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High-grade prostatic intraepithelial neoplasia as an exposure biomarker for prostate cancer chemoprevention research

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There is a tremendous need for exposure biomarkers, which need to function as intermediate end-points in cancer chemoprevention studies. Likely candidates include process biomarkers, atypical adenomatous hyperplasia, and a newly identified atrophic state. High-grade prostatic intraepithelial neoplasia (HGPIN) has the potential to be a useful exposure biomarker, having substantial predictive value for prostate cancer in chemoprevention trials. A limitation of the use of HGPIN as a biomarker is accessibility, since it requires the use of a highly invasive procedure that would not normally be applied unless malignancy is suspected to be present. However, most other biomarkers of prostatic tissue are similarly invasive. The HGPIN lesion appears to be highly measurable; however, problems of sampling coupled with the heterogeneity of the prostate raise questions about the degree to which the presence of HGPIN can be seen to characterize a given person's prostate gland. HGPIN has the advantage that it appears to be quite highly proximal to the development of cancer and to be modifiable. It remains less clear to what degree it reflects the exposures that are believed to alter prostate cancer risk. HGPIN has been identified as a clinical entity only recently and much additional research on the utility of this marker is needed.

Need for biomarkers

Epidemiological research on prostate and other cancers has been plagued by imprecision in the measurement of diet, occupation, physical activity and other exposures (Marshall *et al.*, 1999). Occupational studies have suggested that some industrial exposures, such as cadmium, may be relevant to risk (Ross & Schottenfeld, 1996). Dietary studies have suggested that obesity or perhaps consumption of a diet high in fat or in vegetable fat may increase risk (Giovannucci *et al.*, 1993). It has also been suggested that a diet low in some plant-based antioxidants, especially lycopene, may be a risk factor for prostate cancer (Giovannucci *et al.*, 1995; Clinton & Giovannucci, 1998). For both etiological and epidemiological research on diet and prostate cancer, biological markers of exposure would be useful, if it can be shown that they provide greater accuracy in exposure assessment. It would be preferable to link these markers to long-term exposure. Prostate cancer is believed to involve an etiology that may span several decades. The most likely premalignant lesion for prostate cancer — high-grade prostatic intraepithelial neoplasia (HGPIN) — begins to appear in men in the third or fourth decade of life, three decades before prostate cancer becomes highly incident (Sakr *et al.*, 1996; Sakr, 1998). Research on smoking as a risk factor for prostate cancer also suggests effects that only appear after several decades of exposure (Giovannucci *et al.*, 1999). Clearly, we need biomarkers of exposure that span long periods of time.

Also needed are markers of premalignant change that might be related to risk-enhancing exposures. As an indicator of premalignant change, HGPIN could be useful as a marker of carcinogenic exposure. There is evidence that HGPIN is related to substantially increased risk of prostate cancer (Bostwick & Brawer, 1987; Davidson *et al.*, 1995). Studies which might link this premalignant lesion to exposure would be extremely valuable.

HGPIN could serve as a highly useful exposure biomarker, perhaps as an intermediate biomarker of prostate cancer risk.

Possible candidates

A number of process indicators proposed as possible exposure biomarkers are associated with increased risk of prostate cancer (Kelloff *et al.*, 1997). Among these are markers of change related to excess cellular proliferation. A deficiency of apoptosis (programmed cell death) has been prominently mentioned. Another likely exposure biomarker would be an indicator of oxidative damage or of excessive oxidation. Markers of differentiation or of mutagenesis might be useful. As markers of advanced carcinogenesis or of tumour progression, angiogenesis indicators could be informative (Kelloff *et al.*, 1997).

In addition, several nonmalignant disease states could provide important information. Adenomatous atypical hyperplasia (AAH) has been proposed (Sakr & Grignon, 1998). The limitation of AAH as an exposure marker is that there is, at present, only weak evidence that AAH is causally linked to increased risk of the majority of prostate cancer. More recently, a form of prostatic inflammatory atrophy has been proposed as related to increased risk (De Marzo *et al.*, 1999), although research on this condition is not yet fully developed.

HGPIN has been proposed as a biomarker. The evidence linking HGPIN to elevated risk of prostate cancer is strong enough that researchers have claimed that HGPIN is almost certainly the premalignant lesion out of which prostate cancer arises (Brawer, 1992; Davidson *et al.*, 1995). There are some prospective data supporting this assertion. On the other hand, a recent study by O'Dowd *et al.* (2000) suggests that the importance of HGPIN as a risk factor for prostate cancer among high-risk populations may have been overestimated.

Access

Given the importance of HGPIN as a risk factor for prostate cancer, it holds tremendous promise for research. However, one of the major limitations to its use is that it can be evaluated only by means of prostatic biopsy. The probability of complications, especially of infection due to prostatic biopsy, is non-trivial and this rules out using evaluation of HGPIN except as part of clinical care. Metabolites isolated from blood or seminal fluid may permit more accessible and valid indirect examination of the prostate. Environmental factors do not have direct access to the prostate, so that any tissue-based marker may be subject to substantial metabolic modification.

There is also some debate about the prevalence of HGPIN. Early reports suggested that the prevalence of this condition in populations at elevated risk could be as high as 15–16% (Bostwick, 1996a). More recent data suggest that it is only a third of that level and that many of the high-risk individuals with HGPIN have synchronous cancer (Weinstein & Epstein, 1993; and van der Kwast, this volume). Research based on large numbers of patients from a range of clinical practices throughout the United States indicates that the probability of detecting HGPIN and no cancer is in the vicinity of 4% of all prostatic biopsies performed (O'Dowd *et al.*, 2000; Weinstein & Epstein, 1993). These figures could change as the treatment of prostatic disease evolves over time. They are also subject to the vagaries of pathology: HGPIN may be occasionally overlooked, or it may be diagnosed as cancer. The incidence of asymptomatic clinical prostate cancer is less than 10%, and only 3% of men in western industrial societies die as a result of prostate cancer. The frequency of the joint presence of HGPIN and prostate cancer is substantially higher than that of HGPIN alone, in keeping with the suspected etiological significance of HGPIN.

Measurability

Whether HGPIN will be useful as a biological marker depends in large part on our ability to identify the extent of its presence in men who are at substantial risk of prostate cancer (Bostwick & Brawer, 1987; Bostwick, 1996b). HGPIN is usually characterized as present in a gland or in a series of glands (Epstein *et al.*, 1995). However, while there is good agreement on the presence or absence of HGPIN in a gland or a limited region of prostatic tissue, there is less agreement about the characterization of an entire prostate or patient in terms of the extent to which HGPIN is present. Clearly, one could consider the number of HGPIN lesions present, the percentage of area within a biopsy occupied by an HGPIN lesion, the extent to which HGPIN lesions are dysplastic, the percentage of

non-stromal tissue taken up by HGPIN, or perhaps the percentage of ductal tissue taken up by HGPIN (Montironi *et al.*, 1995). The extent to which any of these characteristics of HGPIN can be used to characterize the prostate of an individual has not been well defined or studied. The lesion itself can be quantified; one of the marks of HGPIN is degradation of the basal cell layer within the prostatic ducts and glands (Bartels *et al.*, 1998a; Sakr & Grignon, 1998), so that the percentage of the circumference of the basal cell layer that is degraded can be quantified. Grouping this information for a larger region of prostatic tissue, though, is not straightforward. Another potential marker of the extent of HGPIN is nuclear karyometry reflecting dysplasia in different cells (Bartels *et al.*, 1998b, c). There is substantial variation in the extent of dysplasia or cancer that is present. Means to evaluate sampling variability and incorporate this information into an attempt to characterize the tissue are required. A marker of the extent of HGPIN in a gland is the extent to which the circumference of the basal cell layer is eroded. However, the implications of a duct that is badly eroded, as opposed to several that are mildly so, for cancer risk is not clear.

Proximity to the causal pathway

As Schatzkin *et al.* (1990, 1996) and Kelloff *et al.* (1997) have made clear, the usefulness of a biological marker for chemoprevention depends on three characteristics of that biomarker. First is the degree to which it is dependent on a factor that increases risk of the disease. Ideally, the biomarker would be perfectly correlated with (a perfect marker of) exposure to the risk factor. The second characteristic is that the biomarker is predictive of disease. As has already been mentioned, HGPIN is an excellent predictor of prostate cancer risk (Brawer, 1992; Bostwick, 1996a; Davidson *et al.*, 1995), although O'Dowd *et al.* (2000) provide somewhat more equivocal evidence. There is a need for additional analyses of the differences in the results obtained by these studies as to the relevance of HGPIN to prostate cancer risk. The third criterion of biomarker usefulness is the degree to which the biomarker explains the association between exposure and risk. As Schatzkin *et al.* (1990, 1996) have pointed out, controlling statistically for a biomarker which explains the association of exposure and disease will eliminate the association between the exposure and disease. In other words, if the only path from exposure to disease is the one that is transmitted by the biomarker, statistical control for the biomarker would eliminate that pathway or association.

Modifiability

An important characteristic of a biological marker of exposure as relates to the development of chemoprevention strategies is that the marker be modifiable. Removal of the exposure which increases risk, or application of a chemopreventive agent, should decrease the extent of the marker. Thus, for evaluation of HGPIN as an exposure biomarker, it is of interest to determine whether the presence of HGPIN, as a neoplastic structure, is modified by removal of the risk-enhancing exposure or by the application of a chemopreventive agent. There is good evidence that HGPIN is modifiable. Androgen blockade or irradiation administered to individuals with prostate cancer appears to decrease the extent of HGPIN lesions (Ferguson *et al.*, 1994; Montironi *et al.*, 1994; Bostwick, 1996a). However, the extent to which the treatment of HGPIN lesions actually 'normalizes' the tissue, beyond shrinking the volume of lesions, has not been established. Whether the genotypic or phenotypic structure of neoplastic growth is decreased by application of either androgen blockade or irradiation requires additional study. The cells themselves may have undergone repair or the severely dysplastic cells may have undergone apoptosis and replacement by normal cells.

Limitations

The use of HGPIN as an exposure biomarker for use in studies of chemoprevention of prostate cancer appears to be highly promising. Prostate cancer is in part environmentally mediated. It is likely that HGPIN, an important predictor of prostate cancer risk, is similarly environmentally mediated. Nonetheless, it must be recognized that there is no easily accessible path to the prostate from environmental exposures except through a series of extensive metabolic pathways. Therefore, any exposure biomarker that is to be extracted from prostatic tissue has undergone substantial metabolic processing. Since metabolic factors and

processes have multiple opportunities to alter any effect of environmental exposures on tissue characteristics, the prostate is highly unlikely to passively record the effects of exogenous exposures. This may substantially limit the degree to which HGPIN can be used as an exposure biomarker. At present, the necessary epidemiological data on HGPIN that would allow linking of exposure to the presence or absence of HGPIN are lacking.

As noted, the high predictive value of HGPIN in relation to prostate cancer indicates that it may represent a very late post-initiation phase of carcinogenesis. Thus, while HGPIN may function as an important exposure biomarker, it may not develop until several decades after exposure. In addition, coming as it does before completion but after initiation of carcinogenesis, it may be difficult to alter its course by chemopreventive agents. As noted, androgen deprivation seems to suppress it. Whether the application of other agents will have the same effect remains to be seen. It may be necessary to identify earlier biomarkers of exposure that can be altered by chemopreventive agents. A low-grade prostatic intraepithelial neoplasia has been recognized, and it has been assumed that progression of this condition leads to HGPIN. It would thus make sense that this could be related to prostate cancer risk (Bostwick, 1996a). However, there is little evidence, to date, that this lower-grade lesion is indeed related to increased prostate cancer risk. Additional research is needed to clarify the link between low-grade intraepithelial neoplasia and prostate cancer.

It is important to consider the way in which HGPIN as an exposure biomarker uniquely identifies and quantifies a subject with respect to the risk of prostate cancer. The case of another supposedly important biomarker, the index of crypt proliferation in colonic mucosa, is instructive. Crypt proliferation has been used for over two decades as a supposed marker of increased colon cancer risk and as a marker of exposure to carcinogens. Recently, however, it was shown that the degree to which crypt proliferation actually characterizes an individual is so low as to render markers of crypt proliferation virtually useless for most epidemiological and chemopreventive evaluation purposes (McShane *et al.*, 1998). Thus, the degree to which an individual can be characterized with respect to HGPIN and thus prostate cancer risk needs to be established, if measurement of HGPIN as a biomarker is to be interpretable.

An example of the use of HGPIN in chemoprevention of prostate cancer

In a chemoprevention clinical trial, prostate cancer risk was unexpectedly decreased by over 60% following the administration of 200 μg per day of selenium to men in a region with low soil levels of selenium (Clark *et al.*, 1996). All of these men had been diagnosed with basal- or squamous-cell cancer of the skin. These findings led Colditz (1996) to recommend that replication studies be undertaken. This appears to be a situation in which a protective agent has been identified, but the biological mechanisms by which that agent acts are not well understood (Ip, 1998). Combs and Gray (1998) suggested that none of the common forms of selenium (selenite, selenate, selenomethionine or selenocysteine) is likely to be the most biologically active protective form. Among possible protective mechanisms, selenium could contribute to antioxidant protection, immune enhancement or cell-cycle or apoptosis regulation (Combs & Gray, 1998) and it might also block the angiogenesis that is critical to neoplastic growth.

Clearly, another clinical trial of selenium and prostate cancer is needed to replicate the findings of Clark *et al.* (1996). In addition, it will be necessary to identify processes within the prostate that are affected by elevated intake of selenium; indeed, the biology of selenium needs to be delineated in a great deal more detail (Ip, 1998). For these purposes, a population of high-risk individuals would be useful; it would be preferable that, for these individuals, sampling of prostatic tissue should be a part of standard clinical care.

We have initiated a two-armed study, comparing three-year prostate cancer rates among HGPIN patients treated with 200 μg per day of selenium with the rates among HGPIN patients treated with placebo. Each patient will be randomized to one of the two treatment arms within strata of race, baseline selenium, baseline α-tocopherol and baseline prostate-specific antigen (PSA) levels. A total of 470 patients, 235 in each arm, will be randomized. Before randomization, there will be an enrolment period of three months, during which the patient

will undergo a second biopsy in order to rule out prostate cancer. Approximately 1100 patients may need to be enrolled, chiefly to compensate for pre-randomization withdrawals due to prevalent prostate cancer not discovered until the second biopsy. The enrolment period will not include a placebo run-in, since very few noncompliant patients are identified by such a run-in (Feigl *et al.*, 1995); most will be identified by their failure to obtain the second biopsy or to forward the necessary pre-registration materials and information.

There will be two registrations for this study. The first will be at official study enrolment and the second at randomization. Central pathological verification of the presence of HGPIN and the absence of prostate cancer will be documented before study enrolment. Blood will be drawn. At the second registration, the patient's continued willingness to participate will be assessed. Between the first and second registrations, a second biopsy will be conducted; the absence of prostate cancer in the second biopsy will have to be confirmed before the second registration. The patient will then be randomized.

Treatment will be for three years, unless the patient is taken off the study due to toxicity, withdrawal from treatment or diagnosis of prostate cancer. Patients taken off treatment due to toxicity or for other reasons will remain on study for regular follow-up including the biopsy three years after randomization. The study goal is to follow and biopsy every participant at three years (whether on treatment or off) for ascertainment of end-point data. The only exceptions will be men who die, are diagnosed in the interim with prostate cancer, or in the interim request no more contact for study purposes.

At the first visit, the patient will be informed of the slight risk of mild toxicity associated with long-term use of selenium at the study dose (200 μg selenium). He will also be informed that participation in this study requires two additional biopsies, one before randomization and the other three years later, at the end of the study. He will be informed that the purpose of the first additional biopsy will be to confirm that he does not in fact have cancer, and that the second will be to provide a definitive evaluation of whether cancer is present after three years of treatment. He will be informed that additional risk accompanies these additional biopsies.

Slides from each patient who is willing to participate, and who remains eligible after first contact, will be forwarded to the study pathologist for confirmation that HGPIN is present and that cancer is not. The first visit will be scheduled so that, before the date of the visit, confirmation can be received that the patient is eligible. At the second visit, the patient's continued interest will be evaluated. If his second biopsy confirms that he does not have cancer, he will be randomized and provided with his first supply of pills. The study pathologist will review the second biopsy to ensure that the patient does not have cancer. Both the patient and the clinic will be blinded to the treatment assignment.

Each patient will be monitored, in the clinic at which he was initially identified, every six months, from 6 to 36 months after randomization. At each six-month visit, he will be evaluated by a blood PSA test, and he will receive a digital rectal examination (DRE). Compliance will be monitored by pill counts; the patient will be asked to bring his pill packets with him to the clinic. At each visit, blood samples will be drawn for evaluation of selenium. Between the six-month visits, each patient will be contacted by telephone about pill-taking, toxicity and health status. At each visit, the patient will be physically examined and evaluated for evidence of selenium toxicity, including an examination for lens opacity, as well as for the development of serious illness. Any patient whose PSA level increases by 50% or more above his previous level will have a new blood sample drawn and evaluated. If the second sample confirms a rise in PSA of over 50%, or if the DRE is abnormal, the patient will be scheduled for a new sextant biopsy.

Circumstances that will cause the patient to leave the study include a biopsy-based diagnosis of prostate cancer and request by the patient for no further contact. Other circumstances may cause a patient to discontinue study medication but remain on study and in active follow-up. Every effort will be made to obtain three-year outcome data, including biopsy, from these patients. Off-treatment but on-study conditions include, for example, unacceptable toxicity (i.e., drug-related > grade 3) and failure to pick up study drugs. If a patient does not comply with the schedule for clinic visits, blood samples etc., or is persistently non-compliant with respect to pill taking (< 75%

or > 125% of assigned dose), he will be counselled appropriately and kept on study. Of course, a patient may withdraw from chemopreventive treatment or the study at any time of his own volition.

At the 36-month evaluation at the end of the study, the patient will be examined by sextant biopsy, informed of the results, and provided the opportunity to ask questions. The data collection and treatment schedule is summarized in Figure 1.

Drop out rates will be assessed and noted at the time of randomization and during chemopreventive treatment. The proportion of initially enrolled patients who are not randomized and the proportion of randomized patients who do not complete the study will be noted. The proportions of randomized patients who either leave the study or are dropped will be calculated at each one-year interval.

Prostate cancer is the primary outcome to be evaluated in this trial. Data analysis will be based upon intention to treat: the focus of analysis will be prostate cancer among those assigned to treatment compared with that among subjects assigned to placebo. Thus, the three-year risk of prostate cancer among experimental subjects, relative to that among control subjects, will be the central conclusion of the study. The randomization and stratification of the trial will tend to ensure that experimental and control subjects are alike in terms of demographic and disease characteristics: age, race, PSA, and amount of HGPIN in biopsy material. However, in the event of unexpected imbalance, it will be possible to adjust estimates of the treatment effect using multiple covariate control procedures.

This trial will provide the opportunity to study several surrogate end-point biomarkers of prostate

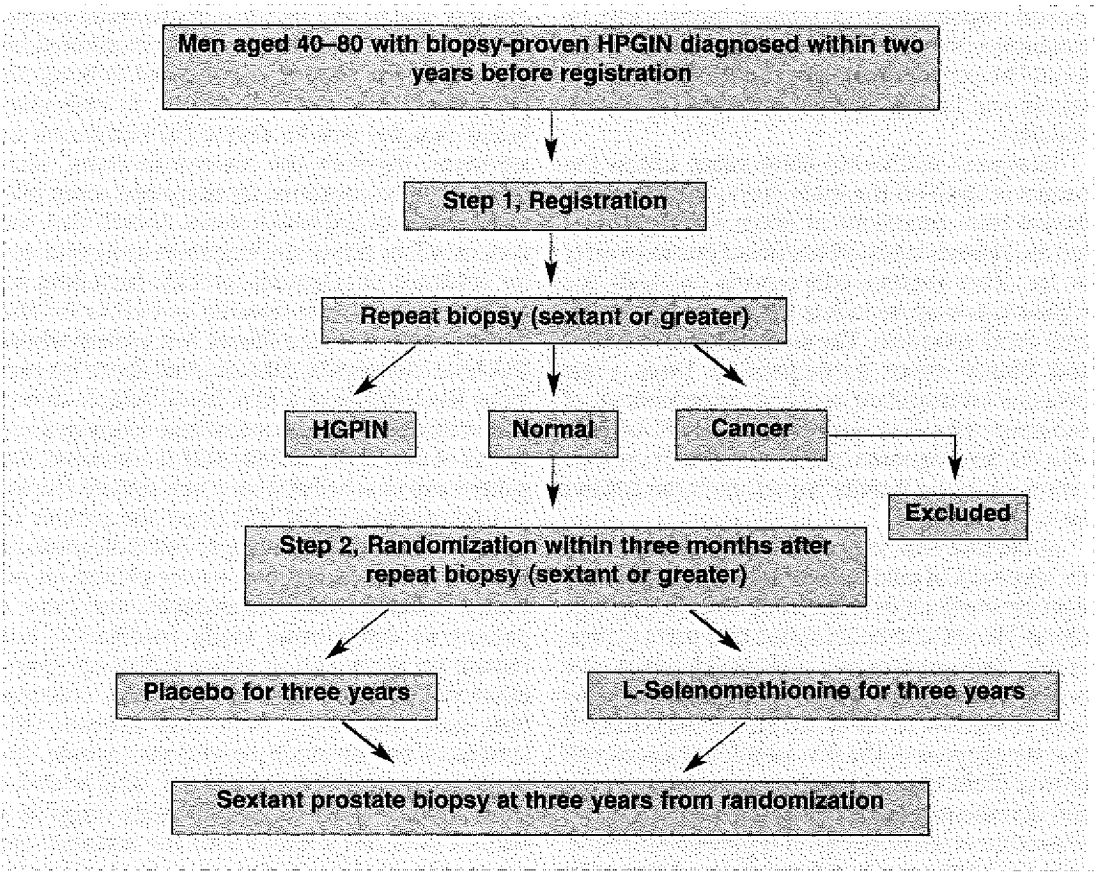

Figure 1. Scheme of trial for selenium chemoprevention of prostate cancer

cancer risk: proliferation as measured by Ki-67 (Raymond *et al.*, 1988); apoptosis as measured by TUNEL (Gavrieli *et al.*, 1992); and karyometry as measured by an automated machine vision system (Bartels *et al.*, 1995; 1996; 1998a,b,c). Use of these biomarkers will add significantly to understanding the means by which selenium exerts any effect on the risk of prostate cancer. It will also help to describe the nature of HGPIN as an exposure and risk biomarker. The first analytical task for examination of the biomarkers will be to evaluate the extent to which each of these biomarkers distinctly characterizes the subject. The model for this approach will be the proliferation biomarker analysis published by McShane *et al.* (1998). With the biomarkers indicating altered proliferation and apoptosis, as well as distorted nuclear chromatin patterns, it will be of interest to assess whether, within categories of treatment or placebo, these biomarkers predict progression to prostate cancer. Given their possible relevance to the risk of progression, it will be important to assess whether treatment has any impact upon changes in the biomarkers.

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