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Development of Chemopreventive Agents for Bladder Cancer

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Abstract The term *cancer chemoprevention* refers to the prevention or prolongation of carcinogenesis by intervention with drugs prior to the malignant (*i.e.*, invasive) stage. The development of chemopreventive drugs is the major objective of the Chemoprevention Branch of the National Cancer Institute. Neoplastic lesions of the urinary bladder present a unique opportunity for evaluating chemopreventive agents because of (1) the accessibility of the lesions to observation and biopsy, and (2) those patients who have been successfully treated for a primary lesion represent a population at unusually high risk for recurrence and/or progression.

Although 70–80% of bladder cancers initially present as superficial, papillary transitional cell neoplasms with limited potential for invasion, the incidence of recurrence is high after resection (60–75%). Recurrent tumors are highly unpredictable, and may be of higher grade or stage (progression). Although recurrence is responsible for high treatment-related morbidity, progression represents the greatest potential for mortality. Thus, potential chemopreventive agents *considered* here would modulate bladder carcinogenesis from initiation of normal-appearing tissue through progression of superficial tumors.

Clinical trials of chemopreventive drugs involve healthy target populations, and the endpoints are reduced cancer incidence or mortality, reduced/eliminated precancerous lesions or increased latency, with none to minimal toxicity. Since cancers may not appear for 20–30 years, two of the most difficult aspects of testing these drugs in intervention trials are the long observation periods and large study populations required to measure cancer incidence reduction. However, observing the regression or recurrence of superficial bladder lesions (TIS, T1, Ta) requires relatively short time periods. Thus, these lesions lend themselves to the investigation of *intermediate biomarkers*, defined as morphologic and/or molecular alterations in tissue between initiation and tumor invasion. It is hypothesized that modulation of one or more biomarkers would interrupt carcinogenesis and result in a decrease in cancer incidence. Thus, evaluation of biomarkers as surrogate endpoints would allow bladder trials to be of even shorter duration, use fewer subjects and be lower in cost. In addition, intermediate biomarkers could predict which superficial lesions (or normal-appearing tissue) have the greatest potential for neoplastic progression. Development of strategies for the design of intervention trials for bladder cancer and review of the current status of intermediate biomarkers in the bladder, and methods for their validation, are major objectives of this workshop.

Key words: bladder cancer, chemoprevention, intermediate biomarkers, intermediate endpoint biomarkers, surrogate endpoints

The development of cancer chemopreventive drugs is the major objective of the Chemoprevention Branch of the National Cancer Institute (NCI). The term *cancer chemoprevention* refers to prevention or prolongation of carcinogenesis by intervention with drugs prior to the malignant (*i.e.*, invasive) stage of carcinogenesis [1]. It is important to understand the conceptual differences between the development of drugs for cancer chemoprevention and those for cancer treatment. For cancer treatment, the goal is to kill cancer cells or to increase survival in cancer patients. In chemoprevention, the goal is reduced cancer incidence or mortality, or increased latency in a healthy population,

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although this may be a population at high risk for a specific cancer. Since cancer may not develop for up to 20–30 years and at a relatively low incidence in healthy subjects, clinical chemoprevention trials require long observation periods and large study populations. A major objective of this workshop is the development of strategies for design of intervention trials which circumvent this problem.

Bladder cancer is expected to be the fourth most prevalent cancer in males in 1992, with 38,500 new cases estimated for the year [2]. Of diagnosed bladder cancers which are histologically confirmed (98%), 93% are transitional cell carcinomas (TCC) [3]. The majority of these (70–80%) initially present as superficial, papillary TCC which have a limited potential for invasion [4,5,Farrow, this proceedings]; however, the risk of distant recurrence within 2–5 years is high (60–75%) following treatment [5,6]. Although recurrence of the same type of lesion is responsible for high treatment-related morbidity, these lesions are highly unpredictable and may recur at a higher grade or stage (*i.e.*, progression). Possible progression following an initial presentation of a superficial tumor represents the greatest potential for mortality and provides a unique opportunity for intervention. The development of potential chemopreventive drugs as considered in this workshop has been expanded to include modulation of bladder carcinogenesis from initiation of normal-appearing tissue through progression of superficial tumors.

Thus, bladder carcinogenesis provides a unique opportunity for investigating the efficacy of potential chemopreventive drugs for three reasons. First, the tissue is relatively accessible to observation and biopsy; second, those patients who have been successfully treated for a primary lesion represent a population at unusually high risk for recurrence and/or progression; and, third, observing this population for recurrence and/or progression requires a relatively short time period (2–5 years).

CHEMOPREVENTIVE DRUG DEVELOPMENT AT NCI

The Drug Development Program at the Chemoprevention Branch, NCI, has been described previously [7,8] and is outlined in Figure 1. Briefly, the process begins with the identification of potential drugs (pharmaceuticals, natural products or minor dietary constituents) from surveillance and analysis of the literature and from the NCI Testing Program. Data on both efficacy (*i.e.*, biological activities that either

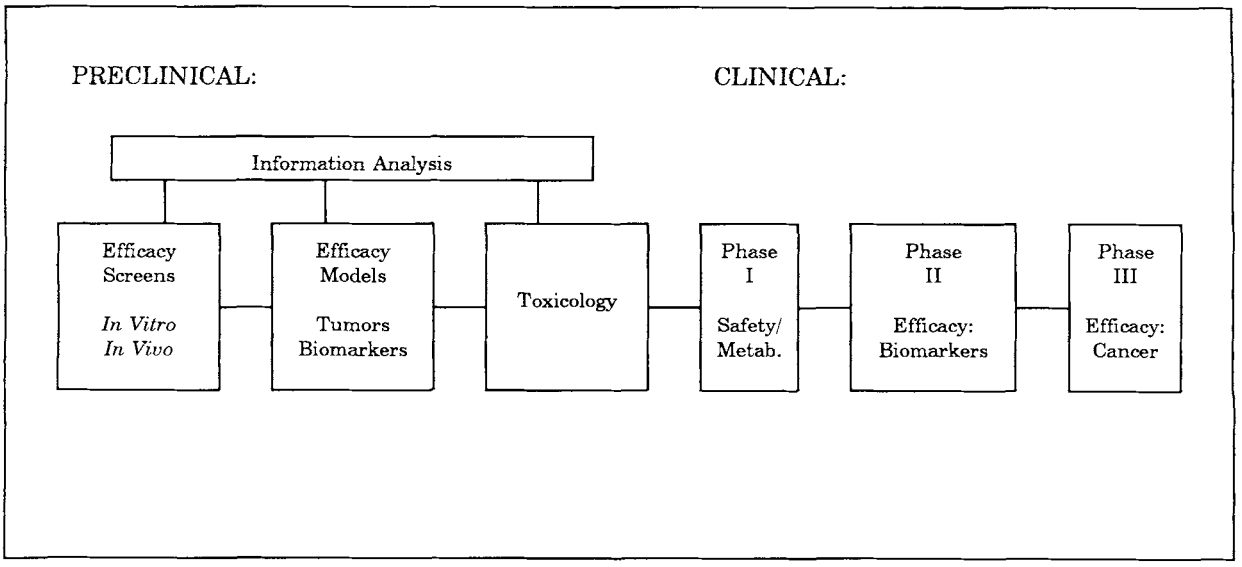

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Fig. 1. Chemopreventive agent drug development strategy.

directly or indirectly indicate inhibition of carcinogenesis) and toxicity are gathered from both sources.

In the NCI Preclinical Testing Program, a battery of *in vitro* screens using human and animal cells is used to select promising agents for *in vivo* testing (see Tables I and II). A panel of animal screening assays which are target organ-specific are used to assess efficacy *in vivo*, *e.g.*, the *N*-butyl-*N*-(4-hydroxybutyl)nitrosamine (OH-BBN)-induced mouse bladder TCC model (Dr. Moon, these proceedings). Traditional preclinical toxicity tests are also performed in two species, especially if the agent is not a pharmaceutical. The science and rationale for all of the systems used in the Chemoprevention Branch program have been described previously [9,10]. Although the information flow appears to be linear, an empirical approach may also be used which involves gathering efficacy and toxicity data in accepted models and protocols where available, while continuing to be aware of mechanistic data.

After qualifying for the clinical phase of testing, potential chemopreventive drugs enter Phase I, II, and III trials. Phase I trials are designed to determine human dose-related safety, pharmacokinetics, and metabolism of the drug. Both Phase II and III trials are for determination of cancer chemopreventive efficacy. Phase II trials are small scale, placebo-controlled studies which may include modulation of intermediate biomarkers as study endpoints, as discussed below. Phase III trials involve a large target population, with cancer incidence reduction as the endpoint.

The drug development effort at NCI has been in progress for about 6 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 [11,12].

IMPORTANCE OF INTERMEDIATE BIOMARKERS IN CLINICAL CHEMOPREVENTION TRIALS

For chemopreventive 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 biomarkers for evaluating clinical efficacy. *Intermediate biomarkers* are biological alterations in tissue occurring between initiation and tumor invasion. It is hypothesized that modulation of one or more intermediate biomarkers by a chemopreventive agent(s) would interrupt carcinogenesis. Validation of a biomarker as an intermediate endpoint would 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 bladder 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-related, differentiation-related, genetic, or biochemical. This classification scheme has been applied to biomarkers in various tissues such as colon [7] and prostate [8]. Table III is a representative listing of potential intermediate biomarkers in the bladder classified in this manner. However, it should be noted that the terms "biomarker" and "marker" in the cancer literature can refer to several concepts, which should be distinguished from that of intermediate biomarkers. These appear in Table IV and have been discussed previously [7].

In chemopreventive drug development strategy, histological precancerous lesions are an important starting point. As described recently

TABLE I. Chemopreventive Drug Development Progress: DFMO

In Vitro Screens: A427 (+) JB6 (+) RTE (NE) MMOC (+)

In Vivo Screens:

Hamster Trachea (MNU)	NE
Rat Mammary Glands (DMBA)	+
Mouse Bladder (OH-BBN)	+
Mouse Colon (MAM Acetate)	NE
Hamster Pancreas (BOP)	OT

Efficacy Models (Single Agent):

Rat Colon (AOM)	+
Hamster Lung (DEN)	NE
Rat Mammary Glands (MNU)	+
Mouse Bladder (OH-BBN)	+
Mouse Skin (BP)	OT
Rat Prostate (MNU, Testosterone Propionate)	OT

Efficacy Models (Agent Combinations):

Rat Colon (+ *Piroxicam*)	+S
Hamster Lung (DEN) (+ *β-Carotene*)	+A
Mouse Bladder (+ *Oltipraz*)	+S

Preclinical Toxicology:

Dog 1-Year	Complete
Rat 1-Year	Complete

Clinical Trials:

Phase I	Complete
Phase II	In preparation

Additional Tasks Complete:

Drug Availability
Formulation
Stability Studies
Pharmacokinetics
Clinical Chemistry (Assay for Serum Levels)

Screens: A427, Human lung tumor A427 cell line; JB6, Mouse epidermal cells (TPA); RTE, Rat tracheal epithelial cells (BP); MMOC, Mouse mammary organ culture (DMBA). **Results**: +, significant tumor inhibition ($p \leq 0.05$); +A, additive effect; +S, synergistic effect; NE, no effect; OT, on test.
Chemicals: AOM, azoxymethane; BP, benzo(*a*)pyrene; BOP, *N*-nitrosobis(2-oxopropyl)amine; DEN, diethylnitrosamine; DFMO, α-difluoromethylornithine; DMBA, 7,12-dimethylbenz(*a*)anthracene; MAM Acetate, methylazoxymethanol acetate; MNU, *N*-methyl-*N*-nitrosourea; OH-BBN, *N*-butyl-*N*-(4-hydroxybutyl)nitrosamine; TPA, 12-*O*-tetradecanoyl-phorbol-13-acetate.

TABLE II. Chemopreventive Drug Development Progress: Oltipraz

In Vitro Screens: A427 (+) JB6 (NE) RTE (+) MMOC (+)

In Vivo Screens:

Hamster Trachea (MNU)	+
Rat Mammary Glands (DMBA)	+
Mouse Bladder (OH-BBN)	+
Mouse Colon (MAM Acetate)	+

Efficacy Models (Single Agent):

Rat Colon (AOM)	+
Hamster Lung (DEN)	+
Rat Mammary Glands (MNU)	+
Mouse Skin (BP)	+
Rat Prostate (MNU, Testosterone Propionate)	OT
Hamster Pancreas (BOP)	OT

Efficacy Models (Agent Combinations):

Rat Colon (+ *DFMO*)	+S
Hamster Lung (DEN) (+ *β-Carotene*)	+S
Hamster Lung (DEN) (+ *4-HPR*)	+S
Mouse Bladder (+ *DFMO*)	+S

Preclinical Toxicology:

Dog 1-Year	Complete
Rat 1-Year	Complete

Clinical Trials:

Phase I	OT

Additional Tasks Complete:

Drug Availability
Formulation
Stability Studies
Pharmacokinetics
Clinical Chemistry (Assay for Serum Levels)

Screens: A427, Human lung tumor A427 cell line; JB6, Mouse epidermal cells (TPA); RTE, Rat tracheal epithelial cells (BP); MMOC, Mouse mammary organ culture (DMBA).
Results: +, significant tumor inhibition ($p \leq 0.05$); +A, additive effect; +S, synergistic effect; NE, no effect; OT, on test.
Chemicals: AOM, azoxymethane; BP, benzo(*a*)pyrene; BOP, *N*-nitrosobis(2-oxopropyl)amine; DEN, diethylnitrosamine; DFMO, α-difluoromethylornithine; DMBA, 7,12-dimethylbenz(*a*) anthracene; MAM Acetate, methylazoxymethanol acetate; MNU, *N*-methyl-*N*-nitrosourea; OH-BBN, *N*-butyl-*N*-(4-hydroxybutyl)nitrosamine; TPA, 12-*O*-tetradecanoyl-phorbol-13-acetate.

TABLE III. Examples of Potential Intermediate Biomarkers in the Bladder by Class

Histological and Premalignant Lesions
Transitional Cell Carcinoma *in Situ* (TIS) (a non-papillary lesion)
Transitional Cell Carcinoma, Stage Ta (a papillary lesion)
Proliferation-Related
EGF-R Distributed to Superficial Cell Layers
Differentiation-Related
Altered Blood Group-Related Antigens (*e.g.*, Increased Lewis^X)
Altered Cytoskeletal Proteins (*e.g.*, Increased G-Actin Expression)
Altered Cell Surface Protein Expression (*e.g.*, Integrins)
Tumor-Associated Antigens, Early Expression (*e.g.*, M344)
Genetic
Nuclear Morphometry
DNA Content (*e.g.*, Aneuploidy, DNA Index)
Loss of Heterozygosity (*e.g.*, Chromosome 9q)
Activated Proto-Oncogenes and Inactivated Tumor Suppressors (*e.g.*, Rb Gene Inactivation)

[13], 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. In the bladder, a possible histological biomarker is transitional cell carcinoma *in situ* (TIS). Drs. Koss and Farrow in these proceedings discuss this flat, often high grade lesion and its significance in bladder carcinogenesis.

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 latency period. For example, proliferation-related markers appear to be very important in the colon [14], but the slow growth rate in bladder neoplasia may limit the use of some proliferation markers in this organ. However, a shift in expression of the epidermal growth factor receptor (EGF-R) and altered excretion of EGF may precede overt evidence of TCC, as discussed by Drs. Messing and Reznikoff in this volume.

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

TABLE IV. Use of the Term "Marker" in Cancer Literature

Cancer Marker	Description
Intermediate Endpoint Biomarkers	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.

[Adapted from 7]

premalignant states. For example, during abnormal development of colonic epithelial cells, the expression of certain cell surface or secreted carbohydrate conjugates may be altered [15,16]. In the bladder, altered expression of Lewis^X blood group antigens and integrins on cell surfaces has been reported by Drs. Sheinfeld and Grossman (this volume). In addition, Dr. Hemstreet discusses cytoskeletal components, F- and G-actin, as possible intermediate biomarkers in the bladder (this volume).

The accumulation of genetic changes within a single cell has been proposed to be responsible, at least in part, for the development of cancer [17]. The importance of genetic instability is illustrated by the induction of mutations and chromosomal aberrations by most carcinogens [18], the detection of karyotypic variation in many solid tumors [19], and the higher incidence of cancer in individuals with compromised DNA repair [20]. Gross genetic changes which may be useful intermediate biomarkers include alterations in cellular DNA content (aneuploidy, DNA index), nuclear aberrations, and altered patterns of gene expression. In these proceedings, Dr. deVere White discusses aneuploidy in superficial TCC, and Dr. Sandberg outlines the chromosomal abnormalities seen in premalignant and early malignant neoplasms. Other changes, such as mutations, may take place at the gene level. Dr. Benedict addresses the role that the loss of function of specific genes may have in bladder carcinogenesis, *i.e.*, retinoblastoma gene (Rb).

Biochemical markers such as increased levels of enzymes and other proteins have also been associated with early stages of carcinogenesis. An obvious example is the increase in serum levels of prostate specific antigen (PSA) in the presence of prostatic intraepithelial neoplasia. Although no human bladder markers of this class are discussed in these proceedings, foci of increased nonspecific esterase activity and decreased alkaline phosphatase activity that precede TCC in the OH-BBN-induced rat model of bladder cancer have been described previously [21,22].

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 [7]: 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 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 will probably 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 lesions must serve, at least initially, as the gold standard for validation of the other markers.

To further develop intermediate biomarkers, the NCI is using some as surrogate endpoints in both preclinical models and Phase II trials (*e.g.*, oral leukoplakia, squamous metaplasia of the lung, etc.), and anticipating many more such trials in the next few years. Four agents [aspirin, α-difluoromethylornithine (DFMO), *N*-(4-hydroxyphenyl)retinamide (4-HPR), and piroxicam] will be evaluated this year as modulators of dysplasia and other intermediate biomarkers in bladders of OH-BBN-treated female Fischer 344 rats. Two Phase II trials are planned for DFMO and 4-HPR as modulators of biomarkers in patients with resected superficial bladder cancer. It is hoped that modulation of intermediate biomarkers will correlate with decreased cancer incidence and, thus, validate their use as surrogate endpoints in future chemoprevention trials.

DRUGS UNDER DEVELOPMENT FOR CHEMOPREVENTION OF BLADDER CANCER

In the NCI Testing Program, nine agents have been effective against OH-BBN-induced bladder cancer in male BDF mice. Two of these drugs, DFMO and oltipraz, have made significant progress into clinical trials and their status will be briefly reviewed here. Drs. Loprinzi and Kensler will discuss DFMO and oltipraz, respectively, in more depth in this volume.

DFMO is an irreversible inhibitor of ornithine decarboxylase (ODC), the rate-limiting enzyme in the biosynthesis of polyamines (putrescine, spermine, spermidine). It has been demonstrated that polyamine concentrations are highly regulated [reviewed in 23] and that polyamines are capable of noncovalent interaction with macromolecules such as nucleic acids and proteins [24]. This suggests that polyamines play a role in normal cell proliferation and differentiation. In neoplastic tissues, polyamine levels are high and associated with increased cell proliferation [23–25]. DFMO is believed to act as a chemopreventive by interference with polyamine biosynthesis in the post-initiation stages of carcinogenesis [24,26], although one study suggests action at the initiation stage in OH-BBN-induced male mouse bladder carcinogenesis [27]. The results of preclinical testing of this agent in the NCI program are summarized in Table I. DFMO appears to have broad chemopreventive efficacy, which includes rat bladder [28], mammary glands and colon, and mouse bladder and skin. The agent is currently on test in hamster pancreas and rat prostate assays. In the clinic, two Phase I trials have been completed and doses for intervention trials have been suggested. Phase II clinical trials measuring tumor recurrence in patients with previously resected superficial bladder cancer are in the initial stages.

Oltipraz is a dithiolthione which has been used pharmaceutically to treat schistosomiasis. The consumption of cruciferous vegetables (cauliflower, Brussels sprouts, cabbage) which contain structurally similar dithiolthiones has been associated with decreased cancer risk in both humans and experimental animals [29,30]. As shown in Table II, oltipraz was effective in the NCI Preclinical Testing Program against tumors of the mouse bladder as well as hamster trachea and lung, mouse skin, and rat mammary glands and colon. This agent appears to exert chemopreventive activity during the initiation phase of carcinogenesis by enhancing enzyme activities that catalyze electrophilic detoxication and maintain reduced glutathione (GSH) pools [31,32]. At high dietary levels, oltipraz elevated the activities of GSH-*S*-transferases, UDP:glucuronyltransferase, GSSG reductase and cytochromes P-450. The overall result is enhanced inactivation and subsequent elimination of chemical carcinogens. In addition, there are data from animal studies indicating that the agent may also affect promotion and progression (R. Moon, personal communication). Due to its extensive efficacy and low toxicity in animals and lack of significant side effects in humans, oltipraz is on test in Phase I clinical trials.

TABLE V. Chemoprevention Branch Preclinical Testing Program
Summary of Progress: Listing by Agent
(Covering Contractors' Reports Received by December 15, 1992)

A. *In Vitro* and *in Vivo* Efficacy Tests — Results

Agent	*IN VITRO*				*IN VIVO*										
					Lung		Colon			Mammary					
	A427	JB6	RTE	MMOC	DEN	MNU	Crypts	Mouse	Rat	DMBA	MNU	Blad	Pros	Panc	Skin
N-Acetyl-*l*-cysteine	NE	NE	+	+P		+	(NE)		+		+	+			
Bismuthiol I	NE	NE	+	NE	+		(NE)				NE	+			
Ibuprofen	+	+	NE	NE		NE	(+)		+		(+)	+			
Indomethacin	NE	+	NE	+	NE		(+)	(NE)			NE	+			
2-Mercaptoethane-sulfonate, Sodium Salt	NE	NE	+	NE			(NE)	NE			NE	+			
Molybdate, Sodium	+	NE	NE	+		NE	(NE)	NE			(+)	+			NE
Piroxicam	+	(NE)	NE	NE	NE		(+)		+		(+)	+			+

Assay Class (*in vitro*): **A427**, Human lung tumor A427 cells; **JB6**, Mouse JB6 epidermal cells; **RTE**, Rat tracheal epithelial cell focus assay; **MMOC**, Mouse mammary gland organ culture assay.
Assay Class (*in vivo*): Lung/DEN, Hamster lung (DEN-induced); **Lung/MNU**, Hamster lung/trachea (MNU); **Colon/Crypts**, Foci of aberrant crypts in rat colon; **Colon/Mouse**, Mouse colon (MAM); **Colon/Rat**, Rat colon (AOM); **Mammary/DMBA**, Rat mammary glands (DMBA); **Mammary/MNU**, Rat mammary glands (MNU); **Blad**, Mouse bladder (OH-BBN); **Pros**, Rat prostate (MNU/hormone); **Panc**, Hamster pancreas (BOP); **Skin**, Mouse skin (DMBA).
Chemical Agents: AOM, azoxymethane; BOP, *N*-nitrosobis(2-oxopropyl)amine; DEN, diethylnitrosamine; DMBA, 7,12-dimethylbenz(*a*) anthracene; MAM, methylazoxymethanol; MNU, *N*-methyl-*N*-nitrosourea; NNK, *N*-nitrosonornicotine; OH-BBN, *N*-butyl-*N*-(4-hydroxybutyl)nitrosamine.
Test Results: +, Chemopreventive activity observed; (+), Chemopreventive activity observed, preliminary result; **+P**, MMOC assay, agent positive during promotion (days 9–24); **NE**, No chemopreventive effect observed; (**NE**), No chemopreventive effect observed, preliminary result.

To date, seven additional agents have inhibited OH-BBN-induced mouse bladder cancer in the NCI Preclinical Testing Program. Three of these agents are nonsteroidal antiinflammatory drugs (NSAIDs): ibuprofen (Motrin®, Nuprin®, Advil®), indomethacin (Indocin) and piroxicam (Feldane®). It has been proposed that the chemopreventive activity of this class may be mediated primarily by inhibition of prostaglandin synthesis; prostaglandins play a role in the control of neoplastic and non-neoplastic cell proliferation (reviewed by Earnest in this volume). In addition, ibuprofen is a free radical scavenger, and indomethacin and piroxicam block induction of ODC activity. All of these activities could contribute to chemopreventive efficacy of NSAIDs during the promotion phase of carcinogenesis. The chemopreventive efficacy of these agents in the NCI Preclinical Testing Program is summarized in Table V.

Three agents categorized generally as thiols have also inhibited mouse bladder cancer: *N*-acetyl-*l*-cysteine, mesna (2-mercaptoethane sulfonate, monosodium salt) and bismuthiol I. *N*-acetyl-*l*-cysteine (Mucomyst®, Parvolex®), a derivative of *l*-cysteine, is a pharmaceutical used as a mucolytic agent and as an antidote against acetaminophen poisoning and the adverse effects of chemotherapeutic agents; it is regarded as safe and without serious side effects. This agent is postulated to inhibit carcinogenesis by several mechanisms, including direct deactivation of electrophilic carcinogens [33], induction of detoxifying enzymes (glutathione-*S*-transferases [34,35], glutathione peroxidases [36], NAD(P)H-quinone reductase [37]), inhibition of ODC activity [36], and scavenging of free radicals [38]. The chemopreventive efficacy of *N*-acetyl-*l*-cysteine has been demonstrated in rat colon, intestines and mammary glands, mouse lung, and hamster lung, as well as mouse bladder (Table V). Mesna is also used therapeutically as a mucolytic [39] and appears to have relatively little toxicity [40]. It is active against cyclophosphamide-induced rat bladder tumors [41] and OH-BBN-induced mouse bladder tumors (see Table V). Finally, bismuthiol I inhibits lipid peroxidation [42] and may be effective during the promotion phase of carcinogenesis. In the NCI Preclinical Testing Program, this agent inhibited both hamster lung and mouse bladder tumors (Table V).

The last agent, molybdenum (sodium salt), is presumed to be an essential trace metal found in several enzymes, including xanthine oxidase (reviewed in 43,44). An epidemiological association between esophageal cancer and low levels of dietary or tissue molybdenum suggested chemopreventive activity [45,46]. In preclinical studies, this agent has been effective primarily against nitrosamine-induced cancers, such as OH-BBN-induced mouse bladder tumors. No specificity for a particular stage of carcinogenesis has been noted.

Currently, sixteen additional agents are on test in the NCI Preclinical Testing Program's OH-BBN-induced mouse bladder system. Although only one of these agents is a retinoid, many of this class have been previously shown in the literature to be effective in animal models of bladder carcinogenesis [*e.g.*, 47,48]. Based on this information, several clinical studies of retinoids are also discussed in this volume. Dr. Decensi discusses a clinical trial with 4-HPR that will begin soon in Italy, and Dr. Prout describes a pilot study of 13-*cis*-retinoic acid conducted by the National Bladder Cancer Group.

CONCLUSION

The objective of this workshop is to clarify what is possible for chemical intervention at the premalignant and early malignant phases of carcinogenesis in the bladder. As mentioned previously, two of the most difficult aspects of testing chemopreventive drugs in intervention trials are the long observation periods and large study populations required to reach an endpoint of cancer incidence reduction. The advantages of the bladder as a target organ in chemoprevention studies include the availability of a high risk population of patients with resected bladder cancer, the relatively short time period for recurrence and/or progression of superficial bladder lesions (TIS, T1, Ta), and the relative accessibility of the organ to observation and biopsy. However, the design of chemoprevention bladder trials also involves several problems, including undependable methods for detecting flat lesions (TIS; which tend to be more invasive and lethal), and the lack of indicators which predict tumor recurrence and/or progression. Obviously, work needs to be done on identifying

intermediate biomarkers to distinguish tissue at high risk of recurrence or progression. Review of the current status of intermediate biomarkers in the bladder, and methods for their validation, are major objectives of this workshop. The goal for this workshop is to use the information gained from deliberations by the experts present to develop relevant strategies for chemopreventive drug development.

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