

Proceedings of the Third International Conference on Recent Advances and Future Directions in Endocrine Manipulation of Breast Cancer: Conference Summary Statement

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Introduction

The Third International Conference on Recent Advances and Future Directions in Endocrine Manipulation of Breast Cancer, cochaired by Steven Come, MD, and Aman Buzdar, MD, was held in Cambridge, Massachusetts, June 21–22, 2003.

The conference was organized with the objective of analyzing recent advances in basic, translational, and clinical research relating to endocrine manipulation of breast cancer, and examining the implications of these findings both for patient management and for future research. Conference attendees were selected based on their contributions to the basic or clinical understanding of breast cancer and included internationally recognized researchers in the fields of molecular biology, pharmacology, and medical oncology.

The conference, which combined brief scientific reports with extended periods of open discussion, focused upon the following issues:

- evolving concepts of nuclear (genomic) *versus* membrane (nongenomic) estrogen receptor (ER) localization and function;
- cross-talk between estrogen and growth factor signaling pathways as a principal mechanism of resistance to endocrine therapy;
- adaptive hypersensitivity to estrogen deprivation;
- the status of preclinical efforts to abrogate or delay resistance to endocrine therapy by targeting growth factor pathways, and identification of additional targets that warrant future investigation;
- the present status of aromatase inhibitors in early breast cancer and in the prevention setting;
- preclinical observations on the protective effect of pregnancy;
- sequencing available therapeutic agents in postmenopausal women: additional data needed and additional approaches that should be evaluated;
- the value and limitations of current preclinical and clinical models in evaluating new agents and management strategies; and
- priorities for future research.

At the conclusion of the conference, the faculty convened to formulate the following summary statement.

The ER, Cross-Talk, and Resistance to Endocrine Therapy. The existence of cross-talk between estrogen and growth factor pathways is now well established. Accumulating evidence suggests that this interaction occurs both in the nucleus and in the cell membrane or adjacent cytoplasm. The interaction between members of the epidermal growth factor (EGF) family, especially HER-1 and HER-2, and the ER is the best studied to date. The spectrum of action of selective estrogen receptor modulators (SERMs), such as tamoxifen, is at least in part determined by the balance of ER coregulatory proteins, coactivators, and corepressors. Overexpression of HER-2 is associated with an increase of coactivators such as AIB1, which stabilize ER α and potentiate the agonist effect of tamoxifen, and thereby clinical resistance. Furthermore, activation of growth factor pathways can, through Akt and mitogen-activated protein (MAP) kinase, lead to phosphorylation of the ER, even in the absence of ligand.

Whereas the mechanisms of action of the SERMs are reasonably well understood, those of fulvestrant are less clear. Whereas fulvestrant is positioned as an ER down-regulator, it also appears to have some ligand function as a steroidal antiestrogen. ER down-regulation is a complex process triggered by a variety of ligands. AIB1 does not appear to be implicated in ER down-regulation by fulvestrant. Whereas ER down-regulation is being used as a surrogate end point for assessing the activity of fulvestrant and determining dosing schedules, this may not in fact be the relevant pharmacodynamic end point. Because the

Grant support: Publication of these proceedings was made possible by unrestricted educational grants from AstraZeneca Pharmaceuticals, Novartis Pharmaceuticals, and Pfizer Oncology. Editorial assistance and Continuing Medical Education Sponsorship were provided by InforMEDICAL Communications, Inc. (Carlisle, MA).

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majority of ER is not needed for transcription, down-regulation may not be the crucial mechanism for fulvestrant, nor an appropriate target for drug development.

Given the clinical ascendancy of third-generation aromatase inhibitors, it is critical to understand the mechanisms of resistance to these agents. The most applicable *in vitro* model is MCF-7 cells grown under long-term estrogen deprivation (LTED cells). Unlike SERMs, aromatase inhibitors have no potential agonist activity; rather, resistance appears to be a consequence of adaptive hypersensitivity of tumor cells to the dramatic reduction in estradiol (E_2) levels induced by these agents. As in the case of resistance to SERMs, however, cross-talk is again important. Up-regulation of growth factor pathway signaling appears fundamental to the adaptation displayed by LTED cells. Activation of both MAP kinase and phosphoinositide 3'-kinase pathways is observed, and most, but not all, of hypersensitivity to E_2 can be reversed with dual inhibition of these pathways. In LTED cells, there is not only an increase in ER α but also an increase in the amount of ER α found in the cell membrane or submembrane cytoplasm (nongenomic ER). There, ER appears to associate with growth factor pathways. Within minutes of exposure to estradiol, activation of MAP kinase occurs, and this effect is much more rapid than the classical (genomic) effect of E_2 on the nuclear ER.

Thus, ER α can be both stabilized and activated by growth factor pathways even in the absence of ligand, whereas growth factor pathway signaling may be maintained by the association with ER. The latter is supported by the observation *in vitro* that degradation of ER by fulvestrant in LTED cells leads to a loss of HER-1 and HER-2 signaling, and of MAP kinase and Akt activation. This effect may be mediated by a reduction in tumor growth factor α , a key HER-1 ligand.

Resistance to endocrine therapy may be *de novo* or acquired. The potential to abrogate or delay this process, respectively, has been investigated in cell lines and in xenografts implanted with MCF-7 cells. Consistent with the observations on the role of EGF family signaling in resistance to endocrine therapy, tamoxifen stimulates MCF-7 cells that have been transfected with HER-2 (representing *de novo* resistance), whereas the combination of tamoxifen and gefitinib inhibits these tumors. Furthermore, initial cotreatment of wild-type MCF-7 with tamoxifen and gefitinib delays the emergence of resistance compared with tamoxifen alone. In this model, estrogen deprivation and fulvestrant display the same interaction with gefitinib. By reducing ligand or by degrading ER, aromatase inhibitors or fulvestrant could be particularly promising in combination with antigrowth factor therapy, interfering with cross-talk at multiple points.

However, in models resistance inevitably emerges to combined treatment with antiestrogens and gefitinib. Signaling through the insulin-like growth factor I receptor appears to stimulate these doubly resistant cells, and preliminary observations reveal efficacy in tamoxifen-resistant cell lines cotreated with gefitinib and an insulin-like growth factor I receptor inhibitor. It is possible that targeting multiple pathways simultaneously, especially as initial therapy rather than at the time resistance emerges, will be a successful strategy in combination with traditional endocrine maneuvers in hormone receptor-positive patients. In addition to HER-1, HER-2, and insulin-like

growth factor I receptor, there are a variety of regulatory nodes, which are central to multiple pathways and provide additional attractive targets. These include insulin receptor substrates, integrins, and cyclin D1. However, the safety of such an approach has not been confirmed in normal cells. Furthermore, in the MCF-7 cell lines, resistance to antiestrogens and then gefitinib is associated with a progressively more malignant phenotype. Hence, this approach may select for a more aggressive malignancy if it is not totally successful.

A possible alternative approach to resistance is provided by observations of LTED cells, which are exposed to increased but still physiological doses of estradiol. Unlike wild-type MCF-7 cell, which are stimulated by E_2 , LTED cells appear sensitized to the proapoptotic effect of estradiol. This process may be mediated through up-regulation of the Fas receptor in LTED cells. Furthermore, LTED cells re-exposed to E_2 show a decrease in MAP kinase activity, suggesting that cross-talk has been diminished. Thus, it is possible that periodic exposure to E_2 after estrogen deprivation or the combination of estrogen deprivation and antigrowth factor therapy would be of clinical benefit by both its proapoptotic effect and by moving surviving cells back toward a less malignant, endocrine-responsive phenotype. Preclinical studies to investigate such sequencing are planned.

Endocrine Therapy: Clinical Update

Adjuvant Therapy. In the setting of early breast cancer, the ATAC (Arimidex, Tamoxifen, Alone or in Combination) trial data have been updated. With a median follow-up of 47 months, the results remain consistent with the initial report confirming the potential of the aromatase inhibitors (AIs) for adjuvant use. A safety update (median treatment duration 37 months) shows no major changes in the incidence of predefined adverse events. Of note, whereas the first analysis showed increased long bone fracture in the anastrozole group compared with tamoxifen, there has been no increase in fracture rates over time in the anastrozole-treated arm. In reviewing the updated data, the American Society of Clinical Oncology Health Services Research Committee noted that the follow-up provided a greater level of assurance regarding the toxicity and efficacy of adjuvant anastrozole. However, the committee maintained the position taken previously by the American Society of Clinical Oncology Cancer Technology Assessment Working Group that tamoxifen should remain the standard pending 5-year data from ATAC and other trials of adjuvant AI use. In clinical practice, however, AIs are rapidly becoming the standard of care for those patients in whom tamoxifen is deemed undesirable because of comorbidities or risk factors.

Numerous studies underway are expected to provide additional data on the role of AIs as adjuvant therapy. At this point, it is uncertain whether the advantage for anastrozole *versus* tamoxifen observed in ATAC applies equally to all patients or whether certain subsets of women derive particular benefit. Furthermore, additional data on sexual function and other quality of life issues during and after therapy with AIs is needed. Three placebo-controlled trials, International Breast Cancer Intervention Study 2 (anastrozole), the National Surgical Adjuvant Breast and Bowel Project (NSABP) B33 study (exemestane),

and the (J)MA17 (letrozole), will be particularly helpful in clarifying bone loss and other toxicity issues.

Advanced Disease. Currently, virtually all hormone receptor-positive women developing advanced breast cancer will have received tamoxifen in the adjuvant setting. For first-line endocrine therapy of advanced disease in postmenopausal women, evidence from large well-conducted trials in patients presumed to still be tamoxifen sensitive (>1 year between cessation of tamoxifen and identification of metastatic disease or no prior tamoxifen) has established a superiority of nonsteroidal AIs over tamoxifen. Data are awaited from a trial comparing steroidal AI to tamoxifen in this setting.

For postmenopausal women considered tamoxifen resistant (≤ 1 year between cessation of adjuvant tamoxifen and identification of metastatic disease or progression on tamoxifen therapy for advanced disease), treatment with either a steroidal or nonsteroidal AI or fulvestrant are options based on available data. Whereas AIs are most commonly used in this setting at present, two Phase III trials comparing fulvestrant to anastrozole have demonstrated comparable efficacy and tolerability. Fulvestrant is a steroidal estrogen antagonist that also produces a reversible down-regulation of ER α . It is Food and Drug Administration approved for use in postmenopausal women with disease progression on antiestrogens. At this time, it is unknown whether there is an advantage for the use of fulvestrant before *versus* after use of AIs. A clinical benefit rate of $\sim 40\%$ has been reported for the use of an AI after fulvestrant therapy. Despite the increasing use of AIs as first-line therapy for metastatic disease in postmenopausal women and the likelihood that these agents will see increasing application in adjuvant and prevention settings, evidence on which to base treatment selection after progression on a nonsteroidal AI is largely limited to retrospective reports with some Phase II data. Whereas use of a steroidal AI, fulvestrant, tamoxifen, and megestrol acetate are all options, objective responses have been reported after AI therapy with pharmacological doses of estrogens. Thus, clinical trials are needed to provide guidance for sequencing of endocrine therapy in metastatic disease.

Endocrine Approaches to Prevention of Breast Cancer.

Preclinical and clinical evidence suggest a major role for estrogen in the promotion of breast cancer and possibly in its initiation. The Breast Cancer Prevention P-1 trial of tamoxifen has established the principle that a reduction in invasive and preinvasive breast lesions can be achieved through ER antagonism. Another SERM, raloxifene, is currently being tested against tamoxifen in the STAR trial of prevention in postmenopausal women. In clinical studies AIs appear to have an improved therapeutic index to the SERMs, and the ATAC data showing a reduction in contralateral breast cancer suggest that the AIs may be an alternative to the SERMs for cancer prevention. They are currently being evaluated as potential preventive agents in small preliminary trials based on risk factors such as mammographic density of tissue or elevated serum estradiol levels. Phase III trials of AIs in breast cancer prevention are now underway, but will not begin reporting results for at least 6 years. The IBIS 2 trial, a follow-up to the ATAC trial, randomizes women at elevated risk to anastrozole or placebo. The Italian ApreS trial (exemestane *versus* placebo) is enrolling BRCA 1/2 carriers who are postmenopausal and have not yet developed breast

cancer. The National Cancer Institute of Canada Clinical Trial Group study MAP3 will randomize women at elevated risk to placebo or to exemestane with or without celecoxib. The inclusion of a cyclooxygenase-2 inhibitor is based on observations that elevated prostaglandin E₂ levels associated with preinvasive and invasive breast lesions increase activity in a number of tumor-promoting pathways. While important and promising, these ongoing studies in postmenopausal women must be complemented by additional investigation beyond the P-1 trial in younger women. The RAZOR study is a small pilot project to determine the feasibility of a larger trial comparing raloxifene plus goserelin to placebo in premenopausal women at heightened risk.

In coming to terms with the influence of estrogen on breast cancer risk, the paradox of the protective effect of early pregnancy needs to be better understood and its implications fully explored. In a murine model developed to mimic and study the effects of pregnancy on breast cancer prevention, short-term exposure to physiological doses of estrogen and progestins in the sexually maturing animal have clear protective effects on subsequent exposure to carcinogens. The mechanism for the protective effect in this model includes activation of the tumor suppressor gene *p53*. If this process can be more thoroughly understood and confirmed in humans, it may be possible to develop a short-term intervention strategy with an agonist SERM that would be a safe and tolerable preventive therapy for young women at increased risk for breast cancer.

In the risk/benefit evaluation of preventive interventions, the long-term implications of estrogen deprivation on end organs and cognitive function needs further study. Better selection tools are needed to identify women at elevated risk. Serum levels of estradiol have been used as a risk marker. The significance of intrabreast estrogen levels as an independent risk factor and marker in postmenopausal women has not yet been thoroughly studied.

Disease Models and Research Priorities. The MCF-7 cell line has been of great and continuing value in understanding the biology of endocrine-responsive breast cancer and in predicting the clinical efficacy of potential therapies. However, data developed using this cell line, or any other cell line derived from a single tumor, may only be applicable to a specific phenotype or subgroup of breast cancer. This limitation may be particularly important in studying mechanisms of resistance and in evaluating interventions with combination therapies. The importance of interactions between tumor cells and stroma is also increasingly recognized as necessary both to our understanding of tumor development and as a source of potential therapeutic targets. Three-dimensional culturing of tumor cells is required to properly replicate this cross-talk in *in vitro* models. Furthermore, molecular studies in tumor cell lines may not translate adequately to an understanding of the biology of normal tissue. For prevention strategies, it will be important to model pharmacological interventions in normal cells, optimally using three-dimensional cultures.

Xenograft models do afford the interaction of tumor and host stroma but, like cell lines, carry the limitation of representing a single tumor or tumor subgroup. As distinct subgroups of human breast cancer are defined, it will be important to develop

cell lines and animal models that can represent this spectrum for preclinical investigation.

Although transgenics have greatly advanced basic research in cancer biology, the available mouse models of breast cancer have some significant limitations in their usefulness for the study of endocrine-responsive disease. This primarily relates to the fact that the promoters used are themselves hormone-responsive, confounding attempts to study the effects of hormones in the system. Transgenic models in other species, such as the rat, may provide opportunities for studying ER-positive tumors, which has been difficult in the mouse. Better models for metastatic disease are needed, as the few currently available do not optimally simulate human disease. There are also few validated animal models representing early phases of disease in which preventive approaches can be explored. Finally, a comparison of gene microarray data from human breast tumors to that of mouse mammary tumors will be critical in defining and developing appropriate mouse models for the preclinical evaluation of individual agents and treatment strategies.

Preclinical findings must ultimately be validated in the clinic. Neoadjuvant studies offer an opportunity to investigate biology and new therapies in early stage human breast cancer. A relatively small neoadjuvant study with defined clinical and biomarker endpoints can provide essential preliminary data to support subsequent larger formal adjuvant studies. Biological insights stemming from these studies can also identify new leads for therapeutic intervention and response prediction. The IMPACT neoadjuvant study of tamoxifen *versus* anastrozole will soon be reporting, and its findings, interpreted in conjunction with the ATAC adjuvant trial, are expected to support the predictive value of neoadjuvant studies.

While the neoadjuvant model is a valuable tool for exploratory investigation, it does have a number of shortcomings. The number of suitable patients is limited by the ever-decreasing size of primary tumors at diagnosis. Furthermore, it is difficult to validate the predictive results of neoadjuvant endocrine therapy for individual patients, even if the overall results of a trial mirror those of traditional adjuvant therapy. The long duration of adjuvant endocrine therapy compared with chemotherapy, which can be completed in ≤ 24 weeks, renders the design of trials to validate neoadjuvant endocrine therapy problematic. A trial could be done in postmenopausal women who undergo an initial biopsy and sentinel node procedure. Those patients in whom chemotherapy was not indicated could then be randomized to either 4 months of neoadjuvant endocrine therapy followed by primary therapy and then continued on the same endocrine therapy, or to initial primary therapy followed by the same adjuvant endocrine therapy. The total duration of endocrine therapy would be balanced in the two arms. For patients in whom chemotherapy was indicated the randomization would be between preoperative chemotherapy and preoperative aromatase inhibitor therapy. For those getting preoperative endocrine treatment, chemotherapy would be administered after surgery (holding the endocrine treatment during chemotherapy administration). This would at least provide some information on the predictive value of the results of neoadjuvant therapy (at the

time of primary surgery) on long-term outcome for individual patients in the first arm and as to whether there was any outcome advantage to neoadjuvant plus conventional adjuvant therapy *versus* conventional adjuvant endocrine therapy scheduling.

Observations in the neoadjuvant setting do not necessarily translate to the behavior of metastatic disease. For example, a relatively small proportion of patients in neoadjuvant trials demonstrate clinically apparent acquired resistance. Also, it is not understood why breast cancer metastasizes in an organ-specific manner in different patients, and whether these differences are biologically important. For the study of metastatic disease, repeat biopsies before, during, and after a therapy to assess changes in biomarkers and gene microarrays may reveal mechanisms of both primary and acquired resistance. These critically important studies can be done on small specimens, making such a protocol feasible and acceptable. Similarly, it will be of great value to obtain and study samples from tumors that develop in participants of prevention trials such as STAR.

In addition to better models, better disease markers and predictors are needed. To accurately assess the benefits of various interventions, it will be important to be able to identify patients who are likely to benefit from that strategy. For example, recent observations suggest that low levels and/or cytosolic mislocalization of the cyclin-dependent kinase inhibitor p27 identify subsets of patients with potential aberrant coexpression of oncogenic kinases who are, thus, more likely to be resistant to endocrine therapy alone. On the other hand, recent data suggest that in patients with ER+ breast cancers and high levels of nuclear p27, adjuvant hormonal therapy is superior to chemotherapy. Therefore, patients with low and/or cytosolic p27 would be good candidates for trials that explore the combined use of antiproliferative factor therapies and endocrine therapies.

In advanced breast cancer, numerous clinical trials evaluating new biological agents that target a variety of cellular pathways have been initiated. These include HER-1 kinase inhibitors, dual kinase inhibitors (HER-1 and HER-2 tyrosine kinase inhibition), vascular endothelial growth factor monoclonal antibody, cyclin-dependent kinase inhibitors, cyclooxygenase-2 inhibitors, farnesyl transferase inhibitors, and rapamycin analogues (mTOR inhibitor). These agents have potential for combined use with antiestrogens or aromatase inhibitors in an effort to block cross-talk in hormone receptor-positive breast cancer. It is possible that drugs with modest or no activity as monotherapy could have substantial benefit when combined with agents acting on different pathways or elsewhere on the same pathway. Although the efficacy of the receptor tyrosine kinase inhibitor gefitinib as monotherapy is currently unclear, when given in combination with tamoxifen, it significantly delays the onset of resistance. It will be a particular challenge to avoid discarding potentially useful agents based on the classical screening approach of single agent efficacy. Improved preclinical models and well-designed clinical trials that can identify appropriate patient populations will be required to define the optimal use of these new agents in conjunction with endocrine therapy.