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The Chemoprevention of Breast Cancer by Reducing Sex Steroid Exposure: Perspectives From Epidemiology

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Abstract Mitogenesis is a major driving force in neoplastic development. Blocking the effect of breast cell mitogens by reducing the actual exposure of the breast to these mitogens is an obvious strategy for breast cancer prevention. The ovarian hormones, estrogens and progesterone, are major effective (direct or indirect) breast cell mitogens. A woman's exposure to ovarian estrogens and progesterone is drastically reduced by the use of combination-type oral contraceptives (COCs), but the synthetic estrogen and progestogen in the COCs effectively replace ovarian estrogens and progesterone, so that breast cell exposure to these hormones is not decreased. Doses of estrogen and progestogen in modern COCs are close to the minimum attainable while still retaining both contraceptive efficacy and ovarian suppression (so that endogenous estrogen and progesterone do not add to the dose of estrogen and progestogen from the COC). Considerably lower effective breast cell exposure to estrogen and progestogen can, however, be achieved by using a gonadotropin-releasing hormone agonist (GnRHA) to suppress ovarian function and compensate for the resulting hypoestrogenemia with low-dose hormone replacement therapy. Compared to modern COCs, estrogen exposure can be reduced by approximately 60%, and progestogen dose by more than 80%. Such a contraceptive is predicted to reduce *lifetime* breast cancer risk by more than 50% if used for 10 years. The possibility that a practical contraceptive could achieve such a major benefit is shown by the dramatic decline in the incidence of both ovarian and endometrial cancer in young women in the U.S. over the last 3 decades—a direct result of COC use.

Key words: Breast cancer, chemoprevention, estrogen, oral contraceptives, progestogen

As Boone and Kelloff [1] have stated, "The two driving forces of neoplastic progression in an epithelium are mutagenesis and mitogenesis. . . . The major strategy of chemoprevention is to block the effects of both mutagens and mitogens." At present, little is known about important breast mutagens (it is possible that there are no predominant exogenous breast mutagens), but much is known about breast cell mitogens. The most obvious method of chemoprevention of breast cancer currently available is to block the effect of breast cell mitogens by reducing their availability to the breast.

Overwhelming evidence indicates that breast cell division is controlled by the ovarian steroids, estrogen and progesterone, and that reducing breast exposure to these mitogens significantly reduces the risk of cancer [2,3]. The most direct evidence of the latter is provided by the epidemiological observation that bilateral oophorecto-

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my before age 35 reduces subsequent breast cancer risk by approximately 70% [4].

A non-surgical reversible bilateral oophorectomy can currently be achieved with a gonadotropin-releasing hormone agonist (GnRHA). Long-term use of a GnRHA will therefore significantly reduce breast cancer risk [5]. Such a medical oophorectomy will be associated with significant side effects, as is a bilateral oophorectomy. We have shown in a pilot trial that such side effects can be avoided with the use of low-dose, add-back sex steroids [6,7]. Epidemiological evidence shows that this therapy will only affect the breast cancer prevention aspect of GnRHA use to a minor extent [4,5].

In this paper we first describe the critical evidence concerning the effects of estrogen and progesterone on breast cell proliferation and breast cancer risk, followed by our pilot trial regimen, its predicted effects on breast cancer risk, and its observed beneficial effects on breast mammographic patterns, and our current approach to developing a practical chemoprevention regimen based on these findings. This approach consists of a single injection given 3 or 4 times a year. If such a regimen is successfully developed, lifetime breast cancer risk could be reduced by more than 50% if the regimen is used for 10 years, and lifetime ovarian cancer risk should be reduced by more than two-thirds.

The use of combination-type oral contraceptives (COCs) has led to a dramatic decline in the incidence of ovarian and endometrial cancers in the U.S. over the last 3 decades [8]. For women below age 50, ovarian cancer incidence has declined by 20% and endometrial cancer incidence has declined by almost 30%, demonstrating that the attempt to develop a practical chemoprevention regimen for breast cancer based on a hormonal approach is a most realistic goal to pursue.

THE PROTECTIVE EFFECT OF EARLY MENOPAUSE

The age-incidences (*i.e.*, annual age-specific rates of occurrence) of common non-hormone-dependent adult cancers rise continuously and increasingly rapidly with age [9]. These curves are plotted on a log-log basis for etiological purposes, because such plots usually approximate a straight line [10]. The incidence at age t, I(t), of such a cancer rises as a power of age (the power is usually between 4 and 6) [10,11].

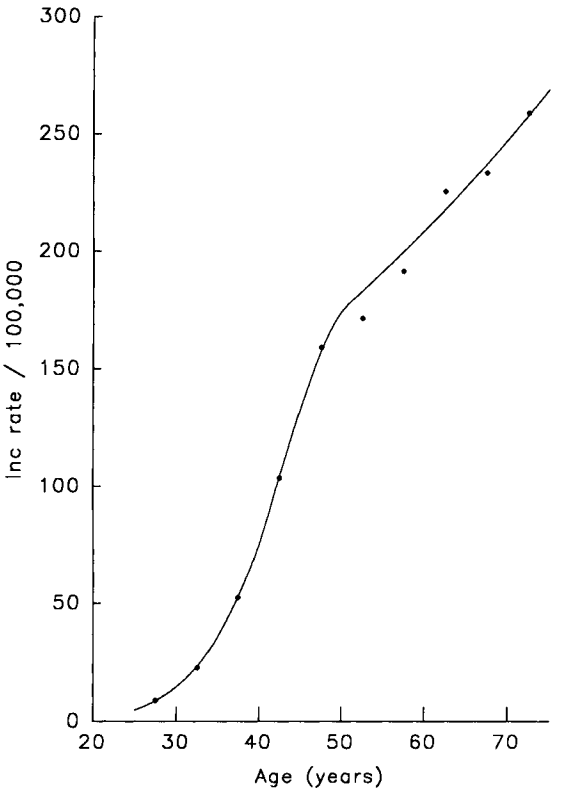

Fig. 1: Age-specific incidence rates (per 100,000) for breast cancer in U.S. white women, 1969–1971.

When plotting age-incidence curves on a log-log scale, deviations from a straight line are immediately obvious and point to some unusual process taking place. Figure 1 shows the age-incidence curve for breast cancer. Breast cancer incidence continues to increase with age, but there is an apparently transitory slowing of the increase from about age 50 to about age 60. A log-log plot shows the situation much more clearly (Fig. 2). There is a distinct slowing of the rate of breast cancer increase around age 50, *i.e.*, around the average age at menopause, and this slowed rate of increase continues from about age 50 on. The important etiologic elements for breast cancer thus appear to be present in premenopausal women, and to be sharply reduced following menopause. This profoundly important conclusion has been verified by direct epidemiological study [12–16], and is the key epidemiological observation on the relationship of ovarian hormones to breast cancer risk.

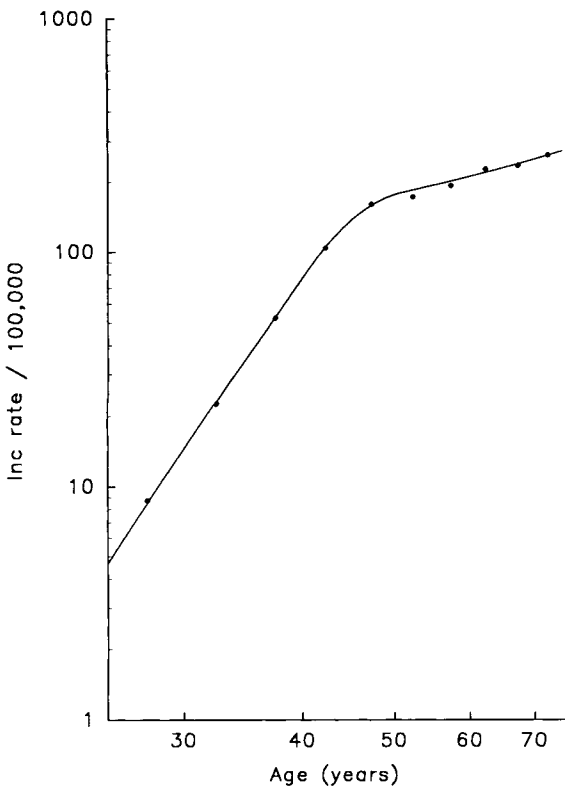

Fig. 2: Log-log plot of age-specific incidence rates (per 100,000) for breast cancer in U.S. white women, 1969–1971.

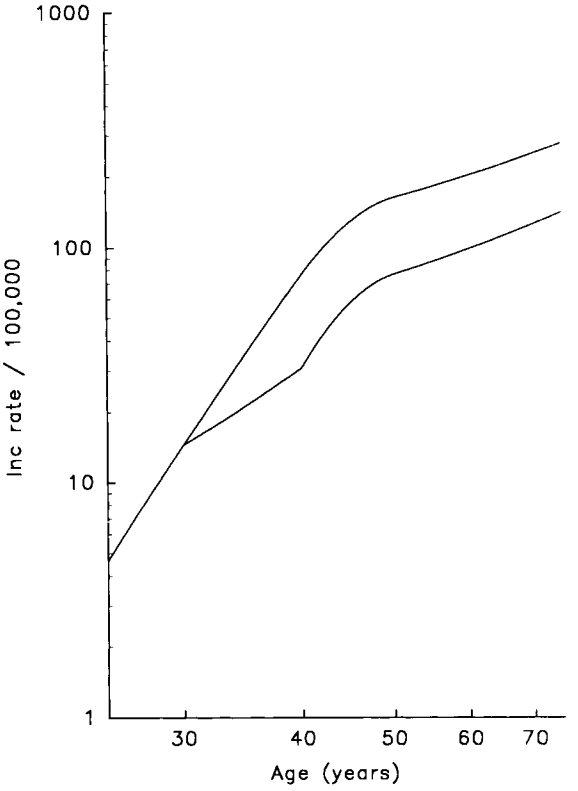

Fig. 3: Log-log plot of breast cancer age-specific incidence rates (per 100,000): 'normal' curve and calculated curve based on using the first prototype contraceptive regimen for 10 years.

Although the rate of increase in breast cancer slows considerably after menopause, the incidence continues to increase in the U.S. and other Western countries. In certain low-risk Asian countries the incidence rate has, until recently, remained constant after menopause; there are no examples of cohort-specific incidence curves going down after menopause. This strongly suggests that whatever happens to increase (or decrease) incidence is, in most instances, not reversible; *i.e.*, that factors which increase (or decrease) risk at any particular time will cause lifelong increased (or decreased) incidence rates.

MITOTIC ACTIVITY IN NORMAL BREAST CELLS

Although the ovary produces other hormones, the ovarian hormones generally considered to play critical roles in affecting breast cancer risk are estradiol and progesterone. The functioning ovary produces relatively large amounts of both estradiol and progesterone in a cyclic fashion, resulting in a distinctive pattern of serum levels of these hormones in premenopausal women (Fig. 3) [17]. In postmenopausal women, serum estradiol levels are approximately constant at roughly one-third of the lowest premenopausal level, and serum progesterone levels are effectively zero.

Tritiated thymidine labeling studies of normal breast epithelial cell division have shown that labeling is 2- to 2.5-fold higher in the luteal phase (second half of the menstrual cycle) than in the follicular phase (Fig. 3), and epithelial cell mitotic counts behave similarly [18]. In the postmenopausal period, when estrogen levels are low and progesterone is absent, rates of breast cell proliferation are very low [19].

The very low rate of breast cell division in the postmenopausal period compared to the follicular phase strongly suggests that estrogen alone induces some breast cell division, and the mitotic

rate pattern over the menstrual cycle suggests that estrogen and progesterone together induce much more cell division. This estrogen-augmented-by-progestogen hypothesis can effectively explain almost all of the known critical breast cancer risk factors.

BREAST CELL PROLIFERATION AND KEY EPIDEMIOLOGICAL EVIDENCE

Several key epidemiological observations [2,18] have been made on the relationship of ovarian hormones to breast cancer risk. Early menopause (natural or bilateral oophorectomy) reduces risk. Postmenopausal obesity increases risk, but premenopausal obesity decreases risk. Menopausal estrogen replacement therapy (ERT) only increases risk to a relatively small extent. COCs do not decrease risk. Depot medroxyprogesterone acetate (DMPA; Depo Provera®, Upjohn, Kalamazoo, Michigan) does not decrease risk.

Early menopause reduces the risk of breast cancer by reducing levels of both estrogen and progesterone.

For older postmenopausal women, breast cancer risk increases with weight [20]. In contrast, in premenopausal women, increasing weight has been associated with a small decrease in risk [20]. The increased anovulation and increased frequency of low progesterone levels in the luteal phase (progesterone values are, on average, approximately half of 'normal' values) associated with premenopausal obesity markedly decrease breast exposure to progesterone, while bioavailable estradiol appears to be almost unchanged during ovulatory cycles [21,22] and decreased during anovulatory cycles. After menopause, the decreased risk associated with premenopausal obesity is gradually eliminated, and an increased risk is finally achieved by the increased levels of bioavailable estrogen in obese postmenopausal women compared with normal weight postmenopausal women. The contrasting effects of obesity in the premenopausal and postmenopausal periods can thus be readily explained by the estrogen-augmented-by-progestogen hypothesis.

Postmenopausal women on ERT have about twice the normal annual rate of increase in breast cancer incidence [23,24]. Data from European studies suggest a slightly larger figure; this can be ascribed to their use of higher doses of estrogen. Except for the study by Bergkvist and colleagues [25,26], these studies have effectively evaluated the effect of estrogen alone, not estrogen plus a progestogen. Bergkvist and colleagues provided some sparse data on the effect of combination estrogen plus progestogen on breast cancer risk. In their study, the relative risks for long-term combination estrogen plus progestogen were considerably higher than any found for users of estrogen alone. The estrogen-augmented-by-progestogen hypothesis predicts that ERT will increase breast cancer risk, and that the addition of a progestogen will increase risk further. In the U.S. (before the recent increases due to screening), breast cancer incidence increased about 2.1% per year of age in the postmenopausal period. This rise can be attributed solely to endogenous estrogens [11]; the breast cancer risk associated with ERT can be predicted by comparing endogenous serum estrogen levels to the serum estrogen levels achieved during ERT. In postmenopausal women, the serum level of bioavailable estradiol is approximately 12 pg/ml [27]. The serum level of non-sex hormone binding globulin (SHBG)-bound estradiol in women taking conjugated estrogens (CEs) is approximately double this [27]. Assuming that estradiol is the most important estrogen both from endogenous sources and from ERT, the incremental increase in breast cancer risk due to ERT should be approximately equal to that due to endogenous estrogens. This is precisely what is observed.

At present, there are insufficient data on the effects of ERT plus progestogen on breast cancer risk. The 10 mg/day of medroxyprogesterone acetate (MPA) usually prescribed as progestogen with conjugated estrogens has been estimated to be equivalent to the usual 1 mg dose of norethindrone (NET) used in COCs [28]. This dose of progestogen, when taken in combination with 30–50 μg of ethinylestradiol (EE2) as a COC, induces as much breast cell division as a normal menstrual cycle [18]. The estrogen-augmented-by-progestogen hypothesis predicts that the effect of postmenopausal estrogen plus progestogen therapy would be greater than that of ERT alone. The sparse available data support this prediction [25,26].

Epidemiological studies of COC use and breast cancer risk have found some evidence of

an increased risk [24], but the effect appears small and possibly confined to young women. One would predict that breast cell proliferation in women taking COCs would be less than, equal to, or greater than that observed during a normal menstrual cycle, depending on the doses of estrogen and progestogen in the particular COC. However, the situation is complicated, because the combined estrogen and progestogen of the COC are present for three-fourths of the 28-day COC cycle, whereas in the natural cycle, *progesterone* is only effectively present for half of the cycle (the luteal phase). COCs also contain the synthetic estrogen ethinylestradiol combined with one of a number of synthetic progestogens, so a direct comparison with normal ovarian steroid levels is not possible. Thus, it is not possible to predict the associated breast cell proliferation rate with any confidence. Direct observational studies of breast cell proliferation suggest that the total breast cell proliferation may be very similar over a COC cycle and over a normal menstrual cycle [18]. This explains why oral contraceptive use is not associated with marked change in breast cancer risk. It appears likely that if total breast cell proliferation in pill-regulated cycles is close to that occurring in normal cycles, then one would predict what has been found in epidemiologic studies, *i.e.*, only a small effect of COC use on breast cancer rates.

DMPA is a long-lasting (3 months) injectable progestogen contraceptive that effectively suppresses ovulation. The two population-based epidemiologic studies of the effect of DMPA on breast cancer risk found some evidence that DMPA may increase breast cancer risk. Neither study found any evidence that DMPA reduces risk [24]. DMPA use is associated with reduced ovarian estrogen levels (serum estradiol levels are somewhat less than normal early follicular levels [29,30]) and very low progesterone levels. These data effectively show that progestogens are breast cell mitogens, since an estrogen-alone hypothesis for breast cell proliferation requires a dose-response relationship between estradiol levels and breast cell proliferation in the low follicular to high follicular (and luteal) range to explain the increased breast cell proliferation in the luteal phase of the cycle. This would imply that the low estradiol levels associated with DMPA use would be associated with reduced breast cancer risk, and this has not been found.

OTHER BREAST CANCER RISK FACTORS

First Full-Term Pregnancy

Late first full-term pregnancy is a major breast cancer risk factor. MacMahon *et al.* [31], in their international case-control study, found that women with a first full-term pregnancy under age 20 had about one-half the risk of nulliparous women, but that nulliparous women did not have as high a risk as women with a first full-term pregnancy after age 35. Increasing parity appears to cause further small decreases in risk.

These complex effects of first birth and parity are not immediately explicable in terms of their effects on breast cell proliferation. Animal models suggest that the effects are a combination of increased cell division during the first two trimesters of pregnancy, and the counteracting effect of a long-term decrease in the number of stem cells, brought about by breast stem cell differentiation during pregnancy, or a change in responsiveness of breast stem cells to hormonal stimuli [32].

International Comparisons

Breast cancer rates in the U.S. have, until recently, been some 4- to 6-fold greater than rates in Japan [11]. Low postmenopausal weight (approximately 50 kg in Japan compared with approximately 67 kg in the U.S.) and late menarche (two years later in Japan) explain approximately half of this difference [11]. Late menarche is associated with a delay in the onset of breast cell proliferation; and the much reduced weight of postmenopausal Japanese women will lead to very low postmenopausal estrogen levels and therefore to a near-zero breast cell mitotic rate. The predicted U.S. breast cancer incidence rate with a two-year delay in menarche and a low postmenopausal weight is, however, still 2.5-fold higher than the Japanese rates. Accounting for this by a mitotic rate hypothesis requires that the premenopausal breast cell mitotic rate of Japanese women be 20% less than that of U.S. women [11]. Such a reduction in premenopausal mitotic rate would likely result from an approximately 20% reduction in "effective" premenopausal hormone levels (averaged in some way over estradiol and progesterone over the menstrual cycle).

MacMahon *et al.* [33] showed in studies done in the early 1970s that urinary CE levels were much reduced in premenopausal Japanese women. Three recent studies of premenopausal women [34–36] showed clearly reduced estradiol levels in Asian women maintaining a lifestyle like that of the Asian women who enjoy a low breast cancer rate. Goldin *et al.* [34] found a 44% reduction of estradiol in Asian women; Bernstein *et al.* [35] found an 18% reduction; and Key *et al.* [36] found a 30% reduction. No differences in progesterone levels were reported in the single study that investigated this [35]. These results show that the premenopausal estradiol levels of Japanese women with 4- to 6-fold lower breast cancer rates could easily have been 20% lower than the levels in Western women. This would provide a complete hormonal (mitotic rate) explanation of the difference between Japanese and U.S. breast cancer rates. There have been no studies of breast cell mitotic rates in Japanese (or other Asian) women.

THE CHEMOPREVENTION OF BREAST CANCER

We have described the basis of the estrogen-augmented-by-progestogen hypothesis for breast cell proliferation (and hence of breast cancer risk). We argued that present day COCs do not protect against breast cancer because they deliver estrogen plus progestogen to the breast in quantities sufficient to replace the actions of the naturally produced estrogen and progesterone of the normal menstrual cycle. The dose of sex steroids in COCs is close to the lowest dose possible for preventing ovulation, and it does not appear possible to achieve a reduction in breast cancer risk by reducing the dose of steroids in conventional COCs.

COCs achieve two separate goals. The first is to prevent ovulation, and the second is to counteract the effects of the hypoestrogenemia caused by the ovarian failure associated with the first goal. The progestogen component of COCs has a vital role in suppressing ovulation, but a minor role (as regards bone metabolism) in dealing with the associated hypoestrogenemia, which is dealt with by the estrogen component of the COC. The lowest estrogen dose in conventional COCs is 30 μg of EE2. If the first goal of COCs, *i.e.*, preventing ovulation, could be achieved by some other means, could this dose of estrogen be reduced further? How much estrogen is required to control menopausal hypoestrogenemia, in particular hot flashes, and adverse changes in serum cholesterol and calcium balance?

CE is the estrogen most frequently used as ERT in the U.S., but CE is not used in contraceptive formulations. EE2 is the only estrogen used in COCS, but it is not often used as ERT; as a consequence, less is known about the dose of EE2 required as ERT. A daily dose of 5–10 μg of EE2 has been suggested as adequate [36,37]. Studies of small numbers of women indicate that 5 μg may be sufficient to control hot flashes [39] and vaginal atrophy [37], and that 10 μg is more than sufficient to achieve the required effect on serum lipoproteins [40]. However, detailed studies of the minimal effective dose of EE2 to prevent loss of bone mineral density (BMD) are lacking. Available studies suggest that the required dose will be in the 5–15 μg range [41,42], *i.e.*, at most half the dose used in current low-dose COCs.

GnRHAs, when given chronically, inhibit pituitary release of follicle stimulating hormone (FSH) and luteinizing hormone (LH), reversibly inhibit ovulation, and reduce ovarian sex steroid production to postmenopausal levels [43]. Thus, the reversible ovulation-inhibiting function of COCs can be achieved by using a GnRHA. This enables one to concentrate solely on finding the combination of add-back sex steroids of greatest benefit to a woman's health. We noted above that a daily dose of approximately 10 μg of EE2 appears likely to be all the estrogen needed. Some progestogen is needed to control any endometrial hyperplasia which may be caused by the EE2; this is likely to be achieved satisfactorily by giving a progestogen for 13 days once every 4 months. It appears possible to significantly reduce the dose of estrogen and progestogen in COCs if a GnRHA is used to block ovarian function.

Our first prototype GnRHA-based contraceptive regimen is shown in Table I, and was placed on pilot clinical trial at our institution some 3 years ago [6]. We used the 28-day depot form of the GnRHA, leuprolide. We chose to use CE rather than EE2, since more data exist for this form of ERT. There appeared to be ample data suggesting that 0.625 mg per day of CE for 24 days per 28-day cycle would prevent hot flashes,

TABLE I. First Prototype Contraceptive Regimen

Agent	Rationale
GnRHA - Leuprolide depot Day 1 every 28-day cycle	Induce a reversible medical oophorectomy, reducing risk of breast, ovarian, and endometrial cancer
Estrogen - CE 0.625 mg po, 24 days per 28-day cycle Monday through Saturday	Prevent bone mineral loss, prevent possible rise in cardiovascular disease risk, prevent menopausal symptoms, and prevent urogenital atrophy
Progestogen - MPA 10 mg po for 13 days, every fourth cycle	Reverse any endometrial hyperplasia, and prevent any possible increased risk of endometrial cancer

loss of BMD [44,45], and induce a beneficial effect on cholesterol [46,47]; 0.625 mg of CE is roughly equivalent to 5–10 μg of EE2 [48]. Administering progestogen only every fourth cycle minimized breast exposure, and preserved the maximum beneficial effects of ERT on cardiovascular disease risk, while maintaining some beneficial effect on the endometrium. We estimated that the low-dose CE, as in this first prototype contraceptive regimen, would be unlikely to lead to endometrial cell proliferation greater than that occurring during a normal menstrual cycle, especially since progestogen was given every fourth cycle [4,5]. The minimum duration necessary to *control* endometrial hyperplasia completely appears to be 12–13 days of progestogen therapy [49,50]. A small proportion of women will develop hyperplasia if progestogens are not given every 28-day cycle, but few will develop symptoms, and there is evidence that a 13-day progestogen course every 4 cycles will eliminate any hyperplasia that has developed [51]. We chose for convenience to use MPA as the progestogen rather than one of the usual progestogens used in COCs. MPA is the usual progestogen used with CE in the U.S. Ten mg of MPA has been estimated to be equivalent to the usual 1 mg dose of NET used in COCs [28].

If we equate 0.625 mg of CE to 10 μg of EE2, then this prototype contraceptive regimen has 38% of the total EE2 dose of a 30 μg EE2 COC; it will also have lower associated endogenous estrogen levels. Low-dose COCs permit some follicle development (and estrogen production), whereas GnRHA use at the dose proposed does not. Similarly, the total progestogen dose of this prototype contraceptive regimen is 15% of that of a 1 mg NET COC. The effect of the prototype contraceptive on breast cancer risk may be estimated from the epidemiological studies discussed above; in particular, studies documenting the substantial protective effect of bilateral oophorectomy and the small increased risk associated with use of CE. Administration of a progestogen every fourth cycle would be expected to have a small additional effect on breast cancer risk.

TABLE II. Predicted Reduction in Lifetime Cancer Risk With First Prototype Contraceptive Regimen

	Duration of Regimen (yrs)		
	5	10	15
Breast	31%	53%	70%
Ovary	41%	67%	84%
Endometrium	18%	33%	45%

We employed a mathematical model to calculate the protective effects of this prototype contraceptive regimen on breast cancer [4,5]; the results are shown in Table II. The regimen is calculated to reduce lifetime breast cancer risk by 31% if used for 5 years, 53% if used for 10 years, and 70% if used for 15 years. Although these figures were calculated from a mathematical model, they can be seen to be close to correct by comparing the 10 and 15 year figures to the known effects of early oophorectomy. Epidemiological studies have found that early oophorecto-

my with no ERT is associated with about a 65–75% reduction in breast cancer risk. The figures we calculated for our regimen are effectively these figures reduced to account for the small increased risk of breast cancer associated with ERT use. Figure 3 illustrates the effect on the breast cancer age-incidence curve with 10 years of use of this regimen.

Table II also shows the predicted relative risks for ovarian cancer using the prototype contraceptive; the regimen is predicted to reduce the lifetime risk of ovarian cancer by 41% if used for 5 years, 67% if used for 10 years, and 84% if used for 15 years. The predicted reduction in risk of endometrial cancer is much less, but still of note (Table II).

Fourteen women were randomized to our contraceptive regimen and 7 women to the control arm [6,7]. We removed one woman randomized to the contraceptive arm from the study following the second dose of GnRHA in view of her poor compliance with the oral CE. All other women have remained on study.

A symptom questionnaire was used to assess tolerance of the regimen; the contraceptive subjects had significantly fewer symptoms associated with the luteal phase of the menstrual cycle, commonly referred to as premenstrual syndrome (PMS) symptoms, on the regimen than before they started it [6]. Cyclical breast symptoms were effectively eliminated, and patients did not note any other changes in their breasts. The few occurrences of hot flashes or vaginal dryness were eliminated by increasing the estrogen dose to 0.9 mg of CE. Unscheduled bleeding or spotting was infrequent and decreased with time on the regimen. A beneficial rise in high density lipoprotein cholesterol was seen in the contraceptive subjects. However, despite the use of an estrogen dose which is known to prevent loss of BMD in normally postmenopausal (non-oophorectomized) women, a small (2–3%) loss of spinal and femoral BMD was seen in the contraceptive regimen women at 1 year.

The reason for this loss of BMD appears to be inhibition of ovarian androgen production by the GnRHA, which may also account for the changes in libido occasionally reported with GnRHA [6]. Women in the contraceptive regimen group had a 62% drop in non-SHBG-bound testosterone (T). In contrast, during the perimenopausal and early naturally postmenopausal period, T levels are stable. This explains why the CE dose we used has been found to prevent bone loss in naturally menopausal women, but not in our volunteers. The data in the literature on the dose of CE required to prevent BMD loss immediately post oophorectomy is sparse; only 6 women have been studied, and although on average they did not lose bone, the variability in the BMD changes in these women is sufficient to make their results compatible with the BMD loss we observed. The study continues with the addition of a small dose of androgen aimed at replacing that lost by the action of the GnRHA; preliminary results suggest that this modified regimen is not associated with any further bone loss.

Mammographic densities of women on the contraceptive regimen have been dramatically reduced (Fig. 4) [7]. Although there is no direct evidence that a reduction in mammographic densities will lead to a reduced risk of breast cancer, we believe this to be the case. There is much direct evidence that increased mammographic densities are associated with increased breast cancer risk [51]; early menopause is known to reduce breast cancer risk, and cross-sectional studies show that menopause is associated with a reduction in mammographic densities [51].

Menopause is associated with reduced breast cell mitotic activity, and we believe that the decreased mammographic densities reflect this. The majority of the breast consists of adipose and fibrous tissue. In the premenopausal breast, less than 15% of breast volume consists of epithelial cells, decreasing to less than 5% by age 60 [52]. The relative amounts of fibrous and adipose tissue are what determine the appearance of the mammographic image, since fibrous tissue is radio-opaque and adipose tissue is radio-lucent. Increased fibrous tissue equates to increased mammographic densities. Since estrogen and progesterone receptors in the breast appear to exist only in epithelial cells [18], the reduced sex steroid levels of postmenopausal women affect fibrous tissue secondarily to their effect on epithelial cells.

The statistically significant reductions in mammographic densities at one year compared to baseline films indirectly suggest that the aim of this first prototype regimen to reduce breast cell mitotic activity has been accomplished [7]. Further studies are planned in the U.S. and are

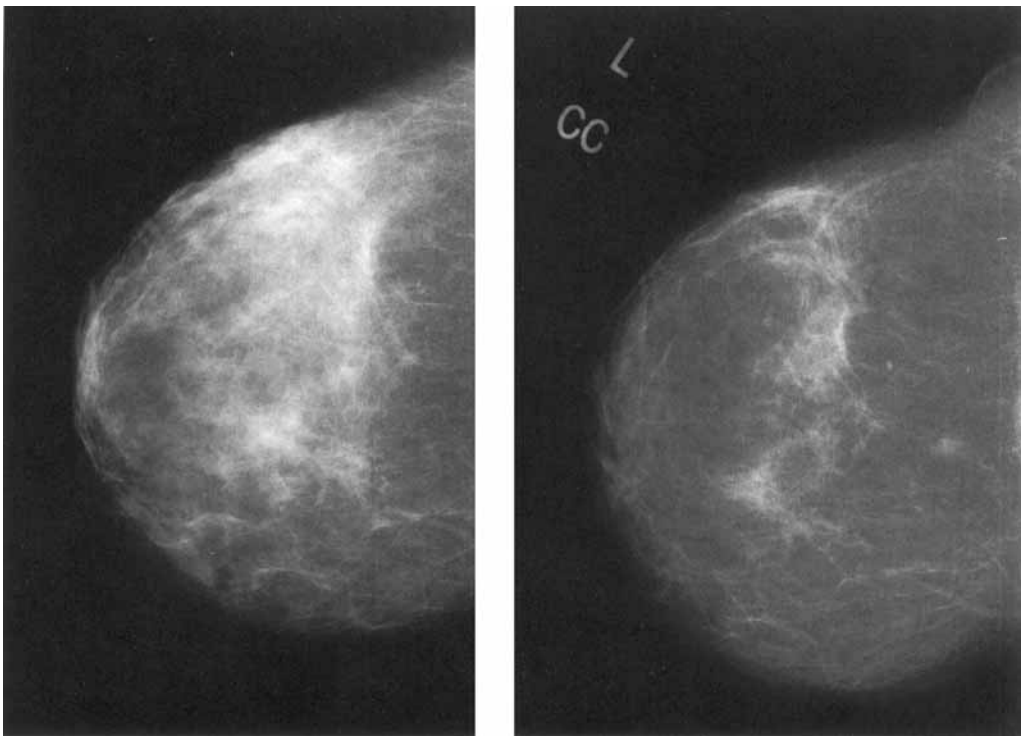

Fig. 4: Mammogram at baseline (left) and of the same breast after 1 year on the first prototype regimen.

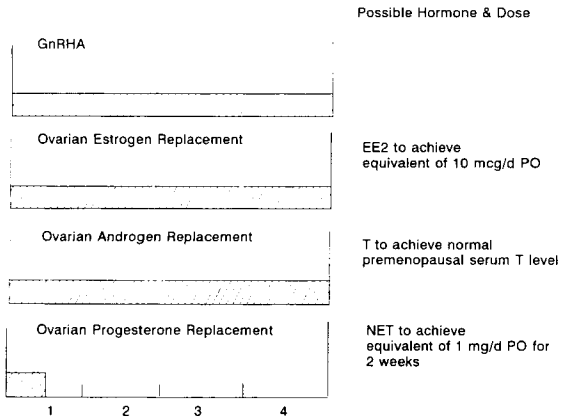

Fig. 5: Proposal for a zero-order release depot formulation of a contraceptive regimen to reduce breast cancer risk.

ongoing in the U.K., in which direct measurement of cell proliferation in the breast will be made.

To make such a breast cancer prevention regimen practical, it will have to be simple and reasonable in cost. The latter does not appear, at least in the long-term, to be a major issue. A regimen such as that illustrated in Figure 5 is likely to be acceptable to many women. This depot contraceptive would deliver all 4 hormone components in a single injection to be given 3 (or possibly 4) times per year. The depot would deliver, with approximately zero-order (constant) release characteristics, the GnRHA, EE2, and T continuously. The NET would be released for 2 weeks at a high enough dose to completely control endometrial hyperplasia. Later versions of such a contraceptive may contain a very low-dose release of NET for a more extended period. This low-dose would not be aimed at achieving luteinization of the endometrium, but only to control endometrial mitotic activity which appears to require only a very low dose of progestogen [28]. This latter regimen would then be predicted to be associated with a much greater reduction in endometrial cancer risk than is shown in Table II.

Disclosure Drs. Pike and Spicer are associated with Balance Pharmaceuticals, Inc., a company

established to develop the contraceptive regimens discussed here.

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