observational studies have associated conventional HT with multiple side effects such as elevated risk of developing breast, ovarian and endometrial cancers, as well as cardiovascular disease and stroke (Nachtigall et al. 1979, Obel et al. 1993, The Writing Group for the PEPI Trial 1995, Hulley et al. 1998, 2002, Johnson 1998, Greendale et al. 1999, Notelovitz et al. 2002, Waters et al. 2002, Writing Group for the Women's Health Initiative Investigators 2002, Million Women Study Collaborators 2003, Barakat et al. 2006, Veerus et al. 2006, Yaffe et al. 2006, Fournier et al. 2008b, Tierney et al. 2009, Schierbeck et al. 2012, Manson et al. 2013, Sood et al. 2014, Clavel-Chapelon 2015, Hodis et al. 2016, Marjoribanks et al. 2017). Considering that breast cancer is the most common cancer in women worldwide and the leading cause of cancer-related deaths in women in developed countries (Ferlay et al. 2015, Torre et al. 2015), the association between HT and breast cancer risk is alarming. Although several studies reported adverse effects associated with HT prior to 2002 (Nachtigall et al. 1979, Obel et al. 1993, The Writing Group for the PEPI Trial 1995, Hulley et al. 1998), it was the findings of the highly publicized Women's Health Initiative (WHI) (Writing Group for the Women's Health Initiative Investigators 2002) that caused alarm and confusion about the safety of HT. The WHI study was a large-scale randomized, controlled clinical trial that evaluated the benefits and risks of CEE alone in hysterectomized postmenopausal women or CEE in combination with MPA in postmenopausal women with a uterus (Writing Group for the Women's Health Initiative Investigators 2002). The results of the trial suggested that CEE-MPA combinations, but not CEE alone, were associated with increased invasive breast cancer risk (Writing Group for the Women's Health Initiative Investigators 2002). In contrast, the Million Women Study (MWS), a cohort study comprising over one million postmenopausal women from across the United Kingdom, found that the use of estrogen alone or estrogen-progestin combinations were both associated with increased invasive breast cancer risk (Million Women Study Collaborators 2003). Interestingly, this study found increased breast cancer risk with all HT preparations investigated, and no difference in risk between specific estrogens (CEE and EE) or progestins (MPA, NET and LNG) (Million Women Study Collaborators 2003).

Many additional studies investigating breast cancer risk associated with HT use have been conducted (Nachtigall et al. 1979, Obel et al. 1993, Herrington...

Notably, although most clinical and observational studies investigating the association between HT and increased breast cancer risk examined the effects of CEE and MPA, a few studies have in fact investigated other estrogens and progestogens. For example, three clinical trials have reported no increased breast cancer risk associated with the use of oral E2 alone or in combination with NET-A (Obel et al. 1993, Tierney et al. 2009, Schierbeck et al. 2012), the latter of which was previously shown to increase breast cancer risk when used in combination with CEE in the MWS (Million Women Study Collaborators 2003). Similarly, at least one other study reported no increased risk with the use of an E2 only patch (Notelovitz et al. 2002), while the Estrogen in the Prevention of Reinfarction Trial found no increased risk with the use of E2 valerate alone (Cherry et al. 2002).

However, the Kronos Early Estrogen Prevention Study found increased breast cancer risk associated with the use of E2 patches in combination with oral micronized P4, while the Early Versus Late Intervention Trial (ELITE) also found increased risk with oral E2 used in combination with a P4 vaginal gel (reviewed in (Marjoribanks et al. 2017)). In contrast to the above-mentioned studies showing increased breast cancer risk with the inclusion of P4, the PEPI Trial reported no increased breast cancer risk in women administered CEE plus oral micronized P4 or CEE plus MPA (The Writing Group for the PEPI Trial 1995). Similarly, the French E3N cohort study found that estrogen (CEE or bE2) alone or in combination with P4 or dydrogesterone (a progestin not used clinically in the USA) was not associated with increased breast cancer risk. Interestingly, results from the same study showed that other estrogen-progestin combinations containing the progestins MPA, NET-A, medrogestone, chlormadinone acetate (CMA), cyproterone acetate (CPA), R5020 or NoMAC, were associated with increased breast cancer risk (Fournier et al. 2008a, Clavel-Chapelon 2015). This French cohort study also suggested that administration of oral versus transdermal E2 does not influence the degree of breast cancer risk (Fournier et al. 2008a, Clavel-Chapelon 2015). Interestingly, a recent Cochrane review examining the adverse side effects of HT compiled the results of 22 clinical studies, including most of the above-mentioned studies, and suggested that estrogen-progestin HT combinations increased breast cancer risk, while use of estrogen-only HT did not (Marjoribanks et al. 2017). It is clear from the above that more clinical and molecular studies investigating the association between different hormones used in HT and breast cancer are needed.

Taken together, the evidence in the literature investigating an association between specific hormones used in HT and increased breast cancer risk is contradictory. However, there are many other hormones used in FDA-approved HT products such as esterified estrogens, E2 acetate, trimegestone and DRSP, or custom-compounded bHT products such as bE2, bE1, and bT, that have not been investigated in large-scale clinical trials or cohort studies, and thus, it is not known whether these steroid hormones are linked to increased breast cancer risk. An added conundrum is whether the increased risk is due to the initiation of new tumors or the promotion of small, pre-existing tumors. Although still a matter for debate, it is most likely due to tumor promotion (Dietel 2010) as women in the WHI and MWS trials developed tumors within the first year of combined HT use, while breast tumors need an average of 7–10 years to grow to a detectable size (Chlebowski et al. 2003). At the molecular level, steroid hormones predominantly elicit their effects by binding to steroid receptors, which are ligand-activated transcription factors belonging to the nuclear receptor superfamily (Griekspoor et al. 2007, Aagaard et al. 2011).
Steroid receptors as mediators of hormone activity and carcinogenesis

When steroid hormones enter the bloodstream, they bind to various serum binding proteins such as sex-hormone-binding globulin (SHBG), corticosteroid-binding globulin (CBG) and/or albumin (Table 4). SHBG predominantly binds estrogen and testosterone, while CBG binds cortisol and P₄ (Yen et al. 2015). The hormones bound to SHBG or CBG are considered unavailable to tissues, while the free, unbound hormones and those bound to albumin are considered biologically available to enter cells of target tissues and elicit a response by binding to a steroid receptor (Pardridge 1981, Kuhnz et al. 1990, Kuhl 2005). As indicated in Table 4, some estrogens and progestogens bind to SHBG and/or CBG, while others do not, resulting in large differences in the availability of estrogens and progestogens used in HT. For example, while approximately 37% of serum E₂ can bind to SHBG, 16% of E₁ binds, only 1% of E₂ binds and EE does not bind at all. As a result, E₂, E₁ and EE are mostly available to enter cells of target tissues (Table 4) suggesting that these estrogens may be more abundant than E₁ in target tissues, and therefore, may compete with E₂ for binding to the ER. In the same way, progestins also differentially bind to SHBG as shown by 35.5% of NET and between 47.5 and 73.6% of LNG binding, while MPA and DRSP do not bind at all (Table 4). Considering that the data in Table 4 indicates that progestogens are mostly available, it is plausible that they may be more abundant in target tissues than endogenous steroid hormones, even when administered at low concentrations, and thus, may compete with these hormones for binding to their cognate steroid receptors.

Steroid hormones can permeate the cell membrane and elicit their effects by binding to steroid receptors, such as the GR and MR, as well as the sex-steroid hormone receptors, the ER, PR and AR (Griekspoor et al. 2007). A high degree of homology exists between the steroid receptor family, and the receptors are organized into four evolutionary-conserved domains (Fig. 2), namely the N-terminal domain containing the ligand-independent activation function 1 (AF-1) region, the highly conserved DNA-binding domain (DBD), a hinge region and a relatively conserved ligand-binding domain (LBD) containing an additional ligand-dependent activation function (AF-2) region (Giguère et al. 1986, Kumar et al. 1987, Mangelsdorf et al. 1995, Faus & Haendler 2006, Griekspoor et al. 2007, Aagaard et al. 2011). Generally, unliganded AR, GR and MR are found in the cytoplasm, the ER and PR-A predominantly in the nucleus (Li et al. 2005, Yen et al. 2015), while PR-B is distributed between the cytoplasm and the nucleus (Li et al. 2005, Griekspoor et al. 2007). Unliganded steroid receptors are associated with chaperone proteins such as heat shock protein (Hsp)90 and Hsp70 (Pratt & Toft 1997), but dissociate from the chaperone proteins upon ligand binding, as the steroid receptors undergo a conformational change (Griekspoor et al. 2007). The ligand-bound cytoplasmic steroid receptor can then enter the nucleus (Griekspoor et al. 2007), where it generally binds as a dimer to semi-palindromic DNA sequences known as hormone response elements (HREs) to activate target gene expression (transactivation) (Faus & Haendler 2006, Aagaard et al. 2011) or as a monomer to negative HREs (nHREs) or other DNA-bound transcription factors such as nuclear factor kappa B (NFκB), to repress target gene expression (transrepression) (reviewed in (Faus & Haendler 2006)). Steroid hormones can also elicit non-genomic effects either by binding to membrane-bound receptors to activate signaling cascades, which ultimately result in the downstream regulation of gene expression or by interacting with membrane kinases to activate rapid signaling pathways (Faus & Haendler 2006, Hammes & Levin 2007, Bennett et al. 2010, Krug et al. 2011, Yang et al. 2011, Vernocchi et al. 2013, Diep et al. 2015, Schwartz et al. 2016).

The ER, PR, AR and GR are expressed in most breast cancers, and it is therefore not surprising that they all play functional roles in breast cancer cell biology (reviewed in Sikora 2016)). Moreover, emerging evidence suggests that their signaling pathways are not always distinct but are in fact extensively intertwined. This too is not surprising considering the high degree of homology between the steroid hormone receptors and their cognate DNA-binding sites (Giguère et al. 1986, Kumar et al. 1987, Mangelsdorf et al. 1995, Faus & Haendler 2006, Griekspoor et al. 2007, Aagaard et al. 2011, Need et al. 2012). In the following sections, we summarize the role of steroid receptors and their interplay in breast cancer, with a focus on the known effects of the estrogens and progestogens via the ER and PR, respectively.

Estrogens and the ER

The first association between estrogen signaling and breast cancer can be traced back to 1896 (Beatson 1896) and since then copious in vivo and in vitro studies have shown that estrogens promote breast cancer development and progression (Lippman et al. 1977, Soto & Sonnenschein 1985, Reddel & Sutherland 1987, Clemons & Goss 2001, Gutendorf & Westendorf 2001, Frasor et al. 2003, Lippert
et al. 2003, Mueck et al. 2003, Yager & Davidson 2006, Watson et al. 2008, Fernandez & Russo 2010, Liu et al. 2015, de Almeida Chuffa et al. 2017). Although the precise mechanisms whereby estrogens promote breast cancer is still an area of ongoing research, it is well established that ER-A is crucial for E_2-induced breast cancer cell growth (Couse & Korach 1999, Brisken et al. 2010). This critical role for ER-A was highlighted by a study showing that E_2 exposure did not cause breast cancer tumor formation in ER-A-knockout mice (reviewed in (Couse & Korach 1999, Brisken et al. 2010)). Considering that the ER is expressed in approximately 75% of breast cancers, current therapies target ER activity or the synthesis of endogenous estrogen (Lanari et al. 2012, Lim et al. 2016, Doan et al. 2017). For example, tamoxifen or fulvestrant are used to antagonize ER-A signaling by blocking or degrading the ER respectively (Santen et al. 2009, Lanari et al. 2012, Lim et al. 2016, McNamara et al. 2016, Doan et al. 2017), while aromatase inhibitors are used to decrease the production of endogenous estrogens by inhibiting the metabolism of testosterone and androstenedione to E_2 and E_1, respectively.

Estrogens and ER signaling lead to the development and progression of breast cancer largely through the regulation of gene expression (Preston-Martin et al. 1990, Dickson & Lippman 1995, Clemons & Goss 2001, Frasor et al. 2003, Yue et al. 2013, Deroo & Korach 2014, Luo et al. 2016). For example, E_2 treatment of the MCF-7 breast cancer cell line results in the upregulation of genes encoding growth factors such as insulin-like growth factor (IGF)-binding proteins and vascular endothelial growth factor (VEGF) (Dickson & Lippman 1995, Ruohola et al. 1999, Mueller et al. 2000, Frasor et al. 2003, Garvin et al. 2006, Walker et al. 2007). Furthermore, genes regulating the cell cycle such as CCND1, CCNA2 and cyclin-dependent kinase 1 (CDK1) are also upregulated by E_2 in MCF-7 cells, as are genes promoting proliferation such as Ki67 (Altucci et al. 1996, Frasor et al. 2003, Welsboren et al. 2009). In contrast, E_1 has previously been shown to downregulate the expression of genes inhibiting proliferation such as CASP9 (Frasor et al. 2003). ChIP-seq analysis mapped ER-binding sites to the promoter regions of the CCND1, CCNA2, CDK1, Ki67 and TGFB3 genes in response to E_2 treatment (Welsboren et al. 2009).
et al. 2009), highlighting the role of the ER in mediating the tumor-promoting effects of E$_2$. However, the ER subtypes, ER-A and ER-B, are known to play different roles in breast cancer (Kuiper et al. 1996, Barkhem et al. 1998, Lazennec et al. 2001, Pettersson & Gustafsson 2001, Platet et al. 2004, Strom et al. 2004, Chang et al. 2006, Boothby & Doering 2008, Treeck et al. 2010, Lattrich et al. 2013, Leygue & Murphy 2013). For example, ER-A has been shown to promote breast cancer pathogenesis by upregulating the expression of cyclin D1, while ER-B inhibited its expression (Liu et al. 2002). Interestingly, the role of ER-B is dependent on whether ER-A is expressed or not. In the presence of ER-A, ER-B can inhibit ER-A-driven proliferation, while in the absence of ER-A, ER-B promotes proliferation (Kuiper et al. 1996, Barkhem et al. 1998, Lazennec et al. 2001, Pettersson & Gustafsson 2001, Platet et al. 2004, Strom et al. 2004, Chang et al. 2006, Boothby & Doering 2008, Treeck et al. 2010, Lattrich et al. 2013, Leygue & Murphy 2013). The differential action of the ER subtypes may in part be due to the regulation of subtype-specific target genes (Kian Tee et al. 2003, Monroe et al. 2003, Platet et al. 2004, Stossi et al. 2004, Chang et al. 2006, Zhao et al. 2008, Paruthiyil et al. 2009), possibly due to differences in the N- and C-terminals of ER-A and ER-B (Arnal et al. 2017). In addition, ER-B has been shown to downregulate the transcriptional activity of ER-A by modulating the recruitment of transcription factors required by the ER-A transcription complex and by increasing ER-A degradation (Matthews et al. 2006). While ER-B is expressed in lobular breast cancers and a subgroup of triple-negative breast cancers (TNBC), its expression is lost early in ductal breast cancer (reviewed in (Warner et al. 2017)). Although the precise role of ER-B in TNBC is not known, it is thought to be a potential target for treating this breast cancer subtype. For example, it has been shown that postmenopausal women with TNBC respond to tamoxifen treatment due to high expression levels of ER-B (Honma et al. 2008). Furthermore, tamoxifen decreased cell growth and increased apoptosis in the ER-A-negative SK-BR-3 breast cell line transduced with ER-B (Treeck et al. 2008). Moreover, the use of ER-B selective agonists has been suggested as treatment for the vascular symptoms experienced by menopausal women (Warner et al. 2017). The reason for this suggestion is two-fold: (i) the hypothesis that vascular effects in menopause are due to increased activin and follicle-stimulating hormone (FSH) levels because of decreased endogenous E$_2$ levels and (ii) the observation that ER-B selective agonists increase ER-B expression. ER-B regulates inhibin, which directly inhibits activin and subsequently FSH, suggesting that ER-B selective agonists could effectively decrease the vascular symptoms of menopause (reviewed in (Warner et al. 2017)).

In addition to the full-length ER subtypes, several ER splice variants have been identified in various cell lines; however, it is not clear whether all of these variants are also expressed in tissue and whether they are functional proteins (reviewed in (Heldring et al. 2007)). An ER-A 46 splice variant which lacks part of the N-terminal domain has in fact been detected in breast tumor tissue, but its function is still unknown (reviewed in (Arnal et al. 2017)). In contrast, several ER-B splice variants are expressed in breast tissue and have been shown to differentially regulate estrogen signaling (Flouriot 2000, Matthews & Gustafsson 2003, Ramsey et al. 2004, Wang et al. 2005, Leung et al. 2006). For example, the ER-B cx splice variant contains a unique sequence in its LBD and although it cannot bind ligand, it forms heterodimers with ER-A, preventing ER-A from activating gene expression (Ogawa et al. 1998). In contrast, a second ER-B splice variant which also cannot bind ligand, forms dimers with either ER-A or ER-B, blocking their activity (Maruyama et al. 1998).

From the above, it is clear that the role of E$_2$ and the ER subtypes in breast cancer is complex. An added complexity is the fact that many different estrogens are used in HT, and it is not clear whether these estrogens will elicit similar effects to E$_2$ on breast cancer. Studies directly comparing the effects of different estrogens on hallmarks of breast cancer such as cell proliferation, migration, invasion and apoptosis are scarce. The limited studies that are available suggest that while both CEE and E$_2$ increase proliferation, E$_2$ increased proliferation to a greater extent (Mueck et al. 2003, Wood et al. 2008). Furthermore, at least three studies have directly compared the proliferative effects of E$_1$, E$_2$ and E$_3$ and although all studies showed that these estrogens increase breast cancer cell proliferation, differences were observed (Lippman et al. 1977, Gutendorf & Westendorf 2001, Lippert et al. 2003). For example, our recent study (Perkins et al. 2017) and that of Gutendorf and coworkers (Gutendorf & Westendorf 2001) reported potencies in the picomolar range and showed that E$_2$ was more potent than E$_3$, while E$_1$ was the least potent. In contrast, Lippman and coworkers (Lippman et al. 1977) reported potencies in the nanomolar range and showed that E$_2$ and E$_1$ were equipotent, and more potent than E$_3$. From these studies, it is evident that E$_2$ is the most potent estrogen; yet, it is not clear how the proliferative effects of E$_1$, E$_2$, and E$_3$ compare to E$_2$. A study by Lippert and coworkers (Lippert et al. 2003) comparatively investigated the effects of E$_2$, E$_1$, and E$_3$ on proliferation, and although they did
not determine potencies, showed no significant difference in the proliferative effects of 10 nM or 100 nM E$_1$, E$_2$ and E$_3$ and showed that while E$_3$ also stimulated proliferation at 1 µM and 10 µM, E$_2$ inhibited proliferation and E$_1$ had no effect. Interestingly, results investigating the proliferative effects of the synthetic estrogen, ER, relative to E$_2$, are contradictory. While we recently showed that EE was less potent than E$_2$, one study from over 20 years ago showed that EE was as potent as E$_2$ (Gutendorf & Westendorf 2001). It is thus evident that further comparative studies are required to clarify this ambiguity. To the best of our knowledge, studies investigating the effects of estrogens on migration, invasion and apoptosis only focused on E$_2$ and while most studies showed increased migration (Zheng et al. 2011, Shang et al. 2015) and invasion (Albini et al. 1986, Thompson et al. 1988, van den Brule et al. 1992, Zheng et al. 2011, Jiang et al. 2013, Tchafa et al. 2013, Shang et al. 2015), as well as decreased apoptosis (Wang & Phang 1995, Song et al. 2001, Fernando & Wimalasena 2004, Kampa et al. 2005, Tchafa et al. 2013) in the ER-A-positive MCF-7 and T47D breast cancer cell lines, a recent study showed that E$_2$ repressed invasion in the ER-A-positive T47D and BT474 cell lines (McFall et al. 2018). In terms of metastasis, we have shown that E$_2$, E$_3$, E$_1$ and EE promote the anchorage-independent growth of the MCF-7 BUS cell line to the same extent, suggesting that there is no difference in the metastatic potential of these estrogens.

In terms of gene expression, we and two other studies have directly compared EC$_{50}$ values of E$_2$, E$_3$, E$_1$, and EE for both ER-A- and and ER-B-mediated transactivation (Bovee et al. 2004, Escande et al. 2006, Perkins et al. 2017); however, the EC$_{50}$ values between the studies differed by up to 750-fold for some estrogens. Although these studies found that E$_2$ and EE were the most potent estrogens, E$_3$ was the least potent estrogen in one study (Bovee et al. 2004), while we and one other study found that E$_1$ was the least potent (Escande et al. 2006, Perkins et al. 2017). These discrepancies may be due to the different model systems or promoter-reporter constructs used, as the first study used yeast cells and a p406-CYC1 yeast expression vector containing two estrogen response elements (EREs) (Bovee et al. 2004), one study used a cell line-derived from HeLa cells and stably expressing a plasmid containing one ERE (HELN cells) (Escande et al. 2006), while we used the MCF-7 BUS cell line (Perkins et al. 2017). In addition to transactivation of gene expression, the estrogen-bound ER can also transrepress gene expression; however, there is a paucity of studies characterizing this mechanism of action. At least two studies have investigated the efficacy and potency of E$_2$ and/or EE for transrepression of gene expression via ER-A (Cerillo et al. 1998, Harnish et al. 2000). However, little was known for other estrogens used in HT or for the transrepressive activities via ER-B until our recent study revealed that E$_2$, E$_3$, E$_1$ and EE have similar efficacies for transrepression via ER-A and ER-B (Perkins et al. 2017). While the potencies of these estrogens were mostly similar, E$_1$ was more potent via ER-A, EE more potent via ER-B and E$_3$ less potent in a model endogenously expressing both ER-A and ER-B (Perkins et al. 2017).

Considering the scarcity of comparative studies and the fact that our recent study was the first to compare the effects of bioidentical hormones to the endogenous human hormones or synthetic estrogens such as EE, it is imperative that more molecular studies are conducted. This is critical considering that some of the estrogens used in HT and bHT have not been tested in large-scale, double-blinded clinical trials and their safety and efficacy is unknown.

Progestogens and the PR

The role of progestogens including natural P$_4$ and progestins in breast cancer is not straightforward. Some progestins have been associated with increased breast cancer risk (Writing Group for the Women’s Health Initiative Investigators 2002, Million Women Study Collaborators 2003, Fournier et al. 2008a, Marjoribanks et al. 2017), while others and P$_4$ have not (Nachtgall et al. 1979, Obel et al. 1993, Herrington et al. 2000, Hulley et al. 2002, Waters et al. 2002, Greenspan et al. 2005, Veerus et al. 2006, Fournier et al. 2008a, Tierney et al. 2009, Schierbeck et al. 2012). In addition, results from in vitro studies investigating the effects of progestogens on breast cancer cell proliferation are also contradictory. For example, while some studies have shown that P$_4$ (Carvajal et al. 2005), R5020 (Hissom & Moore 1987, Moore et al. 2000), MPA (Franke & Verme 2003, Werner et al. 2005) and NET-A (Schoonen et al. 1995, Franke & Verme 2003, Werner et al. 2005) promote proliferation of the MCF-7, ZR75 or T47D breast cancer cell lines, others have shown that these progestogens are anti-proliferative in the T47D cell line (Horwitz & Freidenberg 1985, Musgrove et al. 1991, Botella et al. 1994, Groshong et al. 1997, Formby & Wiley 1999). Some studies have even suggested that P$_4$ and the progestin ORG 2058 are proliferative for one cell cycle, after which they exert anti-proliferative and pro-apoptotic effects (Musgrove et al. 1991, Groshong et al. 1997). Interestingly, the effects of progestogens on proliferation also seem to be dependent on the absence
or presence of estrogen. For example, while progestogens such as P₄, MPA, NET, LNG, GES and RS020, have been shown to promote proliferation of the MCF-7 cell line, they exerted anti-proliferative effects in the presence of E₂ \( (\text{Schoonen et al. 1995}) \). Investigations into the effects of the progestogens on other hallmarks of breast cancer such as apoptosis, migration and invasion are scarce, and results from the limited studies are ambiguous. For example, some studies suggest that P₄ \( (\text{Franke & Vermes 2003, Werner et al. 2005}) \), MPA and NET increase apoptosis \( (\text{Werner et al. 2005}) \), while others suggest that these progestogens inhibit apoptosis \( (\text{Ory et al. 2001, Franke & Vermes 2003, Moore et al. 2006}) \). While it has also been shown that P₄, MPA, NES and DRSP can promote migration \( (\text{Fu et al. 2008, 2010, Diaz et al. 2012}) \) and invasion \( (\text{Kato et al. 2005, Fu et al. 2008, 2010}) \) in the T47D \( (\text{Fu et al. 2008, 2010}) \) and ZR75 \( (\text{Kato et al. 2005, Diaz et al. 2012}) \) breast cancer cell lines, MPA was found to promote migration and invasion of the T47D cell line to a greater extent than P₄, NES and DRSP \( (\text{Fu et al. 2008}) \). Interestingly, P₄, NES and DRSP, unlike MPA, reduced E₂-induced invasion but not migration \( (\text{Fu et al. 2008}) \). Furthermore, in vivo studies have shown that P₄, MPA, NET and LNG promote the progression of T47D and/or BT-474 human breast cancer cell xenografts in nude mice \( (\text{Liang et al. 2007, 2010}) \), via a mechanism requiring expression of the potent angiogenic growth factor VEGF, a protein implicated in the progression of tumor growth and metastasis \( (\text{Hyder et al. 2001, Liang et al. 2007, 2010}) \). Moreover, at least one study has shown that both P₄ and MPA reactivate stem cell-like properties in pre-existing breast cancer stem cells \( (\text{Horwitz & Sartorius 2008}) \), supporting the idea that these progestogens reactivate dormant breast cancer cells or potentiate the effects of small, previously undetectable cancers \( (\text{Horwitz & Sartorius 2008, Eden 2011}) \). In further support of progestins promoting the progression of already established tumors, a recent study showed that MPA increased the expression of CD44 and the activity of aldehyde dehydrogenase, known markers of cancer stem cells, in BT-474 and/or T47D breast cancer cells \( (\text{Goyette et al. 2017}) \). In light of the above, it is clear that more molecular studies are needed, particularly studies directly comparing the effects of the progestogens on hallmarks of breast cancer and the physiology of mammary stem cells. It is well known that P₄ elicits its biological effects primarily by binding to the PR \( (\text{Grimm et al. 2016}) \) and that progestins were designed to mimic the actions of P₄ also by binding to the PR \( (\text{Sitruk-Ware 2004, Sitruk-Ware & Nath 2010}) \). However, it is known that some progestins can also bind to and elicit biological effects via steroid receptors other than the PR, such as the AR \( (\text{Africander et al. 2014, Louw-du Toit et al. 2017}) \) and GR \( (\text{Koubovec et al. 2005}) \). Thus, whether the observed effects of the progestogens on proliferation and other hallmarks of breast cancer are mediated by the PR, or any of the other steroid receptors, is still an area of ongoing research. However, at least one study has provided evidence that PR knockdown using siRNA \( (\text{Wargon et al. 2015}) \) abrogates MPA-induced breast cancer cell proliferation.

Three PR isoforms, PR-A, PR-B and PR-C, transcribed from different promoters of a single gene, have been identified \( (\text{Kastner et al. 1990, Diep et al. 2015}) \). However, only PR-A and PR-B are functional as PR-C lacks a DBD, and thus, cannot bind to DNA to activate gene transcription \( (\text{Condon et al. 2006, Diep et al. 2015}) \). Interestingly, approximately 65% of P₄-regulated genes are regulated only by PR-B, while approximately 4% are regulated only by PR-A and 25% are regulated by both PR isoforms \( (\text{Richer et al. 2002, Lanari et al. 2012}) \). These somewhat unique gene sets result in different biological roles for PR-A and PR-B, where PR-B mediates P₄-induced normal breast cell proliferation \( (\text{Conneely et al. 2003, Mulac-Jericevic et al. 2003}) \), while P₄-bound PR-A is implicated in maintaining ovarian and uterine functions \( (\text{Conneely et al. 2003, Mulac-Jericevic et al. 2003, Lanari et al. 2012}) \). The transcriptional activity of the individual isoforms is also cell specific and is extensively regulated by post-translational modifications including phosphorylation, sumoylation, acetylation and ubiquitination (reviewed in \text{Diep et al. 2015}). However, PR-A is generally more transcriptionally active than PR-B in the absence of ligand \( (\text{Jacobsen et al. 2002}) \), while PR-B is more transcriptionally active in the presence of agonist \( (\text{Faire & Lange 2007, Lanari et al. 2012}) \). The latter may be due to the additional AF-3 region in the N-terminal domain of PR-B, which enables the binding of cofactors to PR-B that cannot bind PR-A \( (\text{Giagrande et al. 1997}) \).

The PR is a well-known ER-target gene and is thus expressed in most ER-A-positive breast tumors \( (\text{Horwitz et al. 1978, Kastner et al. 1990, Dunbier et al. 2010}) \). Although traditionally thought of only as an indicator of active ER signaling pathways in breast cancer tumors, the role of the PR in breast cancer is quite complex and dependent on multiple factors such as the relative ratio of PR-A to PR-B \( (\text{Diep et al. 2015}) \). PR-A and PR-B are generally expressed at equimolar ratios in the normal mammary gland \( (\text{Mote et al. 2002}) \), resulting in the formation of PR-A/B heterodimers that regulate a specific gene set (reviewed in \text{Lanari et al. 2012}). In contrast, PR-A and/or PR-B expression is often increased in
atypical breast lesions, dysregulating the ratio of the PR isoforms (Graham et al. 1995). This dysregulation disrupts normal PR signaling due to the predominance of one PR isoform and the subsequent formation of homodimers that regulate a unique gene set (reviewed in Lanari et al. 2012). Interestingly, PR-A is upregulated in most ductal carcinomas in situ and invasive breast cancers (Graham et al. 1995, Mote et al. 1999, 2002) and is thought to be more stable than PR-B (Faivre & Lange 2007). Although the exact mechanism behind this has not been fully elucidated (Faivre & Lange 2007), it may be due to the fact that PR-B contains six more phosphorylation sites in its N-terminal domain than PR-A, and the increased kinase activity in pre- or early-malignant breast tissue drives PR-B phosphorylation resulting in both PR-B hyperactivity and degradation (Dressing & Lange 2009, Dressing et al. 2009). Moreover, the activity of PR-B, as well as the ER, AR, GR and MR, can also be repressed by PR-A under normal cellular conditions (Vegeto et al. 1993, McDonnell & Goldman 1994, McDonnell et al. 1994, Kraus et al. 1995, 1997, Lim et al. 1999, Conneely & Lydon 2000, Li et al. 2005, Griekspoor et al. 2007), suggesting crosstalk between steroid receptors. Interestingly, steroid receptor crosstalk mechanisms have been described in the breast cancer context.

Interplay between ER-A and the PR

Recent evidence in the literature suggests that crosstalk between ER-A and the PR plays an important role in breast cancer pathogenesis (Giulianelli et al. 2012, Daniel et al. 2015, Mohammed et al. 2015, Singhal et al. 2016). For example, it has been suggested that the potent PR agonist, R5020, promotes breast cancer progression by activating kinase cascades via a mechanism requiring both ER-A and the PR (Migliaccio et al. 1998, Ballare et al. 2003). Furthermore, Giulianelli et al. (2012) provided evidence of an interaction between ER-A and the PR in breast cancer tissue and cell lines and showed that this interaction is required for MPA-induced breast cancer gene expression and cell proliferation in the T47D breast cancer cell line. Subsequent studies have revealed that the PR can modulate the transcriptional activity and chromatin localization of ER-A through the formation of these ER-A-PR complexes (Giulianelli et al. 2012, Daniel et al. 2015, Mohammed et al. 2015, Singhal et al. 2016). For example, Daniel and coworkers (Daniel et al. 2015) showed that the unliganded PR can act as a molecular scaffold, resulting in PR, ER-A, PELP-1 (proline-, glutamic acid- and leucine-rich protein 1) and IGF1 complexes that alter ER-A gene regulation leading to a more aggressive proliferative response upon E2 stimulation of the MCF-7 cell line. A second study by Mohammed et al. (2015) showed that P4- or R5020-bound PR is recruited to the ER-A complex in both MCF-7 and T47D cells and redirects E2-activated ER-A chromatin binding such that the gene expression profile is similar to that of PR alone. A similar mechanism was shown by Singhal et al. (2016) in primary ER-A- and PR-positive human tumors and is reported to lead to decreased proliferation and an improved clinical outcome (Mohammed et al. 2015). The above-mentioned studies suggest that an interaction between ER-A and the PR can be associated with either poor or good prognosis in breast cancer and that the outcome is determined by the absence or presence of PR ligands. Whether this is true for all PR ligands is not known. This is particularly important for progestins used in HT as some progestins from the earlier generations have been implicated in increased breast cancer risk, while clinical trials implicating newer-generation progestins that have a greater affinity for the PR and elicit biological effects more similar to P4 than progestins from the earlier generations (Sitruk-Ware 2004, Sitruk-Ware & Nath 2010, Africander et al. 2011), are mostly lacking. Further studies are thus required to elucidate the role of ER-A-PR crosstalk in breast cancer pathogenesis in response to different progestins. An added complexity in delineating the role of ER-PR crosstalk in response to progestins is the fact that although a few studies report that some progestins and/or their metabolites may bind to the ER (Larrea et al. 2001, Pasapera et al. 2002, Escande et al. 2006, Lemes et al. 2009, Louw-du Toit et al. 2017), conflicting results are often reported, and most of these studies fail to differentiate between the ER subtypes. In a recent study, we have compared the binding of P4, MPA, NET-A, LNG, GES, NES, DRSP and NoMAC to the individual ER subtypes and showed that only NET-A, LNG and GES, which are all derived from testosterone, bind to ER-A, while none of the progestogens bind to ER-B (Louw-du Toit et al. 2017). Interestingly, it has previously been shown that ER-A is required at least for MPA-induced breast cancer cell proliferation (Giulianelli et al. 2012); thus, studies investigating the estrogenic activity of progestins used in HT and whether ER-A is required for the effects of all progestins on proliferation are needed. Furthermore, considering that the ER-B subtype is also expressed in breast cancers, investigations are required to determine whether a similar interaction occurs between ER-B and the PR and what the implications of such an interaction would be. Current molecular studies investigating the role of ER-B in breast cancer cell
biology are however limited, likely due to the lack of an effective commercial antibody for this ER subtype (Nelson et al. 2017). A better understanding of the role of ER-PR crosstalk, and whether it is involved in the increased breast cancer risk associated with some progestins, may help with the design of a progestin that can be used in HT without breast cancer risk.

**Interplay between the ER and AR**

A number of studies have suggested that androgens and the AR play a critical role in breast cancer biology (reviewed in Dimitrakakis & Bondy 2009, Hickey et al. 2012, McNamara et al. 2014, Tarulli et al. 2014, Rahim & O’Regan 2017) and considering that the AR is expressed in about 90% of primary breast tumors (reviewed in Hickey et al. 2012), it is not surprising that AR-targeted treatment for breast cancer is actively being investigated. However, the precise role of the AR in breast cancer is dependent on whether ER-A is present. While the AR generally plays an anti-proliferative role in ER-A-positive breast tumors by inhibiting the activity of ER-A (Dauvois et al. 1991, de Launoit et al. 1991, Hackenberg et al. 1991, Birrell et al. 1995a, Ortmann et al. 2002, Greeve et al. 2004, Peters et al. 2009), the AR can also mimic the role of the ER-A in ER-A-negative breast cancers and promote breast cancer development (reviewed in Rahim & O’Regan 2017). As a result, clinical trials are currently evaluating the use of selective AR modulators in ER-A-positive breast cancer therapies, and anti-androgens for use in ER-A-negative breast cancer therapies (Rahim & O’Regan 2017). One suggested mechanism whereby the AR can attenuate the activity of ER-A is by displacing ER-A from ER-binding sites, either via binding to androgen response elements that are in close proximity to ER-binding sites in estrogen target genes or by competing with ER-A for binding directly to EREs in target genes (Peters et al. 2009, Need et al. 2012). Another mechanism may be an AR-mediated increase in ER-B expression, which is known to inhibit the activity of ER-A, as increased ER-B expression has previously been shown in the presence of natural dihydrotestosterone (DHT) and the synthetic androgen mibolerone in MCF-7 and ZR75 breast cancer cell lines (Rizza et al. 2014).

Interestingly, it has been shown that the first-generation progestin MPA is a potent AR agonist (Africander et al. 2014, Louw-du Toit et al. 2017), and that like DHT, it inhibited the transcriptional activity of ER-A in MDA-MB-231 breast cancer cells overexpressing ER-A and the AR (Peters et al. 2009). However, AR-mediated effects in breast cancer appear to be a double-edged sword. For example, MPA treatment has effectively been used to treat breast cancer (Birrell et al. 1995b) via a mechanism that potentially promotes AR-induced apoptosis. In contrast, MPA used in HT has been associated with increased breast cancer risk (Writing Group for the Women’s Health Initiative Investigators 2002, Million Women Study Collaborators 2003, Fournier et al. 2008a) by a mechanism possibly involving the disruption of normal AR signaling (Kemppainen et al. 1999, Carroll et al. 2016). As some progestins can bind to the AR and elicit androgenic effects, while others elicit anti-androgenic effects, it is important to determine the progestin agonist and antagonist properties for AR-mediated transactivation and transrepression of target genes in the same model system. We have performed these experiments for P₄, MPA, NET-A, LNG, GES, NES, DRSP and NoMAC, and showed that the first (MPA, NET-A), second (LNG) and third-generation progestins (GES) display potent AR agonist activity, similar to that of DHT, for transactivation and transrepression of gene expression (Africander et al. 2014, Louw-du Toit et al. 2017). In contrast, like P₄, the fourth-generation progestins elicit anti-androgenic activity, similar to that of the well-known AR antagonist hydroxyflutamide (Louw-du Toit et al. 2017). Considering that some progestins that have been reported to increase breast cancer risk can elicit androgenic activity, studies are required to determine if ER-A-AR crosstalk plays a role in the mechanism whereby some progestins increase breast cancer risk.

**Interplay between the ER and the GR or MR**

Recent studies have also highlighted roles for the GR, MR and their cognate ligands in breast cancer cell biology. In terms of the GR and its ligands (glucocorticoids), both are involved in mammary gland development during puberty and pregnancy (reviewed in Vilasco et al. 2011, McNamara et al. 2017). Glucocorticoids have been shown to regulate breast cancer cell proliferation (Mattern et al. 2007, Vilasco et al. 2011, Courtin et al. 2012), invasiveness, motility and adhesiveness via GR-mediated upregulation of oncogenes and downregulation of metastasis suppressor genes (reviewed in Moutsatsou & Papavassiliou 2007). Interestingly, although the GR is expressed in approximately 60% of breast cancers (Abduljabbar et al. 2015), no definitive correlation has been found between GR expression and prognosis of breast cancers (reviewed in Vilasco et al. 2011). Some studies, however, suggest that its role may be context dependent as high GR expression has been associated with a good outcome in ER-A-positive...
cancers, while it is associated with a poor outcome in ER-A-negative cancers (Pan et al. 2011). For example, in ER-A-negative MDA-MB-231 cells, GR activation by dexamethasone (Dex) has been shown to increase the expression of genes involved in cell survival, such as serine/threonine protein kinase 1 (SGK1) and dual specificity protein phosphatase 1 (DUSP1) (Pan et al. 2011), while in the mouse xenograft model of MDA-MB-231 cells apoptosis induced by the chemopreventative agent, paclitaxel, was inhibited by Dex (Pan et al. 2011). In the presence of the ER, however, ER-A-GR complexes were formed when MCF-7 cells were treated with E₂ and Dex (Karmakar et al. 2013, West et al. 2016) resulting in the reprogramming of ER-A- and GR-binding sites (Voss et al. 2011, Miranda et al. 2013, West et al. 2016), and the subsequent activation of genes associated with a more favorable breast cancer outcome (West et al. 2016). The reprogramming involved an assisted loading mechanism which entailed the GR altering the chromatin landscape to expose novel binding sites to which ER-A can bind (Voss et al. 2011, Miranda et al. 2013). Moreover, Dex has been shown to antagonize the proliferative effects of E₂ (Zhou et al. 1989), while also inactivating estrogens by sulfation due to the activation of estrogen sulfotransferase (Gong et al. 2008, Vilasco et al. 2011). However, the converse is also true as E₂ has been shown to decrease GR expression and dephosphorylate the GR, thus inhibiting glucocorticoid action (Zhang et al. 2009, Vilasco et al. 2011). Taken together, although it is clear that the interplay between the ER and GR in breast cancer is complex, targeting of the GR as a potential for novel breast cancer therapies should not be excluded.

The MR has been shown to compensate for the absent GR during specific stages of mammary gland development (Kingsley-Kallesen et al. 2002), suggesting that the MR may play a similar role to the GR in breast cancer cell biology (Sikora 2016). Although the MR is expressed in most breast cancer tumors (Martin et al. 1984, Sasano et al. 1997, Yang & Young 2009), little is known about its role in breast cancer pathogenesis. Nevertheless, the MR ligand, aldosterone, has been shown to increase breast cancer cell proliferation and migration via a mechanism requiring the MR and the G-protein estrogen receptor (GPER), also known as the G protein-coupled receptor 30 (GPR30) (Rigiracciolo et al. 2016). This suggests that the MR is also involved in crosstalk mechanisms in breast cancer. Interestingly, at least one study has shown that the MR and ER-A can form a complex in HEK293 cells transiently transfected with cDNA expression vectors for the MR and ER-A (Barrett Mueller et al. 2014), thus it is likely that a similar complex formation may be seen in breast cancer cells. Implications of this putative MR and ER-A crosstalk is not clear. Although beyond the scope of this review, both the GR and MR have also been implicated in crosstalk with the PR (Leo et al. 2004), emphasizing the importance of future studies investigating the cellular mechanisms of the GR and MR in breast cancer, as well as the extensive interactions between different members of the steroid receptor family.

Conclusion

Despite the efficacy of FDA-approved conventional HT in relieving the symptoms of menopause, its association with increased breast risk is a major concern. Although both estrogen only and estrogen-progesterin HT regimens have been associated with increased breast cancer risk, findings from various clinical trials indicate that estrogen-progesterin combinations are associated with a higher risk than estrogen-only HT, suggesting that the progestin component is responsible for the increased risk. These studies, however, only compared a few progestins and considering that many different progestins, some known to elicit differential effects (Happgoed et al. 2004, Stanczyk et al. 2013, Africander et al. 2014), are available it cannot be assumed that all progestins would increase breast cancer risk. However, the alarm surrounding the associated breast cancer risk has caused some women to turn to custom-compounded bHT as an alleged safer, natural alternative. Evidence to support the safety and efficacy of these bHT regimens is however lacking and has resulted in many associations including the NAMS, IMS, EMAS and Endocrine Society recommending against the use of custom-compounded bHT (de Villiers et al. 2013). Considering the large variety of hormones used in HT and bHT, and the confusion as to whether or not any of these regimens are safe in terms of breast cancer risk, or whether they initiate or promote breast cancer, it is clear that more clinical and molecular studies directly comparing their mechanism of action are needed. Molecular studies should include the determination of binding affinities, relative agonist and antagonist potencies and efficacies for transactivation and transrepression of various estrogens and progestins for individual steroid receptors. In addition, studies are required to elucidate the effects of different progestins in modulating mammary stem cells and on the reactivation of stem cell-like properties in pre-existing breast cancer stem cells. Moreover, since estrogen-progesterin combination regimens are associated with a higher risk than estrogen-only regimens and that progestins elicit differential effects via steroid receptors,
it is possible that signaling via multiple steroid receptors may contribute to the observed increased breast cancer risk. Furthermore, recent studies have revealed roles for interplay between many of the steroid receptors in breast cancer cell biology. If the extensively intertwined nature of steroid hormone receptor signaling pathways can be unraveled, it may be possible to elucidate the mechanism behind progestin-induced breast cancer and design novel progestins that do not increase breast cancer risk.

Declaration of interest
The authors declare that there is no conflict of interest that could be perceived as prejudicing the impartiality of this review.

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