Future perspectives of selective estrogen receptor modulators used alone and in combination with DHEA

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Abstract

Breast cancer is the most frequently diagnosed and the second cause of cancer death in women, thus making breast cancer a most feared disease. Since breast cancer metastasizes early and it is unlikely that improvements in the treatment of metastatic disease could permit a cure in most cases in the foreseeable future, it is clear that prevention is essential in order practically to eliminate deaths from breast cancer. Tamoxifen is the only selective estrogen receptor modulator (SERM) currently registered for use in breast cancer prevention; the tamoxifen versus raloxifene study should indicate the efficacy of this compound compared with raloxifene. The recent benefits of aromatase inhibitors over tamoxifen indicate the advantages of a blockade of estrogens more complete than the one achieved with tamoxifen, a SERM having some estrogenic activity in the mammary gland and an even higher estrogenic action in the uterus. However, it is unlikely that the general estrogen ablation achieved with aromatase inhibitors will be acceptable for the long-term use required for prevention. It is thus important to develop SERMs with highly potent and pure antagonistic activity in the mammary gland and uterus while possessing estrogen-like activity in tissues of particular importance for women's health, namely the bones and the cardiovascular system. However, it is expected that a SERM alone will not meet all the requirements of women's health at the postmenopause when ovarian estrogen secretion has ceased and peripheral formation of androgens and estrogens from DHEA by intracrine mechanisms is decreased by 60% or more. One possibility is to combine a SERM with DHEA, a precursor of sex steroids that permits, somewhat like SERMs, tissue-specific formation of androgens and/or estrogens according to the level of expression of the steroidogenic and steroid-inactivating enzymes. DHEA could thus compensate for the important loss of androgens that accompanies aging and could also permit sex steroid formation and action in the brain while breast cancer prevention would be achieved by the SERM.

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Introduction

Breast cancer is diagnosed in more than 1 million women worldwide yearly. Since it is unlikely that improvements in the treatment of advanced breast cancer will permit the cure of most cases in the foreseeable future, and breast cancer has already metastasized in 50–60% of cases at the time of diagnosis, the development of an efficient and well-tolerated prevention strategy is imperative. Tamoxifen has provided proof-of-principle data showing that a selective estrogen receptor modulator

(SERM) is efficacious in both the treatment and the prevention of breast cancer (Fisher *et al.* 1998).

According to the latest Oxford analysis of the Early Breast Cancer Trialists' Collaborative Group (EBCTCG; 2005), 5 years of tamoxifen treatment in the adjuvant setting produced, after 15 years of follow-up, an $11.8 \pm 1.3\%$ absolute reduction in breast cancer recurrence and a $9.2 \pm 1.2\%$ reduction in breast cancer mortality in women with estrogen receptor (ER)-positive tumors. Treatment with the SERM raloxifene caused a dramatic 76% decrease in breast cancer incidence following 3 years of its

administration in osteoporotic postmenopausal women (Cummings *et al.* 1999). For the remaining 24%, it is possible that they represent cancers which had already developed alternative pathways of survival because of an advanced stage. The study of tamoxifen versus raloxifene (STAR trial) should define the relative merits of tamoxifen and raloxifene in preventing breast cancer in the adjuvant setting.

Long-term treatment with tamoxifen, however, is well known to have side-effects related to the partial agonistic activity of this compound, especially the increased risk of endometrial cancer and thromboembolic events (Fisher *et al.* 1994, Jaiyesimi *et al.* 1995, EBCTCG 2005). Moreover, tamoxifen therapy over 5 years does not seem to convey additional benefits and may even bring negative effects (Fisher *et al.* 1996, 2001, Peto 1996). In addition, resistance to tamoxifen is a well-established phenomenon (Clarke *et al.* 2001). The limit of 5 years of tamoxifen administration and its estrogenic side-effects indicate the need to search for improved SERMs.

The ATAC study of anastrozole and tamoxifen alone or in combination study (Baum *et al.* 2003) has recently shown the improved efficacy of anastrozole over tamoxifen, thus suggesting the use of 5 years of anastrozole instead of tamoxifen adjuvant therapy for postmenopausal women with early breast cancer (Howell *et al.* 2005). Moreover, studies with anastrozole, letrozole and exemestane have shown the benefits of switching from tamoxifen to an aromatase inhibitor (Goss *et al.* 2003, Coombes *et al.* 2004, Jakesz *et al.* 2005). It should be mentioned that, despite the relatively short-term duration of the studies performed with aromatase inhibitors, an increased number of fractures have been found with all the aromatase inhibitors compared with tamoxifen (Baum *et al.* 2002, Coleman *et al.* 2004, Coombes *et al.* 2004, Jakesz *et al.* 2005, Lonning *et al.* 2005). Moreover, despite the advantages of switching to an aromatase inhibitor, the advantages obtained are still far from the prevention of recurrence in all patients, and resistance to aromatase inhibitors develops (Goss 2002).

In this context of general estrogen deprivation caused by aromatase inhibitors, estrogen deficiency has been reported to have a negative effect on cognitive status, especially short- and long-term memory (Sherwin 2003, Tralongo *et al.* 2005). Preliminary data have indicated a higher incidence of impaired word finding in women who were treated with the aromatase inhibitor exemestane compared with tamoxifen (Jones *et al.* 2003). Such data raise questions about the long-term effects of general estrogen

deprivation on cognitive function, a problem which could be amplified under the long-term conditions of prevention.

Despite the limitations mentioned above with the presently available drugs, it is well recognized that estrogens play the predominant role in breast cancer development and growth. Therefore, estrogen deprivation should be part of the strategy of prevention. However, as mentioned above, it is unlikely that generalized estrogen deprivation, such as achieved with aromatase inhibitors, will be acceptable for long-term use. It thus seems logical to suggest that SERMs, with their tissue-specific action, are the class of drugs upon which the best hope for an efficient and well-tolerated preventative therapy for breast cancer relies.

One essential characteristic of the SERM(s) chosen for prevention of breast cancer should be that they are compounds free of any agonistic activity in the mammary gland and uterus while exerting estrogen-like activity in other tissues of importance for women's health. It is also possible that the best strategy for global women's health will be a combination of a SERM with a tissue-specific precursor of sex steroids, in order to compensate for the progressive loss of androgens with age, while permitting estrogen formation in the brain in order to protect cognitive function.

Endocrinology of the mammary gland, breast cancer and women's health

Endocrinology of the normal mammary gland: intracrinology

It is remarkable that man, in addition to possessing very sophisticated endocrine and paracrine systems, has vested largely in sex steroid formation in peripheral tissues (Labrie 1991, Labrie *et al.* 1985, 1988, 1997a, Simpson 2000). In fact, while the ovaries and testes are the exclusive sources of androgens and estrogens in lower mammals, the situation is very different in man and higher primates, where active sex steroids are a very large part (androgens) or are wholly (estrogens after the menopause) synthesized locally in peripheral tissues, thus providing target tissues with the appropriate controls which permit adjustment of the formation and metabolism of sex steroids according to local needs (Fig. 1).

The rate of cell-specific transformation of the adrenal precursor steroids DHEA and DHEA sulfate (DHEA-S) into androgens and/or estrogens in each peripheral target tissue thus depends upon

Figure 1 Schematic representation of the role of ovarian and adrenal sources of sex steroids in premenopausal women. After menopause, the secretion of estradiol by the ovaries ceases and then almost 100% of sex steroids are made locally in peripheral target intracrine tissues. ACTH, adrenocorticotropin; DHEA, dehydroepiandrosterone; DHT, dihydrotestosterone; E₂, 17 β -estradiol; LH, luteinizing hormone; LHRH, LH-releasing hormone; CRH, corticotropin-releasing hormone.

the level of expression of the various steroidogenic and metabolizing enzymes in each cell of each tissue (Fig. 2). This sector of endocrinology that focuses on the intracellular hormone formation and action has been called intracrinology (Labrie *et al.* 1988, Labrie 1991).

In women, the role of the adrenal precursors DHEA and DHEA-S in the peripheral formation of sex steroids is even more important than in men. In fact, in men, androgen secretion by the testes continues at a high level throughout life while, in women, estrogen secretion by the ovaries completely ceases at menopause, thus leaving the adrenals as the primary source of precursors of sex steroids. In fact, the best estimate is that the intracrine formation of estrogens in peripheral tissues in women is 100%

after the menopause while the vast majority of androgens are also made locally from DHEA throughout life (Adams 1985, Labrie *et al.* 2003a).

In addition to E₂, another important but still largely unrecognized estrogen is androst-5-ene-3 β ,17 β -diol (5-diol) (Fig. 2). This steroid of adrenal origin has, in fact, been shown to exert direct estrogenic effects in both normal and malignant estrogen-sensitive tissues at concentrations found in the circulation of normal adult women (Adams 1985, Poulin & Labrie 1986, Simard *et al.* 1988). It should be emphasized that aromatase inhibitors do not affect the formation of this estrogen, thus leaving 5-diol free to continue to stimulate breast cancer after inhibition of E₂ formation. As mentioned earlier, the 100-fold higher concentration of DHEA in the mammary gland versus plasma is accompanied by parallel findings for the estrogenic 5-diol (Poortman *et al.* 1983).

The major importance of estrogen formation in peripheral tissues after the menopause is also clearly supported by the finding that tissue levels of E₂ remain elevated at about 200 pg/ml in breast cancer tissue after the menopause while plasma levels of E₂ are decreased below 10 pg/ml (Poortman *et al.* 1983). In fact, in breast cancer tissue, it is remarkable that the concentration of E₂ is similar in pre- and postmenopausal women (Poortman *et al.* 1983). The major role of peripheral estrogen formation in postmenopausal women is well demonstrated by the observation of the major benefits of aromatase inhibitors in advanced breast cancer in postmenopausal women (Nabholtz *et al.* 2000, Goss *et al.* 2003, Mouridsen *et al.* 2003) as well as by the findings of a 76% decrease in breast cancer incidence in postmenopausal osteoporotic women who received the SERM raloxifene for 3 years (Cummings *et al.* 1999).

The role of DHEA in peripheral tissues is well supported by the observation that the concentration of DHEA in retroperitoneal fat in postmenopausal women is approximately 100-fold higher than the serum concentration, thus providing a high level of substrate for the local intracellular formation of androgens and/or estrogens (Szymczak *et al.* 1998). That the high intracellular levels of estrogens after the menopause are not limited to breast cancer is illustrated by the observation that the concentration of E₂ is about five times higher in retroperitoneal fat than in plasma levels in postmenopausal women (Szymczak *et al.* 1998). Such data, showing that relatively high levels of E₂ remain present in peripheral tissues after the menopause, suggest that removal of these

Figure 2 Human steroidogenic and steroid-inactivating enzymes in peripheral intracrine tissues. DHEA, Dehydroepiandrosterone; DHEAS, DHEA sulfate; 5-Diol, androst-5-ene-3 β , 17 β diol; 5-Diol FA, 5-Diol fatty acid; 5-Diol S, 5-Diol sulfate; 3 β -HSD-I, 3 β -hydroxysteroid dehydrogenase-Type I; 4-Dione, Androstenedione; testo, Testosterone; ER, Estrogen Receptor; AR, Androgen receptor; A-Dione, Androstenedione; Ro-DH-I, Ro-dehydrogenase type I; ADT, Androsterone; UGT, uridine glucuronosyl transferase; ADT-G, ADT glucuronide; E₁, Estrone; E₂ 17 β -estradiol.

estrogens is likely to have negative and poorly tolerated consequences in many systems under the long-term conditions of estrogen ablation required for efficient prevention of breast cancer with compounds having an action limited to estrogen formation, as observed with aromatase inhibitors.

Estrogens and breast cancer

As mentioned above, among all the risk factors, estrogens are well recognized to play the predominant role in breast cancer development and growth (McGuire *et al.* 1975, Asselin & Labrie 1978, Davidson & Lippman 1989). Considerable attention has thus focused on the development of blockers of estrogen biosynthesis and action (Wakeling & Bowler 1988, Dauvois *et al.* 1991, de Launoit *et al.* 1991, Lévesque *et al.* 1991, Gronemeyer *et al.* 1992, Labrie *et al.* 1992a, 1995a, Gauthier *et al.* 1997) for the treatment of breast cancer.

In fact, a most important characteristic of the endocrine physiology of the mammary gland is that the normal mammary gland, as well as early breast

cancer, absolutely require estrogens for proliferation and growth. In agreement with the important role of estrogens, inhibitors of estrogen formation and action have shown very positive results in breast cancer therapy, these benefits being accompanied by an exceptionally good tolerance compared with chemotherapy. Moreover, in addition to the well-recognized benefits of the antiestrogens tamoxifen and fulvestrant, these observations also pertain to inhibitors of estrogen formation, namely the aromatase inhibitors anastrozole, letrozole and exemestane (Bonneterre *et al.* 2000, Mouridsen *et al.* 2001, Goss 2002, Goss *et al.* 2003), as well as to medical castration with LHRH agonists (goserelin, leuprolide, decapeptyl and buserelin). While being much better tolerated, these compounds used alone or in combination usually show results superior to chemotherapy, especially in early disease.

Androgens inhibit breast cancer

While it is well recognized that estrogens play the predominant role in the development and growth of

human breast cancer, a series of observations has shown that androgens such as testosterone (Ulrich 1939, Fels 1944, Segaloff *et al.* 1951, Cooperative Breast Cancer Group 1964), fluoxymesterone (Kennedy 1958, Tormey *et al.* 1983, Ingle *et al.* 1991), calusterone (Gordan *et al.* 1973) and anabolic steroids (Gordan 1976, Segaloff 1977) have an efficacy comparable with that achieved with other types of endocrine manipulation.

Evidence from preclinical studies strongly demonstrates the inhibitory effect of androgens on breast cancer (Labrie *et al.* 2006). The above-mentioned clinical data are, in fact, well supported by the observation of a synergistic effect of DHEA and the pure antiestrogen EM-800 (a precursor of acolbifene) in preventing the development of dimethylbenz(a)-anthracene (DMBA)-induced mammary tumors in the rat (Luo *et al.* 1997b). Moreover, DHEA shows an almost exclusive androgenic effect on the histomorphology and structure of the rat mammary gland (Sourla *et al.* 1998), thus suggesting that the inhibitory effect of DHEA is due to its transformation into androgens. Moreover, the effect of androgens as direct inhibitors of breast cancer growth is well supported by the presence of ARs in a large proportion of human breast cancers (Trams & Maass 1977, Allegra *et al.* 1979, Bryan *et al.* 1984, Miller *et al.* 1985). There is also genetic evidence indicating a protective role of androgens against breast cancer (Wooster *et al.* 1992, Lobacaro *et al.* 1993).

A potent and direct inhibitory effect, at physiological concentrations, of androgens has been observed on the proliferation of human breast cancer cells (Poulin *et al.* 1988, 1989a,b, Dumont *et al.* 1989, Simard *et al.* 1989, 1990). In fact, the first demonstration of a potent and direct inhibitory effect of androgens on human breast cancer growth was obtained in the estrogen-sensitive human breast cancer cell line ZR-75-1 (Poulin *et al.* 1988). In that study, DHT not only completely blocked the stimulatory effect of E_2 on cell proliferation but it further reduced cell growth in the absence of estrogens. At low cell density, DHT completely prevented breast cancer cell growth.

It should be added that treatment of ovariectomized monkeys with testosterone decreased the stimulation of mammary epithelial proliferation induced by E_2 by about 40% (Zhou *et al.* 2000). Moreover, it is possible that part of the increased risk of breast cancer in BRCA-1 mutant patients is associated with the decreased efficiency of the mutated BRCA-1 gene to interact with the AR (Park *et al.*

2000). It is also pertinent to mention that female athletes and transsexuals taking androgens show atrophy of mammary gland epithelial tissue (Burgess & Shousha 1993, Korkia & Stimson 1997).

In addition to the direct inhibitory effect of androgens on mammary epithelial cell proliferation, it is increasingly apparent that mammary cells possess complex regulatory mechanisms that allow for the strict control of the intracellular levels of both stimulatory and inhibitory sex steroids. For instance, our data show that DHT favors the degradation of E_2 into estrone (E_1), thus suggesting that the potent antiproliferative activity of DHT in E_2 -stimulated ZR-75-1 human breast cancer cells is, at least partially, exerted on 17β -HSD activity (Adams 1985, Poulin *et al.* 1988, 1989b, Couture *et al.* 1993). Conversely, we have found that estrogens cause a marked increase in the production of the glucuronidated androgen metabolites androstane $3\alpha,17\beta$ -diol glucuronide and androsterone glucuronide in MCF-7 cells, thus decreasing the inhibitory androgenic activity (Roy *et al.* 1992). In fact, since glucuronidation is the predominant route of androgen inactivation, androgen-inactivating enzymes may constitute an important locus of the regulation of breast cancer growth.

The long series of preclinical and clinical data reviewed by Labrie *et al.* (2003a, 2006) indicate that proliferation of both the normal mammary gland and breast cancer results from the balance between the stimulatory effect of estrogens and the inhibitory effect of androgens (Fig. 3). Moreover, the data showing the additive inhibitory action of antiestrogens and androgens suggest that taking advantage of the inhibitory action of androgens on breast cancer proliferation could well improve the efficacy of the currently used and well-tolerated estrogen-deprivation therapies for the treatment and prevention of breast cancer; the most physiological androgen precursor is DHEA which can lead to formation in a tissue-specific manner.

DHEA inhibits breast cancer

Low circulating levels of DHEA and DHEA-S have been found in patients with breast cancer (Zumoff *et al.* 1981) and DHEA has been found to exert antioncogenic activity in a series of animal models (Schwartz *et al.* 1986, Gordon *et al.* 1987, Li *et al.* 1993). In fact, DHEA has been shown, in animal model systems, to be an inhibitor of breast, prostate, lung, liver, skin and thyroid carcinogenesis (Ratko *et al.* 1991, Li *et al.* 1994, McCormick

Figure 3 (A) Schematic representation of the balance between the stimulatory action of estrogens and the inhibitory effect of androgens on mammary gland and breast cancer proliferation. In (B) blockade of the action of estrogens by a SERM and addition of androgens made from DHEA both lead to a reduction in mammary gland and breast cancer proliferation and growth.

et al. 1996, Kohama *et al.* 1997, Lubet *et al.* 1998, Shilkaitis *et al.* 2005). Among a series of data showing its inhibitory effect on breast cancer, DHEA blocked the stimulatory effect of N-nitroso-N-methylurea on mammary tumor development in the rat by a p16-dependent mechanism (Shilkaitis *et al.* 2005). DHEA has also been shown to have immunomodulatory effects *in vitro* (Suzuki *et al.* 1991) and *in vivo* in fungal and viral diseases (Rasmussen *et al.* 1992). On the other hand, a stimulatory effect of DHEA on the immune system has been described in postmenopausal women (Casson *et al.* 1993).

In order to investigate the possibility that DHEA and its metabolites could have a preventive effect on the development of mammary carcinoma, we have studied the effect of increasing circulating levels of DHEA, constantly released from Silastic implants, on the development of mammary carcinoma induced by DMBA in the rat. DMBA-induced mammary carcinoma in the rat has been widely

used as a model of hormone-sensitive breast cancer in women (Asselin *et al.* 1977, Asselin & Labrie 1978, Dauvois *et al.* 1989). Treatment with increasing doses of DHEA delivered constantly by Silastic implants of increasing length and number caused a progressive inhibition of tumor development (Li *et al.* 1993). It is of interest that tumor size in the group of animals treated with the highest dose (6×3.0 cm long implants) of DHEA was similar to that found in ovariectomized animals, thus showing a complete blockade of estrogen action by DHEA. Such data clearly demonstrate that circulating levels of the precursor adrenal steroid DHEA, comparable with those observed in normal adult premenopausal women, exert a potent inhibitory effect on the development of mammary carcinoma induced by DMBA in the rat.

Sex steroids and women's health: marked decline in DHEA with age

There is no medical event related to women's health with a higher negative impact on morbidity (and frequently mortality) than the menopause, a condition closely associated with declining sex steroid availability. The rapid fall in circulating E_2 at the menopause, coupled with the demonstrated beneficial effects of exogenous estrogens on menopausal symptoms (Grady *et al.* 1992, Greendale & Judd 1993, Lomax & Schonbaum 1993, Archer *et al.* 1999) and bone resorption (Weiss *et al.* 1980, Christiansen *et al.* 1982, Genant *et al.* 1990, Harris *et al.* 1991, Grady *et al.* 1992, Field *et al.* 1993, Lindsay 1993, Archer *et al.* 1999, Women's Health Initiative 2002) has focused most of the efforts of hormone replacement therapy (HRT) on various forms of estrogens (estrogen replacement therapy) as well as on combinations of estrogen and progestin (HRT) in order to avoid the risk of endometrial cancer induced by estrogens administered alone. Classical HRT has, however, recently been seriously questioned or even abandoned by many women following data indicating that the combination of Premarin and Provera (Prempro) causes a 26% increase in the incidence of breast cancer at 5.2 years of follow-up with a potential negative impact on cardiovascular events (Women's Health Initiative 2002). Similar concerns have also been raised in the Million Women Study (Beral *et al.* 2005).

The 70–95% reduction in the formation of DHEA and DHEA-S by the adrenals during aging (Labrie *et al.* 1997b) results in a dramatic reduction in the formation of androgens and estrogens in peripheral

target tissues. Among other possible factors, such hormonal changes are believed to play a role in the development of insulin resistance, type 2 diabetes and atherosclerosis (Coleman *et al.* 1984, Schriock *et al.* 1988). In fact, a beneficial effect of DHEA on the increase in insulin resistance that occurs with age has been observed (Diamond *et al.* 1996, Han *et al.* 1998). Moreover, low DHEA has been associated with atherosclerosis (Alexandersen *et al.* 1996), aging (Watson *et al.* 1996), insulin resistance and diabetes (Coleman *et al.* 1984, Schriock *et al.* 1988) as well as obesity (MacEwen & Kurzman 1991, Tchernof *et al.* 1995a). The 70–95% reduction in the formation of DHEA and DHEA-S by the adrenal could also well be involved in the pathogenesis of age-related diseases such as obesity (Nestler *et al.* 1988, MacEwen & Kurzman 1991, Tchernof *et al.* 1995a). DHEA may also be related to insulin resistance and hyperinsulinemia through its association with obesity. In agreement with this possibility, we have observed that statistical control for differences in adiposity and abdominal fat accumulation

eliminated the association between DHEA and glucose tolerance in men (Tchernof *et al.* 1995b).

In fact, since DHEA is transformed to both androgens and/or estrogens in peripheral tissues, such a fall in serum DHEA and DHEA-S explains why women at the menopause not only lack estrogen but also suffer deprivation of androgens for quite a few years. It is of interest to mention that in an early publication by Greenblatt *et al.* (1950), it was indicated that estrogen plus androgen was better than estrogen alone for treatment of the menopause.

SERMs used alone

Role of SERMs in breast cancer

The observation that tamoxifen (Fig. 4) reduces the incidence of contralateral breast cancer (Cuzick & Baum 1985) followed by the study of Powles *et al.* (1989) has opened the way for the clinical trials that led to the approval of tamoxifen by the Federal Drug Administration (FDA) as the first agent to

SERMs

Figure 4 Structure of a series of SERMs.

reduce the risk of breast cancer in high-risk women. Tamoxifen, despite its limitations, thus provided the proof-of-principle data for chemoprevention in breast cancer. Tamoxifen is, in fact, the only FDA-approved drug for breast cancer risk reduction in women who have a minimum 5-year GAIL (Gail score for predicting the risk of developing breast cancer; Gail *et al.* 1989) estimated risk of breast cancer of 1.66% and/or lobular carcinoma *in situ*. The approval was based on the data of the National Surgical Adjuvant Breast and Bowel Project P-1 project where women were randomized to receive 5 years of tamoxifen or placebo. In that trial, women taking tamoxifen had 49% fewer diagnosed cases of breast cancer with 43.9 versus 22.0 cases per 1000 women followed for 69 months (Fisher *et al.* 1998). However, as mentioned above, tamoxifen induced some serious adverse events, namely increased incidence of uterine cancer and blood clots, thus limiting its use.

Most encouraging results have been obtained with raloxifene, a SERM having a very low stimulatory effect on the uterus, thus minimizing or avoiding the risk of endometrial cancer. In fact, a 76% reduction in the risk of diagnosis of breast cancer was observed in osteoporotic women who received raloxifene (Cummings *et al.* 1999). The STAR trial which compares tamoxifen and raloxifene in the adjuvant setting should define the relative merits of the two drugs.

Two classes of aromatase inhibitors, namely steroidal (e.g. exemestane) and non-steroidal (e.g. anastrozole and letrozole) are now available for the treatment of breast cancer. The data obtained indicate that aromatase inhibitors are more effective than tamoxifen as first-line therapy for postmenopausal women with advanced breast cancer (Nabholtz *et al.* 2000, Mouridsen *et al.* 2003, Paridaens *et al.* 2003). Moreover, as mentioned above, aromatase inhibitors used as adjuvant therapy have shown results superior to tamoxifen in preventing recurrence of the disease in the adjuvant setting (Baum *et al.* 2003, Goss *et al.* 2003, Coombes *et al.* 2004).

Aromatase inhibitors decrease the risk of breast cancer with a reduced risk of uterine cancer and blood clots (Baum *et al.* 2002, Goss *et al.* 2003, Coombes *et al.* 2004). Potential limitations of aromatase inhibitors, however, are related to the observation that these compounds do not block estradiol formation completely (Johannessen *et al.* 1997, Geisler *et al.* 2000). Moreover, aromatase inhibitors do not inhibit the formation of the estrogenic 5-diol from DHEA by 17 β -hydroxysteroid

dehydrogenase activity (Fig. 2). 5-diol is present in the blood of pre- and postmenopausal women at the levels of 0.49 ± 0.20 ng/ml and 0.27 ± 0.15 ng/ml respectively. At these concentrations, 5-diol is well known to stimulate the proliferation of human breast cancer cells and other estrogen-sensitive tissues (Adams 1985, Poulin & Labrie 1986). As mentioned above, aromatase inhibitors thus leave 5-diol free to continue to stimulate breast cancer in the presence of a highly efficient but incomplete inhibition of E₂ formation. The long-term effects of aromatase inhibitors, moreover, remain to be evaluated.

As has been well demonstrated in prostate cancer, the more efficient blockade of androgens achieved by combining medical (gonadotropin-releasing hormone (GnRH) agonist) or surgical castration with a pure antiandrogen is more efficient than monotherapy, even at the advanced stage (Denis *et al.* 1993, Caubet *et al.* 1997, Prostate Cancer Trialists' Collaborative Group 2000, Klotz 2001, Labrie *et al.* 2002a, 2005b). It is thus likely that the optimal long-term benefits of estrogen blockade in breast cancer will be achieved with optimal estrogen blockade. For comparison, in localized prostate cancer, monotherapy with GnRH agonists alone achieved a one-third decrease in the death rate from prostate cancer (Peto & Dalesio 2003) while an at least 90% long-term control and possible cure of the disease was achieved when combining a GnRH agonist with a pure antiandrogen (Labrie *et al.* 2002a).

That these observations made in men with localized prostate cancer could apply to breast cancer is supported by recent preclinical data showing that the combination of letrozole and fulvestrant is much more efficient than either compound used alone in inhibiting the growth of human MCF-7 (MCF-7 Ca) tumors in nude mice (Jelovac *et al.* 2005a). In fact, with the combination therapy, tumor size was not only completely blocked but it decreased 45% below baseline. It is noticeable that these important benefits of combined estrogen blockade observed on breast tumor growth were achieved in the presence of no additional inhibitory effect of the combination of the two drugs on uterine weight, thus indicating that the human breast tumor is more sensitive to low levels of estrogens remaining in the tissue after monotherapy with letrozole or fulvestrant than the normal uterus. It should be mentioned that the model used did not show an additive effect of anastrozole plus tamoxifen or letrozole plus tamoxifen (Lu *et al.* 1999,

Long *et al.* 2004), a finding which was confirmed in the trials of arimidex and tamoxifen alone versus their combination in patients with breast cancer (Dowsett *et al.* 2001). Such data stress the importance of recognizing that all SERMs are different and that the data obtained with tamoxifen cannot be extrapolated to other SERMs having less or no estrogenic activity in the mammary gland and uterus and vice versa.

The underlying principle is that, in sex hormone-sensitive cancer, even low sex steroid levels permit continuous cancer cell division with the risk of additional adverse gene mutations and adaptation of other growth pathways, especially the kinase pathways, which more than compensate for the decreased estrogen levels and stimulate cancer cell growth independently of sex steroids. Such an adaptive phenomenon creates resistance to treatment (Schiff *et al.* 2005). In support of this interpretation, the recent preclinical data mentioned above have shown that the combination of an aromatase inhibitor with an antiestrogen is much more efficient than either compound used alone to inhibit the growth of human breast tumors in nude mice (Jelovac *et al.* 2005a). The resistance to treatment observed with aromatase inhibitors in advanced breast cancer may, in fact, be related to the incomplete blockade of estrogens achieved with these compounds combined with the residual estrogenic stimulus of 5-diol.

SERMs under development

Among the SERMs under development, arzoxifene, an analog of raloxifene (Fig. 4) has shown a 10.3% response rate at the 20 and 50 mg doses in tamoxifen-resistant patients while 26.1% and 8.0% response rates were seen at the same doses in tamoxifen-sensitive patients (Buzdar *et al.* 2003). On the other hand, in another phase II trial, a 30% response rate was observed with the 20 mg dose in previously untreated patients with a further 17% of patients showing stable disease (Baselga *et al.* 2003). A low 8% response rate was, however, seen with the 50 mg dose. The compound has moved to phase III where it is compared with tamoxifen (Johnston 2005).

Another SERM in development is acolbifene. Possibly the most important property of acolbifene recognized so far is that it causes the disappearance or cure of 60% of human breast cancer tumors in nude mice (Gutman *et al.* 2002). Based upon the data obtained with tamoxifen, the effect of hormone

therapy was so far thought to be limited to a tumorostatic action. In other words, following the results originally obtained with tamoxifen (Gottardis *et al.* 1988), the effect of hormonal therapy has classically been believed to be limited to a slowing of tumor growth or a tumorostatic action. The tumorocidal action of acolbifene is thus a new and most important paradigm of hormone therapy which most likely results from a more complete blockade of the estrogen receptor. Furthermore, acolbifene is the most potent of all available antiestrogens and SERMs in inhibiting the stimulatory effect of estrogens on the proliferation of human breast cancer cells *in vitro* (Simard *et al.* 1997a, Labrie *et al.* 2001a, 2002b). Among seven antiestrogens tested, acolbifene is also the most potent inhibitor of the stimulatory effect of estrogens on the growth of human breast cancer tumors in nude mice (Gutman *et al.* 2002). Moreover, while resistance to treatment is a major problem in cancer therapy, no resistance is observed with acolbifene in human breast cancer tumors in nude mice (Gutman *et al.* 2003).

Acolbifene possesses no estrogenic activity *in vitro* in human Ishikawa endometrial carcinoma cells while all other SERMs tested stimulate alkaline phosphatase activity in these cells (Simard *et al.* 1997b, Labrie *et al.* 2001a). Similarly, in the rat, acolbifene has no stimulatory effect on the endometrium, contrary to raloxifene which exerts a significant stimulatory effect (Sato *et al.* 1998, Martel *et al.* 2000, Labrie *et al.* 2002b). In fact, acolbifene blocks the stimulatory effect of all tested SERMs on alkaline phosphatase activity in human Ishikawa endometrial carcinoma cells (Simard *et al.* 1997b, Labrie *et al.* 2002b).

The phase II/III clinical program consisted of two studies (ERC-103 and C/197-042) evaluating the efficacy of acolbifene in the treatment of breast cancer in patients who had failed tamoxifen. In study ERC-103, 43 postmenopausal women were enrolled: five patients (one complete response, four partial responses) responded to therapy with acolbifene (Labrie *et al.* 2004) with a response rate of 12% while seven (16%) patients had stable disease for more than 6 months. These results are numerically comparable with the activity seen with the aromatase inhibitors anastrozole, letrozole and exemestane in the same category of patients.

In the phase III study, the primary objective was to compare the progression-free survival (PFS) between acolbifene and anastrozole treatment in patients with advanced disease who had progressed with tamoxifen. An interim analysis based on a total of 110 events (progression or death) was

performed. Both the 20 mg and 40 mg doses of acolbifene were compared with anastrozole. Median PFS survival times were 3.19, 4.11 and 4.01 months for acolbifene (20 mg), acolbifene (40 mg) and anastrozole respectively. With such similar findings in the three groups, it was unlikely that acolbifene could be shown to be superior to anastrozole in this category of patients. Based on the results of this interim analysis demonstrating that it was unlikely to meet the original objective of superiority, this study was terminated. An overview of the safety analysis demonstrated a similar toxicity profile to anastrozole with the majority of adverse events being related to disease rather than to treatment. A phase III study in previously untreated metastatic breast cancer patients is planned for this category of patients who are more likely to benefit from the treatment.

While not being a SERM, the preclinical characteristics of fulvestrant have clearly shown that the compound is a pure antagonist of estrogen action in the mammary gland and uterus but is without bone protection or other estrogen-like activity (Howell *et al.* 2000). *In vitro* and *in vivo* studies have shown that tamoxifen-resistant cell lines and tumors remain sensitive to fulvestrant (Hu *et al.* 1993, Osborne *et al.* 1995). Fulvestrant, a steroidal pure antiestrogen, at the dose used, has been shown to be equivalent to tamoxifen on the initial response or primary treatment of advanced breast cancer (Howell *et al.* 2004). On the other hand, fulvestrant has been shown to lead to a longer median time to progression compared with anastrozole in patients who had progressed with prior endocrine therapy (Howell *et al.* 2002, Osborne *et al.* 2002). Encouraging results have also been observed with fulvestrant in patients progressing under treatment with aromatase inhibitors (Johnston 2005), a phenomenon possibly related to the upregulation of growth factor-signaling pathways (Jelovac *et al.* 2005b, Sabnis *et al.* 2005).

Benefits of SERMs on bone

The US Surgeon General's report on bone health and osteoporosis estimates that over 12 million women and men over 50 years of age will have osteoporosis in the USA by 2010, increasing to 14 million by 2020. Among women over 50 years of age, the life-time risk of fracture is 40% and 13% in men over 50 years of age.

Tamoxifen has been shown to decrease osteoporotic bone fractures (Fisher *et al.* 1998). Among

Table 1 SERMs in development

1	TSE 424; bazedoxifene (Wyeth)
	(a) Osteoporosis (phase III, USA)
	Two phase III trials vs raloxifene
	(b) HRT in combination with Premarin (phase III, USA; expected NDA 2007)
2	ERA 923; pipendoxifene (Wyeth)
	Breast cancer (phase II, February 2005)
3	Acolbifene (Endoceutics)
	(a) Breast cancer (phase III)
	(b) HRT (phase III)
4	LY381; arzoxifene (Lilly)
	(a) Bone fractures (phase III, USA; expected NDA 2010)
	(b) Uterine cancer (phase III, prevention)
	(c) Breast cancer (compared with tamoxifen)
5	Lasofloxifene (Pfizer)
	Osteoporosis (preregistration, USA)
	Vaginal atrophy
6	PSK 3471; Prostakan (Sanofi–Aventis)
	Osteoporosis

*Partial list; NDA, National Drug Administration.

the SERMs in development (Fig. 4 and Table 1), all of them show beneficial effects on bone loss, at least at the preclinical level. In fact, arzoxifene, bazedoxifene and PSK 3471 are at various stages of clinical development for the prevention and/or treatment of osteoporosis or for the prevention of osteoporotic fractures. While the action of each SERM on other systems, especially the uterus, must be evaluated carefully, it is likely that prevention of bone loss will be achieved with each of these compounds. However, while it is likely that all known SERMs have a potential protective effect on bone in women, one must choose the SERM(s) most likely to have the best protective effect on the breast and endometrium, namely SERM(s) having pure antagonistic activity at the level of the mammary gland and uterus (Labrie *et al.* 1999, 2001b, 2002c).

SERMs used in combination with DHEA

Declining androgens with age

We feel that the increased understanding of androgen and estrogen formation and action in peripheral target tissues (Labrie 1991, Labrie *et al.* 1992b,c, 1994, 1995b, 1996b, 1997a,b,c,d, Luu-The *et al.* 1995), as well as our recent observations indicating the predominant role of androgens over that of estrogens in the prevention of bone loss after

ovariectomy in the rat (Martel *et al.* 1998) and the observation of a stimulatory action of DHEA on bone mineral density (BMD) in postmenopausal women (Labrie *et al.* 1997c) have paved the way for timely and potentially highly significant progress in the field of sex steroid replacement therapy at the menopause. This is particularly important at the present time because of the issues raised by the use of estrogens and progestins in this setting (Morales *et al.* 1994, Diamond *et al.* 1996, Labrie *et al.* 1997c, Baulieu 1999, Stomati *et al.* 2000, Women's Health Initiative 2002, Beral *et al.* 2005).

Normal women produce an amount of androgen equivalent to about two-thirds of that secreted in men but their production of androgen has already decreased by 60% at the menopause (Labrie *et al.* 1997a). As mentioned above, the pool of androgens decreases progressively from the age of 30 years in parallel with the decrease in serum concentrations of DHEA and DHEA-S (Labrie *et al.* 1997b). Consequently, it appears logical to use SERMs combined with tissue-specific androgenic/estrogenic replacement therapy around and after the menopause. This appears to be the best way to maintain a physiological balance between these two classes of sex steroids in each cell and tissue, an objective which can only be met by the local formation of androgens and estrogens in peripheral tissues from exogenous DHEA, a tissue-specific precursor of androgens and estrogens.

As mentioned above, the almost exclusive focus on the role of ovarian estrogens has removed attention from the dramatic 70% fall in circulating DHEA which already occurs between the ages of 20–30 and 40–50 years (Migeon *et al.* 1957, Vermeulen & Verdonck 1976, Vermeulen *et al.* 1982, Orentreich *et al.* 1984, Bélanger *et al.* 1994, Labrie *et al.* 1997b, Beral *et al.* 2005). Since DHEA is transformed to both androgens and estrogens in peripheral tissues, such a fall in serum DHEA and DHEA-S explains why all women at the menopause not only lack estrogens but are also deprived of androgens. While men are protected from the age-related fall in serum DHEA by the continuous high rate of testosterone secretion by the testicles, the low amount of testosterone of ovarian and adrenal origins has a much less protective effect on the marked fall observed in serum DHEA with age in women.

Synergistic effects on bone

Since acolbifene and DHEA act by two different mechanisms following interaction with the estrogen

and androgen receptors respectively, their combination appears to be well justified for the prevention and treatment of osteoporosis. In fact, in the ovariectomized rat, acolbifene is about ten times more potent than raloxifene in protecting against bone loss (Martel *et al.* 2000). On the other hand, we have found that DHEA exerts beneficial effects on bone in both intact and ovariectomized female rats (Luo *et al.* 1997c). Thus, in intact female rats, treatment with DHEA increases BMD of total skeleton, lumbar spine and femur (Luo *et al.* 1997c). Moreover, we have observed that the combination of a sex steroid precursor (DHEA) and acolbifene not only maintained the stimulatory effect of DHEA on bone formation, but also potentiated the inhibitory effect of the SERM on bone turnover and resorption, as demonstrated by the further decrease in urinary hydroxyproline and calcium excretion (Luo *et al.* 1997c).

Our preclinical data clearly indicate that DHEA can provide the beneficial effects which are lacking with the use of a SERM alone. While a SERM has effects limited to inhibition of bone resorption, the addition of DHEA stimulates bone formation (an effect not found with a SERM, a bisphosphonate, an estrogen or calcitonin) and further reduces bone resorption above that achieved with acolbifene alone. Moreover, the antiresorptive therapies do not improve all the characteristics of the normal bone, especially the microarchitecture. While the effects of acolbifene on bone have so far only been observed preclinically, treatment with DHEA has been found to increase BMD in postmenopausal women (Labrie *et al.* 1997b, Baulieu *et al.* 2000). The observed stimulatory effects of DHEA on BMD in postmenopausal women and the increase in serum osteocalcin, a marker of bone formation, are of particular interest for the prevention and treatment of osteoporosis and indicate a unique activity of DHEA on bone physiology, namely on bone formation (Labrie *et al.* 1997b).

Synergistic effects on breast

With regard to the breast, DHEA is known to prevent the development (Luo *et al.* 1997b) and to inhibit the growth (Li *et al.* 1993) of dimethylbenz(a)anthracene mammary tumors in the rat. DHEA, in addition, inhibits the growth of human breast cancer xenografts in nude mice (Couillard *et al.* 1998). Thus, contrary to estrogens and progestins which exert stimulatory effects, DHEA is expected, from a series of preclinical studies, to

inhibit both the development and the growth of breast cancer in women (Labrie *et al.* 2003a).

Because of its highly potent and pure antiestrogenic activity, acolbifene should not only eliminate the risk of breast, uterine and ovarian cancer associated with estrogen use (Lacey *et al.* 2002, Riman *et al.* 2002, Rodriguez *et al.* 2002, Women's Health Initiative 2002, Beral *et al.* 2005) but it may also reduce the spontaneous incidence of these cancers which are diagnosed in 13.3% (breast cancer), 2.7% (endometrial cancer) and 1.7% (ovarian cancer) of women during their lifetime.

Other beneficial effects of the combination of SERM and DHEA

In addition to an increase in bone formation, DHEA has also been shown, in postmenopausal women, to stimulate vaginal maturation and decrease skin dryness (Labrie *et al.* 1997b, Baulieu *et al.* 2000). It is also possible that SERMs could exert additional beneficial effects in postmenopausal women. It seems appropriate to mention some preclinical data obtained with acolbifene which could be very useful if found in women. These data pertain to the reduction of cholesterol and triglycerides, reduced weight gain and increased insulin sensitivity (Luo *et al.* 1997a, Picard *et al.* 2000, Lemieux *et al.* 2003, 2005). The inhibitory effect of acolbifene on serum cholesterol has been found to be due to an increase in the level of the low density lipoprotein receptor in the liver (Lemieux *et al.* 2005). Acolbifene has also been shown to increase nitric oxide synthesis in endothelial cells (Simoncini *et al.* 2002).

Conclusion

Since, as mentioned above, breast cancer metastasizes early compared with prostate cancer (Labrie *et al.* 2002a, 2005b), prevention is essential in order to achieve a marked decrease in deaths. Without prevention, the majority of breast cancers will continue to be diagnosed at a true advanced stage, despite being clinically localized. For the purposes of comparison, in the field of prostate cancer, more than 99% of cases can be diagnosed with appropriate screening at the clinically localized stage when cure is a possibility (Labrie *et al.* 1996a, 2002a, 2005b). Unfortunately, there is no marker for breast cancer equivalent to prostatic-specific antigen for prostate cancer and, much too frequently, breast cancer has already spread at distant sites at the time of diagnosis, thus explaining the recurrence after efficient local

therapy by surgery and/or radiotherapy.

Knowing that estrogens play such a crucial role in breast cancer, the two most obvious choices for prevention of the disease are an aromatase inhibitor to block the formation of estrogens (Goss & Strasser 2001) or a SERM to block the action of estrogens and exert additional benefits of importance for women's health (Jordan 2001). The use of a SERM having multiple actions is now a reality with raloxifene which has been found to treat and prevent osteoporosis while reducing the risk of breast cancer in osteoporotic women (Cummings *et al.* 1999).

Since the generalized estrogen ablation caused by aromatase inhibitors is unlikely to be acceptable for prevention of breast cancer because of the adverse effects expected under long-term conditions in tissues other than the breast and uterus, it seems clear that major efforts should be devoted to the development of SERMs which have a potent and pure antiestrogenic activity in the mammary gland and uterus while having beneficial effects in other systems of importance for women's health, especially the bones, the cardiovascular system and metabolic processes. As mentioned above, all SERMs are different, and detailed assays must be performed at the preclinical level to ensure the best chances of success of the new compounds in the clinic. It should be remembered, however, that clinical data provide the ultimate and only appropriate proof of efficacy.

In addition to the need to have a SERM with pure antiestrogenic activity in the mammary gland and endometrium, it is also clear that a SERM alone will not meet all the hormonal requirements of women after the menopause. It thus appears reasonable to develop the combination of a SERM with DHEA, a tissue-specific precursor of both androgens and estrogens (Labrie *et al.* 2005a). That SERMs can efficiently block the effect of estrogens in peripheral tissues has been well demonstrated with studies of acolbifene in the rat (Labrie *et al.* 2003b); this eliminates the risk of breast and uterine cancer and thus strongly supports the combination approach.

Tissue-specific HRT achieved with the combination of SERM and DHEA could then help to control hot flashes and improve cognitive function and memory (Yaffe 1998), while preventing breast cancer, uterine cancer, ovarian cancer and bone loss as well as fat accumulation and metabolic deterioration (Table 2). One objective is thus to develop a novel strategy for the benefit of peri- and

Table 2 Effects of a SERM, DHEA and their combination

End organ	DHEA	SERM	DHEA and SERM ^a
Bone density	+	+	+
Cholesterol	+	+	+
Hot flushes	+	tbd	tbd
Breast cancer	+	+	+
Endometrial hyperplasia	–	+	+
Uterine bleeding	–	+	+
Thromboembolic events	tbd	tbd	tbd

+, beneficial effect; –, no effect; tbd, to be determined.

^aAnticipated net effect of DHEA and SERM on target organs.

postmenopausal women, namely a tissue-specific HRT, i.e. DHEA plus acolbifene or another SERM having the minimal characteristics of being a completely pure antagonist of estrogens in the mammary gland and uterus.

Disclosure

The author is president of Endorecherche, the company responsible for the development of acolbifene and has patents on medical uses of DHEA and its combination with SERMs.

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