Reproductive Cancer and Hormone Replacement

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Introduction

The application of steroid hormones to otherwise healthy postmenopausal women has become a domain of preventive medicine. Its primary intention is the treatment of climacteric symptoms. Long-term, it is directed towards the metabolic consequences of estrogen deficiency such as osteoporosis, cardiovascular disease and neuroendocrine aging. Similar to oral contraception, there is an impact of hormone replacement therapy (HRT) on incidence and mortality of reproductive cancer.

Gusberg, already in 1947, defined adenomatous hyperplasia as the morphologic precursor lesion of endometrial cancer [1]. Together with Kaplan, he described 191 patients with adenomatous hyperplasia in a prospective follow-up investigation [2]; in 90 of these patients, immediate hysterectomy was performed; in those surgical specimens he found 20% of the cases had coexistent cancer, in 30% borderline lesions were detected. Among the remainder of 101 women, 8 (11.8%) developed endometrial cancer within an average follow-up of 5.3 years. In the control group of 202 women with postmenopausal bleeding, but without any sign of hyperplasia or cancer, in the primary DC specimen, only one woman developed endometrial cancer within the same time period. Gusberg and Kaplan calculated the cumulative risk of a transition of adenomatous hyperplasia to develop into endometrial cancer to be one-third within 9 to 10 years, a concept that was contradicted by others. In the meantime, Bert Vogelstein [3] could demonstrate convincingly that cancerogenesis can be considered a developmental process of hyperplasia to adenomatosis to in-situ cancer to infiltrating cancer depending on specific and multiple genomic defects, as demonstrated with colon cancer. Kurman et al [4] looked at "untreated" hyperplasia of endometrial cancer in 170 women and demonstrated hyperplasia and neoplasia to represent two separate and biologically different phenomena; the morphological discriminant is cellular atypia.

Apparently more than 40% of all newly diagnosed female cancers are hormone-dependent. In addition to endometrial cancer these mainly are breast and ovarian cancer. What is our current view on the way hormones influence the oncogenetic cascade?

This cascade of cancerogenesis is related to an accumulation of intracellular genetic mutations as well as epigenetic abnormalities in controlled gene expression. Hormones can indeed influence the development of various cancers as demonstrated in the clinical experiment and in epidemiol-

ogy. Hypotheses have been formulated to show a relation of specific female reproductive tumours (breast, endometrium, ovary) to hormonal signaling; the same holds true for the male prostate gland. Therapeutic induction of ovulation has been discussed as being related to the morphogenesis of ovarian cancer. Breast cancer with its incremental incidence is epidemiologically associated with a complex of family history as well as reproductive and environmental factors. While early menarche and late menopause for example have been defined as risk factors, loss of ovarian function at younger age appears to be protective; this also relates to first full-term pregnancy at younger age, while primiparity beyond 35 certainly increases breast cancer risk. Such epidemiologic information, very often of borderline significance, asks for intensified research in order to provide a basis for biological plausibility of the importance of any of those inferred epidemiological impacts. Recently, research focussed on genetically programmed cell death (apoptosis) as a determinant of tumour growth. There is a lot of information as to the hormone dependence of cellular regression.

Experimental experience with respect to control and quantitation of apoptosis has provided a growing insight into the development of hormone-dependent tumours [5]. Apoptosis is the genetically programmed process of active cellular self-destruction and this way clearly distinguished from necrosis. Apoptosis is under the control of intrinsic and extrinsic factors (hormones, growth factors); it can be activated in tissues, during embryonic development or normal cyclicity of endocrine-related organs. Loss of apoptotic mechanisms can foster tumour development [6]. Bursch experimented on estrogen-induced kidney tumour transplants in hamsters. When diethylstilbestrol (DES)-pretreated hamsters are inoculated with cellular suspensions of estrogen-induced kidney tumours, within 2 to 3 weeks these hamsters developed solid tumours; is DES withdrawn, the tumour in the recipient animal will regress within a few days by 80 to 90% of its mass. Following this estrogen withdrawal, tumour regression occurs within four days. The mitotic activity of these tumours, within a period of 24 hours, returned to the level of what it was before DES withdrawal. The extent of areas of necrosis remained unchanged during the experiment. The inoculated tumour regained its original volume within two days after re-uptake of DES treatment. These observations point to a clear dependence of programmed cell death in kidney tumour cells from DES in an inhibitory, and following its discontinuation, in a supportive manner. Functional and morphologic variation characterizes the typical demise of tumour cells as apoptosis. These observations can also be interpreted as indicating an important mechanism in which, independent of its mitogenic capacities, hormones like DES can alter tumour growth.

Contemporary cancer research has provided a "multiple-hit" theory for the phenomenon of cellular transformation to the malignant phenotype as being dependent on a sequence of distinct genetic alterations [7, 8]. Successful breeding of transgenic mice provided excellent proof for the importance of inborn dominant oncogenes as causative for cancer at younger age. Although these oncogenes are present in all existing cell types, not all of these cells will be transformed. Additional genetic defects turned out to be a prerequisite for tumour development.

In a classic experiment with two variants of transgenic mice, when they

were carrier of either activated ras-gene or a deregulated myc-gene in breast tissue, they developed clonal breast tumours from these cell lines within three months. Their offspring, according to Mendelian mode, in one quarter were carrier of both the ras- and myc-oncogene; these developed the same clonal breast tumour within one month. All of these tumours developed into focal lesions, their further progression to infiltrating tumours would require additional genetic events. These observations represent a strong experiment in favor of the validity of a "multiple-hit" theory of cancerogenesis.

The deregulated production of a normal myc-protein can induce cellular transformation. In order to further delineate such myc-protein related transformation processes, a hybrid gene from myc-sequences was constructed with sequences which encode the hormone-binding domain of the estrogen receptor. This domain acts like an intracellular switchboard which inactivates its associated protein domains in the absence of the ligand and will keep it active with the ligand present. These genetic chimeras, when located in a retroviral vector, can infect normal cell cultures. With no estrogen present, the myc-protein was inactive, and cellular replication was operating normally. The addition of an estrogen, however, induced the accumulation of active myc-protein in the cellular nucleus with a resultant complete transformation of the cell. Current research on the hormone dependence of the cell cycle puts its focus on regulator genes. These are DNA strands which code for the expression of cell-specific hormone receptors and their products. The specific profile of such regulator genes will be responsible for the organ- and cell-specific hormone signaling.

Our current experimental knowledge points to a hormone dependence of proliferation as well as cellular apoptosis as the means by which hormones can interfere with tumour growth. On the other hand, there are also indications for hormone-induced cellular expression of oncogenes as well as cell-specific expression of variable hormone receptors.

This knowledge will allow further insight into the way in which a hormonal stimulus may promote or inhibit tumourigenesis long-term and uni-directional. Independent of environmental and dietetic factors, age is a clearly defined risk factor of cancer. Breast cancer manifests predominantly at post-menopausal age. Breast development and differentiation, tumourigenesis as well as growth and progression are influenced by sex hormones. Generally speaking, excessive endocrine stimulation of specific organs will induce incremental cell division and thereby lead to accidental accumulation of genetic defects with accelerated cell cycle turnover. As a result, there will be an accumulation of neoplastic phenotypes [2, 3, 4, 9].

In view of clinical practice, the fact as to whether HRT will influence hormone-dependent tumours can only be answered by randomized, controlled long-term epidemiological investigation.

Can we introduce steroid hormones for replacement in peri- and postmenopausal women independent of or bound to their family history of cancer? For proper decision-making, we would have to consider HRT with respect to general cancer risks and in addition in such women who survived endometrial, cervical, vaginal or ovarian cancer and require further medical intention.

ENDOMETRIAL CANCER

Not taking breast and colon cancer into account, the adenocarcinoma of the endometrium is the most frequent malignancy in women from western industrialized countries. Rather infrequent before menopause, endometrial cancer will only account for 7.5% of all cancers before the age of 50. Between the ages of 40 and 67, the incidence will increase rapidly, after which it will persist at a constant plateau. Experimental and observational studies associate endogenous and exogenous estrogens with an elevated risk of endometrial hyperplasia and endometrial cancer. Clinical situations in which elevated serum estrogen levels are experienced long-term with a concurrent deficit in cyclical secretion of progesterone as seen in adipose women, the syndrome of polycystic ovaries or various ovarian tumours such as granulosa or theca-cell tumours, are associated with an increased risk of endometrial cancer.

Epidemiology

The risk of endometrial cancer increases dose- and time-dependently with estrogen monotherapy (table 1). These observations date back to the thirties and forties of the last century when the importance of progesterone for endometrial transformation was first observed; nevertheless, the addition of a progestogen was not seriously taken into account. Progesterone replacement certainly was more common in Europe, but finally, the USA enforced progestin therapy in the beginning nineties. Today, many European and American detailed investigations demonstrated the protective effect of progestogens against the estrogen-dependent elevated risk of endometrial cancer.

Estrogens at higher levels and long-term elevate the risk of endometrial cancer to fivefold and beyond [21]; however, it should be noted that this elevated cancer risk is restricted to early stages and well-differentiated tumours [22]. Complete remission following estrogen-induced endometrial cancer arrives at 95%. Less well differentiated endometrial cancers differ, however, with respect to hormone dependence, a phenomenon as yet not well understood. On the other hand, estrogens influence the transformation process of normal to precancerous endometrial cells not only via estrogen-binding domains with genetic code activation, but also via possible immune-suppressive mechanisms [22].

Importance of progestogens

The effect of progestogens on the endometrium also is dose- and time-dependent and not so much influenced by the type of progestogen. As a result, those progestogens in clinical use will have the same preventive effect on

Table 1. Estrogen monotherapy and risk of endometrial cancer in postmenopausal women

Authors	Cases (n)	Relative Risk
Quint, 1975 [10]	291	1.8
Smith et al., 1975 [11]	317	4.5
Ziel and Finkle, 1975 [12]	94	7.6
Mack et al., 1976 [13]	63	8.0
McDonald et al., 1977 [14]	145	4.9
Gray et al., 1977 [15]	205	3.1
Horwitz and Feinstein, 1978 [16]	119	1.7
Antunes et al., 1979 [17]	451	6.0
Jick et al., 1979 [18]	67	20.0
Shapiro et al., 1985 [19]	425	3.5
Persson et al., 1989 [20]	74	1.8

endometrial cancer [23]. In traditional clinical investigation, progestogens have been applied in a rather sequential or cyclic fashion. A continuous-combined mode of estrogen and progestogen therapy has gained preference particularly at later postmenopausal years; at this age, two-thirds of all women prefer amenorrhea, and the risk of dysfunctional bleeding by residual endogenous ovarian estrogen secretion can be neglected. Whitehead's group investigated the inter-individual variation of endometrial reaction to progestogens. A protection of the endometrium is seen in nearly all women with a minimal dose of 0.7 mg norethisterone, 250 µg levonorgestrel, 200 mg progesterone, 10 mg medroxyprogesterone acetate or 20 mg dydrogesterone (table 2). Estrogen-dependent endometrial hyperplasia will be prevented by lower than the typical progestogen transformation dose, provided co-medication is offered for a minimum of 12 days [24]. Sturdee [25] objected to this concept and considered 10 days as sufficient duration of progestogen co-medication. Certainly, we do observe individual variation of endometrial transformation all the way from 7 to more than 12 days.

Table 2. Effective dose of progestogens for endometrial protection in sequential HRT

	mg/day
oral:	
Progesterone (micronized)	200–300
Medroxyprogesterone acetate (MPA)	5–10
Medrogestone	5
Dydrogesterone	10–20
Cyproterone acetate (CPA)	1
Norethisterone acetate (NETA)	1–2.5
dl-norgestrel (NORG)	0.15
Levonorgestrel (LNG)	0.075
Desogestrel	0.15
transdermal:	
Norethisterone acetate (NETA)	0.25

Estrogen action not balanced by an appropriate dose and duration of progestogens is a common determinant of many risk factors of endometrial cancer. Among these are obese women with their enlarged capacity to aromatize androstendione to estrone, who carry a threefold risk [26]. In an overweight situation of more than 25 kg, the risk will be tenfold. Gambrell could demonstrate already in 1977 a second most frequent incidence of endometrial cancer to exist in non-substituted postmenopausal women who abstained from hormone replacement because they never experienced any menopausal symptoms [27]. In this group, many obese and nulliparous women are found [28, 29]. For that reason, Gambrell suggested the application of progestogens in non-estrogen substituted women of various risk groups in order to oppose the endogenous overproduction of estrogens [30]. As long as the gestagen test is positive, a progestogen should be provided for a period of 10 to 14 days each month; a negative gestagen test should be repeated within a year.

This risk group should not only consist of postmenopausal obese women, but also of higher-weight young women with polycystic ovaries in which a fivefold increased endometrial cancer risk was found [29, 31]. In those women with polycystic ovaries, the risk of breast cancer is also elevated sixfold [29], the mechanism of which still remains unclear.

Our current management of postmenopausal hormone replacement is based on a continuous application of estrogens with an additional cyclical or continuous combination of progestogens. Pharmacokinetic and pharmacodynamic variability both of the estrogen as well as the progestogen component and – more importantly – the inter-indi-

vidual variation do not favor the concept of one estrogen-progestogen combination for all indications [29]. Varying degrees of blood supply and hormone sensitivity as well as other factors can cause aberrant endometrial development. For this reason, morphological variations of endometrial regeneration as seen from endometrial biopsies or DNC will not always be completely verified. Hysteroscopy-guided biopsies as a "gold standard" of endometrial surveillance during HRT as well as endometrial ultrasound are the most reliable ways of morphological control in women at risk. In non-hysterectomized women, hormone replacement should be started with a combined estrogen-progestogen preparation in order to prevent uncontrolled bleeding, unless vaginal ultrasound proves an endometrial thickness of less than 5 mm (double-layer).

The low estrogen dose regimen

There are a lot of low-dose estrogen HRT products on the market, raising the question as to the efficiency and safety of these products targeting the endometrium, breast tissue, vasomotor symptoms, bone metabolism or lipid profiles. Genant et al [32] could induce endometrial hyperplasia with oral conjugated estrogens in a dose-dependent manner. After two years of treatment with a low-dose preparation (0.3 mg per day of conjugated estrogen), only one case of endometrial hyperplasia (1.7 % of the investigated cases) was observed; this is identical with the incidence in the placebo group. Notelovitz et al [33] differentiated a low-dose treatment group of postmenopausal women who were administered 0.3, 0.625 and 1.25 mg of conjugated estrogens orally per day. They found clear evidence of low-dose estrogens being associated with a profound reduction of endometrial cancer risk. This has also been confirmed by a 12-week investigation of transdermal application of 25, 50 and 100 µg estradiol per day versus placebo. Utian et al [34] demonstrated estrogen trophicity in vaginal PAP smears of originally atrophic vaginal epithelium. In the 25-µg-per-day treatment group, only one case of endometrial hyperplasia was observed in a total of 14 probands as compared to 10 cases out of 22 with the 50 µg-per-day dose and a total of 88 cases in the 100 µg-per-day application.

Estrogen in survivors of endometrial cancer

The low estrogen dose not only provides a very low risk of endometrial cancer, but also good bleeding control and thereby excellent patients' compliance. These advantages raise the question as to estrogen replacement in survivors of endometrial cancer. The clinical-scientific information at hand is clearly limited. Three retrospective studies have analyzed estrogen replacement following surgery of endometrial carcinoma. One investigation by Creasman et al as published in 1986 referred to 21 low-risk women with endometrial cancer stage Ia and Ib, grade I and II, of which 47 (22 %) were estrogen-substituted for a mean for 26 months. In this investigation at Duke University, hormone replacement was started between 0 and 81 months following definitive cancer treatment with a mean interval of 15 months. As this was a retrospective analysis, the interval was ranging rather widely. The investigators did not find an increased risk of recurrence when controls were adjusted to tumour size, myometrial invasion, lymph-nodes spread, peritoneal cytology and age; a total of 47 estrogen-

substituted women was compared to 174 women without hormone treatment [35]. Very surprisingly, the risk of recurrence in the non-treated group was higher (15 %) as compared to the treatment group (2 %). Also mortality risk in the treatment group with 26 cases (16 as a result of cancer and 10 of intercurrent disease) was higher as compared to tumour-dependent mortality of estrogen-treated women. Lee et al [36] confirmed this experience in 1989, when they investigated 44 women with a history of endometrial cancer stage I and a subsequent hormone replacement for a mean of 64 months. The majority of these women started estrogen replacement therapy (ERT) within the first postoperative years.

No recurrence or mortality was observed in the treatment group. A selection bias is incurred by the inclusion of the group of women with a better prognosis. Later on in 1996, another retrospective analysis on 123 women post endometrial cancer surgery was reported which did not experience any negative influence on survival when ERT women were compared to non-users [37]. Two additional observational studies support the contention that endometrial cancer is not any longer a contraindication to ERT. Baker reported on a small group of 31 women with ERT. A very stringent selection resulted in a group of very-low-risk women [38]. In a "letter to the editor", Bryant reported on oral ERT in 20 women following treatment of endometrial cancer treatment starting 10 to 24 months following surgical intervention with a dose of 0.625 mg conjugated estrogens [39]. These reports took care of all feasible selection criteria. No observations were ever published on exacerbations of endometrial cancer in women on estrogen replacement.

The big majority of women with endometrial cancer stages I and II can be cured with a long-term survival rate of more than 80 %. Beresford et al [40] calculated a relative risk of endometrial cancer of 1.3 (CI 0.8–2.2) when a progestogen was added to ERT in otherwise healthy postmenopausal women. With five or more years of estrogen and progestogen replacement therapy, the risk will be 2.5 (CI 1.1–2.5). Persson et al [20], in their cohort analysis, found a relative risk of 1.0 (CI 0.7–1.4) in women on combined estrogen-progestogen replacement. These investigations clearly point to a protective effect of an added progestogen. The question whether women following surgical therapy of endometrial cancer should be hormone-replaced cannot be answered without referring to the overall benefit-risk equation of combined progestogen and estrogen treatment. As all such investigations so far are of retrospective nature, this would require additional prospective randomized studies in order to gain therapeutic safety.

OVARIAN CANCER

Epithelial ovarian carcinoma is the leading cause of death from gynecologic cancer. There is an estimate of 14 ovarian cancers in 100,000 women, representing one woman in about 70 to develop ovarian cancer in her lifetime, and one woman in 100 to die from this disease.

The incidence of ovarian cancer increases with age and peaks in the eighth decade. While epithelial ovarian cancer is infrequent in women below age 40, it will increase from 15 to 16 per 100,000 at ages 40–44 and peak at a rate of 57 per 100,000 at age 70–74. The median age of diagnosis is 63,

and almost half of the patients will be 65 years or older [41]. In our country, about 70% of ovarian cancer will be diagnosed at advanced stages III–IV of the disease. At present, less than 30% of stage II–IV patients survive five years or longer, for stage III, this will be 10–25%, stage IV less than 5%.

Genetic and environmental factors

The molecular events leading to the development of epithelial ovarian cancer are grossly unknown. Epidemiologic studies have identified reproductive, environmental, and genetic factors as important in the carcinogenesis of ovarian cancer.

a. Reproductive factors

The bulk of epidemiologic evidence favors parity as the most important risk factor for ovarian cancer. Women who were ever pregnant have 30–60% less risk of ovarian cancer than do nulliparous women [42]. Multiple pregnancies exert an increasingly protective effect. One to two pregnancies infer a relative risk of 0.49–0.97 as compared to 1.0 for nulliparous women. More than three pregnancies further decrease the relative risk to 0.35–0.76.

In a collaborative analysis of twelve US case-control studies [43], each month of breast-feeding was associated with an additional risk reduction, although no consistent relationship could be established between lifetime months of breast-feeding and decreased risk. The same investigation demonstrated only weak trends of decreasing risk with increasing age at menarche. These trends were stronger in young than in older women. Considering age at natural menopause, when younger ages are compared to those of 55 years and more, no clear patterns were evident.

The risk of developing epithelial ovarian cancer of all histologic subtypes in users of oral contraception is reduced by 40% compared to that of non-users [43, 44]. This protective effect increases with duration of use and continues for at least 10–15 years after discontinuation of the pill. This protection, which is already seen with as little as 3–6 months of oral contraception, reaches an 80% reduction in risk with more than ten years of use, and is a benefit associated with all monophasic formulations, including the low-dose products [45]. Oral contraception is particularly protective in women at high risk of ovarian cancer (nulliparous women and women with a positive family history) [46]. By that token, continuous use of oral contraception for ten years by women with a positive family history for ovarian cancer can reduce the risk of epithelial ovarian cancer to a level equal to or less than that experienced by women with a negative family history [46]. The same magnitude of protection has been observed in a case-control study of women with BRCA1 or BRCA2 mutations [47].

The protective effect of parity, multiple births, history of breast-feeding and oral contraceptive use supports the "incessant ovulation" hypothesis for the aetiology of ovarian cancer [48]. From this hypothesis, ovarian cancer develops from an aberrant repair process of the surface epithelium, which is ruptured and repaired during each ovulatory cycle. The likelihood of ovarian cancer to develop will therefore be a function of the total number of ovulatory cycles, together with a genetic predisposition and other, not well defined environmental factors.

b. Genetic factors

One important indicator of an individual woman's probability of develop-

ing ovarian cancer is family history. From a clinical point of view it is helpful to separate the genetic risk for ovarian cancer into familial and hereditary aspects. Compared to a lifetime risk of the general population of 1.6%, a woman with a single family member affected by ovarian cancer has a 4–5% risk [49]. This risk will increase to 7% when two relatives are affected by ovarian cancer. A woman with at least two first-degree relatives with ovarian cancer is defined as hereditary ovarian cancer syndrome and has a lifetime probability as high as 50% of developing ovarian cancer [50]. It is estimated that between 1% and 5% of all ovarian cancer patients will be part of hereditary ovarian cancer syndromes [43].

Three distinct genotypes of hereditary ovarian cancer have been identified [50, 51]:
1. Breast-ovarian cancer syndrome, when ovarian cancer is associated with early-onset breast cancer.
2. Ovarian cancer-only syndrome, which is rarer than the breast-ovarian syndrome and characterized by multiple cases of ovarian cancer in the affected kindreds.
3. As part of the Lynch type II cancer family syndrome, which is characterized by the inheritance of non-polyposis colo-rectal cancer, endometrial cancer, and, to a lesser extent, ovarian cancer.

The majority of patients with early-onset breast cancer and with two or more cases of ovarian familial-hereditary cancer will carry a mutated BRCA1 allele [52]. Various functional activities of BRCA1 – including its ability to regulate progression through the cell cycle, apoptosis, DNA repair events and the maintenance of genomic integrity – may contribute to the biologic activity of this gene as a tumour suppressor [53].

However, these so-called "care-taker" activities of BRCA1, some of which parallel the activities of the p53 tumour suppressor gene, do not appear to be cell-type-specific. Over-expression of a wild-type BRCA1 gene was recently found to inhibit signaling by the ligand estrogen receptor (ERα) in various human breast and prostate cancer cell lines [53]. Thus, wild-type BRCA1 can suppress estrogen-dependent transcriptional pathways related to the proliferation of epithelial cells, and mutation of BRCA1 can result in the loss of this ability, contributing to tumourigenesis. In addition, BRCA1 transcription can be induced through the mitogenic activity of estradiol in cells expressing estrogen receptors [54].

Furthermore, mutations of the p53 gene as well as abnormalities of dominant oncogenes frequently found in ovarian cancer involve c-myc, H-ras, Ki-ras and the erb-B2 oncogenes. The identifiable molecular changes have not produced a unifying model to account for how the observed alterations in oncogenes and tumour suppressor genes lead to the development and progression of ovarian cancer. No precursor lesion has been identified, and controversy persists as to whether there is progression from an adenoma to a borderline malignancy to an invasive epithelial cancer [55].

c. Environmental factors

In addition to the epidemiologic information on the reduction in ovarian cancer incidence following hormonal contraception, other studies suggested association of risk with environment of industrialized western countries. A diet high in meat and animal fat has been reported in some studies to be associated with an increased risk of ovarian cancer. Others failed to demonstrate an

alteration of risk in association with fat, protein, fiber, or vitamins A and C. Obesity has also been associated with a slight increase in relative risk [55].

Cramer et al [56] developed the hypothesis that ovarian cancer is a consequence of hypergonadotropic hypogonadism, as they found an association of dietary galactose consumption and decreased levels of transferase with hypergonadotropic hypogonadism.

Other dietary factors such as coffee and tobacco usage have not been associated with ovarian cancer, though there appears to be a slightly increased risk with alcohol consumption [55].

Definite associations with industrial exposure to carcinogens or to diagnostic and therapeutic radiation have not been established. Conflicting reports refer to the association of the use of talcum powder (shown to contain asbestos) to the development of ovarian cancer. This aspect refers to the passage of such materials through the vaginal reproductive tract to the ovaries.

Impact of hormone replacement

Estrogen plays a key role in many biological phenomena such as cellular differentiation, homeostasis and reproduction. This is in line with the multitude of different pathological conditions associated with changes in the production of estrogen and/or the cellular response to these stimuli. The recent discovery that an additional estrogen receptor subtype (ERβ) is present in various human tissues has significantly advanced our understanding of the mechanisms underlying estrogen signaling [57]. ERβ is found in the human ovary, uterus, endometrium and breast. In the ovary, the receptor is localized to the stroma of the cortex as well as to the granulosa cells. The granulosa cells apparently contain only ERβ mRNA.

ERβ is thus likely to play an important role in the regulation of follicular growth and oocyte development.

Animal studies, epidemiological data, receptor analyses and therapeutic trials with hormones all suggest that not only normal ovaries, but also many ovarian tumours, benign as well as malignant, can be considered as endocrine-related and hormone-dependent. Patients with ovarian cancer who do not reach a complete response after standard initial surgery and chemotherapy, particularly in those with well-differentiated tumours, may benefit from the last resort hormone therapy [58].

The impact of oral contraceptive use was not particularly variable by invasiveness of tumour (invasive vs. borderline) or by histologic type (serous, mucinous, endometrioid, clearcell, or other). No clear difference in the reduction of ovarian cancer risk was seen following high- or low-dose pill use [59]. This would support the hypothesis of sufficient reduction of gonadotropin levels as an important mechanism of protection. Since exogenous estrogens reduce the high gonadotropin levels during the menopausal transition, replacement with estrogens and progestogens could conceivably reduce the risk of ovarian cancer. However, from an epidemiological point of view, this does not seem to be the case. In an European review, HRT use for five years or shorter had no influence on the RR for ovarian cancer, whilst long-term ERT increases the risk of cancer of the ovaries with a RR varying from 0.52–1.71 [60]. Literature on the interaction of hormone replacement with ovarian tumourigenesis is relatively scarce. Epidemiological data are inconsistent. A moderate and often non-significant excess risk of ovarian cancer in HRT users was reported in a multicentric US case-control study [61]. Other studies con-

ducted in Australia [62] and North America [43] did not show an excess risk; pooled RR for ovarian cancer for ever-HRT users were 0.9 (95% CI 0.7–1.3) in hospital-based studies and 1.1 (95% CI 0.9–1.4) in population-based ones without any duration-risk relationship. In a companion study [63], the RR of borderline ovarian tumours based on 327 cases was also 1.1 (95% CI 0.7–1.9).

To that point, the available data exclude a strong association between HRT and epithelial ovarian cancer, though a moderate association remains open to debate. To provide further information on the issue, a collaborative re-analysis of four European case-control studies, two conducted in Greece and one each in Italy and the United Kingdom, was performed; this analysis included a total of 1,470 ovarian cancer cases and 3,271 hospital controls [64]. Information on duration of HRT use and time since these substances were last used was not available for all women who reported having used them. This limited the analysis to women with information on all measures of HRT use [64]. The resulting estimates are presented in table 3. This re-analysis revealed a weak positive association with duration, the RR increasing from 1 (baseline) for those who had never used them to 1.67 (95% CI 1.11–2.51) for those who had used them for less than two years and to

1.79 (95% CI 0.91–3.54) for those who had used them for two years or more. There was also some evidence that the excess RR for ovarian cancer declined with time since last use, being 1.96 (95% CI 1.20–3.21) among recent users (< 10 years) and 1.45 (95% CI 0.86–2.52) among those who had stopped using HRT for more than ten years.

The association between HRT and ovarian cancer was of similar magnitude in the four data sets considered [65–68]. The RR ranged between 1.67 and 1.78 when exclusion was made, in turn, of one study. The association was observed in various age groups and seemed to persist several years after menopause in parous and nulliparous women and in OC users and never-users. In particular, the pooled RR was 1.69 (95% CI 1.27–2.24) among never OC users. Likewise, allowance for family history of ovarian and breast cancer for the two studies which include the information did not materially modify the estimates. It is important to note that the first Greek study of Tzonou [65] collected information on HRT for menopausal women in 112 cases and 188 controls; the second study by Polichronopoulou [66] included 152 postmenopausal cases and 129 controls with a participation rate close to 90%. The third study by Parazzini [67] was hospital-based, case-controlled and included a total of 971 patients below 75 years of age; the comparison

Table 3. Distribution of ovarian cancer cases and controls according to study center and use of HRT [64]

HRT use					Study					
	Greece 1		Greece 2		Italy		UK		total	
	Cases	Controls	Cases	Controls	Cases	Controls	Cases	Controls	Cases	Controls
Never	100	175	146	125	915	2421	200	404	1361	3125
Ever	12	13	6	4	56	82	35	47	109	146
OR (95% CI) ever use	1.77 (0.76–4.15)		1.40 (0.38–5.19)		1.66 (1.16–2.37)		1.68 (0.99–2.80)		1.71 (1.30–2.25)	

group included 2.503 women admitted to the same network of hospitals. The fourth study by Booth et al [68] was hospital-based, conducted in the UK and based on 235 cases below 65 years of age with histologically confirmed epithelial ovarian cancer and 451 controls of comparable age.

Case-control studies are always limited by their comparators; controls are selected for certain criteria which never include the full array of possible confounders. Earlier case-control studies reported decreased risk, no association or increased risk. More recent and larger case-control studies have suggested increased risk, particularly with long duration of estrogen use. However, even the largest of these investigations have had limited statistical
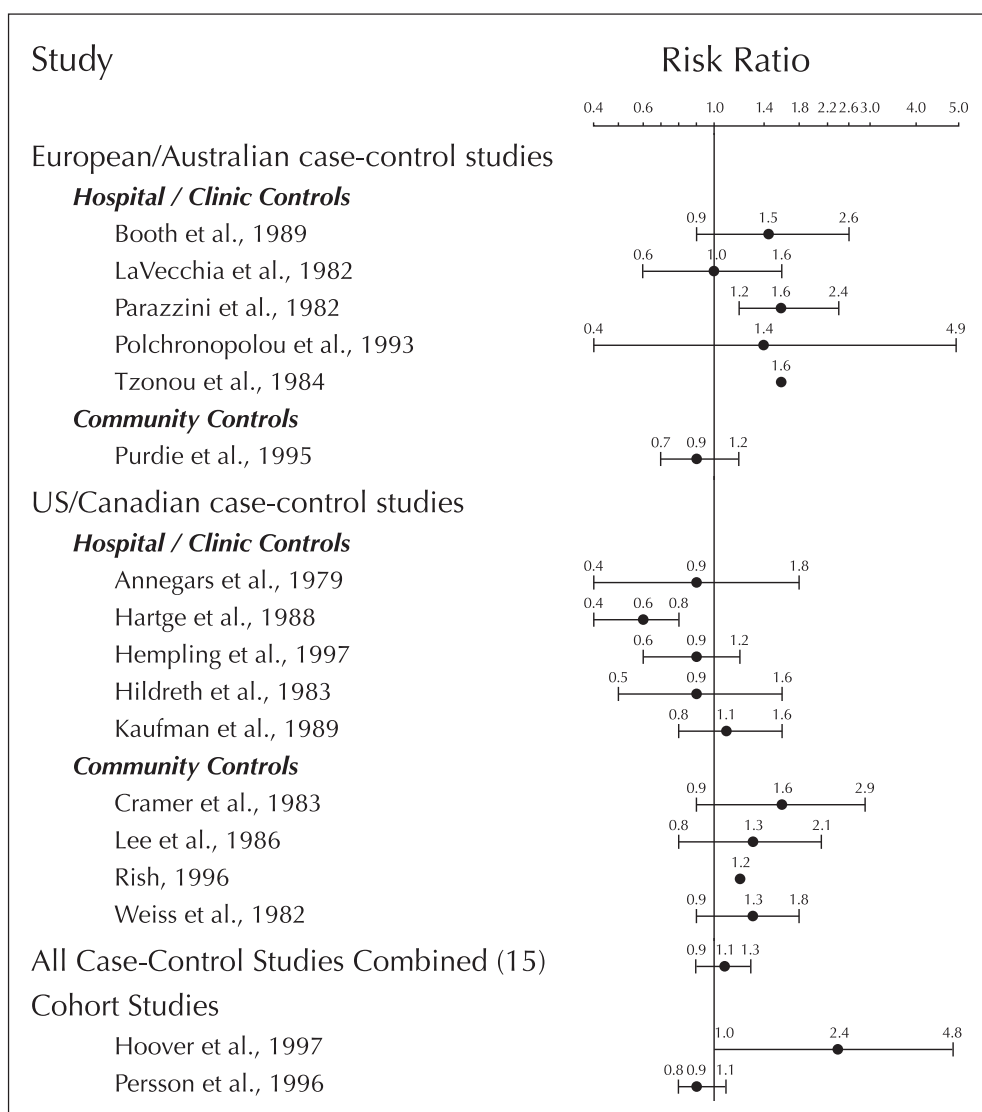
Study

Risk Ratio

Figure 1. Summary of risk ratios and 95 % confidence intervals from case-control and cohort studies of estrogen replacement therapy and risk of epithelial ovarian cancer (redrawn acc. to [74])

power to assess the risk associated with long duration of estrogen use (figure 1).

The latest report on estrogen replacement therapy and ovarian cancer mortality is by the American Cancer Society's Cancer Prevention Study II, a prospective US cohort study with mortality follow-up from 1982 to 1996. This investigation had a total of 211,581 postmenopausal women who completed a baseline questionnaire in 1982 and had no history of cancer, hysterectomy, or ovarian surgery at enrollment [69]. The main outcome measure was ovarian cancer mortality, compared among never-users, users at baseline, and former users as well as by total years of use of estrogen replacement therapy. The results are presented in table 4 and table 5. A total of 944 ovarian cancer deaths was recorded in fourteen years of follow-up. Women who were using ERT at baseline had higher death rates of ovarian cancer than never-users (RR 1.51; 95% CI 1.16–1.96). The risk was slightly, but not significantly increased among former estrogen users. Duration of use was associated with increased risk in both baseline and former users. Baseline users with ten or more years of use had an RR of 1.59 (95% CI 1.13–2.25). The authors did adjust ovarian cancer death rates for annual age per 100,000 women and found 64.4 for baseline users with ten or more years of use, 38.3 for former users with ten or more years of use, and 26.4 for never-users. Among former users with ten or more years of use, risk decreased with time since last use reported at study entry.

Mortality certainly is the most pertinent clinical endpoint, and the American Cancer Society's Cancer Prevention Study II certainly proposes some link between postmenopausal estrogen use for ten or more years with increased risk of fatal ovarian cancer [69]. This is a situation somewhat comparable to our more recent experience with HRT and breast cancer risk. It would appear that there is some risk

Table 4. Ovarian cancer mortality by estrogen use and duration and recency of estrogen use, Cancer Prevention Study II, 1982–1996 [69]

Estrogen Use	No. of Deaths	No. of Person-Years	Rate Ratio (95% CI)*	Rate Ratio (95% CI)#
Never	689	2,185,876	1.00 (Referent)	1.00 (Referent)
Ever	255	625,984	1.21 (1.05–1.41)	1.23 (1.06–1.43)
Recency of use				
Baseline	62	151,880	1.45 (1.11–1.88)	1.51 (1.16–1.96)
Former	193	474,103	1.15 (0.98–1.36)	1.16 (0.99–1.37)
Years of use, baseline users				
< 10	31	110,379	1.07 (0.74–1.54)	1.14 (0.79–1.65)
≥ 10	31	41,396	2.13 (1.48–3.06)	2.20 (1.53–3.17)
Years of use, former users				
< 10	158	416,823	1.09 (0.92–1.30)	1.10 (0.92–1.31)
≥ 10	35	57,281	1.55 (1.10–2.18)	1.59 (1.13-2.25)

* Rate ratio estimates adjusted for age and race. CI indicates confidence interval.

Models adjusted for age at baseline, race, duration of oral contraceptive use, number of live births, age at menopause, body mass index, age at menarche, and tubal ligation.

population which needs to be defined. It may be the catabolism of estrogens, with the known implication of catechol metabolites of estrogens in carcinogenic and cytotoxic effects, particularly when further metabolized to quinones, acting via lipid peroxidation, consumption of reducing equivalents, oxidation of DNA, and DNA single-strand breaks. Or on the other hand the problem of tissue-specific responses in the presence of polymorphisms of cytochromes CYP17, CYP1A1 and COMT needs to be defined in a way as it is done in order to delineate the individual and increased risk of breast cancer [70]. Women with individual genotypic risks of reproductive cancer may respond to hormone therapy, provided local tissue hormone metabolism is affected.

Following treatment of ovarian cancer, women will usually be considerably distressed. Not only did they have surgery, chemotherapy or radiotherapy, but also will they have to adapt to the rapid onset of hormonal deficiency. Remarkably few of these women are offered HRT to relieve their general discomfort. In a study of conservative treatment of early ovarian cancer in premenopausal women (younger than 40 years of age), a group of Italian investigators [71] found that pregnancy did not affect survival in women who conceived after conservative treatment. Very little information is available about HRT given to women treated for ovarian cancer. A British study [72] failed to demonstrate any difference in outcome between 78 women who received HRT following treatment of ovarian cancer and 295 women who were not treated. However, there was a tendency for women who had endometrioid or clear-cell tumours to do better on hormonal therapy than women who were not given estrogen and progestogen. The results from this paper were reassuring in that while HRT did not have any adverse effects on outcome, it certainly improved the quality of life for those women who were taking hormones.

In a summary of the literature, Rao and Slotman [73] concluded that in

Table 5. Ovarian cancer mortality among former estrogen users, by duration and time since last use, Cancer Prevention Study II, 1982–1996 [69]

Years since last Estrogen Use*	No. of Deaths	No. of Person-Years	Rate Ratio (95 % CI)#	Rate Ratio (95 % CI)+
Duration of Use < 10 y				
Never	689	2,185,876	1.00 (Referent)	1.00 (Referent)
Former	158	416,823	1.09 (0.92–1.30)	1.10 (0.92–1.31)
Use within 15 y	45	160,278	1.17 (0.85–1.59)	1.17 (0.85–1.60)
No use for ≥ 15 y	113	256,545	1.06 (0.87–1.31)	1.07 (0.87–1.32)
Duration of Use ≥ 10 y				
Never	689	2,185,876	1.00 (Referent)	1.00 (Referent)
Former	35	57,281	1.55 (1.12–2.18)	1.59 (1.13–2.25)
Use within 15 y	19	30,887	1.98 (1.25–3.15)	2.05 (1.29–3.25)
No use for ≥ 10 y	16	26,394	1.27 (0.77–2.10)	1.31 (0.79–2.17)

* Years since last use as reported at study entry.

Rate ratio estimates adjusted for age and race. CI indicates confidence interval.

+ Rate ratio estimates adjusted for age at baseline, race, duration of oral contraceptive use, number of live births, age at menopause, body mass index, age at menarche, and tubal ligation.

cases of ovarian cancer, while classical estrogen receptors (ER) are seen in about 60% of cases, progesterone receptors (PR) were documented in about 50% and androgen receptors (AR) in about 70%. PR presence in well-differentiated ovarian cancer is correlated with an improved survival. However, the presence or absence of receptors has not been shown to predict reliably which patients might respond to hormonal therapy. In general, both high-dose estrogen and progestogen therapy and anti-estrogen therapy have been used to treat metastatic ovarian cancer with variable percentages of patients responding [58]. There was a suggestion that in pre-selected cases with well-differentiated cancer of the ovary, some women may nevertheless benefit from hormonal therapy.

Conclusions

Lifetime risk of ovarian cancer is low. While protection against ovarian cancer is one of the most important benefits of oral contraception, it appears just another enigma that HRT would produce opposite effects. There is contradiction in case-control studies worldwide. Taking large prospective studies of ovarian cancer mortality into particular account, these findings add to the inconsistency of previous published data. The US Center for Disease Control (CDC) recently reported the results of a meta-analysis of data from fifteen case-control studies that provided data on ERT and risk of epithelial ovarian cancer [74]. This meta-analysis did not find a significant association of ERT with epithelial ovarian cancer. Furthermore, the CDC evaluation found no clear evidence of an increased risk of ovarian cancer based on increasing dose or lack of estrogen use. The recent report by Carmen Rodriguez et al [69]

associated postmenopausal estrogen use for ten or more years with increased risk of ovarian cancer mortality that persisted up to 29 years after cessation of use. Such data need to be confirmed. Any increase in risk of ovarian cancer mortality due to long-term estrogen use must be considered in the overall balance of potential risks and benefits.

BREAST CANCER

Ever since 1896, when George Beatson, a Scottish surgeon, reported about his experience of a remission of breast cancer following bilateral oophorectomy in premenopausal women [75], the possible relationship of ovarian function and mammary tumourigenesis never escaped our clinical conscience. During that same year of 1896, there were three reports in the German literature on minced extracts of bovine ovaries by F. Mainzer [76], dried extracts from bovine ovaries compressed as tablets by R. Mond [77] as well as aether or aethanol extracts with powder remnants of bovine ovaries compressed to 0.2 g pills by R. Chrobak [78]. These preparations were administered orally to symptomatic postmenopausal women; symptoms clearly tended to disappear and, upon introduction of minced bovine meat as placebo control, recurrence of flushes was observed. Thus, about a hundred years ago, the first successful HRT from ovarian extracts was introduced in parallel to the clinical breast cancer benefit observed in lieu of ovarian ablation. With all the favorable risk-benefit equations attributed to HRT during the last century, the possible relation between estrogen and the risk of breast cancer has remained an oncologic enigma.

Epidemiology and biological plausibility

Several reports on the results of population-based case-control studies in California or Sweden as well as prospective cohort studies (The National Cancer Institute of the US) or nationwide American Breast Cancer Screening Programs have been analyzed in a study subtitled "A clinical response to epidemiological reports" [79]. From this critical review, it was quite apparent that estrogen-alone regimens did not result in a significantly increased risk of breast cancer, even with increasing duration of use up to more than 15 years (odds ratio = 1.06; CI 0.97–1.15). No difference was found comparing current users with past users. Sequential or daily estrogen-progestin regimens were not associated with different responses of localized or advanced disease of any major proportion.

Even those studies that detect an increased risk of breast cancer in hormone users indicate a paradoxical better outcome. It is established that screening facilitates the early detection of breast cancer which might otherwise remain clinically silent for many years. Mammography, our most effective screening tool, advances the time of diagnosis such that in women exposed to estrogens and progestins, screening would likely have resulted in the selective identification of an excess of cases that might otherwise not have been diagnosed or only after the studies were completed. However, lower-grade tumours are present even when there is no difference in the prevalence of mammography, when hormone users and non-users are compared, or when the data are adjusted for the method of detection [80–82]. In the American Breast Cancer Detection Project, current hormone use was associated with a 40 to 60 percent reduc-tion in breast cancer mortality for twelve years after diagnosis [80]. This effect persisted even after correction for cases detected at screening intervals and when in-situ tumours were excluded, indicating the exclusion of detection or surveillance bias. This Project also presented data of protection against breast cancer mortality associated with hormone use that could not be attributed to tumour size, age at diagnosis, BMI, tumour histology, or node status; what may be affected is grade of disease, tumour differentiation and aneuploidy. An access of grade-I tumours has been seen both in users of estrogen alone and of combined estrogen and progestin [83].

In his critical review, Speroff [79] was uncertain about whether or not there is a slight risk of breast cancer (in lean women) with long exposure to estrogen-progestogen and whether or not this conclusion may be imprecise due to bias and small numbers of investigated women. Criteria to strengthen any conclusion of epidemiological findings would be

a. *the strength of the association:*
   the relative risks of the case-control and cohort studies with postmenopausal estrogen-progestin treatment are recognized by epidemiologists as rather weak associations;

b. *consistency, uniformity, and agreement* among many studies are rather scarce, indicating either very small effects or the impact of confounding biases;

c. *a dose-response relationship* is seen after increasing the dose and time of exposure; this aspect may have the best supporting evidence;

d. *temporal relationship* the outcome data with respect to improved survival rates in hormone users support the contention that hormonal treatment promotes the detection of pre-existing tumours.

This epidemiological dilemma enforces questions as to our current insight into tumour biology and breast tissue hormone metabolism. Pathobiology may provide better understanding of the role of hormones in the development and growth of breast cancer. In which way do genetic and environmental factors influence estrogen homeostasis and tissue-specific exposure to estrogen and its metabolites? Ideally, a relation between exposure to
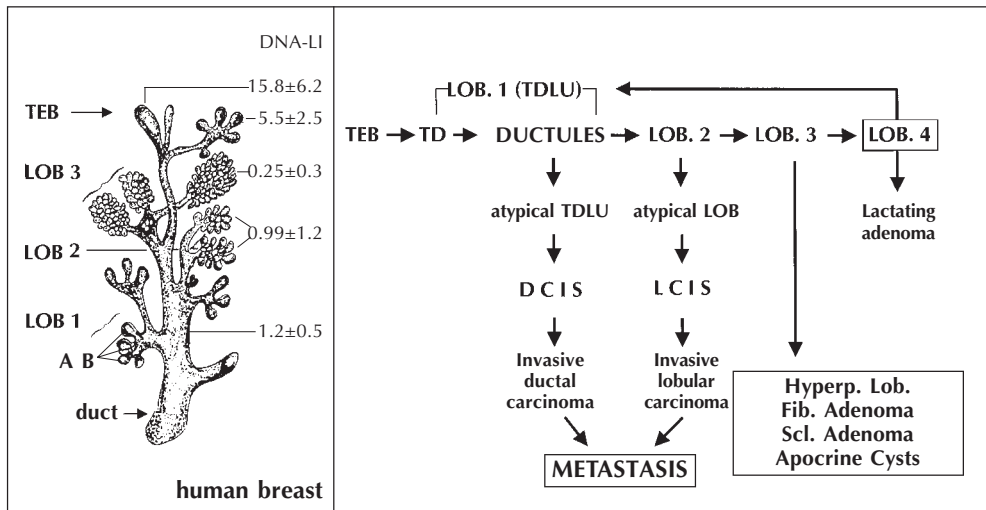

Figure 2. Experimental model of breast cancer carcinogenesis (redrawn acc. to Russo et al [84, 98]). TEB: terminal bud, LOB 1: lobules of I°, LOB 2: lobules of II°, LOB 3: lobules of III°, LOB 4: lobules of IV°, TD: terminal duct, TDLU: terminal ductulo-lobular unit, DCIS: ductal carcinoma in situ, LCIS: lobular carcinoma in situ
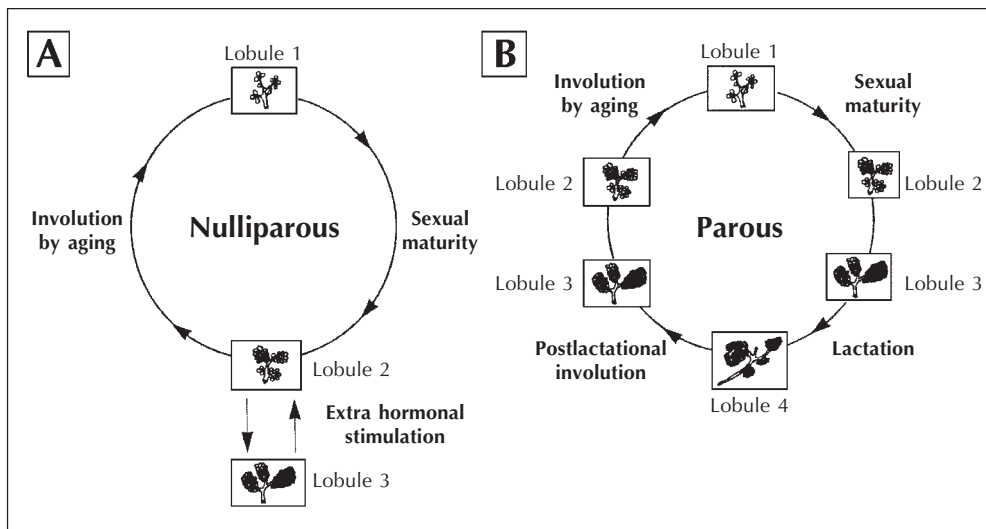

Figure 3. Cyclical development and differentiation of the glandular breast [85]
A: Cyclic variation of breast tissue in nulliparous women
B: Cyclic variation in parous and lactating women

estrogen and risk of breast cancer can be identified in specific groups of women and may allow us to predict risks in the individual.

Estrogen and carcinogenesis of glandular breast

Many lines of evidence suggest that exposure to estrogen is a major risk factor for the development of breast cancer. The response of an organ to the proliferative effects of a hormone may be a progression from normal growth to hyperplasia to neoplasia.

The group of Russo et al from Fox Chase Center in Philadelphia created a plausible experimental model of breast carcinogenesis (figure 2). The message of these investigations points to the biological importance of terminal pregnancy for cellular differentiation of breast tissue and further the preventive character of lactation with its promotion to type IV lobules which will regress at a later phase (figure 3) [84, 85]. The further differentiated a glandular breast will be, the less prone it is to experimental cancerization [9].

In general, the risk of breast cancer could be determined by the cumulative exposure of breast tissue to estrogen [86]. Individual reproductive history supports this contention in that early menarche, late first full-term pregnancy, and late menopause are associated with an increased risk of breast cancer in contrast to the reduced risk seen with early menopause. The relative risk of these hormonally mediated indicators is listed in table 6. The predictive value of these factors is increased

Table 6. Reproductive Indicators of the Risk of Breast Cancer

Indicator	Risk Group		Relative Risk	Reference
	Low	High		
Sex	Male	Female	150.0	Hulka, 1997 [87]
Age (yr)	< 50	≥ 50	6.5	Ries et al., 1999 [88]
Age at Menarche (yr)	≥ 14	< 12	1.2–1.5	Rockhill et al., 1998 [89] Bruzzi et al., 1985 [90] Gail et al., 1989 [91]
Age at birth of first child (yr)	< 20	≥ 30	1.9–3.5	Hulka, 1997 [87] Leon et al., 1995 [92] Madigan et al., 1995 [93] Ramon et al., 1996 [94] Lambe et al., 1996 [95]
Breast-feeding (mo)	≥ 16	0	1.37	Enger et al., 1998 [96]
Parity	≥ 5	0	1.4	Hulka, 1997 [87] Madigan et al., 1995 [93] Ramon et al., 1996 [94] Lambe et al., 1996 [95]
Age at Menopause (yr)	< 45	≥ 55	2.0	Hulka, 1997 [87]
Estrogen therapy	Never	Current	1.06–1.4	Speroff, 2000 [79] Grodstein et al., 1997 [97]
Estrogen-Progestin therapy	Never	Current	1.4	Grodstein et al., 1997 [97]
Postmenopausal BMI	< 22.9	> 30.7	1.6	Hulka, 1997 [87]
Family history of Breast Cancer	No	Yes	2.6	Madigan et al., 1995 [93]

by combining them. As an example, individual age and age at first full-term birth would not only reflect the total exposure to estrogen but also the effect of sex-steroids on final differentiation of the glandular breast induced by pregnancy and lactation as major determinants of susceptibility to cancer [98].

Other contributing factors to individual variation in exposure to estrogen are obesity in postmenopausal women, differences in exercise and dietary intake of certain nutrients. Among the latter, studies of intakes of alcohol, fat, antioxidant vitamins, and fiber have produced conflicting results. Phytoestrogens with their structural similarity to physiologic estrogens, when ingested, have both estrogen agonist and antagonist effects in humans. Flaxseed, a source of mammalian lignans and alpha-linoleic acid, has been shown to exert antiestrogenic effects by binding to estrogen receptors and inhibiting the synthesis of estrogen. The incidence of breast cancer is lowest in regions where the intake of soy, an abundant source of phytoestrogens, or of flaxseed is high; whether or not this inverse relation is direct or only indicative of other influencing factors, is a matter of debate [99].

Breast tissue metabolism of estrogen

There is a significant amount of information showing that breast cancer tissues contain all the enzymes necessary for the formation of estradiol from circulating precursors, including aromatase, sulfatase, and 17β-hydroxy steroid dehydrogenase (17β-HSD) [100–102]. Two main pathways are implicated in estradiol formation in normal breast and breast cancer tissues. The "aromatase pathway" which transforms androgens into estrogens and the "sulfatase pathway" which converts estrone sulfate

(E_1S) into estrone (E_1) which is then transformed into E_2 by the reductive 17β-HSD activity.

Autocrine and paracrine regulation of local estrogen biosynthesis in normal and tumour breast tissue is via growth factors acting upon aromatase activity; this enzyme is preferably expressed in the tumour-bearing quadrant of the breast as compared to distant areas of the same quadrant or other quadrants. Apparently, aromatase regulation operates against a concentration gradient of estrogens, comparing peripheral plasma to local tissue levels both in pre- and postmenopausal women [103, 104].

The fact that estradiol levels in breast tumours of postmenopausal women remain as high as in the premenopause, while plasma levels decrease, clearly points to the discrepancy between these two compartments. It would implicate the necessity of mechanisms that require local factors. In addition, these data, which have been confirmed by several other studies, are important to the hypothesis that local production of estradiol is the source of this steroid at breast tissue level [104]. By the same token, androstendione was found at lower concentrations in the tumour as compared to fatty tissues of all quadrants whereas testosterone did not show this difference. Finally, E_1S is highly concentrated in the tumour.

As androstendione is the major precursor for local estrogen synthesis, this is in accordance with the importance of local aromatase activity. The latter was comparable in all tissues, whereas changes could be seen in the activity of the 17-OH HSD. We measured this enzyme by substrate-to-product conversion, no specific type has been distinguished. One should bear in mind, however, that these laboratory esti-

mates cannot easily be extrapolated to real activity in the tissue because stimulatory and inhibitory factors do play additional roles. Furthermore, the promoter for aromatase in tumours is different from that in fatty tissues. Quantitative evaluation indicates that in human breast tumours, E_1S via sulfatase is 100 to 500 times higher a precursor for E_2 than are androgens via aromatase [105, 106].

Biosynthesis of estrogen

The precise mechanisms controlling estrogen production in postmenopausal women are still unclear. Both cytochrome CYP17 (encoding P-450 17-alpha-hydroxylase) and cytochrome CYP19 (encoding P-450-aromatase) are involved in estrogen biosynthesis; polymorphisms of both genes have been identified in the general population [107, 108]. Women who are heterozygous or homozygous for a cytochrome CYP17 polymorphism have been shown to produce high serum estradiol concentrations; however, this polymorphism is not unequivocally associated with increased risk of breast cancer [99]. There are, however, ongoing studies demonstrating a link between polymorphisms of the P-450-aromatase gene with increased risk of breast cancer [107] (table 7). The estrogen production may also be influenced by variation in tissue-specific promoters of aromatase gene expression [109]. A detailed investigation on the expression of aromatase in human breast tumours [110] has demonstrated a change of promoter I.4 in normal breast tissue to promoter PII and PI.3 in breast cancer, which are more active and may result in increased synthesis of aromatase mRNA.

Promotor functional studies by functional analysis will be essential for a

clear understanding of the control of aromatase expression in breast tumours and its role in cancer development and may involve transcription factors specific to breast cancer cells contributing to the growth of breast tumours in an autocrine or paracrine fashion. The aromatase gene may finally act as an oncogene that initiates tumour formation in breast tissue [99].

Breast tissue sensitivity to estrogen

Estrogens may diffuse passively through cellular and nuclear membranes. On the other hand, specific cells and tissues express estrogen receptors to which estrogen would bind and form a ligand-receptor complex in order to activate specific sequences in the regulatory region of genes responsive to estrogen, known as estrogen-response elements. These genes in turn regulate cell growth and differentiation.

New discoveries about the mechanism of estrogen action represent one of the most important scientific advances of today. Not only do estrogens behave differently from tissue to tissue and from cell to cell, but there are also variations among individual women. Physiologically active doses in one in-

Table 7. Genotype polymorphisms of estrogen-metabolizing genes and risk of breast cancer* [108]

RISK FACTOR	Odds Ratio	95 % CI
CYP 17 encoding P-450 17α-hydroxylase	1.23	0.67–2.28
CYP 1A1 encoding cytochrome P-450 IAI	1.79	0.86–3.78
COMT	4.02	1.12–9.08
TWO PUTATIVE HIGH RISK GENOTYPES	3.52	1.06–12.4

* association higher with prolonged estrogen exposure years

dividual may produce less of an effect in another. Estrogen-receptor levels are low in normal breast tissue, and high levels have been directly correlated with an increased risk of breast cancer [111]. Receptor levels increase with age in some ethnic groups and apparently are higher in white women as compared to black or Japanese women. This phenomenon may be related to the function of a tumour-suppressor gene, the loss of which may result in failure to down-regulate estrogen receptors with resultant defects of the cell cycle and finally driving breast carcinogenesis [112].

The human estrogen receptor (ER) belongs to the nuclear receptor superfamily of ligand-inducible transcription factors. The recent identification of ERβ has indicated that the cellular responses to ER ligands are far more complex. ERα and ERβ interact with the same DNA response elements and exhibit similar, but not identical ligand-binding characteristics. ERβ binds estrogens with a similar affinity to ERα and activates the expression of reporter genes containing estrogen response elements in an estrogen-dependent manner. In vitro, the α and β receptors form heterodimers with each other, and the β receptor decreases the sensitivity of the α form to estrogen, thereby acting as a physiologic regulator of the proliferative effects of the α receptor [113].

In order to evaluate the role of differentiated ERs in breast cancer, the expression of both ER isoforms in normal and malignant breast tissue has been investigated [114]. In normal breast tissue, expression of ERβ predominated, with 22% of samples exclusively ex-
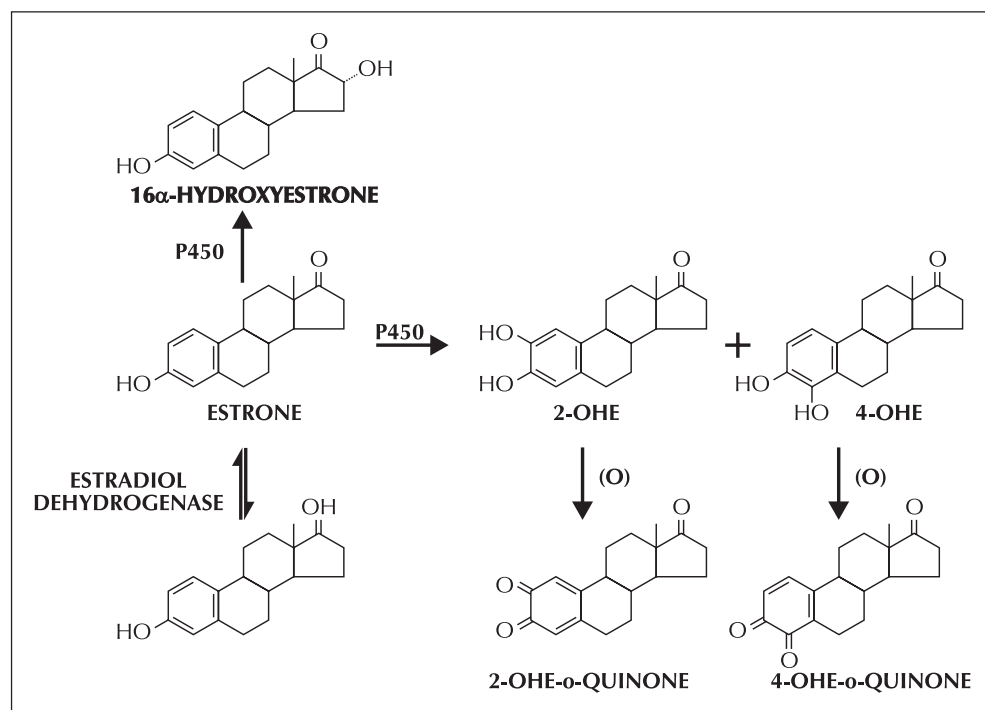

Figure 4. Major metabolites of estrone and 17β-oestradiol [115]. [0] refers to oxidative enzyme or metal ion.

pressing ERβ; this was not observed in any of the breast tumour samples. Most tumours expressed ERα, either alone or in combination with ERβ.

Catabolism of estrogens

Estrogens are catabolized predominantly by hydroxylation with a resultant formation of 2-hydroxy-oestrone and 2-hydroxy-oestradiol, 4-hydroxy-oestrone and 4-hydroxy-oestradiol, and 16α-hydroxy-oestrone and 16α-hydroxy-oestradiol (figure 4) [115]. The 2-hydroxy and 4-hydroxy metabolites are converted to anticarcinogenic methoxylated metabolites (2-methoxy-oestrone and 2-methoxy-oestradiol, 2-hydroxy-oestrone and 2-hydroxy-oestradiol-3-methylether, 4-methoxy-oestrone and 4-methoxy-oestradiol, and 4-hydroxy-oestrone and 4-hydroxy-oestradiol-3-methylether) by catechol O-methyl-transferase (COMT). The catechol metabolites of estrogens are implicated in the carcinogenic and cytotoxic effects of these compounds (figure 5) [115]. They are further metabolized to electrophilic quinoids, such as o-quinones, which can isomerize to their tautomeric p-quinone metides; the roles of these quinoids in mediating the adverse effects of estrogens have not been investigated in detail. It is possible for these electrophilic and redox active quinoids to cause damage within cells by a variety of pathways. Catechol estrogen-mediated redox cycling can cause lipid peroxidation, consumption of reducing equivalents, oxidation of DNA, and DNA single-strand breaks [115].

Postmenopausal women with a variant allele that codes for a COMT with low activity have a higher risk of breast cancer than women with a wild-type allele [116]. On the other hand, 17β-
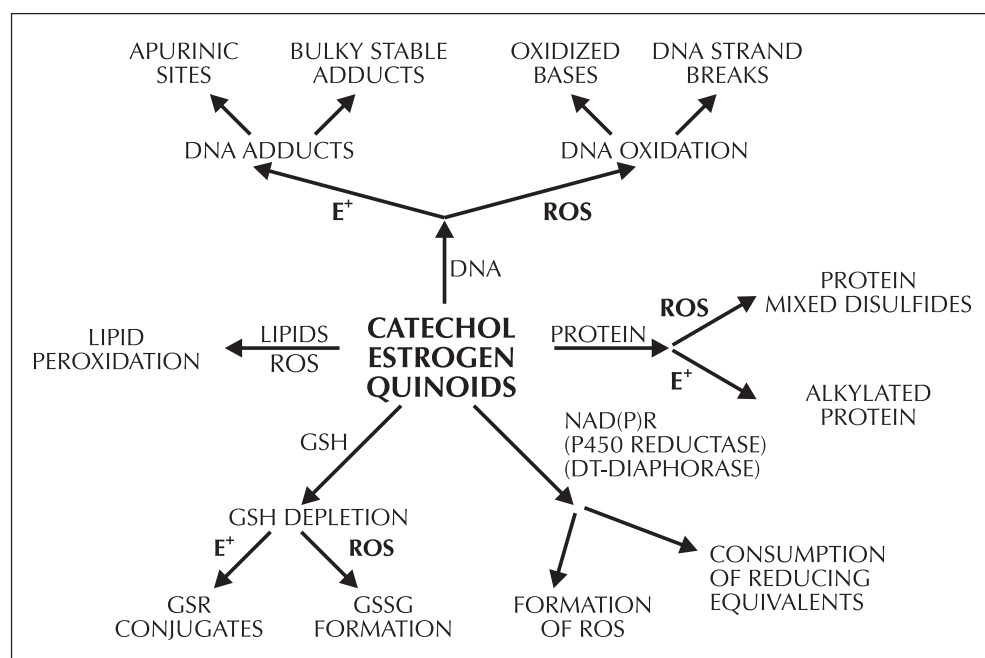

Figure 5. Potential cytotoxic and genotoxic mechanisms of catechol estrogens in vivo [115]. E+ = damage due to alkylation by catechol estrogen quinoids. ROS = oxidative damage by reactive oxygen species

hydroxysteroid dehydrogenase activity is higher in breast tumours than in normal breast tissue [104].

Taking these tissue-specific variations of estrogen production and catabolism into consideration, there is reason to believe that cumulative exposure to estrogen and its metabolites may vary distinctly within individual women. Polymorphisms of cytochrome CYP17, CYP1A1 and COMT are found to be associated with increased risk of breast cancer (table 7) [108]. To identify high-risk genotypes in women may delineate the individual at increased risk of breast cancer.

Clinical response of breast cancer tissue to hormone exposure

In order to further depict normal and cancerous tissue response to hormone exposure, we investigated over 100 postmenopausal women with breast cancer [117]. During cancer surgery, tissue samples were preserved for laboratory work-up in terms of homogenization by microdismembranation, suspension with trasylol, extraction with ethanol-acetone, evaporation of liquid phase and separation, defatting, addition of tracers for recovery and extraction for determination of estrone and estradiol by highly specific radio-immunoassays [118]. Local estrone and estradiol concentrations in terms of fmol/g were compared in cancer tissue versus adjacent or distant normal control tissue. Then these estimates were evaluated in never-users of HRT versus ever-users of HRT. As this investigation is still in progress and will further involve the expression of local enzyme activities as well as the production of steroid metabolites and of estrogen receptors, we can only report about our preliminary experience. While estrone and estradiol levels, as seen before, did not vary in cancer tissue as compared to neighbouring normal breast tissue, it was also not evident that HRT would produce any remarkable difference in local estrogen concentration. The modes of HRT included sequential and combination-type regimens.

Such observations would suggest that oral hormone replacement, given a concentration gradient of plasma versus breast tissue levels of more than an order of magnitude, would not have any demonstrable impact on local breast tissue estrogen metabolism. There is good reason to abstain from over-interpretation of such preliminary data. However, such observations would reconcile our clinical experience with any endocrine therapy. It would only result in tumour regression when local breast tissue hormone metabolism is affected as seen with SERMs or aromatase inhibitors.

Effect of HRT on mortality and in breast cancer survivors

If estrogen replacement were of any major harm to women who survived breast cancer and its treatment, one would expect an unfavorable prognosis in women who developed breast cancer during estrogen replacement. However, women diagnosed while on hormone replacement have a better prognosis [119]. Women with a diagnosis of breast cancer within one year following discontinuation of estrogen treatment will survive longer than non-hormone users or women who last took their estrogens for longer than one year [120]. In this investigation, Gambrell pointed to the fact of breast cancer mortality of 22% being diagnosed while under ERT as compared to 46% in non-users (p < 0.002). In that situation, 57% of hormone users were lymph-node-negative as compared to

42% of non-users; within the lymph-node-negative group, mortality rated 8% for hormone users and 25% for non-users (p < 0.05). Henderson and co-workers [121] confirmed this experience and reported on a 19% reduction of breast cancer mortality among 4,988 women using ERT as compared to 3,865 non-users who later on developed breast tumours. Relative breast cancer mortality in women while under HRT is documented from nine different studies in figure 6.

Conclusion

Estrogen is important in the maturation and differentiation of normal breast tissue and is associated with most of the epidemiological risk factors of breast cancer. Estrogens will proliferate normal ductal epithelia in the non-cancerous breast during the menstrual cycle and in pregnancy and will act on these cells via two distinct estrogen receptors. Genetic and environmental factors influence estrogen homeostasis and tissue-specific exposure to estrogen and its metabolites. Whether or not cumulative life-time exposure to estrogen
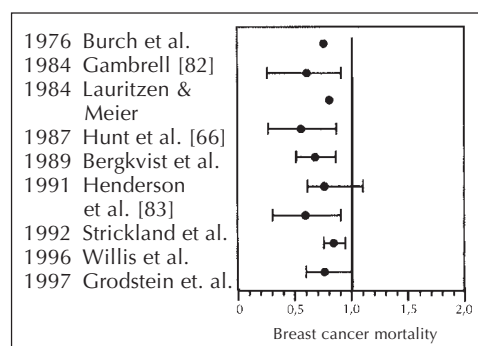

Figure 6. Breast cancer mortality in current HRT users: results of mortality from nine studies. The points indicate relative breast cancer mortality when the disease was diagnosed while under HRT. 95% confidence intervals are given as far as available [122]

has any bearing on breast tissue metabolism remains unclear. A large body of data supports the hypothesis that estrogen and its metabolites may be related to the promotion of pre-existing breast cancer. Genetic disposition to polymorphisms of key metabolic enzymes may dispose the individual to the formation of estrogen metabolites which are toxic to DNA strands. Only that way can breast cancer initiation by estrogen be postulated. There is, however, no indication so far of generally practiced oral hormone replacement therapy to specifically alter local breast tissue estrogen metabolism both in normal or cancer tissue.

CANCERS OF OTHER REPRODUC-TIVE ORGANS

This will be summarizing reports on our clinical experience with other uterine, vulvar and vaginal tumours.

Cervical cancer

Annual incidence rates of cervical cancer per 100,000 women vary widely between 48.2 in Columbia and 3.8 in Israel, whereby developing countries present with higher incidences as compared to industrial nations. In Germany, about 20 of 100,000 will be diagnosed the disease within a year. This refers to about 6% of all female neoplasias. Its age-related maximum is with 45–54 years, and in-situ lesions will peak at ages 35–44.

Although the papilloma virus is considered to be the primary initiator of this tumour, there has always been a debate as to whether those 10% of invasive tumours which are of adenomatous type, could be hormone-depend-

ent. However, no correlation of HRT with cervical cancer could be demonstrated (table 8).

Although the endocervical epithelium does contain estrogen and progestogen receptors and varies according to female steroid hormone influences, a correlation between steroid hormones and cervical cancer has never been shown [60]. There is a report 120 women treated for stage I and stage II cervical cancer [123]. Neither recurrence nor survivor rates over a 5-year period were influenced negatively by HRT. Smith et al [124] reported on a group of 105 women receiving HRT; the incidence of human papilloma virus in the HRT users was not any different from a control group while hormone treatment might theoretically influence steroid receptor expression in the adenomatous epithelium of the endocervix, no correlation was seen with HRT or oral contraceptives [125].

Cancer of the vagina and vulva

Morphologically, the upper two-thirds of the vagina are of Mullerian origin, the lower third is ectodermal squamous epithelium of the sinus urogenitalis. The vaginal epithelium contains estrogen as well as progestogen receptors and its response to estrogens is well known; estrogen replacement will produce optimal short-term effects.

The incidence of vaginal cancer is 1–2% of all gynecological malignomas,

it varies widely over ages 25–84 and will peak at ages 60–70. There is a papilloma virus predisposition.

Carcinoma of the vulva represents 3–5% of all genital malignancies with an age peak of 65 years, however, 15% will be seen in women younger than 40 years. Most of these tumours (90%) are of squamous epithelial origin, melanomas represent 4.8%, adenocarcinoma only 0.6%.

A summarizing statement is listed in table 9. No information is available about any relation of HRT with cancers of the vagina and vulva [60].

Uterine sarcoma

Finally, we would like to point to sarcomas of the uterus which represent 2–4% of all malignant uterine tumours. Histologically speaking, there are carcinosarcomas, leiomyosarcomas, endometrioid sarcomas and adenosarcomas. They all are fast-growing tumours and very often are mistaken for uterine fibroids. Prognosis is rather bad with a 5-year survival rate of 20–30%.

Therefore, it would be of particular interest to know whether any of the histologic types of uterine sarcomas may be hormone-dependent. It has been suggested that the hormonal status is correlated to survival of sarcomas since premenopausal women have a better survival (50%) compared to post-menopausal (30%). Sarcomatous tissues of the uterus contain estrogen or

Table 8. HRT and cervical cancer

- Endocervical epithelium is ER- and PR-positive; cyclic variation has been observed, but does not correlate with cancer
- Invasive tumours are 90% squamous epithelium, 10% adenomatous; no response of squamous epithelium to estrogens
- Infection with human papilloma virus not affected by HRT [60]

Table 9. HRT and vaginal and vulvar cancer

- Almost entirely of squamous cell origin
- Vaginal   0.5 per 100,000
  Vulva   2.0 per 100,000
- Vaginal epithelium both ER- and PR-positiv
- No correlation with HRT use [60]

progestogen receptors [126]. It has been suggested to determine estrogen and progestogen receptors in leiomyosarcomas in order to consider anti-estrogenic treatment in receptor-positive patients [127]. There is a report by Schwartz et al [128] who found a positive, non-significant association (RR 1.7, 95% CI 0.7–4.1) between oral contraceptive use and leiomyosarcomas of the uterus in women who were on OCs even 15 or more years prior to diagnosis. There are, however, no reports on HRT affecting the risk of leiomyosarcoma. Even if we look at these carcinomas as endocrine-related like endometrial cancer, HRT would not be contraindicated in patients treated for leiomyosarcoma of the uterus. Consequently, HRT should consist of estrogen and progestogen combination therapy (table 10).

Colon and rectal carcinoma

Recently, there was a series of publications on colon and rectal carcinomas and their relation to HRT [129]. Gustafsson and Enmark [57] have reported on ERβ expression along the mucosal lining of the gastro-intestinal tract. ERβ expression with its potential of inhibiting ERα activity might provide tumour protection. The incidence of colon cancers in man and woman is about 6%. When on HRT, women will

Table 10. HRT and sarcoma of the uterus

- 1% of all gynecological malignancies
- Survival rates at:
  premenopause 50%
  postmenopause 30%
- Sarcomatous uterine tissue is ER-positive and PR-positiv
- No reports on any influence of HRT
- Estrogen and progestogen replacement should be considered in survivers of uterine sarcoma of the endometrioid type

experience a 20% reduction of colon cancers and a 15% reduction of rectal cancers.

CONCLUDING REMARKS

This review on hormone replacement in malignancies of reproductive organs should demonstrate that estrogens are important in the growth and differentiation of hormone-responsive tissue. While they will proliferate surface epithelia of the ductal breast, the vagina and others, there is no doubt that genetic and environmental factors influence estrogen homeostasis and tissue-specific exposure to estrogen and its metabolites. Accumulative life-time exposure to estrogen may have a bearing on the metabolism of hormone-responsive organs. Genetic disposition to polymorphism of key metabolic enzymes with a resultant formation of toxic metabolites may be one of the reasons why in some individuals, estrogen exposition might involve cancerogenesis.

Clinical experience, however, points to major benefits in that mortality is reduced in breast cancer women with HRT exposure; in other words, women who develop breast cancer within a year of discontinuing estrogen replacement will survive longer than non-users.

The increased risk of endometrial cancer following long-term exposure to estrogens only can successfully be counteracted by the appropriate addition of progestins. The apoptotic potential of progestins particularly of the 19-norprogesterone variety might also apply to breast tumours.

In the presence of a low life-time risk of ovarian cancer, while these tumours are still diagnosed at later stages and therefore produce unfavorable outcomes, it should be taken into account

that literature is still inconsistent with respect to ovarian cancer incidence as related to HRT. The recent report of the CDC of an association of long-term postmenopausal estrogen use with increased risk of ovarian cancer mortality needs to be confirmed.

It is reassuring to note that other reproductive tumours such as cancers of the cervix, vulva and vagina do not seem to be related to HRT use. Taken altogether, overall cancer mortality is reduced in current or ever HRT users.

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MENOPAUSE
ANDROPAUSE

Hormone replacement therapy through the ages
New cognition and therapy concepts