Journal of Steroid Biochemistry & Molecular Biology 142 (2014) 52–61

Contents lists available at ScienceDirect

Journal of Steroid Biochemistry and Molecular Biology

journal homepage: www.elsevier.com/locate/jsbmb

Menopausal hormone therapy and breast cancer

Richard J. Santen*

University of Virginia Health Sciences System, Charlottesville, VA 22908-1416, USA

ARTICLE INFO

Article history:
Received 15 October 2012
Received in revised form 13 June 2013
Accepted 28 June 2013
Available online 16 July 2013

Keywords:
Breast cancer
Menopausal hormone therapy
Mechanisms
Estradiol
Progestogen

ABSTRACT

Observational and randomized controlled trial data have extensively examined the relationship between menopausal hormone therapy (MHT) and risk of developing breast cancer. A highly influential study from the Women's Health Initiative (WHI) in 2002 reported that a MHT regimen of *conjugated equine estrogens and medroxyprogesterone acetate* increased the risk of breast cancer by 26%. Later reports from the WHI indicated that a MHT regimen with *conjugated equine estrogens* alone decreased the risk of breast cancer by 23%. Critical re-examination of the WHI study noted that the average participant age was 63, that few women had symptoms, and that the WHI results might not apply to younger, symptomatic women shortly after menopause. Since the original publications, several post hoc analyses and observational studies have stimulated reconsideration of the WHI findings. Emphasis has been directed toward risks in younger women just entering the menopause, the subgroup who are most likely to be considering MHT use.

The goal of this treatise is to integrate available mechanistic and clinical information related to the use of estrogen alone or estrogen plus a progestogen for five years or less. These data suggest that estrogen alone neither decreases nor increases risk in younger women initiating therapy close to the time of menopause but decreases risk in older women. Both younger and older women experience an excess risk with estrogen plus a progestogen. The attributable risk in younger women is less in those with a low underlying Gail Model risk score. Effects of MHT on risk largely reflect actions on pre-existing, occult, undiagnosed breast cancers. Tumor kinetic models suggest that the pro-proliferative effects of estrogen plus a progestogen on occult tumors provide a mechanistic explanation for the increased risk with this therapy. Pro-apoptotic effects of estrogen alone may explain the reduction of breast cancer in women starting this therapy at an average age of 63 as reported in the WHI study.

This article is part of a Special Issue entitled 'Menopause'.

1. Introduction

The role of menopausal hormone therapy (MHT) to potentially increase the risk of breast cancer is an important issue for recently menopausal, symptomatic women considering use of this therapy. Over a period of two decades, observational studies have reported conflicting findings regarding breast cancer risk related to MHT which has resulted in substantial controversy [1]. However, a large meta-analysis of observational data published in 1997 provided convincing evidence of increased risk and identified five important factors which had confounded previous individual studies [2]. These confounding factors included: (a) The relative risk of breast cancer from MHT is small and large studies with a long duration of follow-up are required to minimize Type I and Type II statistical errors. (b) The risk of breast cancer increases linearly with duration of MHT use. Accordingly, comparisons of "ever users" with "never users" are invalid since duration of estrogen use is not considered. (c) The increased risk of breast cancer imparted by MHT dissipates within 4 years of cessation of therapy. Therefore, only women using MHT within four years of study might be found to be at increased risk. (d) Breast cancer risk also diminishes over a four year period following the menopause, presumably as a reflection of decreased estrogen and progesterone levels. As a result, analyses of observational studies need to match users versus non-users as to time following menopause. (e) The increased risk of breast cancer from MHT is higher in lean women. Inclusion of a large proportion of obese women in a single study might then obscure associations between MHT use and breast cancer risk.

The large, randomized Women's Health Initiative trials (see Box 1) then confirmed the conclusions of the meta-analysis and added additional information about individual subgroups. First published in 2002 the WHI reported that menopausal hormone

* Tel.: +1 434 924 2961; fax: +1 434 924 1284.
E-mail address: rjs5y@virginia.edu

0960-0760/$ – see front matter © 2013 Elsevier Ltd. All rights reserved.
http://dx.doi.org/10.1016/j.jsbmb.2013.06.010

Box 1

Characteristic	Estrogen alone arm	Estrogen alone arm	Estrogen plus progestogen arm	Estrogen plus progestogen arm
Hormone/placebo	Placebo	Conjugated equine estrogen (CEE) 0.625 mg	Placebo	CEE 0.625 mg plus medroxy-progesterone acetate
Number randomized	5429	5310	8102	8506
Average age	63.6	63.6	63.3	63.2
BMI	30.1	30.1	28.5	28.5
Prior hormone use	39%	38%	26%	26%
Study duration years	6.8	6.8	5.2	5.2
Drop out rate	45%	45%	28%	31%
Drop in rate	8%	7%	9%	5%

References: E alone arm [3,51].

therapy (MHT)[1] with estrogen plus a progestogen increased the risk of breast cancer by 26% (RR 1.26, CI 1.00–1.59) [3]. Subsequent to that time, the WHI findings have been re-analyzed and new data obtained which have modified the overall conclusions of both the estrogen alone and combined estrogen plus progestogen arms of the studies. Concomitantly, extensive molecular, biologic and pathophysiologic studies have added clarity to our understanding of the mechanisms underlying the WHI findings.

Re-interpretation of the MHT data after its initial publication in 2002 was considered necessary since the average age of women entered into the WHI trials was 63 and only a limited number of women experienced symptoms and particularly those related to vasomotor instability [3–6]. Most importantly, the original data did not separately assess risks and benefits in the group of women most likely to be considering MHT, those just entering menopause and experiencing significant symptoms. It should be emphasized that the WHI utilized only oral conjugated estrogens plus one synthetic progestogen (MPA) and not estradiol given orally or transdermally nor progesterone or other synthetic progestogens. Finally the populations of women in the estrogen (E) alone and E plus progestogen (E + P) studies differed. The E alone study entered women who had previously undergone a hysterectomy. Nearly half of these women had a concomitant bilateral oophorectomy. Accordingly, the differences reported between the two studies could reflect the differences in populations as well as hormones used.

This treatise will review mechanistic data as well as recent risk/benefit data in women closer to the onset of menopause and specifically those <60 years of age or <10 years post-menopausal. The initial sections of this review will examine the biology and pathophysiology underlying the risks of breast cancer from MHT.[1] The later sections will focus on studies published since the original WHI publication which address these risks in younger women.

2. Biology and pathophysiology

2.1. Breast cancer

2.1.1. Background

The associative relationship between estrogens and breast cancer is supported by experimental evidence in a variety of animal systems and by epidemiologic data in women [7,8]. While the causes of breast cancer are diverse and include hormonal, genetic, environmental, and aging factors, estrogens play a key role. The two strongest pieces of associative evidence implicating estrogen in women are that: (a) removal of both ovaries before the age of 35 reduces the lifetime risk of breast cancer by 75% [9,10] and (b) women have a 100 fold higher risk of breast cancer than do men. This evidence is considered associative since the ovary also secretes progesterone and the differences between men and women could be related to a protective effect of testosterone. Additional epidemiologic data suggest that an increase in risk of breast cancer is associated with enhanced estrogen exposure during a woman's lifetime. Pertinent factors include breast density [11]; plasma estradiol levels [12,13]; age at menopause, first live birth and menarche; BMI; and MHT [14,15] (Fig. 1).

2.1.2. Mechanism of breast carcinogenesis

How estrogens cause breast cancer is not precisely known. Both ER *dependent* and *independent* effects likely play contributory roles [8,16] (Fig. 2). A widely accepted concept is that increased cell proliferation results in enhancement of genetic mutations and insufficient time for DNA repair [18,19]. Accumulation of a sufficient number of "driver" gene mutations results in de novo cancers [20]. Estrogen, acting through ERα, directly stimulates proliferative genes and growth factor production which collaborate to enhance the rate of cell growth. The effects of ERα occur through both nuclear and extra-nuclear actions [21].

Evidence is accumulating that ERα independent effects contribute to the carcinogenic process but definitive proof is as yet lacking. Through Cyp1A1, estrogens are enzymatically converted to 2-OH-catechol metabolites that damage DNA by forming stable DNA adducts (Fig. 2). By an alternate CYP1B1 pathway,

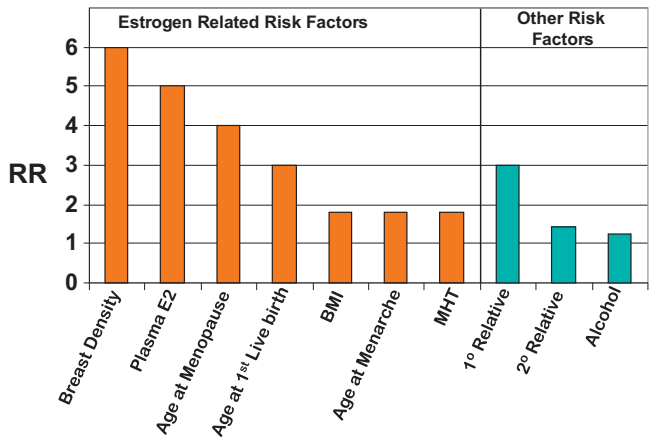

Fig. 1. Estrogen related risk factors for breast cancer. Risk factors for breast cancer related to clinical aspects that are associated with an increased chronic exposure to estradiol and expressed as relative risks (RR).

Figure adapted from the review of E. Amir et al. [83] and published in the article by Yager et al. [16]. Reproduced with the permission of the Endocrine Society.

[1] The author has chosen to utilize the term menopausal hormone therapy (MHT) in preference to HRT (hormone replacement therapy). Since "replacement therapy" generally implies that all women should be given a hormone that is missing. The author and most societies agree that the term HRT may not be appropriate.

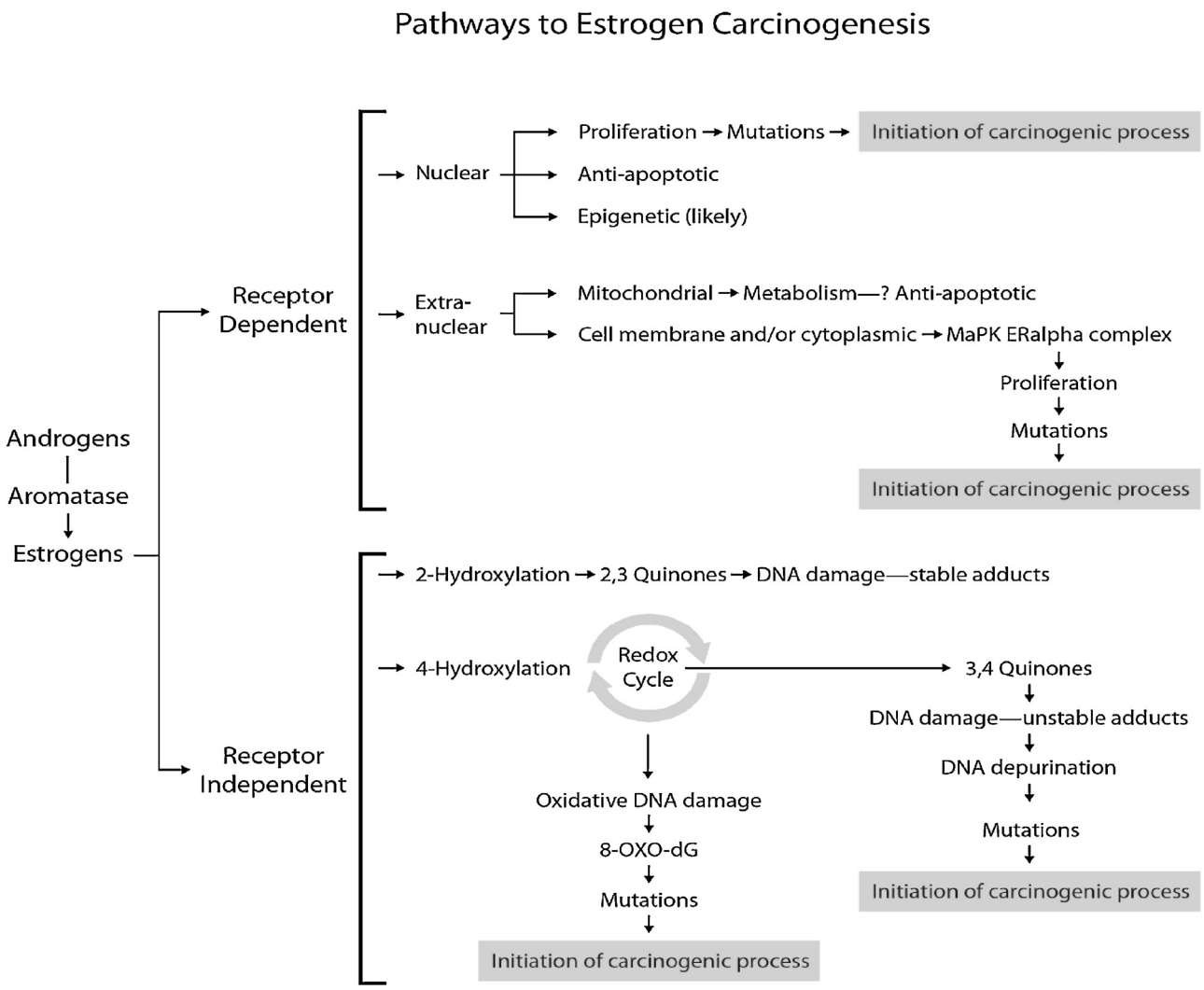

Fig. 2. Estrogen dependent and independent mechanisms of estradiol induced carcinogenesis.
Taken from the article by Yager et al. [16] and reproduced with permission of the Endocrine society.

estrogens are hydroxylated to 4-OH-catechol-metabolites which form unstable adducts by binding to adenine and guanine on the DNA backbone. These adducts rapidly undergo cleavage from their respective DNA sites, a process called depurination [17]. This leaves "naked" sites on DNA which result in point mutations through error prone DNA repair. Via another mechanism, 4OH-estradiol undergoes redox cycling, generating oxygen free radicals [8]. Direct DNA damage results from formation of 8-OXO-dGuanine (8-OXO-dG) which itself is unstable, leading to depurination and point mutations.

Extensive data supporting the ER *independent* genotoxic pathway are provided by experimental studies demonstrating that estradiol and 4-OH-estradiol can induce mutations, anchorage independent colony growth, loss of DNA heterozygosity, and transplantable tumors in ER negative benign breast cell lines [17–21]. The strongest experimental evidence involves the administration of E2 to ovariectomized, ERα-knock out/Wnt-1 double transgenic mice. In a dose–response fashion, E2 enhances the time of onset and incidence of tumor formation in these animals [22].

Data from women also suggest a role for direct carcinogenic effects of estrogen metabolites. The breast preferentially converts estradiol to the 4-OH-genotoxic metabolites in situ [23] in normal women. Increased urinary and plasma levels of these metabolites are associated with an enhanced risk of breast cancer [24–26]. In women with BR Ca 1 mutations, oophorectomy reduces cancer incidence even though the tumors are primarily ER. In the WHI E + P arm, MHT increased ER− as well as ER+ tumors as evidence of an ER independent effect [27]. In the EPIC study, estradiol levels in the top quintile were associated with an increased risk of ER−/PR− breast cancer (RR 2.11 CI 1.00–4.46) as well as ER+/PR+ (RR 2.91 CI 1.63–5.23) [28]. Taken together, the data suggest a combined role of ER *dependent* and ER *independent* mechanisms to induce mutations. As suggested by Hanahan and Weinberg [29], accumulation of a sufficient number of mutations in key carcinogenic pathways results in neoplastic transformation [29,30]. Genetic, environmental, and other factors not related to estrogen undoubtedly play a role in the carcinogenic process as well.

2.1.3. *Protective effects of estrogen*

The WHI E alone study reported a significant reduction in breast cancer risk in women taking conjugated equine estrogen (CEE) for 7 years and then stopping for an additional 4 years [31]. As described in detail later in this manuscript, this effect may be due to estrogen induced apoptosis in occult breast tumors too small to be detected by mammography at the start of the study.

2.1.4. *Progestogens*

The mechanistic role of progestogens in mammary carcinogenesis is less clear since cell culture and animal experiments demonstrate both proliferative and anti-proliferative effects of

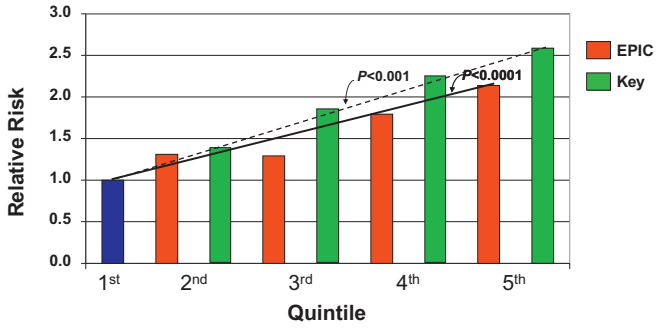

Fig. 3. Level of free plasma estradiol and breast cancer risk. Free estradiol refers to non-sex-hormone binding globulin (SHBG)-and albumin bound estradiol and not to unconjugated levels. Estradiol levels are divided into quintiles from the lowest (first quintile) to the highest quintile (fifth quintile).

The data are taken from two studies from Key et al. [12] and the EPIC study [13]. Reproduced with the permission of the Endocrine Society.

these hormones [32]. In women, however, synthetic progestogens and particularly the CEE/MPA combination are pro-proliferative [32]. As evidence, the terminal duct lobular unit, thought to be the site of cancer initiation, is stimulated to a greater extent by CEE plus MPA than by CEE alone in women [33]. Recent data suggest that progestogens may also promote the conversion of differentiated cancer cells into cancer stem cells and thus amplify the carcinogenic process [34].

2.2. Mechanisms explaining epidemiologic links of estrogen to breast cancer

Obesity is associated with an increased risk of breast cancer in *post-menopausal* women [7,35]. This effect is attributed to increased aromatase activity in adipose tissue with consequent enhancement of estrogen production. Mechanistic data suggest that increased leptin associated with obesity reduces AMP-kinase activity [36] and through downstream mechanisms involving CRTC2 increases aromatase transcription. In *pre-menopausal* women, obesity disrupts cyclic menstrual function, lowering integrated estradiol production, and thus is not associated with increased breast cancer risk [35]. The elevated insulin levels associated with obesity have also been linked to breast cancer as insulin is a growth factor for breast cancer cells [37].

2.2.1. Plasma estrogens

Epidemiologic studies have shown an increased risk of breast cancer with increasing levels of free estradiol [12,13] (Fig. 3). In an important new study, Tworoger and Hankinson et al. demonstrated that a weighted score, incorporating several estrogens and their metabolites provided increased power to predict the development of breast cancer than individual hormone measurements [38]. The relative risk of breast cancer in the top quintile versus bottom quintile with this integrated score was 3.0 (CI 1.8–5.0) and for ER+ disease 3.9 (CI 2.0–7.5). This is to be compared with RRs of 2.4 (CI 1.4–4.1) for all cancers and 2.9 (CI 1.4–5.9) for ER+ disease when plasma E2 alone is measured. While potentially very useful, confirmation of this method in much larger studies is now required.

2.2.2. Tissue estrogen levels

Production of estrogen directly in the breast rather than uptake from plasma may be important in the carcinogenic process in post-menopausal women. The ratio of tissue to plasma estradiol is higher in post-menopausal than in pre-menopausal women [39] suggesting a role for in situ production [40–44]. A recent study correlated the estrogen message signature by c-DNA array analysis in breast with the level of plasma E2 [45] and suggested an equal contribution from local synthesis and ER mediated uptake. This issue is biologically important since it may be possible to develop breast tissue specific inhibitors of aromatase [36].

2.2.3. Mammographic density

Breast density is associated with a greater risk of breast cancer than family history or other known factors (Fig. 4) [11]. Estrogen alone and to a greater extent, estrogen plus a progestogen, cause an increase in breast density [46]. The mechanism for the association with breast cancer risk with breast density is unknown but recently was postulated to result from an increase in tissue synthesis of estrogen from aromatase in dense tissue [47].

2.3. Re-assessment of the risks and benefits of MHT

2.3.1. Weighing of evidence

Substantial flaws resulting primarily from unappreciated biases frequently confound interpretation of observational studies. Well-designed randomized controlled trials (RCTs) allow definitive answers to clinical questions but flaws in their design can also complicate interpretation. Plausible, comprehensive, and replicated observational data and meta-analyses can provide substantial support for less than definitive RCTs. Based on this reasoning, assessment of data from studies of MHT requires careful weighing of the type and quality of available evidence [48,49]. In this treatise, the well characterized GRADE system is utilized for assessing level of evidence (see Box 2).

2.3.2. Expression of data as excess benefits and risks

The specific methods used to describe the endpoints reported in published studies of MHT confound the process of benefit/risk assessment. Various publications have described relative, absolute, or excess risks and examined time-frames ranging from one to 20 years. The initial WHI trial [3] and observational studies [14] primarily reported relative risks (RR), a statistic relating the percent increment in an event in one population to that in another. Other studies report absolute risk which describes the number of events in a population of given size. Relative risk provides meaningful information to an individual patient only when absolute risk is high but not when low. As an illustrative example, the risk of dying from a plane crash might have risk of one in 1,000,000 flights. The relative risk from taking five plane trips would increase by 500% but the absolute risk would be trivial. Excess (or attributable) risk subtracts the absolute risk in an untreated control group from that in a treated population [50]. For an individual woman, excess risk or benefit is a more meaningful statistic since it represents an estimate of what she can expect to experience as a result of taking MHT. Based on this reasoning, the majority of data in this treatise are converted to excess risk or benefit [14]. The duration of use is also a key component in analysis. As women choosing MHT at the time of menopause can be expected to continue this therapy for a period of about 5 years, the statistics in this treatise are calculated on the assumption of 5 years of use.

2.4. WHI and other data

2.4.1. Level of evidence

The WHI E alone and E + P trials enrolled women whose average age was 63 (Box 1). Only 3.5% had recently entered menopause. Specifically, only 574 of the 16,608 women were in the 50–54 year old age range in the WHI E + P trial [14]. Because the average age was 63, few women were symptomatic [5]. Drop-out rates approximated 30% in the E + P trial [3] and 45% in the E alone arm [51]. Drop-in rates (i.e. those in the placebo arm who initiated MHT outside of the trial) approximated 5–9% [3,51]. In addition, 26% of women in the E + P trial and 38–39% in the E alone trial had used

Box 2: Level of evidence by GRADE scale [48]

Grade	Clarity of risk/benefit	Description of supporting evidence	Implications
A	Benefits clearly outweigh risk or vice versa	Consistent evidence from well-performed or exceptionally strong evidence from unbiased observational Studies.	Recommendations apply to most patients under most circumstances. Further research is unlikely to change confidence in the estimate of effect
B	Benefits clearly outweigh risks or vice versa	Evidence from RCTs with important limitations (inconsistent results, methodologic flaws, indirect or imprecise evidence) or unusually strong evidence from unbiased observational studies.	Recommendations apply to most patients under most circumstances. Further research is likely to have an impact on confidence in estimate of effect and may change the estimate.
C	Benefits clearly outweigh risk or vice versa	Evidence for at least on critical outcome from observational studies, from RCTs with serious flaws, or indirect evidence.	Recommendations may change when higher quality evidence becomes available. Further research is very likely to have an important impact on confidence in the estimate of effect and likely to change the estimate.
D	Benefits clearly outweigh the risks or vice versa	Evidence for at least one of the critical outcomes from unsystematic clinical observations or very indirect evidence.	Recommendations may change when higher quality evidence becomes available. Any estimate of effect, for at least one critical outcome, is very uncertain.

MHT previously and then underwent a 3 month wash out period prior to study entry. Notably, outcomes differed in those never previously receiving hormone therapy ("MHT naïve") when compared to prior or current users [52]. These considerations, taken together, led the Endocrine Society in its Scientific Statement to downgrade the WHI E alone and E+P trial results to level B evidence [14]. Nonetheless, with level B evidence, the overall conclusions are not expected to change with additional studies, only the magnitude of the effects.

3. Breast cancer and MHT

3.1. Original findings of the WHI E+P arm

The first publication of the WHI E+P study in 2002 reported a 26% increase in relative risk of breast cancer in women previously exposed to hormone therapy. Surprisingly, no increase was reported for the 74% who had never taken hormone therapy prior to randomization (i.e. "MHT naïve" group) (RR 1.09 CI 0.86–1.40) [52] (Fig. 5). Tumors diagnosed in women receiving E+P in the WHI were larger than those in women receiving placebo with a higher percent with nodal involvement [52,53]. It is likely that a "masking effect" provided at least a partial reason for this observation. MHT increases breast density, an effect which masks the ability to detect smaller tumors. The increase in nodal involvement remains unexplained but has been suggested to represent an effect of progestogens to increase tumor invasiveness. This possibility is supported by in vitro and in vivo xenograft data but remains unproven in women [34]. The 11 year follow-up of the WHI E+P arm also reported a slight increase in death rate in the MHT group (i.e. 0.63 extra deaths per 1000 women over 5 years). All-cause mortality was also slightly higher in the E+P group (1.07/1000/5 years) [54]. These effects could reflect a promotional effect of E+P, enhancement of the number of stem cells, an increase in invasiveness or a combination of these factors.

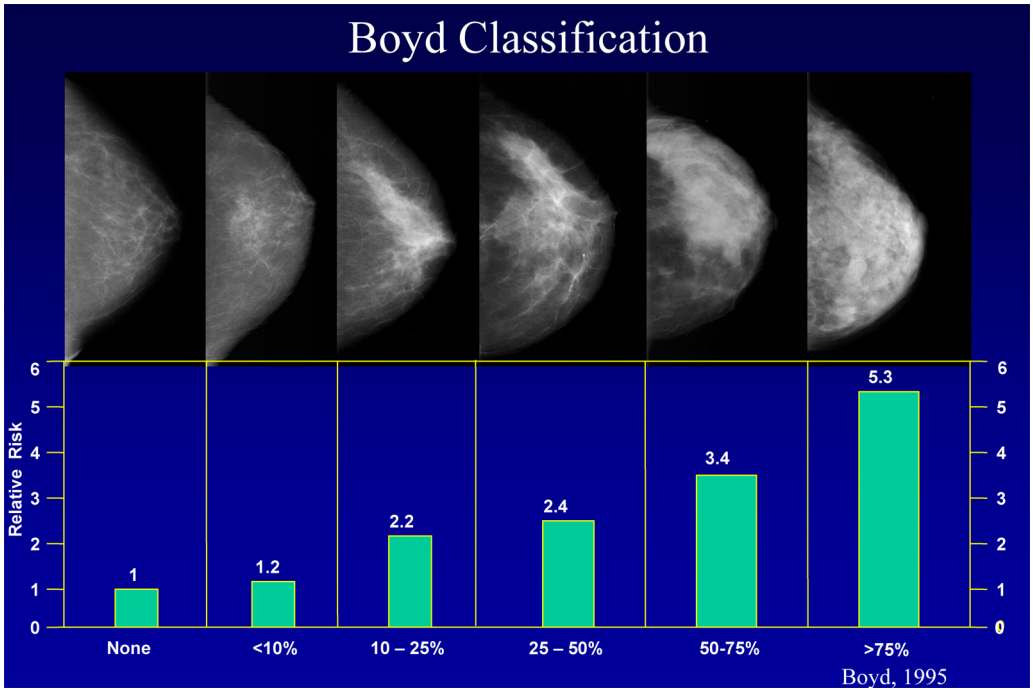

Fig. 4. Relative risk of breast cancer as a function of the degree of mammographic density. Relative risks represent the top quintile versus the bottom quintile of values in the population studied. *$P<0.001$. Data derived from the study of Boyd et al. [84].

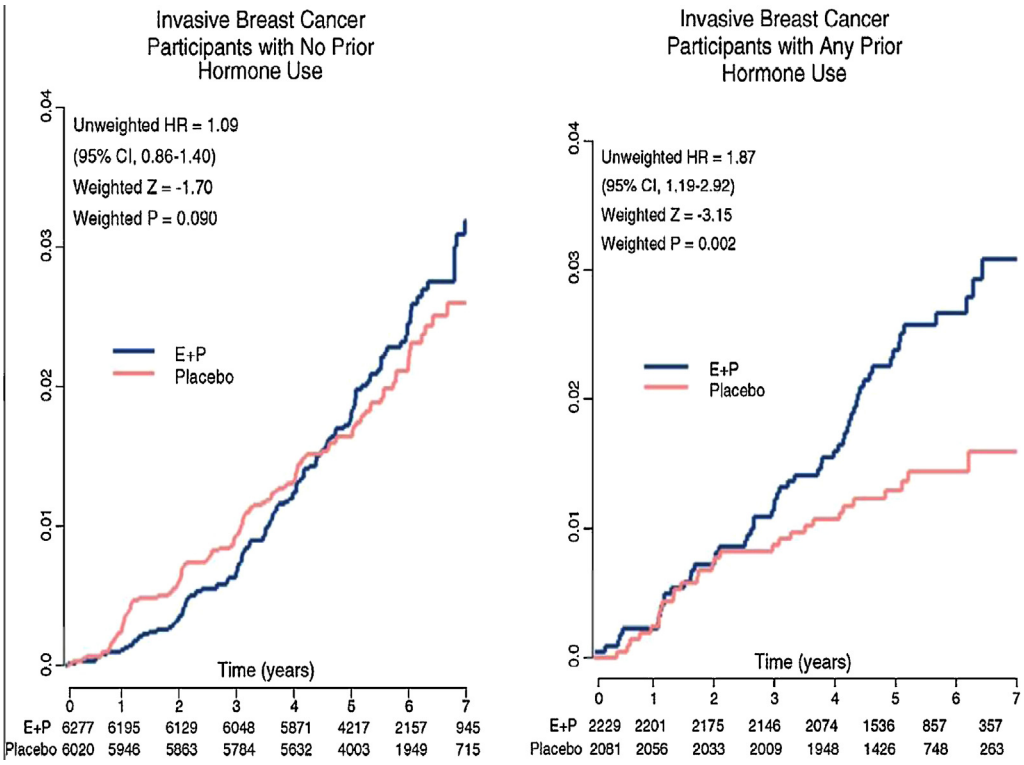

Fig. 5. Incidence of breast cancer as a function of prior hormone therapy. Risk of breast cancer in WHI participants who were "MHT naïve" (i.e. no prior hormone use) in the left panel and with prior hormone use in the right panel [52].
Reproduced with the permission of the authors and publisher.

3.2. Original findings in the WHI E alone arm

This study, limited to women with a prior hysterectomy, paradoxically reported a non-statistically significant trend toward reduction in breast cancer risk (RR 0.80 CI 0.62–1.04) [4]. While initially considered a chance finding, post hoc subset analyses found that the reduction was statistically significant in three subgroups: (a) women adherent to taking the assigned medication (RR 0.67 CI 0.47–0.97), (b) those developing in situ tumors (RR 0.69 95% CI 0.51–0.95) and (c) ductal tumors (RR 0.71 CI 0.52–0.99). After 11 years of follow-up in women taking E alone for 7 years and then stopping for 4 years, the reduction became statistically significant for the entire group (RR 0.77 CI 0.62–0.95) [31]. Interestingly, this reduction in risk was not seen in those who had taken prior hormone therapy (RR 0.98 CI 0.67–1.44) [4,52]. These findings provide reassurance that estrogens alone would be safe for hysterectomized patients, at least for a period of 7 years of use (i.e. the period of observation in the WHI study).

3.3. Perspective

Much emphasis has been placed on the risk of breast cancer from MHT. However, it is important to put these risks into proper perspective and consider the magnitude of the excess risks if MHT is taken over a five year period. For E + P, 7 extra breast cancer cases would be expected in 1000 women. For E alone, no extra breast cancer cases would be predicted. On this basis, the breast cancer risks from E + P for 5 years can be considered relatively uncommon and from E alone, non-existent. It is pertinent to point out that the overall data from the WHI are not very relevant to clinical practice since the average age of women in the WHI was 63. In clinical practice most women initiate MHT shortly after the menopause. No pre-designed, randomized, controlled data are as yet available to determine breast cancer risks in women of this age group.

3.4. Younger women

As the average age of women in the WHI was 63, a key question arose whether women starting E + P shortly after the menopause and at a younger age would experience similar risks. Data suggested that the risk might be higher in younger women. The term "GAP time" was introduced to describe the duration between onset of menopause and start of MHT. Short GAP referred to the initiation of MHT within 5 years after menopause onset and long gap, 5 or more years [55]. In the "MHT naïve "group (i.e. no prior hormone therapy), the RR was 1.77 CI 1.07–2.93 in those with a short gap (i.e. <5 years since menopause) and 0.99 (CI 0.74–1.31) in those with a long GAP (i.e. ≥5 years). The onset of an increase in breast cancer occurred within 3–5 years of initiation of use [14]. For E alone, no reduction in risk occurred in the Short Gap "MHT naïve" patients (RR 1.12 CI 0.39–3.21) as was observed in the Long Gap "MHT naïve" women (RR 0.58 CI 0.36–0.93) [56]. Individual observational studies and meta-analyses generally support the WHI data in younger women [14,57,58]. The level of evidence for the women initiating MHT shortly after menopause is considered level B based on the consistency of observational data.

3.5. Effects of other factors on risk

Observational data suggest that obese women experience a lesser risk from MHT than their lean counterparts. The Million Women Study reported a relative risk of breast cancer of 2.30 CI 2.12–2.53 in women with a BMI of <25 kg/m^2 versus 1.78 CI 1.64–1.94 in those with a BMI of >25 [59]. The large collaborative group observational study reported a similar effect [2]. It has been suggested that this might explain the somewhat higher relative

risks reported from European studies in women who are generally leaner than their North American counterparts [14]. Other studies suggest that the risk of breast cancer is increased in women who experience breast pain on initiation of MHT [60].

3.6. Effects of underlying risk of breast cancer on individual risk

The *relative risks* of breast cancer as determined by the Gail model have been found to be similar among women at low, intermediate or high *absolute* risk. For example, reported RRs were 1.19, 1.29, and 1.25 for those with Gail scores of <1.25, 1.25–1.75, and >1.75% respectively [27]. However it is important to consider absolute or attributable risks rather than relative risks. This finding is highly relevant when counseling women in the clinical setting. For example, if the underlying *absolute* risk as determined by the Gail model is 1% at five years (10/1000 women) and the relative risk is 1.29, then the excess risk is 2.9/1000. With a Gail model score of 5% (50/1000) the excess risk is 16/1000. This reasoning supports the recommendation to calculate risk of breast cancer in all women considering the use of MHT.

3.7. Class effects

The risk of breast cancer from estrogen or progestogens has generally been considered a class effect since nearly all types of estrogen and progestogen have been associated with increased risk in observational studies. For example, in the EPIC study, no difference in breast cancer risk was observed between CEE and estradiol (RR 1.15, CI 0.78–1.69) [61]. However, French investigators have long held the view that progesterone itself has certain different properties than the synthetic progestogens. "The E3N study reported that estradiol plus progesterone or dydrogesterone used for five years or less were not associated with increased breast cancer risk (RR 1.00 CI 0.83–1.22 for estrogen plus progesterone and 1.22 CI 0.83–1.72 for estrogen plus dydrogesterone). In contrast, women using estrogen plus other progestogens experienced an increased risk (for example, RR 1.69 CI 1.50–1.91 for estrogen plus medroxyprogesterone acetate) (level of evidence C). Longer use of estrogen plus progesterone, however, did appear to increase risk [62]. While these results require confirmation in other studies, the data argue against a class effect and suggest that progesterone or dydrogesterone may be associated with a reduced risk of breast cancer compared to synthetic progestogens, With respect to the estrogen components, no studies in women have directly compared the risk of breast cancer from conjugated equine estrogens (CEE), estradiol, estrone or other synthetic estrogens. While studies in mice and in monkeys suggest that CEE has less of a proliferative effect on breast than estradiol [63,64], no difference in breast cancer risk has been observed in women [61].

3.8. Lowering of breast cancer incidence after cessation of MHT

After publication of the WHI, the percentage of women using MHT worldwide dropped precipitously [65–69]. Concordant with this reduction, the incidence of breast cancer in many but not all countries fell in parallel [65,70]. This has suggested a cause and effect relationship based on a decreased stimulatory effect of E + P on occult tumor growth. It has been pointed out that many other factors could explain the decline in incidence such as the implementation of breast cancer screening and change in types of MHT used [65,71]. Some studies report a recent increase in breast cancer incidence even though there has been no contemporaneous enhancement of MHT use, Nonetheless, after reviewing all data, the Scientific Statements of the Endocrine Society and IMS concluded that at least a component of the decline was caused by the cessation of MHT [14,72].

3.9. Initiation versus promotion

A key question regarding MHT and breast cancer is whether estrogens with or without progestogens initiate the process of breast cancer or merely promote the growth of pre-existing tumors. A series of 8 autopsy studies in women ages 40–80 reported a mean prevalence of occult, undiagnosed breast cancers in 7% of otherwise healthy women (1% invasive and 6% occult) [14]. The time required for these tumors to reach the size needed for diagnosis on mammograms depends on their doubling time [73]. The detection threshold for mammographic detection ranges from 1.44 cm in women less than age 40 who have dense breasts to 0.88 cm in those >70 who have predominantly fatty breasts [73]. Bailey et al. reviewed existing literature and calculated doubling times for women older than age 40 [73]. The median doubling time approximated 200 days with 25–75% ranges from 100 days to 400 days. Using these data, we calculated that only 6% of de novo tumors would reach the detection threshold by 7 years, the duration of the WHI studies [74] (Fig. 6). Accordingly, the major effect of MHT is likely to be exerted on pre-existing occult, undiagnosed tumors, representing a promotional effect rather than initiation of de novo tumors. On this basis, MHT with E + P stimulates pre-existing tumors to grow faster and to reach the diagnostic threshold earlier as a mechanism for the increased breast cancer risk. Our model best describes the actual data when assuming that E + P increases tumor growth rate from doubling times of 200 days to 150 days (Fig. 7). While most of the effects of MHT are on promotion, it should be emphasized that animal studies indicate that estrogens can also exert an initiation effect of breast cancers as well [16].

The explanation for a reduction in breast cancer with E alone may also rest on the biologic effects on occult, pre-existing tumors. Data accumulated over the past ten years suggest that E can induce apoptotic cell death in tumors deprived of estrogen long term [75–78]. As the women in the WHI were of average age 63, 12 years past the menopause, their occult tumors were likely somewhat deprived of estrogen long term. We utilized our model to predict the effect of estrogen alone on tumor incidence in the WHI trial (Fig. 8). The assumptions were that estrogen alone caused apoptosis in 30% of the ER positive tumor cells. The model accurately predicts the observed findings. Of interest is that fact that only the Long Gap "MHT naïve" women experienced a reduction of breast cancer with E alone whereas those previously treated did not [52]. The Long Gap, "MHT naïve" group can be considered to have undergone long term estradiol deprivation. These data suggest the hypothesis that estrogen induced apoptosis of pre-existing tumors may have been the mechanistic explanation for the reduced risk observed [14].

3.10. Effects of routes of administration of MHT

Regarding breast cancer, the large EPIC study found no differences between oral and cutaneous delivery systems [61]. Vaginal estrogens exert local effects preferentially and are more efficacious in relieving symptoms of urogenital atrophy than systemic administration [14,79]. However, some estradiol is absorbed systemically, even from the vaginal ring which delivers 7.5 μg of estradiol daily [80–82]. The small amount absorbed systemically can exert biologic effects since changes in bone density have been observed in response to low dose vaginal estradiol but insufficient data on breast cancer risk are available.

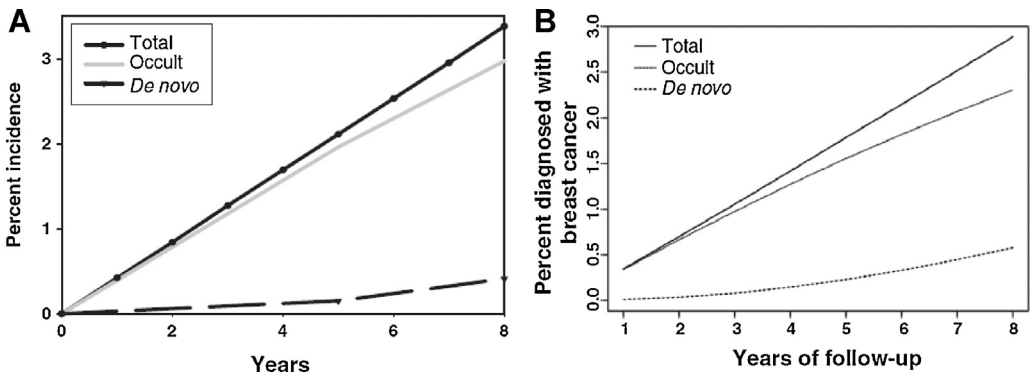

Fig. 6. Menopausal hormone therapy stimulates proliferation of occult breast tumors. (A) The incidence of total tumors expressed as a percentage of the population which reached the detection threshold over a period of 8 years compared with the incidence of de novo and occult tumors. The estimates are based on a biology based occult tumor growth model [74]. (B) The incidence of total tumors expressed as a percentage of the population which reached the detection threshold over a period of 8 years compared to the incidence of de novo and occult tumors as calculated by a computer based model [74]. The calculations were designed to assess the incidence of de novo and occult tumors reaching the diagnostic threshold in women who were similar in age to those randomized to the WHI E alone and E + P trials.

Reproduced from Santen et al. [74] with the permission of the authors and publisher.

3.11. Dose–response effects

The relationship of dose to increased risk from MHT on any endpoint is unclear with no randomized, controlled trial evidence. The large, observational, collaborative group analysis found no statistically significant relationship between dose and breast cancer risk [2]. These data merit a level of evidence of C. Information regarding MHT dose and heart disease or stroke are minimal.

3.12. Method of administration

New Epic study data on continuous versus episodic progestogens examined the use of MHT and risk of breast cancer in a large number of women in the European community [61]. This observational study provided evidence that the use of continuous E + P regimens is associated with a greater risk of breast cancer than sequential regimens (level of evidence C).

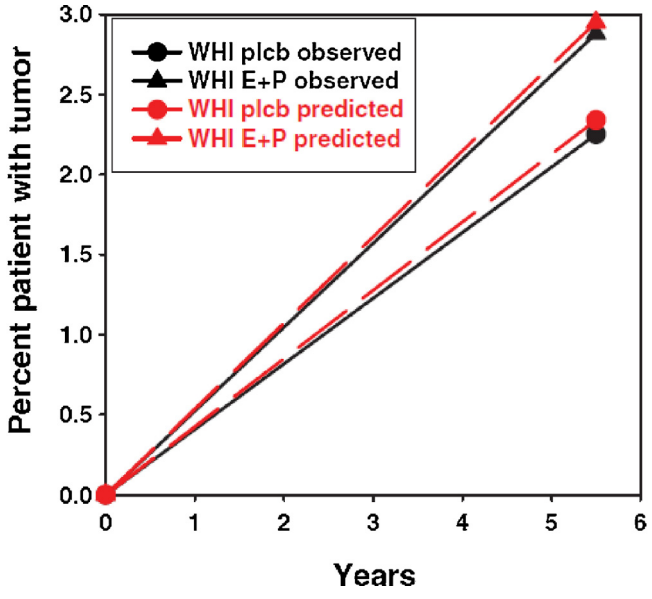

Fig. 7. Observed versus predicted breast cancers with combined hormone therapy. The incidence of breast tumors predicted in the placebo and hormone arms of the WHI E + P trial over a 5.2 year period as assessed by the biology based prediction model. In comparison are shown the observed data in the placebo (plcb) and hormone treated arms of the WHI E + P trial.

Data reproduced from Santen et al. [74] with the permission of the authors and publisher.

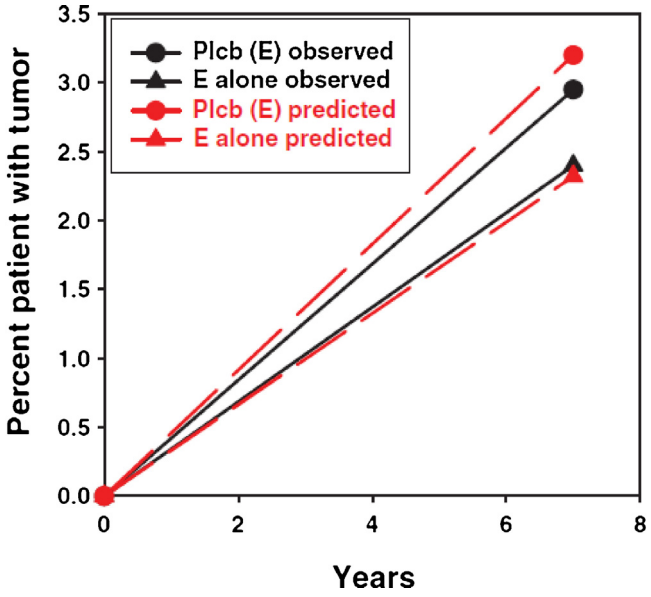

Fig. 8. Observed versus predicted breast cancers with estrogen alone. The incidence of breast tumors predicted in the placebo and hormone treatment arms of the WHI E alone trial over a 7.2 year assessment period using the biology based tumor model. In comparison are shown the observed data in the placebo and hormone treatment arms of this study.

Data reproduced from Santen et al. [74] with the permission of the authors and publisher.

4. Summary

The effects of MHT on breast cancer which occur in women on MHT for five years or less primarily reflect hormonal actions on pre-existing, occult, undiagnosed tumors. Biological based and computer generated models suggest that only 6% of tumors diagnosed over a 5–7 year period occur de novo from initiation of a new cancer. The remaining 94% represent small, occult, undiagnosed tumors which grow to a size exceeding the diagnostic threshold. Estrogen plus a progestogen increases the risk of breast cancer in women initiating MHT at the time of menopause or only a few years afterword. Estrogen alone does not increase this risk if taken for 5–7 years. In contrast, women initiating estrogen alone for the

first time ten or more years after the menopause experience a 23% reduction in risk. The attributable risk from E + P in the younger women is relatively small, approaching 7 women per thousand taking E + P for 5 years. Attributable risk from E + P is higher in those with a higher underlying risk based on breast cancer risk prediction methods. A reasonable conclusion is that women considering E + P as MHT should have their underlying risk routinely assessed before making a therapeutic decision. Unconfirmed data suggest that progesterone itself when combined with an estrogen might impart a lower risk of breast cancer than use of synthetic progestogens. Data obtained since the original WHI reports suggest a nuanced approach in treating menopausal symptoms and that treatment decisions should be based factors present in that individual patient.

References

[1] T.L. Bush, M. Whiteman, J.A. Flaws, Hormone replacement therapy and breast cancer: a qualitative review, Obstetrics & Gynecology 98 (3) (2001) 498–508 (Review).

[2] Breast cancer and hormone replacement therapy: collaborative reanalysis of data from 51 epidemiological studies of 52,705 women with breast cancer and 108,411 women without breast cancer. Collaborative Group on Hormonal Factors in Breast Cancer, Lancet 350 (9084) (1997) 1047–1059 (see comment; erratum appears in Lancet 1997 Nov 15;350(9089):1484).

[3] J.E. Rossouw, G.L. Anderson, R.L. Prentice, et al., Risks and benefits of estrogen plus progestin in healthy postmenopausal women: principal results. From the Women's Health Initiative randomized controlled trial, Journal of the American Medical Association 288 (3) (2002) 321–333 (see comment).

[4] M.L. Stefanick, G.L. Anderson, K.L. Margolis, et al., Effects of conjugated equine estrogens on breast cancer and mammography screening in postmenopausal women with hysterectomy, Journal of the American Medical Association 295 (14) (2006) 1647–1657.

[5] V.M. Barnabei, B.B. Cochrane, A.K. Aragaki, et al., Menopausal symptoms and treatment-related effects of estrogen and progestin in the Women's Health Initiative, Obstetrics & Gynecology 105 (5:Pt 1) (2005), t-73.

[6] Anonymous. UK. Public Assessment Report Hormone-Replacement Therapy. Safety update.

[7] M. Clemons, P. Goss, Estrogen and the risk of breast cancer, New England Journal of Medicine 344 (4) (2001) 276–285 (erratum appears in N Engl J Med 2001 Jun 7;344(23):1804; Review).

[8] J.D. Yager, N.E. Davidson, Estrogen carcinogenesis in breast cancer, New England Journal of Medicine 354 (3) (2006) 270–282 (see comment; Review).

[9] M. Feinleib, Breast cancer and artificial menopause: a cohort study 776, Journal of the National Cancer Institute 41 (2) (1968) 315–329.

[10] D. Trichopoulos, B. MacMahon, P. Cole, Menopause and breast cancer risk, Journal of the National Cancer Institute 48 (3) (1972) 605–613.

[11] N.F. Boyd, H. Guo, L.J. Martin, et al., Mammographic density and the risk and detection of breast cancer, New England Journal of Medicine 356 (3) (2007) 227–236.

[12] T. Key, P. Appleby, I. Barnes, G. Reeves, Endogenous Hormones and Breast Cancer Collaborative Group. Endogenous sex hormones and breast cancer in postmenopausal women: reanalysis of nine prospective studies 828, Journal of the National Cancer Institute 94 (8) (2002) 606–616.

[13] R. Kaaks, S. Rinaldi, T.J. Key, et al., Postmenopausal serum androgens, oestrogens and breast cancer risk: the European prospective investigation into cancer and nutrition 72, Endocrine-Related Cancer 12 (4) (2005) 1071–1082.

[14] R.J. Santen, D.C. Allred, S.P. Ardoin, et al., Postmenopausal hormone therapy: an Endocrine Society scientific statement, Journal of Clinical Endocrinology & Metabolism 95 (7 Suppl. 1) (2010), Suppl-Sus. 66 (Review).

[15] R.J. Santen, N.F. Boyd, R.T. Chlebowski, et al., Critical assessment of new risk factors for breast cancer: considerations for development of an improved risk prediction model 400, Endocrine-Related Cancer 14 (2) (2007) 169–187.

[16] J.D. Yager, R. Santen, Mechanisms relating estrogen to breast cancer, Translational Endocrinology and Metabolism 3 (1) (2012) 75–93.

[17] E. Cavalieri, D. Chakravarti, J. Guttenplan, et al., Catechol estrogen quinones as initiators of breast and other human cancers: implications for biomarkers of susceptibility and cancer prevention, Biochimica et Biophysica Acta 1766 (1) (2006) 63–78 (Review).

[18] M.L. Mello, B.C. Vidal, I.H. Russo, M.H. Lareef, J. Russo, DNA content and chromatin texture of human breast epithelial cells transformed with 17-beta-estradiol and the estrogen antagonist ICI 182,780 as assessed by image analysis, Mutation Research 617 (1-2) (2007) 1–7.

[19] M. Saeed, E. Rogan, S.V. Fernandez, F. Sheriff, J. Russo, E. Cavalieri, Formation of depurinating N3Adenine and N7Guanine adducts by MCF-10F cells cultured in the presence of 4-hydroxyestradiol, International Journal of Cancer 120 (8) (2007) 1821–1824.

[20] J. Russo, I.H. Russo, The role of estrogen in the initiation of breast cancer, Journal of Steroid Biochemistry & Molecular Biology 102 (1–5) (2006) 89–96 (Review).

[21] G.A. Balogh, I.H. Russo, B.R. Balsara, J. Russo, Detection of chromosomal aberrations by comparative genomic hybridization during transformation of human breast epithelial cells in vitro, International Journal of Oncology 29 (4) (2006) 877–881.

[22] W. Yue, J.P. Wang, Y. Li, et al., Effects of estrogen on breast cancer development: role of estrogen receptor independent mechanisms, International Journal of Cancer 127 (8) (2010) 1748–1757.

[23] E.G. Rogan, A.F. Badawi, P.D. Devanesan, et al., Relative imbalances in estrogen metabolism and conjugation in breast tissue of women with carcinoma: potential biomarkers of susceptibility to cancer, Carcinogenesis 24 (4) (2003) 697–702.

[24] N.W. Gaikwad, L. Yang, S. Pruthi, et al., Urine biomarkers of risk in the molecular etiology of breast cancer, Breast Cancer 3 (2009) 1–8.

[25] N.W. Gaikwad, L. Yang, P. Muti, et al., The molecular etiology of breast cancer: evidence from biomarkers of risk, International Journal of Cancer 122 (9) (2008) 1949–1957.

[26] B.J. Fuhrman, C. Schairer, M.H. Gail, et al., Estrogen metabolism and risk of breast cancer in postmenopausal women, Journal of the National Cancer Institute 104 (4) (2012) 326–339.

[27] R.T. Chlebowski, G.L. Anderson, M. Gass, et al., Estrogen plus progestin and breast cancer incidence and mortality in postmenopausal women, Journal of the American Medical Association 304 (15) (2010) 1684–1692.

[28] R.E. James, A. Lukanova, L. Dossus, et al., Postmenopausal serum sex steroids and risk of hormone receptor-positive and -negative breast cancer: a nested case–control study, Cancer Prevention Research 4 (10) (2011) 1626–1635.

[29] D. Hanahan, R.A. Weinberg, Hallmarks of cancer: the next generation, Cell 144 (5) (2011) 646–674 (Review).

[30] L.D. Wood, D.W. Parsons, S. Jones, et al., The genomic landscapes of human breast and colorectal cancers, Science 318 (5853) (2007) 1108–1113.

[31] A.Z. LaCroix, R.T. Chlebowski, J.E. Manson, et al., Health outcomes after stopping conjugated equine estrogens among postmenopausal women with prior hysterectomy: a randomized controlled trial, Journal of the American Medical Association 305 (13) (2011) 1305–1314.

[32] R.J. Santen, Risk of breast cancer with progestins: critical assessment of current data, Steroids 68 (10–13) (2003) 953–964 (Review).

[33] L.J. Hofseth, A.M. Raafat, J.R. Osuch, D.R. Pathak, C.A. Slomski, S.Z. Haslam, Hormone replacement therapy with estrogen or estrogen plus medroxyprogesterone acetate is associated with increased epithelial proliferation in the normal postmenopausal breast, Journal of Clinical Endocrinology & Metabolism 84 (12) (1999) 4559–4565.

[34] K.B. Horwitz, C.A. Sartorius, Progestins in hormone replacement therapies reactivate cancer stem cells in women with preexisting breast cancers: a hypothesis, Journal of Clinical Endocrinology & Metabolism 93 (9) (2008) 3295–3298.

[35] Z. Huang, S.E. Hankinson, G.A. Colditz, et al., Dual effects of weight and weight gain on breast cancer risk, Journal of the American Medical Association 278 (17) (1997) 1407–1411.

[36] K.A. Brown, E.R. Simpson, Obesity and breast cancer: progress to understanding the relationship, Cancer Research 70 (1) (2010) 4–7 (Review).

[37] C. Pichard, Plu-Bureau, Neves-E Castro, A. Gompel, Insulin resistance, obesity and breast cancer risk, Maturitas 60 (1) (2008) 19–30 (Review).

[38] S.S. Tworoger, B.A. Rosner, W.W. Hankinson SE, The combined influence of multiple sex and growth hormones on risk of postmenopausal breast cancer: a nested case control study, Breast Cancer Research 13 (2011) R99.

[39] M.A. Blankenstein, I. Maitimu-Smeele, G.H. Donker, J. Daroszewski, A. Milewicz, J.H. Thijssen, On the significance of in situ production of oestrogens in human breast cancer tissue, Journal of Steroid Biochemistry & Molecular Biology 41 (3–8) (1992) 891–896.

[40] A. Lipton, S.J. Santner, R.J. Santen, et al., Aromatase activity in primary and metastatic human breast cancer, Cancer 59 (4) (1987) 779–782.

[41] A.A. Larionov, L.M. Berstein, W.R. Miller, Local uptake and synthesis of oestrone in normal and malignant postmenopausal breast tissues, Journal of Steroid Biochemistry & Molecular Biology 81 (1) (2002) 57–64.

[42] M.J. Reed, P.A. Beranek, M.W. Ghilchik, V.H. James, Estrogen production and metabolism in normal postmenopausal women and postmenopausal women with breast or endometrial cancer, European Journal of Cancer & Clinical Oncology 22 (11) (1986) 1395–1400.

[43] P.E. Lonning, H. Helle, N.K. Duong, D. Ekse, T. Aas, J. Geisler, Tissue estradiol is selectively elevated in receptor positive breast cancers while tumour estrone is reduced independent of receptor status, Journal of Steroid Biochemistry & Molecular Biology 117 (13) (2009) 31–41.

[44] B.P. Haynes, A.H. Straume, J. Geisler, et al., Intratumoral estrogen disposition in breast cancer, Clinical Cancer Research 16 (6) (2010) 1790–1801.

[45] A.K. Dunbier, H. Anderson, Z. Ghazoui, et al., Relationship between plasma estradiol levels and estrogen-responsive gene expression in estrogen receptor-positive breast cancer in postmenopausal women, Journal of Clinical Oncology 28 (7) (2010) 1161–1167.

[46] G.A. Greendale, B.A. Reboussin, S. Slone, C. Wasilauskas, M.C. Pike, G. Ursin, Postmenopausal hormone therapy and change in mammographic density, Journal of the National Cancer Institute 95 (1) (2003) 30–37 (see comment).

[47] K. Ghosh, K.R. Brandt, C. Reynolds, et al., Tissue composition of mammographically dense and non-dense breast tissue, Breast Cancer Research & Treatment 131 (1) (2012) 267–275.

[48] B.A. Swiglo, M.H. Murad, H.J. Schunemann, et al., A case for clarity, consistency, and helpfulness: state-of-the-art clinical practice guidelines in endocrinology using the grading of recommendations, assessment, development, and evaluation system, Journal of Clinical Endocrinology & Metabolism 93 (3) (2008) 666–673.

[49] V.M. Montori, GRADE: A System for Assessing Level of Evidence, 2009.
[50] R.J. Santen, G.R. Petroni, Relative versus attributable risk of breast cancer from estrogen replacement therapy, Journal of Clinical Endocrinology & Metabolism 84 (6) (1999) 1875–1881 (Review).
[51] G.L. Anderson, M. Limacher, A.R. Assaf, et al., Effects of conjugated equine estrogen in postmenopausal women with hysterectomy: the Women's Health Initiative randomized controlled trial, Journal of the American Medical Association 291 (14) (2004) 1701–1712 (see comment).
[52] G.L. Anderson, R.T. Chlebowski, J.E. Rossouw, et al., Prior hormone therapy and breast cancer risk in the Women's Health Initiative randomized trial of estrogen plus progestin, Maturitas 55 (2) (2006) 103–115.
[53] R.T. Chlebowski, S.L. Hendrix, R.D. Langer, et al., Influence of estrogen plus progestin on breast cancer and mammography in healthy postmenopausal women: the Women's Health Initiative Randomized Trial, Journal of the American Medical Association 289 (24) (2003) 3243–3253 (see comment).
[54] R.T. Chlebowski, L.H. Kuller, R.L. Prentice, et al., Breast cancer after use of estrogen plus progestin in postmenopausal women, New England Journal of Medicine 360 (6) (2009) 573–587.
[55] R.L. Prentice, R.T. Chlebowski, M.L. Stefanick, et al., Estrogen plus progestin therapy and breast cancer in recently postmenopausal women, American Journal of Epidemiology 167 (10) (2008) 1207–1216 (see comment).
[56] R.L. Prentice, Women's health initiative studies of postmenopausal breast cancer, Advances in Experimental Medicine & Biology 617 (2008) 151–160 (Review).
[57] J.A. Collins, J.M. Blake, P.G. Crosignani, Breast cancer risk with postmenopausal hormonal treatment, Human Reproduction Update 11 (6) (2005) 545–560 (see comment; erratum appears in Hum Reprod Update. 2006 May–Jun;12(3):331; Review).
[58] G.K. Reeves, V. Beral, J. Green, T. Gathani, D. Bull, S.C. Million Women, Hormonal therapy for menopause and breast-cancer risk by histological type: a cohort study and metaanalysis, Lancet Oncology 7 (11) (2006) 910–918 (see comment).
[59] V. Beral, S.C. Million Women, Breast cancer and hormone-replacement therapy in the Million Women Study, Lancet 362 (9382) (2003) 419–427 (see comment; erratum appears in Lancet. 2003 Oct 4;362(9390):1160).
[60] C.J. Crandall, A. Karlamangla, M.H. Huang, G. Ursin, M. Guan, G.A. Greendale, Association of new-onset breast discomfort with an increase in mammographic density during hormone therapy, Archives of Internal Medicine 166 (15) (2006) 1578–1584 (erratum appears in Arch Intern Med. 2006 Nov 13;166(20):2176).
[61] K. Bakken, A. Fournier, E. Lund, et al., Menopausal hormone therapy and breast cancer risk: impact of different treatments. The European Prospective Investigation into Cancer and Nutrition, International Journal of Cancer 128 (1) (2011) 144–156.
[62] A. Fournier, Estrogen–progestagen menopausal hormone therapy and breast cancer: does delay from menopause onset to treatment initiation influence risks? Journal of Clinical Oncology (2009), online publication Sept 14, 2009.
[63] Y.Y.W.W.J.S.R. Song, The effect of a tissue specific estrogen complex (TSEC) on breast cancer xenografts in mice: rationale for use of a tissue selective complex, Proceedings of the Endocrine Society (2012) (abstract).
[64] C.E. Wood, T.B. Clarkson, H. Chen, et al., Comparative effects of oral conjugated equine estrogens and micronized 17beta-estradiol on breast proliferation: a retrospective analysis, Menopause 15 (5) (2008) 890–898.
[65] A. Gompel, Plu-Bureau. Is the decrease in breast cancer incidence related to a decrease in postmenopausal hormone therapy? Annals of the New York Academy of Sciences 1205 (2010) 268–276 (Review).
[66] S.A. Tsai, M.L. Stefanick, R.S. Stafford, Trends in menopausal hormone therapy use of US office-based physicians, 2000–2009, Menopause 18 (4) (2011) 385–392.
[67] A.S. Robbins, C.A. Clarke, Regional changes in hormone therapy use and breast cancer incidence in California from 2001 to 2004, Journal of Clinical Oncology 25 (23) (2007) 3437–3439.
[68] K. Canfell, E. Banks, M. Clements, et al., Sustained lower rates of HRT prescribing and breast cancer incidence in Australia since 2003, Breast Cancer Research & Treatment 117 (3) (2009) 671–673.
[69] R.M. Martin, What was the immediate impact on population health of the recent fall in hormone replacement therapy prescribing in England/Ecological Study. Wheeler BW MCGD, editor, Journal of Public Health (2010), http://dx.doi.org/10.1093/pubmed/fdq021, Advance Access.
[70] P.M. Ravdin, K.A. Cronin, N. Howlader, et al., The decrease in breast-cancer incidence in 2003 in the United States, New England Journal of Medicine 356 (16) (2007) 1670–1674 (see comment).
[71] A. Gompel, R.J. Santen, Hormone therapy and breast cancer risk 10 years after the WHI, Climacteric 15 (3) (2012) 241–249.
[72] D.W. Sturdee, A. Pines, International Menopause Society Writing Group, et al., Updated IMS recommendations on postmenopausal hormone therapy and preventive strategies for midlife health, Climacteric 14 (3) (2011) 302–320.
[73] S.L. Bailey, B.M. Sigal, S.K. Plevritis, A simulation model investigating the impact of tumor volume doubling time and mammographic tumor detectability on screening outcomes in women aged 40–49 years, Journal of the National Cancer Institute 102 (16) (2010) 12631271.
[74] R.J. Santen, W. Yue, D.F. Heitjan, Modeling of the growth kinetics of occult breast tumors: role in interpretation of studies of prevention and menopausal hormone therapy, Cancer Epidemiology, Biomarkers & Prevention 21 (7) (2012) 1038–1048.
[75] R.X. Song, G. Mor, F. Naftolin, et al., Effect of long-term estrogen deprivation on apoptotic responses of breast cancer cells to 17beta-estradiol [see comment], Journal of the National Cancer Institute 93 (22) (2001) 1714–1723.
[76] R.X. Song, Z. Zhang, G. Mor, R.J. Santen, Down-regulation of Bcl-2 enhances estrogen apoptotic action in long-term estradiol-depleted ER(+) breast cancer cells, Apoptosis 10 (3) (2005) 667–678.
[77] R.X. Song, R.J. Santen, Apoptotic action of estrogen, Apoptosis 8 (1) (2003) 55–60 (Review).
[78] J.S. Lewis, K. Meeke, C. Osipo, et al., Intrinsic mechanism of estradiol-induced apoptosis in breast cancer cells resistant to estrogen deprivation, Journal of the National Cancer Institute 97 (23) (2005) 1746–1759.
[79] J. Suckling, A. Lethaby, R. Kennedy, oestrogen for vaginal atrophy in postmenopausal women, Cochrane Database of Systematic Reviews 2006 (4) (2003) CD001500 (update of Cochrane Database Syst Rev. 2003;(4):CD001500; PMID: 14583935; Review).
[80] G. Schmidt, S.B. Andersson, O. Nordle, C.J. Johansson, P.O. Gunnarsson, Release of 17-betaoestradiol from a vaginal ring in postmenopausal women: pharmacokinetic evaluation, Gynecologic & Obstetric Investigation 38 (4) (1994) 253–260.
[81] I.S. Fraser, R. Ayton, E. Farrell, E. Weisberg, G. Darling, A. Murkies, A multicentre Australian trial of low dose estradiol therapy for symptoms of vaginal atrophy using a vaginal ring as delivery system, Maturitas 22 (Suppl.) (1995).
[82] A. Kendall, M. Dowsett, E. Folkerd, I. Smith, Caution: vaginal estradiol appears to be contraindicated in postmenopausal women on adjuvant aromatase inhibitors, Annals of Oncology 17 (4) (2006) 584–587.
[83] E. Amir, O.C. Freedman, B. Seruga, D.G. Evans, Assessing women at high risk of breast cancer: a review of risk assessment models [Review], Journal of the National Cancer Institute 102 (10) (2010) 680–691.
[84] N.F. Boyd, J.W. Byng, R.A. Jong, et al., Quantitative classification of mammographic densities and breast cancer risk: results from the Canadian National Breast Screening Study, Journal of the National Cancer Institute 87 (9) (1995) 670–675.