*Breast Cancer Research and Treatment* **70:** 213–219, 2001.


*Conference report*

# Expedition inspiration consensus 2001

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The fifth Expedition Inspiration conference was held on March 1–3, 2001. While there are other conferences that concentrate on a particular facet of breast cancer, the design and goals of this conference are unusual. In order to maximize interaction of investigators and clinicians the meetings are small, invited, and private. The participants include both senior and junior physicians and scientists involved in clinical and basic research as well as clinical practice. The meetings serve four purposes:

1. Active discussion among participants who do not usually interact.
2. Develop consensus as to the state of our knowledge as well as an action plan to stimulate future studies.
3. Develop collaborative projects among the meeting participants.
4. Foster new investigations by participants as well as others.

This year the role of hormones in the etiology, prognosis, prevention and treatment of breast cancer were the subject of the discussion. Investigators studying breast cancer biology, endocrinology, molecular biology, epidemiology and clinical management participated in a stimulating discussion resulting in the consensus and action items which follow:

## Selective estrogen receptor modulators (SERMs), aromatase inhibitors, and ovarian ablation

A number of new hormonal agents are becoming available for the treatment of breast cancer. These include pure antiestrogens, such as faslodex, new SERMs that appear not to increase the risk of endometrial cancer, such as raloxifene, and aromatase inhibitors. At least two aromatase inhibitors have shown efficacy equal to or greater than that of tamoxifen in the treatment of postmenopausal women with receptor positive metastatic breast cancer.

One challenge is to determine whether incorporating the new hormonal agents into adjuvant regimens can improve long term disease free survival. Several trials using aromatase inhibitors in the adjuvant therapy of postmenopausal women are ongoing or have completed accrual.

Young women (under the age of 35) with receptor positive tumors who are treated with CMF-type chemotherapy and do not receive endocrine therapy have been reported to have a worse prognosis than older premenopausal patients with receptor positive tumors as well as a worse prognosis than equally young women with receptor negative tumors. These very young women are those who are least likely to undergo menopause as a result of chemotherapy, and it may be that continued hormonal stimulation of tumor cells contributes to their poor prognosis.

Numerous trials have shown that ovarian ablation produces results similar to those produced by chemotherapy in premenopausal women with receptor positive tumors. A large part of the benefit of chemotherapy in young women may be the result of chemotherapy-induced menopause. One important question is the value of chemotherapy when added to optimal hormonal therapy in premenopausal women. Including quantitation of levels of ER and PR positivity in such trials might allow for estimation of differential benefit in patient subgroups.

A second question is the nature of the optimal hormonal regimen. Ovarian ablation and tamoxifen have been found to produce similar results in the setting of advanced disease. Recent results in premenopausal women with receptor positive metastatic breast cancer have suggested that the combination of tamoxifen plus ovarian ablation (via GnRH agonist) may produce a survival advantage over the use of either agent alone. Aromatase inhibitors cannot be used in premenopausal women except in the setting of ovarian ablation, and should they prove superior to tamoxifen in postmenopausal women, their use in younger women will need to be addressed. Young women have more side effects from hormonal therapy than do older women, including infertility, osteoporosis, vasomotor symptoms, and urogenital atrophy. Clinical trials addressing hormonal therapy need to include careful assessment of relevant toxicities.

*Action Items.* Clinical trials of hormonal therapy in receptor positive premenopausal women are needed. Questions to be addressed include the optimal agents, duration of therapy, and importance of chemotherapy in the setting of optimal hormonal treatment. Such trials should report both ER and PR status. Careful assessment of relevant toxicities is crucial.

## Clinically relevant subsets of ER-positive breast tumors

Overexpression of the estrogen receptor (ER, alpha isoform) has long been recognized as a breast cancer prognostic marker and even a stronger predictive marker of response to endocrine therapy. Three emerging lines of evidence now support the likelihood that ER-positive breast tumors can be divided into at least two clinically relevant subsets, one with good responsiveness to endocrine therapy and relapse-free patient survival, and the other with much poorer endocrine responsiveness and patient outcome.

1. The independent prognostic value of ER-positivity has formerly been shown to be time-dependent; that is, associated with better patient prognosis (relapse-free survival) than ER-negativity especially within the first 5 years after primary tumor diagnosis and excision. However, when stage-matched and untreated ER-positive primary tumors are carefully followed-up over much longer time intervals, two distinct prognostic subsets emerge based on patient menopausal status. For postmenopausal women, ER-positive and ER-negative breast tumors appear to be associated with comparable longterm patient survival. In contrast, ER-positive breast tumors in premenopausal patients not treated with adjuvant therapy are associated with a significantly worse patient survival than comparably staged and untreated ER-negative tumors.

*Action item.* Further study of archived ER-positive tumors from early-staged premenopausal patients not treated with adjuvant therapy to validate this provocative observation; and multivariate analysis of other established breast tumor prognostic markers (e.g., PR, S-phase, ErbB2, p53) in stage-matched ER-positive tumors from premenopausal versus postmenopausal patients that might explain the prognostic differences of ER-positivity based on menstrual status.

2. RNA samples from a small group of primary T3 and T4 breast tumors from patients with long term follow-up, analyzed by expression microarrays (9,000 cDNA clones per array) and clustering programs, identified two ER-positive tumor subsets having luminal gene expression characteristics (Types A and B). Survival analyses indicated that Type A ER-positive breast tumors are associated with significantly better patient survival (following adjuvant treatment) than Type B ER-positive tumors, in which the poorer patient survival was comparable to that of a third subset of ER-negative tumors that overexpress ErbB2 (*HER2/neu*).

*Action item.* Independent microarray and clustering analyses are needed from a larger sample of patients to confirm the existence of Type A and Type B ER-positive breast tumor subsets with their significantly different patient outcomes, and to verify that differences in responsiveness to endocrine adjuvant therapy accounted for these outcome differences. Further immunohistochemical analysis of the Type A versus B ER-positive tumors with probes not represented on the microarray (e.g., PR) may confirm and further characterize these microarray-determined breast tumor subsets.

3. Progesterone receptor (PR) status has formerly been shown to predict two subsets of ER-positive metastatic breast cancer: ER-positive/PR-positive tumors that have a response rate to endocrine therapy nearly 2-to-3-fold higher than that of ER-positive/PR-negative tumors. Using a well-validated PR assay and looking at the longterm outcome of ER-positive primary breast tumors treated with adjuvant endocrine therapy (primarily tamoxifen), it now appears that pa-

tients with ER-positive/PR-negative primary tumors also have significantly worse relapse-free and overall survival compared to those with ER-positive/PR-positive primaries. Furthermore, while increasing patient age is associated with increasing likelihood of an ER-positive primary, the proportion of poorer-risk ER-positive/PR-negative breast tumors also increases with age.

*Action item.* Since the poor-risk predictive value of ER-positive/PR-negative breast tumors has largely been determined after endocrine therapy with the partial ER antogonist, tamoxifen, the poor-risk predictive value of ER-positive/PR-negative tumor status after treatment with other endocrine therapies should also be evaluated; in particular, tumor response rates and patient survival for these two ER-positive tumor subsets should be compared after ovarian ablation (surgical, medical), aromatase inhibition, and treatment with a pure estrogen antagonist (e.g., faslodex).

## Mechanism of action of ER$\alpha$ and ER$\beta$ in relation to SERMs and the development, progression and prevention of breast cancer

Human mammary epithelial cells and many breast carcinomas express one or both of the two estrogen receptors, ERα and ERβ. Undoubtedly, the single most important marker of estrogen sensitivity and response to tamoxifen therapy in breast cancer is the presence of ERα in tumor cells. ERα is also considered a significant target for breast cancer prevention by selective estrogen receptor modulators (SERMs) like tamoxifen and raloxifene, as demonstrated by the NSABP P1 and MORE trials. Considerably less is known at this point about the expression and function of ERβ in normal breast epithelium and in breast cancer. Estrogens and SERMs regulate diverse cellular activities via one or both of the two ER subtypes (ERα and ERβ) in hormone responsive tissues and cancers. Liganded ERs can interact with a complex mix of coactivators, corepressors and other signaling molecules that differ in expression and importance from tissue to tissue. In addition, different SERMs may alter the affinity and/or selectivity of one or both ERs for these coregulators, allowing for tissue selective responses. There is also suggestive evidence that the two ER subtypes may oppose each other in different cell contexts. It is certainly true that ERα and ERβ can have different actions in the same tissue and that they can interact with each other, which may result in complex estrogenic/antiestrogenic responses to a given ligand. Thus, ERβ can stimulate AP1-activated genes in the presence of SERMs like tamoxifen, while inhibiting the same genes in the presence of full estrogens like estradiol and DES, whereas ERα appears to enhance AP1-responsive genes in the presence of both agonists and antagonists. Recently, partial insight into the molecular basis of estrogen agonism and antagonism has been revealed by the crystal structures of ERα and ERβ ligand binding domains (LBDs) complexed with several ligands, including estradiol (E2), diethylstilbestrol (DES), raloxifene (RAL), 4-hydroxytamoxifen (OHT), the phytoestrogen genistein (GEN) and the so-called complete antagonist ICI 182,780 (faslodex). This information has helped define and predict some of the properties and behaviors one might expect from different SERMs. However, our knowledge of ER-mediated responses to diverse natural and synthetic SERMs is far from complete. Added to this complexity is an increasing body of evidence to suggest that rapid, nongenomic actions of SERM-ER complexes may be an important and largely unappreciated mechanism by which SERMs regulate proliferation, apoptosis and other cell-specific responses to SERMs. Thus, the current challenges in this area are:

1. Understanding the molecular mechanisms by which SERMs elicit tissue-selective agonist or antagonist responses via one or both ER subtypes.
2. Understanding the roles of ERα or ERβ expression and modification in breast cancer genesis and progression to hormone independence.

*Action items.* Additional molecular and structural information is needed to improve our knowledge of how the two ER subtypes (ERα and ERβ) mediate individual and collective responses to SERMs, especially in concert with multiple coactivators and corepressors. More information is also needed about the expression and possible roles of both ERs and their isoforms, especially ERβ, in normal breast epithelium and in progressive breast disease. Both correlative and mechanistic approaches are required to define the roles of ERα and ERβ in these processes and to selectively target each ER and/or associated signaling pathways, such as over- or under-expressed coregulators (e.g., AIB1 or NCoR), for therapeutic or preventive intervention. DNA array technology should be well suited for identifying complex hormone signaling pathways in normal and diseased tissues and for identifying altered gene profiles in hormone responsive and un-

responsive breast cancers as well as patient responses to neoadjuvant therapy like tamoxifen.

### Estrogens and other hormones as specific explanations for recognized hormonal risk and prognostic factors, and their underlying mechanisms of action

Many conditions associated with elevated estrogen and progestin levels are established breast cancer risk factors. Less clear are the mechanisms through which these hormones operate. For a number of conditions associated with elevation in these hormones (hormone replacement therapy (HRT), obesity, pregnancy, perhaps moderate alcohol consumption), increased risk appears almost instantaneously following exposure and dissipates rapidly following cessation of exposure. The protection associated with oophoretomy and use of selective estrogen receptor modulators (SERMs) appears equally as rapidly. These observations imply an important role for these hormones acting relatively late in carcinogenesis, perhaps involving tumor growth promotion.

Even less clear are the mechanisms of action of hormonal exposures associated with risk factors that operate earlier in carcinogenesis (e.g., height, the timing of menarche and of first full-term birth, geographic and ethnic differences, birth weight, being the product of a toxemic pregnancy). Speculation on mechanism has included hormonal influences on size of stem cell populations, breast duct mass, mitogenesis, cellular differentiation, and DNA damage and repair processes.

While most focus has been on the role of estrogen and progestin in explaining breast cancer risk factors, the full range of hormones involved is unknown. There is increasing laboratory evidence for a role for other hormones, many of which are also strongly associated with recognized breast cancer risk factors. Recently, both laboratory and epidemiologic findings have raised particular interest in androgens, glucocorticoids, insulin and insulin-like growth factors (IGFs), and prolactin.

Many of the conclusions with respect to carcinogenesis apply as well to issues of treatment and prognosis. Obesity, ovarian function, pregnancy and hormone-based therapies all influence the natural history and prognosis of breast cancer. While much of the emphasis has rightfully been on the role of estrogen and progestin, the range of hormones potentially involved is unknown, and likely to be broader. Accumulating clinical evidence of a potential prognostic role for the IGFs, and recent laboratory findings implicating glucocorticoid receptor activation in inhibiting mammary epithelial cell apoptosis are particularly provocative.

*Action Items.*

1. Interdiction of effective exposure to increased levels of estrogen and progestin relatively late in carcinogenesis can reduce breast cancer risk. Behavioral approaches to this include weight loss, reduction of alcohol consumption and termination of HRT, even at older ages. Use of SERMs are also effective but development of new SERMs with lower frequency of serious side effects will be required in order to make them more applicable to a broader range of high-risk women.
2. Future opportunities for prevention and individualized treatment based on hormonal influences will require a better understanding of the range of hormones involved and their underlying mechanisms of action in breast tissue. Among the ways to achieve this are interdisciplinary efforts to understand hormonal risk and prognostic factors. Such studies should include evaluation of a broad range of hormones (estrogens, progestins, androgens, insulin and IGFs, glucocorticoids, prolactin, and others) and be focused not only on identifying which hormones are involved when, but also on the underlying mechanisms likely to be responsible for their effects.

### It is uncertain how much benefit is derived from treatment with adjuvant chemotherapy in women with receptor positive tumors who have also been given an optimal adjuvant endocrine therapy

This questions has arisen, in part, from the results of two large trials in which patients were randomized to either four cycles of cyclophosphamide plus doxorubicin (CA) or the same regimen followed by four cycles of paclitaxel. In both studies, a reduction in the hazard of recurrence or death from adding paclitaxel were seen only in those patients whose tumors were estrogen and/or progesterone receptor negative. This result was unexpected because prior adjuvant studies have generally demonstrated that chemotherapy is effective in patients with receptor positive tumors, and combinations of chemotherapy plus tamoxifen have been shown to be better than tamoxifen alone. In overviews or meta-analyses of trials in which paclitaxel

was not used, the reduction in annual odds of recurrence or death from adjuvant chemotherapy is smaller when the chemotherapy is given with tamoxifen than when it is given alone. These differences do not reach levels of conventional statistical significance, but the duration of tamoxifen in the studies included in the overviews varied from 1 to 5 years and not all patients had receptor positive tumors. We now know that 5 years of tamoxifen is significantly better than shorter durations. Since several large trials have demonstrated that non-paclitaxel regimens plus 5 years of tamoxifen are more effective than 5 years of tamoxifen alone in postmenopausal patients with receptor positive tumors, the differences in the duration of tamoxifen is not, by itself, sufficient to explain the differences in outcome between the adjuvant paclitaxel studies and those included in the overviews. However, the variable results from adding chemotherapy to tamoxifen in patients with receptor positive tumors are sufficient to generate a testable hypothesis. Adjuvant chemotherapy may not improve disease-free and overall survival in patients whose tumors are strongly receptor positive and who have been given optimal adjuvant endocrine therapy.

*Action Item.*

1. Adjuvant chemotherapy trials should stratify patients on the basis of receptor positivity. This will help physicians and patients with receptor positive tumors determine whether the added time free of recurrence or the added survival, if any, is worth the additional toxicity associated with chemotherapy compared to using optimal endocrine therapy alone.
2. The effects of chemotherapy should be correlated with different levels of estrogen receptor positivity, the presence of both an estrogen and progesterone receptor, and newer markers of tumors responsiveness to endocrine therapy.

## The egf receptor family of growth factors and ligands as targets for anticancer therapy

The egf receptor (efgr) family of growth factors and ligands has emerged as one of the most attractive areas for the development of anticancer therapy. There are multiple bases for this assertion.

First, a wealth of information has suggested that these gene products play pathophysiologic roles in oncogenesis. Mutations in the transmembrane domain of the egfr are responsible for multiple chemical carcinogen induced tumors in model systems. Mutations resulting in truncated, spontaneously activated forms of the egfr contribute to the pathogenesis of gliomas in humans and similar altered gene products resulting from alternative spling contribute to breast and other solid tumors. Overexpression due to gene amplification of *HER2/neu* [ErbB2] occurs in varying proportions of many human tumors including breast, ovary, bladder, gastric and lung cancers.

Second, extensive prognostic studies suggest that overexpession of family members is associated with a worse prognosis in many human cancers including breast, ovary, lung, gliblastoma and gastric carcinomas. Whilst not proving a pathogenetic role in these tumors, work from animal models would strongly support such a contention. Overexpression of the egfr and ErbB2 in breast cancer are both associated with a greater liklihood of hormone independence as well.

Third, and most excitingly, at least two independent strategies aimed at altering signaling through these receptor systems have shown encouraging efficacy in humans with cancer. These studies place on a firm foundation the notion that these receptors are involved in disease pathogenesis. Antibodies directed against the egfr are capable of inducing profound anticancer effects in squamous cell tumors of the head and neck particularly when combined with either standard chemotherapy or irradiation. Similarly, antibodies directed against ErbB2 have now been approved for human use in breast cancer where their combination with taxanes has been proven to both increase response rate and prolong survival in metastatic disease. This latter approach is now being explored in the adjuvant setting. In addition to antibody approaches several groups and pharmaceutical companies have developed potent inhibitors of the intracellular kinase domain of these receptors which are required for their activity. Early clinical trials suggest significant grounds for encouragement. Thus it is clear that the egfr family is critically involved in many human cancers.

## Hormone replacement therapy as a risk factor

Postmenopausal estrogen-progestin replacement therapy increases breast cancer risk to a significantly greater extent than does estrogen replacement therapy. Postmenopausal estrogen replacement therapy (ERT) at the dose usually administered in the U.S. increases

breast cancer risk approximately 2% per year of use (relative risk, RR, of 1.02 per year of use). Five years of ERT use increases risk by approximately 5 times this amount (RR $\sim$ 1.10 per 5 years of ERT use), and longer use increases the risk proportionately (e.g., RR $\sim$ 1.20 per 10 years of ERT use).

ERT is associated with much greater increased relative risks for endometrial cancer (RR $\sim$ 2.0 per 5 years of ERT use). The endometrial cancer risks were established in the mid 1970s, and, in response to the greatly increased risks, progestins were added to ERT (estrogen-progestin replacement therapy; EPRT). The progestin is either added for 10–12 days per month in a sequential fashion (sequential estrogen-progestin replacement therapy; SEPRT), or estrogen and a lower dose of progestin are always taken together (continuous-combined estrogen-progestin replacement therapy; CCEPRT).

Recent breast cancer case-control studies have shown that EPRT use increases breast cancer risk to a much greater extent than ERT use. These studies suggest that the relative risk per year of EPRT use is approximately three times that associated with ERT use, that is RR $\sim$ 1.06 per year of EPRT use. Five years of EPRT use increases risk by approximately five times this amount (RR $\sim$ 1.30 per 5 years of EPRT use), and longer use increases the risk proportionately (e.g., RR $\sim$ 1.60 per 10 years of ERT use). Evidence that these effects are directly due to the EPRT use comes from the randomized trial (the PEPI trial) that showed substantial increases in mammographic densities in women taking EPRT, much greater increases than were seen in the ERT arm of the trial. Further evidence comes from the finding of greatly increased breast cell proliferation in women using EPRT.

Progestins clearly need to be given to protect the endometrium from the carcinogenic effects of unopposed ERT. They need to be delivered to the endometrium in a manner that will have a minimal effect on the breast. There is good evidence that this can be accomplished by using a 'direct' endometrial route of administration with use of an intra-uterine device (IUD) containing progesterone (Progestasert), that was designed as an intra-uterine contraceptive device (IUCD) for premenopausal women. Use of this device is associated with a very low serum progesterone concentration of $< 1.5$ nmol/l (follicular phase of the menstrual cycle). The recently FDA-approved levonorgestrel containing IUCD (Mirena) will probably also provide endometrial protection with very little effect on the breast, although this would need to be demonstrated. Alternatively, it may be possible to administer micronized progesterone by an intravaginal tablet at a dose that will provide adequate endometrial progestin levels with low blood levels so that the effects of the progesterone on the breast should be small. If these routes of administration are unacceptable to a woman then giving progestins for 10 days every 3 to 4 months should provide satisfactory protection of the endometrium with much less effect on the breast than current forms of EPRT. Two clinical trials of 10 mg per day of medroxyprogesterone acetate (MPA) given for 14 days every 3 months have been published, in which the dose of estrogen was conjugated estrogens at 0.625 mg/day. These two studies suggest that this approach may be satisfactory in that the extent of associated endometrial hyperplasia was minimal. A further trial did not show satisfactory control of hyperplasia, but in this trial a much higher dose of estrogen was given.

It is not clear how the relative risks for breast cancer associated with EPRT use in the form of conjugated estrogens as measured in the two U.S. studies apply to other forms of EPRT that use a transdermal (patch) delivery system or use different progestins in different schedules.

*Action Items.*

1. Encourage the development of an IUD device specifically designed for postmenopausal women. The device needs to deliver just sufficient progestin to the endometrium to block the action of replacement doses of estrogen. The device needs to be designed to only need replacing very infrequently.
2. Encourage studies of the vaginal route of administration of progesterone with ERT, in order to find the minimum dose and duration of therapy adequate to protect the endometrium. Since the dose-duration response relationship of progestin with ERT on the breast is so poorly understood, it is necessary with both of the above Items to incorporate studies of their actions on the breast (e.g., using mammographic density or other biomarkers of action).
3. Encourage the FDA to make measurement of the effects on the breast part of the licensing of any new forms of EPRT. Encourage the FDA to require of manufacturers of all forms of EPRT currently licensed in the U.S. to make measurements of their effects on the breast.

**Breast cancer prevention via a hormonal or dietary mechanism**

The role of hormones in the initiation of breast cancer remains unclear. However, reduction in estrogen levels by bilateral ovariectomy or inhibition of estrogen receptor action by tamoxifen significantly reduces breast cancer risk. These effects are present for the risk of developing breast cancer in either pre- or postmenopausal women as well as those who are at high familial risk (i.e., *BRCA1*/*BRCA2* mutation carriers). Surprisingly, no apparent association has been found between increased estrogenicity during reproductive years and premenopausal breast cancer risk. Further, dietary components or life-style factors associated with altered estrogenicity, such as dietary intake of fat or phytoestrogens (esp. soy) or physical activity, have not been clearly linked to breast cancer risk. High estrogen levels characterizing pregnancy actually reduce breast cancer risk. Additionally, increased levels of adipose tissue -derived estrogens during childhood and premenopausal years are associated with reduced risk of developing breast cancer, while postmenopausal obesity increases the risk. Accumulating evidence indicates that the timing of hormone and dietary exposures might play as an important role in affecting breast cancer risk as life-time exposures. For example, both human and animal data suggest that high *in utero* estrogenicity, including that induced by dietary means, may pre-initiate breast cancer. This could result from changes in the morphological development of the breast and altered expression of genes that play a critical role in estrogen's signal transduction pathways. Virtually nothing is known about estrogen exposures during childhood and breast cancer risk, or the ability of childhood diet to alter the risk.

*Action items.*

1. Explore associations between *in utero* and childhood estrogenic exposures and breast cancer risk. Include women who are at high familial risk, such as *BRCA1*/*BRCA2* mutation carriers to these studies.
2. Identify modifiable factors that can alter estrogenicity *in utero* and during childhood. These factors are likely to be linked to diet and physical activity.

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