ELSEVIER

EXECUTIVE SUMMARY OF THE NATIONAL CANCER INSTITUTE WORKSHOP: HIGHLIGHTS AND RECOMMENDATIONS

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ABSTRACT

Prostate cancer chemoprevention represents a relatively new and promising strategy for reducing the immense public health burden of this devastating cancer of men in the United States and Western societies. Chemoprevention is defined as the administration of agents (drugs, biologics, and natural products) that modulate (inhibit) one or more steps in the multistage carcinogenesis process culminating in invasive adenocarcinoma of the prostate. In 2000, there were an estimated 170,000 new cases of prostate cancer and 31,000 deaths in the United States. During the past decade, the National Cancer Institute (NCI) organized the chemoprevention research program and began testing the first generation of promising agents (eg, 4-(hydroxy)-fenretinide [4-HPR], difluoromethylornithine [DFMO], antiandrogens) in high-risk cohorts and launched the first-large scale US phase 3 primary prevention trial, known as Prostate Cancer Prevention Trial (PCPT-1), in 18,000 average-risk men (age more than 55 years and prostate-specific antigen [PSA] less than 3 ng/mL) treated for 7 years with finasteride or placebo. In the summer of 1998, the *NCI Prostate Cancer Progress Review Group (PRG) Report* to the director of NCI was published in response to the leadership of the prostate cancer advocacy community in conjunction with Congress. To further elucidate and address critical issues identified in this report and to develop a research agenda for the newly created Prostate and Urologic Cancer Research Group in the Division of Cancer Prevention at NCI, the NCI organized the workshop "New Clinical Trial Strategies for Prostate Cancer Chemoprevention." The major objectives were to promote understanding and cooperation among the NCI, US Food and Drug Administration (FDA), academia, pharmaceutical industry, and the public regarding new opportunities for clinical prevention trials for prostate cancer. The workshop was divided into three concurrent breakout panels and a fourth joint integrative panel. The workshop addressed multiple key areas identified in the PRG report in the following panels: (1) Molecular Targets and Promising Agents in Clinical Development; (2) Intermediate Endpoint Biomarkers for Prevention Trials; (3) High-Risk Study Populations for Prevention Trials, and (4) Preventive Clinical Trial Designs and Regulatory Issues. Expert panelists were drawn from leading academic, pharmaceutical, and government scientists in basic research and clinical investigation. Key pharmaceutical, biotechnology, academic, and National Institutes of Health scientists presented overviews of their new agents and products in clinical development (representing the next generation of promising agents). Senior FDA physicians from the Center for Drugs and Center for Biologics presented on current standards for new drug and biologic approval for chemoprevention efficacy. Some of the key topics included recent advances in the state of knowledge of promising agents in the clinic based on molecular targets as well as bottlenecks in drug development for pharmaceutical sponsors; strategic modulable biomarkers that can serve as primary endpoints in phase 1/2 trials to assess preventive efficacy; high-risk cohorts with precancer (high-grade prostatic intraepithelial neoplasia) and representative clinical trial designs that are ready for immediate translation into efficient prevention trials, such as Bayesian sequential monitoring for early assessment of biologic activity and factorial designs for assessment of multiagent combinations. Finally, each expert panel generated recommendations for areas of future research emphasizing opportunities and infrastructure needs. UROLOGY **57** (Suppl 4A): 4–27, 2001.

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The concept of cancer chemoprevention represents a new direction for the United States' war on cancer. It is defined as the administration of agents that inhibit one or more stages in the multiple-step process of carcinogenesis. In this context, carcinogenesis, like atherosclerosis, should be viewed as a continuum representing different subclinical and clinical disease states that progress over many decades. Surgery, radiation, and chemotherapy, together with early detection of cancer, have been the mainstays of cancer treatment for the past 3 decades. However, starting in the early 1980s, the National Cancer Institute (NCI) and organizations in the private sector (American Cancer Society [ACS], American Association of Cancer Research [AACR], and American Society of Clinical Oncology [ASCO]) began a major investment in public health education aimed at modifying high-risk behaviors—including smoking, obesity, and high-fat, low-fiber diets—and promoting healthy lifestyles, that is, increased consumption of fruits and vegetables and weight reduction. This new effort was patterned after the successful public health initiatives adopted a decade earlier for reducing morbidity and mortality from cardiovascular diseases.

The introduction of drug-based therapies, such as antihypertensives and lipid-lowering agents, and their ability to significantly modulate surrogate endpoints for risk reduction (hypertension and high cholesterol) and improve survival, has been a major stimulus to the NCI and the extramural cancer research community to develop effective agents and surrogate markers for risk reduction and cancer prevention. This led to the launch of the first generation of chemoprevention trials testing hypotheses that antioxidant dietary supplements—such as β-carotene, vitamin A, vitamin E, selenium, calcium, and megadoses of vitamins—can prevent the development of various epithelial cancers. Secondary analyses of two of these trials generated provocative hypotheses of the preventive efficacy of vitamin E and selenium (see The Selenium and Vitamin E Chemoprevention Trial [SELECT] below).

Prostate cancer chemoprevention underscores a relatively new and promising strategy for reducing the immense public health burden of this devastating cancer of men in the United States and Western societies. It is a medical intervention approach guided by the use of well-characterized agents (drugs, biologics, and nutrients) with a wide therapeutic index and intermediate and potential surrogate endpoints for clinical outcomes, such as cancer incidence reduction in at-risk target populations. During the past decade, the NCI organized the chemoprevention research program and began testing the first generation of promising agents (eg, 4-(hydroxy)-fenretinide [4-HPR], difluoromethylornithine [DFMO], antiandrogens) in phase 1/2 clinical trials in high-risk populations. Development of agents for cancer prevention is guided by principles jointly set forth by the NCI and the US Food and Drug Administration (FDA). The developmental pathway encompasses preclinical studies of pharmacology/toxicology and animal models of efficacy, and extends to clinical testing in phase 1 to 4 clinical trials.

In the early 1990s, NCI initiated the first large-scale phase 3 trial, the Prostate Cancer Prevention Trial (PCPT), using finasteride in over 18,000 healthy men. Although definitive results will not be available for several more years (eg, 2003), PCPT has clearly demonstrated the feasibility of large-scale trials for prostate cancer prevention. More recently, NCI has initiated the second major phase 3 prostate cancer prevention trial, called SELECT, which will test the preventive efficacy of the antioxidant micronutrients vitamin E (D-α-tocopherol acetate) and selenium (as L-selenomethionine) in over 32,000 healthy men at risk for prostate cancer.

In addition, several NCI-supported clinical trials in high-risk populations for prostate cancer (eg, subjects with high-grade prostatic intraepithelial neoplasia [HGPIN] and subjects with elevated prostate-specific antigen [PSA] and negative biopsy samples) are now in progress testing antiandrogens, antiestrogens, vitamin D analogs, selective cyclooxygenase (COX)-2 inhibitors, and a wide array of antioxidants (selenium, lycopene, soy isoflavones). Thus, the modern era of prostate cancer chemoprevention has begun to come of age.

In the summer of 1998, the *NCI Prostate Cancer Progress Review Group (PRG) Report* (hereafter called the *PRG Report*) to the director of NCI (Dr. Richard Klausner) was published. The impetus for the *PRG Report* was the inspired leadership of the prostate cancer advocacy community working with Congress. It summarized the current levels of NCI extramural support for prostate cancer research and articulated the most critical needs and opportunities in the form of research questions linked to the categories of biology, etiology/prevention, diagnosis and early detection, systemic/local treatment, outcomes research, and resources. As a result of the leadership of the director of NCI, the *PRG Report* now serves as a de facto program announcement and guides the research community regarding priority areas. Critical research areas and questions applicable to prostate cancer prevention were identified and led to the implementation of this workshop.

NATIONAL CANCER INSTITUTE WORKSHOP: OBJECTIVES, ORGANIZATION, AND OUTCOMES

To further elucidate and address these critical issues and generate a focused research agenda for the new Prostate and Urologic Cancer Research Group (PUCRG) of the Division of Cancer Prevention (DCP), the NCI organized the workshop on "New Clinical Trial Strategies for Prostate Cancer Chemoprevention" held August 8–9, 1999, in Baltimore, Maryland. The mission was to promote understanding and cooperation among the NCI, FDA, academia, the pharmaceutical industry, and the public regarding prostate cancer prevention science. The workshop was divided into three concurrent breakout panels (1–3) followed by a joint integrative panel on trial designs (panel 4). Leading investigators drawn from basic and clinical research programs in academia served as chairs and discussion leaders for each panel. Key pharmaceutical and biotechnology scientists participated in all panels and presented overviews of their new agents/products in clinical development emphasizing both opportunities and "bottlenecks" in early drug development and the approval of new agents for prostate cancer prevention. The major objectives were to examine current trials and devise new clinical trial strategies and define research priorities. The expected outcomes included dissemination of the summary panel recommendations and progress toward the formulation of developmental pathways for the investigation and FDA approval of new agents for prostate cancer prevention.

The workshop addressed multiple critical areas identified in the *PRG Report* in the following four panels:

Panel 1. Molecular Targets and Promising Agents in Clinical Development: What are the most important mechanism(s) of action of potential chemopreventive agents/interventions?

Panel 2. Intermediate Endpoint Biomarkers (IEB) for Prostate Cancer Prevention Trials: What IEB are the most appropriate to use when designing prostate cancer prevention trials?

Panel 3. High-Risk Study Populations for Prostate Cancer Prevention Trials: What is the appropriate target population for a prevention trial?

Panel 4. Prevention Clinical Trial Designs and Regulatory Issues: How can prevention trials be designed with fewer patients and shorter time frames?

Highlighted below are some of the key advances in the state of knowledge of promising agents in the clinic, established and new biomarkers under development, clinical cohorts, and trial designs that are ready for immediate translation into prevention trials, as well as emerging molecular targets and agents, innovative technologies for new biomarker assays, and cohort identification and quantitative risk models that require further development. Recommendations for areas of future research emphasis—opportunities, infrastructure needs, and resources—are listed after each panel.

SUMMARY OF PANEL 1: MOLECULAR TARGETS AND NEW AGENT DEVELOPMENT

Panel 1 focused on agents currently in clinical testing that have the potential to inhibit, reverse, or modulate the natural history of prostate carcinogenesis, that is, the transition from normal prostatic epithelium to precancer (eg, HGPIN) to invasive cancer to clinically active systemic disease. New leads come from epidemiology (eg, soy isoflavones, lycopene), clinical experience in neoadjuvant and advanced prostate cancer (eg, antiandrogens, antiestrogens), secondary analyses from recent randomized phase 3 prevention trials (selenium, vitamin E), and experimental models (DFMO, 4-HPR). As noted, three agents have reached phase 3 testing for prostate cancer prevention (finasteride, selenium, and vitamin E).

The list of novel agents in the clinic is rapidly expanding owing to the identification of new molecular targets, for example, steroid hormone receptors (estrogen receptor [ER]-β, vitamin D receptor), apoptotic pathways (caspases, poly-ADP-ribose polymerase-3 [PARP-3]), angiogenesis factors (vascular endothelial growth factor [VEGF]/KDR), prostaglandin synthetic pathways (COX-2, lipoxygenase (LOX), growth factor signal transduction pathways (ras farnesylation, tyrosine kinases, insulinlike growth factor–1 [IGF-1]), proliferation/differentiation targets (ornithine decarboxylase [ODC], retinoid X receptors [RXR], peroxisome proliferator-activated receptor [PPAR]), and oxidative stress (methylation of glutathione-S-transferase-π [GSTP1], DNA adducts). This molecular targeted approach is being accelerated by the discovery of new genes coming from the NCI Cancer Genome Anatomy Project (CGAP) and the high throughput chemical/pharmacogenomic screens being deployed by academia and pharmaceutical/biotechnology industry collaborations. Furthermore, the development and application of new functional imaging technologies (positron emission tomography [PET], magnetic resonance imaging [MRI]/magnetic resonance [MR] spectroscopy) will facilitate qualitative and quantitative assessments for validating molecular targets and drug action in vivo.

TABLE I. *Candidate prostate cancer prevention agents*

Sex steroid signaling	5-α-reductase inhibitors (finasteride)
	Antiandrogens (receptor antagonists)
	Selective estrogen receptor modulators (SERM)
Differentiation/antiproliferation	Retinoids (RAR, RXR, selective agonists)
	Vitamin D analogs
	Ornithine decarboxylase inhibitors (DFMO)
Growth signaling pathways (angiogenesis)	PDGF receptor antagonists
	VEGF receptor antagonists
	FGF receptor antagonists
	Famestyl-protein transferase inhibitors
	Protein tyrosine kinase inhibitors (soy isoflavones)
Arachidonic acid–associated signaling (proapoptosis)	Nonselective cyclooxygenase inhibitors (NSAIDs)
	Selective cyclooxygenase-2 inhibitors (celecoxib, rofecoxib)
	5-lipoxygenase inhibitors
	Other anti-inflammatory agents (R-flurbiprofen)
	PPAR modulators (sulindac sulfone)
Gene therapy	Genetically modified vaccines
	In situ delivery of immunostimulatory genes
	In situ delivery of cytotoxic genes
	Replication-restricted cytolytic viruses
Growth factors	Endothelin-1 antagonists
	Matrix metalloproteinase inhibitors
	IGF-1 pathway inhibitors
	PSA protease inhibitors
	PPARγ modulators (glitazones)
Antioxidants	Vitamin E
	Selenium
	Carotenoids
	Others (green tea polyphenols)

PSA = prostate-specific antigen, IGF = insulin-like growth factor, PDGF = platelet-derived growth factor, RAR = retinoic acid receptor, VEGF = vascular endothelial growth factor, PPAR = peroxisome proliferator activated receptor, FGF = fibroblast growth factor, RXR = retinoid X receptor.

The Prostate Cancer Chemoprevention Pipeline

There are a surprisingly large number of heterogeneous agents directed at a diverse array of targets at various stages of clinical development with potential for prostate cancer prevention. All of the agents discussed in panel 1 are either FDA approved or in phase 1 to 3 clinical trials. Although apoptosis and angiogenesis are common critical pathways for many agents, these mechanisms are especially linked to anti-inflammatory agents and growth factor signaling, as shown in Table I. Furthermore, some agents identified as proapoptotics exert significant effects on angiogenesis (eg, selective COX-2 inhibitors). There is increasing recognition that combinations of noncytotoxic agents will be the standard approach in the future. Issues of agent dose, schedule, sequence, mechanism of action/resistance, and nonoverlapping toxicity will become critical for the optimization of combinations. A set of key factors to be considered in evaluating candidate agents is described in Table II. Hopefully, attention to these 10 critical questions in the preclinical phase and in early clinical testing of candidate agents might expedite the process of early drug development and ascribe priority to new agents for advancement to clinical phase 3 efficacy testing.

There is consensus that many candidate agents will likely be tested first in the treatment of established prostate cancer, such as watchful waiting, adjuvant settings after prostatectomy/radiation therapy, and biochemical recurrence (rising PSA). With this approach, critical data regarding drug side effects as well as the anti–prostate-cancer efficacy of the agent can be gained relatively quickly, and these data can be used to determine whether further development for prevention will be warranted. One of the most difficult challenges will involve candidate preventive agents (eg, antioxidants such as lycopene or sulforaphanes) that may interfere with prostate carcinogenesis (reduce oxidative stress) but do not inhibit the growth of established prostate cancer. The development of these agents will require new phase 1/2 trial designs such as the presurgical model (preprostatectomy) and new biomarkers that can be used in small trials to help define dose and schedule for phase 2/3 trials. For these agents, modulating

TABLE II. ***Factors in the development of a preventive agent for prostate cancer***

- Agent
- Mechanism
- Model activity
  - In vitro
  - In vivo
- Phase
  - 1
  - 2A
  - 2B
  - 3
- Maximum tolerated dose
- Dosage limiting toxicity
- Administration schedule
- Formulation
- Anticancer activity
- Biologic markers
- Prostate cancer markers
- Target population
- Minority population

biomarkers will need to serve as surrogates for prevention activity (efficacy).

Pharmaceutical development programs, concerned that FDA approval and marketing of a prostate cancer preventive agent will require a prolonged and expensive series of clinical trials, have generally focused on established prostate cancer treatment. The panel agreed that to maximize the effect of new drug discovery and commercial development programs on the prevention of prostate cancer, not only will new drugs be required, but new validated surrogate and strategic clinical trial endpoints and well-defined high-risk clinical trial cohorts will also be needed (see panels 2 to 4).

Sex Steroid Signaling: Antiandrogens and Antiestrogens

Sex steroid hormone signaling is being actively targeted by a number of FDA-approved drugs, developed for benign and malignant diseases. Antiandrogens that antagonize or modulate the production and actions of testosterone and its most potent metabolite dihydrotestosterone (DHT) are the lead agents for prostate cancer prevention. The steroid 5-α-reductase inhibitor finasteride, FDA-approved for treatment of benign prostatic hyperplasia (BPH) and alopecia, is the subject of the first large phase 3 primary prostate cancer prevention trial and involves more than 18,000 average-risk men. Androgen-receptor antagonists (bicalutamide, flutamide) are being evaluated as adjuvant monotherapy for those at high risk of recurrence after surgery or radiation and in high-risk cohorts with HGPIN.

In addition, there is mounting evidence supporting an estrogen hypothesis for prostate cancer prevention. Selective estrogen receptor modulators (tamoxifen, SCH 57050, GTx-006, raloxifene, arzoxifene, ERA923) are under active development by several pharmaceutical companies. Arzoxifene (LY35381), which binds both ER subtypes, has demonstrated activity against the human prostate cancer cell line LNCaP growing in a mouse xenograft model. The NCI has initiated a phase 2 trial in early prostate cancer evaluating the effect of a selective estrogen receptor modulater (SERM) alone and in combination with antiandrogens on HGPIN and prostate cancer.

Antiproliferation/Differentiation: ODC Inhibitors, Retinoids, and Vitamin D Analogs

Some agents that modulate neoplastic cell proliferation or differentiation pathways show promise as prostate cancer prevention agents. DFMO, an inhibitor of ODC, a critical enzyme in polyamine metabolism, has shown chemopreventive activity in preclinical models of prostate cancer (eg, TRAMP) and is under clinical development for the prevention of prostate cancer and other epithelial cancers (colon, bladder, cervix, skin, breast, esophagus). Pilot clinical trials have demonstrated that DFMO modulates polyamines in the prostatic tissue of subjects with prostate cancer. Recently, FDA-approved retinoids, such as 9-*cis*-retinoic acid (Panretin; Ligand Pharmaceuticals Inc, San Digeo, CA), a pan-agonist for retinoic acid receptors (RAR) and RXR, and the RXR selective agonist Targretin (Ligand), have been shown to modulate growth of prostate cancer cells. Vitamin D analogs (eg, Hectorol [doxecalciferol; Bone Care International, Madison, WI], FDA-approved for the treatment of secondary hyperparathyroidism) have been shown to be active in both animal models and patients with hormone-refractory prostate cancer. The most useful vitamin D agents for prevention will likely bind to the vitamin D receptor and cause minimal changes in serum calcium.

Arachidonic Acid Signaling/Proapoptotics: Nonsteroidal Anti-Inflammatory Drugs, COX-2, LOX Inhibitors, and Nuclear Factor–κB Modulators

Evidence from epidemiologic studies, preclinical models, and randomized clinical trials support an association between nonsteroidal anti-inflammatory drugs (NSAIDs), arachidonic acid pathways, and decreased incidence of prostate cancer. Several pharmaceutical companies with FDA-approved selective inhibitors (celecoxib, rofecoxib) have selectively targeted COX-2, a key enzyme in the synthesis of proinflammatory prostaglandins (PGE_2), for cancer prevention. In humans, COX-2 is not only upregulated in colorectal cancer and polyps from

familial adenomatous polyps (FAP) subjects, but also is upregulated in precancer (prostatic intraepithelial neoplasia [PIN]) and in prostatic carcinomas. Novel NSAID (*R*-flurbiprofen [MPC-7869; Myriad Pharmaceutical Company, Salt Lake City, UT]) and a metabolic derivative of sulindac (Exisulind; Cell Pathways Inc., Horsham, PA), which lacks COX activity and inhibits cGMP phosphodiesterases (II/V), have demonstrated activity against prostate cancer in animal models and in phase 1/2 clinical trials. Elucidation of the role of 5- and 12-lipoxygenases (5-LOX, 12-LOX) as mediators of cell fate in prostate cancer cells in vitro provides a rationale for the evaluation of 5-LOX and 12-LOX inhibitors as well. Novel natural product inhibitors of 5-LOX and 12-LOX, derived from the green-lipped mussel (lyprinol) and approved in Australia for the treatment of arthritis, are now undergoing clinical evaluation in prostate cancer cohorts.

Growth Signaling Pathways/Angiogenesis: Protein Tyrosine Kinase Inhibitors, Farnesyl Protein Transferase Inhibitors, and Soy Isoflavones

Protein tyrosine kinase inhibitors (PTKIs), targeting the platelet-derived growth factor (PDGF) receptor (SU-101), VEGF receptor Flk-1 (SU-5416), and pan-inhibitors of PDGF, VEGF, and fibroblast growth factor (FGF) receptors (SU-6668) have all entered phase 1 to 3 clinical trials. Upregulation of these receptors involved in angiogenesis has been demonstrated in rodent models of prostate cancer and in human prostatic cancer. SU-101 has demonstrated activity against advanced prostate cancer in early clinical trials. Several farnesyl protein transferase inhibitors (FPTIs) that can interrupt *ras*-mediated signal transduction are under development by a number of pharmaceutical companies (SCH 66336, R115777). These agents have been shown to be active in prostate cancer cell lines independent of ras mutation status. Soy isoflavones are PTKIs that are under development for cancer prevention. These agents have been shown to be active in prostate cancer cells and in animal models of prostate cancer. Two genistein-enriched isoflavone compounds, PTI-G2535 and PTI-G4660 (PTI), which are also PTKIs, have entered phase 1/2 clinical trials in subjects with early prostate cancer.

Growth Factors and Other Novel Molecular Targets: Endothelin-1, Matrix Metalloproteinases, IGF-1, Bowman-Birk Inhibitor Concentrate, and PPAR

Various other growth factors and associated molecular targets may provide new agents for prostate cancer. Endothelin-1 (ET-1) levels are elevated in men with prostate cancer and appear to function in an autocrine growth pathway. The ET-1 antagonist ABT-627, which has a favorable side effect profile, is now in phase 2/3 clinical trials in hormone-refractory subjects. Matrix metalloproteinase (MMP) inhibitors, such as marimastat, have the potential to arrest the progression of prostate cancer and are under clinical development. IGF-1 has been implicated as a serologic risk factor and proliferation/antiapoptotic modulator of prostatic carcinogenesis. Several candidate preventive agents for prostate cancer may modulate IGF-1 or IGF-binding proteins (IGFBP) (eg, vitamin D analogs, SERM, synthetic retinoids, lycopene). Interestingly, PSA itself, a serine protease cleaves IGFBP to increase the local activity of IGF-1 in prostatic cells. In addition, the Bowman-Birk inhibitor concentrate (BBIC), an orally bioavailable protease inhibitor that modulates PSA levels and oncogene activity, is in phase 1/2 trials. PPAR modulators include FDA-approved type-2 antidiabetic agents ("glitazones"), a ligand for PPAR-γ, and also include such agents as sulindac sulfone (PPAR-δ) and retinoids. Activation of PPAR-γ-2 by a novel endogenous fatty acid metabolite, 15-deoxy-prostaglandin J_2 (15d-PGJ_2), the terminal metabolite of the prostaglandin J series, is associated with type 2 (autophagocytic) nonapoptotic cell death in prostate cancer cells.

Antioxidation and Carcinogen Defense Mechanisms

Epidemiology has provided evidence for an inverse association between exposure to antioxidant nutrients (lycopene, soy isoflavones, selenium, vitamin E, green tea polyphenols) and prostate cancer incidence and mortality. Several lines of evidence suggest that prostate cancer may be fueled by oxidative stress in the context of deficient cell defense. Most prostate cancers lose expression of GSTP1, a major carcinogen detoxifying enzyme, as a result of extensive hypermethylation in CpG islands in this gene's promoter region. However, the most provocative leads for identifying promising agent(s) come from secondary analyses of two randomized controlled primary prevention trials, that is, the large Alpha-Tocopherol Beta-Carotene (ATBC) trial of α-tocopherol (vitamin E) and the nutritional prevention of cancer trial of selenium in subjects with a history of skin cancer. These unexpected findings have led to a large randomized trial (the SELECT) in 32,400 men at risk for prostate cancer. In addition, a variety of phase 1 to 3 clinical trials are in progress evaluating the effect of selenium, lycopene, genistein, and antioxidant cocktails on the modulation of biomarkers and the progression of high-risk populations to invasive prostate cancer. Several of these ongoing trials are discussed in panel 3.

Panel 1

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Gene-Based Interventions: Granulocyte-Macrophage Colony-Stimulating Factor, Interleukin-2, and p53

There are a variety of gene therapy approaches being evaluated for treating established prostate cancer that have potential applications for prostate cancer prevention. The promising approaches include vaccines of prostate cancer cells genetically modified to secrete immunomodulatory cytokines (eg, granulocyte-macrophage, colony-stimulating factor [GM-CSF]) or formulations of immunomodulatory genes, such as interleukin-2 (IL-2), for direct injection into prostate cancer in situ, and these have entered early clinical testing. Cytoreductive gene therapies in which gene transfer is attempted in vivo to trigger target cell death is under active clinical development. Examples include early phase 1 trials of intraprostatic injection of adenovirus-mediated p53 gene transfer, which has exhibited promising anticancer activity, and intraprostatic injection of the replication-restricted cytolytic adenovirus CN706, which is expressed selectively in PSA-producing cells. Depending on the efficacy/toxicity profiles observed, these approaches may be considered for evaluation for prostate cancer prevention activity in high-risk cohorts.

Panel 1 Recommendations and Research Opportunities

1. Recommended Criteria for Candidate Chemopreventive Agents. Critical questions that need to be considered in evaluating candidate agents include:

- Does the agent have a known mechanism of action?
- What is the activity of the agent in prostate cancer models?
- Has the agent completed phase 1 human testing?
- What are the side effects associated with agent administration?
- Is there an adequate formulation for the agent suitable for prostate cancer prevention?
- Does the agent have known activity against established prostate cancer in humans?
- Are there biomarkers that can serve as strategic clinical trial endpoints to test proof-of-principle for agent mechanism of action?
- Are there surrogate endpoints for prostate cancer that can be used to monitor drug activity and anticipate drug efficacy?
- Is there a suitable population for testing the efficacy of the agent for prostate cancer prevention?
- Are there characteristics of the agent or its

mechanism of action that warrant focused clinical testing in a specific special population?

2. New Molecular Approaches for Identifying Novel Targets, Mechanisms, and Biomarkers for Prostate Cancer Prevention Agents. It is recommended that putative prostate cancer preventive agents be evaluated in vitro, and later in vivo, using cDNA microarray technology.

- Recently developed cDNA microarray technology can be used to gain insights into gene expression induced by putative preventive agents by cataloging expression changes in multiple cellular pathways.
- More complete characterization of agents will allow identification of potential biomarkers of response, both class-specific responses and those responses that lie in common pathways.
- Since virtually all prostate cancers lose expression of GSTP1, a major carcinogen-detoxifying enzyme, a promising strategy for preventing prostate cancer development and its progression is to globally upregulate expression of enzymes of carcinogen defense.
- It is recommended that more research be focused on testing synthetic and diet-derived agents that have been documented to induce phase 2 enzymes of carcinogen defense.
- Increased understanding of the mechanisms of these agents will significantly augment the design of rational combinations of agents and individualization of prevention strategies..
- Genes induced after exposure to agents could be assayed in strategic, short-term early clinical trials as a measure of the pharmacokinetics and biologic activity (efficacy) of potential agents in high-risk cohorts.
- Such trials should speed the selection of the most promising agents that then may be tested in larger clinical trials of efficacy.

3. Infrastructure and Resources Required to Advance Prostate Cancer Preventive Agent Development. It is recommended that the newly created Rapid Access Prevention Intervention Development (RAPID) program in DCP be used and expanded to support promising agents for prostate cancer. RAPID will foster critical collaborations between NCI and the originating academic laboratory. The following tasks illustrate the capabilities of RAPID:

a) conduction of in vitro and in vivo preclinical pharmacology and efficacy studies,

b) development of analytical methods for quantifying agent in plasma and tissue,

c) conduction of Investigational New Drug–directed toxicology studies, and

d) support for early phase 1 pharmacokinetic-pharmacodynamic and safety studies in healthy volunteers and high-risk subjects.

It is recommended that the newly created Quick Trials program of NCI be used and expanded to support promising preventive agents, especially "antioxidants" and anti-inflammatory agents, and rational combinations for prostate cancer. The panel recognizes that phase 2 evaluation of promising agents represents a major bottleneck in new chemopreventive agent development for the pharmaceutical industry, academia, and the NCI.

The NCI should develop and support an "accelerated" developmental clinical trial program (mechanism) to evaluate promising agents (drugs, biologics, and natural products) for the prevention of prostate cancer that are ready for phase 2 trials. There is a major need to test combinations of preventive strategies (eg, drugs and gene-based vaccines) in short-term phase 2 trials using factorial designs and biomarker (pharmacodynamic and molecular target) endpoints. This program should provide a critical link between RAPID and the successful use of traditional investigator-initiated research mechanisms (RO1 grants). Collaborative interdivisional studies involving the new NCI clinical trial units (CTU) should be encouraged for the evaluation of promising noncytotoxic agents for secondary chemoprevention of high-risk subjects after primary management of early prostate cancer.

It is recommended that newly created NCI programs, such as the "Interdisciplinary Research Teams for Molecular Target Assessment" and the "Molecular Target Drug Discovery for Cancer: Exploratory Grants," be used to support promising preventive agents for prostate cancer. These new programs seek the discovery and validation of molecular targets and the development of molecular assays, molecular and cellular imaging, and other tools that provide information on the extent to which molecular targets are affected by in vivo interventions in preclinical models and in proof-of-principle early clinical trials.

In addition, the new NCI small grants (RO3) program, which provides limited support for studying novel ideas in model systems and translational research, offers a critical bridge between the discovery of new molecular targets/pathways and applications in early clinical trials of promising preventive agents and new biomarkers.

4. Support Collaboration with Industry. The NCI should continue to actively collaborate with the pharmaceutical/biotechnology industry through confidentiality agreements (CDA), clinical trial agreements (CTA), and collaborative research and development award (CRADA). These partnerships provide mutual benefits for all parties and have led to the development and approval of new preventive agents (eg, celecoxib). Opportunities for extending the number of pharmaceutical (novel agents) and biotechnology (novel biomarkers)

partners interested in working with NCI were identified during the course of the NCI workshop and have been translated into new CDA and CTA.

5. Support the Development of Decision Network Committees (DNC) in DCP. These committees would function to help prioritize clinical trials of promising preventive agents, including those for prostate cancer, that cover discovery, early development, phase 1/2 trials, and phase 3 trials.[1]

6. Support the Development of Specific Criteria and Guidelines for Agent Development. The new DNC should develop specific criteria and guidelines for agent prioritization and clinical trial designs for each clinical phase of development relevant to prostate cancer.

7. Support Research and Development of New Animal Models for Prostate Cancer Prevention. There should be increased linkage between prostate cancer research and the new NCI Mouse Models of Human Cancers Consortium.

8. Support New Research Initiatives for Clinical Trials. There should be new initiatives to develop clinical trials of prostate cancer prevention that are directly linked to the NCI program in molecular targets of prevention and gene-environmental interactions.

9. Support the Development of Workshops on Pharmaceutical-Related Topics. These workshops should cover pharmacogenomics, molecular genetics of prostate cancer, functional imaging of drug action, and novel drug delivery approaches.

10. Support the Development of New Prostate Cancer Consortia for the Testing and Evaluation of Promising Agents. Current support mechanisms, such as the phase 1/2 master agreement awards, should be continued and expanded. Members could include NCI cancer centers, Prostate SPORES, cooperative cancer groups (Cancer Community Oncology Program [CCOP]), the Veteran's Administration (VA), the Department of Defense (DOD) prostate centers, national oncology networks, and government (Department of Agriculture) and private nutrition-science–based organizations.

SUMMARY OF PANEL 2: INTERMEDIATE ENDPOINT BIOMARKERS

Panel 2 focused on identifying those objectives that would help advance the field of prostate cancer prevention and better define the role of biomarkers in prevention trials. There was an emphasis on the need to identify the most appropriate IEB for immediate application in prevention trials and work on those that need further development. A major objective of prevention research and biomarker development is the identification of the molecular changes that are causally related to or correlated with the transition from normal epithelium to premalignant status to invasive prostatic carcinoma (Figure 1). The important role of HGPIN as a major precursor of some prostatic cancers (aggressive lesions in the peripheral zones) was underscored. Furthermore, heterogeneity in HGPIN is recognized. The other leading candidate risk markers/precursors for prostate cancer are atypical small acinar proliferation (ASAP) changes associated with PIN and image-analysis–defined malignancy-associated changes (MAC) in normal-appearing prostatic epithelium, which is also associated with PIN. The role of chronic inflammation and the release of highly reactive molecules (hydrogen peroxide and nitric oxide) in prostate carcinogenesis was also discussed. There is increasing evidence that inflammation is found in the same zones as HGPIN and cancer and is associated with proliferative inflammatory atrophy (PIA). In addition, it is also recognized that an angiogenic switch may be associated with HGPIN and progression to invasive cancer. These new concepts in the pathogenesis of prostate cancer provide a context for the identification of new biomarker endpoints for prevention trials.

The primary use of IEB in serving as indicators of biologic response for proof-of-principle trials and as surrogate endpoints for cancer incidence reduction was emphasized. IEB can be subdivided into tissue- and nontissue-based categories.

Tissue-Based Markers

Histopathology: HGPIN, other morphologic markers (ASAP, MAC). This also includes the potential role for PIA.

Markers assessed in tissue using immunostaining or in situ hybridization: proliferation, apoptosis, angiogenesis, telomerase, and alterations in oncogenes or tumor suppressor genes.

Computer-assisted quantitative image analysis: nuclear and chromatin structures, MAC.

Radiologic imaging and biomarkers: MRI/MR spectroscopy with metabolic profiles, bioelectrical impedance.

Laser capture microdissection, cDNA microarrays, and tissue proteomics: prostate expression databases (PEDB), CGAP.

Nontissue-Based Markers

PSA: forms, velocity, free and total, etc.

Markers of oxidative stress (oxidized DNA bases, products of lipid peroxidation)

Exfoliated cells for cytology in biologic fluids (urine, semen, etc.)

Statistical and Standardization Issues: Validation, Monitoring, and Quality Control

Five major areas (classes) were proposed as the most promising candidates to fulfill the objectives

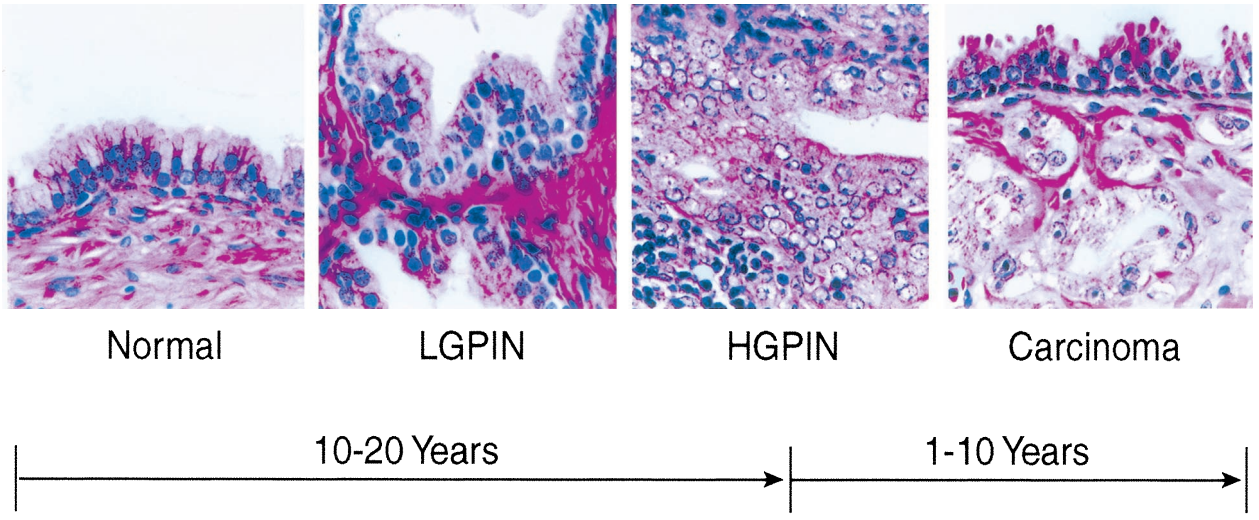

FIGURE 1. *The stages and timeline of human prostate carcinogenesis are shown. The natural history reflects a pathobiologic continuum, starting with normal-appearing glandular epithelium that evolves over time into dysplasia/prostatic intraepithelial neoplasia and finally progresses to invasive adenocarcinoma. HGPIN = high-grade prostatic intraepithelial neoplasia; LGPIN = low-grade prostatic intraepithelial neoplasia.*

of panel 2 (Table III). These include: (1) diagnostic histologic changes (PIN); (2) machine-assisted image analysis of tissue-based alterations (MAC and "tumor fingerprinting"); (3) radioimaging modalities (MRI, MR spectroscopic imaging [MRSI]) for assessing tissue changes; (4) markers of oxidative stress (DNA adducts, lipid peroxidation) assessed in serum and in target organ tissue, including fluids obtained by prostate massage; and (5) tissue-specific markers measured in serum and tissue (PSA, human kallikrein-2 (HK-2), IGF-1, and novel markers derived from genomics/proteomics). Many new assays and targets are under active development as IEB and their relation to stages in epithelial carcinogenesis.[2]

The panel recognized other approaches for the classification of biomarkers in the context of developing neoplasia or predicting its biologic course, including: carcinogen exposure (to potentially carcinogenic injury); cancer risk (associated with increased probability of developing certain cancers); and neoplastic progression potential (a wide array of prognostic markers). Furthermore, there is interest in classifying biomarkers according to mechanistic action(s) or molecular targets. Relevant to prevention trials is the identification of biomarker endpoints that can be modulated by various agents and reflect early neoplastic transformation and progression through the stages of prostate carcinogenesis. The biologic model of neoplastic development shown in Figure 1 most likely corresponds to at-risk target populations identifiable by histologic features and molecular changes in biomarker expression.

Markers Assessed by Routine Histology in Tissue Sections. The most established IEB in this category is HGPIN, a risk marker for the presence or development of cancer and arguably the most likely precursor lesion for higher-grade prostate cancer. There is a need for achieving diagnostic consistency, maximizing the use of the limited biopsy material for molecular studies for which validation and standardization issues must be established. Equally important is the identification of cohorts of patients with this lesion in whom the natural evolution of HGPIN or its modulation by intervention can be assessed through clinical follow-up. Other potential precursors, including ASAP and certain histologic changes associated with inflammation and atrophy (PIA), should be explored further.

Markers Assessed in Tissue Using Such Techniques as Immunohistochemical Staining, Fluorescence In Situ Hybridization (FISH), etc. There is a growing number of markers that assess pathways or alterations in cellular/subcelluar domains and gene products believed to be associated with the development or progression of neoplasia. The list includes proliferation, differentiation, and apoptosis markers, the angiogenesis pathway, cell regulatory markers, growth factors and their receptors, tumor suppressor genes, and chromosomal probes to assess DNA deletions and gains by FISH.

Telomerase function and angiogenesis have gained recent interest in studies dealing with prostatic neoplasia. Telomerase, a ribonucleoprotein that plays a critical role in cell immortality, could be a useful biomarker for prostate cancer. It has

TABLE III. *Major classes of intermediate endpoint biomarkers*

Type of Biomarker	Strengths	Weaknesses	Notes
Early histological changes (high-grade PIN) and markers assessed in tissue by FISH and immunostaining	Predictive value for cancer; presence or development; may help predict progression	The need to obtain tissue samples; diagnostic reproducibility	Could serve as markers in high-risk groups to target chemoprevention
Machine-assisted tissue-based changes; nuclear signature and chromatin texture	Objective and reproducible data collection	Technically demanding, need for standardization	Has the potential of overcoming sampling error by capturing early changes in morphologically normal cells in the field
Imaging modalities; grayscale, color Doppler, MRI, MRSI ([choline + creatine]/ citrate ratio)	Enhancing the ability to identify and sample suspicious areas and higher grade cancer	Technically demanding and requires highly trained operators	Combines imaging with the metabolic characteristics of tissue enhancing detection of earliest changes
Markers of DNA damage and other serum and nontarget tissue–related markers	Eliminating the need for tissue sampling, easier to monitor agent-induced modulation	Difficult assays to perform, wide "normal" ranges, specificity issues	If standardized, these would be ideal assays, with frequent sampling before, during, and after interventions
Tissue-specific markers (prostate-specific antigen, new genomic/ proteomic markers)	Extensive clinical experience for screening early detection, staging and monitoring posttherapy	Sensitivity and specificity issues; inability to detect precursor lesions (PIN) at present	Continuous potential for fine tuning and improvement; better tissue-based markers are potentially identifiable

FISH = fluorescent in situ hybridization, MRI = magnetic resonance imaging, HGPIN = high-grade prosthetic intraepithelial neoplasis, MRSI = magnetic resonance spectroscopy.

been detected in tumor specimens of all cancer types, as well as in urine, blood, and fine-needle aspirates. It has also been found in some HGPIN lesions, but it is generally not present in normal prostate tissues or BPH specimens. There is evidence that serum PSA levels in telomerase-positive patients were found to be significantly higher than those of telomerase-negative patients, suggesting that the telomeric repeat amplification protocol (TRAP) assay could become a biological marker for prostate cancer. Newer assays based on immunohistochemistry are under active development and are needed to monitor biologic responses in prevention trials.

There is a strong rationale for using angiogenesis as a target for preventive approaches in prostate cancer. There is need for a molecular throughput screening assay to monitor and measure angiogenesis as a modulable IEB in clinical trials. Many cancers, including prostate cancer, require the induction of angiogenesis as a necessary step for growth and progression. These events appear to transpire early in tumorigenesis, before the preneoplastic stage via a so-called angiogenic switch. Currently, angiogenesis is measured by microvessel density (MVD) using immunostaining with anti–factor-8 or anti-CD31. HGPIN is associated with an increase in MVD. Key factors in the angiogenic process include growth factors (eg, VEGF, FGF), growth factor receptors, adhesion receptors (integrins), proteases (matrix metalloproteinases), and protease inhibitors (eg, tissue inhibitors of metalloproteinases [TIMP]). Newer methods based on reverse transcriptase/polymerase chain reaction (RT/PCR) amplification of mRNA have great potential for quantifying the expression of multiplex gene function (ie, growth factors and receptors).

Apoptosis and methods for its quantitation are a very active area of biomarker development. Although the two most common methods used for detecting apoptosis in fixed tissues are morphologic changes and DNA fragmentation (eg, TUNEL), a variety of new markers for detecting caspase-mediated events may provide more reliable methods. Immunohistochemical detection of "neoepitopes" resulting from caspase cleavage events may be an earlier event in the execution phase of apoptosis. Potential markers include antibodies recognizing active caspase 3 and neoepitopes on fragment caspase substrates, such as PARP, and cytokeratin 18. Combining these methods could detect both an early (eg, PARP labeling) and late (eg, TUNEL labeling) apoptotic event, reducing the possibility of false positives or negatives. The advantage of using immunohistochemical markers is that the methods are based on stan-

dard techniques. An important principle is that the measurement of apoptosis needs to be confirmed by more than one method. One of the recommendations that panel 2 considered is to combine morphologic observations (TUNEL) with an immunohistochemical marker assay. Moreover, new concepts in programmed cell death point to a nonapoptotic cell death pathway associated with S-phase arrest of prostate cancer cells and type 2 autophagocytosis mediated by the PPAR-γ ligand, 15d-PGJ_2.

Markers Assessed in Tissue by Machine-Assisted Analysis. The ability to establish a "fingerprint" of transformed, morphologically normal epithelium (MAC) in the field at risk is extremely important in an organ system with the sampling problems encountered in the prostate. Developing a reliable profile of the early neoplastic changes (dysplasia) preceding visible morphologic changes has the great potential of overcoming the sampling limitations. Determining this profile in serial prostate biopsies by computer-assisted image analysis (CAIA) can help assess the response to prevention agent(s). Preliminary findings suggest that lesions visually given the same diagnostic grade (eg, HGPIN) may exhibit very different "lesion signatures" when analyzed by CAIA. There is a need for further investigations to establish the changes in nuclear texture and other parameters during preventive interventions. Because these changes are quantified as a morphometric index (mean and SD), providing a continuous biologic response variable for endpoint assessment, CAIA is one of the most promising approaches for the development of clinically meaningful IEB and valid surrogate endpoints of cancer risk reduction.

In vivo Imaging. MRI, MRSI, and bioelectrical impedance imaging represent new modalities with great potential, but they need to be validated in prospective studies. Preliminary studies with MRI/MRSI suggest an improved sensitivity and specificity of sextant biopsies for the early detection of prostate cancer through the detection of metabolic profiles of choline, creatine, and citrate. Although this technology is currently geared to maximize the ability of detecting smaller and higher-grade tumors, it has the potential of expanding into the detection of premalignant lesions (HGPIN) for cohort identification and in monitoring the metabolic changes induced by intervention. Electrical abnormalities arising from premalignant and malignant breast epithelium can be reversed by such agents as tamoxifen. The application of this new technology (bioelectrical impedance) in radical prostatectomy specimens could reduce the false-negative rate of transurethral ultrasound (TRUS)–guided sextant biopsies as well as monitor and localize proliferative areas for prostate prevention. Determining ways to increase the cost-effectiveness of this technology would promote its use in large clinical trials, while defining a uniform protocol would ensure that these results are standardized.

Oxidative Stress Markers. Oxidative stress is believed to contribute to the development of cancer and may represent a biologic link responsible for the association between inflammation and other forms of tissue injury/damage and carcinogenesis. A great advantage to this approach would be the ability to avoid sampling the "lesional" tissue or even the need for sampling the target organ. These markers are traditionally assessed in the serum. However, an extremely promising area would be to determine oxidative stress markers in the prostate and biologic fluids (eg, semen, urine) for cohorts with and without prostate cancer, as well as in those considered high risk. Recent studies suggest that urine cytology can be used to assess GSTP1 expression status in exfoliated prostate cancer cells. GSTP1, a major carcinogen detoxifying mechanism, is inactivated early in prostate carcinogenesis. Monitoring the changes of these markers (GSTP1 levels, oxidized DNA bases, and the products of lipid peroxidation) with such relative ease should enhance follow-up of patients in prevention trials. A central reference laboratory, highly experienced with these assays, a central clearinghouse to streamline sample collection, and a quality assurance protocol, similar to those suggested for the PIN consortium, were recommended.

PSA and Novel Tissue-Specific Markers Derived from Genomics/Proteomics. PSA continues to be a cornerstone in the early detection and monitoring of prostate cancer after treatment. Refinements in the use of PSA isoforms (percent free) and PSA metrics of longitudinal changes (velocity and doubling time) have improved the predictive value for early detection and recurrence. Longitudinal studies suggest that percent free PSA may predict tumor aggressiveness as much as a decade before the initial diagnosis of prostate cancer and thus could provide useful information for evaluating the effect of chemopreventive agents in at-risk cohorts. HK-2 is a prostate-specific, PSA-like kallikrein that cleaves pro-PSA to generate the active form of PSA. The results of several recent studies have demonstrated that a ratio of percent free PSA to total HK-2 provides unique information for the detection of prostate cancer and enhances specificity. In addition, studies suggest that HK-2 is differentially expressed (higher) in PIN and cancer compared with benign epithelium. However, there is a paucity of information regarding the utility of serum biomarkers, including PSA and HK-2, as IEB for prevention trials. An important caveat is that some agents,

such as finasteride, artificially lower PSA levels, and others classified as differentiation agents (phenyl-butyrate and vitamin D analogs) can transiently elevate PSA in subjects with prostate cancer, thus limiting their value in short-term studies. In addition, several other serologic markers show promise as markers of increased risk, such as IGF-1/IGFBP (eg, IGFBP-3). Several preventive agents (eg, SERM, 4-HPR, vitamin D analogs, and lycopene) can modulate serum IGF-1 levels. There is a need to further evaluate the association between serologic factors of risk, the progression to prostate cancer, and the modulation of serologic factors by preventive agents.

Gene Expression Databases: The Prostate Expression Database and the NCI Cancer Genome Anatomy Project. The inherent heterogeneity of prostate cancer and the diversity of promising agents suggest that it is unlikely that a single biomarker can provide the specificity for characterizing genetic alterations that occur in neoplasia and assessing a specific treatment response. Efforts have been directed toward simultaneous assays that measure multiple biomarkers at the DNA, RNA, or protein level to generate differential patterns of expression. One such comprehensive approach involves the use of DNA arrays to quantitatively detect changes in the expression of thousands of genes. As described below, gene expression databases have been developed for the construction and analysis of cDNA expression arrays, providing a virtual archive of thousands of genes expressed in prostatic tissue. In all of these efforts, the incorporation of laser capture microdissection (LCM), with its ability to improve the specificity of tissue sampling, is strongly suggested.

The focus of the PEDB is to define the prostate transcriptome, and the focus of the NCI CGAP is the comprehensive molecular characterization of normal, precancer, and cancer cells for each major tumor type including prostate cancer. The transcriptome can be defined as the identity of every expressed gene in a particular tissue and its level of expression. It is a dynamic link between the genome and "proteome," which defines the cellular phenotype. In PEDB, expressed sequence tags (EST) derived from more than 40 human prostate cDNA libraries are organized into distinct groups that are annotated with information from the GenBank, dbEST, and Unigene public sequence databases. The developers of PEDB have reported on a recent analysis of 1536 different prostate cDNA microarrays in search of genes that are androgen-regulated as a prototype for identifying drug-induced changes. They described a new clone called "prostase" that is similar to PSA and represents a potential new candidate marker. In addition, the CGAP database reports data on the expression of 10,000 genes in normal prostate tissue with 724 genes being considered unique to normal prostate tissue.

TABLE IV. ***Intermediate endpoint biomarker development process***

IEB Candidates	Review by a strategic planning team
Application	Detection, diagnosis, prognosis, monitoring
Indication	Patient stratification for treatment option (ie, surgery, radiation, chemotherapy, gene therapy, chemoprevention, watchful waiting, etc.)
Evaluation	Selection of IEB based on molecular mechanisms with a focus on specific clinical utility (literature and research experience)
Reduction to practice	Model development and establishment of test specifications (ie, retrospective training and testing)
Clinical trials	Model validation (prospective) and regulatory approval process
Publish	

IEB = intermediate endpoint biomarkers

CGAP and PEDB provide sequences for all potential biomarkers. To date, CGAP has discovered approximately 30,000 genes (29,685) and is the driving force in gene discovery. These databases provide unique opportunities for identifying pathways of expression, such as the action of agents downstream and other markers that are involved in a metabolic pathway. Once this is known, it is feasible to define polymorphisms, that is, single nucleotide polymorphisms (SNP), that may correlate with a given response. Among their many applications, database and array-based methods of genetic analysis can be useful for the identification, acquisition, and assessment of candidate molecular markers that could be used as surrogate endpoints for assessing preventive interventions.

Tissue Proteomics. The most widely used method involves the separation of proteins by charge and by mass on conventional 2-D gel electrophoretic platforms. There is a pressing need for a high throughput methodology to look at clinical endpoints that directly measure specific proteins or protein circuits in actual microscopic cell populations. The NCI and FDA have jointly undertaken a Tissue Proteomics Initiative and evaluated several promising methods. One approach involves the coupling of LCM with sensitive chemiluminescent immunoassays. This has produced a 10-fold higher level of detection of PSA in stained human prostate tissue compared with standard immunoassays. Surface Enhanced Laser Desorbtion Ionization (SELDI) is a new affinity-based technique in which the population of tissue cell proteins binds to spe-

Panel 2

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cial capture bait on the surface of a chip and is then detected by a laser beam. The biomarker pattern profiles reveal changes in protein expression as epithelial cells change from normal to PIN to invasive cancer. SELDI appears to be an important technology for the discovery of disease-related proteins, assessment of biologic response to therapy, and toxicity monitoring. Progress using SELDI (protein fingerprints can be obtained from 25 to 50 cells) suggests that it will now be feasible to start looking at PIN lesions, for example its protein profile.

Standardization, Quality Control, Analytical/Statistical Validation Issues. Table IV describes a standardized approach to the development of new IEB and predictive models that use a product development strategic team comprised of internal medical specialists, scientists, and external advisory panels. This team aligns biomarker selection with clinical situations of importance to the physician for disease management. The patient sample dimensions and clinical trial design must also align with the targeted clinical situation and often are retrospective testing and modeling trials followed by prospective validation trials of the new IEB or predictive algorithm.

Candidate IEB for prostate cancer prevention trials (eg, PSA, HGPIN, morphometric markers) represent phenotypes driven by a constellation of genetic lesions. Additional research is needed to permit the incorporation of genetic or molecular measures (genetic alterations in HGPIN that predict progression), which may enhance the utility of IEB. One of the most important considerations discussed by panel 2 was the need for standardization of methods for measuring IEB. Variability in methods for measuring particular IEB and lack of data on the limits of normal variation for IEB in the prostate pose perhaps the greatest limitation to informative use of IEB in prevention trials. In this respect, one only has to consider that the current generation of markers (proliferation, apoptosis, morphometric markers) and the emerging set of molecular markers have yet to demonstrate consistent results in prostate cancer. Methodologic problems and models of SE validation in prostate prevention trials are discussed in detail in the article by Trock.[4]

Panel 2 Recommendations and Research Opportunities

1. The panel identified the need to establish centers and a consortium for PIN. Such a resource would include a biorepository, laboratory facilities, including microdissection equipment, the

ability to use microarray technology, etc., to maximize the yield of a limited lesion, and clinical and laboratory follow-up. This consortium should coordinate its efforts with the NCI Early Detection Research Network, Prostate SPORES, and special recruitment mechanisms, such as the CCOP, VA, DOD, oncology networks, and the new NCI Clinical Trial Gateway Website. Subjects with PIN represent the highest risk cohort for prostate cancer progression, and PIN consortia will facilitate the identification and enrollment of these subjects into efficiently designed prevention trials for new agent registration by the FDA.

2. The panel supports research into the natural history of PIN prior to HGPIN and the identification of earlier markers of prostate carcinogenesis, such as PIA and its association with HGPIN. Linkage to current NCI program announcements on the Molecular Epidemiology of Prostate Carcinogenesis and the NCI Prostate SPORES program is important.

3. CAIA is a promising and objective tool that can establish numerous data points quickly and reproducibly. It is especially helpful to be able to assess these parameters in morphologically normal epithelium (MAC) before, during, and after preventive interventions. The panel identified the need for further investigation into this technology.

4. Oxidative stress/DNA damage markers in serum and leukocytes allow for easier and frequent access to samples from participants in prevention trials. The possibility of evaluating these markers in prostate fluids for cohorts with and without prostate cancer and in high-risk cohorts should also be explored.

5. Research into the development and validation of molecular throughput screening assays (RT/PCR) to monitor and quantitate angiogenesis in clinical trials should be supported.

6. Research into the development and validation of combined panels of markers for measuring apoptosis/cell death in clinical trials should be encouraged. The most promising methods include morphologic, DNA fragmentation, and immunohistochemical methods based on the caspases. Novel pathways of nonapoptotic cell death involving synthetic prostaglandin ligands for PPAR-γ need to be explored.

7. More research into the development and validation of in vivo functional imaging, such as MRI/MRSI, PET, and bioelectrical impedance/light scattering spectroscopy for the detection of early cancers and HGPIN and for monitoring the response to preventive therapy is needed.

8. There is a need to support the development of technology (proteomics) that directly measures specific proteins or protein pathways, in actual microscopic cell populations that may be undergoing disease progression or responding to preventive interventions (eg, SELDI). New high-throughput methods for the identification (automated tandem mass spectrophometry) and quantitation of proteins (isotope-coded affinity tags) are in development and will allow global analysis of new marker protein patterns for disease status and response to treatment.

9. There is a need to support the continued development of multiplex microarray technology/gene expression for risk stratification of the cohort, molecular classification of precancer cells, response monitoring, and statistical analysis of the complex data patterns generated after preventive agent intervention. Linkage and collaboration with the NCI CGAP and intramural programs (Advanced Technology Center) is important.

10. More research and development of IEB and predictive algorithms through linkage to the Early Detection Research Network (EDRN) biomarker developmental/validation laboratories/clinical epidemiology centers is needed.

SUMMARY OF PANEL 3: IDENTIFICATION AND RECRUITMENT OF HIGH-RISK POPULATIONS

Panel 3 considered six general research areas: (1) identification of appropriate target populations (cohorts) for prevention trials; (2) PSA and its role in risk stratification/identification of high-risk individuals for prevention trials; (3) clinical models for evaluating the activity of preventive agents; (4) identification of other risk groups; (5) other modifiers of the risk of developing prostate cancer (diet and supplements); and (6) new recruitment opportunities for prevention trials involving the VA and national oncology networks. There was consensus that multiple potential preventive agents should be tried in all risk groups and clinical models so as to best define which agents appear promising for large-scale trials. This concept is based on the hypothesis that the specific molecular mechanisms that underlie the development of or progression of disease in each risk group and clinical model may be different within a specific group such that different agents may be useful for different settings.

Target Populations

Three major risk groups were identified: (1) low/average-risk states (general population); (2) intermediate-risk states, including African American men (AAM), familial/hereditary kindreds at risk, individuals with elevated PSA without cancer; and (3) high-risk states, including subjects with HGPIN. Subgroup stratification and the advantages and disadvantages of each target group are

TABLE V. *Target populations for prevention*

Risk Group	Specific Population	Advantages	Disadvantages
Low/average	General population	Easily definable Readily available Results widely applicable	Rate of progression slow Requires large study population and long follow-up interval Studies costly
Intermediate	African Americans	Higher risk than general population	Difficult to define Difficult to recruit because of perceived bias
	Genetic		
	Family history	Double or greater the risk of prostate cancer	Ascertainment bias Risk varies with number of affected family members, age of onset, and degree of relatedness Likely to be genetically heterogeneous
	HPC-1–linked	Genetically homogeneous	Identification invasive and costly Affected subjects rare
	Other genes	Genetically homogeneous	Identification invasive and costly Affected subjects rare Risk of progression undefined
	Elevated PSA/negative biopsy	Well-defined histologic endpoint Intermediate risk of progression	Heterogeneous population Sampling error
High	High-grade PIN	Highest known risk Reduced sample size Reduced study duration	Sampling error Diagnosis subjective

HGPIN = high-grade prosthetic intraepithelial neoplasia; HPC = hereditary prostate cancer; PSA = prostate-specific antigen.

described in Table V. The demographics, epidemiology, and biology of prostate cancer in AAM were reviewed, including the earlier age of onset of HGPIN, high-fat diets, and a shorter CAG repeat length (associated with increased androgen stimulation) in the androgen receptor gene.

The contributions of genetics to risk was discussed. Although only a few "cancer genes" for prostate cancer have now been identified (eg, HPC-2/ELAC2 on chromosome 17), population-based studies have clearly identified an increased risk based on family history and segregation analysis. Furthermore, risk–age studies have suggested that family history, including an autosomal dominant germline mutation, may be responsible for a significant portion of early-onset prostate cancer (approximately 10% to 15%). Several different loci on chromosome 1, including HPC-1 (1q24-25), 1p36, 1q42.2-43) and loci on the X chromosome (HPC-X), have been reported in linkage studies of families with prostate cancer. There are several important studies in genetic cohorts underway. One study is recruiting African American families to further confirm the chromosomal findings mentioned above. Currently, 46 families have been recruited for this study. The average number of men diagnosed with prostate cancer per family is 5.1. The outcome of these genetic studies may provide molecular targets for blocking gene expression and preventing forms of hereditary prostate cancer. Another investigation involves a detailed study of brothers and first cousins of young prostate cancer probands. It includes a phase 2B randomized controlled trial of DFMO versus placebo to evaluate modulation of potential surrogate endpoint biomarkers (PIN and PSA) in these at-risk subjects.

PSA and Risk Stratification

Recently obtained data on PSA levels from the Army/Navy Serum Repository (over 20 million samples) have been reported. Several important conclusions emerged, such as the observations that in the youngest age cohort (20 to 29 years old), African Americans (as a group) had significantly higher PSA values than whites, the variability in values was very low at younger ages, and the rate of change with age was lower in African Americans. Similar findings in a study with the Army War College (Carlisle, PA) also have been reported. These observations may allow the development of an early warning strategy for identifying individuals at high risk for prostate cancer as well as identifying a unique group for prevention trials.[11] The need to develop PSA guidelines to screen not just for cancer but for curable cancer was emphasized.

TABLE VI. *Clinical models for testing preventive agents*

Model	Advantages	Disadvantages
Elevated PSA/negative biopsy sample	Well-defined histologic endpoint Intermediate risk of progression	Heterogeneous population Sampling error
Presurgical (watchful waiting)	Early stage disease Readily available study population Pre- and posttreatment tissue available for biologic study	Treatment period short
Adverse pathology after RP	High risk of progression	More advanced disease Clinical endpoint and PSA velocity
Rising PSA after RP or RT	High risk of progression	Most advanced disease Clinical endpoint and PSA velocity

RP = radical prostatectomy; RT = radiation therapy; PSA = prostate-specific antigen.

CLINICAL MODELS

There are a surprisingly large number and variety of ongoing clinical chemoprevention trials, many supported by the NCI. Five major clinical models were used in the reported trials: (1) subjects with elevated/borderline PSA/negative biopsy results for cancer; (2) HGPIN; (3) preprostatectomy (short and extended interval from diagnosis to surgery); (4) subjects with adverse pathology and poor prognostic factors before/after surgery (eg, Partin tables); and (5) rising PSA after prostatectomy. The latter three are models of secondary prevention. Subjects with early-stage prostate cancer (low volume and low Gleason score) who elect not to have surgery or radiation therapy (so-called watchful waiting) are part of the presurgical category in Table VI. They represent up to 25% of all subjects with early-stage disease and exhibit an increasing rate of progression to clinically aggressive disease that requires intervention. The preprostatectomy model offers the best opportunity to compare and validate the utility of the sextant diagnostic biopsy with the whole gland and concurrently to evaluate the effect of the chemopreventive agent on the histology and molecular targets in the index cancer, HGPIN, and adjacent normal-appearing epithelium in the field at risk. As reviewed by Lopaczynski *et al.*[4] elsewhere in this supplement, preprostatectomy is an ideal model for studying the stromal–epithelial interaction and differential effects of preventive agents. The advantages and disadvantages of these models are listed in Table VI. Collectively, these models of secondary prevention should be fertile ground for identifying promising agents that may have activity in primary prevention settings and also may yield useful information on the management of patients with disease in specific clinical situations (ie, adjuvant or secondary prevention).

In most current studies, a panel of IEB are being followed, ranging from proliferation assays (proliferation cell nuclear antigen [PCNA] labeling in tissue) to MRI/MRSI of the prostate (choline to citrate metabolic ratios). Three completed phase 2 studies in presurgical/watchful waiting subjects were reported using 4-HPR, lycopene, and a regimen involving a low-fat diet with added selenium, soy, and lycopene. Three weeks of 4-HPR did not affect IEB, but the study may have been confounded by biopsy-induced changes in biomarker expression. Four weeks of lycopene supplementation appeared to exert a biologic effect (increased connexin 43 and reduced PSA), but the small sample size and unbalanced stratification for Gleason grade confounds interpretation. The 12-month dietary intervention study (low fat, soy, selenium, etc) showed a trend toward PSA decline and incorporated MRI/MRSI to monitor response. A follow-up randomized controlled preprostatectomy trial employing a low-fat diet with dietary supplements is now in progress.

Other ongoing trials include: HGPIN (low-dose flutamide versus placebo); preprostatectomy (DFMO, vitamin D analogs, COX-2 inhibitors, sulindac sulfone); and postprostatectomy including subjects with rising PSA (soy supplementation, sulindac sulfone, and troglitazone). The most mature of these trials, a phase 2 randomized placebo-controlled trial of sulindac sulfone showed an effect on stabilizing the rate of rise of PSA in high-risk subjects, based on a 6-month interim analysis that has been confirmed at 12 months.

The biology and epidemiology of selenium and its relation to prostate cancer was extensively ad-

dressed as well in the ongoing trials using this compound. Currently, two major forms of organoselenium formulations are in clinical trials, selenized brewer's yeast and purified L-selenomethionine. A wide variety of studies are in progress, including the evaluation of selenium in randomized controlled trials with different risk groups and clinical models: general population (SELECT), negative biopsy and elevated PSA, HGPIN, preprostatectomy, and watchful waiting. Changes in PSA levels, PSA velocity, biopsy-proven prostate cancer, or time to clinical progression are used as primary endpoints depending on the trial. The design and status of the ongoing PCPT of finasteride versus placebo in the general population of men at risk (age >55 years, PSA <3 ng/mL) was reviewed as well as the rationale and design of the recently initiated SELECT trial (2 × 2 factorial design with vitamin E (400 IU of *dl*-α-tocopherol acetate) and organic selenium (200 μg of L-selenomethionine) in the general population. In PCPT, African Americans are underrepresented, but in SELECT a concerted directed effort to increase accrual of this group is being made by targeting VA participants, who are overrepresented by AAM. Using this recruitment approach, the ongoing Prostate Intervention Versus Observation Trial (PIVOT) trial has accrued 25% African Americans.

Identification of Other Risk Groups

Recognizing the current limitations of risk assessment, panel 3 also considered whether additional risk groups could be defined. To date, little has been done in this area. These other risk groups would include a multivariate Gail-like model, metabolic gene polymorphisms/phenotype, genetically isolated populations, and dietary and personal habits. Most of the discussion focused on the idea of constructing a multifactorial Gail-like model as used in chemoprevention trials of breast cancer for prediction of risk stratification. If validated, such a model could reduce the number of subjects needed for a prevention trial while still generating results that are widely applicable to the general population. Several prototypes are under development. The most useful model would probably include a combination of genetic (inherited and somatic) risk markers, family history of early onset prostate cancer, histology (abnormal biopsies including HGPIN), and environmental factors (diet and lifestyle). Modifying metabolic genes in colon, breast, and other cancers are being increasingly defined, and it is clear that risk can be more precisely estimated by including their effect. It would be important to study the effect of single nucleotide polymorphisms and variations (polymorphisms) in the androgen receptor, 5α-reductase, estrogen receptor, sex-steroid metabolizing enzymes (eg, cytochrome p450 3 A4), redox regulators, and the fatty acid metabolism pathway (PPAR).

Potential Modifiers of Risk for Developing Prostate Cancer

Panel 3 also considered the numerous epidemiologic observations in the literature suggesting associations between various dietary, lifestyle, genetic, dietary supplement, and nontraditional factors (alternative and complementary medicines) and prostate cancer. Because there is no clear agreement regarding what other data should be collected routinely in epidemiologic and clinical trials, it was further suggested that NCI should address this by requiring a set of information (eg, questionnaires, tissue) that would be collected in all studies. There was general agreement that detailed information on diet, vitamins, supplements, nontraditional medicine, and family history (with genetic linkages where possible) should be collected as well as blood, lymphocytes, and tissue for DNA, RNA, and proteomic analysis.

Special Recruitment Opportunities: The VA and Oncology Networks

There are many aspects of the VA healthcare system that make it a unique setting for the study of prostate cancer, and these were reviewed. The VA has developed a multicenter research infrastructure, including the Cooperative Studies Program and new epidemiology and information centers (eg, the National Blood and Tissue Storage Facility). The VA national cancer registry reports that there were about 5000 newly diagnosed prostate cancer patients per year between 1995 and 1997. Due to the large number of AAM users of the VA system, the veteran population provides an excellent resource for studying health issues in a minority population. Approximately 20% of new cases of prostate cancer were AAM veterans.

Because prostate cancer research is a high priority, the VA has now become engaged in several large-scale, multicenter research efforts. A number of projects are underway, including observational studies of factors that predict incident disease, follow-up studies for predictors of poor outcome (involving 1000 subjects), studies of quality of life (involving 600 veterans with prostate cancer), and randomized trials testing treatment strategies (eg, PIVOT involving 500 subjects). These VA studies involve active collaboration with other groups, such as the DOD, NCI, and Southwest Oncology Group (SWOG) (related to SELECT). The VA is planning to expand the SELECT cohort for a follow-up study from 1000 to 10,000 subjects.

The organization and scope of US Oncology, a large physician practice management company

Panel 3

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and network recently formed in a merger between the Physician Reliance Network and American Oncology Resources, was reviewed. It is organized similarly to NCI cooperative groups and includes seven CCOPs within its network. Currently there are 750 medical oncologists in the network in 25 states caring for about 15% of the US cancer population. Approximately 25% of its members actively participate in cooperative group studies, including breast and prostate prevention trials. The panel examined how US Oncology could work best with the NCI over the next 5 to 10 years in conducting prevention trials, including prostate cancer. One of the most important initiatives is the establishment of a central institutional review board, which has already been accepted by the Office for Human Research Protection (OHRP) at the National Institutes of Health (NIH), that would facilitate participation in the NCI Expanded Participation Project. The network is poised to collaborate with clinical cooperative groups, such as SWOG, and participate in large phase 3 prevention trials (eg, SELECT), with incidence and mortality endpoints. Within the network, there are 60 community cancer centers providing a research infrastructure facilitating close collaborations between medical oncologists and other subspecialists. US Oncology offers a promising new mechanism for identifying eligible patients, actually registering them in studies, and facilitating the acquisition of precancerous tissue for prevention trials in high-risk subjects for prostate cancer.

Panel 3 Recommendations and Research Opportunities

1. There was general agreement to strongly support the evaluation of multiple promising prevention agents in all risk groups and clinical models to best define which agents appear most promising for large-scale prevention trials. This includes a wide variety of natural and synthetic agents.

2. The panel supports the development of a Gail-type male model/algorithm to predict prostate cancer risk and identify high-risk individuals for prevention trials. Collaborations and workshops between the NCI, the pharmaceutical industry, and academic investigators should be pursued to expedite development.

3. Defining the natural history of PSA and progression to clinical prostate cancer in different normal ethnic and racial populations is an important

goal, both for devising strategies for early detection and for identifying appropriate high-risk subsets for prevention trials. A collaborative research effort between DOD/VA and NIH/NCI would seem the most efficient way to achieve this goal.

4. The panel supports research aimed at identifying whether new PSA isoforms and other serologic factors (HK-2, IGF-1/IGFBP-1/3, etc.) can be used to distinguish occult from clinically significant and curable prostate cancer.

5. The overall issue of prostate cancer in African American men is a complex one, and to make progress will require the interaction of individuals from a diversity of disciplines, multiple investigators, and probably multiple institutions. A joint NIH Office of Special Populations–NCI Program Project mechanism would be a good way to stimulate the field. Use of previously successful targeted interdisciplinary research program projects focusing on genetic and acquired risk factors and prevention trials in genetically defined cohorts at risk for prostate cancer should be supported.

6. The panel supports research into the best way to identify and recruit familial/hereditary, African American, or other high-risk cohorts for prevention trials.

7. The panel supports enhanced recruitment strategies for high-risk cohorts for prevention trials through closer linkage to NCI infrastructures (Clinical Cooperative Groups, Prostate SPORES, CGN, IPCC, EDRN, clinical trials network), VA/DOD, and national community oncology networks. In particular, the VA offers unique opportunities for augmenting minority recruitment in prevention trials. National oncology networks offer a new mechanism for developing collaborations between oncologists and subspecialists (urologists) to conduct prevention trials in subjects with precancer.

8. The panel supports the evaluation of new educational strategies for informed decision making to encourage individuals to participate in prevention clinical trials. Pilot randomized studies of the use of educational material to augment patient recruitment should be supported.

9. The panel supports more research into the natural history of PIN before HGPIN, including the identification of earlier disease alterations and disease states of prostate carcinogenesis. This will ease the indentification of cohorts, such as proliferative inflammatory atrophy.

10. The panel supports more research into the role of other risk factors, such as metabolic polymorphisms, single nucleotide polymorphisms, and other exogenous risk modifiers in the natural history of prostate cancer.

11. The panel supports the collection of standardized data sets that capture relevant epidemiologic information (diet, lifestyle, genetic, and nontraditional supplement use).

12. The panel supports research related to the identification of genetically isolated populations for prostate cancer.

SUMMARY OF PANEL 4: PREVENTION CLINICAL TRIAL DESIGNS

The scope of panel 4 comprised virtually all of the other three workshop panels. The goal was to incorporate the most promising agents, biomarkers (endpoints), and cohorts into well-designed prevention trials. Building on the major advances in recent years in our understanding of prostate cancer pathobiology and the critical issues identified in the *PRG Report*, the NCI, the pharmaceutical industry, and the academic research community are searching for better clinical trial methods to test and identify effective prostate cancer prevention agents. One of the keys is to design more efficient prostate cancer prevention trials that minimize study size and duration without losing the ability to generate valid evidence of preventive efficacy. Topics from the other panels that weighed heavily in panel 4's discussions included appropriate study populations, endpoints (definitive and intermediate), and molecular targeting of potential preventive agents. Critical for the molecular targeting approach is the use of validated biomarkers as strategic clinical trial endpoints to evaluate and confirm proof-of-principle and proof-of-efficacy trials. Panel 4 also considered the design of early and intermediate prevention trials, statistical methods to minimize trial sample size and study duration (eg, Bayesian interim analysis and factorial designs), current FDA standards for prevention efficacy, the current major large-scale initiatives in prostate cancer prevention, quality-of-life endpoints, and validation of surrogate endpoints.

Bayesian Monitoring and Analysis of Phase 2 Trials

As a result of the large number of promising agents for prostate cancer prevention in the pipeline and limited resources and patients, the NCI has been evaluating methods that could improve the efficiency and throughput of new agent evaluation in phase 2, a critical decision point in agent development. One such approach is the use of a Bayesian algorithm for monitoring progress, ie, interim analysis with as few as 30 subjects. The Bayesian stopping rule is conditioned on the choice of prior distribution for the treatment effect size so that one can stop quickly for ineffective agents (eg, neutral prior) but stop more slowly for active agents (skeptical prior). Before implementing this method in phase 2 prostate trials, the per-

formance of the Bayesian method was shown to compare favorably to three standard group sequential methods in simulated randomized controlled trials.

The Bayesian method has now been applied to several prospective phase 2 prevention trials. For example, this approach was used for the analysis of a recent randomized trial of 4-HPR versus placebo in subjects scheduled for prostatectomy.[5] With as few as 30 subjects, the Bayesian method compared favorably with the standard methods, showing that 4-HPR at 200 mg/day is inactive in this clinical model of prostate cancer. Furthermore, the Bayesian method retains simplicity of interpretation and provides flexibility in monitoring as the data accumulates without the need to adjust for type 1 error at each interim look.

Target Populations for Early and Intermediate Prevention Trials

It will be critical to identify high-risk populations in order to reduce the sample size and duration of prostate cancer prevention trials. Panel 3 delineated target populations at risk and clinical models for conducting primary and secondary prevention trials. In addition, panel 4 outlined several well- (and lesser-)known risk factors for developing prostate cancer, including older age, African American ethnicity, elevated PSA with negative biopsy sample, abnormal digital-rectal examination (DRE), HGPIN, family history of prostate cancer, risk for progressive rather than latent or indolent prostate disease, and presence of certain serologic/cellular/molecular risk markers (as detailed in panels 1 to 3). Panel 4 noted that some models have achieved an 80% power to predict prostate cancer (in smaller model and external [validation] populations) based on the risk factors of PSA, DRE, race, and age.[6] The development of similar models in larger populations would provide a simple and effective way to identify high-risk individuals for prostate cancer chemoprevention trials (as has been done by Gail *et al*.[7] for breast cancer). Other potential risk factors include prior biopsy, histology of prior biopsies (eg, HGPIN), family history of prostate cancer, age of puberty, caloric intake at adolescence, PSA free/total/velocity, serologic factors (HK-2, IGF-1, oxidized DNA bases, androgen/estrogen ratio), and SNP of relevant genes (5-α-reductase, CYP 3A4, ELAC-2, etc.).

Other appropriate target populations for prostate cancer prevention study include prior cancer patients, who can be studied for preventing early recurrence (biochemical progression versus primary prevention involving populations without cancer history), patients with early localized prostate cancer who opt for watchful waiting, patients with latent or indolent prostate cancer, patients with rising PSA after surgery or radiation and who, along with patients scheduled for prostatectomy, can be recruited to phase 2 trials to evaluate toxicity and biologic activity and validate the mechanism of action of agents with promising preclinical profiles. Evidence for regulatory decisions and salient design features for phase 1 to 3 prostate prevention trials are shown in Table VII.

Large-Scale Primary Prevention Trials and Definitive Trial Designs

Because the most definitive prevention endpoint of survival is prohibitively expensive to assess in clinical trials, cancer incidence has been the focus of large-scale definitive trials in prostate cancer prevention. Two long-term, large-scale trials currently are using the definitive cancer-incidence endpoint—the PCPT and SELECT. Started in 1993, the PCPT is a 10-year two-arm trial that successfully accrued (in 3 years) its full complement of over 18,000 average-risk men randomized to either finasteride or placebo. The results of this trial are expected in 2003. SELECT is a 12-year study involving 32,400 at-risk men randomized to selenium and vitamin E in a 2 $\times$ 2 factorial design scheduled to start in Spring 2001.

The PCPT and SELECT illustrate that definitive trials using the cancer-incidence endpoint (rather than the even more costly survival endpoint) may be very expensive, large, and long projects. Not many such trials are feasible. Therefore, considerable effort is being expended to design trials with surrogate endpoints. The purpose of a surrogate endpoint study in the prostate or any other site is to validate surrogate endpoints that can replace currently definitive endpoints (ie, that modulation of the surrogate endpoint correlates with the long-term cancer-incidence reduction or survival improvement). Promising surrogate endpoints include prostate premalignancy (HGPIN), image-analysis–derived features from nuclear morphometry, tissue-related markers of prostate carcinogenesis (apoptosis, angiogenesis, metabolic profiles), drug effect markers, and serologic risk factors (PSA isoforms, IGF-1/IGFBP-1, DNA adducts). There is intense interest in using well-defined precancerous lesions, such as HGPIN, as surrogate endpoints, as illustrated by the recent AACR Task Force on Intraepithelial Neoplasia[8] and the World Health Organization Conference on Premalignant Lesions of the Genitourinary Tract (D. Bostwick, personal communication). Because many patients with HGPIN progress to malignancy within 5 years, this is an excellent candidate for potential validation as a surrogate endpoint for testing prevention agents. Representative definitive phase 3 trial designs for prostate cancer incidence reduction and surrogate endpoint validation are shown in Table VIII.

TABLE VII. ***Evidence and designs for scientific/regulatory decisions for phases 1, 2, and 3 prostate prevention trials***

Phase (sample size)	Cohort	Design	Endpoint	Issues
1	Normal, high risk	Dose ascending	PK/safety	MSD = phase 2 dose
(25–50)	Early PCA (WW)	(1 month)	PD/IEB	Grade 2 toxicity
2A	High risk*	RCT/DR	Biologic	Interim analysis
(50–100)	HGPIN/PCA	(1–2 months)	Change	Biodistribution/PSA
			PD/IEB	Drug effect
			Histology	Proof of principle
			PK/Safety	
2B	High risk	RCT/DR	Histology (HGPIN)	Histology
(100–300)	(HGPIN)	(6–12 months)	PD/IEB	SE = accelerated approval
	Early PCA (WW)		PSA	Cancer incidence
				PSA velocity
3	Intermediate risk	RCT	Clinical benefit	Validate SE
(200–1500)	High risk (HGPIN)	(1–5 years)	Cancer incidence	PSA velocity
	Posttreatment		Quality-of-life	Time-to-event
				Proof of efficacy

Intermediate to high-risk: Subjects with elevated PSA and negative biopsy sample for cancer, subjects with strong family history of prostate cancer, subjects with high-grade PIN.*
PK = pharmacokinetics, RCT = randomized control trial, PCA = prostate cancer, MSD = maximum safe dose, DR = dose-response, PD = pharmacodynamics, PSA = prostate-specific antigen, WW = watchful waiting, IEB = intermediate endpoint biomarker, HGPIN = high-grade prosthetic intraepithelial neoplasia, SE = surrogate endpoint.

TABLE VIII. ***Representative chemoprevention trial designs for prostate cancer incidence reduction and surrogate endpoint validation***

Cohort	Study Size	Statistical Power (α)	Treatment Effect	Primary Endpoint
Average risk				
Risk based on age >50, or 55 years (PSA <4 or <3 ng/mL, negative DRE)	18,000–32,000	90% (0.05)	25% decrease in cancer incidence	PCA incidence at 7 years of treatment (mandatory vs routine exit biopsy)
Increased risk				
Family history of first-degree relatives (PSA <4 ng/mL)	1500	80% (0.05)	33% decrease in cancer incidence; 33% decrease in proportion with elevated PSA velocity	5-year cumulative risk of PCA
Intermediate risk				
PSA >4 ng/mL (no cancer detected in biopsy sample)	700–1000	80% (0.05)	50% decrease in cancer incidence; 50% decrease in PSA trajectory	PCA incidence at 4–5 years of treatment
High risk				
HGPIN (with no cancer detected on two sextant biopsies)	200–450	93% (0.05)	33/40% decrease in cancer incidence	PCA incidence at 1–3 years of treatment

All studies randomized, controlled, and blinded.
DRE = digital rectal exam, PSA = prostate-specific antigen, HGPIN = high-grade PIN, SE = surrogate endpoint, PCA = prostate cancer, PIN = prostatic epithelial neoplasia.

Quality-of-Life Endpoints

Panel 4 also considered methods involved with ascertaining quality-of-life (QOL) endpoints, such as patient stress and fatigue levels, which are of growing importance in cancer prevention and therapy research. Recent study in the PCPT indicated that individuals who enroll in prostate chemoprevention trials are likely of better health, lifestyle, QOL, and socioeconomic status than the general population, demonstrating that QOL endpoints can be successfully obtained in a large-scale prostate chemoprevention trial. Therefore, a QOL endpoint study might provide strong prognostic factors for characterizing study subjects' potential for clinical benefit.

Factorial Designs

The factorial design is a natural choice for evaluating multiple agents in the same prevention set-

ting, because it allows the treatment effect of each agent and their combinations to be assessed. Factorial designs are efficient in estimating the treatment effect when there is a positive interaction (additive or synergistic effect) or no interaction and no overlapping toxicities among the different agents. A factorial design reduces the percentage of patients receiving placebo in a 2×2 design evaluating two agents, as only one-quarter of the patients are randomized to the placebo. The Physicians' Health Study (aspirin and β-carotene) and the ATBC Cancer Prevention Study are examples of the successful implementation of 2×2 factorial designs. The increased attention to rational combinations of chemoprevention agents, especially antioxidants, will significantly enhance the value and role of factorial designs in both early (phase 2), intermediate, and large-scale prevention trials.

FDA Approval of Agents That Prevent Prostate Cancer

Panel 4 also considered the ultimate goal of prostate cancer prevention research—to gain FDA approval for agents that can improve public health by reducing prostate cancer incidence and mortality. To date, no agent has received FDA approval for chemoprevention of prostate cancer. In the shorter term, it is anticipated that both the PCPT and SELECT have the potential to achieve positive results (with finasteride [PCPT] or selenium or vitamin E [SELECT]) that may lead to FDA approval for these agents for prevention. Longer-term, however, it is hoped that more efficient trials employing SE that can reduce the size, duration, and expense may qualify future promising prostate-cancer preventive agents. The cancer endpoint is an FDA-accepted measure of clinical benefit. Nevertheless, FDA has accepted surrogate endpoints for granting approval, such as regression of actinic keratosis (skin cancer) by fluorouracil (5FU) and colonic polyps (familial colon cancer syndromes) by celecoxib.[9] Certain preinvasive lesions, such as polyps in familial adenomatosis polyposis and PIN lesions in high-risk subjects for prostate cancer, can be evaluated under an accelerated approval (subpart H) mechanism, which, if granted by the FDA, is provisional on the commitment to conduct a phase 4 validation study to confirm the clinical benefit in reducing cancer risk or mortality. The suppression of preinvasive lesion surrogate endpoint including PIN offers the advantage of reducing or delaying clinical surveillance, therapy, and associated morbidity and ultimately cancer and mortality. A three-step (trial) schema and developmental pathway for efficient new agent registration for prostate cancer chemoprevention is presented in the article by Lieberman.[10]

Validation of Surrogate Endpoints

Future studies should attempt to identify the most promising agents through molecular targeting and to validate prevention surrogate endpoint models using either HGPIN or panels of cellular and molecular biomarkers to test promising agents. These studies will require efficient statistical methods for analyzing multiple trial endpoints, including molecular/cellular markers of risk and drug effects on carcinogenesis (surrogate endpoint). To compare the surrogate endpoint with the gold standard, a validation trial essentially requires the same duration as that of a trial using definitive endpoints.

The large currently definitive trial designs, such as those of the PCPT and SELECT, will remain valuable for assessing long-term or less obvious toxic effects, other multiple outcomes/endpoints of cancer chemoprevention study, such as QOL, translational endpoints, including molecular risk markers, and the validation of surrogate endpoints of prevention efficacy. The future direction of FDA registration studies include the use of molecular progression models. These models must have a tight link to cancer development, and the associated surrogate endpoints require validation. Nevertheless, the development of such models are underway in the current studies of PIN. Two trials in PIN cohorts that have definitive endpoints of cancer incidence reduction were discussed in panel 3. Statistical methods, frequently encountered problems, and chemoprevention clinical models for surrogate endpoint validation are discussed in detail by Trock.[4]

Panel 4 Recommendations and Research Opportunities

1. The panel supports increased use of factorial designs, especially in proof-of-principle and intermediate (efficacy) trials of combinations of agents.

2. The panel supports the increased use of Bayesian monitoring and interim analysis to expedite decision making in phase 2 (effective versus ineffective agents).

3. The panel supports the development of a "Gail" prostate model to identify high-risk individuals and improve the efficiency of study designs. This should incorporate inherited and somatic genetic factors, such as single nucleotide polymorphisms, ELAC-2 in CYP 450 metabolizing enzymes, methylation patterns of GST, etc.

4. The panel supports validation studies of HGPIN and other high-risk states (elevated PSA combined with negative biopsy samples) as surrogate endpoint(s) for prostate cancer incidence reduction in definitive trials.

5. The panel supports validation studies of po-

Joint Panel 4

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All participants from panels 1, 2, and 3 included in panel 4.

tential surrogate and QOL endpoints in large-scale primary prevention trials (PCPT and SELECT).

6. The panel supports the development of methods (statistical models) for analyzing multiple primary endpoints, that is, panels of biomarkers including multiplex gene expression arrays and proteins.

7. The panel supports the development and validation of molecular progression models of prostate carcinogenesis. Reversing molecular alterations could constitute a surrogate endpoint for decreased cancer incidence. This includes the development of predictive multivariate models, which can identify high-risk subjects for early recurrence (eg, 50% in 2 years) after prostatectomy that incorporate conventional adverse pathologic features with new genetic assays for polymorphisms (ELAC2, CYP3A4, etc.).

8. Increased use of state-of-the-art technologies, such as genomics, cDNA microarrays, proteomics, and functional imaging, promise to greatly advance molecular modeling studies.

9. The panel supports molecular modulation trials in high-risk cohorts, especially in subjects with histologically defined lesions (dysplasia, PIN), as these studies would require many fewer subjects and fewer years to complete than the current phase 3 definitive trials.

10. There should be continuing efforts by NCI, academia/professional organizations (AACR), the FDA, the pharmaceutical industry, and the public to develop a consensus on pathways and mechanisms for expediting validation of surrogate endpoints and more efficient trial designs for approving new preventive agents for prostate cancer. Future workshops on developmental pathways for new agent approvals should be conducted.

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