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Cancer Chemoprevention: Progress and Promise

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Gary J. Kelloff has been at the National Cancer Institute since 1968. His early research focused on oncogenes, particularly on oncogenic retroviruses (serving as Chief of the Viral Immunology Section for many years). Since 1983, he has been directing cancer chemoprevention research as Chief of the Chemoprevention Branch in the Division of Cancer Prevention. His primary focus has been designing and managing chemoprevention research and drug development programmes, encompassing all aspects from drug discovery through clinical trials.

Cancer chemoprevention is the use of agents to inhibit, delay or reverse carcinogenesis. The focus of chemoprevention research in the next millennium will include defining the genotypic and phenotypic (functional and histological) changes during carcinogenesis, the cancer risk conferred by these changes, their modulation in preclinical experimentation and randomised clinical trials by chemopreventive drugs, dietary agents and regimens and treatments resulting from early detection. The key elements of this research effort will be basic and translational risk evaluation programmes; chemopreventive and dietary agent drug discovery and development; development of transgenic animal models; required safety and pharmacology studies; well-designed phase I, II and III chemoprevention studies; and much expanded early detection programmes. The large number of chemoprevention research programmes now ongoing ensures that the promise of chemoprevention will continue to be realised in the next decade.

Key words: chemoprevention, surrogate endpoints, risk evaluation, molecular carcinogenesis, intraepithelial neoplasia
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INTRODUCTION

CARCINOGENESIS HAS been and will continue to be the subject of intense experimental, epidemiological and clinical research on its molecular, cellular, tissue and clinical aspects. Chemoprevention is the use of agents to inhibit, delay or reverse this process. Over the past decade, advances in understanding carcinogenesis have made possible the identification of candidate chemoprevention drugs that are being developed to hit key molecular targets [1–4]. Carcinogenesis at the

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cellular and tissue level is characterised by accelerating mutagenesis and proliferation, and drug development strategies involving modulation of these activities are also proving successful. In the next decade, exponential gains are anticipated which will allow even better definition of the mechanisms of carcinogenesis, more precise early detection and estimates of cancer risk, and quantitative histopathological evaluation of precancerous tissues. All these technologies will contribute to the major aspects of cancer chemoprevention: (1) discovery and characterisation of chemopreventive agents; (2) evaluation and validation of carcinogenesis biomarkers as surrogate endpoints for cancer incidence that are useful for evaluating chemopreventive efficacy; and (3) definition of individual, as well as population-based cancer risks, for selecting cohorts benefiting from chemopreventive strategies and suitable for evaluating these strategies.

The rapid sequencing of the human genome, estimated to contain approximately 100 000 genes, the identification of the several hundred of these genes that are involved in carcinogenesis and, in particular, the progress in functional cancer genomics deriving from this effort may provide the most immediate contribution to advancing chemoprevention. The genes involved in carcinogenesis and their products will provide rational targets for preventive agents, and will provide an additional dimension for estimating an individual's risk. Rare genetic syndromes have already provided the experimental leads to identifying oncogenes and tumour suppressor genes. Subtler risks such as those conveyed by modifier genes are also being identified. Examples include polymorphisms in the genes that control oestrogen [5] and androgen [6] metabolism and, therefore, have a role in breast, prostate and other cancers, as well as polymorphisms of enzymes that metabolise endogenous and exogenous human carcinogens (for example, glutathione-*S*-transferases (GST)) [7].

Epithelial carcinogenesis, which accounts for more than 80% of the human cancer burden, is a multi-year (sometimes decades) process of clonal selection and evolution of genetically damaged cells, leading to the abnormal precancer phenotype that eventually becomes invasive cancer [1, 2, 4]. The genetic progression models that are continually being refined for major human cancers, for example, colon [8] and head and neck [9], show that the sequence of genetic damage is multiple choice, multiple path and by nature stochastic. Therefore, integrating genomics with tissue histomorphometry and imaging technology provides the best means of defining human risk of later cancer development, and also provides measurable parameters (biomarkers) that, when modulated by drugs, provide compelling evidence that the drug will reduce cancer incidence. Because of the shorter latency to intermediate biomarker endpoints and the smaller cohorts required for treatment, developing surrogate endpoints for cancer incidence is critical to the progress of chemoprevention and for cost-effective development of chemopreventive agents. In addition, chemoprevention studies in transgenic animal models of human carcinogenesis will allow validation of surrogate endpoints by comparing drug versus placebo modulation of these endpoints and their correlation to cancer incidence reduction.

Many classes of agents have shown promising chemopreventive activity including anti-oxidants, anti-inflammatories, anti-oestrogens and anti-androgens [4, 10]. These examples, especially the anti-oxidants and anti-inflammatories, suggest that strategies and drug classes for cancer chemoprevention are relevant to prevention of other chronic disorders of aging such as cardiovascular, neurodegenerative, and rheumatoid diseases. The recent cancer incidence reductions by tamoxifen [11] and vitamin E [12] for breast and prostate cancers, respectively, also suggest the tremendous public health impact possible from chemoprevention following the development of more effective drugs. Currently, more than 50 candidate chemoprevention drugs are under clinical development in phase II trials, and these will provide a few more high priority drugs for definitive phase III cancer incidence reduction trials while progress is made in surrogate endpoint characterisation and validation [1, 2, 4]. A few years ago, the National Cancer Institute (NCI) and Food and Drug Administration (FDA) defined a process for incremental accumulation of drug efficacy and safety information needed to secure chemopreventive drug marketing approvals ultimately based on cancer incidence reduction, but also considering biomarker surrogate endpoints [13]. As further data are accumulated, additional strategies may be documented using these surrogate endpoints of cancer incidence as a rapid basis for drug approval. It is also likely that proving chronic safety in chemopreventive settings will be more challenging than proving efficacy, since these drugs may be prescribed to large populations at relatively low absolute risk of developing cancer.

Notwithstanding progress made in the last decade and high prospects, major issues and challenges exist, in aspects of chemoprevention ranging from agent discovery and preclinical efficacy evaluation to surrogate endpoint characterisation and validation, identification of target populations based on risk, clinical trial design and public health impact. In the following review, we cite these, together with a description of the progress that has been made and the expectations for the next decade.

DISCOVERY OF CHEMOPREVENTIVE AGENTS AND TARGETS: CURRENT KNOWLEDGE AND FUTURE ADVANCES IN DEFINING EARLY EVENTS IN CARCINOGENESIS CRITICAL TO PROGRESS IN CHEMOPREVENTION

Carcinogenesis can be viewed as a process of progressive disorganisation. At the cellular level, this disorganisation is the loss of proliferation controls and is characterised by increasing aneusomy and heterogeneity. By the time cancer has developed, this heterogeneity leaves few, if any, reliable molecular targets for intervention [4]. Standard therapy's limited success in improving survival from cancer of the major epithelial target organs is probably due to this chaotic nature of cancer. For cancer therapy, the possibility remains of customising therapy to an individual once a molecular genotype is determined with a pathway that proves amenable to induction of cell death; defining such pathways is now an intense research effort in the oncology community.

The outlook for chemoprevention, where precancers are the targets, is more promising in this regard. Carcinogenesis is progressive, and this progression in precancer has been mapped by the appearance of specific molecular and more general genotypic damage associated with increasingly severe dysplastic phenotypes [1, 14, 15]. Progression models have been developed by Vogelstein, Sidransky and their colleagues, the seminal work being that by Fearon and Vogelstein in colon [8]. These researchers have also described carcinogenesis in brain [16], bladder [17–20, and Simoneau and Jones, 21] and in head and neck [9, 22]. Lam, Gazdar and

their colleagues have described early analyses of chromosomal loss correlating to dysplasia grade and appearance of non-small cell lung cancer (NSCLC) [22, 23]. In addition, Larson and colleagues described accumulating chromosomal loss at defined loci in cervical intra-epithelial neoplasia (CIN) [24]. Unlike cancer, significant portions of the signal transduction pathways are still functional and possible to control in precancer. Chemoprevention will be able to take advantage of new technology for identifying and evaluating such early molecular targets for intervention, as well as for quantitative evaluation of cancer risks and tissue- and cell-based changes in these early stages of carcinogenesis.

For example, Brown and Botstein [25] have recently reviewed the potential of functional genomics in biology—the utility ranging from analysing a mutant phenotype to subcellular localisation of gene products (that is, relative expression in specific tissues), to uncovering patterns of expression along signal transduction pathways. Genomic chip arrays being produced for analysing specific target organs number in some cases thousands of genes and the volume of data being produced will require very sophisticated data analysis to define and quantify contributors to risk. To this end, proven cases from archival specimens from properly designed tissue banks will help provide the endpoints and validation necessary. Further, the use of specimens from preclinical and clinical chemoprevention interventions where known effective drugs are compared with placebo controls may provide insights as to which single moieties or patterns in this pleiotropic process are the most important. In this regard Lippman and coworkers [26] have recently presented the results of quantitative morphometric analysis in breast, correlating morphometric changes to dysplasia severity.

Besides gene chip technologies, new cell and organ culture technologies and newer information on genetic susceptibility to cancer will be used to evaluate chemopreventive agents and targets. The primary objective is to develop models that closely mimic human carcinogenesis. For example, raft cultures, which allow evaluation of stromal–epithelial interactions, cells from transgenic mice and cells from subjects carrying known cancer-predisposing genes (for example Li–Fraumeni syndrome, APC mutations) are being explored. Currently, adequate animal models do not exist for evaluating chemopreventive efficacy in some major cancer sites—for example lung (squamous cell carcinomas), prostate, ovary, brain, pancreas and oestrogen receptor negative (ER^-) breast cancer. An important area of research in the near future will be development of animal models (transgenics or carcinogen-induced) for these cancers.

Transgenic and gene knock-out mice carrying well-characterised genetic lesions predisposing to carcinogenesis are already appropriate models for evaluating chemopreventive efficacy and determining appropriate biomarkers. For example, the multiple intestinal neoplasia *(Min)* mouse [27] and other strains carrying lesions in the *Apc* gene [28] may be the best characterised. The *Min* mouse has an *Apc* mutation qualitatively similar to that in human FAP patients, which predisposes the mice to colorectal adenomas and carcinomas. Jacoby and associates [29] have found a strong correlation between inhibiting prostaglandin synthesis and preventing adenoma formation in this strain. Also, a human papilloma virus (HPV)-infected (K14-HPV16 heterozygote), oestradiol-treated mouse develops cervical squamous carcinomas that result from the progression of CIN-like lesions [30]. These lesions can be inhibited by the antiproliferative and potential chemopreventive agent, DFMO.

Closer approximations to human carcinogenesis may be possible by manipulating two or more carcinogenesis-associated genes, including modifier genes, in a single animal. For example, it might be feasible to knock out p53 in an animal that already carries another tumour suppressor defect (for example *Apc* or p16). Studies are now being conducted on *Min* and *Apc*1638 mice also carrying genes allowing error-prone DNA repair [28]. A key contribution to future development of such animal models will be identification of specific cancer-related genes (for example, in the Cancer Genome Anatomy Project) which can be applied to the construction of animal models for evaluating chemopreventive efficacy.

The treatment of transgenic and gene knock-out mice with carcinogens may prove to be particularly effective as a strategy for modelling human carcinogenesis at specific cancer targets. For example, You and colleagues [31] are evaluating chemopreventive effects of various agents in p53 mutant and gene knock-out mice also treated with carcinogens (for example, benzo(*a*)pyrene (B(*a*)P) or *N*-nitrosonornicotine (NNK) to induce lung tumours, or dimethylhydrazine to induce colon tumours).

CHEMOPREVENTIVE AGENTS: A WIDE VARIETY OF AGENT CLASSES WITH CHEMOPREVENTIVE ACTIVITY HAVE BEEN IDENTIFIED. FUTURE RESEARCH WILL FOCUS ON AGENT STRATEGIES TO OPTIMISE THE RISK–BENEFIT PROFILE

Signal transduction modulators, for example, growth factor, receptor inhibitors (epidermal growth factor receptor (EGFR) inhibitors), oncogene inhibitors (Ras farnesylation inhibitors) and retinoids, are among the agent classes that have already shown significant chemopreventive activity [10]. Steroidal hormones are strongly implicated in breast, ovary, prostate and possibly other cancers [10]. Anti-oestrogens and aromatase inhibitors are highly promising chemopreventive agents at these targets [10]. Inflammation and oxidative damage are associated with carcinogenesis in most epithelial tissues, particularly colon, bladder, oesophagus, head and neck and lung. Agents with several different anti-inflammatory mechanisms have shown chemopreventive activity—non-steroidal anti-inflammatory drugs (NSAIDs) which inhibit cyclo-oxygenases (COX-1 and COX-2); selective COX-2 inhibitors, which retain NSAID anti-inflammatory activity with less toxicity than drugs which inhibit both COX-1 and COX-2; inducible nitric oxide synthase (iNOS) inhibitors; and lipoxygenase (LOX) inhibitors. Many anti-oxidants are dietary products, which could be suitable for use in the general population, and which appear to have preventive potential in many diseases of aging besides cancer (particularly, cardiovascular disease, arthritis and Alzheimer's disease). The anti-oxidant antimutagens have significant potential, particularly in tissues like lung and colon where there are high levels of carcinogen exposure. Inducers of enzymes involved in carcinogen detoxification (for example, GST and NAD(P)H:quinone reductase) are promising antimutagens. Tea polyphenols and the combination of selenium with vitamin E have received much attention recently. Table 1 summarises many of the cellular chemopreventive mechanisms,

Table 1. *Mechanisms for chemoprevention: possible molecular targets and promising agents**

Mechanism	Possible molecular targets	Representative agents
Antimutagenesis		
Inhibit carcinogen uptake	Bile acids (bind)	Calcium
Inhibit formation/activation of carcinogen	Cytochromes P450 (inhibit)	PEITC, tea, indole-3-carbinol, soy isoflavones
	PG synthase hydroperoxidase, 5-lipoxygenase (inhibit)	NSAIDs, COX-2 inhibitors, lipoxygenase inhibitors, iNOS inhibitors, glucocorticoids
	Bile acids (inhibit)	Ursodiol
Deactivate/detoxify carcinogen	GSH/GST (enhance)	Oltipraz, NAC, sulforaphane
Prevent carcinogen–DNA binding	Cytochromes P450 (inhibit)	Tea
Increase level or fidelity of DNA repair	Poly(ADP-ribosyl)transferase (enhance)	NAC, protease inhibitors (Bowman–Birk)
Antiproliferation/antiprogression		
Modulate hormone/growth factor activity	Oestrogen receptor (antagonise)	SERMs, soy isoflavones
	Androgen receptor (antagonise)	Bicalutamide, flutamide
	Steroid aromatase (inhibit)	Exemestane, vorozole, arimidex
	Steroid 5α-reductase (inhibit)	Finasteride, epristeride
	IGF-I (inhibit)	SERMs, retinoids
	AP-1 (inhibit)	Retinoids
	Peroxisome proliferator activated receptor (activate)	Retinoids, NSAIDs
Inhibit oncogene activity	Farnesyl protein transferase (inhibit)	Perillyl alcohol, limonene, DHEA, FTI-276
Inhibit polyamine metabolism	ODC activity (inhibit)	DFMO
	ODC induction (inhibit)	Retinoids, NSAIDs
Induce terminal differentiation	TGFβ (induce)	Retinoids, vitamin D, SERMs
Restore immune response	COX (inhibit)	NSAIDs, COX-2 inhibitors, tea, curcumin
	T, NK lymphocytes (enhance)	Selenium, tea, NSAIDs, COX-2 inhibitors
	Langerhans cells (enhance)	Vitamin E, NSAIDs, COX-2 inhibitors
Increase intercellular communication	Connexin 43 (enhance)	Carotenoids (lycopene), retinoids
Restore tumour suppressor function	p53 (inhibit HPV E6 protein)	–
Induce apoptosis	TGFβ (induce)	Retinoids, SERMs, vitamin D
	RAS farnesylation (inhibit)	Perillyl alcohol, limonene, DHEA, FTI-276
	Arachidonic acid (enhance)	Retinoic acid
		NSAIDs, COX-2 inhibitors, lipoxygenase inhibitors
	Caspase (activate)	Retinoids
	Guanosine monophosphate diesterase (inhibit)	NSAIDs, suilindac sulphone
Inhibit angiogenesis	FGF receptor (inhibit tyrosine kinase)	Soy isoflavones, COX-2 inhibitors
	Thrombomodulin (inhibit)	Retinoids
Correct DNA methylation imbalances	CpG island methylation (enhance)	Folic acid
Inhibit basement membrane degradation	Type IV collagenase (inhibit)	Protease inhibitors (Bowman-Birk)
Inhibit DNA synthesis	Glucose 6-phosphate dehydrogenase (inhibit)	DHEA, fluasterone

*Adapted from [4]. PEITC, phenethylisothiocyanate; PG, Prostaglandins; NSAIDs, non-steroidal anti-inflammatory drugs; COX, cyclo-oxygenase; iNOS, inducible nitric oxide synthase; GSH, glutathione; GST, glutathione-*s*-transferase; NAC, *N*-acetyl-*l*-cysteine; SERMS, selective estrogen receptor modulators; IGF-I, insulin-like growth factor-1; AP-1, (transcription) activator protein-1; DHEA, dehydro-epiandrostenedione; ODC, ornithine decarboxylase; DFMO, 2-difluoromethylornithine; TGFβ, tumour growth factor beta; NK, natural killer cells; HPV, human papilloma virus; FGF, fibroblast growth factor; CpG, cytosine-guanosine.

molecular targets for inhibiting these mechanisms, and corresponding agents/agent classes currently being explored for chemopreventive efficacy.

Newer and future research on chemopreventive agents will include pharmacodynamic modelling, agent combinations and systematic development of dietary components.

Pharmacodynamic modelling

A recent example of pharmacodynamic modelling—the topical application of chemopreventive agent to target tissue to avoid systemic metabolism and toxicity—confirmed the chemopreventive potential of aerosolised steroids [32]. In this study Wattenberg used aerosolised budesonide in B(*a*)P-treated mice to establish the principle of topical delivery to precancerous epithelia with multifocal (field defect) damage as a chemoprevention strategy. The approach has particular promise for the lung, but is applicable to several target organs, the primary advantage being to improve therapeutic index (in the case of corticosteroids about 1/30). Retinoids formulated and delivered by this means could well improve efficacy without toxicity, being applicable eventually to large populations. A pilot study of one of the currently approved aerosolised steroids is in progress in patients with pre-cancerous lesions in the bronchus.

Agent combinations

One strategy to improve efficacy and reduce toxicity is agent combinations [2, 4]. In some combinations of two agents with different presumed mechanisms of activity, synergistic or additive activity may be seen. Such improved activity may allow either or both the agents to be administered at lower doses, thereby reducing potential toxicity. For example, synergistic activity has been observed in rat colon studies with combinations of DFMO and the NSAID

piroxicam [33] and in rat mammary and prostate cancers with combinations of retinoids and anti-oestrogens [34–36], and these strategies are now being tested clinically. Thus, the identification and evaluation of potentially effective agent combinations will be an ongoing research effort for chemoprevention. Another combination strategy is the use of a second agent to counter the toxicity of a known effective chemopreventive agent. An example is co-administration of the prostaglandin E_2 (PGE_2) analogue, misoprostol, to counter the gastro-intestinal toxicity associated with administration of NSAIDs.

Development of dietary components

Because of their expected safety for long-term administration to healthy people, diet-derived compounds are of high interest as potential chemopreventive agents. Dietary components with chemopreventive activity typically start as complex mixtures. The primary strategy for developing these substances is preparation and characterisation of optimal standardised mixtures and purification of the active substance. Hopefully, the standardised mixtures will retain chemopreventive efficacy without increasing toxicity. The purified active substance may prove to be a more promising agent than the mixture, but may also be used primarily as proof of principle for establishing health claims. For example, the preclinical efficacy of curcumin has been determined with food-grade agent, which is a mixture of curcuminoids, ranging from 40 to 85% curcumin. A purified curcumin, micronised for increased bioavailability is now being evaluated. Similarly, tea polyphenol extracts have been well characterised for evaluation in preclinical studies, and epigallocatechin gallate (EGCG), which appears to be a primary active component is being developed in parallel. EGCG is very expensive to manufacture; it may be used only to demonstrate the potential efficacy of tea. The standardised mixtures, since they resemble food more than medicine, might also be more palatable to the population. For example, two soy isoflavone mixtures containing genistein, other isoflavones (primarily daidzein), fat and carbohydrate are being developed. One is nearly 'pure', containing 90% genistein; the second more closely resembles a natural soy product, containing less than 50% genistein. These preparations may be targeted to populations at different risks—high-risk or previous cancer patients might receive the more 'drug-like' 90% genistein preparation, while subjects with normal risk might be given the more 'food-like' 50% genistein mixture. The effort to confirm dietary leads is expected to burgeon over the next few years. The US FDA has proposed guidelines for identifying and evaluating heterogeneous botanicals such as the tea and isoflavone mixtures, and the number of publications on chemopreventive effects of characterised dietary components is increasing, for example, many on tea polyphenols, curcuminoids, selenised garlic/selenomethyl-cysteine, and broccoli compounds (sulforaphane). The Dietary Supplement Health Education Act (DSHEA), 1994 and the Nutrition Labeling and Education Act, 1990 provide mechanisms for possible health claims on dietary components based on a body of published scientific data (not necessarily specifically cancer chemoprevention studies). It is expected that the increasing level of sophistication in analysing epidemiological data, particularly, molecular epidemiological data, will lead to many more new chemopreventive hypotheses regarding diet-derived substances.

SURROGATE ENDPOINT BIOMARKERS (SEBS): CONTINUED CAREFUL RESEARCH WILL BE REQUIRED TO CHARACTERISE EFFICACY BASED ON SEBS AND VALIDATE THEM AS SURROGATES FOR CANCER INCIDENCE

Intermediate biomarkers of cancer are the phenotypic, genotypic and molecular changes that occur during carcinogenesis; many are potentially surrogate endpoints (SEBs) for cancer incidence and understanding their inter-relationship in this regard is very important to chemoprevention. For example, intra-epithelial neoplasia (IEN), as phenotypic precancers are the most promising SEBs. However, recent analyses of head and neck tissue [37, 38] have underscored the need to evaluate genotypic changes in normal-appearing epithelia surrounding dysplastic lesions with the finding that the phenotype of the whole tissue does not always reflect underlying genotypic changes (already present in normal-appearing tissue) that contribute to new primary tumours. Progressive genomic instability as measured by loss of heterozygosity (LOH) or amplification at specific microsatellite loci was used by Sidransky and colleagues [16] to characterise some of these genotypic changes during head and neck carcinogenesis. These genotypic biomarkers are potential surrogate endpoints in head and neck, and may also prove useful in other tissues where microsatellite instability is a predominant feature of carcinogenesis—for example, in hereditary non-polyposis colorectal cancer (HNPCC)-associated and some sporadic colorectal cancers. As for all biomarkers, it is highly desirable to measure modulation of these genotypic changes quantitatively as the difference (Δ) between the biomarker value at the end of treatment and baseline. Thus, baseline biopsies or other baseline tissue measurements are important. New technology such as computer-assisted pathology, high-volume gene-chip-based assays and improved diagnostic tools such as confocal microscopy, the light-induced fluorescence endoscope (LIFE) scope for visualising bronchial tissue and the magnifying endoscope for colorectal monitoring will be critical in ensuring the adequate development of SEBs for chemoprevention studies.

Quantitative endpoints—computer-assisted image analysis (CAIA)

The quantitative evaluation of surrogate endpoints is important, since it is very likely that qualitative discrete measures such as complete and partial response (CR and PR, respectively) will be too crude and lack the reproducibility to detect carcinogenesis-associated changes in small samples. Biomarkers measured by CAIA, including both nuclear and nucleolar morphometry and cytophotometry, should prove valuable in this regard. For example, the study in breast [26] cited above shows that CAIA can be used to describe the grade/severity of dysplasia [26]. Adequate performance ensures that a small trial with limited tissue availability will produce meaningful results. Quantitative nuclear and nucleolar morphometric changes (that is changes in size, shape and texture of nuclear material) are used to describe the histopathology that characterises progression of IEN. Their promise is based on gradient changes associated with increasing IEN severity. Several computer-assisted imaging systems are commercially available. These systems essentially consist of a light microscope, light sensor, digitiser to convert the light to computer-readable form, and a computer with appropriate software to analyse the tissue measurements.

Examples of these measurements are nuclear size, shape, texture and pleomorphism, and nucleolar number, size, shape, position and pleomorphism. CAIA is also useful for cytometry including measurements of cellular proliferation and DNA ploidy that typify IEN histopathology.

Gene-chip technology, immunochemistry and quantifying effects at molecular targets

The supporting technology as well as the data generated from the Human Genome Project and the Cancer Gene Anatomy Program have provided the means to look quantitatively at general genetic damage as well as specific genetic changes at the molecular level. Much of the work in this area has involved gene sequence comparisons. For example, the measurement of microsatellite instability using reverse transcription polymerase chain reaction (RT-PCR) at pre-defined markers has been cited above for detection of gene expression changes at loci relevant to carcinogenesis [9]. However, although quantitative, this technique looks only at DNA sequences and not at specific gene functions. Comparative gene hybridisation (CGH) is being used to approach the evaluation of functional changes. In this technique, gene chips with specific gene sequences (cDNA) and mutations are made (for example wild-type and well-characterised mutations in p53). Corresponding changes in genes in tissue undergoing carcinogenesis can be evaluated by hybridising the tissue DNA to the specialised chips. Many different commercial gene chip packages are now made. More importantly, the capability for making individualised gene chips with specific genes is now available. Besides evaluating changes in tissues undergoing carcinogenesis, these chips may be designed to evaluate subjects at risk, for example those carrying specific germline mutations and genetic polymorphisms.

Fluorescence *in situ* hybridisation (FISH) and particularly chromosomal *in situ* hybridisation (CISH) are also powerful techniques to quantify potential functional changes in carcinogenesis-related genes. Both techniques involve labelling specific gene products related to carcinogenesis. FISH applies a fluorescent label to a gene product of interest. CISH applies different labels (different colours) to wild-type and mutant gene products, allowing the comparison of different relative amounts at different stages of carcinogenesis both before and after treatment with chemopreventive agents. Hittelman and associates [39] have applied CISH in characterising head and neck carcinogenesis.

Value of animal models for validation of surrogate endpoints

As suggested above, animal models which mimic specific characteristics of human carcinogenesis are useful in evaluating biomarkers as surrogate endpoints for cancer incidence. Particularly, the correlation of surrogate endpoint modulation to effects on cancer incidence in such models can provide strong evidence for validating the surrogate endpoint. This correlation can strengthen efficacy claims prior to definitive clinical validation. Transgenic and gene knock-out mice which carry well-characterised genetic lesions predisposing to carcinogenesis are proving to be good models for biomarker evaluation.

Biomarker research and development is also being carried out in carcinogen-induced animals. For example, Boone and associates [40] have recently described CAIA morphometric measures to follow skin carcinogenesis in B(*a*)P-induced SENCAR mice and oesophageal cancer in nitrosamine-induced rats. They also have shown that the chemopreventive agents DFMO and phenethylisothiocyanate (PEITC), respectively, inhibit early lesions as well as cancers in these models.

Sampling issues—tissue difficult to visualise

Precancer (IEN) is currently detected primarily through biopsies. Advances in *in vivo* imaging modalities provide promise for less invasive early detection. The challenge for early detection by these technologies differs among key human cancer target organs. Access by direct visualisation of colon, upper aerodigestive, head and neck, lung, bladder, cervix/endometrium and skin provides an advantage over less accessible targets such as breast, prostate, pancreas, ovary and liver. In some cases, the amount of abnormal tissue can be quantified; therefore, not only can risk estimates be improved but since statistical sampling is not as much of an issue, the extent of modulation by chemopreventive drugs can also be quantified. Advances in the specificity and sensitivity of detecting moieties present in the serum from microscopic disease are becoming more likely and practical from the rapid advances occurring in genomics and proteomics.

Difficulty in detecting prostatic intra-epithelial neoplasia (PIN) is an example of the sampling issues that must be addressed in chemoprevention studies using surrogate endpoints, particularly in tissues difficult to visualise [41]. In men aged $\geq$50 years, high-grade PIN (HGPIN) incidence is 50%. However, of all sextant prostate biopsies taken in this subpopulation for any reason, when no cancer is present, only 5% HGPIN incidence is detected. In the general population, $<$1% HGPIN incidence is detected in sextant prostate biopsies. These discrepancies are most probably due to the inability to ensure adequate tissue sampling in the prostate and call for standardised measurement methods. The number and location of samples from invasive cancer, IEN, and adjacent normal-appearing tissue, as well as the thickness/number of histological sections processed and scored are important parameters that affect variability, accuracy and reproducibility in all tissues. In prostate and other tissues, current approaches for refining the measurement of prevalence and extent of IEN involve calculating the area and volume of IEN, and digital imaging of the tissue.

New technology such as computer-assisted pathology (CAIA as described above), high-volume gene chip-based assays and improved diagnostic tools such as the confocal microscope, the LIFE scope for visualising bronchial tissue and the magnifying endoscope for colorectal monitoring will be critical to assuring the adequate development of surrogate endpoints for chemoprevention studies.

DEFINING CANCER RISK—INDIVIDUAL AND POPULATION-BASED STUDIES: CONTINUAL REFINEMENT OF CANCER RISK ESTIMATIONS IS ESSENTIAL FOR DETERMINING APPROPRIATE TARGET POPULATIONS AND INTERVENTION STRATEGIES

Excepting the rare genetic syndromes where the relative risk of an individual developing cancer can be very high (subjects with FAP, Li–Fraumeni, *BRCA*, etc.), measurable risk factors defining any population usually confer relative risks of developing cancer in the units or tens. Examples to put this in perspective include studies of tobacco smoking risk showing relative risks of 30% for 30 pack-years and the presence of atypical ductal hyperplasia in the breast of adult

females conferring a risk of 4–5 of developing breast cancer. The small relative risks and the multifactorial aetiology of most human cancer makes randomised intervention trials, where the effects on a single variable are evaluated and the confounding variables are controlled, the only reliable method for determining chemopreventive efficacy. The impracticality of conducting more than a handful of large human intervention trials to test various hypotheses emphasises the importance of more accurately predicting an individual's absolute risk and developing the methods to monitor that risk with functional genomics, histomorphometry and imaging.

REGULATORY ISSUES: ADVANCES ARE BEING MADE IN GAINING APPROVAL OF CHEMOPREVENTION AGENTS BASED ON SURROGATE ENDPOINTS OF CANCER INCIDENCE AND ON IMPROVEMENTS IN QUALITY OF LIFE

Proof of chemopreventive drug efficacy based on cancer incidence reduction can require up to 45 000 subjects over a period of more than 10 years depending on the risk of the population under study. The new technologies that allow more precise, individual risk evaluation, trials in already known high-risk cohorts, and the use of surrogate endpoint biomarkers will potentially reduce the size and duration of some cancer incidence reduction trials to as few as 500–1000 patients over a period of 3 years. The number of candidate chemoprevention drugs in the developmental pipeline, the high dollar cost and time of cancer incidence reduction trials, the scarcity of eligible and willing subjects, and the opportunity provided by the wealth of scientific data and technology make the safe approval of chemopreventive drugs for selected populations on the basis of well-characterised surrogate endpoints a desirable and practical necessity. A challenge for the future will be to define this process. Besides reducing the cancer burden, chemoprevention drugs could provide a sound basis for improvement of quality of life in some clinical situations. For example, in high-risk subjects with precancers, improved quality of life could result from delaying the need for and reducing the morbidity due to surgical treatments and invasive screening procedures.

PUBLIC HEALTH AND CHEMOPREVENTION: CONTINUED EFFORTS TO DEVELOP SAFE CHEMOPREVENTIVE AGENTS CRITICAL FOR EARLY INTERVENTION

The promise of chemoprevention is evidenced by the increasing number of clinical strategies and studies at most of the major cancer target organs. At this point in time, cancers in at least 11 organ systems have been evaluated for development of chemopreventive agents—prostate, breast, colon, lung, head and neck, bladder, oesophagus, cervix, skin, liver and multiple myeloma [1, 3, 4].

The science of toxicity evaluation of drugs developed for chronic human use is well established, and guidance specific for chemopreventive agent development has been published [13]. Volumes of animal and human data as well as multi-year experience with drugs given chronically to humans over 40 or more years have provided only a few unanticipated safety problems. The preclinical toxicology evaluation of candidate drugs involves chronic administration of 10-fold the starting doses in human safety evaluations, and then pushing drug administration to doses creating detectable preclinical toxicities so that the profile of expected problems are identified (carcinogenicity potential is evaluated by essentially lifetime administration of drug to rodents, and specialised—for example reproductive—toxicity studies are carried out routinely). Safety evaluation in humans is performed incrementally and often begins in cohorts at high risk for disease, and thus at greater potential for benefit. The decision for a drug approval for a given indication is made on a case-by-case basis after a rigorous analysis of efficacy and safety and the risk–benefit profile of the intended recipients. The public health impact of chemopreventive drug intervention will also develop incrementally, as drugs are found effective in higher-risk cohorts and, in the absence of safety problems, are justifiably qualified for use in lower-risk populations. Postmarketing surveillance will be critical and will continue to find the few unanticipated safety problems that may emerge. The availability of the postmarketing surveillance process itself makes possible the approval of drugs where the weight of the clinical evidence has clearly determined that the drug will make an impact on the reduction of human cancer risk, before the effects of long-term human exposure can be evaluated. The rapid progress being made in detection of genetic polymorphisms as it relates to drug-metabolising enzymes, as described above, as well as more sophisticated evaluation of risk will define subsets of the population most likely to benefit from drug intervention.

PUBLIC HEALTH AND CHEMOPREVENTION IN THE NEXT DECADE, WIDESPREAD EDUCATION THAT CANCER IS PREVENTABLE AND INSURANCE COVERAGE FOR PROACTIVE PREVENTION ARE NEEDED

The past two decades have seen a tremendous increase in media coverage, publications (both trade and consumer) and educational programmes on disease prevention, which are directed at the general population. Most provide information on cancer prevention, usually not exclusively, but in context with other chronic diseases (particularly cardiovascular disease). A common theme is behaviour modification to create a healthy lifestyle, such as by smoking cessation (ASSIST) or general dietary improvement (Five-A-Day). Others advocate surveillance and early detection (for example, guidelines for mammographic screening). A future challenge is educating the population on the potential additional benefits of active intervention, whether it is by prescribed drug or specific dietary substances. Important aspects of the educational process will be the methods for fully characterising an individual's risk and incorporating such risk estimates into early detection programmes, so that the most appropriate intervention can be planned. Although the information needed to calculate individual risk is for the most part only now beginning to emerge, the 'risk disk' recently developed by the NCI for evaluation of breast cancer risk and required for use in prescribing tamoxifen for breast cancer risk reduction is a prototype for this effort. To ensure that the benefits of prevention are available widely, a further challenge must be met—that is, gaining recognition for proactive prevention as a standard of care. Preventive care in the general population, such as early detection screening (for example, annual mammograms), has only recently begun to have limited insurance coverage. Widespread coverage will be required so that all who could benefit have access to such care.

Basic and applied prevention research defining the genotypic and phenotypic changes during progression of normal tissue to precancer to cancer in humans (and experimental animals), the risk conferred by these changes, and the modulation of these changes in preclinical experimentation and randomised clinical trials by active intervention with chemopreventive drugs, dietary agents and regimens, and treatments resulting from early detection provides the main scientific focus for a cancer chemoprevention effort in the next millennium. The key elements of this research effort are too numerous to list in detail but should include comprehensive basic and translational tissue, cell and serum-based risk evaluation programmes; chemopreventive and dietary agent drug discovery and development; further development and testing of transgenic animal models; required safety and pharmacology studies; phase I, II and III chemoprevention and appropriate nutriceutical and diet-based studies with rigorous criteria established for prioritising large studies and proactive planning to ensure accrual; and much expanded early detection programmes. The promise of cancer prevention is here, and the growing number of research programmes in chemoprevention sciences ensures that this promise will continue to be realised in the next decade.

1. Kelloff GJ, Hawk ET, Crowell JA, *et al.* Strategies for identification and clinical evaluation of promising chemopreventive agents. *Oncology (Huntington)* 1996, **10**, 1471–1480, 1484.
2. Kelloff GJ, Hawk ET, Karp JE, *et al.* Progress in clinical chemoprevention. *Semin Oncol* 1997, **24**, 241–252.
3. Hong WK, Sporn MB. Recent advances in chemoprevention of cancer. *Science* 1997, **278**, 1073–1077.
4. Kelloff GJ. Perspectives on cancer chemoprevention research and drug development. *Advances Cancer Res* 1999, **78**, 199–334.
5. Henderson BE, Ross R, Bernstein L. Oestrogens as a cause of human cancer: The Richard and Hinda Rosenthal Foundation Award Lecture. *Cancer Res* 1988, **48**, 246–253.
6. Ross RK, Pike MC, Coetzee GA, *et al.* Androgen metabolism and prostate cancer: establishing a model of genetic susceptibility. *Cancer Res* 1998, **58**, 4497–4504.
7. Miller MS, McCarver DG, Bell DA, Easton DL, Goldstein JA. Genetic polymorphisms in human drug metabolic enzymes. *Fundamental Appl Toxicol* 1997, **40**, 1–14.
8. Fearon ER, Vogelstein B. A genetic model for colorectal tumorigenesis. *Cell* 1990, **61**, 759–767.
9. Califano J, van der Riet P, Westra W, *et al.* Genetic progression model for head and neck cancer: implications for field cancerization. *Cancer Res* 1996, **56**, 2488–2492.
10. Kelloff GJ, Boone CW, Steele VE, *et al.* Mechanistic considerations in chemopreventive drug development. *J Cell Biochem* 1994, (Suppl. 20), 1–24.
11. Fisher B, Costantino JP, Wickerham DL, *et al.* Tamoxifen for prevention of breast cancer: Report of the National Surgical Adjuvant Breast and Bowel Project P-1 Study. *J Natl Cancer Inst* 1998, **90**, 1371–1388.
12. Heinonen OP, Albanes D, Virtamo J, *et al.* Prostate cancer and supplementation with α-tocopherol and β-carotene: incidence and mortality in a controlled trial. *J Natl Cancer Inst* 1998, **90**, 440–446.
13. Kelloff GJ, Johnson JJ, Crowell JA, *et al.* Approaches to the development and marketing approval of drugs that prevent cancer. *Cancer Epidemiol Biomarkers Prevent* 1995, **4**, 1–10.
14. Sporn MB. The war on cancer. *Lancet* 1996, **347**, 1377–1381.
15. Schipper H, Turley EA, Baum M. A new biological framework for cancer research. *Lancet* 1996, **348**, 1149–1151.
16. Sidransky D, Mikkelsen T, Schwechheimer K, *et al.* Clonal expansion of p53 mutant cells is associated with brain tumour progression. *Nature* 1992, **355**, 846–847.
17. Sidransky D, Messing E. Molecular genetics and biochemical mechanisms in bladder cancer. Oncogenes, tumour suppressor genes, and growth factors. *Urol Clin N Am* 1992, **19**, 629–639.
18. Sidransky D, Frost P, Von Eschenbach A, *et al.* Clonal origin of bladder cancer. *N Engl J Med* 1992, **326**, 737–740.
19. Rosin MP, Cairns P, Epstein JI, *et al.* Partial allelotype of carcinoma *in situ* of the human bladder. *Cancer Res* 1995, **55**, 5213–5216.
20. Mao L, Schoenberg MP, Scicchitano M, *et al.* Detection of primary bladder cancer by microsatellite analysis. *Science* 1996, **271**, 659–662.
21. Simoneau AR, Jones PA. Bladder cancer: the molecular progression to invasive disease. *World J Urol* 1994, **12**, 89–95.
22. Thiberville L, Payne P, Vielkinds J, *et al.* Evidence of cumulative gene losses with progression of premalignant epithelial lesions to carcinoma of the bronchus. *Cancer Res* 1995, **55**, 5133–5139.
23. Kishimoto Y, Sugio K, Hung JY, *et al.* Allele-specific loss in chromosome 9p loci in preneoplastic lesions accompanying non-small-cell lung cancers. *J Natl Cancer Inst* 1995, **87**, 1224–1229.
24. Larson AA, Liao S-Y, Stanbridge EJ, *et al.* Genetic alterations accumulate during cervical tumorigenesis and indicate a common origin for multifocal lesions. *Cancer Res* 1997, **57**, 4171–4176.
25. Brown PO, Botstein D. Exploring the new world of the genome with DNA microarrays. *Nature Genet* 1999, **21**(Suppl.), 33–37.
26. Sneige N, Lagios MD, Schwarting R, *et al.* Interobserver reproducibility of the Lagios nuclear grading system for ductal carcinoma *in situ*. *Human Pathol* 1999, **30**, 257–262.
27. Su L-K, Kinzler KW, Vogelstein B, *et al.* Multiple intestinal neoplasia caused by a mutation in the murine homolog of the APC gene. *Science* 1992, **256**, 668–670.
28. Edelmann W, Yang K, Kuraguchi M, *et al.* Tumorigenesis in MLH1 and MLH1/Apc1638N mutant mice. *Cancer Res* 1999, **59**, 1301–1307.
29. Jacoby RF, Marshall DJ, Newton MA, *et al.* Chemoprevention of spontaneous intestinal adenomas in the *Apc*Min mouse model by the nonsteroidal anti-inflammatory drug piroxicam. *Cancer Res* 1996, **56**, 710–714.
30. Arbeit JM, Howley PM, Hanahan D. Chronic oestrogen-induced cervical and vaginal squamous carcinogenesis in human papillomavirus type 16 transgenic mice. *Proc Natl Acad Sci USA* 1996, **93**, 2930–2935.
31. Matzinger SA, Crist KA, Stoner GD, *et al.* K-*ras* mutations in lung tumours from A/J and A/J X TSG-p53 F1 mice treated with 4-(methylnitrosamino)-1-(3-pyridyl)-1-butanone and phenethyl isothiocyanate. *Carcinogenesis* 1995, **16**, 2487–2492.
32. Wattenberg LW, Wiedmann TS, Estensen RD, *et al.* Chemoprevention of pulmonary carcinogenesis by aerosolized budesonide in female A/J mice. *Cancer Res* 1997, **57**, 5489–5492.
33. Reddy BS, Nayini J, Tokumo K, *et al.* Chemoprevention of colon carcinogenesis by concurrent administration of piroxicam, a nonsteroidal antiinflammatory drug with D,L-α-difluoromethylornithine, an ornithine decarboxylase inhibitor, in diet. *Cancer Res* 1990, **50**, 2562–2568.
34. Anzano MA, Byers SW, Smith JM, *et al.* Prevention of breast cancer in the rat with 9-cis-retinoic acid as a single agent and in combination with tamoxifen. *Cancer Res* 1994, **54**, 4614–4617.
35. Anzano MA, Peer CW, Smith JM, *et al.* Chemoprevention of mammary carcinogenesis in the rat: combined use of raloxifene and 9-cis-retinoic acid. *J Natl Cancer Inst* 1990, **88**, 123–125.
36. Lucia MS, Anzano MA, Slayter MV, *et al.* Chemopreventive activity of tamoxifen, N-(4-hydroxyphenyl)retinamide, and the vitamin D analogue Ro24-5531 for androgen-promoted carcinomas of the rat seminal vesicle and prostate. *Cancer Res* 1995, **55**, 5621–5627.
37. Westra WH, Sidransky D. Phenotypic and genotypic disparity in premalignant lesions: of calm water and crocodiles. *J Natl Cancer Inst* 1998, **90**, 1500–1501.
38. Mao L, El-Naggar AK, Papadimitrakopoulou V, *et al.* Phenotype and genotype of advanced premalignant head and neck lesions after chemopreventive therapy. *J Natl Cancer Inst* 1998, **90**, 1545–1551.
39. Hittelman WN, Kim HJ, Lee JS, *et al.* Detection of chromosome instability of tissue fields at risk: *in situ* hybridization. *J Cell Biochem* 1996, (Suppl. 25), 57–62.
40. Boone CW, Bacus JW, Bacus JV, *et al.* Properties of intraepithelial neoplasia relevant to cancer prevention and to the development of surrogate endpoints for clinical trials. *Proc Soc Exp Med Biol* 1997, **216**, 151–166.
41. Kelloff GJ, Lieberman RL, Steele VE, *et al.* Chemoprevention of prostate cancer: concepts and strategies. *Eur J Urol* 1999, **35**, 342–350.