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Introductory Remarks: Development of Chemopreventive Agents for Prostate Cancer

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Abstract The term "cancer chemoprevention" refers to the prevention of cancer by intervening with drugs prior to the malignant (*i.e.*, invasive) stage of carcinogenesis. The development of chemopreventive drugs is the major objective of the Chemoprevention Branch at the National Cancer Institute.

The testing of drugs for cancer chemoprevention differs from testing of those for cancer treatment. Chemopreventive drug trials involve healthy target populations, and the endpoints of reduced cancer incidence or mortality, reduced/eliminated precancerous lesions, or increased latency must be achieved with little or no drug toxicity.

The design of cancer chemoprevention trials for prostate presents several problems, such as the age of the study population and undependable methods for detecting microscopic foci by sequential sampling. A major motivation for organizing this workshop is the development of strategies for the design of chemopreventive intervention trials for prostate cancer.

One of the most difficult problems of chemoprevention drug testing is the necessity of lengthy trials due to the long developmental period of many cancers. This is especially true for prostate cancer. A major solution to the problem is the use of intermediate biomarkers, defined as morphological or molecular intraepithelial changes that can constitute short-term endpoints in chemoprevention clinical trials. They are categorized as histological, genetic, proliferation-related, and differentiation-related. Modulation of intermediate biomarkers, instead of cancer incidence, as trial endpoints would allow chemoprevention trials to be of shorter duration, to use fewer subjects, and to be of lower cost. Review of the current status of prostatic intermediate biomarkers, and methods for identifying and validating them, are also major reasons for convening this workshop.

Key Words: chemoprevention, clinical trials, intermediate biomarkers, prostate

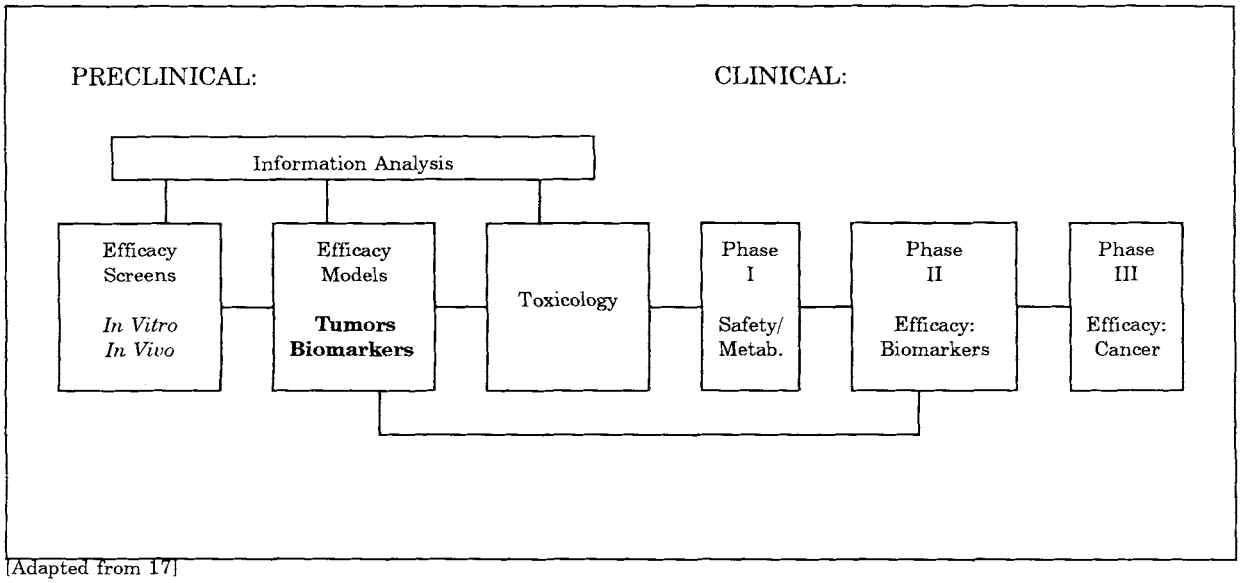
In 1991, the prostate became the site of the highest cancer incidence (22%) and the second leading cause of death attributable to cancer (12%) in U.S. males [1]. However, since these numbers refer primarily to clinically evident tumors, the incidence problem is actually underestimated. Microscopic or latent foci of adenocarcinoma have been detected in serial sections of "normal" prostates at autopsy of men dying from other causes. The frequency of organs with latent tumors has been shown to increase substantially with each decade of life from the 50s (5.3–14%) to the 90s (40–80%) [2]. Thus, it has been estimated that 9 of 10 prostatic cancers will remain undetected and clinically silent [3].

Exogenous factors appear to contribute more to the disparity between latent and clinical prostate cancer than hereditary factors, since the incidence of latent adenocarcinomas does not vary widely between populations. The prevalence of microscopic lesions at autopsy was 20.6, 28.8, and 36.9 per 100,000 in Japanese, Germans and African-Americans, respectively; however, rates of clinical cancer were 2.7, 21.1, and 67.1 per 100,000 in these same populations, respectively [4]. Furthermore, the clinical cancer rate of Japanese immigrants to the U.S. approaches that of U.S. Caucasians within two generations [5,6]. This suggests that the progression of latent cancers to clinical cancers can be modulated.

Cancer chemoprevention or prevention of cancer by the use of chemical agents, the subject of this workshop, generally involves intervention with agents prior to the malignant stage of carcinogenesis. In the prostate, however, the slow progression of latent cancers may afford an additional opportunity for altering the development of clinically evident or metastatic prostate tumors. Thus, potential chemopreventive drugs considered in this workshop would modulate prostate tumorigenesis from the initiation of normal-appearing tissue through the progression of latent cancers.

It may be useful to describe the chemoprevention drug development process and status at the National Cancer Institute (NCI) as an introduction to this meeting. Drug development in the NCI Chemoprevention Branch (Fig. 1) begins with information analysis. From continued surveillance of the literature, we have identified more than 1700 agents with some activity that inhibits carcinogenesis [*e.g.*, 7]. These agents include pharmaceuticals, natural products, and nutrients. The NCI is interested in both pharmaceuticals and the remaining types of agents; each type has advantages and disadvantages. Pharmaceuticals have the benefit of well-defined protocols for obtaining regulatory approval. Often, the pharmaceutical sponsor has completed much of the testing for toxicity and preclinical efficacy by the time the NCI places the agent in clinical chemoprevention trials. In the pharmaceutical industry, it now takes 12 years from discovery of a new agent to approval for the clinic; any strategy which shortens this interval is of value to the NCI.

The theoretical definition of candidate chemopreventive agents is more difficult than it first appears. Identification of chemical structures, biological activities, and biochemical or molecular targets that form the selection criteria for effective chemopreventives is sometimes empirical. Examples of biological activities which may confer chemopreventive properties include inhibition of ornithine decarboxylase [*e.g.*, difluoromethylornithine (DFMO)], induction of Phase II metabolic enzymes (*e.g.*, oltipraz), and inducers of differentiation (*e.g.*, retinoids). In addition, a potential agent may have many functions that appear to be important; determining which is mechanistically most important for chemoprevention is often problematic. For example, retinoids have numerous reported activities [8]; one is induction of transforming growth factor β (TGF-β), for which more than 100 biological activities have been reported in the literature. Also, oncogenes may appear to be appropriate targets for chemopreventives;



[Adapted from 17]

Fig. 1. Chemopreventive Agent Drug Development Strategy

however, inhibition of oncogene expression or product function might suppress normal cell function and create a level of toxicity that cannot be tolerated in chemoprevention. So, the empirical approach involves gathering efficacy and toxicity data in accepted animal models or toxicology protocols, while continuing to be aware of mechanistic data. The science and rationale for all the systems used in the Chemoprevention Branch program have been described elsewhere [9].

The drug development effort at NCI has been in progress for about five years. Approximately 200 agents are on test in *in vitro* screens; more than 100 agents are on test in animal efficacy screens. There are approximately 20 agents for which reasonable toxicity data are already available or for which NCI is evaluating toxicity. The best of these agents are coming into Phase I and Phase II clinical trials [10,11]. The proof of the empirical approach in modeling human cancer will be in the outcome of the clinical trials.

CANCER CHEMOPREVENTION VERSUS CANCER TREATMENT

It is important to understand the conceptual differences between the development of drugs for cancer chemoprevention and the development of those for cancer treatment. The differences in the testing of these two types of potential drugs in the clinic include the target populations, the goals and endpoints, and the acceptable levels of toxicity.

For cancer treatment, cancer patients are the target population; for chemoprevention, target populations include the general, healthy population as well as individuals at high risk for cancer, persons with precancerous lesions, and those who have had a previously treated cancer, but are currently disease-free. These target populations have profound implications for design of chemoprevention clinical trials: recruitment is more difficult; more subjects are required; monitoring is more arduous and complex; and compliance is an issue since the drugs are typically administered in an outpatient setting.

Second, the goals and endpoints of cancer chemoprevention and cancer treatment are different. In treatment, the goal is to kill cancer cells or to increase patient survival; the measurable endpoints are decrease or disappearance of tumors and increase in disease-free survival. In chemoprevention, the goal is the inhibition of carcinogenesis (*i.e.*, initiation, promotion, and progression); the measurable endpoints are reduced cancer incidence, reduced cancer mortality, reduced/eliminated precancerous lesions, and increased latency period.

Third, the levels of acceptable toxicity are lower in cancer chemoprevention as compared with cancer treatment. In cancer treatment and advanced disease, severe toxicity is acceptable; in an adjuvant setting, moderate and acute toxicity are acceptable. However, in cancer chemoprevention, none or minimal acute and chronic toxicity are acceptable. This difference really determines which agents can be used as chemopreventives.

CHEMOPREVENTION DRUG EVALUATION IN CANCER SETTINGS

Despite the differences between cancer chemoprevention and cancer treatment, the point should be made that cancer is a continuum and there are certain clinical cancer settings where chemopreventive drug development for precancer can be augmented. For example, retinoids are well-documented cancer chemopreventive agents; basic mechanistic studies have appeared in the literature for more than 15 years [*e.g.*, 12–16]. As studies of retinoids moved into the clinic, most of the early trials involved cancer patients. These early clinical trials were not very successful, but they yielded a large body of data on mechanisms and toxicity. With such information, agents could ethically be tested in cohorts with lesser disease. This progression is not unusual in drug development. For example, the development of methotrexate for arthritis depended on early clinical trials in end-stage rheumatoid arthritis. The drug was not very effective, but regimens were developed based on the toxicity and efficacy data from these early trials that allowed methotrexate to be evaluated in cohorts with less severe arthritis. By this process, methotrexate was found to be very useful in the treatment of earlier stages of rheumatoid arthritis. Thus, despite the

contrasts between cancer chemoprevention and treatment, the information obtained may be complementary.

IMPORTANCE OF INTERMEDIATE BIOMARKERS IN CLINICAL CHEMOPREVENTION TRIALS

For chemoprevention drug development, one of the most difficult aspects is the long period required for many cancers to develop, and, consequently, the apparent requirement for long clinical trials to test the efficacy of chemopreventives. One approach to this problem is the identification of intermediate endpoint biomarkers for evaluating clinical efficacy, especially in the prostate. *Intermediate endpoint biomarkers* are biological alterations in tissue between initiation and tumor development. It is hypothesized that modulation of one or more intermediate endpoint markers by a chemopreventive agent(s) will interrupt carcinogenesis. Validation of a marker will be obtained when the final endpoint, cancer incidence, is also decreased as a result of this modulation.

Evaluation of intermediate biomarkers instead of cancer incidence as trial endpoints allows chemoprevention trials to be of shorter duration, use fewer subjects, and be lower in cost. They may also allow use of serum or a small tissue sample to monitor response. In addition, they provide effective doses for Phase II trials and rationale for Phase III trials, and may provide basic scientific contributions to understanding the mechanisms of carcinogenesis. Clearly, much work remains to be done in identifying and validating appropriate intermediate biomarkers. Review of the current status of early markers and development of research strategies for identifying and validating intermediate biomarkers for prostate cancer is one of the main reasons for convening this workshop.

To model the role of intermediate biomarkers in cancer it is useful to classify them into the following groups: premalignant lesions/histologic changes, proliferation, differentiation, genetic, or biochemical. This classification scheme has been applied to biomarkers in various tissues such as colon, bladder, and cervix. Table I is a representative listing of potential intermediate biomarkers in the prostate classified in this manner. However, it should be noted that various types of markers have been associated with cancer in the literature, but not all are useful in chemoprevention. These appear in Table II and have been discussed previously [17].

In chemoprevention strategy, histological precancerous lesions are an important starting point. As described recently [18], they may provide a measurable endpoint for clinical trials, as well as a high risk tissue in which other intermediate biomarkers can be developed and validated. For prostate, a possible histological marker is prostatic intraepithelial neoplasia (PIN). Bostwick describes this lesion and its relationship to cancer elsewhere in this Proceedings.

Loss of control of cellular proliferation is a basic component of carcinogenesis. In most experimental models of carcinogenesis, decreasing the proliferation rate results in decreased cancer incidence, decreased tumor multiplicity, or lengthened latent period. For example, proliferation markers appear to be very important in the colon [19]; however, the slow growth rate in prostatic neoplasia may limit the use of some proliferation markers in this organ. In these proceedings, increased S-phase is discussed as a potential marker by Nagle, and T. Thompson discusses the association between elevated TGF-β_1 and onset of prostatic cancer.

As cells differentiate, a specific pattern of expression of cellular components, such as proteins and carbohydrates, occurs. Since cancer cells undergo aberrant patterns of differentiation, it is likely that cellular components characteristic of differentiation will be modified in premalignant states. For example, during abnormal development of colonic epithelial cells, the expression of certain cell surface or secreted carbohydrate conjugates may be altered [20,21]. Thus, differentiation markers also may prove useful in chemoprevention clinical trials. Nagle discusses some of the possible differentiation markers in the prostate (*e.g.*, decreased expression of vimentin and increased expression of cytokeratins) elsewhere in this volume.

The accumulation of genetic changes within a single cell has been theorized to be responsible, at least in part, for the development of cancer. The importance of genetic instability is illustrated by the induction of mutations and

Table I. Examples of Prostatic Intermediate Endpoint Biomarkers by Class

Histological and Premalignant Lesions	
	Prostatic Intraepithelial Neoplasia (PIN)
	Atypical Adenomatous Hyperplasia (AAH)
Proliferation	
	Increased S-Phase Fraction
	Elevation of TGF-β
Differentiation	
	Loss of High Molecular Weight Cytokeratins (50–64 kDa)
	Altered Blood Group-Related Antigen Expression (*e.g.*, H Antigen)
	Decreased Expression of Vimentin
Genetic	
	Nucleolar Prominence
	DNA Content (*e.g.*, Aneuploidy)
	Loss of Heterozygosity (*e.g.*, Chromosome 10q)
	Oncogenes and Tumor Suppressors (*e.g.*, p53)
Biochemical	
	Increased Secretion of Prostate Specific Antigen (PSA)
	Expression of Type IV Collagenase

chromosomal aberrations by most carcinogens, the detection of karyotypic variation in many solid tumors, and higher incidence of cancer in individuals with decreased DNA repair syndromes. Gross genetic changes include alterations in cellular DNA content (aneuploidy, DNA index), nuclear aberrations, and altered patterns of gene expression. In these proceedings, Lieber and Montironi discuss aneuploidy in prostatic neoplasia.

Biochemical markers such as increased levels of enzymes and other proteins have also been associated with early stages of carcinogenesis. Examples in the prostate include prostate specific antigen (PSA) which is reviewed in these proceedings by Oesterling and type IV collagenase which is addressed by Nagle.

Once potential intermediate biomarkers are identified, it is important to establish criteria for selecting those to be used in clinical trials. Some of the major considerations are as follows [17]: "Is the marker differentially expressed in normal and high-risk tissue?" "Can the marker be modulated by chemopreventive agents?" "At what stage of carcinogenesis does it appear?" "Does the assay for the marker provide acceptable sensitivity, specificity, and accuracy?" "How easily can the marker be measured?" "Can it be

Table II. Types of Biomarkers in Cancer

Cancer Marker	Description
Intermediate Endpoints	Biological alterations in tissue between initiation and tumor development. Includes premalignant lesions, histological changes, cell proliferation markers, cell differentiation markers, and genetic alterations leading to cancers.
Risk Factors (Markers)	Lifestyle factors, disease states, genetic predisposition, previous primary tumor.
Exposure Markers	A subset of risk factors. Includes measures of carcinogen exposure such as carcinogen-DNA adduct formation.
Drug Effect Markers	Effects produced by a drug which may or may not be directly related to carcinogenesis. An example is prostaglandin synthesis inhibition.
Tumor Markers	Traditional markers of cancer. These markers appear in frank cancers.

[Adapted from 17]

Table III. Chemoprevention Trials (Phase II) Protocol Strategy

Phase IIa	Phase IIb
Few Subjects, Non-randomized	Randomized, Blinded Trial
Short-term Trial (3–6 Months)	2 Arms, 50–60 Subjects Each Group
	Use Dose Established in Phase IIa
Endpoint: Determine Measurable Biological Effect	**Endpoint:** Determine Measurable Biological Effect, Agent vs. Placebo
If Biological Effect Is Noted, Dose/Response Study Can Be Initiated	
Endpoint: Determine Minimum Dose, Maximum Safety With Measurable Biological Effect	

obtained by non-invasive techniques?" "Is it technically difficult to measure?" For most organs, it is hard to find many markers that fill some or all of these criteria. This lack of validated markers obviously means that more development is needed. It also suggests that batteries of markers probably will be used until more are validated. Ideally, modulatable biomarkers for chemoprevention should occur as early in carcinogenesis as possible. Paradoxically, the earlier in carcinogenesis that the marker is measured, the less predictive value the marker is likely to have. This suggests that histologic precancer must serve, at least initially, as the gold standard for validation of the other markers.

Despite these current limitations in the use of intermediate biomarkers, the NCI is using some (*e.g.*, oral leukoplakia, squamous metaplasia of the lung, etc.) in Phase II trials, and anticipating many more such trials in the next few years. The typical Phase II protocol outlined in Table III is very familiar. Phase IIa is a dose-finding study. Using information from previous

efficacy and toxicity studies, the lowest dose that demonstrates significant modulation of the marker is selected. This dose is then used in Phase IIb, a randomized blinded trial. Usually, a relatively small number of subjects participate in these trials (50–60 per study group). More subjects may be used if they are required for reliable evaluation of the effects on the intermediate biomarker.

CHEMOPREVENTION PROGRESS AND APPROACHES IN PROSTATE CANCER

The NCI has several ongoing preclinical efficacy studies in rat prostate models. Seven agents are on test: all-trans-*N*-(4-hydroxyphenyl)retinamide (4-HPR), DFMO, dehydroepiandrosterone (DHEA), liarozole, lovastatin, oltipraz, and finasteride (Proscar®). These agents are expected to show the highest promise in the clinic; the results will provide information on possible mechanisms of chemopreventive activity in the prostate. Kadmon, Geller, Gormley, Chung, and Isaacs address various mechanisms in detail in this proceedings. NCI is planning additional studies, both in animal models and in the clinic.

Designing clinical chemoprevention trials for the prostate presents several unique problems. First, the lengthy natural history of prostate cancer increases the duration and cost of clinical efficacy trials over that of other tissues. Participants must receive the chemopreventive agent and be monitored for appearance of cancer over a longer period. In addition, the latent stage of prostate cancer may require a transformation step or proliferative stimulus before becoming clinically apparent [22]. Although this may represent a further opportunity for intervention with drugs, it may also increase the time period necessary for clinical trials. With this in mind, the value of intermediate markers in clinical efficacy trials becomes even more apparent.

Second, selection of a study population is problematic. Use of an older population with attendant higher risk for prostate cancer also entails a higher attrition rate due to death from other causes, thus increasing the number of participants necessary to begin the trial. In addition, the high incidence of microscopic prostatic cancer in older populations as discussed previously further complicates study design. Placement of cancer-free participants into the study group cannot be done with assurance using present detection methods. Identification of an alternate study population, such as men at high risk of cancer development due to the presence of intermediate biomarkers (*e.g.*, PIN), is hindered by the same detection problem. This also relates to a third problem: tracking the tissue response to the chemopreventive agent during the trial. Sampling by repeated biopsy may miss tissue of interest, and may also introduce problems of compliance in a "healthy" population. Transurethral ultrasound (TRUS), digital rectal exam (DRE) and PSA have their own shortcomings, although both Lee and Oesterling have suggested refinements to increase sensitivity (in this volume).

Finally, the alternate modalities available today for clinical chemopreventive intervention are limited. Luteinizing hormone releasing hormone antagonists, which produce androgen blockade, have side effects such as loss of libido and gynecomastia which would not be acceptable in a healthy population. Other hormonal interventions, such as androgen receptor blockers, also have unacceptable side effects and may eventually select for a cell population which has escaped hormonal control. Proscar, a 5α-reductase inhibitor, is discussed by Gormley and Geller as an alternative since it interferes with metabolism of testosterone by the prostate and avoids the problems of androgen ablation.

A strategy under consideration by the NCI which takes into account some of these problems is a chemoprevention trial evaluating Proscar. This study plans to accrue 18,000 to 20,000 men of $\geq$50 years of age (although age $\geq$60 years is a possibility); this large sample size allows for the high death rate in this age group. Following evidence of a normal prostate (including benign prostatic hyperplasia) by DRE and a serum PSA below 4 ng/ml (as measured by Tandem, Hybritech), the men will be randomized to a placebo or Proscar (5 mg/day) group for the 10 year duration of the study. The outcome will be a comparison of prostatic adenocarcinoma incidence and mortality between the two groups.

Another strategy which provides the potential for more timely progress is an ongoing approach presented by Fair. This strategy involves a

clinical trial population which consists of patients (B1–B2 lesions, primarily) who are scheduled for radical prostatectomy. A baseline biopsy is performed prior to a 3–4 month drug intervention protocol. Any of a number of promising chemopreventive agents could be examined by this protocol. Evaluation endpoints include a decrease in the clinical lesion size and/or in the incidence of microscopic adenocarcinomas as compared with an untreated patient population. In addition, intermediate markers in normal-appearing tissue, which is by definition at high risk of transformation due to the presence of cancer, could be evaluated in the biopsies and at prostatectomy for chemopreventive agent effect.

Samuel Broder, director of NCI, has stated that the Institute has targeted prostate cancer research as a high priority in 1992 [23]. As noted above, the subject of this workshop is to clarify what is possible for chemical intervention at the premalignant and early malignant phases of carcinogenesis in the prostate. From NCI's perspective, the goal is to use this information to identify relevant strategies for chemopreventive drug development.

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