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A Phase II Chemoprevention Trial Design to Identify Surrogate Endpoint Biomarkers in Breast Cancer

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Abstract Surrogate biomarkers for risk assessment and efficacy of potential chemopreventive agents are needed to improve the efficiency and reduce the cost of conducting chemoprevention trials. In addition to criteria of sensitivity, specificity, quantifiability, and reproducibility applicable to most potential biomarkers, there are additional specific constraints in developing biomarkers for specific organ sites. In the case of breast tissue, these difficulties include lack of a consensus on the nature of premalignant lesions and the histologic criteria used to define them; even when such a consensus can be evolved, there are limitations in visualizing such lesions without invasive biopsies. Also, knowledge of specific genetic and biochemical changes in premalignant lesions is limited. In addition, the physiology of breast tissue is cyclic; no proven, relevant markers can be studied in a randomly obtained needle aspirate. The earliest determinate lesion that can be recognized in breast tissue is ductal carcinoma *in situ* (DCIS). At the University of Texas M.D. Anderson Cancer Center, we have initiated a study to develop biomarkers for tamoxifen and 4-hydroxyphenylretinamide by administering one or both of these drugs to women with DCIS or small invasive lesions in the interval between the initial diagnostic core biopsy and definitive surgery. The treatment is to be administered for 2–4 weeks. Proposed biomarkers to be studied include: (a) markers associated with neoplastic phenotypes, *e.g.*, excessive proliferation, alterations of nuclear morphology and angiogenesis; (b) proteins likely to be required for response to the putative chemopreventive agents, *e.g.*, estrogen receptor, nuclear retinoid receptors; (c) markers indicative of intact downstream response pathways, *e.g.*, progesterone receptors; (d) oncogenes and tumor suppressor genes regulated by the proposed chemopreventive agents, *e.g.*, *neu*, TGF-β; and (e) potential novel markers of genetic instability that could be studied in randomly obtained needle aspirates, *i.e.*, random chromosomal gains and losses in high risk mammary epithelium. The experience gained in designing and conducting this trial is expected to facilitate development of future chemoprevention trials of breast, as well as other organ site cancers.

Key words: Biomarkers, breast cancer, chemoprevention, clinical trial, ductal carcinoma *in situ*, Phase II, retinoids, tamoxifen

Effective preventive strategies are needed to reduce the continuing high morbidity and mortality from breast cancer. This requires identification of potentially active interventions (pharmacologic or behavioral) followed by well-designed clinical trials to test their efficacy [1]. However, clinical trial designs based on conventional means of risk assessment, which use cancer occurrence as an endpoint, require a large number of subjects and long periods of follow-up to provide meaningful results [1]. Novel strategies using biological markers as surrogate endpoints (SEBs) may provide a more cost-effective and rapid means of testing chemopreventive interventions.

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THE CHALLENGE

A large number of genetic, biochemical and phenotypic alterations occur during the development of breast cancer [2–4]. Many of these can be considered potential biomarkers for risk assessment and/or efficacy in prevention trials. Previously, we described general criteria to evaluate any candidate alteration as a potential biomarker [5]. These include sensitivity, specificity, quantifiability, modulation by the proposed intervention, and technical feasibility in the available tissue specimen. Several additional, organ-specific constraints are encountered in trying to identify SEBs for breast cancer. For instance, it is difficult to identify premalignant lesions in the breast without a directed, invasive biopsy. Many investigators feel that such premalignant lesions as atypical hyperplasia are merely predictors of subsequent neoplasia risk, not true premalignant lesions [reviewed in 6]. There is a dearth of well-characterized, specific genetic or biochemical changes in premalignant lesions [5]. Finally, cyclical hormonal influences on breast epithelium lead to significant alterations in the expression of many growth regulators during the menstrual cycle [7].

DUCTAL CARCINOMA *IN SITU* (DCIS) AS A CHEMOPREVENTION TARGET

To address the above problems and develop strategies for design of Phase II trials, an NCI-sponsored workshop, "Chemoprevention of Breast Cancer: Surrogate Endpoints and Agents in Short-Term Clinical Trials," was held October 5–10, 1993 in Lake Tahoe, California (see *J. Cell. Biochem.* Suppl. 17G, 1993). Following the deliberations, a consensus statement identified DCIS as a chemoprevention target. Also recognized was the short window of opportunity that exists between initial diagnosis of DCIS on a needle biopsy and definitive surgery, and that this period could be used to test modulation of potential SEBs by chemopreventive agents.

There are several reasons for targeting women with DCIS for short-term intervention trials. DCIS constitutes 15–20% of screen-detected breast cancers in recent series in contrast to the 0.8–5% incidence in older literature [8]. Complete excision (*e.g.*, by simple mastectomy) results in a virtual 100% cure rate. However, incompletely excised or treated with a lumpectomy alone, DCIS has up to a 30–40% recurrence rate [9–11]. Furthermore, recurrences following DCIS are frequently invasive, usually at or near the site of excision [12,13]. Most important, perhaps, is the realization that DCIS is the earliest lesion with specific genetic changes and defined biologic behavior that can be recognized in the breast tissue and for which there is a fairly good agreement among pathologists in establishing a histological diagnosis [6,14].

At present, a major limitation of using DCIS as a prevention target is the inability to rule out the presence of associated invasive disease without removing the whole lesion. Until this can be accomplished, SEB trials in DCIS will have to be limited to short duration treatments.

DESIGN OF A SHORT TERM, PHASE II SEB TRIAL FOR TAMOXIFEN AND 4-HYDROXYPHENYLRETINAMIDE (4-HPR)

In accordance with the consensus statement of the breast cancer chemoprevention workshop, the NCI invited proposals for short-term, Phase II trials of tamoxifen and 4-HPR. The University of Texas M.D. Anderson Cancer Center successfully competed for the funding of one such protocol. The design of this trial and the proposed biomarkers to be investigated are discussed below.

The study subjects will be women presenting with small breast lumps (T_1N_0 by TNM staging criteria) or mammographic calcifications suspicious for malignancy. Core biopsies (five biopsies obtained stereotactically from mammographically visualized lesions, or at least two biopsies obtained under ultrasound guidance from palpable lesions) as well as fine-needle aspirates (to obtain whole cells) will be performed. Following confirmation of histologic diagnosis, participants will be randomized to receive 20 mg tamoxifen, 200 mg 4-HPR, or a combination of both. Treatment will be continued until the time of definitive surgery (planned duration of treatment 3 ± 1 weeks). An effort will be made to plan definitive surgery on all participants on day 21 to eliminate any confounding effect of the variability of treatment duration on SEBs. Toxicity will be carefully monitored in all participants and compliance will be ensured by measuring blood levels of the study drugs and their metabolites, as well as by

pill counts. Tissue obtained at the time of definitive surgery will be compared with the measurements on pretreatment diagnostic biopsies to assess modulation of biomarkers. Accrual of 50 subjects is planned for each arm.

PROPOSED BIOMARKERS

The biomarkers proposed to be studied in this trial are listed in Table I and have been chosen because they fulfill one or more of the following criteria: they are relevant to the development of neoplasia, either phenotypically (*e.g.*, proliferation, angiogenesis, and nuclear morphometric features) [15,16] or mechanistically (*e.g.*, molecular markers such as *neu*); they are likely to be required for response to the proposed chemopreventive agent (*e.g.*, estrogen receptor, retinoid receptors) [17]; or they are relevant to breast carcinogenesis and are likely to be modulated by the proposed intervention (*e.g.*, tamoxifen treatment may be expected to lead to upregulation of estrogen receptor, progesterone receptor, *neu*, and TGF-β with a concomitant decrease in proliferation and angiogenesis [15,18–22], whereas retinoids may lead to upregulation of TGF-β and retinoid receptors [23] but downregulation of *neu* [24] (Fig. 1).

In addition, we plan to investigate novel markers of genetic instability, *i.e.*, numerical chromosomal aberrations in histologically normal epithelial cells. In future trials, these could be studied in randomly obtained fine-needle aspirates, thus eliminating the need for a directed biopsy and the prerequisite of the presence of an index lesion before a patient can be enrolled in a short-term SEB trial.

An important objective of this trial is to perform a detailed quantitative assessment of the biomarkers by taking advantage of the rapidly improving image analysis techniques. We will quantitate immunohistochemical staining using appropriate positive and negative internal controls, and quantitate morphologic and textural features of the nuclei in the index lesion and adjacent normal epithelium using the Cyto-Savant™ image analysis systems (Xillix, Vancouver, B.C., Canada) [25].

RELEVANCE OF THE BREAST BIOMARKER TRIAL TO GYNECOLOGIC CANCER PREVENTION TRIALS

The experience gained in designing the above trial for breast cancer may help with the design of similar trials for gynecologic cancers which share many of the same risk factors and often harbor many of the same genetic alterations found in breast cancers. The general criteria for selection and validation of potential biomarkers are common to all organ sites [5]. Similarly, many of the proposed biomarkers, *e.g.*, quantitative nuclear morphology [26], proliferation, and angiogenesis, are potentially useful irrespective of the specific chemopreventive intervention employed. Perhaps more important is the impact of cyclical changes in the hormonal milieu which will need to be taken into account when evaluating biomarkers in gynecologic target organs, just as in breast cancer [7,27]. Limitations of tissue

TABLE I. Proposed SEBs for Phase II Trial of Tamoxifen and 4-HPR

(a)	Markers of neoplastic phenotype
	• Quantitative histology — nuclear morphometry
	• Proliferation — Ki-67 immunostaining
	• Angiogenesis — Factor VIII immunostaining
(b)	Markers of intact response pathways
	• Estrogen receptor
	• Progesterone receptor
	• Nuclear retinoid receptors
(c)	Inducible growth regulatory molecules
	• Transforming growth factor-β
	• NEU oncoprotein
(d)	Markers of genetic instability
	• Numerical chromosomal aberrations

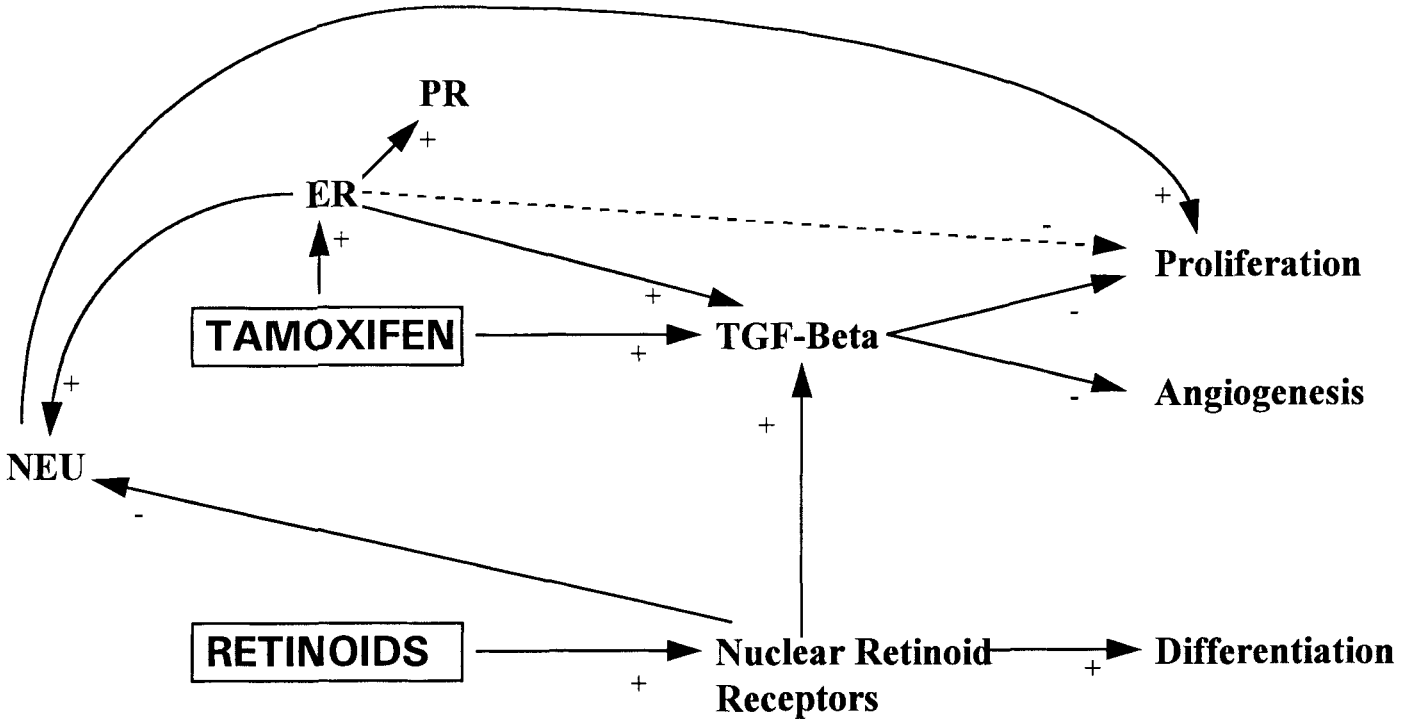

Fig. 1. Potential interactions among proposed biomarkers for tamoxifen and retinoids. +, upregulation; –, downregulation. Broken line indicates that the mechanism of the proposed effect is unknown.

availability are also equally applicable to gynecologic sites, especially the ovary. Most importantly, this trial design takes into account the concerns of participants who have newly diagnosed cancers and are likely to be unwilling to participate in a short-term prevention trial if it interfers with their standard, definitive, and timely local therapy.

CONCLUSIONS

The development of SEBs for chemoprevention trials is still in its infancy. It is hoped that these early trials will not only assist in identifying SEBs, but also in understanding the *in vivo* mechanisms of action of the chemopreventive agents, so that future chemoprevention trials can be designed in a more efficient and rational fashion.

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