

©**Combined Hormone Replacement Therapy and Breast Cancer**  
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**March 2008**

**SUMMARY**

On the basis of a systematic and thorough review of the published scientific literature, and our collective scientific investigations, clinical experience and scientific knowledge, we are of the opinion that the inclusion of the synthetic progestin medroxyprogesterone acetate (MPA) in combination hormone replacement therapy (cHRT) contributes to the development of breast cancer.

We believe that:

1. cHRT that contains MPA contributes to the development of breast cancer in women by disrupting the normal steroid hormone interactions required for a balance of growth within breast tissue.
2. While MPA has been beneficially used in the treatment of a particular subgroup of breast tumors, this does not imply that it will not have an adverse effect on normal breast tissue when used in a different pharmacological context, namely cHRT. Specifically, the apparently widespread acceptance of the safety of MPA in cHRT based on its use as a breast cancer therapy is misguided.
3. MPA should no longer be prescribed for use in systemically-administered cHRT, because its disease-promoting risks outweigh its therapeutic benefits.
4. Oral micronized progesterone (OMP) is a progestin in current clinical use that is structurally and functionally bio-identical to natural progesterone. Unlike MPA, OMP does not disrupt normal steroid hormone interactions in the breast, but effectively opposes the action of estrogen on the endometrium. As such, OMP is a safer alternative to the use of MPA in systemically-administered cHRT.
5. Local administration of a synthetic progestin (such as levonorgestrel) to the uterus by implantation of intrauterine devices is another option when considering alternatives to systemic administration of the progestin component of cHRT.

**INTRODUCTION**

The breast is a dynamic organ that can undergo dramatic changes repeatedly throughout a woman's lifespan [1]. During puberty the immature breast undergoes a period of growth and morphogenesis that prepares it to become a functional mammary gland. Throughout the reproductive years, additional growth will occur in the luteal phase of each menstrual cycle in anticipation of

pregnancy. When pregnancy occurs, the breast begins an extensive growth phase that greatly increases the amount of breast tissue and ultimately alters its entire architectural structure. In the later stages of pregnancy, the newly expanded mammary glands undergo functional changes that confer the ability to produce milk. Following pregnancy and lactation, the breast regresses through a process called involution that returns it to a state of readiness similar to that of early pregnancy, but the tissue will now possess a different architecture and an altered genetic program that persists throughout the reproductive years. In the total absence of a full-term pregnancy, the breast structure remains in a state similar to that of the pubertal breast, although exposure to exogenous hormones (e.g. contraceptives) can stimulate a small portion of the breast tissue to adopt a state similar to that in early pregnancy. At menopause, the breast fully regresses to an immature state in all women, whether they had a full-term pregnancy or not in their reproductive years.

Regulation of breast growth and development requires a specific interaction between three sex hormone signaling pathways: estrogen, progesterone, and androgen. The actions of these hormones mainly target epithelial cells that line the mammary ducts and form the mammary lobules that produce milk in the breasts of pregnant women. Proliferation of these cells is essential for breast growth, and this is tightly regulated in a healthy state by a careful balance of hormone activities that either encourage or inhibit survival and proliferation. These breast epithelial cells are also those that typically undergo transformation to form breast tumors. In order to understand how this transformation occurs, the action of all three sex hormones must be considered.

### ***Estrogen action in the breast***

Over 100 years ago it was demonstrated that removal of the ovaries could suppress the growth of breast cancer [2]. Since then, multiple lines of scientific evidence have shown that exposure to the female sex hormone estrogen is involved in the development of breast cancer [3]. Breast epithelial cells depend on functional estrogen signaling for growth and survival, and this characteristic is maintained in most breast cancers. Sex hormone signaling involves interaction of the hormone with specific receptors within cells. Estrogen signals through the estrogen receptor (ER), which exists in two forms, ER $\alpha$  and ER $\beta$ , that arise from two distinct genes. Studies involving female mice that lack either ER $\alpha$  or ER $\beta$  have revealed that the structural and functional development of the mammary gland is primarily controlled by the action of ER $\alpha$  [4]. Therefore, current therapies for breast cancer aim to limit estrogen signaling in the breast by inhibiting estrogen synthesis or specifically blocking the action of ER $\alpha$  [5].

### ***Progesterone action in the breast***

Progesterone is another sex hormone that contributes to normal development of the mammary ducts and lobules. Estrogen causes breast epithelial cells to become responsive to progesterone by inducing expression of the progesterone receptor (PR). Like ER, PR has two forms, PR-A and PR-B, but unlike the two

ER forms, they arise from the same gene [6]. Studies of female mice lacking one or the other of these receptors indicate that PR-B alone is necessary for proliferation of breast epithelial cells, particularly that associated with increased mammary duct and lobule growth during early pregnancy [7]. In late pregnancy progesterone inhibits growth and promotes the production of milk proteins, partly by causing cells to become responsive to the pituitary hormone prolactin.

The action of progesterone in breast tissue is complex and involves both growth-promoting and growth-inhibiting actions that vary with age, stage of the menstrual cycle, and stage of pregnancy [6]. Epithelial cells of the juvenile breast all possess ER and PR, a profile that corresponds to very low proliferative activity [8]. Following puberty, some epithelial cells lose PR expression, so that the glands contain cells that have both ER and PR and cells that only contain ER. The ER and PR-containing cells do not proliferate, but upon exposure to progesterone, they secrete factors that stimulate proliferation of cells containing only ER. During a normal menstrual cycle, progesterone secreted by the ovaries during the luteal phase stimulates some proliferation in the ducts and lobules of the breast [9], and in the event of pregnancy the rate of proliferation dramatically increases coincident with the rise in estrogen and progesterone characteristic of early pregnancy. If pregnancy does not occur, serum progesterone levels drop and some breast epithelial cells die before the breast returns to a more quiescent state until the next luteal phase of the menstrual cycle [9].

Since progesterone is able to stimulate proliferation of breast epithelial cells, PR signaling is likely to contribute to the development of breast cancer. Indeed, studies of PR-deficient mice show that this steroid signaling pathway is necessary for known mammary carcinogens to induce breast tumors [7]. In these animal studies, tumor formation is associated with abnormal PR expression patterns and pathways of signaling that substantially boost the ability of progesterone to stimulate breast epithelial cell proliferation [8]. Similar characteristics have been observed in human breast tumors and some human breast cancer cell lines, but on the whole the contribution of abnormal PR signaling to the development of human breast cancer remains undetermined [10, 11].

### ***Progesterone action in the uterus***

In contrast to the variable actions of progesterone in the breast, the action of progesterone in the uterus is consistently anti-proliferative [6]. In the uterus, progesterone inhibits the proliferative action of estrogen on endometrial cells and induces the secretory phase necessary for successful pregnancy. If a woman is exposed to estrogen without subsequent exposure to progesterone, the endometrium is predisposed to develop cancer. In part, this was made evident in the mid 1970s by studies clearly showing an increased rate of uterine cancer in women on HRT comprised solely of estrogen [12, 13].

### ***Synthetic progestins in hormone therapies***

With the recognition in the mid 1970s that unopposed estrogen increases the rate of endometrial cancer, combined hormone replacement therapy with estrogen plus a synthetic progestin (i.e. cHRT) was introduced for postmenopausal women with an intact uterus [14]. Synthetic progestins have structural modifications that make them more stable and have higher bioavailability than natural progesterone, hence their preference for pharmaceutical applications [15-17]. These include cHRT as well as various forms of female contraception, where the synthetic progestin is administered either alone or in combination with estrogen.

At the time hormone pharmaceuticals containing a synthetic progestin were developed and brought into widespread use, two general assumptions prevailed:

1. Progesterone opposes estrogen activity in all body organs
2. Synthetic progestins globally mimic the action of progesterone

Both of these assumptions proved to be false. First, the action of progesterone in different reproductive tissues depends on the relative activities of PR-A and PR-B [18]. While normal uterine function is dependent on PR-A, normal breast function is dependent on PR-B, and this appears to be the reason why progesterone inhibits estrogen-induced proliferation in the uterus but enhances estrogen-induced proliferation in the breast. Second, while synthetic progestins do bind to both PR forms, and can elicit responses similar to progesterone in some target tissues, their diverse chemical structures can also result in responses that are quite different from those associated with native progesterone [15-17, 19-21]. In part, this is due to the fact that synthetic progestins can promiscuously bind to other steroid hormone receptors and either stimulate or inhibit their activity [22, 23]. In contrast, binding of natural progesterone or bio-identical molecules such as OMP to the PR is very specific, and there is very little or no binding of progesterone to other steroid receptors at physiological concentrations of the ligand.

Continuous exposure to a synthetic progestin as part of cHRT or female contraception is like being in a perpetual luteal phase of the menstrual cycle or state of early pregnancy, which in the breast corresponds to a time of increased proliferation of breast epithelial cells, particularly in the breast lobules. Therefore, the breast tissue of women using these hormone pharmaceuticals is excessively stimulated to proliferate over a long period of time, uninterrupted by the non-proliferative phases of a menstrual cycle, or the differentiation that occurs with advancement of pregnancy and lactation. This is of particular concern for postmenopausal women since involution of breast tissue after menopause results in a breast structure that is more hormone responsive than adult pre-menopausal breast tissue [1]. To prevent uncontrolled growth and cancer transformation in such a situation would require constant and robust activity by the normal

processes within the breast that maintain a balance of growth. Although progesterone fulfills that role in the uterus, this is not the case in breast tissue. Instead, it is the action of hormones commonly thought of as male hormones (i.e. androgens) that keeps breast growth in a state of equilibrium.

### ***Androgen action in the breast***

Although estrogen and progesterone were historically considered the main functional sex hormones in women, females also produce significant quantities of androgen hormones [24]. Androgens not only serve as precursors for estrogen production but also exert direct effects in female tissues through the androgen receptor (AR). The importance of AR signaling in female tissues was not recognized for a long time and therefore it is not as well described or understood as ER and PR signaling. However, study of female mice that lack AR has definitively proven that many female-specific body functions rely on normal AR signaling, including mammary gland growth and development [25]. There are many androgen hormones, but only two have a high affinity for the AR and are considered the major drivers of AR activity within a tissue: testosterone and 5 $\alpha$ -dihydrotestosterone (DHT).

Testosterone (T) is one of the main circulating androgen hormones in female serum. This androgen is unique for being a potent agonist of the AR as well as a substrate for the steroidogenic enzymes that are able to convert T to either estradiol (E2), the major ER agonist, or an even more potent AR agonist, DHT. Unlike T, DHT is not a precursor of E2 and therefore its biological effects are considered to be mediated predominantly by the AR. In contrast, the biological effects of T can be mediated directly through the AR or indirectly through ER following conversion of T to E2. Testosterone is secreted by the ovaries and adrenal glands and can be taken up by body tissues which not only vary in terms of their capacity to metabolize T, but also have variable steroid receptor profiles. The ultimate response of a tissue to T depends on all of these variables.

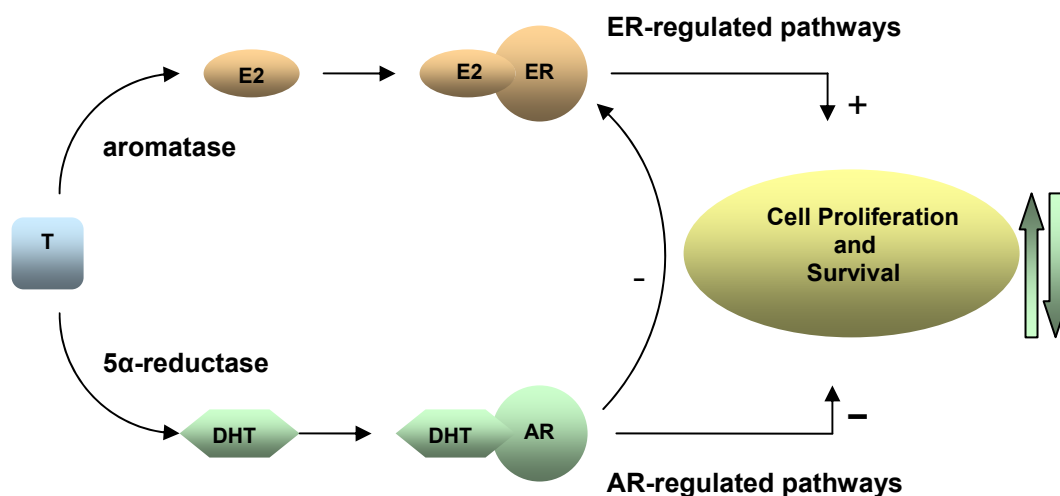
Breast tissue has the capacity to metabolize T to either E2 (via the enzyme aromatase) or to DHT (via the enzyme 5 $\alpha$ -reductase), and breast epithelial cells possess both ER and AR [26]. Therefore, the conditions are present for these cells to be regulated by both steroid receptor signaling pathways. Collectively, studies of specific AR signaling in relation to normal breast growth and breast cancer indicate that androgens acting via the AR inhibit estrogen-stimulated proliferation of breast epithelial cells (reviewed in [27, 28] and discussed on pages 15 to 16 below).

### ***Hormonal homeostasis model***

Based on scientific evidence developed in our own and other laboratories, we believe that androgen activity is the main opponent of estrogen activity in normal breast tissue, and that a balance of AR and ER signaling is necessary to maintain growth equilibrium in the breast. According to this hormonal homeostasis model (Figure 1), suppression or disruption of AR signaling would

upset the regulation of normal breast growth by allowing unopposed estrogen activity and thereby predispose the tissue to development of breast cancer [29]. These concepts are described in more detail on pages 17 to 19 below.

In accordance with the hormonal homeostasis model and accumulating epidemiological, clinical, and experimental evidence, we believe that chronic exposure to MPA in cHRT disrupts normal AR signaling in the breast [29]. This disruption would be expected to result in unrestrained estrogen stimulation of breast tissue, thereby promoting the development of breast cancer. The evidence for this proposition is discussed in detail below, starting with the epidemiological evidence before moving on to emerging biological validation through scientific studies.



**Figure 1.** The opposing actions of estrogens and androgens in the breast: the hormonal homeostasis model. T=testosterone; E2=estradiol; DHT= 5α-dihydrotestosterone; ER = estrogen receptor; AR = androgen receptor.

### **BREAST CANCER RISK ASSOCIATED WITH MPA**

Since its formulation in 1958, MPA has been used extensively in hormone pharmaceuticals throughout the world, mainly for the following clinical applications:

1. Treatment for secondary amenorrhea: induces menstruation following progestin withdrawal
2. Treatment for dysfunctional uterine bleeding (endometriosis, endometrial cancer): causes atrophy of the uterine lining

3. Female contraception: inhibits gonadotropin release by the brain and thereby inhibits ovulation; also thins the endometrium thereby reducing possibility of embryo implantation
4. Second-line breast cancer therapy: used at high doses for breast cancer that fails to respond to chemotoxic or endocrine therapies
5. Hormone replacement therapy: employed as the progestin component of cHRT to counter the deleterious effects of unopposed estrogen on the endometrium; also prescribed as prophylactic treatment for osteoporosis and cardiovascular disease as well as other age-related female health problems

In the USA, an estimated 6 million women were using cHRT by the year 2001, and at least 80% of prescriptions contained MPA [30]. Although cHRT use declined to an estimated 2.5 million by 2003 due to public fear of adverse health consequences, cHRT continues to be used by millions of postmenopausal women and MPA remains the most commonly prescribed synthetic progestin in the USA [30].

#### ***Observational studies and historical perceptions of MPA as a carcinogen***

In the late 1970s MPA began its history of prescriptive use for postmenopausal women on HRT who had an intact uterus, with the intent of preventing development of uterine cancer [14]. At this time, MPA was not formally licensed for this clinical application, but because of its anti-proliferative effects on the endometrium and its accepted use for other clinical applications, it was generally considered safe. Therefore, use of MPA in cHRT rose steadily and remained high throughout the late 1980s and 1990s [30]. This occurred despite warnings from US scientists in 1988 that cHRT would increase the incidence of breast cancer in post-menopausal women [31, 32], and subsequent clinical studies (discussed below) that supported this concept.

Beginning in 1989 and continuing into the 21<sup>st</sup> century, numerous large observational studies examining cHRT use in postmenopausal women were published in the scientific literature and cumulatively supported the same conclusion: that continuous use of cHRT for 5 years or more increased breast cancer risk (reviewed in [33]). The vast majority of women on cHRT involved in these studies were given MPA as the synthetic progestin, since this is the most widely prescribed drug used for this purpose in the USA. The Million Women study, conducted between 1996 to 2001, was one of the largest studies of this genre and reported an additional 6 cases of breast cancer per 1000 women with HRT use versus an additional 19 cases of breast cancer per 1000 women with cHRT use, compared to non-users [34]. The large size of this study enabled sub-analysis of the effect of specific synthetic progestins in cHRT and found MPA to be associated with the highest risk of breast cancer.

In tandem with these studies of cHRT in post-menopausal women, studies of pre-menopausal women using hormone contraceptives (again, mostly containing MPA) were raising the concern of increased breast cancer risk and contributed to the World Health Organization's (WHO) declaration in 1991 that hormone contraceptives increase the risk of developing breast cancer in women [35].

### ***FDA Licensing of MPA***

Throughout the 1980s and early 1990s, cHRT was given as two separate preparations (usually an estrogen tablet and a synthetic progestin tablet) because there was no formal licence to market them as a single preparation. Indeed, during this time pharmaceutical companies were actively applying for US Federal Drug Administration (FDA) approval to market a single preparation containing both cHRT components, but received repeated rejections. This same pattern was occurring with applications to approve the use of MPA administered alone in depot injection form as a long-acting contraceptive. In both instances this lack of success occurred for similar reasons:

1. Lack of adequate clinical trials ascertaining efficacy and safety
2. Evidence that MPA caused benign mammary tumors in dogs [36]

In 1992, MPA was licensed by the FDA for use as depot contraception on the basis of WHO-sponsored trials in Thailand, Kenya, Mexico and New Zealand that demonstrated efficacy, despite the fact that women under 35 years who had used MPA for 2 to 6 years or more within the trials had a greater than 2-fold increased risk of developing breast cancer [37]. Two years later, in 1994, the FDA approved a combined preparation of estrogen and MPA for use in peri- and postmenopausal women as cHRT. Although the WHO contraceptive trials involved pre-menopausal women taking MPA alone, they had raised concern regarding the carcinogenic effects of this synthetic progestin in humans before the FDA's approval for use in a single preparation of cHRT. The FDA did consider the accumulating evidence that MPA is carcinogenic to human breast tissue, and thus approval was provided with the recommendation that proper clinical trials to assess safety be undertaken. There is no published evidence of such trials ever being initiated by a pharmaceutical company.

### ***The Women's Health Initiative***

In 1993, against a backdrop of criticism that it would be a waste of time and money, the National Institute of Health (NIH) initiated a "gold-standard" clinical trial in which 16608 postmenopausal women aged 50-79 years with an intact uterus at baseline were recruited by 40 US clinical centers during the years between 1993 and 1998. This trial, called the Women's Health Initiative (WHI), was designed to specifically examine the ability of HRT or cHRT to prevent chronic disease in post-menopausal women, with a planned duration of 8.5 years follow-up after recruitment [38].

While the WHI trial was still in progress, the WHO-sponsored International Agency for Research on Cancer (IARC) declared cHRT “possibly carcinogenic” in humans [39]. This statement reflected lingering doubt due to the continuing lack of clinical trials specifically assessing the health benefits and risks of cHRT. At this time there was still strong belief that HRT or cHRT provided benefit to cardiovascular and bone health in post-menopausal women, which was formally being tested by the WHI trial.

The WHI trial was the first prospective, randomised interventional study specifically involving MPA in cHRT. The main aim of the trial was to assess chronic disease prevention in women taking HRT and cHRT, and a wide spectrum of health parameters were routinely assessed, including incidence of breast cancer. After a mean of 5.2 years of follow-up, WHI trial investigators reported a 40% increase in heart disease and a 24% increase in invasive breast cancer among women in the cHRT group compared with the placebo group [38]. On May 31, 2002, the independent Data and Safety Monitoring Board recommended stopping this arm of the WHI trial before its planned completion because the test statistic for invasive breast cancer exceeded the stopping boundary for this adverse effect and the global index statistic supported risks exceeding benefits. With formal release of this announcement, a huge public response ensued that ultimately resulted in a 38-68% reduction in cHRT use in the USA in the following year [40, 41].

Although recent commentaries have highlighted a number of shortcomings in the design and analysis of the WHI trial, its data combined with the weight of evidence from multiple observational studies conducted in the USA and Europe published before and after the trial, cemented the view that women taking cHRT have an increased risk of developing breast cancer (meta-analysis by [42]). In light of this accumulating evidence against the safety of cHRT in postmenopausal women, in 2005 the IARC amended its original classification of cHRT as “possibly carcinogenic in humans” to “carcinogenic in humans” [43, 44].

***MPA contributes to the development of hormone responsive breast cancer***

In 2003, the US National Cancer Institute (NCI)-sponsored Breast Cancer Surveillance Consortium reported a 72% increased risk of ER-positive invasive breast tumors among long-term users of cHRT compared to non-users [45]. The same study did not find an increased risk of breast cancer associated with estrogen-alone HRT, thus highlighting the cancer-promoting effect of synthetic progestin as part of menopausal hormone therapy. This finding confirmed earlier concerns that the escalating use of cHRT in the US population during the 1980s was causing the disproportionate increase in ER-positive compared to ER-negative invasive breast tumors (131% versus 22-27%, respectively) during this same time period [46].

Among the different types of breast cancer, invasive lobular carcinoma has the strongest positive association with cHRT use, and this association is evident after

only 3 years of continuous exposure [47]. Invasive lobular carcinoma is second to invasive ductal carcinoma in terms of overall incidence, but the reported change in frequency of these two types of breast cancer markedly differed in the USA from 1987-1999, when diagnosis of invasive lobular carcinoma increased by 96% but diagnosis of invasive ductal carcinoma increased by only 3% [47]. Invasive lobular carcinomas raise a higher degree of public health concern than invasive ductal carcinomas because they are less likely to be detected by screening mammography and are therefore more likely to be advanced-stage tumors at time of diagnosis [48]. Therefore, the association between cHRT use and invasive lobular carcinoma is consistent with the observation that women taking cHRT are more likely to have advanced-stage disease and an unfavourable prognosis at diagnosis [38].

### ***Changing incidence of invasive, hormone responsive breast cancer***

Among women in the USA aged 50 or more, breast cancer incidence rose steadily from the late 1970s to the late 1990s, a trend largely attributed to a combination of the following three factors [49-54]:

1. Increasing rates of screening mammography
2. Increasing use of menopause hormone therapies
3. Changes in reproductive behaviour over time

While the relative contributions of these three factors to the increasing incidence of breast cancer can not be absolutely determined, the disproportionate increase in hormone sensitive invasive breast tumors during this time was an indication that hormone therapies were having a substantial effect, since increases in screening mammography should proportionally increase the incidence of hormone-sensitive and insensitive types of breast tumors [46, 49].

Between approximately 1998 to 2001 the incidence of breast cancer in the USA began to plateau, with a statistically non-significant yearly trend toward decline from 1999-2000 that was predicted to occur when screening mammography achieved saturation [49-54]. In addition, the trend toward reduced incidence may have been influenced by a reduction in the use of menopausal hormone therapy following the release in 1998 of null results from the Heart and Estrogen/Progestin Replacement Study (HERS) contra-indicating HRT and cHRT as beneficial to cardiovascular health in postmenopausal women and the subsequent restrictive guidelines for their prescription issued by the American Heart Association [50].

The trends in breast cancer incidence in the USA from the late 1970s to 2001 were consistent with concurrent epidemiological trends and therefore not unduly surprising. In contrast, the steep, statistically significant 7-14% decline in the incidence of breast cancer among populations of peri- and post-menopausal-age women in the USA that occurred from 2001 to 2003, and continued into 2004,

caused an international stir (first reported in [54]). This was the first major drop in breast cancer incidence that had occurred in the USA over several decades. In fact, the decline reduced breast cancer rates to a level similar to that observed in the mid 1980s, before the expansion of cHRT use among post-menopausal women. The phenomenon drew public, scientific and medical practitioner attention back to considering the powerful effect of menopausal hormone therapy as a fuel for breast cancer.

### ***Changes in cHRT use and the reduction of breast cancer incidence***

Many studies support the view that the dramatic decline in breast cancer incidence in the USA during 2001-2003 and beyond was strongly influenced by the coincident dramatic decline in cHRT use following cessation of the cHRT arm of the WHI trial in 2002 [40, 49, 50, 52, 54-57]. The major argument against this view is that rates of screening mammography also generally declined by 1-4% in the USA during this period, and may have resulted in a reduced tumor detection rate [30, 58, 59]. However, this small reduction in screening frequency is unlikely to cause such a precipitous decline in overall breast cancer incidence, much less a specific decline of small, ER-positive invasive breast cancers which constituted the bulk of cases in decline among all studies. Moreover, the initial report of a decreased incidence of breast cancer in the USA during 2001-2003 involved a broad, mixed population cohort that lacked specific data on rates of mammography screening and menopausal hormone therapy use among the participants [54]. Since then, however, several regional studies with more comprehensive data have been published, all of which discount reduced screening mammography rates as a major causal factor in the decline [49, 56, 57].

Compelling evidence for the influence of cHRT discontinuation on breast cancer incidence among post-menopausal women comes from a study involving approximately 3 million non-Hispanic white women aged 45-74 years living throughout California's 58 regional counties. The incidence of breast cancer in this population declined by 8.8% in counties with the smallest reductions in cHRT use compared to a decline of 22.6% in counties with the greatest reductions in cHRT use from 2001-2004 [57]. Importantly, the rates of screening mammography throughout the state did not change during this time, and even increased in certain areas such as Marin county, where discontinuation of cHRT and breast cancer incidence showed the highest rates of decline [55, 57]. The California study is supported by another large regional study in the state of Oregon, where the incidence of invasive breast cancer dropped by 18% from 2001-2004 despite a constant rate of screening mammography during the same period [49].

Kerlikowske et al [56] took another approach to examine the issue of declines in menopausal hormone therapy versus screening mammography in the causality of decreased breast cancer incidence in the USA after the year 2001: they examined breast cancer incidence rates in women undergoing routine

mammography screening from 2001-2003. They report a statistically significant 5% decline in the annual rate of invasive breast cancer overall, and specifically an 18% annual decline in ER-positive tumors after the start of a coincident decline in HRT (25%) or cHRT (38%) use among women aged 50-69 years.

Although some have commented that the decline in cHRT use seems to be a counterintuitive cause of reduced breast cancer incidence, many others assert that in fact, this pattern would be wholly expected given the dependence of ER-positive breast tumors on the hormones that fuel their growth [60]. Indeed, this very feature is exploited by current therapies for hormone-responsive breast cancers, which rapidly respond to hormone withdrawal or blockade. It is also the rationale for using ER blockade therapy as a means of preventing breast cancer in women at high risk [61].

In the absence of any other epidemiological factor that dramatically changed in such a diverse sampling of the US population during 2001-2003, the weight of scientific evidence supports the view that the decrease in incidence of breast cancer in the USA during this time period was due to the parallel decline in cHRT use, not to changes in screening mammography. That such a population-wide event occurred is further testament to the carcinogenic nature of MPA as part of cHRT (given that MPA is the most widely used progestin in cHRT in the USA) and provides an indication of the scale of benefit that replacement of this form of menopausal therapy with a safer alternative could have within the US population.

### **BIOLOGICAL ACTION OF MPA IN THE BREAST**

In the endometrium, MPA, like natural progesterone, effectively opposes the proliferative action of estrogen and thereby prevents development of uterine cancer in women taking estrogen-based hormonal therapies. Used alone as a contraceptive, MPA also behaves in a progesterone-like manner, presumably acting on the hypothalamus and pituitary gland to inhibit follicle-stimulating hormone release, which stops egg maturation and ovulation within the ovaries. In contrast, experimental models have demonstrated that MPA enhances the proliferative effects of estrogen on breast epithelial cells, consistent with the action of progesterone in breast tissue during the luteal phase of the menstrual cycle or during early pregnancy [62, 63]. This finding is supported clinically where MPA as part of cHRT was shown to increase breast epithelial cell proliferation [64]. MPA use in post-menopausal women also increases breast density, which is associated with breast epithelial cell proliferation as well as reduced mammographic detection of smaller tumors and is therefore an established risk factor for breast cancer [65, 66].

Although the biological effects of MPA described above are thought to be mediated by the PR, in most instances this has yet to be experimentally proven. Indeed, in a gene profiling study using breast cancer cell lines, the action of MPA did not mimic that of endogenous progesterone [67]. This is an important point, because while native progesterone acts mainly through its specific receptors,

synthetic hormones are more promiscuous: in addition to binding the PR isoforms, MPA also binds with high affinity to the AR and the glucocorticoid receptor (GR) [22, 23], and can either activate or inhibit their natural biological activities. This cross-talk with other steroid receptor signaling pathways can be complex and cause a diverse range of biological effects in various body organs. Therefore, it cannot be assumed that the biological effects of MPA in women will mimic the action of progesterone or work via the same signaling pathways.

In the 1970s, the clinical use of MPA extended to second-line treatment of breast cancer that failed to respond to first-line chemotoxic and anti-estrogenic hormone therapies [68, 69]. The rationale for this clinical practice was the prevailing dogma that progesterone inhibits growth of breast epithelial cells. The drug was administered at doses at least 100-fold higher than that used in female contraception or cHRT, in part to mimic the high levels of progesterone in late pregnancy which stimulate terminal differentiation of breast epithelial cells. This therapy proved to be effective in some cases, and again this was assumed to have occurred by MPA mimicking the action of progesterone signaling through the PR.

However, clinical studies by our laboratory and others have demonstrated that the response of breast tumors to MPA therapy is dependent on the level of AR, not the level of PR [70]. Moreover, the interval within which there is no progression of breast cancer in response to treatment with MPA is directly proportional to the level of AR in the primary tumor [70]. Further, inactivating mutations in the AR gene in breast tumors are associated with failure of second-line MPA therapy [71]. Like DHT (the most potent natural ligand for the AR), MPA at a high dose can inhibit the proliferation of AR-positive, but not AR-negative, breast cancer cell lines and AR antagonists can reverse these inhibitory effects, consistent with stimulation of AR-regulated pathways by MPA [22]. These clinical and experimental effects all depend on the application of high doses of MPA (100 nM) and occur in the context of breast cancer, where normal steroid signaling pathways are often disrupted.

To delineate the means by which MPA as part of cHRT promotes breast cancer growth in post-menopausal women, the biological action and steroid receptor signaling pathways affected by MPA in normal breast tissue must be determined. Experimental studies show that a high dose (100 nM) of MPA, similar to that used for breast cancer therapy stimulates proliferation and survival of breast epithelial cells within cultured explants of normal human breast tissue, and this occurs both with MPA alone and in the presence of estrogen [63, 72]. These studies suggest that chronic exposure to MPA could promote breast cancer development and growth by excessively stimulating breast epithelial cell proliferation. However, the high doses of MPA employed by these experimental studies are not consistent with the lower doses characteristic of cHRT use or the levels of progesterone in the luteal phase of the menstrual cycle. It is also unknown to what degree the actions of MPA in this experimental system are

mediated by PR. In fact, a high dose of MPA decreases PR and increases AR expression within 24 hours in similarly cultured normal breast explant tissues [26].

We have shown that at lower doses similar to those achieved with cHRT (1-10 nM), MPA disrupts rather than stimulates normal AR signaling in breast cancer cells [29]. We believe this action occurs in normal breast epithelial cells and that disruption of AR signaling by MPA upsets the balance of breast growth maintained by the opposing actions of estrogen and androgen in breast tissue. The evidence for this belief is explained in detail below.

## **ANDROGENS AND THE BREAST**

The realization that androgen activity opposes estrogen activity in the breast stemmed from the emerging evidence that androgens play a critical protective role in breast cancer growth (reviewed in [29]). In particular, clinical observations have strongly supported the concept that AR activity inhibits growth and progression of breast cancer. Historically, fluoxymestrone, a potent androgenic pharmaceutical that has no estrogenic activity, was used as hormonal therapy for advanced breast cancer, demonstrating an efficacy comparable to that of tamoxifen, a selective estrogen receptor modulator that specifically inhibits ER $\alpha$  action in breast tissue [73-75]. However, as women on fluoxymestrone experienced virilising side effects (such as hirsutism), the use of androgenic compounds as hormonal therapy for breast cancer was discontinued, particularly following the therapeutic success of estrogen blockade therapies. Laboratory studies show that androgens predominantly inhibit the growth of breast cancer cells, both *in vitro* [76] and *in vivo* [77] by acting through the AR. This inhibition may involve the induction of cell death through a process called apoptosis. In addition, AR signaling inhibits the ability of estrogen to stimulate proliferation of cultured breast cancer cells [78]. Enforced expression of an active AR also results in complete abrogation of estradiol-induced breast cancer cell proliferation [79].

### ***AR expression in breast tumors***

The AR is the most common steroid receptor present in primary breast tumors, occurring at a higher frequency (60-95%) than either ER $\alpha$  (70–80%) or PR (50–70%) [80-87]. Increasing tumor grade is positively associated with loss of ER, PR, and AR, but of the three, AR is more frequently retained in grade 3 tumors and thus AR appears to be the last steroid receptor to be lost with progression of disease [88]. AR is expressed in about 50% of ER-negative primary breast cancers [83], and is present in up to 75% of metastatic breast cancer deposits, which predominantly lack other steroid receptors. Indeed, one in four metastatic breast lesions express AR as the sole steroid receptor [80]. Loss of AR has also been implicated in the transition from in-situ carcinoma to invasive malignancy [89].

Collectively, these studies support the assertion that AR may be a useful prognostic marker in the assessment of breast cancer and a potential target for therapy [86, 88]. Indeed, multiple studies report that the presence of AR in breast tumors is associated with better patient outcomes in univariate analyses, and that AR may be a useful means of identifying patients at risk of disease progression at time of primary surgery, particularly for women with invasive cancers [84, 86, 88]. Although some studies indicate that AR is not an independent prognostic marker in multivariate analyses, these findings are mostly generated using data that includes tumors with relatively low AR levels (a minimum of 10% positive cells). Studies that stratify AR levels suggest that high levels of AR are independently prognostic of better outcomes for breast cancer patients [86].

This is corroborated by our recent study involving a large cohort of 219 invasive breast cancers [79]. Our data shows that AR levels (median % positive cells  $\geq 75$ ) are significantly associated with ER $\alpha$  levels ( $\chi^2$ ; P value  $< 0.0001$ ) and PR levels ( $\chi^2$ ; P value = 0.001): in patients with ER $\alpha$  positive tumors, coincident AR positivity was independently associated with relapse-free survival and overall survival, while low AR levels ( $< 75\%$  positive cells) were associated with a 2.3 fold increased risk of relapse (P value = 0.024) and a 4.8 fold increased risk of cancer-related death (P value = 0.002).

Our data are consistent with another recent study which reported that co-expression of AR and 5 $\alpha$ -reductase, the enzyme that converts testosterone to DHT in breast carcinomas, is an independent prognostic marker for disease-free and overall survival in multivariate analyses, where either factor alone was not [90]. This study also reports an inverse correlation between co-expression of AR and 5 $\alpha$ -reductase and tumor size or proliferation index, indicating that intra-tumoral synthesis of DHT is an important means of activating AR to reduce growth of breast cancer cells.

In sum, the large body of evidence derived from multiple research laboratories using various methods of scientific enquiry strongly upholds the concept that AR signaling regulates estrogen-stimulated growth of breast tumor cells and AR is emerging as an important prognostic biomarker in breast cancer.

Collectively, the studies discussed above are consistent with the hormonal homeostasis model described on pages 5 and 6, and support two conclusions:

1. AR is an important regulator of breast cancer cell growth; and
2. An agent that disrupts AR signaling in the breast could promote tumor progression.

### ***AR action in normal breast tissue***

To better understand the role of AR in breast tumorigenesis, it is important to examine its action in normal breast tissue. Normal human breast epithelial cells have high levels of AR that do not fluctuate with the menstrual cycle as do ER and PR, and do not to change with menopause or increased age [91]. Clinical observations of women with pathological disorders of androgen excess (e.g,

congenital adrenal hyperplasia) or women who use anabolic steroids typically report diminished breast development (reviewed in [28]). These conditions involve circulating androgen concentrations in the male range, which is likely to stimulate robust AR activity.

Studies in rodents and primates where specific AR agonists and antagonists were employed demonstrate that AR signaling inhibits estrogen-stimulated breast epithelial cell proliferation (reviewed in [91]). In particular, these studies also show that androgen-induced inhibition of breast epithelial cell proliferation occurs through a decrease in ER $\alpha$  levels, which in turn corresponds to a decrease in the genes regulated by ER $\alpha$  that stimulate breast epithelial cell proliferation (e.g. c-myc, a proto-oncogene) and survival (e.g. bcl-2, anti-apoptotic factor) [77, 92]. In addition, stimulation of specific AR-signaling decreases levels of anti-apoptotic factors (e.g. bcl-2) and increases levels of pro-apoptotic factors (e.g. Bax) [93, 94]. Therefore, growth in normal breast tissue is kept in balance by ER $\alpha$  signaling that stimulates breast epithelial cells to survive and proliferate and AR signaling that inhibits proliferation and promotes cell death (Figure 1).

A major limitation in the study of normal human breast tissue to date has been a lack of suitable experimental models that adequately reproduce its hormone responsiveness, which is dependent on an interaction between breast stromal and epithelial cells *in vivo*. Breast epithelial cells removed from their stromal neighbors quickly lose steroid receptor expression and adopt abnormal behavior when cultured *in vitro*. In contrast, a testimony to the abnormal nature of breast cancer cells is the fact that they frequently maintain steroid receptor expression *in vitro* (the profile of which varies between different breast cancer cell lines), and therefore study of steroid receptor responses in these cells may not be representative of normal breast epithelial cells.

Our research group employs a novel methodology to study the behaviour of normal breast tissue removed from women undergoing reduction mammoplasty or having tissue removed for cancer. The technique involves culturing pieces of human breast tissue explants on gelatine sponges as previously reported [26] to maintain normal cellular morphology, tissue architecture and expression of sex steroid receptors (ER, PR and AR). Using this method we have demonstrated that normal breast epithelial cells maintain functional AR signaling in the presence of DHT [95-97]. We have now begun to use this model to study whether MPA disrupts AR signaling in normal breast epithelial cells, and determine what the mechanistic and biological consequences of this disruption are.

On the basis of current knowledge and our own data, we have generated a hormonal homeostasis model depicting the interaction of ER and AR signaling in the maintenance of normal breast epithelial cell proliferation (Figure 1). The balance between these pathways is critical for the regulation of breast growth and development (reviewed by [28, 29]). Moreover, we assert that AR signaling

protects breast epithelial cells from becoming cancerous and progressing to aggressive disease [29].

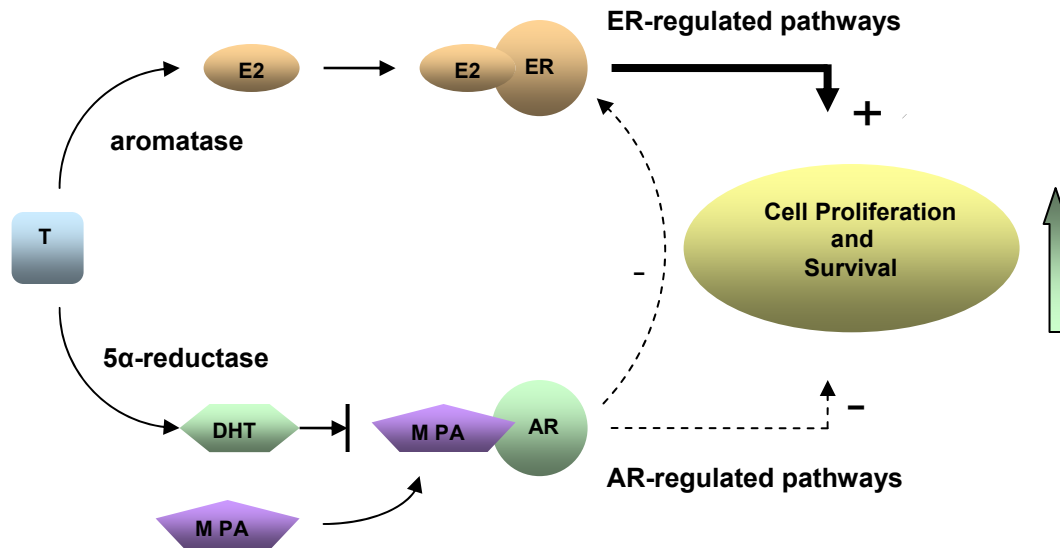
### **MPA AS AN ENDOCRINE DISRUPTOR OF AR SIGNALING**

There is little question as to the importance of estrogenic stimulation of breast tissue in the development of breast cancer. It logically follows that any reduction in a homeostatic mechanism that opposes the stimulatory effects of estrogen on breast tissue or any mechanism that enhances estrogen activity in the breast will play an important role in the development of breast cancer. Extensive evidence from our investigations and that of others demonstrates that exposure to MPA at low doses consistent with that associated with a combined contraceptive or CHRT use contributes to increased risk of breast cancer due to two distinct activities:

1. Disruption of AR signaling that allows unrestrained estrogen activity (as described in the preceding section); and
2. Stimulation of PR signaling that enhances estrogen activity (as described on page 4).

MPA thus exerts a “double-whammy” effect in the promotion of breast epithelial cell proliferation, and it is not surprising that use of this synthetic progestin has been associated with an increased risk of breast cancer. In particular, our studies have focused on the disruption of AR signaling by MPA in breast tissues [29]. This signaling pathway is present and active in breast tissue in women throughout their lifespan, and represents an important homeostatic mechanism that counter-balances the proliferative stimulus of estrogen on normal and malignant breast epithelial cells.

As a result of our own and other studies, we now have evidence that MPA has the capacity to disrupt the normal endocrine function of endogenous T or DHT in the breast tissue of both pre- and post-menopausal women (see Figure 1). As such, MPA should be considered an *endocrine disruptor*, which is defined as an exogenous substance that acts like a hormone in the endocrine system but disrupts the physiologic function of endogenous hormones [98]. This concept is depicted in Figure 2 and the evidence is described in detail in the following paragraphs.



**Figure 2.** MPA disrupts normal AR signaling in the breast: the endocrine disruption model. In accordance with the hormonal homeostasis model, this disruption of AR signaling would be expected to result in unrestrained estrogen stimulation of breast tissue. T= testosterone; E2 = estradiol; DHT = 5α-reductase; MPA = medroxyprogesterone acetate; ER = estrogen receptor; AR = androgen receptor.

### ***MPA interaction with AR***

The exact structure of a protein is a critical determinant of its ultimate function and even small changes to the structure of a protein can result in dramatic functional differences. For example, the albumin protein present in uncooked egg-white changes structure upon heating and turns from a viscous liquid into a firm solid. Steroid receptors are proteins that similarly change function with change of structure, and activation of these receptors by their native cognate hormones generally involves a switch from an inactive to an active form.

All steroid hormone receptors have the following functional domains: N-terminal trans-activation domain (NTD), DNA-binding domain (DBD), and a ligand-binding domain (LBD). Crystal structure analysis of the AR has revealed that the binding of its most potent ligand, DHT, to the LBD results in a distinct structural change in this domain that results in formation of a cleft called the activation function 2 (AF2) surface [99]. Formation of AF2 enables the AR to interact either with itself or with other similarly-activated AR molecules, and with proteins called nuclear co-regulators (co-activators and co-repressors) [100-103]. These interactions regulate the ability of hormone-bound AR to stimulate transcription of target genes.

The AR is unique among other steroid receptors because the AF2 surface preferentially binds to a region within its own NTD, thus forming what is called an

N-C interaction [100, 101, 104]. The N-C interaction can either occur within one AR molecule (intramolecular interaction) or between two AR molecules (intermolecular interaction), the latter forming a head-to-tail dimer. These interactions stabilise AR and regulate its interaction with other nuclear co-factors. Collectively, laboratory studies and clinical observations of men who have mutations in the AR that abolish or compromise its ability to form an N-C interaction, indicate that it is a critical determinant of AR action as a transcription factor [105].

### ***Molecular interactions between MPA and AR***

MPA binds AR with high affinity, nearly equivalent to that of the most potent native androgen, DHT [22]. However, our studies indicate that it does not induce the same conformational changes in receptor structure [29]. MPA is a more bulky hormone than DHT and upon binding to AR this extra bulk forces displacement of a key amino acid residue, Phe874, such that it projects abnormally into the AF2 cleft, thereby forming an altered AF2 surface structure that influences its function. Indeed, we and others have demonstrated that low concentrations of MPA (1-10 nM) do not induce an N-C interaction, consistent with MPA binding AR but forming an abnormal AF2 surface [29, 101]. However, of greater importance was the finding that at these same low doses, MPA potently antagonised the ability of physiological levels of DHT to induce an N-C interaction, perhaps through competitive binding [29, 101]. This inhibition of DHT activity by MPA was significantly greater than that achieved by either progesterone or the specific AR antagonist, hydroxyflutamide. In contrast, higher doses of MPA (100 nM) can induce a moderate level of N-C interaction in the AR, consistent with high doses stimulating AR activity [29, 63]. In terms of AR-regulated gene expression, we believe the following based on the above evidence:

1. No or low AR-regulated gene expression with exposure to 1-10 nM of MPA.
2. Low to moderate AR-regulated gene expression with exposure to 100 nM MPA.
3. Reduced DHT-stimulated AR-regulated gene expression in the presence of 1-10 nM MPA.

We have recently experimentally corroborated some of these predictions using a culture system that eliminates the confounding influence of MPA action through PR [106]. For example, of the 195 genes up-regulated by 1 nM DHT in a breast cancer cell line that has AR but lacks PR, approximately 18% were up-regulated by 1 nM MPA and approximately 35% by 100 nM MPA. We are currently using the human breast explant technique (described in the previous section) to examine selected candidate genes for further analysis. With this system, we have produced the first solid evidence that MPA can disrupt the expression of an AR-regulated gene in a physiological context, consistent with a disruption of AR function [96].

The hormonal homeostasis model also predicts why cHRT use has been most strongly associated with an increased risk of invasive lobular carcinomas, a type of breast cancer that has markedly risen since the introduction of cHRT [47]. The AR is particularly abundant in lobular tumors and is positively associated with low to intermediate tumor grade and a low proliferation index [85]. Conversely, loss of AR in grade 3 ILC tumors independently predicts shorter breast cancer-specific survival and disease-free intervals [107]. In the human breast explant culture system, low doses of MPA can inhibit the ability of DHT to increase AR levels and thus inhibit the expression of an AR-regulated gene [95]. It is highly probable that MPA acts similarly *in vivo*, and with chronic exposure could upset the balance of AR and ER signaling that maintains normal breast tissue growth (Figure 2).

In summary, the scientific studies of MPA action in normal breast tissue are beginning to biologically validate the epidemiological evidence that cHRT employing MPA contributes to the development of breast cancer in post-menopausal women. The mechanism involves an endocrine disruption of AR signaling in the breast which normally opposes estrogenic stimulation of breast epithelial cell proliferation and thereby keeps breast tissue growth in balance.

#### **ALTERNATIVES TO MPA**

MPA use is much less common in Europe than in the USA. In Europe, oral micronised (natural) progesterone (OMP) is more commonly prescribed, as are other synthetic progestins (e.g., levonorgestrel, NETA). In France the majority of women taking cHRT receive natural progesterone in the form of OMP rather than a synthetic progestin. Large French observational studies show that cHRT with OMP does not increase the risk of breast cancer, whereas the use of synthetic progestins do [108, 109]. Other synthetic progestins used in Europe have, like MPA, been associated with increased breast cancer risk in observational and randomized controlled trials comparing HRT with cHRT [110-113], but the extent of the risk increase varies among the different synthetic steroids [108, 109, 114, 115]. These studies highlight the fact that the actions of synthetic progestins can be very different from those of the native hormone progesterone. In support of the clinical impression that OMP is a more favourable progestin supplement than MPA, experiments on post-menopausal monkeys (in which menopause is induced by ovariectomy) show that estrogen in the presence of MPA, but not OMP, stimulates breast epithelial cell proliferation [116].

It is now clear that systemically-administered cHRT containing MPA in particular (and potentially synthetic progestins in general) represents an unacceptable health risk in postmenopausal women. Although some argue that the risk is relatively small, we agree with others who have contended that the risk is totally unnecessary and that the current challenge is to develop forms of cHRT with less adverse health risks [65, 117, 118]. Although it is necessary to systemically increase the levels of estrogen to alleviate menopausal symptoms, this is not the

case for progestin, which was only added to HRT to reduce the risk of endometrial cancer. Thus, it is clinically and scientifically intuitive that delivery of the progestin directly into the uterine cavity would reduce systemic effects and be efficacious in reducing the risk of endometrial cancer. A levonorgestrel-releasing intrauterine device is one such system. This system delivers high levels of the drug to the uterine cavity, but systemic levels remain low [119, 120]. Use of the levonorgestrel-releasing intrauterine system is not associated with an increased risk of breast cancer [121-123]. Other alternatives that could be explored include vaginal delivery of progestin or systemically-administered sequential cHRT treatment regimens that employ lower doses of progestin for shorter time intervals (i.e. 10 days every 3-4 months) [65].

### **POTENTIAL IMPACT OF MPA IN cHRT**

Disruption of hormonal homeostasis in the breast by MPA as part of cHRT has serious implications for the current and future health of women, whose lifestyle is rapidly changing. Increasing numbers of women in the USA and other countries wait to bear children until later years or remain childless, both factors being established risk factors for breast cancer. To prevent pregnancy, many of these women will use hormone contraceptives that may confer an added risk of developing breast cancer. Then, at menopause, many women will elect to use cHRT to relieve themselves of debilitating menopausal symptoms or protect themselves from age-related declines in health. This cHRT use can span a decade or more. During extended exposure to hormone therapies, breast tissue is chronically given the stimulus to proliferate and thus becomes vulnerable to malignant transformation, particularly in post-menopausal women in whom the breast tissue exists in a state ready to proliferate in response to a hormone stimulus. Current changes in world population dynamics predict that by 2045-2050, women 50 years or older will comprise 18% (1.6 billion) of the world's population [124]. Thus, changes in reproductive epidemiology combined with the continued use of cHRT regimes that contribute to increased breast cancer risk have the potential to substantially increase the incidence of breast cancer to epidemic proportions.

### **CONCLUSION**

The synthetic progestin MPA as currently used in cHRT increases breast cancer risk in women. The hormonal homeostasis model claims, and the evidence now clearly demonstrates, that AR signaling acts to balance the proliferative action of estrogen in normal breast tissue. We believe and have scientific evidence to support the concept that chronic exposure to MPA in cHRT disrupts normal AR signaling in the breast, as described in the endocrine disruption model. Disruption of AR signaling by MPA can be expected to result in unrestrained estrogen stimulation of breast tissue. Thus, MPA acts as an endocrine disruptor of the balance between estrogen (stimulatory) and androgen (inhibitory) signaling in the normal breast, and thereby contributes to the development of breast cancer.

Synthetic progestins currently used in cHRT are largely administered systemically and are therefore available to alter the normal interactions of sex steroid signaling pathways in the breast. With continuous exposure to cHRT over a duration of 3 years or more, breast epithelial cells can undergo malignant transformation. Despite a marked initial decline in the use of MPA following its formal recognition as a mammary carcinogen, there continues to be widespread use of MPA in cHRT among women in the USA and other parts of the world. Several safer alternatives to MPA use in cHRT are available and in clinical use. Thus ongoing promotion of MPA in cHRT and the advocacy of suitable alternatives are major public health issues.

### **DISCLAIMER**

We believe all of the opinions expressed in this report are true to a reasonable degree of scientific certainty. We have applied the same degree of scientific rigor to our analysis here that we use in treating patients, conducting research and expressing opinions to our professional colleagues.

Stephen Birrell MD PhD has had 20 years of experience on the drug advisory boards of AstraZeneca Pty and Novartis Corporation. In this role he has given expert opinions on many drug trials and safety issues in relation to hormonal therapies. He has made representations to the Therapeutics and Drugs Administration in Australia (the equivalent of the FDA) and is currently the owner and CEO of Chavah Pty Ltd, a small biotech company that undertakes Phase I and II trials of hormonal therapies in breast cancer. Stephen Birrell has been an expert witness in many breast cancer malpractice lawsuits in Australia and the UK. He also was an expert witness at the Australian House of Representatives' inquiry into breast cancer in 1994.

Wayne Tilley, PhD is the foundation Professor of the Dame Roma Mitchell Cancer Research Laboratories of the University of Adelaide/Hanson Institute. These laboratories specifically investigate the role of hormones in the development and treatment of breast and prostate cancer. He is the Chief Scientific Officer of Chavah Pty Ltd and has been the main scientific advisor to Stephen Birrell's Flinders and Burnside Breast Cancer Clinics. Professor Tilley is a frequent speaker at international meetings on hormone action in breast and prostate cancer, and is an advisor and speaker for many pharmaceutical companies, specifically AstraZeneca, Bristol Myers Squibb and Novartis. Currently he is a member of the Board of Trustees of the National Breast Cancer Foundation in Australia, and is the Chair of the Research Advisory Committee of the Foundation. He is also a Board member of Andrology Australia, the Australian Government's peak advisory group on the use of androgens and male sexual health. Professor Tilley has no prior experience as an expert legal witness.

Drs Birrell and Tilley are engaged as expert witnesses by Williams Love O'Leary & Powers, P.C; their fee for this service is US\$550 per hour.

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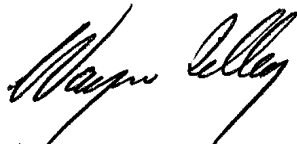
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April 01, 2008



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