Evaluation of Cytophotometric DNA Content Abnormalities in Premalignant and Preinvasive Breast Lesions

Daniel W. Visscher, MD, John D. Crissman, MD, and Mark Micale, PhD

Harper Hospital, Department of Pathology, Wayne State University School of Medicine, Detroit, MI 48201

Abstract Progression of breast neoplasia is characterized by a variety of causal and nonspecific molecular, karyotypic, and cellular level genetic alterations. These include allelic losses, chromosomal rearrangements, and aneusomies, as well as widely divergent clonal DNA content aberrations. Establishment of the sequence and the pathologic significance of individual changes has been challenging owing to disease heterogeneity, protracted natural history, and difficulties in sampling and localizing precursor lesions. These limitations have driven attempts at cytophotometric evaluation of premalignant/preinvasive proliferations using histologic (*i.e., in situ*) microdissection of intact paraffin-embedded tissue sections. Using image cytophotometric measurements in preserved sections, clonal DNA content abnormalities are identified in up to three-fourths of preinvasive breast carcinomas. The incidence of DNA aneuploidy is grade-dependent and similar in degree to invasive lesions. Comparison of ploidy determinations between preinvasive and corresponding invading populations, however, suggests host tissue permeation is accompanied by measurable DNA content shifts in many cases. Image cytophotometric DNA content abnormalities are also detectable in florid/atypical proliferative lesions, albeit less frequently (~25% of cases) and to a lesser extent (*i.e.*, near diploid) than cytologically malignant lesions. Although the sensitivity of cytophotometric ploidy assessments in tissue sections is limited by nuclear sectioning, the presence of genomic instability in premalignant lesions is supported by evidence of individual chromosome aneuploidy, demonstrated by interphase cytogenetics with fluorescent centromere-specific probes. These data suggest cytophotometrically detectable DNA content anomalies may precede unequivocal morphologic transformation in breast neoplasia. Further, clonal DNA content heterogeneity in breast carcinoma may accompany biologically critical steps in neoplastic progression of breast tumors.

Subjects and Recruitment Strategies for a Short-Term Phase II Chemoprevention Trial of Breast Cancer Using Surrogate Endpoint Biomarkers

Victor G. Vogel, MD, MHS

University of Texas M.D. Anderson Cancer Center, Department of Breast and Gynecologic Oncology, Houston, TX 77030

Abstract No validated serum markers of breast cancer risk have been identified. Therefore, the identification of women for a clinical trial of surrogate endpoint biomarkers (SEBs) is complicated by the need for repeated sampling of breast parenchyma to determine the biologic effect of the chemopreventive agent. Criteria for the ideal study population include: (1) a rapidly identifiable high-risk profile;

(2) the presence of a histologic lesion known to be associated with an increased risk of developing invasive breast cancer; and (3) clinical and ethical justification for repeated breast biopsies. The model of Gail *et al.* [1] accurately predicts the subsequent incidence of breast cancer for women who are being examined annually with mammography [2], but less than 10% of women younger than 50 years with elevated Gail-model risk scores have sufficient risk to enter the Breast Cancer Prevention Trial (BCPT). While not all eligible women will accept randomization, we have published data showing that more than 80% of women with two or more relatives with breast cancer perceive their personal risk for breast cancer to be high [3]. As many as half of these women say they are willing to participate in chemoprevention trials (data submitted for publication), but the requirement for repeated sampling of breast tissue in an SEB trial may prevent their enrollment. While it is possible to sample breast tissue without biopsy using fine needle aspiration, sampling errors occur due to random technical misses of the breast parenchyma in 25% of women with risk factors. Reliable, reproducible sampling necessitates either open or needle core biopsy. Therefore, women with a first biopsy done for clinical reasons are the most appropriate candidates for an SEB trial. We have estimated there are more than 1.2 million US white *women age* 50 or older with a history of biopsy showing proliferative benign breast disease; another 10,000 biopsies showing proliferative changes are done each year in US white women. Approximately 20% of women with proliferative disease also have atypical hyperplasia [4]. In addition, post-menopausal women with Stage I breast cancers can be considered for inclusion in an SEB trial because of the lack of consensus regarding adjuvant therapy. Sampling the contralateral breast at the time of breast cancer diagnosis followed by a trial of a chemopreventive agent is feasible in these women. Including patients with ductal carcinoma *in situ* (DCIS) does not interfere with existing trials. SEBs can be studied in the original biopsy specimen, and the chemopreventive agent can be administered for a short duration before definitive radiotherapy is employed. Repeated sampling of breast tissue with open biopsy following a course of a chemopreventive agent is justifiable in this group with a 10-year risk of developing invasive breast cancer that approaches 40% in the absence of radiotherapy. We have shown that eligible and willing subjects for a chemoprevention trial can be recruited efficiently following screening mammography and rapid risk assessment (data submitted for publication). A similar strategy can be extended to pathologic data bases. We have also used group informed consent techniques with success for the BCPT. In this technique, groups of eligible women are educated together about the trial with a yield of 18% of eligible subjects enrolling. This strategy can be employed in a trial of SEBs. Because women with lobular carcinoma *in situ* are eligible for BCPT, they should not be studied in an SEB trial. In summary, women with proliferative disease with or without atypia, those with DCIS, post-menopausal women with Stage I disease, and possibly, women with increased multivariate risk for breast cancer constitute the ideal study populations for a trial of SEBs in breast cancer.

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