



Strategies for chemoprevention of prostate cancer

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Because prostate cancer has a long latency and high incidence, it is a good target for chemoprevention by agents such as retinoids, antiandrogens, antiestrogens, and vitamin D analogs. Phase II chemoprevention trials are frequently conducted on cohorts of patients with previous cancers or premalignant lesions who are scheduled for prostate cancer surgery; such trials are currently in progress with several agents. Prostatic intraepithelial neoplasia (PIN) can be used as a surrogate endpoint biomarker for prostate cancer incidence. Studies of men with high-grade PIN (HGPIN) are particularly useful in that they require a much smaller cohort of 200–400 patients instead of the 18 000 patients required for typical Phase III trials. Even with a smaller sample size, statistically significant evidence of cancer prevention is achieved due to the high probability of HGPIN progressing to cancer (35–55%). A Bayesian sequential monitoring system allows interim analysis of biomarker modulation as early as the completion of 30 patients. Putting all these strategies together will help inhibit, delay, or modulate the natural history of prostate carcinogenesis.

Keywords: chemoprevention; HGPIN; clinical study design

Introduction

Chemoprevention is the administration of agents (drugs, biologics and nutrients) to prevent induction of, inhibit, or delay progression of cancers. Because of its long latency and high incidence, prostate cancer is an important target for chemoprevention.^{1,2} As for other cancers, development of rational prostate chemopreventive strategies requires knowledge of major mechanisms of carcinogenesis in the tissue and identification of agents that interfere with these mechanisms.^{3–6} Also, to identify suitable populations for evaluation of potential chemopreventive agents and for future chemopreventive intervention, such risk factors as the presence of early premalignant lesions and genetic predisposition should be well-characterized. Because carcinogenesis takes place over a long time period, requiring large cohorts for evaluable studies, cancer incidence is usually not a feasible endpoint for chemoprevention clinical trials.^{3–5} Therefore, chemopreventive agent development depends on identifying and characterizing early intermediate end-

points and validating them as surrogate endpoints for cancer incidence in clinical chemoprevention trials.^{4,6} Table 1 summarizes the various aspects of chemoprevention in prostate cancer.

Promising chemopreventive agents in prostate

An agent requires experimental or epidemiological data showing chemopreventive efficacy, safety on chronic administration, and a mechanistic rationale for the chemopreventive activity observed.^{3–5} On this basis, promising chemopreventive agents in prostate include retinoids (for example, all-*trans*-*N*-4-(hydroxyphenyl)retinamide (4-HPR) and 9-*cis*-retinoic acid), RAMBA (retinoic acid metabolism blocking agent), vitamin E, organoselenium, lycopene, soy derivatives such as isoflavones (for example, genistein), 2-difluoromethylornithine (DFMO), steroid 5 α -reductase inhibitors (for example, finasteride, dual type 1 and 2 inhibitors), apoptosis inducers (for example, perillyl alcohol), and differentiation agents (for example, vitamin D analogs). Of the agents proposed as chemopreventives in prostate, the best known is finasteride

Table 1 Prostate cancer: considerations and factors for chemoprevention

The Problem
 In USA most common cancer in men
 32% (209 900) of total new cancer cases (of top 10 cancer sites) in men (estimated 1997)
 14% (41 800) of cancer deaths in men (estimated 1997)

Risk factors/risk markers
 Age >50 y
 Familial history of prostate cancer
 High serum testosterone
 High fat diet/high red meat consumption
 Population/geographical background (highest incidences in Canada and northwest Europe)
 Prostatitis
 Genetic polymorphisms (for example, in SRD5A2, gene for steroid 5 α -reductase)
 Low micronutrient levels (for example, selenium, carotenoids, vitamin D)

Proposed chemopreventive mechanisms/chemical classes
 Steroid 5 α -reductase inhibitors (for example, finasteride)
 Retinoids (for example, 9-*cis*-retinoic acid)
 RAMBA (retinoic acid metabolism blocking agent)
 Antiproliferatives (for example, DFMO, DHEA analogs)
 Apoptosis inducers (for example, perillyl alcohol and congeners)
 Differentiating agents (for example, vitamin D analogs)
 Antioxidants (for example, vitamin E, selenium, lycopene)
 GSH-enhancing agents (for example, oltipraz)
 Antiestrogens (for example, toremifene, tamoxifen, raloxifene, SERM-3)
 Aromatase inhibitors (for example, vorozole)
 Antiandrogens (for example, leuprolide, flutamide)
 Angiogenesis inhibitors (for example, linomide)
 Signal transduction regulators (for example, genistein)

Preclinical models of prostate cancer
 Lobund-wistar rats treated with MNU
 Wistar rats treated with MNU then chronic testosterone
 Noble rats treated with testosterone and estradiol (develop PIN)
 Transgenic mouse with C3(1) and SV40 T-antigen
 Rodents with human prostate xenografts (for example, LNCaP)

Intermediate endpoint biomarkers
 Histological: PIN (nuclear morphometry, nucleolar morphometry, nuclear texture, DNA ploidy)
 Proliferation: PCNA, Ki-67 antigen expression
 Differentiation: loss of high molecular weight cytokeratins (50–64 kD), altered blood group antigens (for example, Lewis^Y antigen), vimentin
 Genetic/regulatory: *c-erbB-2*, TGF α , p53, *bcl-2/bax*, *pc-1*, chromosomal loss or gain (for example, 8p, 9p and 16q), TGF β , IGF-1
 Biochemical: PSA levels, PAP levels
 Angiogenesis: microvessel density, vWF, VEGF

Clinical cohorts phase II
 Patients scheduled for radical prostatectomy
 Patients with PIN
 Patients with cancer on biopsy, treated by watchful waiting
 Patients at high risk for biochemical failure or rising PSA post radical prostatectomy
 Subjects with positive family history

Clinical cohorts phase III
 Patients with PIN
 Men at high risk (for example, PSA >4 ng/ml and negative biopsy)
 Men from general population, Age >55 y, normal PSA and DRE

(Proscar[®]), which inhibits the enzyme testosterone 5 α -reductase.⁷ In progress is a large Phase III clinical trial of finasteride in chemoprevention of prostate cancer which has enrolled 18 000 subjects (see Table 2).⁸ Early experimental data suggested that retinoids such as 4-HPR or agents which inhibit retinoid metabolism such as RAMBA will be chemopreventive in the prostate, provided sufficient tissue levels are achieved.⁹ The enzyme ornithine decarboxylase (ODC), which is associated with cellular proliferation, is found at high levels in the prostate and prostate neoplasms;¹⁰ DFMO, an ODC inhibitor and potent antiproliferative agent, appears to be a promising chemopreventive agent. In a cohort with prior non-melanoma skin cancer, selenium in the form of selenized brewer's yeast (200 μ g Se/d) was associated with a 63% reduction in prostate cancer compared with placebo controls.¹¹ Epidemiological studies suggest that increased

serum levels of lycopene, the most abundant serum carotenoid, are associated with a decreased relative risk of prostate cancer.^{12,13}

Rationale for antiandrogens and antiestrogens as chemopreventive agents in prostate cancer

Testosterone's association with prostate cancer risk is well known;¹⁴ both experimental and clinical data suggest antihormonal activity is a potential chemopreventive mechanism in the prostate. Chemopreventive strategies are being developed using antiandrogens (for example, flutamide and bicalutamide, as well as steroid 5 α -reductase inhibitors) and antiestrogens (for example, tamoxifen, toremifene, raloxifene, SERM-3 and steroid aromatase inhibitors). Bostwick and his colleagues¹⁵ have recently described clinical protocols for using antiandrogens as

potential chemopreventive agents. At therapeutic doses, for example, antiandrogens produce unwanted side effects such as impotence, loss of libido, gynecomastia and diarrhea. Combining antiandrogens with different targets, such as an androgen antagonist with a 5 α -steroid reductase inhibitor, may provide efficacy at lower, less toxic doses. The strategies are also partially based on the observation that significant levels of the active form of testosterone, dihydrotestosterone (DHT), are produced directly in prostate tissue from adrenal precursors. Therefore, eliminating gonadal testosterone by surgical or chemical castration is not likely to be sufficient or the most effective way to prevent progression of prostatic intraepithelial neoplasia (PIN) and early prostatic carcinoma. For example, Labrie *et al*¹⁶ have shown that while castration reduced serum testosterone concentrations by 90–95%, DHT concentrations in prostate were only reduced 50–70%.^{16,17} Bostwick and his colleagues¹⁵ are currently carrying out a Phase II randomized controlled trial to test the hypothesis that coupling testicular with prostatic androgen blockade will be effective in slowing progression of PIN to prostate cancer. In this study, an LHRH antagonist (leuprolide) is administered in combination with an antiandrogen (flutamide) for 12 weeks prior to prostatectomy, in a cohort with localized prostate cancer and concurrent high grade PIN (HGPN). This treatment is used in some centers to shrink the prostate prior to surgery. The chemoprevention study compares grade, PIN area, and other biomarkers of prostate glands from patients who received the drug combination with glands from untreated controls. Bostwick and his colleagues suggest that the benefits of inducing PIN regression could outweigh the toxicity of this relatively short treatment period in high-risk subjects with HGPN.

Vitamin D₃ analogs and prostate cancer inhibition

Both epidemiological and experimental studies have implicated vitamin D₃ in controlling the progression of prostate cancer. Epidemiological data show general correspondence between cancer incidences and low or deficiency levels of vitamin D,^{18–20} as well as specific correlations of low vitamin D levels to subsequent diagnoses of prostatic carcinomas.²¹ Studies in prostatic carcinoma cell lines (for example, LNCaP, DU-145, PC-3, ALVA-31) show that the cancer inhibitory effects of vitamin D₃ are its antiproliferative and differentiation-inducing activities. Furthermore, these potentially chemopreventive activities appear to be mediated via the vitamin D receptor (VDR).^{22–24} That is, inhibitory activity is higher in prostatic carcinoma cells with significant levels of VDR and the capability of converting vitamin D₃ to its inactive metabolite 1,24,25-trihydroxyvitamin D₃.²⁵ Some data suggest that cell cycle arrest in G₁ is an important mechanism for the antiproliferative activity²⁶ with induction of the cell cycle-dependent kinase inhibitor p21 being the molecular target of the VDR complex.²⁷ Another potential target is the suppression of Id gene expression (Id is an inhibitor of differentiation).²⁸

Induction of hypercalcemia is a well-known toxicity of vitamin D₃, which limits its usefulness as a chemopreventive drug and has led to the development of synthetic analogs with greater differentiating potency and lower or

equivalent hypercalcemic activity. For example, Hedlund *et al*²⁹ tested 13 less toxic vitamin D analogs. Three 23-trienyl analogs of 1 α ,25-dihydroxyvitamin D₃ (Ro 23-7553, Ro 24-5531, and Ro 25-6760), two of which also contain 25,26-hexafluoro substituents (Ro 24-5331 and Ro 25-6760), were consistently effective inhibitors of prostate cancer cell growth. These analogs required VDR for activity and caused carcinoma cells (LNCaP) to differentiate as measured by induction of prostate-specific acid phosphatase (PAP) and prostate specific antigen (PSA).

Intermediate biomarkers/surrogate endpoints of prostate cancer

Biomarkers must fit expected biological mechanisms (that is, differential expression in normal and high-risk tissue, on or closely linked to the causal pathway for the cancer, modulated by chemopreventive agents, and short latency compared with cancer), may be assayed reliably and quantitatively, measured easily, and correlate to decreased cancer incidence.^{3–5} Table 1 lists potentially important intermediate biomarkers for prostate cancer. As in other cancers, two types of prostate biomarkers, measures of the histological precancerous lesion PIN³⁰ and indicators of cellular proliferation kinetics, stand out in regard to their high correlation to cancer and their ability to be quantified. Measurements made by computer-assisted image analysis (CAIA) that are potentially useful as assessments of chemopreventive efficacy include PIN nuclear polymorphism comprising nuclear size, shape (roundness), and texture (DNA distribution patterns); nucleolar size and number of nucleoli/nuclei; DNA ploidy; and proliferation biomarkers such as S-phase fraction, DNA labeling index, Ki-67, and proliferating cell nuclear antigen (PCNA).⁵ Other possible biomarkers are associated with differentiation (for example, blood group antigens, vimentin), genetic damage (for example, chromosomal gain or loss), signal transduction regulators (for example, TGF α , *c-erbB-2* expression), and biochemical changes (for example, PSA levels).

PIN as SEB for prostate cancer

The promise of PIN as a premalignant lesion and hence potential surrogate endpoint biomarker (SEB) for prostate cancer, and the characteristics of PIN progression, have been described recently by Brawer, Bostwick and their colleagues^{30–38} Figure 1 depicts the progression of prostate epithelia from normal to early low grade PIN (LGPIN) to HGPN to carcinoma. The evidence that PIN is a precancer includes morphology; the atypia observed in HGPN is virtually indistinguishable from invasive cancer, except that in HGPN the basal membrane is still intact. As PIN progresses, the likelihood of damage to the basal cell layer increases. PIN and prostate cancer share other phenotypic parameters. For example, certain cytoskeletal proteins, secreted proteins and degree of glycosylation are shared by PIN and cancer, but not by benign prostatic hyperplasia or normal prostate epithelium. Also, PIN is associated spatially and temporally with prostate cancer, both being found primarily in the

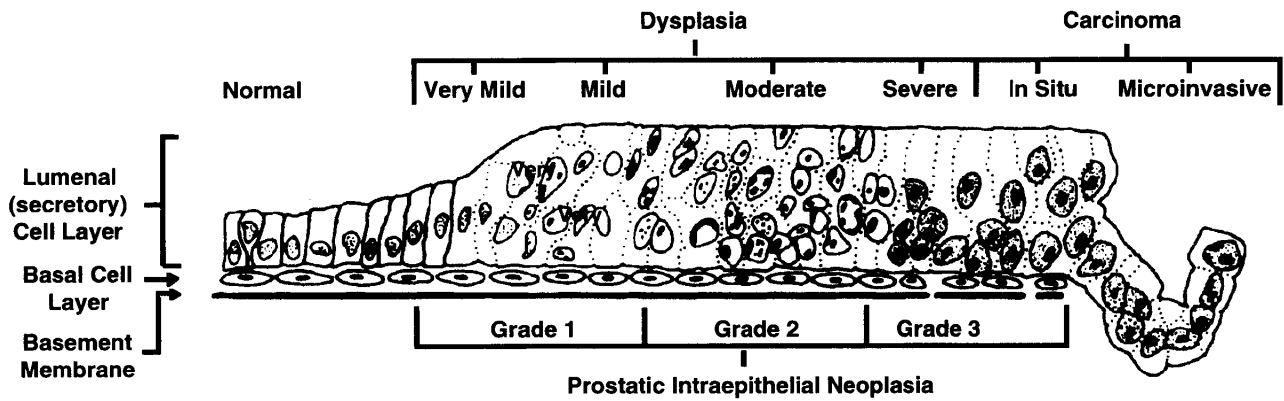


Figure 1 Progression from normal prostatic epithelium through PIN to microinvasive cancer. Reprinted with permission from Bostwick and Brawer³².

Table 2^a Grading criteria for PIN

	Cytologic Features				Associated features	
	Architectural Features	Nuclei	Chromatin	Nucleoli	Basal cell layer	Basement membrane
PIN 1 (LGPIN)	Epithelial cell crowding, stratification, and irregular spacing	Enlarged with marked variation in size	Normal	Infrequent	Intact	Intact
PIN 2 (HGPIN)	Similar to PIN 1 with more cell crowding and stratification	Enlarged with some variation in size	Increased	Occasional large and prominent	Intact	Intact
PIN 3 (HGPIN)	Similar to PIN 2 with occasional luminal bridging	Markedly enlarged with some variation in size	Markedly Increased	Frequent, large, and similar to invasive carcinoma	May show some disruption	May show some disruption

^aFrom Lipski et al, 1996, reference 36.

peripheral zone, and with far less prevalence in the transition zone. Based on several studies in which whole prostates were obtained, HGPIN was seen in 32% (of 876) of specimens without cancer and in 73% (of 731) specimens with concomitant cancer. Increasing rates of aneuploidy and angiogenesis from low grade PIN to HGPIN to cancer also are evidence that PIN is a precancer. Table 2 shows grading criteria for PIN. Quantitative assessments of these features using CAIA (for example, for measuring nuclear pleiomorphism) may be particularly useful as SEB for prostate cancer.

Clinical evaluation of chemopreventive drugs in prostate

Table 3 summarizes clinical prostate chemoprevention studies currently sponsored or funded by the National Cancer Institute, and Tables 4 and 5 summarize potential cohorts and study designs. Phase II trials are critical for evaluating chemopreventive efficacy, particularly for establishing intermediate endpoints as surrogate cancer endpoints.³⁻⁵ Cohorts in these trials should be suitable for measuring chemopreventive activity of the agent and intermediate biomarkers chosen as endpoints. Also, many cohorts proposed for Phase II trials are composed

of patients with previous cancers or premalignant lesions. For such patients, the trials are conducted within the context of standard treatment. For example, a cohort currently used in Phase II prostate cancer chemoprevention trials are patients scheduled for prostate cancer surgery. These patients are treated with the chemopreventive drug during the two- to eight-week period between diagnostic biopsy and prostatectomy. As shown in Table 3, changes in various biomarkers between the diagnostic biopsy tissue and biopsy tissue taken after prostatectomy are evaluated. Trials following this protocol are currently in progress with DFMO, 4-HPR, flutamide, finasteride, flutamide + finasteride, toremifene and selenomethionine/selenized yeast. A study of flutamide + leuprolide in the presurgical cohort with prostate cancer was described above. Another cohort for Phase II and early Phase III trials is patients with HGPIN without prostatic carcinoma. In this cohort, the primary endpoint is PIN regression and cancer incidence reduction, and the treatment period is up to three years. A trial of antiandrogen monotherapy (flutamide) is now in progress in this PIN cohort. A Phase III trial of finasteride was cited above.⁸ The cohort for this trial is essentially normal (PSA \leq 3 ng/ml, no evidence of cancer on DRE), but high-risk (age \geq 55 y) men. Bostwick has described these and other potential cohorts for Phase II and III chemoprevention trials.³⁴ The other cohorts are men with prostate cancer for

Table 3 NCI chemoprevention branch-sponsored or funded phase II/III clinical chemoprevention trials: prostate cancer

Agent	Cohort (treatment period)	Endpoint(s)
Phase II		
DFMO	Scheduled for prostate cancer surgery (4–8 Weeks)	Histopathology (PIN grade, nuclear Polymorphism, nucleolar polymorphism, ploidy), proliferation biomarkers (PCNA, Ki-67)
	Scheduled for prostatectomy (stage A or B prostatic carcinoma or bladder cancer without prostatic carcinoma and scheduled for cystoprostatectomy) (14 d)	Drug effect measurements: ODC activity (skin and prostate), polyamine levels (prostate). histopathology (TRUS-guided biopsies). Biochemical biomarkers: PSA, PAP, testosterone
	Serum PSA 3–10 ng/ml (includes patients with prostatic carcinoma and PIN) (14 d–1 y)	Drug effect measurements: ODC activity (skin and prostate), polyamine levels (prostate). Histopathology (TRUS-guided biopsies). Biochemical biomarkers: PSA, PAP, testosterone
Selenium	Scheduled for prostate cancer surgery (28 d)	Histopathology (PIN grade, nuclear polymorphism, nucleolar polymorphism, ploidy). Proliferation biomarkers (PCNA, Ki-67), genetic/regulatory biomarkers (p53, <i>bcl-2</i> , <i>pc-1</i> , chromosome 8p loss)
Flutamide	Patients with high-grade pin (12 Months)	PIN grade and incidence, cancer incidence, nuclear polymorphism, nucleolar size, ploidy. Other endpoints: PCNA, angiogenesis, apoptosis, LOH chromosome 8, growth factors, PSA
Flutamide/ leuprolide	Scheduled for radical prostatectomy (12 weeks)	PIN grade and incidence, nuclear polymorphism, nucleolar size, ploidy. Other endpoints: PCNA, angiogenesis, apoptosis, LOH chromosome 8, growth factors, PSA
Flutamide/ Finasteride	Scheduled for radical prostatectomy (4–8 weeks)	PIN grade and incidence, nuclear polymorphism, nuclear sized, ploidy
4-HPR	Biopsy-proven non-metastatic prostate adenocarcinoma, scheduled for radical prostatectomy (4 weeks)	Genetic/regulatory biomarkers: TGFβ, <i>cmyc</i> , p53, plasminogen activators (tPA, uPA) apoptosis
4-HPR	Scheduled for prostate cancer surgery (4–8 weeks)	Histopathology: PIN grade, nuclear polymorphism, nucleolar polymorphism, ploidy. Proliferation biomarkers: PCNA, Ki-67. Differentiation biomarkers: Lewis ^y antigen. Genetic/regulatory biomarkers: p53, EGFR, TGFα
Soy protein	Patients at high risk for biochemical failure post surgery	Rising PSA, circulating prostate cancer cells (PSM-RT/PCR)
Phase III		
Finasteride	Mean ≥ 55 y or age with normal DRE and PSA <3.0 ng/ml (7 y)	Prostate cancer incidence (grade and stage), BPH incidence and severity, overall and prostate-specific mortality, TURP, PSA levels
Selenized yeast	Skin cancer (melanoma, non-melanoma) patients, low Se areas in USA (≅ 1 y)	PSA levels

Table 4 Representative cohorts and trial design for clinical chemoprevention efficacy trials in prostate^a

Cohort	Study size	Statistical power	Treatment effect	Primary endpoint
Risk based on age >55 y (PSA <3 ng/ml, negative DRE)	18 000 subjects	90% (0.05)	25% decrease in cancer incidence	Prostate cancer incidence at 7 y of treatment
HGPIN (with no cancer detected on two sextant biopsies)	200–400 patients	93% (0.05)	33/40% decrease in cancer incidence 0.25 s.d. change in intermediate biomarker	Prostate cancer incidence at 1–3 y of treatment
PSA >4 ng/ml (no cancer detected on biopsy)	700 patients	80% (0.05)	50% decrease in cancer incidence 50% decrease in PSA trajectory	Prostate cancer incidence at 4–5 y of treatment
Post-radical prostatectomy, rising PSA	120 patients	80% (0.05)	25% decrease PSA trajectory (0.13 s.d. change)	PSA level after 2 y of treatment

^aAll studies randomized, controlled and blinded.

whom ‘watchful waiting’ rather than surgery is prescribed and men at high risk because of elevated PSA (>4 ng/ml).

Cancer risk and cohort selection

The cancer risk of the cohort or target population affects most aspects of the clinical study design—including

choice of investigational agent, control regimen, primary endpoint, sample size and statistical power (see Table 5). For example, the Phase III finasteride trial requires a very large cohort (18 000 men aged >25 y) to detect the clinical endpoint of 25% reduction in cancer incidence by seven years at 90% power. In contrast, Phase II/III studies in subjects with HGPIN may require only 200–450 subjects to detect the endpoint of 33–40% reduction in prostate cancer incidence within 1–3 years at 90% power. The

Table 5 Evidence for scientific/regulatory decisions based on clinical chemoprevention trials in prostate

Phase	Cohort	Design	Endpoint	Issues
I	Normal risk ($N = 20$) High risk ($N = 25-75$)	Dose-ascending (single-dose) Randomized, controlled, concurrent multidose treatment (3-6 months treatment)	Safety, pharmacokinetics Pharmacodynamics, intermediate biomarkers, drug effect measurements	Multiple doses MTD = Grade 2 toxicity (Phase II dose)
IIa	Precancer/cancer scheduled for prostate surgery ($N = 100$)	Randomized, controlled, double-blinded (4-8 weeks treatment)	Pharmacodynamics, intermediate biomarkers, safety, pharmacokinetics	<ul style="list-style-type: none"> • Interim analysis • Establish dose-response effect on biomarkers • MTD = Grade 2 toxicity
IIb	Precancer ($N = 100-300$)	Randomized, controlled, double-blinded (3-12 months treatment)	Intermediate biomarkers (SEB)	<ul style="list-style-type: none"> • Evaluate histology as primary SEB • Possibility of accelerated approval
III	High risk ($N = 450-18000$)	Randomized, controlled, double-blinded (3-7 y)	Cancer incidence Intermediate biomarkers (SEB)	<ul style="list-style-type: none"> • PIN or PSA as SEB Validate SEB

marked reduction in sample size required to see a statistically significant reduction in cancer incidence arises from the high probability of HGPIN progressing to cancer (35-55%).³⁰⁻³⁸

Data analysis

The level and type of evidence required by regulatory agencies (particularly the FDA) to establish efficacy against prostate cancer and safety in the prostate cancer risk cohorts also determines the appropriate chemoprevention trial design. Phase I chemoprevention trials test safety and pharmacokinetics and also explore effects of the agent on selected intermediate biomarkers and/or drug effect measurements. Phase II trials develop evidence of dose-response modulation of intermediate biomarkers (both prevention and regression), and Phase III trials evaluate chronic safety, efficacy against cancer incidence, and validation of intermediate biomarkers (which may in the future be used as SEBs for cancer incidence). Studies in all phases are typically randomized, double-blinded and placebo-controlled. Some Phase I trials, mostly for new investigational drugs, may not be placebo-controlled or randomized.

As discussed above, a major thrust of chemoprevention drug development is the validation of intermediate biomarkers as SEBs for cancer incidence. This validation hinges on several factors:

- (1) correlation between the effect on the SEB and cancer incidence;
- (2) proportional magnitudes of changes in SEB and cancer incidence;
- (3) explanation by the SEB of a significant fraction (50-75%) of the treatment effect.³⁹

To improve the efficiency of new agent evaluation in randomized, controlled Phase II studies, we have adapted a Bayesian sequential monitoring scheme⁴⁰ for interim analysis of the modulation of selected intermediate biomarkers⁴¹ as early as the completion of 30 patients. The Bayesian method is designed to stop quickly for ineffective agents but more slowly for active agents than conventional group sequential methods (for example, O'Brien-Fleming⁴²). The Bayesian approach allows interpretation to be more flexible and simpler, but requires

prior knowledge of the intermediate biomarker (mean and variance) and the typical treatment effect (mean and variance).

Definition of endpoints

Statistical classification of study endpoints (continuous *vs* discrete) and nature of the endpoint sampling (for example, use of paired samples, measuring change from baseline to posttreatment in the same sampling area, or use of unpaired samples in the posttreatment groups) are critical to achieving reliable evaluations. The number and location of samples from the invasive cancer, HGPIN, and adjacent normal-appearing tissue, as well as the thickness/number of histologic sections processed and scored are important parameters that affect variability, accuracy and reproducibility.⁴³

Since HGPIN is the best candidate surrogate endpoint for prostate cancer incidence in chemoprevention trials, the standardization of PIN assessment is critical. Current approaches for measuring effects on the prevalence and extent of HGPIN involve calculating the percent of patients with PIN.⁴³ Other promising methods include mapping area and volume of PIN (Bostwick, personal communication) and digital imaging of the prostate whole mount (Becich and Trump, personal communication).

Opportunities for chemopreventive intervention in prostate carcinogenesis

As suggested by the discussion above, there are many opportunities for inhibiting, delaying or modulating the natural history of prostate carcinogenesis. Examples of possible cohorts for chemopreventive intervention in Phase II and III trials are defined by risk factors and include (by increasing risk): age >50y; genetic polymorphism in 5 α -steroid reductase 2 (SRD5A2); elevated PSA with no other indication; and HGPIN. SEBs are also defined by these risk factors. The expected benefits of using intermediate biomarkers in chemoprevention trials include enhanced identification of high-risk groups, more rapid assessment of efficacy based on modulation of the

biomarker, smaller sample size (important for pivotal Phase II/III studies), a Bayesian method for interim analysis, and a quantitative estimate of the effect of intervention.

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