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PANEL STATEMENT

The Most Promising Surrogate Endpoint Biomarkers for Screening Candidate Chemopreventive Compounds for Prostatic Adenocarcinoma in Short-Term Phase II Clinical Trials

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Abstract Surrogate endpoint biomarkers (SEBs) are needed in clinical chemoprevention trials to avoid the excessively long study periods and high costs associated with the use of cancer incidence reduction as an endpoint, particularly with relatively slow-growing tumors such as prostatic adenocarcinoma. SEBs should be directly associated with the evolution of neoplasia, and develop with high frequency in abnormal cells of susceptible individuals. If SEBs can be modified by a particular intervention regimen in short-term studies, the rationale for carrying out long-term studies may be strengthened. The consensus panel identified a small and manageable group of biomarkers measured in tissue or serum as the most promising in prostate cancer chemoprevention, including (1) prostate specific antigen (PSA); (2) morphometric markers, such as nuclear size and roundness; (3) proliferation markers, such as MIB-1 and PCNA; (4) nuclear DNA content (ploidy); (5) oncogene c-*erb*B-2 (HER-2/*neu*) expression; (6) angiogenesis; and (7) high-grade prostatic intraepithelial neoplasia (PIN). Information regarding many of these and other biomarkers is limited, calling for further investigation. Also, these factors, chosen chiefly for their proven or proposed utility as prognostic factors, may be less useful as SEBs. It was agreed that concurrent study of numerous markers rather than single markers allows comparison of their relative utility, including assessment of ease of quantitation and the sensitivity, specificity, and positive and negative predictive value.

Key words: Biomarkers, chemoprevention, premalignancy, prostate cancer, prostatic intraepithelial neoplasia, risk stratification

Cancer chemoprevention refers to the inhibition, reduction, or prevention of invasive neoplasia with drugs or chemicals. The use of surrogate endpoint biomarkers (SEBs) promises rapid results in clinical prevention trials, but progress in chemopreventive drug development is slowed by lack of agreement on which SEBs can be substituted for cancer incidence reduction. SEBs make it possible to carry out many studies on fewer subjects for shorter periods of time. Useful SEBs are directly associated with the evolution of neoplasia, and develop with high frequency in abnormal cells of susceptible individuals. A useful surrogate biomarker should be measured easily and reliably and with sufficient precision, correlated strongly to the true outcome of reduced cancer incidence and mortality, and modifiable by intervention. If SEBs are modified by a particular intervention regimen in short-term studies, this strengthens the rationale for carrying out long-term studies.

Chemoprevention trials differ from standard chemotherapeutic regimens, requiring innovative study designs and strategies. Phase II clinical trials begin with small, short-term, efficient studies to determine the dose of a given chemopreventive agent that exhibits a pharmacodynamic effect on an SEB, to determine the minimum dose at which this biological effect is observed, and to confirm the maximum safe dose. Phase II trials often conclude with randomized, blinded studies of small groups of subjects using a measurable biological effect of the agent but not of the placebo as an endpoint. These studies should improve future research designs, provide a better biological understanding of the agent, and provide more quantitative endpoints.

A variety of factors are available to identify and evaluate risk modulation in selected target populations by chemopreventive agents. These factors include reversal of abnormal cytology, prevention of nuclear aberrations or aneuploidy, inhibition of selected enzymes such as ornithine decarboxylase or prostaglandin synthetase, and changes in cell proliferation. Markers of precancerous lesions may also be useful to define populations that may benefit from chemoprevention trials; however, more information is required concerning the ability of such markers to predict and/or modulate cancer incidence. The development of sensitive and accurate SEBs should greatly enhance the ability to design effective cancer risk reduction trials.

A good SEB should reflect changes at various stages in carcinogenesis, and may be chosen from among morphologic, morphometric, genomic, proliferative, regulatory, and differentiation markers, or other categories of markers. Concurrent study of numerous markers rather than single markers allows comparisons of their relative utility, including assessment of ease of quantitation and the sensitivity, specificity, and positive and negative predictive value. The SEB may be derived from study of biopsy tissue as well as blood, fine needle aspiration cytology (if indicated), and urine and semen analysis as appropriate for the SEB under study. The rationale for each marker may be based on relevant animal studies, *in vitro* studies, or by analogy with known SEBs in other organ systems (*e.g.*, genomic changes in colonic carcinogenesis).

PROMISING SEBs IN PROSTATE CANCER

The following seven biomarkers are considered promising as surrogate endpoints for screening chemopreventive compounds for prostate cancer in short-term Phase II trials. This panel of candidate SEBs was considered by the consensus committee as most likely to show an effect in short-term trials, preferably within 3 years or less. Each is measured easily and accurately in serum or in tissue specimens such as formalin-fixed, paraffin-embedded needle biopsies, and may be modifiable by intervention. Also, the efficacy of each for prognosis has been established and confirmed. It should be noted, however, that none of these markers has been tested as a surrogate biomarker in prostate cancer, so this consensus statement is based on incomplete data.

Prostate Specific Antigen

Prostate specific antigen (PSA) is the most important, accurate, and clinically useful biochemical prostate marker; produced by and specific for prostatic tissue, it is an excellent candidate SEB for chemoprevention trials, and may be the most practical because it is measured from serum rather than from tissue. This 34 kD serine protease is manufactured by the epithelial cells and secreted into the prostatic ductal system, where it catalyzes the liquefaction of the

seminal coagulum after ejaculation. Serum levels are normally below about 4.0 ng/ml, but vary according to patient age [1,2]; any process which disrupts the normal architecture of the prostate allows diffusion of PSA into the stroma, where it gains access to the blood through the microvasculature. Elevated serum PSA levels are seen with prostatitis, benign prostatic hypertrophy (BPH), and transiently following biopsy, but the most clinically important elevations are seen with prostatic adenocarcinoma [3]. Although cancer produces less PSA per cell than benign epithelium, the greater number and density of malignant cells, and the associated stromal disruption, accounts for the elevated serum PSA levels. The clinical utility of PSA has recently been reviewed [4].

The major form of measurable PSA in the serum is a complex between the PSA molecule and α-1-anti-chymotrypsin; there is a higher proportion of complexed PSA in the serum of patients with cancer than in other patients, and this serum fractionation may be diagnostically useful [5]. New microassays for serum PSA allow detectability as low as 0.1 ng/ml.

In tissue sections of normal and neoplastic prostate, PSA expression is easily demonstrated immunohistochemically, and helps the pathologist distinguish high-grade prostate cancer from transitional cell carcinoma, colonic carcinoma, granulomatous prostatitis, lymphoma, and other histologic mimics [6]. It also allows the site of tumor origin to be identified in metastatic adenocarcinoma. PSA expression is usually greater in low-grade tumors than in high-grade tumors, but shows significant heterogeneity from cell to cell. Up to 1.6% of poorly differentiated cancers will be negative for both PSA and prostatic acid phosphatase [6–9].

Morphometric Markers

Numerous morphometric markers have provided valuable prognostic information in prostate cancer, including size and number of nucleoli; nuclear texture, shape, and roundness; and the number of apoptotic bodies. Morphometric studies should employ objective, quantitative morphometric techniques, preferably computer-assisted, although manual methods can be used when other methods are not available. A recent study successfully separated prostate cancers with favorable and unfavorable prognoses based on a discriminant function derived from five chromatin texture-related features [10]. The consensus panel recognizes that there are no accepted standards for morphometric studies, and considers this an important and significant area for future investigation.

Proliferation Markers

The rate of cell proliferation is a useful prognostic factor and SEB in many cancers. The consensus panel felt that proliferation should be included, and preferred the markers MIB-1 and proliferating cell nuclear antigen (PCNA) rather than others such as mitotic index, S-phase fraction, Ki-67, topoisomerase II, DNA polymerase-alpha, and ^{3}H-thymidine or 5-bromo-2′-deoxyuridine incorporation. The utility of proliferation fraction as an SEB and prognostic factor in prostate cancer is limited by the low and narrow range of growth fractions, varying from 0.4% to 9.1% in one study [11].

Monoclonal antibody Ki-67 recognizes a human nuclear antigen expressed in the S, G_2, and M phases of all cycling human cells and absent in G_0 and early G_1; although Ki-67 remains popular to evaluate proliferative activity in frozen tissue, it has been replaced in archival studies by its counterpart, MIB-1, which provides accurate and reproducible immunohistochemical results in paraffin-embedded sections. Ki-67 expression weakly correlated with time to tumor progression after hormonal therapy [12,13]. Results with MIB-1 in prostate cancer are just emerging, but appear to be comparable to Ki-67 in other cancers.

PCNA (or cyclin) is a nonhistone nuclear protein, an accessory of DNA polymerase. Closely linked to the cell cycle, its expression is maximal during S phase. Nemoto *et al.* [14] showed a PCNA labeling index in prostate cancer of 1.6%–15.0%, with heterogeneity in expression in different parts of the tumors. Montironi *et al.* [15,16] described decreasing expression of PCNA in normal epithelium, prostatic intraepithelial neoplasia (PIN), and cancer in successive cell layers from the gland periphery to the lumen, suggesting progressive terminal differentiation as cells move toward the lumen.

Nuclear DNA Content (Ploidy)

DNA content analysis of prostate cancer by flow cytometry and static image analysis may provide independent prognostic information to supplement histopathologic examination. Patients with diploid tumors have a more favorable outcome than those with aneuploid tumors; for example, among patients with lymph node metastases treated with radical prostatectomy and androgen deprivation therapy, those with diploid tumors may survive 20 years or more, whereas those with aneuploid tumors die within 5 years [17]. However, the ploidy pattern of prostate cancer is often heterogeneous, creating potential problems with sampling error. An international DNA Cytometry Conference [18] reviewed the literature and concluded that the clinical significance and biologic basis of DNA ploidy needs further investigation.

Our consensus panel felt that the evidence linking nuclear DNA content and prognosis was sufficiently compelling to recommend it as a useful SEB in chemoprevention trials, although the technique is limited by inexact quality standards and interpretative differences. The relative merits of flow cytometry and static image analysis are important considerations beyond the scope of this session.

Oncogene c-*erb*B-2 (HER-2/*neu*) Expression

The c-*erb*B-2 oncogene codes for a transmembrane growth factor receptor with 43% homology to epidermal growth factor receptor (EGFR), but is distinct from EGFR in its chromosomal location and specificity for signal transduction and ligand binding. The function of the c-*erb*B-2 oncoprotein is uncertain; it is thought to play a role in cell growth and differentiation, possessing an intracellular domain with tyrosine specific kinase activity and an extracellular domain. Results of immunohistochemical studies have been variable, ranging from 0–92% staining in hyperplastic prostatic tissue and 0–100% of prostate cancers; the discordant results are attributed to differences in tissue handling and antibody reagents. The consensus panel acknowledged the work of two of its members in endorsing c-*erb*B-2 expression as an SEB; Veltri *et al.* [19] found expression to be a strong univariate predictor of cancer progression in a series of 124 cases followed for a mean of 8.6 years, and Grizzle *et al.* [20] and Myers *et al.* [21] observed coarse and punctate cytoplasmic and membrane staining in a significant number of cancers and in PIN.

Other studies have suggested that c-*erb*B-2 is overexpressed in human prostate cancer, although this has been refuted [22–30]. Activated oncogenes such as *ras* and c-*erb*B-2 appear infrequently in early prostate cancers, but increased expression seems to be correlated with higher tumor grade and aneuploid status [29]. One or more tumor suppressor genes are apparently involved in prostatic carcinogenesis; existing data are not yet mature enough for the panel to recommend use of a genetic marker as an SEB at this time [31,32]. Inactivation of p53, a tumor suppressor gene on chromosome 17p, occurs in up to 25% of advanced primary prostate cancers, and up to about 50% of metastases, but is rare in early cancers, suggesting that it may play a role in late progression [33–36]. Loss of expression of the retinoblastoma gene on chromosome 13q is seen in a minority of prostate cancers, usually in advanced stages [37].

Angiogenesis

Angiogenesis (neovascularity or vessel density) is a necessary prerequisite for tumor growth and progression in most cancers, including prostatic adenocarcinoma. It appears to be stimulated by factors released from cancer cells, inflammatory cells, and the extracellular matrix. Vessel density is increased in PIN and cancer compared with normal and hyperplastic prostatic epithelium [38], and is an independent predictor of pathologic stage, malignant potential [39], and metastasis [40,41]. Significant differences remain in evaluating vessel density [immunohistochemical stain employed, selection criteria for area to be counted, and method of quantitation (manual versus automated)], but the consensus panel expects that standards will eventually be adopted to allow rational comparison of results from different centers.

High-Grade PIN

PIN represents the putative precancerous end of the morphologic continuum of cellular proliferation within prostatic ducts, ductules, and acini

[42–44]. Low-grade and high-grade PIN have been identified; high-grade PIN is considered the most likely precursor of invasive carcinoma, according to a recent consensus conference of the American Cancer Society [45]. In high-grade PIN (formerly PIN 2 and 3), the epithelial cells lining ducts and acini are heaped up, crowded, and irregularly spaced, with pronounced cell stratification. There is less variability in nuclear size than in low-grade PIN because the majority of nuclei are enlarged; the presence of prominent nucleoli, often multiple, is of great diagnostic value. PIN is ideally suited to be an SEB because of its probable role as a precursor for many prostate cancers; unfortunately, current imaging techniques are unable to detect it with precision [46,47], so biopsy is necessary for detection.

The continuum which culminates in high-grade PIN and early invasive cancer is characterized by basal cell layer disruption, progressive loss of markers of secretory differentiation, increasing nuclear and nucleolar abnormalities, increasing proliferative potential, and increasing variation in DNA content (aneuploidy) [48–50]. Clinical studies suggest that PIN predates carcinoma, with low-grade PIN first emerging in men in the third decade of life. PIN is often found in the vicinity of carcinoma; its identification in biopsy specimens of the prostate warrants further search for concurrent invasive carcinoma.

PIN offers promise as an intermediate endpoint in studies of chemoprevention of prostatic carcinoma. Recognizing the slow growth rate of prostate cancer and the considerable amount of time needed in animal and human studies for adequate follow-up, the noninvasive precursor lesion PIN is a suitable intermediate marker predictive of cancer in select cases [51].

PROTOCOL: RANDOMIZED DOUBLE BLIND PLACEBO-CONTROLLED STUDY

Patient Eligibility

Inclusion criteria. Phase II clinical chemoprevention trials should explicitly specify the selection criteria for inclusion in the study. Criteria should minimally include age, health status, clinical laboratory tests including PSA and method of PSA analysis, precise clinical and pathologic diagnosis and extent of the process, previous medical history, and previous therapy. The target populations suitable for Phase II chemoprevention trials in prostate cancer are discussed elsewhere in this volume.

Exclusion criteria. The selection criteria should explicitly specify which patients are excluded from study. Criteria should at the least exclude cases with initial biopsy findings suspicious for, but not diagnostic of, the SEB being studied (equivocal pathologic findings), noncompliance, drug interactions which might influence chemopreventive agent efficacy, including hormone replacement or hormone deprivation therapy, prior cancer, prior thromboembolic event, and life expectancy of less than the time span of the study (3 years).

Statistical considerations. The initial group size, projected size of the final group of evaluable subjects, drop-out estimate, and projected response rate should be used to calculate the number of subjects required to produce a study power of 80–90% or more. Any stratification criteria should be specified, and any periods of compliance "run-in" should be specified.

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