

UC Irvine

UC Irvine Previously Published Works

Title

Progress in Chemoprevention Drug Development: The Promise of Molecular Biomarkers for Prevention of Intraepithelial Neoplasia and Cancer—A Plan to Move Forward

Permalink

<https://escholarship.org/uc/item/5kx8v97f>

Journal

Clinical Cancer Research, 12(12)

ISSN

1078-0432

Authors

Kelloff, Gary J
Lippman, Scott M
Dannenber, Andrew J
et al.

Publication Date

2006-06-15

DOI

10.1158/1078-0432.ccr-06-1104

Copyright Information

This work is made available under the terms of a Creative Commons Attribution License, available at <https://creativecommons.org/licenses/by/4.0/>

Peer reviewed

Progress in Chemoprevention Drug Development: The Promise of Molecular Biomarkers for Prevention of Intraepithelial Neoplasia and Cancer—A Plan to Move Forward

Gary J. Kelloff,¹ Scott M. Lippman,² Andrew J. Dannenberg,³ Caroline C. Sigman,⁴ Homer L. Pearce,⁵ Brian J. Reid,⁶ Eva Szabo,¹ V. Craig Jordan,⁷ Margaret R. Spitz,² Gordon B. Mills,² Vali A. Papadimitrakopoulou,² Reuben Lotan,² Bharat B. Aggarwal,² Robert S. Bresalier,² Jeri Kim,² Banu Arun,² Karen H. Lu,² Melanie E. Thomas,² Helen E. Rhodes,² Molly A. Brewer,⁹ Michele Follen,² Dong M. Shin,¹⁰ Howard L. Parnes,¹ Jill M. Siegfried,¹¹ Alison A. Evans,⁷ William J. Blot,¹² Wong-Ho Chow,¹ Patricia L. Blount,⁶ Carlo C. Maley,⁸ Kenneth K. Wang,¹³ Stephen Lam,¹⁴ J. Jack Lee,² Steven M. Dubinett,¹⁵ Paul F. Engstrom,⁷ Frank L. Meyskens, Jr.,¹⁶ Joyce O'Shaughnessy,¹⁷ Ernest T. Hawk,¹ Bernard Levin,² William G. Nelson,¹⁸ and Waun Ki Hong² for the AACR Task Force on Cancer Prevention

Abstract This article reviews progress in chemopreventive drug development, especially data and concepts that are new since the 2002 AACR report on treatment and prevention of intraepithelial neoplasia. Molecular biomarker expressions involved in mechanisms of carcinogenesis and genetic progression models of intraepithelial neoplasia are discussed and analyzed for how they can inform mechanism-based, molecularly targeted drug development as well as risk stratification, cohort selection, and end-point selection for clinical trials. We outline the concept of augmenting the risk, mechanistic, and disease data from histopathologic intraepithelial neoplasia assessments with molecular biomarker data. Updates of work in 10 clinical target organ sites include new data on molecular progression, significant completed trials, new agents of interest, and promising directions for future clinical studies. This overview concludes with strategies for accelerating chemopreventive drug development, such as integrating the best science into chemopreventive strategies and regulatory policy, providing incentives for industry to accelerate preventive drugs, fostering multisector cooperation in sharing clinical samples and data, and creating public-private partnerships to foster new regulatory policies and public education.

In most epithelial tissues, accumulating mutations (i.e., genetic progression) and loss of cellular control functions cause progressive phenotypic changes from normal histology to early precancer [intraepithelial neoplasia (IEN)] to increas-

ingly severe IEN to superficial cancer and finally to invasive disease. This process can be relatively aggressive in some settings (e.g., in the presence of a DNA repair-deficient genotype) but generally occurs relatively slowly over years and decades. Cancer chemoprevention can be defined as the prevention of cancer or treatment of identifiable precancers (defined as histopathologic or molecular IEN). The long latency to invasive cancer is a major scientific opportunity but also an economic obstacle to showing the clinical benefit of candidate chemopreventive drugs. Therefore, an important component of chemopreventive agent development research in recent years has been to identify earlier (than cancer) end points or biomarkers that accurately predict an agent's clinical benefit or cancer incidence-reducing effect. In many cancers, IEN is an early end point. In 2002, the AACR IEN Task Force recommended focusing chemopreventive drug development on IEN because of the close association between IEN and invasive cancer and because reducing IEN burden can benefit patients by reducing cancer risk and/or the need for invasive interventions (1). The IEN Task Force proposed several practical and feasible clinical trial designs for developing new agents to treat and prevent precancer in nine cancer target organs.

Authors' Affiliations: ¹National Cancer Institute, Bethesda, Maryland; ²University of Texas M.D. Anderson Cancer Center, Houston, Texas; ³Weill Medical College of Cornell University, New York, New York; ⁴CCS Associates, Mountain View, California; ⁵Eli Lilly & Co., Indianapolis, Indiana; ⁶Fred Hutchinson Cancer Research Center, Seattle, Washington; ⁷Fox Chase Cancer Center; ⁸The Wistar Institute, Philadelphia, Pennsylvania; ⁹University of Arizona, Tucson, Arizona; ¹⁰Emory University, Atlanta, Georgia; ¹¹University of Pittsburgh, Pittsburgh, Pennsylvania; ¹²International Epidemiology Institute, Rockville, Maryland; ¹³Mayo Clinic, Rochester, Minnesota; ¹⁴British Columbia Cancer Agency, Vancouver, British Columbia, Canada; ¹⁵University of California at Los Angeles, Los Angeles, California; ¹⁶University of California at Irvine, Irvine, California; ¹⁷Baylor Sammons Cancer Center, Texas Oncology, PA, U.S. Oncology, Dallas, Texas; and ¹⁸Johns Hopkins University, Baltimore, Maryland

Received 5/8/06; revised 5/10/06; accepted 5/10/06.

Note: A complete list of members of the AACR Task Force on Cancer Prevention can be found at <http://www.aacr.org/default.aspx?p=2694>.

Requests for reprints: Gary J. Kelloff, National Cancer Institute, Executive Plaza North, Room 6058, 6130 Executive Boulevard, Rockville, MD 20852. Phone: 301-594-0423, Fax: 301-480-3507; E-mail: kelloffg@mail.nih.gov.

© 2006 American Association for Cancer Research.

doi:10.1158/1078-0432.CCR-06-1104

Knowledge of the molecular basis of carcinogenesis has increased exponentially through research elucidating signaling and metabolic pathways and defining genetic progression models. New technologies in genomics and proteomics and in functional and molecular imaging have spurred this research. As suggested in the 2002 article, this knowledge provides a basis for developing early biomarkers in IEN that will allow discovery of new chemopreventive agents, identify subjects at risk for cancer, and serve as end points for evaluating chemopreventive efficacy.

This report by the AACR Cancer Prevention Task Force provides perspectives on the current state of chemoprevention science and on the possibilities for future rapid advances. We discuss prospects for use of biomarkers in chemopreventive agent development, mechanism-based strategies for chemoprevention, and progress in the clinical development of agents to prevent cancer and prevent or treat IEN. The report concludes with recommendations for accelerating the progress of chemopreventive drug development.

Molecular Biomarkers in Chemoprevention

There are opportunities for using molecular biomarkers in all aspects of chemoprevention. For example, these biomarkers may be molecular targets used for identifying new agents or optimizing lead agents. They can be cancer risk markers for selecting cohorts for chemopreventive studies, and their presence may predict response to mechanism-based interventions. In addition, modulation of these biomarkers in animal and early clinical studies is useful in determining the delivery of biologically effective doses. Because many chemopreventive agents are likely to be used chronically by essentially healthy people, assuring safety on long-term drug treatment is critical. Molecular biomarkers of potential toxicity, such as patterns of activity of drug-metabolizing enzymes, could become very useful in evaluating candidate agents in preclinical development and in monitoring subjects in clinical trials.

Characteristics of an ideal molecular biomarker

Generally, the more closely a biomarker resembles the carcinogenic process it is modeling, the more effective it will likely be in chemoprevention studies. For example, single genes and proteins that are overexpressed, mutated, or masked in precancers or cancers compared with normal tissue may be biomarkers of cancer risk and targets for modulation if they are indicators of a biological process associated with neoplastic progression. Cyclooxygenase-2 (COX-2) is such a target. It is overexpressed in many cancers and precancers (2), and it is a biomarker of inflammatory response to growth factors and other cellular stimuli (3–5). Although more complicated to interpret, increases in expression and activity of growth factor receptors or kinases at critical points in signaling pathways are similarly associated with early neoplastic activities, such as cellular proliferation, survival, and angiogenesis; examples are vascular endothelial growth factor (VEGF) receptors and *ras* and *rho* oncogene expression (6).

Because they are detecting overall changes in cells undergoing carcinogenesis, gene microarray, proteome, and immunogen analyses provide tools for closely modeling the process of neoplastic progression and may be the ultimate biomarkers themselves or a source of individual or coordinated clusters of

molecular biomarkers. Identification of the biomarkers involves analysis of multiple coordinated molecular activities to identify those most important for cancer or alternatively to analyze pathway activation bypassing interpatient differences in activation mechanisms and feedback loops. Gene set enrichment analysis (7) provides one method for identifying critical pathway targets, in which gene occurrence is mapped to discriminate between genes that are affected or not affected in the cancer tissue. Effects on the target may be measured at one or many of the multiple possible intermediate points on the pathway(s) represented by the gene set enrichment analysis map. Gene set enrichment analysis has been used to identify tissue-specific molecular targets affected by deletion of the tumor suppressor PTEN (7, 8). As a second example, Troester and Perou have designed a strategy for applying gene expression profiling (using hierarchical cluster analysis) in breast cancer chemoprevention as risk and end-point biomarkers (9). This approach included a phase II trial design based on Fabian's model (10) with genomic analysis of fine-needle aspiration (FNA) tissue comparing high-risk women at baseline and after 6 months of treatment with either chemopreventive agent or placebo.

Changes in an ideal end-point biomarker would link to clinical benefit, directly or indirectly related to chemopreventive potential, and modulation would be associated with low toxicity (1, 11). For example, in addition to their potential effects in cancer prevention, targets of antioxidants and anti-inflammatory drugs and of cholesterol-lowering drugs are associated with clinical benefit for other diseases of aging—arthritis and cardiovascular disease, respectively. Phase II metabolic enzymes, such as the glutathione S-transferases, are targets for dietary antioxidants. Agents targeting these enzymes may provide clinical benefit by virtue of their pleiotropic chemoprotective effects, and they are likely to have low toxicity (3). Finally, ideal biomarkers can be quantified directly [e.g., epidermal growth factor receptor (EGFR) tyrosine kinase activity (12)] or via a closely related activity, such as inhibition of a specific kinase upstream or downstream from the target [e.g., S6 kinase activity or phosphorylation of 4-EBP or pS6 to measure mammalian target of rapamycin (mTOR) inhibition (13)]. In addition, measurement of these biomarkers should be reliably done on clinical specimens that are obtained as noninvasively as possible. Novel and developing molecular imaging techniques allow noninvasive assessment of the activation state of multiple signaling pathways and functional outcomes, thus offering the potential for serial analysis of effects of chemopreventive agents on the tissue of interest.

Genetic progression models/intrinsic properties of neoplasia

A key concept supporting use of molecular biomarkers for developing chemopreventive agents is that cancer is a disease of genetic progression. Progression has been mapped in tissues by the appearance of specific molecular and more general genotypic damage associated with increasingly severe histologic phenotypes (14–17). Early, critical steps include inactivation of tumor suppressor genes, such as *APC* (15, 16, 18), *BRCA1* or *BRCA2* (19), and *PTEN* (13, 20, 21), and activation of oncogenes, such as *ras* and *PI3KCA* (22). In some cases, progression has been correlated to the appearance of a cluster of genetic defects, such as point mutations and loss of heterozygosity (LOH) in *p16* and *p53* genes and *p16* promoter methylation

in esophagus (23, 24). Progression may also be influenced by factors specific to the host tissue's environment, such as production and action of hormones and growth factors in stroma in the microenvironment of the developing epithelial tumor; activation of VEGF receptor leading to angiogenesis and macrophage-mediated inflammatory response are examples (25–27).

These events, manifested in cells and tissue as intrinsic properties of neoplasia, were described by Hanahan and Weinberg as six acquired characteristics of cancer in their landmark review (26). Because of the imputed association with neoplastic progression, cellular signaling pathways with genetic lesions producing these effects are a rich source of potential molecular targets. Table 1 lists the six properties of neoplasia along with candidate molecular targets for intervention.

Molecular biomarkers for monitoring safety of chemopreventive agents

The expected long-term administration of chemoprevention agents to healthy populations drives the concern to uncover and monitor safety risks. It is likely that early predictors of toxicity will include effects at the molecular level that can be monitored by sequencing, single nucleotide polymorphism arrays, transcriptional profiling, or protein expression profiles. Drug-induced cytochrome P450 expression profiles and cytochrome P450 gene polymorphisms have already been used for many years to evaluate drug toxicity and sensitivity. A recent relevant example compared the chemopreventive agents indole-3-carbinol and its metabolite 3,3'-diindolylmethane (28). Indole-3-carbinol induces reversible liver damage in rats, but 3,3'-diindolylmethane does not. Interestingly, treatment with the two agents induced different cytochrome P450 expression patterns. Rigorous postmarketing surveillance will also contribute to ensuring safety. A standardized set of molecular indicators of potential toxicities specific to the chemopreventive agent or commonly seen in the target population could be incorporated into this surveillance and into earlier stages of clinical development to facilitate comparison and early detection of toxicity.

Mechanism-Based Chemopreventive Agent Development

Table 2 lists many of the promising mechanism-based molecular targets that have been identified over the past three

decades of cancer prevention research along with associated cancer target organs and agents. These individual targets, because they are often risk and/or progression biomarkers, form the basis for identifying and developing many candidate agents but are only part of the process. The knowledge provided by the increasing understanding of genetic progression in cancer, the role of the tumor microenvironment, and the molecular bases for other diseases of aging as well as the emerging technologies in genomics/proteomics and molecular imaging has brought forth new thinking on the development of molecular target-based chemoprevention strategies. Comprehensive review of the scientific strategies and data that are available for the >30 molecular targets that are listed in Table 2 is beyond the scope of this article. However, Table 2 provides the basis for a brief discussion of some of the major research efforts as summarized below.

Inhibition of signal transduction pathways

For more than a decade, both cancer therapy and cancer chemoprevention research have investigated molecular targets in signal transduction pathways leading to cell proliferation and tumor growth. Growth factor receptors or their ligands have been primary targets, with notable well-known successes—erlotinib and cetuximab (EGFR), trastuzumab (HER-2/*neu*), and bevacizumab (VEGF; ref. 29). However, regulation of the signal transduction pathways is complex and inhibition of these single targets may not always be effective (e.g., because of mutations in genes downstream from the receptor and because of alternate pathways to proliferation and survival or induction of feedback loops) or may lead to unwanted side effects (e.g., because of high doses required for inhibition leading to off-target activity or interference with normal cellular functions). As described above, gene expression profiling methods and functional proteomics approaches are now available for looking at the effects of modulating multiple targets and pathways and designing chemopreventive intervention strategies based on effects across pathways and networks. In this regard, several chemoprevention approaches are being evaluated that use combinations of targeted therapies. These are described in Combination strategies.

There is increasing evidence from epidemiologic, experimental, and clinical data suggesting that inhibition of insulin-like growth factor (IGF) signaling, particularly via phosphatidylinositol 3-kinase (PI3K)/AKT-activated pathways, may be a target

Table 1. Characteristics of neoplasia and associated molecular biomarkers

Characteristics of neoplasia	Possible molecular targets
• Self-sufficiency in cell growth	Epidermal growth factor, platelet-derived growth factor, MAPK, PI3K
• Insensitivity to antigrowth signals	SMADs, pRb, cyclin-dependent kinases, MYC
• Limitless replicative potential	hTERT, pRb, p53
• Evading apoptosis	BCL-2, BAX, caspases, FAS, tumor necrosis factor receptor, DR5, IGF/PI3K/AKT, mTOR, p53, PTEN, <i>ras</i> , interleukin-3, NF- κ B
• Sustained angiogenesis	VEGF, basic fibroblast growth factor, $\alpha_v\beta_3$, thrombospondin-1, hypoxia-inducible factor-1 α
• Tissue invasion and metastasis	Matrix metalloproteinases, MAPK, E-cadherin

NOTE: Data from Hanahan and Weinberg (26).

Table 2. Molecular targets and agents for chemoprevention

Molecular target	Clinical target	Representative agents
Anti-inflammatory/antioxidant		
COX-2	Multiple (colon, bladder, esophagus, lung, head and neck, breast, cervix, liver)	Celecoxib, rofecoxib, NSAIDs
EP ₁₋₄	Breast, colon, head and neck	ONO-8711
Inducible nitric oxide synthase/nitric oxide	Colon, prostate, bladder, head and neck	NO-NSAIDs
LOX	Lung, colon, esophagus	Zileuton, zafirkulast, licofelone
NF- κ B	Prostate, colon, head and neck, multiple myeloma, liver	Bortezomib, <i>R</i> -flurbiprofen, curcumin, tea polyphenols, statins, NSAIDs
Antioxidant response element (Nrf2)	Lung, head and neck	Dithiolthiones
Glutathione <i>S</i> -transferase	Lung, liver, head and neck	Dithiolthiones, PEITC
Nkx3.1	Prostate	Tea polyphenols
Prostacyclin	Lung	Iloprost
Epigenetic modulation		
DNA methylation	Prostate, lung	Azacytidine, folic acid
Histone deacetylase	Breast, colon	SAHA
Hormonal/nuclear receptor	Modulation	
5 α -Steroid reductase	Prostate	Finasteride, dutasteride
AR	Prostate	Flutamide, bicalutamide, 3,3'-diindolymethane
Aromatase	Breast, prostate	Exemestane, letrozole, anastrozole
ER- α	Breast, prostate, colon	Tamoxifen, toremifene, arzoxifene, raloxifene, soy isoflavones, acolfibene, indole-3-carbinol, 3,3'-diindolymethane
ER- β	Prostate, colon, breast, ovary	Resveratrol, TAS-108
Peroxisome proliferator-activated receptor- γ	Breast, colon, head and neck, liver	Rosiglitazone, pioglitazone, GW7845, CDDO, LGD100268
Retinoic acid receptor- β	Breast, ovary, colon, head and neck	Fenretinide, 9- <i>cis</i> -retinoic acid
Retinoic acid receptor/retinoid X receptor	Breast, skin, head and neck	9- <i>cis</i> -Retinoic acid
Retinoid X receptor	Breast	Targretin, LGD100268
VDR	Colon, prostate	Vitamin D3 analogues
Signal transduction modulation		
BCL-2	Colon, prostate	ABT-737
Cyclic guanosine 3',5'-monophosphate PDE	Prostate, colon	Exisulind
Cyclin D1	Head and neck, esophagus	
EGFR	Lung, bladder, breast, colon	Gefitinib, erlotinib, EKB569, cetuximab
HMGCoA reductase	Colon, skin (melanoma), breast, prostate	Statins
IGF/IGF receptor	Breast, colon, prostate	
MAPK	Head and neck, lung, breast, bladder	
Matrix metalloproteinases	Colon	Marimistat, prinomastat
mTOR	Prostate	RAD-001
Ornithine decarboxylase, polyamine synthesis	Colon, bladder, skin	DFMO
p53	Lung, esophagus, head and neck	CP31398
PI3K/AKT, PTEN	Head and neck, lung	Deguelin, LGD100268
<i>ras</i>	Colon, pancreas, lung	Tipifarnib, perillyl alcohol
Transforming growth factor- β /SMADs	Breast	CDDO
VEGF/VEGF receptor	Colon, breast	Bevacizumab

for chemoprevention. IGF levels are increased in many cancers, and levels of IGF-binding protein-3 (which, when bound to IGF, inhibits IGF signaling) are decreased (30–37). For example, clinical studies have shown that retinoids and selective estrogen receptor (ER) modulators (SERM) lower the IGF-I/IGF-binding protein-3 ratio in breast and that this activity is associated with antiproliferative chemopreventive activity (38–42). mTOR signaling is also a potential target for chemo-

prevention, because mTOR integrates signals from a host of environmental factors, including amino acids, energy, hormones, and growth factors, to regulate cell cycle. For example, AKT is upstream of mTOR, and activation of AKT1 in transgenic mice leads to the rapid development of high-grade prostatic IEN (HGPIEN). The mTOR inhibitor RAD-001 (a rapamycin analogue) reversed the PIN phenotype, and this effect was associated with increased apoptosis (8).

Oncogene pathway addiction and tumor suppressor hypersensitivity

As described by Weinstein and others (43–46), oncogene addiction is physiologic dependence of cancer cells on the continued activation or overexpression of single oncogenes for maintaining the malignant phenotype. This dependence occurs in the milieu of the other changes that mark neoplastic progression. For example, addiction has been observed in mice transgenic for *ras* in melanoma; BCR-ABL in leukemia; HER-2/*neu* in breast; and c-MYC in pancreas, skin, and leukemia. Addiction has also been observed in many human cancer cells [e.g., *ras* in pancreas; EGFR in lung; the PI3K pathway in multiple tumor types; and cyclin D1 in esophageal, colon, and pancreatic adenocarcinomas (44, 46)]. Because of the selective sensitivity of addicted cells to inhibition of the oncogene or its pathway, these are good candidates for chemoprevention. Particularly relevant to chemoprevention, damping rather than eliminating activity may be effective as was seen for cyclin D1 in esophageal cancer cells (43, 44). Absence of tumor suppressors may confer a similar pro-cancer addiction. For example, APC, p53, and Rb have shown selective antiproliferative and growth inhibition when inserted into cells in which they have been inactivated (43). Clinical studies characterizing precancerous lesions by microarrays and other new technologies are confirming the value of inhibiting these sensitive targets (43).

Infection/inflammation and vaccines

Abundant epidemiologic (47–50) and experimental (51–54) data implicate infection and inflammation as factors in neoplastic progression via production of oxygen and nitrogen radical oxidants, production of growth-promoting cytokines, tumor suppressor inhibition, and stimulation of signal transduction pathways. Some of the prominent infective carcinogens are human papillomaviruses (HPV; ref. 55), EBV (56), human herpesvirus-8 (57), human hepatitis viruses [hepatitis B virus (HBV) and hepatitis C virus (HCV; ref. 58)], schistosomes (59), and *Helicobacter pylori* (60, 61). Because of the frequency of these infections, their prevention or treatment is a potentially fruitful cancer prevention strategy (Fig. 1). Progress in the development of anti-infectives and vaccines that target the carcinogenic mechanisms of these agents has been significant (60). For example, HBV and HCV infections are prominent causes of chronic liver disease, including hepatocellular carcinoma, one of the most common cancers worldwide (58). HCV infection may also be a risk factor for other cancers, including non-Hodgkin's lymphoma and multiple myeloma (62). EBV is a ubiquitous human herpesvirus that is associated with a spectrum of malignant diseases, including Burkitt's lymphoma and nasopharyngeal carcinoma (63).

A major recent advance in chemoprevention research is the development of treatment and prevention vaccines for HPV (55). HPV infections are a leading cause of virus-associated cancers of the anogenital, oropharyngeal, and cutaneous epithelium, attributed to the viral oncogenes E6 and E7 (64, 65). There are >100 different subtypes of HPV known; of these, at least 15 are known high-risk types associated with cervical cancer, with HPV-16 being the dominant type in most parts of the world (55, 64, 66). Approximately half of all tonsillar cancers contain HPV; epidemiologic and molecular pathology studies have suggested that HPV infection may also be associated with other head and neck cancers (55, 67). Many

nonmelanoma skin cancers, especially cutaneous squamous cell carcinoma, contain HPV DNA (30–60%; ref. 55). Novel approaches to the production of virus-like particles (VLP) in plants and second-generation vaccine approaches, including viral and bacterial vaccine vectors as well as DNA vaccines, are being examined.

Prevention of HPV infection can be achieved by induction of capsid-specific neutralizing antibodies. One of the most advanced vaccines of this type is Gardasil, an experimental vaccine targeting the four most common strains of sexually transmitted HPV that cause cervical cancer or genital warts (68, 69). This vaccine was 89% effective in preventing infection with the viral strains and 100% effective in preventing precancerous lesions [cervical IEN (CIN)] or genital warts in a phase II trial. In a phase III trial, Gardasil raised antibodies in >99% of 1,529 people who received a three-dose regimen over a 6-month period. The long-term effects on prevention of cervix cancer and the applicability to third world countries where cervix cancer is particularly prevalent are under evaluation.

Treatment of bacterial infection with antibiotics, thus reducing associated chronic inflammation, is another promising chemopreventive strategy exemplified by treatment of *H. pylori*. The 2005 Nobel Prize in medicine was awarded to Barry J. Marshall and J. Robin Warren for their discovery of the role of *H. pylori* in gastritis (inflammation of the stomach), gastric ulcers, and more severe lesions resulting from chronic inflammation. Individuals with gastric atrophy and intestinal metaplasia are at risk for developing cancer of the stomach, and chronic *H. pylori* infection is one of the most important factors in the development of these precancerous gastric lesions (61). In addition to bacterial factors, polymorphisms in the host cytokine genes that modulate inflammatory responses have a synergistic effect on the development of gastric cancer and precancerous lesions. Individuals with a positive family history of gastric cancer and/or proinflammatory polymorphisms of the interleukin-1 and tumor necrosis factor- α genes and who are infected by *H. pylori* virulent strains (cagA-positive, vacA s1-positive, vacA m1-positive, and babA2-positive) have been found to have a high risk of gastric cancer development.

Inflammation and oxidation

Inflammation not associated with infection may be associated with cancer risk (11, 51, 70–74) in gastrointestinal tract (53, 75), bladder (76, 77), skin (78), lung (78–80), head and neck (81), breast (82, 83) and prostate (84, 85). Moreover, agents modulating molecular targets of inflammation, such as COX (53, 86), inducible nitric oxide synthase (87, 88), and lipoxygenase (LOX; refs. 89, 90), have shown promising chemopreventive activity. Particularly, COX inhibitors, including aspirin, traditional nonsteroidal anti-inflammatory drugs (NSAID), and COX-2 selective inhibitors, have shown chemopreventive efficacy in epidemiologic analyses as well as in clinical studies and are being evaluated in numerous cancer targets where COX-2 overexpression or inflammation is observed. Nuclear factor- κ B (NF- κ B), a protein induced during inflammation that serves as a transcription factor regulating genes for other inflammatory and tumor-promoting proteins [such as COX-2, BCL-2, interleukin-1, interleukin-6, interleukin-8, interleukin-10, tumor necrosis factor- α , LOX, inducible nitric oxide synthase, cyclin D1, cell adhesion molecules, c-MYC, matrix metalloproteinase-9, VEGF, survivin, and telomerase

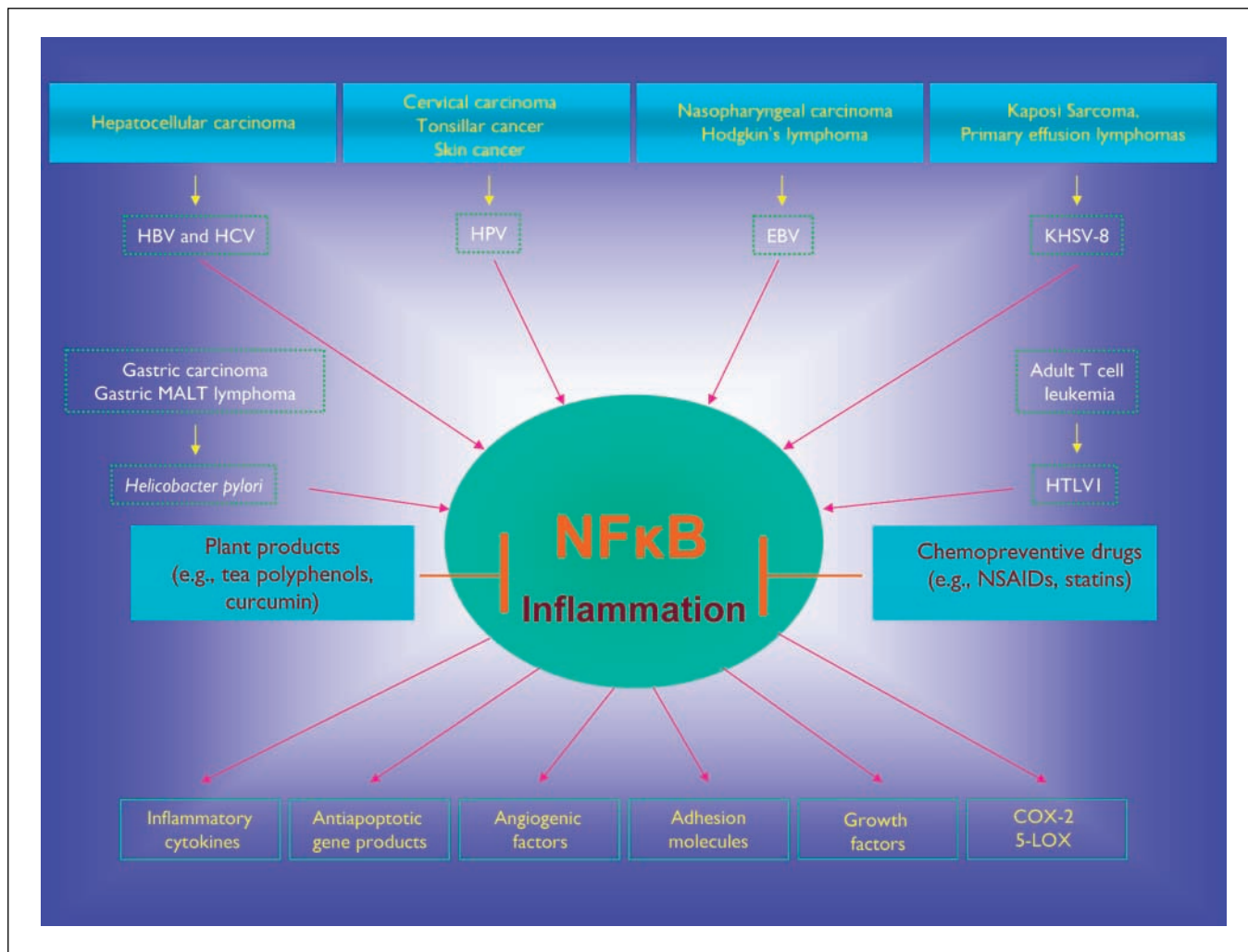


Fig. 1. Inflammation is an important target for cancer prevention and NF- κ B is an important molecular target. Evidence suggests that NF- κ B, a proinflammatory transcription factor, has a role in carcinogenesis and cancer progression. Inflammatory agents, carcinogens, tumor promoters, and the tumor microenvironment activate NF- κ B. Both NF- κ B and proteins regulated by it have been linked to cellular transformation, proliferation, apoptosis suppression, invasion, angiogenesis, and metastasis. Constitutively activated NF- κ B occurs in many tumors (27).

(hTERT)] could prove to be a key molecular target for chemoprevention (25, 27, 91).

Many natural antioxidants (e.g., green tea polyphenols, lycopene, resveratrol, curcumin, and sulforaphane) have broad-spectrum anti-inflammatory and free radical trapping activities. These agents have shown chemopreventive activity in animal models (92, 93), are associated with lower cancer risk in human studies, and seem to be good candidates for development. The green tea polyphenols are particularly noteworthy. These catechins have shown potential chemopreventive activity in numerous animal models (92). Moreover, a recent clinical study showed that tea could potentially prevent prostate cancer in men with HGPIN (94). The characterization of molecular targets of antioxidant activity has been difficult because of the pleiotropic activities of these agents. Attention is now being directed to Nrf2, a transcription factor that activates genes with products involved in deactivating electrophilic toxic compounds. Nrf2 is sequestered by Keap1 until antioxidant inducers cause a conformational change in the Nrf2-Keap1 complex and release Nrf2, which then interacts with an

antioxidant response element to induce antioxidant gene expression (95–98). Nrf2 can also be activated by phosphorylation [e.g., via signal transduction involving mitogen-activated protein kinase (MAPK), protein kinase C, or PI3K]. Synthetic antioxidants (e.g., oltipraz, CDDO, and its derivatives) also have potential chemopreventive activity via these pathways (97, 98).

Epidemiologic and experimental evidence

Epidemiologic data associating cancer preventive activity or cancer incidence with the use of certain drugs, foods, lifestyles, or the presence of germ-line mutations or gene variations historically have been a primary means for identifying possible molecular targets for chemoprevention. Combining epidemiologic leads with experimental data provides a rationale for use of these targets in chemoprevention. For example, numerous studies associate lower incidence of colon cancers, colorectal adenomas, and colorectal cancer mortality with use of aspirin and NSAIDs (86, 99). These data led to the exploration of COX inhibition, the primary mechanistic activity of these drugs, for

chemoprevention. The compelling body of epidemiologic, experimental, and mechanistic evidence implicating estrogen in promotion of breast and other cancers (100, 101) led to the development of anticancer drugs, such as tamoxifen and exemestane, targeting the ER and steroid aromatase, respectively. Hormonal contraceptives induce at least a 50% decrease in development of life-threatening ovarian cancers. Epidemiologic data associating vitamin A and carotenoids with reduced cancer incidence (102) led to the identification of retinoid receptors as molecular targets for prevention, and epidemiologic data on the association of IGF with cancers of the breast, prostate, and lung raised interest in the IGF signaling pathway as a target for chemoprevention (34, 35, 103). Data associating statin usage with reduced risk of colon, prostate, and breast cancers and melanoma (104, 105) provide valuable leads that are being followed up with experimental investigations, both clinical and preclinical to elucidate the target mechanism(s) for this observed effect. Recent reports of large studies and meta-analyses that show no association between statin usage and cancer risk illustrate the complexity of interpreting epidemiologic data (106–109).

Collateral targets of mechanistically targeted drugs

Collateral targets for chemopreventive agents are molecules in signal transduction and metabolic pathways or networks that are upstream or downstream of the direct target of the agent. These indirect targets are also associated with neoplastic progression, possibly more directly than the mechanistic target. Mechanism-based chemoprevention strategies may involve collateral targets in several ways—that is, in combinations of agents to increase efficacy or reduce toxicity of the individual agents, as new direct mechanistic targets for identifying potential chemopreventive agents, and as chemopreventive targets for which the mechanistic targets of agents are surrogates (where it is difficult to design agents that modulate the chemopreventive target). For example, aromatase can be considered a collateral target of COX-2 in that inhibition of prostaglandin synthesis also inhibits prostaglandin-mediated induction of aromatase, so use of NSAIDs in combination with aromatase inhibitors may allow lower doses of aromatase inhibitors and reduced toxicity in prevention of breast cancers (5, 51). Interaction of COX-2 and EGFR is described in Combination strategies. Chan et al. have suggested arachidonic acid as a collateral and alternative target to COX-2, because inhibition of COX-2 raises the level of cellular arachidonic acid, thereby potentially activating ceramide-mediated apoptosis (110). In addition, evidence that telomerase, which is activated early in prostate carcinogenesis, is regulated by ER and is thus a collateral target of ER (111), provides a rationale for exploring ER as a molecular target for chemoprevention in prostate.

Combination strategies

A major aspect of molecular carcinogenesis research is the identification of multiple targets for combinations of drugs that may have greater efficacy than would single agents. Agent combinations targeting the EGFR and COX-2 signaling pathways exemplify combined-agent development for cancer prevention. The independent and interactive signaling of EGFR and COX-2 has been shown in lung, head and neck, and colon carcinogenesis. Several processes linked to carcinogenesis (cell

proliferation, apoptosis, angiogenesis, and invasiveness) can be influenced by the stimulation of EGFR signaling or enhanced synthesis of prostaglandin E₂ (PGE₂).

Data supporting the cross-talk and potential feedback loops between EGFR and COX-2 strengthens the rationale for combination regimens aimed at both targets (refs. 4, 53; Fig. 2). Mutually independent EGFR and COX-2 effects also are important to the potential efficacy of combined inhibitors of these targets. EGFR and its downstream effectors can be activated independently of COX-2/PGE₂, and COX-2/PGE₂ and its downstream effectors can be regulated independently of EGFR signaling (53). For example, PGE₂ can stimulate cell proliferation by an EGFR-independent mechanism (112). Illustrating the potential benefit of independent plus interactive effects, combined inhibitors of COX and EGFR tyrosine kinase almost completely prevented adenoma development in APC(Min) mice (113) and subsequently were shown to be active in a head and neck cancer xenograft model (114).

These prevention and therapy studies highlight the recent convergence of cancer prevention and therapy at the level of early-phase drug development (115). Similar abnormalities are found in both IEN and cancer (Table 2). Many of the molecular and biochemical events leading to increased proliferation and reduced apoptosis in IEN and early invasive cancer also give cancer cells the ability to invade and metastasize. Therefore, many of the molecular targets relevant to advanced cancer are also relevant to precancer, supporting the early assessment of novel drugs for both prevention and therapy. These targets are potentially useful in all phases of chemopreventive agent development (Table 3).

Although not as mature as combined targeting of EGFR and COX-2, other combinations also are supported by strong preclinical data. For example, matrix metalloproteinase inhibitors modulate the migration, invasion, and/or proliferation of mesenchymal cells and may be effective in combination with EGFR and/or COX-2 inhibitors in the setting of dysplastic oral IEN; farnesyl transferase inhibitors enhance the apoptotic activity of IGF-binding protein-3 *in vitro* and *in vivo* (116); peroxisome proliferator-activated receptor- γ ligands enhance histone deacetylase inhibitor activity (117); combined inhibition of IGF-I and mTOR inactivates a potent feedback loop (118), and the combination of a histone deacetylase inhibitor with a DNA methyltransferase inhibitor is highly active *in vitro* and *in vivo* (119). Single agents targeting each of these classes of molecules are at various stages of clinical development and show promise for combination approaches.

Chemoprevention in Major Cancer Target Organs—Update

Following the work of Vogelstein for colon cancer, genetic data have been developed from IEN histopathologic lesions creating the concept of “molecular IEN.” These molecular lesions can precede the histopathologic abnormality and like IEN can contribute to risk assessment and cohort selection. These lesions can also provide leads for molecularly targeted therapy. In this target organ section, an update of the molecular data since the 2002 publication is provided (Fig. 3) as well as a summary of significant completed trial data, new agents under development, and a discussion of promising future clinical studies.

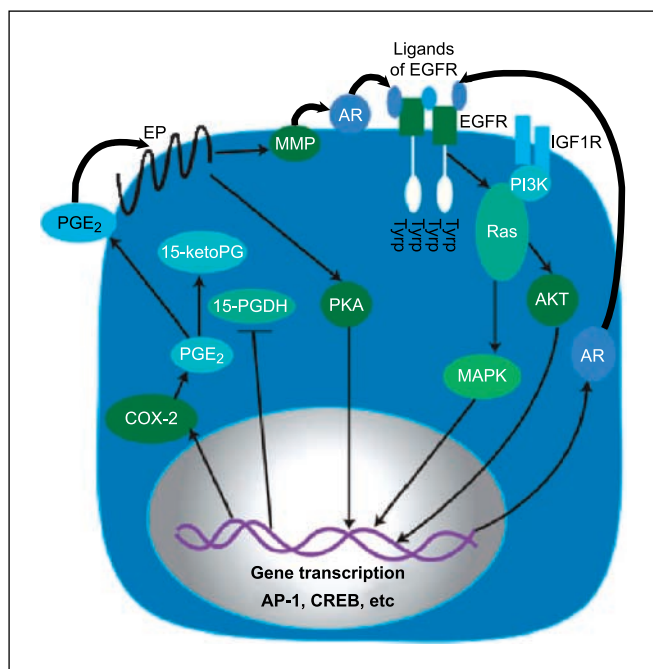


Fig. 2. Cross-talk between EGFR and COX-2 pathways. Activation of EGFR by ligands, including amphiregulin (AR) stimulates MAPK activity, resulting in activator protein-1 (AP-1) – mediated induction of COX-2 transcription and enhanced synthesis of PGE₂ (4). EGFR signaling also inhibits the expression of 15-hydroxyprostaglandin dehydrogenase (15-PGDH), which catabolizes PGE₂ and is suppressed in several tumor types (400–402). PGE₂, in turn, can activate EGFR signaling by a PGE₂ receptor (EP receptor) – dependent mechanism by stimulating the synthesis and release of EGFR ligands. For example, PGE₂ can stimulate protein kinase A (PKA) activity, resulting in cyclic AMP – responsive element binding protein (CREB) – mediated activation of amphiregulin transcription (403). Additionally, PGE₂ can stimulate matrix metalloproteinase activity, leading to release of amphiregulin from the plasma membrane (404). Finally, PGE₂ can transactivate EGFR via an intracellular Src-dependent mechanism (405). Recent data in non – small cell lung cancer cells indicate that PGE₂ can suppress the expression of E-cadherin (a hallmark of the epithelial-to-mesenchymal transition; ref. 406). EGFR tyrosine kinase inhibitors seem to be more active in tumor cells with epithelial properties (e.g., E-cadherin expression; ref. 406). These data suggest that COX-2 inhibitors (by suppressing PGE₂ synthesis and thereby up-regulating E-cadherin) might enhance EGFR tyrosine kinase inhibitor activity (407). Reprinted with permission (407).

Prostate cancer

In 2005, prostate cancer was expected to be diagnosed in 232,090 men and to cause 30,350 deaths, making this the most common and second deadliest cancer in U.S. men (ref. 120; Table 4). The lifetime risk of American men dying of prostate cancer is ~2% to 3%, but the prevalence of latent prostate cancer detected in autopsy series is ~30% (1, 121, 122). The introduction and rapid dissemination of prostate-specific antigen (PSA) screening between 1986 and 1992 caused an increase in the overall age-adjusted incidence of prostate cancer likely due to lead time bias, but thereafter this apparent increase in incidence declined. There is no clear evidence that early detection has reduced prostate cancer mortality because this rate has not declined in association with the use of PSA screening (123).

The natural history of prostate cancer, including prostate premalignancy, is poorly understood (124); however, cellular morphologic changes, such as PIN, are readily identifiable and can be correlated with certain genetic alterations. These alterations include the loss of certain chromosomal regions, their candidate tumor suppressor gene products Nkx3.1 (125,

126) and PTEN (127–129) and cell cycle regulatory genes, such as p27^{kip1} (130). Higher glutathione S-transferase P1 CpG island DNA methylation (131), which stops enzyme activity and other effects resulting from epigenetic changes, are thought to be associated with oxidative stress and the initiation of prostate cancer (132).

Implicated in the multiple pathways that lead to carcinogenesis, proliferative inflammatory atrophy (PIA) is a result of inflammation and dietary influences (85, 133). PIA effects include increased cell death and regenerated cells with DNA damage. It is believed that prostate carcinogenesis progresses from normal epithelium to PIA to PIN to HGPIN and finally to cancer. PIA also is associated with glutathione S-transferase P1 and COX-2 expression. A PIA pathology consensus conference has completed a standardized classification system for PIA lesions, and the future publication of this system should enhance comparisons of PIA research results from different groups and advance the design of preventive interventions.

Spectral imaging and other newer techniques should be investigated and implemented for identifying and characterizing alterations during prostate carcinogenesis. The development of quantitative tools will overcome subjective interpretations and accelerate the understanding of gene regulation within prostate carcinogenesis. New tools for this work include genomics, transcriptional profiling, and proteomics along with novel bioinformatics approaches (such as gene set enrichment analysis cited above). When combined with well-characterized, diverse, and ample clinical samples with long-term clinical follow-up, these tools could lead to a clearer understanding of the molecular biology of prostate cancer.

Completed in 2003, the National Cancer Institute (NCI) – funded Prostate Cancer Prevention Trial was the first large-scale phase III prostate cancer prevention study (134), randomizing 18,882 subjects to receive finasteride (a 5 α -reductase inhibitor) or placebo. The prevalence of prostate cancer over a period of 7 years was 24.8% lower in the finasteride than placebo arm (95% confidence interval, 18.6-30.6; $P < 0.001$). Tumors of a higher grade (Gleason 7-10) were detected 1.25 times more often in the finasteride arm (6.4% of graded tumors) than in the placebo arm (5.1%; $P < 0.001$). Speculation on the reason for the higher grades in the finasteride arm include the possibility that finasteride increases the risk of high-grade cancer through changes in intraprostatic androgen and/or

Table 3. Biomarkers can and should be applied throughout the drug development process for novel chemopreventive agents

- Identify and validate therapeutic targets
- Screen and optimize candidate targeted agents
- Provide proof-of-concept for agents and models
- Enhance mechanistic understanding of drug or drug combination effects (e.g., as clear indicators of target engagement, cell death, and changes in tumor biology)
- Identify optimal target populations
- Predict response, resistance, and toxicity
- Rapidly distinguish responders from nonresponders
- Once validated, serve as surrogate end points for clinical benefit to support drug approvals

estrogen signaling. Higher Gleason grades have been detected in tumors of men with lower testosterone levels (versus normal levels; refs. 135–138), possibly reflecting effects of lower dihydrotestosterone levels, which also occur with finasteride. It also is possible that the increased high-grade disease with finasteride was more apparent than real, either because finasteride caused tumor cell morphologic changes that mimic higher Gleason scores or because finasteride significantly shrank prostate volume, raised the tumor-to-gland ratio (135–137, 139), and thus improved the detection of high-grade tumors. Efforts to explain the high-grade Prostate Cancer Prevention Trial results include extensive analyses of tumor specimens and a NCI-supported follow-up study of long-term outcomes of men diagnosed with high-grade tumors (140).

The Selenium and Vitamin E Cancer Prevention Trial is a large-scale NCI-supported Prostate Cancer Prevention Trial that met its accrual goal with the randomization of 35,534 men between July 2001 and June 2004 (141). Selenium and Vitamin E Cancer Prevention Trial has a prospectively collected

biorepository designed for future research, such as the development of comprehensive models for identifying men at the greatest risk of prostate cancer (especially high-grade prostate cancer) and most likely to benefit from chemoprevention with selenium and vitamin E.

Promising new agents for prostate cancer prevention include antioxidants (e.g., tea polyphenols), SERMs (e.g., toremifene), NSAIDs, soy isoflavones, and statins. For example, recent observational studies suggest that statins are associated with reduced prostate cancer risk. A study involving 34,438 men within the ongoing prospective Health Professionals Follow-up Study (142) indicated that statins and other cholesterol-lowering drugs were associated with a significant 46% reduced risk of advanced prostate cancer, and this association got stronger with longer drug use ($P = 0.008$). Risks of metastatic and fatal disease were reduced 66%. This study did not find an association with reduced overall prostate cancer incidence. Statins were the most likely active agents because they constituted 90% of all cholesterol-lowering treatment at the

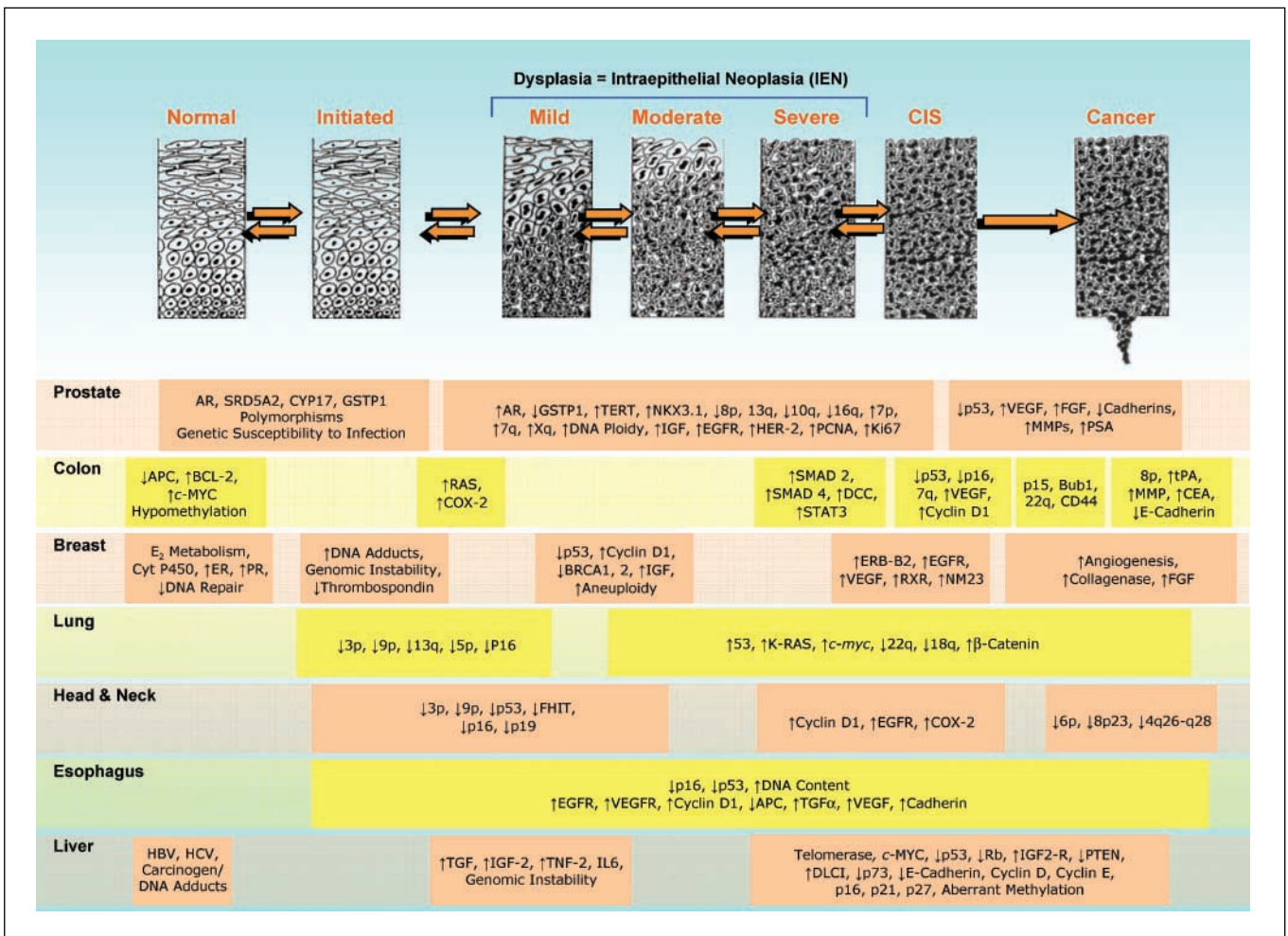


Fig. 3. Molecular biomarkers of carcinogenesis: genetic progression in major cancer target. Carcinogenesis is driven by genetic progression. This progression is marked by the appearance of molecular biomarkers in distinctive patterns representing accumulating changes in gene expression and correlating with changes in histologic phenotype as cells move from normal through the very early stages of precancer, through more severe precancer, to early cancer and finally through early invasive, locally advanced, and metastatic cancer. The figure shows candidate molecular biomarkers of genetic progression in seven target organs: prostate (133, 408, 409), colon (16, 99), breast (1, 410), lung (260–262), head and neck (292–294, 299), esophagus (320, 326, 329), and liver (351). In most tissues, the earliest biomarkers are changes in expression of tumor suppressors and oncogenes, with biomarkers associated with proliferation and uncontrolled growth (e.g., cyclin D1) and invasion usually emerging later. Figure 4 illustrates the elegant work of Reid et al. in describing gene expression changes in development of Barrett's esophagus and how this progression provides opportunities for identifying subjects at risk as well as for intervention.

Table 4. Major cancer target organs and clinical cohorts for evaluation of cancer chemoprevention strategies

	Prostate	Colon	Breast
Cancer burden	In the U.S., most common cancer in men: 32.7% (232,090) of total new cancer cases in men (estimated 2005), 10.3% (30,350) of cancer deaths in men (estimated 2005) 30% Prevalence of latent disease (from autopsy data)	In the U.S., fourth most common cancer overall: 11% (145,000) total new cases; 10% (56,000) total associated deaths (estimated 2005) Estimated 6% of U.S. population will develop invasive colorectal cancer over their lifetimes	In the U.S., most common cancer in women: 15% (211,240) of total new cancer cases, 7% (40,410) of cancer deaths, 15% of cancer deaths on females (estimated 2005)
Clinical cohorts/ end points	Prostate cancer patients scheduled for radical prostatectomy (treated between diagnostic biopsy and surgery, ~ 4-6 wk)/biomarker modulation Patients with HGPIN and other risk factors at high risk for developing prostate cancer (40% over 3 y, 80% over 10 y)/ prostate cancer incidence Patients with organ-confined prostate cancer undergoing watchful waiting (no prostatectomy, radiation or chemotherapy)/biomarker modulation to correlate with clinical end points; time to disease progression Men at high risk (e.g., PSA >4 ng/mL and negative biopsy)/prostate cancer incidence Men at normal risk (age ≥55 y, normal PSA and DRE)/ prostate cancer incidence	FAP patients (treated for ≥6 mo)/prevention or regression of polyps HNPCC patients/carriers (treated for ≥1 y)/biomarker modulation and prevention of colorectal cancers Patients with previous colon cancer or adenomatous polyps (treated for 3 y/ treated and/or followed for up to 6 y)/adenomatous polyp incidence FAP carriers (treated during adolescence)/prevention or delay of polyp occurrence	Patients scheduled for breast cancer surgery (treated between diagnostic biopsy and surgery, ~ 4-6 wk) /biomarker modulation Patients with LCIS or mammographically detected calcifications (DCIS; treated for ≥1 y)/ biomarker modulation, breast cancer incidence High risk with multiple biomarker abnormalities (treated for ≥1 y)/ biomarker modulation, breast cancer incidence Women ≥60, or 35-59 y old with Gail risk factors for 60 y old/breast cancer incidence Patients with previous breast cancer (adjuvant setting)/ breast cancer incidence

(Continued on the following page)

end of study, when the strongest risk reductions were observed. In a case-control study conducted within the Veterans Affairs system, there were significant inverse associations between statin use and prostate cancer, and the inverse associations strengthened with high-grade prostate cancer and longer statin use (143). A significant, duration-dependent inverse association between statin use and prostate cancer risk also was found in another Veterans Affairs case-control study (144). These results are supported by the limited available preclinical data on the effects of statins in prostate carcinogenesis (145). In contrast, as noted above, two recent epidemiologic studies have shown no effect of statins on cancer incidences at multiple

sites, including prostate (106, 107). Tea polyphenols (94) and toremifene (146-148) have both prevented progression of HGPIN to prostate cancer in phase II clinical trials; NSAIDs and soy isoflavones are under evaluation in phase II models.

There is a need for more efficient clinical evaluation of chemopreventive agents that lead to promising models involving serial biopsy, including high-risk men, men with HGPIN, watchful waiting in low-grade cancer patients, and preprostatectomy models. The international, multicenter, double-blind, placebo-controlled Reduction by Dutasteride of Prostate Cancer Events trial is obtaining serial biopsy specimens from at-risk patients being treated with dutasteride or placebo

Table 4. Major cancer target organs and clinical cohorts for evaluation of cancer chemoprevention strategies (Cont'd)

	Lung	Head and neck	Esophagus	
Cancer burden	In the U.S., second most common cancer and leading cause of cancer deaths in men and women; 13% (172,570) of total new cancer cases, 29% (163,510) of cancer-related deaths (estimated 2005)	In the U.S., represents 3% (39,250) of total new cancers, 2% (11,090) of cancer-related deaths (estimated 2005)	In the U.S., all esophageal cancers represent 1% (14,520) of all cases and 2% (13,570) of cancer-related deaths; ~60% of new esophageal cancers are adenocarcinomas (Barrett's dysplasia is precursor)	
Clinical cohorts/ end points	Chronic/former smokers at high risk (e.g., with squamous metaplasia/dysplasia)/biomarker or dysplasia modulation Patients with recently resected stage I lung or laryngeal cancer/lung cancer incidence Men exposed to asbestos or patients with asbestosis, who are chronic or heavy cigarette smokers/lung cancer incidence Patients with previous lung and head or neck cancers/ second primary cancers	Patients with dysplastic leukoplakia/dysplasia regression, oral cancer incidence Patients with previous head and neck cancers/ second primary cancers Subjects at high risk (e.g., smokers and tobacco chewers)/ head and neck cancer incidence	Patients with low-grade, intestinal type Barrett's esophagus with or without dysplasia/Barrett's progression Patients with high grade Barrett's esophagus/Barrett's progression, esophageal cancer incidence Patients at high risk for esophageal adenocarcinoma (e.g., gastroesophageal reflux disease)/esophageal cancer incidence	
	Liver	Cervix	Ovary	Endometrium
Cancer burden	In the U.S., 1% (17,550) of all cancers and rising and 3% (15,420) of cancer-related deaths; much more important worldwide (estimated 2005)	In the U.S., 1% (10,370) of total new cancers in females and 1% (3,710) of total cancer-related deaths in females; much more important worldwide (estimated 2005)	In the U.S., 3% (22,220) of total new cancers in females and 6% (16,210) cancer-related deaths in females (estimated 2005)	In the U.S., 6% (40,880) of total new cancers in females and 3% (7,310) of total cancer-related deaths in females (estimated 2005)
Clinical cohorts/ end points	Patients with previous hepatoma/liver cancer incidence Subjects with environmental exposure (e.g., HBV/HCV or carcinogen)/DNA adducts, biomarker modulation, liver cancer incidence	Patients with CIN III, patients with CIN I, II (sufficiently large lesion)/ CIN regression Patients with HPV infection but without CIN/CIN incidence, cervical cancer incidence High-risk subjects/ prevention of HPV infection	High-risk women (e.g., family history of breast/ovarian cancer, BRCA mutation, Ashkenazi Jewish descent)/ biomarker modulation (e.g., spectral karyotyping)	High-risk women (e.g., HNPCC syndrome, obesity)/biomarker modulation (e.g., CAH and molecular biomarkers, such as PTEN and microsatellite instability)

(149). These patients are established to be free of cancer by a negative 6- or 12-core biopsy taken within 6 months of enrollment and are being monitored for type 1 and 2 5 α -reductase, which are increased in association with prostate

carcinogenesis (150) and are inhibited by dutasteride. The serial biopsy model is expected to help improve the understanding of the natural history of prostate cancer and to facilitate detecting correlations between clinical and pathologic

data. This model also is expected to cut the expenses and accelerate the advances of preventive drug development.

Available data support the conclusion that the presence of PIN on prostate biopsy predicts for an increased risk for prostate cancer and that some PIN lesions give rise to prostate cancers. Thus, PIN lesions detected on prostate biopsy identify men at high risk for developing prostate cancer. However, the limitations of prostate biopsy sampling preclude repeated monitoring of PIN lesions to assess their natural history (138). When a diagnosis of HGPIN is combined with other risk factors, such as serum PSA, age, race, and/or family history, cohorts of men at very high risk for developing prostate cancer are identified who have prostate cancer incidence rates of 40% over 3 years and 80% over 10 years (151, 152). Because extent of PIN cannot be reliably measured by serial sampling, a decrease in the extent of PIN with treatment is not a conclusive efficacy end point. Thus, clinical trials targeted at eliminating or reducing the extent of PIN are not likely to show net clinical benefit without additional data indicating that prostate cancer incidence (risk) has been reduced. Because prostate cancer incidence can be estimated in cohorts of patients with HGPIN, PSA abnormalities, and other risk factors, phase III placebo-controlled trials that have prostate cancer incidence as the primary end point can be conducted with 300 to 500 patients per arm, with the control group having an expected 40% prostate cancer incidence over 3 years. This trial design will more definitively evaluate prostate cancer risk-reduction candidates and will validate extent of HGPIN as a suitable efficacy end point (1, 133, 151, 152). A 30% reduction in prostate cancer incidence in the HGPIN patients who are safely treated with the new agent compared with control patients should likely constitute clinical benefit.

The watchful waiting model involves an arm of no surgery in the setting of low-risk, localized prostate cancer, which is known for its lengthy indolent phase. Consenting patients are put under surveillance with no treatment and evaluated against patients undergoing immediate treatment for organ-confined disease. Surveillance is conducive to embedding phase II chemopreventive (or therapeutic) end points designed to identify potential preventive biomarkers and correlate them with clinical outcomes. The primary objective of surveillance trials is to determine if men with organ-confined disease can avoid or postpone the financial, emotional, and morbidity (e.g., sexual dysfunction) costs of treatment without developing a worse outcome (e.g., progression to advanced disease; refs. 137, 153–156). Furthermore, a 10-year follow-up of a Swedish study of 695 men showed significant differences favoring surgery over watchful waiting in overall mortality (27.0% versus 32.0%; $P = 0.04$), disease-specific mortality (9.6% versus 14.9%; $P = 0.01$), local progression (19.2% versus 44.3%; $P < 0.001$), and distant metastasis (15.2% versus 25.4%; $P = 0.004$; refs. 153, 156). Additional research is needed to determine if these outcomes would parallel outcomes in the United States, where men typically are diagnosed at an earlier stage than were the men in the Swedish study (157) and where different ethnic and behavioral issues may be involved. The ongoing Prostate Cancer Intervention versus Observation Trial is expected to determine whether the Swedish results will be replicated in United States patients (158).

The preprostatectomy model examines effects of chemopreventive agents on biomarker end points in the short interlude between histologic diagnosis and prostatectomy. After

prostatectomy, the whole gland can be examined to help define zonal patterns of prostate carcinogenesis and delineate cell-type characteristics and prostate cancer precursor lesions (159). The short intervention duration (typically from a few days to a month) causes problems for detecting and interpreting biomarkers and meeting accrual goals and creates statistical demands on the biomarker end points that recently have been the subject of systematic investigation, including the development of proteomic end points based on comparison of study patients with subjects without prostate cancer or intervention (160). Efforts at standardization will help meet the accrual goals and statistical demands of evaluating biomarker end points, lessening the risk of false-negative findings in these brief intervention trials. Development and validation of effective molecular imaging approaches that allow assessment of changes during treatment will also facilitate this trial design.

Colorectal cancer

Colorectal cancer is the third most common cancer in both men and women, constituting 10% of new cancer cases in men and 11% in women, and it is the second most common cause of death from cancer in the United States (Table 4). In 2005, there were an estimated 145,000 new cases in the United States and 56,000 related deaths (a rate second only to that of lung cancer; ref. 120). Although the incidence of colorectal cancer decreased between 1998 and 2001 (annual percent change -2.4%) in the United States, $\sim 6\%$ of Americans will eventually develop invasive colon or rectal cancer, and >6 million Americans who are alive today will die of the disease (an individual's lifetime risk of dying from colorectal cancer in the United States has been estimated to be 2.5%). Globally, it is the fourth most common cancer in men and the third most common in women, with mortality paralleling incidence. In the year 2002, there were >1 million new cases of colorectal cancer worldwide (161). Despite evidence that 5-year survival is 90% when colorectal cancer is diagnosed at an early stage, $<40\%$ of cases are diagnosed when the cancer is still localized.

Screening has become a compelling strategy for prevention of colorectal disease. Current evidence indicates that screening for colorectal cancer reduces mortality. This has prompted the U.S. Preventative Services Task Force, the American Cancer Society, the Agency for Health Care Policy Research, and other agencies to recommend that average-risk individuals (those without a family history of colorectal neoplasia or other predisposing conditions, such as inflammatory bowel disease) be screened for colorectal cancer beginning at age 50 years (162, 163). Current evidence-based guidelines provide a menu of options for screening, which includes fecal occult blood testing yearly, flexible sigmoidoscopy every 5 years, the combination of fecal occult blood testing and flexible sigmoidoscopy, colonoscopy every 10 years, or air-contrast barium enema every 5 years. The concept of a menu of choices does not imply equal performance characteristics but rather reflects the need to increase screening compliance. Because millions of individuals yearly in the United States reach screening age, there is a growing need for cost-effective, compliance-driven strategies for screening. Computed tomography (CT) colonography and stool-based genetic testing have recently been explored as options.

CT colonography or "virtual" colonoscopy involves the use of helical CT to generate high-resolution, two-dimensional

images of the abdomen and pelvis. The accuracy and potential of virtual colonoscopy as a screening tool for colorectal neoplasia has been hotly debated because initial studies yielded a wide range of sensitivity. Large recently published multicenter trials continued to fuel this controversy (164, 165). Several key issues will need to be addressed as the use of virtual colonoscopy becomes more widespread. Principal among these is determination of the acceptable size cutoff of a lesion detected by virtual colonoscopy that will necessitate a follow-up colonoscopy.

Specific genetic tests are not currently available for the majority of patients at risk for developing colorectal cancer. A molecular approach to colorectal cancer screening is attractive because it targets genetic changes that are fundamental to the neoplastic process. The feasibility of detecting altered DNA in stool has been shown using a multitarget assay panel of molecular markers (166). A recent multicenter study compared fecal DNA testing with fecal occult blood testing and colonoscopy (167). Although the majority of lesions identified by colonoscopy were not detected by either noninvasive test, multitargeted fecal DNA testing detected a higher proportion of important lesions compared with Hemoccult. Risk models based on these factors are under development (168).

Most sporadic colorectal cancers are believed to develop from a precursor IEN, sporadic adenomas (86, 99). The adenoma-to-carcinoma sequence describes the common pathway taken by neoplasms that arise as the result of the progressive accumulation of genetic changes, which may include alterations in proto-oncogenes, loss of tumor suppressor gene activity, and abnormalities in genes involved in DNA repair. These changes commonly involve chromosomal instability with widespread chromosomal deletions, duplications, and rearrangements that produce aneuploidy (169, 170). Alternatively, increased rates of mutation, often in tandemly repeated DNA sequences known as microsatellites (microsatellite instability) or a form of epigenetic instability called the CpG island methylator phenotype, in which genes are inappropriately silenced by promoter methylation (171), are mechanisms that can lead to progressive multistep carcinogenesis (Fig. 3). Subtle alterations in the regular pattern of the intestinal crypts known as aberrant crypt foci (ACF) are one of the first histologically detectable changes that may be associated with development of colorectal cancers. ACF seem to arise as the result of premalignant genetic alterations; they often show *APC* loss and *K-ras* mutations. The number, size, and dysplastic features of ACF correlate with the number of adenomatous polyps (adenomas). The stool-based genomic panel cited above targets 19 alterations associated with colorectal neoplasia (including mutational hotspots on *K-ras*, *APC*, and *p53* as well as long-fragment DNA).

Over the past several years, several nutrition and drug prevention trials have been completed, including studies of fiber, calcium, and NSAIDs. The potential benefit of low-fat, high-fiber diets based on descriptive epidemiology and case-control studies has generally been accepted, but current data from prospective human trials are thus far equivocal or negative. Two large randomized trials that examined the effects of fiber supplementation on adenoma recurrence failed to show a chemopreventive effect (172). Recently, a prospective double-blind, placebo-controlled trial showed that supplemental calcium (3 g/d calcium carbonate equivalent to 1,200 mg elemental calcium) reduced the incidence and number of recurrent adenomas in subjects with a recent history of these

lesions (173). The effect of calcium was modest (19% reduction in adenoma recurrence and 24% reduction in the number of adenomas over 3 years).

The most promising results come from trials using aspirin and NSAIDs for colorectal cancer prevention. Case-control and cohort studies suggested that the risk for developing of adenoma and carcinoma may be substantially reduced (40-50%) among aspirin and NSAID users compared with controls. A double-blind, placebo-controlled trial studied the effects of celecoxib, a selective COX-2 inhibitor, on colorectal polyps in patients with familial adenomatous polyposis (FAP). Treatment with high doses of this agent for 6 months was associated with a significant reduction from baseline in the number of colorectal polyps compared with placebo (28.0% versus 4.5%; $P = 0.003$; ref. 174). The reduction in polyps was mirrored by the polyp burden representing the sum of polyp diameters. This led Food and Drug Administration (FDA) to approve this drug as an adjunct to standard therapy in patients with FAP. In the sporadic setting, three recently published trials showed that aspirin reduced adenoma recurrence. The magnitude of the effect varied depending on the magnitude of risk in the group studied. Data from a large randomized prospective trial using aspirin in patients with sporadic adenomas showed a modest but significant effect of low-dose (81 mg) aspirin on adenoma recurrence of ~19% (175). A 45% risk reduction in adenoma recurrence was shown using 325 mg aspirin in a higher-risk group of patients with previous colorectal cancer. Data from three large randomized trial, which employed COX-2 inhibitors for chemoprevention of sporadic adenomas, were presented recently. The APPROVe trial showed a highly significant 25% reduction in adenoma occurrence to be associated with intake of 25 mg rofecoxib compared with placebo during 3 years of follow-up in patients with a previous history of colorectal adenomas. Celecoxib showed even more striking efficacy in preventing sporadic adenomas in two studies in similar cohorts. In the Adenoma Prevention with Celecoxib study, in which 2,035 patients were randomized to placebo or 200 or 400 mg celecoxib b.i.d., adenoma incidence at 3 years was reduced by 45% in patients taking celecoxib compared with placebo ($P < 0.0001$; ref. 176). The Prevention of Colorectal Sporadic Adenomatous Polyps observed a similarly highly significant ($P < 0.0001$) reduction in adenoma incidence at 3 years in patients taking 400 mg celecoxib q.d. among 1,561 patients randomized in a 3:2 ratio to treatment or placebo (177). These trials and others found that the use of this class of drugs is associated with an increased cardiovascular risk (178, 179). Future trials, which use this class of agents, will need to assess the potential for risk versus benefit.

Other anti-inflammatory agents have shown promise in preclinical studies and are being evaluated in clinical studies. NO-NSAIDs are particularly interesting in this regard. They are potent chemopreventive agents in mouse models and show low toxicity in clinical settings (88, 180). An alternative downstream pathway from arachidonic acid ends with the leukotrienes. A metabolic product of an enzyme (15-LOX-1) in this pathway down-regulates peroxisome proliferator-activated receptor- δ , thereby possibly inducing apoptosis (181).

In addition to having mostly promising epidemiologic data, statins are efficacious in animal models of colorectal cancer. For example, atorvastatin alone and in combinations with other chemopreventive agents was active in the colon ACF assay

(182). Besides providing a rationale for evaluating the chemopreventive efficacy of atorvastatin in colon, these results also suggest that combinations of atorvastatin with NSAIDs may be an effective chemopreventive strategy, allowing the individual agents to be administered at subtoxic doses (182).

Because the natural history of colorectal cancer is protracted, clinical randomized trials have often concentrated on prevention of colorectal adenomas, the precursors to carcinoma. There has been recent interest in identifying earlier intermediate end points that can be used in chemoprevention trials. Magnifying endoscopy is being used to study and characterize ACF as dysplastic ACFs are thought to be precursors of adenomas in the colon (183). Standardization of techniques to identify and quantify these lesions will be crucial to the successful interpretation of intermediate end-point data. These ACF trials would be followed by the definitive adenoma prevention trials.

Breast cancer

In 2005, breast cancer was expected to be diagnosed in 212,930 women and to cause 40,870 deaths, making this the most common and second deadliest cancer in U.S. women (Table 4). Early detection from widespread mammographic screening has led to earlier diagnosis and a trend to reduced breast cancer mortality. Currently, the presence of atypia (i.e., the abnormal cytologic features primarily of increased nuclear size, abnormal shape, and variation in size or shape in cytologic or histologic specimens) in breast tissue is a known marker associated with breast cancer risk.

Because of their increased risk of developing breast cancer, individuals with abnormal breast histology, including atypical ductal hyperplasia, lobular carcinoma *in situ*, ductal carcinoma *in situ* (DCIS), and BRCA1 or BRCA2 mutations, are candidates for chemopreventive interventions. As cited above, Troester and Perou have designed a strategy for applying gene expression profiling to identifying and associating risk with breast cancer subtypes (9). Significant features of genetic progression in breast IEN (Fig. 3) were discussed in the 2002 review. Briefly, in the early stages of IEN, the expression of tumor suppressor genes is reduced partially due to methylation of gene promoter regions. The predominant phenotype in breast IEN is a progressive increase in the proportion of cells expressing ER- α along with increasing growth factors, receptor tyrosine kinase activity and expression, and diminished apoptosis. Oxidative stress and DNA damage may result in increased expression of wild-type p53 in early IEN, but mutated p53 may not appear until later stages of IEN (DCIS) and invasive cancer. Aneuploidy and LOH have been observed in early-stage IEN and seem to be progressive through late-stage IEN and invasive cancer. Twenty percent to 40% of simple and atypical hyperplasias and 80% of DCIS are found to have LOH regions that are also present in synchronous invasive breast cancers (1).

Biomarkers in breast cancer that have particular relevance to chemoprevention include the following: markers associated with neoplastic phenotypes, such as alterations of nuclear morphology and angiogenesis; expression of mRNAs or proteins likely to be required for response to putative chemopreventive agents (e.g., ERs and retinoid receptors); markers indicative of intact downstream response pathways (e.g., progesterone receptors); oncogenes and tumor suppressor genes regulated by chemopreventive agents (e.g., HER-2/*neu*, transforming growth factor- β , IGF-I, and IGF-II); and markers

of genetic instability, such as microsatellite alterations and DNA methylation in high-risk breast epithelium.

Several recent clinical trials focused on treatment of breast IEN. Fabian et al. reported results of a randomized phase II trial of oral α -difluoromethylornithine (DFMO) using imaging, serum, and urine biomarkers in high-risk women (10, 184). DFMO is an irreversible inhibitor of ornithine decarboxylase, which is a limiting enzyme of polyamine synthesis that is often up-regulated in breast cancer. Eligible women in this trial had random periareolar FNA (RPFNA) cytology that revealed hyperplasia or hyperplasia with atypia but no evidence of breast cancer on clinical examination or mammogram. The women were at high risk for the development of breast cancer based on family history and also had FNA evidence of breast IEN. One hundred nineteen high-risk women were randomized to receive 6 months of oral DFMO (0.5 g/m²/d) versus placebo and underwent repeat FNA of both breasts in a periareolar location at the completion of 6 months of treatment. There was no difference in cytology results at 6 months comparing DFMO to placebo compared with the baseline FNA results. There was also no difference between the DFMO and placebo groups for the secondary end points, including expression of proliferating cell nuclear antigen, p53, and EGFR expression. In addition, there was no difference between the DFMO and placebo groups regarding changes in mammographic breast density or serum IGF-I/IGF-binding protein-3 ratio. A modest reduction in average total urine polyamines was obtained in the DFMO group and there was no reduction in the spermidine-to-spermine ratio, suggesting that the dose of DFMO was too low to definitively affect polyamine synthesis. Although DFMO was not effective, this study design set a precedent for identification of breast IEN in high-risk women using RPFNA and for short-term intervention trials. This clinical trial design has been used to complete a randomized phase II trial of the SERM, arzoxifene versus placebo. Preliminary results from this treatment intervention trial are expected.

Fabian et al. reported the results of a phase I evaluation of biomarker modulation with arzoxifene in breast IEN and early breast cancer patients (185). Arzoxifene has potent antiestrogen activity against breast cancer cell lines and has proven antitumor activity in women with metastatic breast cancer and does not have estrogen activity in the uterus (186). In the phase I multicenter trial, women with newly diagnosed DCIS or T₁/T₂ invasive breast cancer were randomized to receive 10 versus 20 or 50 mg arzoxifene daily in the interval between diagnostic biopsy and definitive surgery. An additional group of patients was enrolled as the no-treatment control group. The phase I experience defined 20 mg arzoxifene as the optimal dose for biomarker modulation. Subsequently, 76 postmenopausal women with DCIS or early-stage breast cancer were randomized to receive 20 mg arzoxifene versus placebo daily during the interval between diagnostic biopsy and definitive surgery. In both trials, increases in serum sex hormone-binding globulin and decreases in IGF-I and the IGF-I/IGF-binding protein-3 ratio were noted. In the dose-finding portion of this study, in 45 evaluable women, decreases in proliferation indices were more prevalent in the arzoxifene-treated group than in the control group. In the 58 evaluable women in the randomized control portion of the study, a decrease in ER expression was observed with arzoxifene compared with placebo. However, no statistically significant difference in the reductions in proliferation was

observed with arzoxifene compared with placebo, a finding felt to be due to the confounding effects of the women stopping hormone replacement therapy at the time of diagnosis. This IEN and early breast cancer treatment "window of opportunity" trial showed the feasibility of conducting such trials in the United States in multiple centers with central pathology assessment and biomarker determination.

Bundred et al. reported the results of their randomized, double-blind, placebo-controlled trial of short-term treatment with gefitinib (Iressa, ZD1839) in patients with DCIS (187). They have shown previously that blockade of EGFR with gefitinib led to reduced epithelial proliferation and increased apoptosis in immunosuppressed mice who were implanted with human DCIS obtained from 16 women (188). Subsequently, 65 patients with intermediate or high-grade DCIS were treated with gefitinib, 250 or 500 mg/d, during the interval from their diagnostic biopsy to definitive surgery. Of the 49 patients assessed, reduction in Ki-67 proliferation was seen in 47% of patients and a >10% decrease in activated nuclear MAPK was seen in 54% of patients (187). The reduction in proliferation correlated with reduced expression of activated MAPK. These results suggest that gefitinib may hold promise in the treatment of intermediate or high-grade DCIS.

Guix and Arteaga et al. showed similar findings with a short course of the EGFR tyrosine kinase inhibitor erlotinib (Tarceva) in women with DCIS or early-stage breast cancer treated between the time of diagnostic biopsy and definitive surgery (189). In these studies, significant reductions in Ki-67 ($\leq 75\%$ inhibition) and substantial reductions in activated MAPK ($\geq 85\%$ inhibition) were shown after 14 days of treatment in ~50% of patients. Interestingly, erlotinib did not affect the expression of total or nuclear phosphorylated AKT, suggesting that AKT, unlike MAPK, may not be under regulation by the EGFR pathway in DCIS and early-stage breast cancer.

Fabian et al. recently reported decreased breast epithelial cell proliferation after 6 months of the aromatase inhibitor letrozole in high-risk women on hormone replacement therapy who had RPFNA evidence of hyperplasia with atypia (190). Twenty-six postmenopausal women, who were on a stable dose of estrogen with or without progestin for at least 6 months before baseline RPFNA and who had evidence of hyperplasia with atypia, were treated with letrozole 2.5 mg/d in addition to continuing their hormone replacement therapy. There were no significant increases in hot flashes or arthralgias with letrozole, although there was an increase in fatigue. At the initial report of this trial, 17 patients had completed the 6 months of letrozole and had undergone a repeat RPFNA. Of the 10 subjects who had a baseline Ki-67 value of $\geq 1.5\%$, all 10 had a reduced Ki-67 expression in their breast epithelial cells at 6 months, with 9 of the 10 showing a reduction in Ki-67 of >50%. The preliminary results from this ongoing trial show that letrozole decreased proliferation in atypical breast epithelial cells in high-risk women who had an elevated Ki-67 even while on hormone replacement therapy.

Although some IEN treatments may induce apoptosis and eradicate IEN in a subpopulation of women, the available evidence suggests that modulation of proliferation, as evidenced by a decrease in Ki-67 in DCIS or hyperplasia with atypia, without evidence of apoptosis, is feasible and is a promising end point for future IEN treatment trials. Although it is possible that short-term treatment of IEN may eradicate high-

risk subclones of IEN in some patients, as carcinogenesis is a multidecade process, prolonged treatment of breast IEN with safe and well-tolerated agents that can chronically suppress proliferation of breast IEN may be a realistic treatment strategy for the future.

The most important advances in the practical application of targeted chemoprevention have focused on the hormonal modulation of breast cancer development. ER has proven to be a primary target for the treatment of breast cancer and the most practical target for chemoprevention of breast cancer (191). Since the publication of the National Surgical Adjuvant Breast and Bowel Project Breast Cancer Prevention Trial P1 in 1998 (192, 193), tamoxifen received supplemental approval from the FDA for risk reduction in high-risk premenopausal and postmenopausal women. Extensive analysis of symptoms data from the National Surgical Adjuvant Breast and Bowel Project study has provided additional practical information for the clinical use of tamoxifen (192, 194–197). Because of concerns about the side effects of tamoxifen, only a small proportion of women eligible for risk reduction consider tamoxifen treatment (198). This led to the Study of Tamoxifen and Raloxifene as a second-generation chemoprevention study with SERMs.

Raloxifene is a SERM that was tested for the treatment and prevention of osteoporosis in the Multiple Outcomes of Raloxifene Trial. Multiple Outcomes of Raloxifene is a multicenter randomized blinded placebo-controlled study that recruited 7,705 women, ages 31 to 80 years in 25 countries, who had been postmenopausal for at least 2 years and who met WHO criteria for having osteoporosis. Participants were randomized into a placebo group or one of two raloxifene groups (60 or 120 mg/d). Results (199, 200) showed that raloxifene reduced the risk of vertebrae fractures. The findings also showed that at 3 years of follow-up (201) the risk of invasive breast cancer had decreased by 76%, and the risk of ER-positive breast cancer had decreased by 90%. There was no significant effect on ER-negative breast cancer (201). At 4 years of follow-up, the incidence of invasive breast cancer was reduced by 72% and the incidence of ER-positive breast cancer was reduced by 84% (202).

The Continuing Outcomes Relevant to Evista Trial was developed to evaluate the efficacy of four additional years of raloxifene. At 7 years, the bone density of Continuing Outcomes Relevant to Evista patients was significantly increased in the lumbar spine and femoral neck compared with patients from the Multiple Outcomes of Raloxifene Trial (203). The incidence of invasive breast cancer and ER-positive invasive breast cancer in the Continuing Outcomes Relevant to Evista Trial was reduced by 59% and 66%, respectively (204).

Raloxifene is only recommended for the prevention of osteoporosis in postmenopausal women, and the Study of Tamoxifen and Raloxifene Trial, which enrolled 19,000 volunteers, is only relevant to postmenopausal women at risk for breast cancer. Preliminary results of the study have been announced recently by the NCI. Raloxifene was as effective as tamoxifen in reducing the incidence of breast cancer, and the women taking raloxifene had fewer uterine cancers and blood clots than those taking tamoxifen. Therefore, the benefits of raloxifene are enhanced bone density and a significant decrease in the incidence of breast cancer as well as reduced side effects compared with tamoxifen. Two other SERMs, lasofoxifene (205, 206) and bazedoxifene (207), are currently

entering clinical trials for the treatment and prevention of osteoporosis. Along with arzoxifene, there is also potential for these new SERMs to be used for the prevention of breast cancer.

An overview of prevention trials of SERMs was published in 2003 (208). In their analysis, Cuzick et al. expressed concern about the cardiovascular side effects of SERMs, especially in their own trial, the International Breast Cancer Intervention I Study (209). The International Breast Cancer Intervention I Study showed an increase in tamoxifen-related deaths, which were due primarily to thromboembolic events that were associated with surgical procedures done during tamoxifen treatment.

The potential side effects of tamoxifen in healthy women have prompted the search for other potential agents for the risk reduction of breast cancer, such as aromatase inhibitors. In several breast cancer adjuvant trials, the use of these agents, in comparison with tamoxifen, have shown a decrease in contralateral breast cancer and a reduction in breast cancer recurrence (210–214). Furthermore, the risks of endometrial cancer and thromboembolic events were also decreased with the use of aromatase inhibitors. Therefore, the International Breast Cancer Intervention organization has instituted an International Breast Cancer Intervention II Study to evaluate the aromatase inhibitor anastrozole as a chemopreventive agent in postmenopausal women. The MAP-3 study of the National Cancer Institute of Canada is also evaluating another aromatase inhibitor, exemestane, as a risk reduction agent in high-risk postmenopausal women (215). A phase II study by Fabian showing that the aromatase inhibitor letrozole reduced cellular proliferation in FNA from high-risk women was described above (190).

The toxicity profile of aromatase inhibitors is better than tamoxifen in adjuvant trials. However, other side effects of these agents, such as risk of osteoporosis and fatal myocardial infarction, have emerged. Only long-term outcome data will help in quantifying the risk versus benefit ratio of these agents.

Several other agents are also currently under investigation (214). For example, NSAIDs, especially selective COX-2 inhibitors, could represent a mechanism-based chemopreventive approach for cancer prevention (216). Epidemiologic studies have reported a relationship between NSAID use and a 30% to 40% reduction in breast cancer (217–219).

A few recent epidemiologic studies have not found a convincing association between statin usage and reduced breast cancer risk (108, 109); however, there is a growing body of epidemiologic, clinical, and experimental data suggesting that statins may have a role in prevention of multiple cancers, including breast. For example, several large, randomized cardiovascular trials have shown that statins reduce not only fatal and nonfatal cardiovascular events but also all-cause mortality (220, 221). Several case-control studies have reported a decreased incidence of a variety of cancers, including breast cancer, among statin users. One nested case-control study of >6,000 Canadian health plan beneficiaries found that statin users were 28% less likely than users of bile acid-binding resins to be diagnosed with any cancer, suggesting that the statins themselves, rather than a more general effect of cholesterol lowering, were responsible for the decrease in cancer risk (222). In particular, female statin users

in the study were 33% less likely than users of bile acid-binding resins to be diagnosed with breast cancer (222). A similar cohort study of >7,000 elderly women reported a 72% relative risk reduction in breast cancer with statin use compared with nonusers (223). Finally, a large case-control study of ~20,000 subjects found a 20% all-cancer risk reduction among users of statins, which reached significance after ~4 years of use (224). Short-term prevention trials involving statins in high-risk women are currently being planned.

Retinoids are a promising group of agents for the prevention of breast cancer. Fenretinide is a synthetic amide of retinoic acid, which induces apoptosis with mechanisms partly involving the retinoid receptors, particularly retinoic acid receptor- β (42). In a large phase III trial, fenretinide decreased second breast malignancies in premenopausal women (225). Another retinoid, bexarotene, a retinoid X receptor selective retinoid, is undergoing analysis in a phase II prevention study (226). Retinoid X receptor can heterodimerize with other retinoid receptors as well as other nuclear receptors, offering the possibility of interfering with multiple gene transcription activities.

Several tissue acquisition models are being used to test drug effects on biomarkers (227). Adequate tissue samples can be obtained by core biopsies directed toward the dense breast area (226) or by RPFNA (228, 229). Ductal lavage is also a potential method for obtaining breast epithelial cells through natural duct openings (226, 228, 230). Currently, there are no validated biomarker end points for breast cancer in chemoprevention trials with invasive cancer as the definitive end point. Because of the shorter latency to intermediate biomarker end points and the smaller cohorts required for treatment, short-term phase I/II breast cancer prevention trials provide an opportunity to identify such end points for use in future larger studies.

It is important to consider who should be included in chemoprevention trials. Models, such as the Gail Risk Model (231) and those that assess hereditary breast cancer risk (232–234), are currently being used. However, each model has its limitations. For example, the Gail Risk Model does not incorporate important hereditary risk factors, such as age of onset of breast cancer in first-degree and second-degree relatives; it also does not factor in family members with ovarian cancer. Patients with a history of breast cancer would also be included because their risk of developing a second primary tumor (SPT) in the contralateral breast is at least twice greater than in women without prior breast cancer (235). Current research efforts are trying to refine risk models by incorporating epidemiologic and hereditary risk factors. The inclusion of tissue risk markers as well as blood markers, such as single nucleotide polymorphisms (236–238), into these risk models could ultimately lead to the identification of individuals who would most benefit from intervention.

Lung cancer

Lung cancer continues to be the most common cause of cancer death in both men and women in the United States. In 2005, 172,570 new cases and 163,510 deaths due to lung cancer were expected, accounting for 13% of all new cancer diagnoses and 29% of all cancer deaths (120, 239). The incidence rate has decreased significantly in men over the past

two decades; in women, the incidence rate decreased for the first time between 1998 and 2001. The 5-year survival rates after the diagnosis of lung cancer have improved marginally from the 1970s (13%) until currently (15%).

It is estimated that the lifetime risk of lung cancer is one in 13 for men and one in 18 for women (239). Cigarette smoking is by far the most important risk factor, with ~85% of lung cancers being tobacco related. In the United States in 2003, there were 91.5 million current and former smokers (240). In the United States 21.6% of adults were current smokers. Smoking prevention and cessation are essential to decrease lung cancer incidence. Former smokers also remain at elevated risk for years after smoking cessation (241) and approximately half of lung cancers occur in former smokers. Smoking cessation is associated with a reduction in lung cancer mortality. In the Lung Health Study, death from lung cancer was 2.2 times more common in current smokers than in sustained quitters after 14.5 years of follow-up of a population who were randomized to an intensive smoking cessation program with or without ipatropium or usual care (242). This decrease in lung cancer mortality was not seen until after 5 years of follow-up, consistent with the theory that smoking-induced damage to the bronchial epithelium is persistent and that former smokers remain at risk.

Lung cancer mortality is high because the majority of cancers are diagnosed after regional (37%) or distant (39%) spread (243). If surgically resected at an early stage, the 5-year survival approaches 70% (244). Thus, early detection has become a major focus for research. Historically, attempts to use chest X-rays or sputum cytology for early detection were unsuccessful (summarized in ref. 245). As the demographics of lung cancer have shifted from predominance of centrally located squamous cell cancers to peripherally located adenocarcinomas, the value of chest X-ray screening in reducing mortality from lung cancer is being readdressed by the ongoing Prostate, Lung, Colorectal, and Ovarian Cancer Screening Trial. Recently published baseline screening results show a detection rate of 6.3 cases/1,000 screens in current smokers, 4.9 cases/1,000 screens in former smokers, and 0.4 cases/1,000 screens in never-smokers (246). Although an encouraging 44% of lung cancers were stage I, long-term results comparing the screened and unscreened populations will be needed to determine if chest X-ray screening reduces mortality.

Multiple nonrandomized studies showed the potential for low-dose spiral CT screening to identify more lung cancers than chest radiographs, with a lung cancer prevalence ranging from <0.5% to >3% depending on the risk of the studied populations (summarized in refs. 246, 247). Among cancers detected by screening, the frequency of stage I cancers in these studies and the randomized Lung Screening Trial (248) ranged from 48% to 100%, whereas the incidence of noncalcified nodules (the majority of which are noncancerous) in screened populations ranged from 7% to 69% depending on the population and type of CT scanner used. The National Lung Screening Trial, which randomized 50,000 participants to spiral CT or chest X-ray, will determine whether the increased detection of early lung cancers by spiral CT will translate to decreased lung cancer mortality. However, spiral CT does not detect central airway lesions well. Therefore, the combination of spiral CT with other modalities that address the central airway is also being studied. McWilliams et al. showed that the

addition of fluorescence bronchoscopy to spiral CT screening in a population with sputum atypia identified via automated image cytometry increased the lung cancer detection rate from 1.8% to 3.1% (249).

It is widely recognized that lung cancer is the result of progressive phenotypic and genotypic abnormalities. The sequence of histologic events is well described for central airway squamous carcinogenesis but not nearly as well understood for peripheral adenocarcinomas or other histologic types. Saccomanno et al. showed that squamous lung cancer developed through a series of stages from mild, moderate, and severe dysplasia to carcinoma *in situ* and eventually to invasive lung cancer over the course of many years (250). In the peripheral lung, atypical alveolar hyperplasia is considered to be a precursor to invasive adenocarcinomas (251), but precursors to other histologic types are not well described.

The relative risk of lung cancer in high-risk individuals with ≥ 30 pack-years of smoking and chronic airway obstruction is 3.18 for those with moderate or greater sputum atypia compared with high-risk individuals with normal sputum cytology (252). The progression rate to cancer is particularly high with severe sputum atypia, with cancer diagnosis in 40% to 45% of subjects within 2 to 10 years (253–255). Nevertheless, it remains difficult to calculate the risk for subsequent lung cancer in any given individual. Bach et al. have developed a risk model using outcomes from the 18,000 subject β -Carotene and Retinol Efficacy Trial cancer prevention study that incorporates age, sex, asbestos exposure, and smoking history (256). This model does not incorporate any molecular characteristics.

The incidence of mild, moderate, and severe bronchial dysplasia documented by fluorescence bronchoscopy in current and former smokers with a ≥ 30 pack-years smoking history is 44%, 14%, and 4.3%, respectively (257). The natural history of these lesions is difficult to assess because some are completely removed during bronchoscopy, but studies using serial autofluorescence bronchoscopic biopsies suggest that ~3.5% of low or moderate dysplasias progress to severe dysplasia, 37% of severe dysplasias remain or progress, and ~50% of carcinomas *in situ* progress to invasive carcinoma within a 2- to 3-year follow-up period (258, 259). It is not known at what frequency atypical alveolar hyperplasias progress to cancer.

The bronchial epithelium of current and former smokers contains smoking-induced genetic damage as well as histologic changes. Genetic abnormalities common in invasive lung cancers, such as loss of regions of chromosomes 3p and 9p, deletions of chromosomal arm on 5p, and mutations of p53 and *K-ras*, occur with differing frequencies in precursor lesions (summarized in ref. 260). 3p and 9p lesions are recognized as occurring early, whereas p53 and *K-ras* lesions occur during late stages of preneoplasia and in overt cancers. Clonal patches of genetic damage (as evidenced by LOH or microsatellite alterations) also occur in the histologically normal bronchial mucosa of both current and former smokers, persisting for years after smoking cessation (261, 262). The progressive accumulation of such molecular abnormalities without the capacity to repair the genetic damage helps to explain why former smokers remain at increased risk despite smoking cessation.

Substantial data suggest that inflammation plays a crucial role in the genesis of lung cancer and that various

anti-inflammatory compounds can prevent cancer development. Wattenberg and others showed that both systemic and inhaled steroids, which inhibit the generation of arachidonic acid from membrane phospholipids by phospholipase A₂, inhibit the development of lung adenomas and carcinomas in mice (263, 264). Based on this rationale, Lam et al. conducted a phase IIb randomized placebo-controlled trial of inhaled budesonide in persons with bronchial dysplasia (265). Participants were selected based on central airway pathology (bronchial dysplasia) but underwent monitoring of both their central and peripheral lung via autofluorescence bronchoscopy and spiral CT. Although the 6-month treatment did not result in regression of the central airway dysplasias (the primary study end point), there was an increased rate of resolution of CT-detected peripheral nodules (a secondary end point). Because the animal models correspond to events occurring in the peripheral lung, these human and animal data suggest that future prevention trials should focus on the peripheral lung.

Similar animal data exist for inhibitors of formation of products of arachidonic acid metabolism by the enzymes 5-LOX and COX (both COX-1 and COX-2 isoforms), which give rise to multiple downstream products, including leukotrienes, hydroxyoctadecadienoic acids, and prostaglandins that have been implicated in various aspects of lung carcinogenesis (266, 267). Inhibitors of these enzymes, zileuton (5-LOX), celecoxib (COX-2), and sulindac (COX-1 and COX-2), are currently under study for prevention of lung cancer in multiple phase IIb studies. In addition, studies of combinations of celecoxib and EGFR inhibitors are also undergoing clinical development based on a strong preclinical rationale showing a positive feedback loop between EGFR and COX-2 signaling (113). EGFR is an important target for second-line and third-line therapy of non-small cell lung cancer (268), although the applicability of this class of agents to prevention remains to be determined. EGFR mutations, which are correlated with response to therapy, occur primarily in nonsmokers; thus, further work is needed to determine if EGFR inhibitors treatment can abrogate early carcinogenesis in smokers. Furthermore, toxicity from the EGFR inhibitor gefitinib precluded prevention trials in curatively treated aerodigestive cancer patients.

Another downstream product of the COX-2 pathway, prostacyclin (prostaglandin I₂), is antineoplastic. Mice over-expressing prostacyclin synthase, which catalyzes prostacyclin formation, have a lower tumor incidence and multiplicity than wild-type littermates exposed to tobacco smoke (269). A phase II clinical trial assessing the chemopreventive potential of iloprost, a prostacyclin analogue, is currently under way.

Other agents that are being explored in the clinic include green tea polyphenols, *myo*-inositol, and the Chinese herbal preparation ACAPHA (antitumor B; refs. 270, 271). Selenium is in a phase III second primary cancer prevention trial based on the observation in the Nutritional Prevention of Cancer trial, designed to assess the efficacy of selenium in reducing nonmelanoma skin cancers, that selenium resulted in a 44% decrease in lung cancers (272). Preclinical data also suggest that estrogen signaling could serve as a target for lung cancer prevention, with intriguing data from a clinical trial of the aromatase inhibitor exemestane that reported a decreased incidence of primary lung cancer in breast cancer patients treated with exemestane after 2 to 3 years of tamoxifen therapy (4 cases) compared with continued tamoxifen (12 cases;

refs. 213, 273). Animal data also exist supporting the use of farnesyl transferase inhibitors, but the development of these agents for chemoprevention has been complicated by an unfavorable toxicity profile (274).

Historically, lung cancer prevention trials have fallen into three categories: prevention of lung cancer in high-risk smokers (phase III studies), prevention of second primary cancers in subjects with curatively treated aerodigestive cancers (phase III studies), and preliminary efficacy phase II trials in high-risk smokers using intermediate end points. The prototypes of the primary prevention trials, the α -Tocopherol β -Carotene Study and the β -Carotene and Retinol Efficacy Trial, both using β -carotene either alone or with vitamin E or vitamin A, showed increased lung cancer incidence with β -carotene (275, 276). These two studies accrued 29,133 and 18,314 participants, respectively. Prototypes of second primary cancer prevention studies, the European Study on Chemoprevention with Vitamin A and *N*-Acetylcysteine and the Lung Intergroup Trial using low-dose 13-*cis*-retinoic acid (13cRA), showed no benefit to the interventions and a possible increase in recurrence and mortality for 13cRA in current smokers (277, 278). These trials accrued 2,592 (1,023 of whom had prior lung cancer) and 1,166 participants, respectively. Prototypes of the third trial design, using intermediate end points, are the phase IIb trial by Kurie et al. using 9-*cis*-retinoic acid alone or with α -tocopherol to reverse the loss of retinoic acid receptor- β expression that occurs so frequently during lung carcinogenesis and the phase IIb trial by Lam et al. using inhaled budesonide to assess the effect of intervention on bronchial dysplasia (265, 279). The former showed a small but statistically significant effect on retinoic acid receptor- β expression, whereas the latter was negative. These trials accrued significantly fewer participants, 220 and 112, respectively.

The appeal of an intermediate end point-driven phase II trial design is the ability to rapidly identify effective agents using a relatively small number of participants before large phase III cancer prevention trials. Identification of high-risk individuals who might benefit from treatment and identification of informative end points predictive of cancer development underlie the success of such a strategy. Current phase II lung cancer prevention trials focus on individuals with heavy smoking exposure or curatively treated aerodigestive cancer patients, frequently using these cohorts to further facilitate the identification of individuals who have intraepithelial bronchial lesions at higher risk for progression to overt cancer. A typical trial design would be to identify smokers with bronchial dysplasia verified by bronchoscopy, treat for 3 to 6 months (preferably in a placebo-controlled setting given the spontaneous resolution of some lesions and the removal of others during bronchoscopy), and repeat bronchoscopy with biopsy of known areas of abnormality as well as any new suspicious areas. Such a strategy requires considerable screening, first with sputum cytology and then by bronchoscopy, to identify the higher-risk individuals who actually have bronchial lesions that can then be followed with serial biopsies. Because bronchial dysplasia is a known precursor to cancer, it is considered to be an informative end point with regard to subsequent cancer incidence. Intermediate end-point trials that focus on molecular abnormalities rather than IEN have the disadvantage of using a less high-risk cohort (without IEN) and a less informative end point.

Although prior phase II trial designs have selected participants with central airway abnormalities within the reach of a bronchoscope, the relevance of this to peripheral adenocarcinoma has not been clear. Animal models leading to both squamous and adenocarcinoma showed differential effectiveness of specific agents in central versus peripheral lung cancer models.¹⁹ The recent availability of spiral CT to follow peripheral nodules that may represent precursors to adenocarcinoma allows the assessment of interventions on the peripheral lung. A new trial design, focusing on high-risk smokers with nodules identified by spiral CT that are neither clearly malignant nor benign, thus becomes possible. The disadvantage is that these nodules are generally too small to biopsy; thus, their histology cannot be determined. Most nodules are likely to be benign or inflammatory rather than premalignant. However, if done in a randomized, placebo-controlled manner, this trial design should be able to identify highly effective interventions for phase III testing. Furthermore, the natural history of the frequently identified spiral CT-detected nodules is yet to be determined; thus, phase IIb placebo-controlled intervention trials could be informative.

As less toxic targeted agents are developed for lung cancer treatment, another opportunity arises to simultaneously address their potential for prevention in the bronchial epithelium assessed via bronchoscopy. If there is a biological rationale for expecting efficacy during early stages of carcinogenesis and the toxicity profile is favorable, when such agents are late in development for metastatic disease or in the adjuvant setting, pretreatment and posttreatment bronchoscopies could address their effect on bronchial dysplasia. This would considerably speed up new prevention agent development by giving an early indication of effectiveness.

The two most significant challenges ahead are to better understand the biology of early carcinogenic events so that specific targeting could arrest the process and to identify those individuals who are at the highest risk so that interventions could be delivered only to those most likely to benefit. Advances in noninvasive imaging that better identify and characterize premalignant lesions would considerably enhance our ability to identify and follow the truly high-risk individuals, both for clinical prevention trials and for routine clinical practice.

Head and neck cancer

Head and neck squamous cell carcinoma (HNSCC), the most common epithelial malignancy arising in the upper aerodigestive tract, is an important public health problem (Table 4). The overall survival rates for these cancers (~45%) have only marginally improved over the last three decades (120, 280). The main reasons for treatment failure are the development of SPTs for patients with early-stage disease (stages I and II) and the development of local recurrence and metastasis for patients with locally advanced HNSCC (280, 281). There is a constant and continuing SPT risk of from 2.7% to 4% yearly in the aerodigestive tract and other sites following initial treatment.

HNSCC is associated with tobacco smoking and alcohol use and is the result of a multiyear, multipath disease process of progressive genetic and associated tissue damage (280–283).

A large placebo-controlled, randomized trial of 13-*cis*-RA to prevent SPTs was conducted in 1,190 patients with curatively treated HNSCC (282). At randomization, the probability of developing a smoking-related SPT was highest among patients who were current smokers. Whether these patients ceased smoking or not during the 10-year follow-up, they were nearly thrice more likely to develop smoking-related SPTs than were patients who never smoked. Former smokers and recent quitters at baseline were ~1.5 times more likely to develop SPTs compared with patients who never smoked. Other contributing factors associated with SPTs include genomic instability and genotypic abnormalities (including cyclin D1, cytochrome P450, glutathione S-transferase, and p53) and mutagen sensitivities (284–287).

Several chemoprevention trials used reversal of IEN as their ultimate end point based on studies that revealed that oral IEN appearing as white patches, or oral leukoplakia, carries a 17.5% risk of malignant transformation at 8 years or 36.4% in cases of dysplastic oral IEN (288). However, the realizations that carcinogenesis is multifocal and multiclonal even within the same lesion, that not all IEN progresses to cancer or can be readily detected and measured, and that no specific drug can target all genetic changes have made more precise definitions of risk (versus risk defined by histologic IEN only) necessary to proceed with chemoprevention trials.

LOH studies have pointed out the power of LOH as predictors of oral cancer development. Several allelic losses have been shown to be early events in head and neck tumorigenesis (289–291). LOH at 3p and 9p is not only frequent but also a predictor of progression to invasive cancer (289–291). LOH at 3p and 9p is a very powerful predictor of a second oral malignancy at previously treated oral cancer sites (292). Moreover, the concept of field cancerization has been genetically confirmed by LOH findings showing the clonal relationship of transformed cells in large areas of mucosa (289, 293). Microsatellite analysis at the 3p, 9p, 17p, 8p, 13q, and 18q chromosomal regions and mutation analysis for p53 have shown that genetically altered mucosa remains after treatment in a significant proportion of HNSCC patients (294). These and earlier findings confirmed the feasibility of using p53 alterations as a tool for molecular staging and fingerprinting of head and neck tumors and underscore the need for a molecular basis for SPT prevention (289).

Retinoid prevention studies highlighted the predictive power of polymorphisms of the cyclin D1 gene, which are often used as a marker of resistance and a predictor of shorter time to cancer development (295). Cyclin D1 has a central role in the G₁-S transition; gene amplification and protein overexpression have been described in 40% to 60% of HNSCC (296) associated with poor prognosis. Retinoid-dependent cyclin D1 proteolysis has been suggested as a candidate intermediate marker for effective retinoid chemoprevention (297). EGFR overexpression occurs frequently in HNSCC, is a poor prognostic factor, and is detected early during head and neck carcinogenesis (298), constituting a potential target. COX-2 has a significant role in head and neck carcinogenesis; COX-2 is overexpressed in the oral mucosa of active smokers through tobacco smoke-stimulated EGFR tyrosine kinase activity. This leads to enhanced transcription and thus provides the rationale for combined inhibition of COX-2 and EGFR (299).

¹⁹ Dr. Lee Wattenberg (University of Minnesota), personal communication.

Current and future development of biomarkers are concentrating on molecular profiling based on established head and neck cancers, where genomics and proteomics are being used to define molecular signatures (gene/protein expression and DNA alterations, including gene promoter hypermethylation, single nucleotide polymorphisms, microsatellite instability, and chromosome aberrations) that could then be validated in premalignancy. Other technologies include comparative genome hybridization and spectral karyotyping for chromosomal changes, identification of circulating cancer cells and endothelial cells and precursors, use of surrogate materials, such as saliva and buccal brushes, and development of nanotechnologies and molecular imaging.

Two areas of focus have been studied historically, reversal of premalignant lesions and prevention of SPTs. Retinoids have an established effect in reversing early premalignant lesions but are associated with mucocutaneous toxicity. After Hong et al. (300) showed in their 1986 randomized trial that high-dose 13-*cis*-RA for 3 months had a 67% response rate versus 10% for placebo ($P = 0.002$), several other trials confirmed retinoid and vitamin A activity in oral premalignant lesions (127, 301, 302). Because one retinoid was not sufficient to reverse moderately and severely dysplastic lesions (which are more prone to transform than are hyperplastic lesions), a combination of IFN- α , α -tocopherol, and 13cRA was evaluated. A study of this combination showed its activity in head and neck IEN and also revealed that genetic abnormalities persist at the site of IEN with a complete clinical and histologic response (303).

SPTs in curatively treated patients occur with an annual rate ranging from 1.2% to 4.7% in retrospective studies and from 4% to 7% in prospective studies. An early randomized trial in this setting suggested the potential effect of high-dose 13-*cis*-RA (304). SPTs developed in significantly fewer 13-*cis*-RA-treated patients (4%) than in patients receiving placebo (24%; $P = 0.005$). Although active, high-dose 13-*cis*-RA had intolerable toxicity, and the overall annual decrease in SPTs has lessened over time since the one-year intervention was completed. Studies of lower, less toxic doses of 13-*cis*-RA (305) or other retinoids (277) showed a lack of activity. Twelve months of treatment with combined 13-*cis*-RA, IFN- α , and α -tocopherol (306, 307) has been tested in a phase II trial to decrease SPTs and recurrence and improve overall survival in 45 locally advanced (stage III/IV) HNSCC patients. After 49.4 months of median follow-up, 7 (15.6%) patients are no longer alive and 9 (20%) patients have experienced progressive disease. Only one SPT (acute promyelocytic leukemia) occurred during follow-up, and no aerodigestive SPT occurred. The estimated 5-year disease-free and overall survival rates were 80% and 81%, respectively (307). These results are significantly better than the historical 5-year overall survival of advanced HNSCC (~40%), and a phase III randomized study is ongoing to these results. Retinoid-associated toxicity and the need for long-term targeted prevention strongly point to the need for developing other classes of agents.

Current prevention studies in the settings of oral IEN and SPT prevention involve interventions targeted toward p53 abnormalities as well as use of selective COX-2 and EGFR tyrosine kinase inhibitors and the epigallocatechin gallate component of green tea. Based on recent findings of cross-talk between COX-2 and EGFR, combination studies are in progress. Preclinical

studies suggest that the combination of COX-2 and EGFR tyrosine kinase inhibitors is active (114, 308). Combining these inhibitors might lead to improved efficacy with reduced toxicity. Other agents include curcumin due to its effects on NF- κ B pathways, agents targeting the PI3K/AKT pathway, antiangiogenesis agents, green tea extracts, and vaccines.

Defining risk is a critically important area of head and neck cancer prevention (289). Currently, information, such as LOH at critical loci, should be included in trial screening or stratification criteria, with more standard criteria. New studies should integrate exploratory analyses of both risk markers, ideally target-specific, and pharmacodynamic markers of efficacy and/or resistance to the intervention. Future trial designs could include cohorts of former cancer patients with stratification for continued tobacco use and smoking cessation, randomization to active intervention in a 2:1 ratio, active follow-up, and profiling of study dropouts. Once a more complete picture of molecular profiling emerges, patients would be allocated to different trial arms based on their profile.

Esophageal cancer

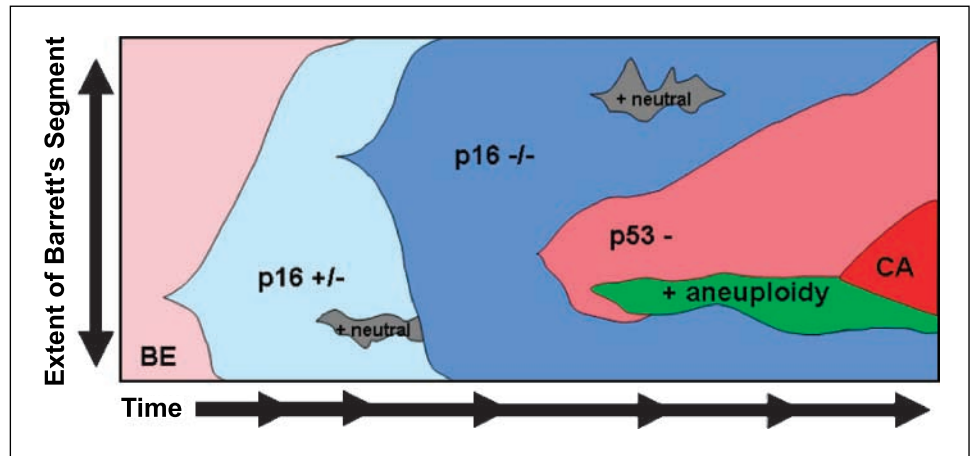
The incidence of esophageal adenocarcinoma in the United States in 2005 is estimated as >8,700 (Table 4) and has been increasing at an alarming rate in the United States and many other western countries for the past 30 years (309). All stage 5-year survival for patients with esophageal adenocarcinoma is only 13.7%. Barrett's esophagus is the only known precursor to esophageal adenocarcinoma, and control of this lethal cancer depends on development of models to assess risks and benefits of emerging screening, surveillance, and preventive options (Fig. 4).

Population-based risk assessment (risk model 1). Incidence rates of esophageal adenocarcinoma rise progressively with age, with an exponential increase until about age 70 years and a slower increase thereafter; rates are highest among White men (310). Risk factors for esophageal adenocarcinoma are derived from several population-based, case-control studies, although data from cohort studies also have emerged. Tobacco smoking is an established risk factor for esophageal adenocarcinoma; increases in risk are relatively modest, but the excess risk persists up to 30 years after smoking cessation (310). These findings suggest that smoking exerts an early-stage effect in the initiation of adenocarcinoma and may explain to some extent the minimal effect of the declining prevalence of smoking on incidence trends of this cancer over the past three decades. Elevated body mass index is also a significant risk factor (311, 312). It is likely that the increases in obesity have contributed to the parallel trends in esophageal adenocarcinoma (313).

The majority of esophageal adenocarcinoma cases are thought to arise in Barrett's esophagus (specialized intestinal metaplasia). Most cases of Barrett's esophagus arise in the context of long-standing gastrointestinal esophageal reflux of gastric acid and bile salts and alkaline duodenal contents (314). Epidemiologic studies have consistently reported increases in risk of esophageal adenocarcinoma associated with symptomatic reflux and hiatal hernia (312).

In a U.S. multicenter study, there were 50% to 60% reductions in the risk of esophageal adenocarcinoma among current users of aspirin and other NSAIDs (311). Further, NSAID reduced the risk of flow cytometric abnormalities and 17p LOH among patients diagnosed with Barrett's esophagus (313). The reduction in these markers of tumor progression has

Fig. 4. An example of clonal evolution in Barrett's esophagus that may be used for risk stratification and monitoring of prevention trials. *X axis*, time; *Y axis*, Barrett's segment. Clones with p16 abnormalities arise early and expand rapidly during neoplastic progression in Barrett's esophagus. p53 abnormalities arise in a p16-deficient genetic background, undergo clonal expansion, and predispose to the development of aneuploidy and esophageal adenocarcinoma. Because these abnormalities undergo clonal expansion, they are easier to detect by endoscopic biopsies than dysplasia, which can be patchy and focal. They can also persist after interventions that downgrade dysplasia and may be used for monitoring.



raised hope that NSAIDs may protect against esophageal adenocarcinoma among high-risk patients.

A reduced risk of esophageal adenocarcinoma among those serologically positive for *H. pylori* infection has been reported in case-control studies (315). It is possible that the rising incidence of esophageal adenocarcinoma may be related in part to the declining prevalence of *H. pylori* infection due to improvements in sanitation and widespread use of antibiotics (60).

The U.S. multicenter study estimated that 79% of esophageal adenocarcinoma may be attributed to a combination of four established and modifiable risk factors: tobacco use, obesity, gastroesophageal reflux disease, and low intake of fresh fruits and vegetables (310). About 30% of the cases were related to symptomatic gastroesophageal reflux disease and 40% to smoking or high body mass index; ascertainment of gastroesophageal reflux disease was likely incomplete in that study. The percentage of the cases attributed to low intake of fruits and vegetables was modest (15%). It is possible to estimate the probabilities of developing esophageal adenocarcinoma among individuals with particular characteristics considered to be causally related to esophageal adenocarcinoma and employing national data on the age-, sex-, race-, and calendar time-specific incidence of the cancer. Such modeling may identify population subsets at sufficiently high risk for Barrett's esophagus or early esophageal adenocarcinoma to merit endoscopic screening, entry into screening trials, and participation in prevention trials.

Barrett's esophagus risk stratification (risk model 2). The annual incidence of esophageal adenocarcinoma in patients with Barrett's esophagus is estimated at 0.5% to 1% (316, 317). Histopathologic classification of dysplasia grade in Barrett's esophagus [negative, indefinite, low-grade dysplasia (LGD; ref. 318), and high-grade dysplasia (HGD; ref. 319)] is the standard method of risk stratification. Surprisingly, only one study has reported rates of progression to esophageal adenocarcinoma stratified by the full spectrum of baseline dysplasia grades (320). Several studies quantified the heterogeneous behavior of dysplasia; some cases progress to esophageal adenocarcinoma, whereas others regress or remain stable. LGD confers a low risk of progression to esophageal adenocarcinoma, and there were no significant differences among negative, indefinite, and LGD in the only study comparing baseline dysplasia grade to progression to esophageal adeno-

carcinoma (320), consistent with other studies (320–322). Controlling the progression of LGD was a stated objective of the previous AACR Task Force, but these recent data suggest that adequately powered randomized trials will require many patients treated with very safe interventions. HGD has the highest morphologic association with progression to esophageal adenocarcinoma; the magnitude of risk over 5 years varies from 15% to 59% in prospective studies, and many patients seem to regress spontaneously (320, 321, 323, 324).

Risk model 2 for patients with Barrett's esophagus would ideally depend on reproducible measurements of biomarkers with estimates of the risks associated with those biomarkers and their interactions validated by prospective or retrospective studies (ref. 325; Fig. 4). Estimates of risk are difficult because the Barrett's segment is characterized by evolving populations of cells where, unlike constitutive genotypes, component molecular biomarkers are likely to change within patients over time. The current state of the research includes several somatic genetic risk factors that have been shown to associate with dramatically increased risk (relative risk >10) in prospective trials (325). These include LOH in p53 and the presence of aneuploid and tetraploid (4N fraction >6%) cells, all of which provide a detection window of risk stratification extending at least to 5 years for most patients (23, 326–328). Biomarker panels will be essential to overcome the inherent tradeoff between sensitivity and specificity for any single biomarker. A preliminary report of a panel of p16, p53, and DNA content is promising for predicting future risk of cancer in patients with Barrett's esophagus with high sensitivity and specificity (329). A similar biomarker panel has been reported for oral leukoplakia (330). Recently, a small phase III trial, a retrospective longitudinal study of epigenetic biomarkers, including methylation of p16, RUNX3, and HPP1, has been reported, but the univariate odds ratios were relatively low and the epigenetic window of detection was only 2 years compared with 5 years for the genetic panel (331), suggesting their lesser utility for screening for high-risk disease.

Intervention in Barrett's neoplastic progression is feasible at several different times and by several different means depending on risk-to-benefit ratio. Primary prevention, which tries to preserve normal structure and function of the squamous epithelium, is an obvious ultimate goal. Secondary prevention, which focuses on eliminating preinvasive neoplastic

clones or suppressing their progression in patients by medical means with Barrett's esophagus/dysplasia or by surgical removal, has been a more approachable, near-term goal. In available animal models of cancer, indomethacin (101, 332–334) and sulindac (335) have yielded conflicting data; however, NSAIDs tend to reduce cancer incidence and burden in a variety of organs. In addition, observational data have identified strong and consistent inverse associations between esophageal adenocarcinoma and NSAID use as summarized in a recent meta-analysis of observational studies (47, 311, 315, 336). A large phase III trial (ASPECT) of omeprazole 20 versus 80 mg/d, with or without aspirin 300 mg/d, proposes to randomize 9,000 men with Barrett's esophagus in the United Kingdom. The trial will evaluate esophageal adenocarcinoma incidence and all-cause mortality, given the substantial comorbidities often affecting men with Barrett's esophagus (337).

Selective COX-2 inhibitors may reduce the esophageal adenocarcinoma, although their cardiovascular risks may be limiting (179). To explore their potential more fully, Heath et al. are conducting a phase IIb trial of 200 mg/d celecoxib versus placebo in 200 patients with Barrett's esophagus (338). The primary outcome measure is change from baseline to 1 year in the proportion of biopsies exhibiting dysplasia.

DFMO (339) reduces the incidence of esophageal tumors in rodents (340) and modulates proliferation in human esophageal biopsy samples (316). A phase IIb randomized, double-blind, placebo-controlled trial of 900 mg/d DFMO for 6 months in 174 patients with Barrett's esophagus or LGD was reported in abstract form (341). The trial failed to meet its accrual goals, having only randomized 77 patients over 5 years. All measured variables, including mucosal proliferation, cyclin D1 expression, and polyamine levels, were unchanged, although it is unclear if this was due to intrinsic lack of efficacy of DFMO, dose, or the selected end points.

Preclinical studies suggest that selenium may function as a chemopreventive through antioxidative or apoptotic mechanisms. In one randomized placebo-controlled trial of 200 µg/d high-selenium yeast, treatment for an average of 4.5 years resulted in a 67% reduction in esophageal cancer incidence, although this was only a secondary end point and too few cases were observed to stratify by histologic subtype (272).

The most promising endoscopic methods for esophageal adenocarcinoma prevention in Barrett's esophagus have been photodynamic therapy and endoscopic mucosal resection. Since the 2002 publication of the AACR IEN review (1), a randomized, prospective, multicenter, photodynamic therapy trial was completed (323). After 24 months, 77% of those patients in the treatment arm and 39% of patients in the control arm had regression of HGD to a lower grade of dysplasia or normal-appearing epithelium ($P < 0.001$). Cancers were also reduced in the treatment group (13%) compared with the control group (28%; $P < 0.006$). In August 2003, FDA approved and granted orphan drug designation to a photosensitizing porphyrin mixture (Photofrin) in conjunction with photodynamic therapy (Axcan Pharma, Inc., Quebec, Canada) for ablation of HGD in patients with Barrett's esophagus who cannot or choose not to undergo esophagectomy. The safety profile showed 94% of patients in the photodynamic therapy group and 13% of patients in the control group had treatment-related adverse events. Complications of photodynamic therapy

included mild phototoxicity (68%) and significant stricture formation (36%) as well as vomiting, chest pain, constipation, and pyrexia. This trial required participation from ~25 centers from the United States, Canada, and Europe to complete recruitment.

Endoscopic mucosal resection is able to obtain large tissue samples that can be analyzed histologically. Initial mucosal resections were primarily used for diagnostic purposes; however, since 2002, reports have surfaced using mucosal resection for the treatment of HGD and superficial cancers (342). In one study of 115 patients followed for a mean of 3 years, metachronous lesions developed in 23% of the patients in residual metaplastic tissue, although only one patient died of esophageal malignancy (343).

Esophagectomy is the most aggressive form of cancer prevention therapy for select patients with Barrett's esophagus, and this option is generally reserved for otherwise healthy individuals who are found to have HGD. The rationale for prophylactic esophagectomy is that undetected esophageal adenocarcinoma may be found in 30% to 40% of surgical specimens obtained from such cases (344). However, longitudinal studies report that 40% to 85% of patients with HGD remain cancer-free for periods up to at least 5 to 8 years (319–321, 324, 345). Thus, not all experts advocate immediate resection for noninvasive disease because operative morbidity and mortality rates may be unacceptably high (346).

Liver cancer

Hepatocellular carcinoma is the fifth most common solid tumor worldwide, the third most common cause of cancer death, and 5-year survival rates for patients with advanced disease are <5%. Incidence and mortality are roughly equal. Eighty percent of new cases occur in developing countries, but the incidence is rising in Japan, western Europe, and the United States (347). The International Agency for Research has projected that there would be 667,000 cases worldwide and 17,550 cases in the United States in 2005 (239). The vast majority of hepatocellular carcinoma cases are attributable to underlying HBV and HCV infection, but several other risk factors exist as well. There are estimated to be 350 million carriers of HBV and 170 million carriers of HCV worldwide (58). HBV-related hepatocellular carcinoma predominate in Asia and sub-Saharan Africa, whereas HCV-related cases are more common in western countries.

Currently, liver transplantation and surgical resection are considered the only curative treatment modalities, yet <20% of hepatocellular carcinoma patients are surgical candidates because of tumor size, multifocality, vascular invasion, or hepatic decompensation. For those undergoing resection, the recurrence rates can be as high as 50% within several years of surgery (348, 349); thus, a large number of patients will seek systemic therapy. A 1997 meta-analysis of 37 randomized clinical trials of systemic and regional chemotherapy in 2,803 hepatocellular carcinoma patients concluded that nonsurgical therapies were ineffective or minimally effective at best (350). Most studies of chemotherapy report response rates of 0% to 25% and do not prolong survival.

Hepatocellular carcinoma is molecularly complex and several excellent reviews summarize the state of knowledge of the most common and important molecular aberrations (351, 352). No

consistent pattern of genetic damage at different evolutionary stages of hepatocellular carcinoma has been described probably because the molecular pathways leading to hepatocellular carcinoma likely differ according to etiology. Hepatocarcinogenesis is a 5- to 30-year multifactorial, multistep process in which external stimuli induce genetic changes in mature hepatocytes leading to cellular proliferation, cell death, and production of monoclonal populations.

Transmission of HBV and HCV via blood product transfusions has been largely eliminated through screening of high-risk donors, viral antibody testing, and abolishing payments for blood product donations. However, a large pool of patients exists who previously acquired HBV or HCV infection. Many of the noninfectious risk factors associated with hepatocellular carcinoma, including excessive alcohol consumption, obesity, and iron overload, can be modified. Potential approaches to prevention include prevention of the primary underlying liver disease (e.g., viral hepatitis), prevention of cirrhosis in patients with established viral infection, prevention of cancer in patients with cirrhosis, and secondary prevention of recurrence in patients who have undergone liver transplantation or resection for hepatocellular carcinoma.

Hepatitis B virus. The most effective means available for avoiding HBV-associated hepatocellular carcinoma is preventing the initial viral infection. A vaccine against HBV has been available since 1982; it was the first vaccine designed to prevent a major human cancer and remains the only such vaccine in wide use. In Taiwan where universal newborn vaccinations began in 1984, hepatocellular carcinoma incidence has begun to decline in young people (353, 354). Clinical trials showed 85% to 95% efficacy in preventing chronic HBV infection, and this response rate can reduce the prevalence of chronic HBV infection to <2% in children living in HBV endemic regions (355). Large-scale efforts are under way to make the HBV vaccine available to children in the poorest countries in addition to middle and upper income countries (356). If successful, this should lead to a dramatic reduction in deaths due to HBV-induced hepatocellular carcinoma and cirrhosis. Treatment of HBV infection can slow progression of liver disease (357, 358). Five antiviral medications, IFN- α , lamivudine, adefovir dipivoxil, entecavir, and pegylated IFN- α -2a, are approved by FDA for use against chronic HBV infection. Although treatment cannot eradicate HBV from cells that are already infected, inhibiting viral replication reduces ongoing inflammation and necrosis in the liver and facilitates the host immune response. Treatment of patients with chronic hepatitis B and advanced liver disease with lamivudine for a median of ~32 months reduced hepatocellular carcinoma incidence and liver disease progression by ~50% despite incomplete suppression of HBV replication (358).

Hepatitis C virus. Several clinical trials indicate that treatment of acute HCV infection with IFN- α can prevent chronic infection (359) and, in some studies, modestly reduce the risk of developing hepatocellular carcinoma (360, 361). Preexisting cirrhosis and concurrent alcohol use decrease the effectiveness of anti-HCV therapy. The current recommendation in the United States for treatment of HCV is combination therapy with pegylated IFN and oral ribavirin for 6 to 12 months depending on the HCV genotype. Therapy leads to sustained viral response in 50% to 55% of HCV patients (362, 363). There is currently no approved or effective second-line

therapy of HCV. Treatment of HCV that results in viral clearance (normalization of alanine aminotransferase) has been shown to reduce the rate of progression to cirrhosis and hepatocellular carcinoma.

Several newer therapeutic strategies are undergoing clinical investigation (364). These consist of modifications of IFN to improve efficacy and reduce side effects, including conjugation with albumin, liposome encapsulation, and polyamino acid-based oral delivery systems. Other potential agents include broad-spectrum antiviral agents, inhibitors of HCV NS3 serine protease, RNA-dependent DNA polymerase inhibitors, ribozymes that cleave specific RNA sequences, antisense oligonucleotides that recognize the noncoding region of HCV RNA, monoclonal antibodies against HCV envelope protein, and agents, such as thymosin α -1 and the natural killer cell activator histamine dihydrochloride, to enhance immune activity (365).

The acyclic retinoid, polypropenoic acid (3,7,11,15-tetramethyl-2,4,6,10,14-hexadecapentaenoic acid), inhibits chemically induced hepatic carcinogenesis in rats and spontaneous hepatocellular carcinoma in mice and suppresses human hepatoma cell growth and α -fetoprotein production *in vitro*. The exact mechanism of action of polypropenoic acid is uncertain; however, preclinical studies suggest that this agent can induce both differentiation and apoptosis of hepatocellular carcinoma cells. A 12-month course of oral polypropenoic acid significantly reduced both recurrent and second primary hepatomas at 38 months; however, follow-up was insufficient to establish an effect on survival (366). A phase II/III study of polypropenoic acid as adjuvant therapy following surgery or ablation of hepatocellular carcinoma in HCV patients is currently under way.

Several studies investigated the expression of COX-2 in human hepatocellular carcinoma to evaluate the possible use of COX-2 inhibitors in the prevention and treatment of this malignancy (367). COX-2 was overexpressed in 75% to 100% of hepatocellular carcinoma specimens and in 47% to 70% of the adjacent noncancerous tissue; well-differentiated hepatocellular carcinoma expresses COX-2 more frequently and strongly than less differentiated tumors. A significant increase in COX-2 levels occurs in liver tissue in parallel with disease progression from chronic hepatitis to cirrhosis (368). Hepatitis B protein HBx, often the only HBV protein detected in HBV-associated hepatocellular carcinoma, induced COX-2 (369). Preclinical evidence supports the potential use of COX-2 inhibitors to reduce the risk of hepatocellular carcinoma. Specifically, the COX-2 inhibitor JTE-522 was highly effective in reducing the development of both cirrhosis and hepatocellular carcinoma in a rat model (370). These agents have not yet been thoroughly evaluated in human studies.

Hepatocellular carcinoma occurs largely in individuals with known risk factors, and significant reduction in hepatocellular carcinoma incidence has been shown through prevention of HBV transmission. In this respect, hepatocellular carcinoma is unique in contrast to most other major cancers, for which risk factors can only be identified at the population level. Thus, hepatocellular carcinoma represents a prime example of an incurable cancer that can indeed be prevented. To advance prevention of hepatocellular carcinoma, clinical trials are needed in the following areas: (a) more effective HBV therapies; (b) new agents to increase the HCV sustained viral response, and identification of factors associated with lack of

response to existing anti-HCV therapies; (c) randomized clinical trials in second-line HCV therapy for primary non-responders and patients who relapse; (d) clinical trials of agents, such as retinoids and COX-2 inhibitors, as secondary prevention for patients undergoing surgical resection, liver transplantation, and ablative therapies. Depending on the results in secondary prevention, consider trials of these agents in primary prevention; and (e) randomized clinical trials to identify the optimal methods and interval for screening populations at risk for hepatocellular carcinoma and to determine whether screening identifies enough treatable tumors to improve long-term outcome and reduce medical care costs associated with advanced hepatocellular carcinoma.

Also needed are (a) a HCV culture system to expand basic research into the pathogenic mechanisms underlying hepatic fibrosis and investigate of new antiviral agents; (b) to investigate the role of fatty liver, obesity, and diabetes in the natural history of hepatocellular carcinoma to identify patients most at risk of developing hepatocellular carcinoma; and (c) to elucidate the mechanisms of viral response and clearance during IFN and ribavirin therapy to develop strategies for the use of these agents in combination with newer agents in HCV.

Gynecologic cancers

Cancers of the cervix, endometrium, and ovary remain a significant public health issue in the United States and worldwide (Table 4). Research in the prevention of these cancers has progressed steadily. Cervical cancer remains a significant problem in developing nations, where access to screening with the Papanicolaou smear is limited. In the United States, preinvasive disease is common, but cervix cancer is less common. Endometrial cancer is the most common gynecologic cancer in the United States and is tightly linked to obesity. There are emerging efforts aimed at weight loss and also chemoprevention of endometrial cancer precursors. Ovarian cancer, although less common, is highly lethal. Recent efforts have focused on chemopreventive and screening strategies for women who carry a BRCA1 or BRCA2 mutation, given their 15% to 50% lifetime risk for developing the disease.

Cervical cancer. This disease remains a significant cause of mortality for women in developing countries. In western Europe, North America, and Japan, screening with the Papanicolaou smear and treatment of precursor lesions have reduced the incidence and mortality. The WHO declared HPV a human carcinogen in 1995 (371). New screening methods for the detection of high-risk HPV showed that some subtypes are more likely to persist and cause lesions than others (372). Even in the United States where screening programs are adequate, the incidence of high-grade squamous intraepithelial lesions is increasing. The current treatment of preinvasive disease focuses on ablation or excision of the transformation zone. This is costly and leaves the potential for recurrence. Newer approaches to the management of HPV-related disease have focused on the development of both prophylactic and therapeutic vaccines and chemopreventive agents that reverse precancerous lesions as well as optical technologies that make a diagnosis.

The chemoprevention strategy most often employed is to choose patients with IEN detected with colposcopically directed biopsy and treat with a chemopreventive. Other high-risk cohorts include patients with HPV or HPV and HIV who have

not yet developed a lesion. CIN is an ideal disease state to address with chemopreventive agents. CIN has a well-defined preclinical phase and is easily recognized from the Papanicolaou smear, colposcopy, and biopsy, and large lesions are unlikely to undergo spontaneous remission. Pharmaceutical agents and micronutrients that have been investigated include folic acid, β -carotene, indole-3-carbinol, fenretinide, retinoic acid derivatives, ornithine decarboxylase inhibitors, and COX-2 inhibitors.

No significant response was found in studies that evaluated the chemopreventive role of folic acid or β -carotene. Earlier studies with retinoic acid showed promising results in regression of dysplasia, but these have not been reproduced by more recent larger trials (373). A randomized phase II trial of indole-3-carbinol showed regression of CIN in 50% of patients, and a larger randomized trial will be conducted by the Gynecologic Oncology Group (374). A small phase I trial using DFMO showed a partial or complete response in 15 of 30 patients treated with dose-deescalating therapy but failed to show a statistically significant response compared with placebo in the follow-on phase II trial (375). Additional preliminary data with COX-2 inhibitors showed no worsening of disease in 20 women (376).

The well-established relationship between HPV and cancer of the cervix has led to the development of vaccines. Both prophylactic and therapeutic vaccine strategies are under investigation. Prophylactic vaccines focus on the induction of effective humoral immunity, increasing antibody responses to HPV in patients naive to the virus. Therapeutic vaccines aim to stimulate cellular immune responses to eliminate virally infected cells and would thus be appropriate for patients who are already infected. The two oncoproteins produced by HPV are E6 and E7. E6 binds to p53, targeting the tumor suppressor for ubiquitin-mediated degradation (64). E7 binds to the retinoblastoma protein, causing the release of E2F and cell cycle progression (65). E6 and E7 are highly expressed in cancers and are therefore ideal immunotherapy targets.

Prophylactic vaccines focus on the ability of the L1 and L2 capsid proteins to assemble into VLPs. VLPs stimulate a potent immune response but do not contain the potentially harmful oncogenes. Harro et al. showed the safety and immunogenicity of HPV-16 VLP vaccine in 2001. The majority of the vaccine recipients achieved serum antibody titers that were ~40-fold higher than what is observed in natural infection (377). In a double-blind study published in 2002, women vaccinated with HPV-16 VLP did not show evidence of persistent HPV-16 infection, showing that VLPs could provide type-specific protection from HPV infection and disease (378). Chimeric VLP vaccines combine a L1 capsid VLP with an E7 gene linked to the carboxyl terminus. Chimeric vaccines have been shown to elicit neutralizing antibodies in addition to T-cell responses to L1 and E7. This may lead to the clearing of HPV-infected basal cells in addition to blocking infection.

Many of the first-generation vaccines have been single-type specific VLP vaccines. However, because ~20 subtypes of HPV have been linked to cervix cancer, a VLP vaccine should aim to provide immunity for more than one subtype. In 2000, a phase II trial was initiated to test a bivalent HPV-16/HPV-18 VLP vaccine (379). A vaccine efficacy of 91.6% (95% confidence interval) against incident infection and 100% efficacy against persistent infection with HPV-16 and HPV-18 were reported. As described above, Merck is developing quadrivalent vaccine

against HPV-6, HPV-11, HPV-16, and HPV-18 that has shown promising results in clinical trials (69). Interestingly, Boursarhin et al. showed that as high as 40% of VLP vaccine recipients developed low-titer neutralizing antibodies against types other than those included in the vaccine (380). Finally, even with multitype HPV vaccines, it is theoretically possible for other high-risk HPV types to emerge.

Several issues need to be clarified before initiating worldwide vaccination programs. One of the difficulties in developing a HPV vaccine is that the virus is difficult to culture effectively. Other vaccine vectors delivering HPV proteins and alternative manufacturing processes may help to overcome this problem. Route of administration, which gender to vaccinate, and at what age to vaccinate have not been adequately addressed in preliminary trials. In 5 years' time, the results of several phase III efficacy trials will be known. The costs of funding clinical trials and manufacturing the vaccines will also need to be addressed. The major challenge will be to make the vaccines available in the developing world where they are needed most.

Ovarian cancer. Epithelial ovarian cancer has the highest mortality rate of any of the gynecologic cancers, with a 5-year survival rate of no more than 30%. This dismal prognosis results from an inability to detect ovarian cancers at an early, curable stage, from the lack of effective therapy for advanced disease, and from our incomplete understanding of both the early changes in the ovary that predate cancer and the initiators of these changes. Although radical surgery and new methods of chemotherapy have improved the disease-free interval following therapy, the overall 5-year survival rate has stayed essentially the same over the last 20 years. Thus, early intervention with chemopreventive agents merits serious consideration.

The risk factors for ovarian cancer include age, obesity, early menstruation, late parity, late menopause, use of fertility drugs, a family history of cancer, personal history of breast cancer, talcum powder, and possibly hormonal therapy. Ovarian cancer may be more likely to occur in those women with more ovulatory events. The ovarian epithelium is a hormonally responsive target organ that expresses receptors for most members of the steroid hormone superfamily, including estrogen, progestin, retinoids, vitamin D, and androgens. In addition, the ovarian epithelium contains COX. Thus, there is the potential for reproductive and environmental factors to affect ovarian cancer risk via a direct biological effect.

The difficulty in detecting precancerous lesions of the ovary complicates trial design. The challenge of obtaining statistically significant and clinically meaningful results from chemoprevention trials is even more complicated for ovarian cancer than for cancers that can be easily biopsied. Difficult access to the organ for repeated tissue sampling, an undefined early natural history of the disease, and the absence of an established screening technique has hampered studies. Optical technologies that can be easily implemented through minilaparoscopes or through the cul de sac may dramatically improve detection and the measurement of modulation of these precancerous lesions. This is important as the low incidence of ovarian cancer makes it an impractical end point for chemoprevention trials. For ovarian cancer chemoprevention trials, the targeted population should include high-risk

women with a strong family history of breast/ovarian cancer, with or without a BRCA mutation, or with Ashkenazi Jewish descent. Although only 10% of ovarian cancers are attributable to germ-line mutations, this high-risk population is a reasonable place to try for preliminary chemoprevention studies because of the higher disease prevalence. Women with BRCA1 mutation have ~40% to 60% risk of developing ovarian cancer (237).

It is difficult to detect precursor lesions of ovarian cancer (381). The evidence for the premalignant lesion, noted on quantitative histopathology, is based on the increased numbers of inclusion cysts and areas of proliferation noted in the ovaries of high-risk women (382). A recent publication (383) showed that histologically normal-appearing ovarian epithelium from cases harboring ovarian lesions exhibits so-called malignancy-associated changes characterizing preneoplastic lesions that cannot be perceived by standard light microscopy but are computationally detectable through karyometric analysis of nuclear abnormalities. This finding was most profound in normal nuclei adjacent to cancer. However, the nuclei from women at increased risk of developing ovarian cancer showed similar findings, suggesting that there may be a lesion that could be used to predict risk of developing cancer and to measure the effects of chemopreventive agents.

There are two ongoing phase II prevention trials. Fox Chase Cancer Center in conjunction with the Gynecology Oncology Group has a trial using fenretinide in high-risk women. Baylor and the University of Arizona have begun trials in high-risk women using fenretinide and in low-risk women with both oral contraceptives and fenretinide.

Observational data suggest that oral contraceptives reduce ovarian cancer risk by ~10% for each year of use leading to a total reduction around 40% after 4 to 5 years (384). Much of the preventive effect is thought to correspond to apoptosis of cells at risk for malignant transformation. Apoptosis is induced in up to 25% of cells in the ovarian epithelium in a primate model receiving levonorgestrel (385). The Cancer and Steroid Hormone Study showed a dose-response relationship with exposure to progestins of higher potency even for a short duration (386).

Retrospective observations from a breast cancer adjuvant trial suggest that fenretinide may reduce ovarian cancer incidence (38, 387, 388), and other receptor-independent, apoptosis-inducing retinoids are being tested. SERMs (389), COX-2 inhibitors (390), and tyrosine kinase inhibitors are also of potential interest (391).

Once women are designated at risk by genetic testing, they usually elect immediate oophorectomies, thus making accrual and completion of chemoprevention trials challenging.

Endometrial cancer. Endometrial cancer is the most common gynecologic cancer in the United States (Table 4), yet there has been little attention focused on prevention of this disease. Recently, chemopreventive strategies for certain cohorts of women at increased risk, including women with Lynch syndrome (hereditary nonpolyposis colorectal cancer syndrome) and obese women, are being considered.

Lynch syndrome is an autosomal dominant cancer susceptibility syndrome caused by a germ-line mutation in repair genes. Women with Lynch syndrome have a 40% to 60% lifetime risk for developing colon cancer or endometrial cancer

(392); therefore, they represent an ideal cohort to study endometrial cancer chemoprevention. A multicenter chemoprevention trial was initiated in 1999, wherein women were randomized to oral contraceptive or to depo-medroxyprogesterone acetate. Multiple studies, including the Cancer and Steroid Hormone Study, found the oral contraceptive decreases the risk of endometrial cancer by 50%. The presumptive mechanism is that oral contraceptives keep the endometrial lining in a quiescent state without the cyclic proliferation, differentiation, and shedding that occurs in the normal menstrual cycle. Progestins, such as medroxyprogesterone, have been shown histologically to reverse both early endometrial cancers and atypical endometrial hyperplasia, the premalignant precursor. This study is close to completing accrual, and the end points are histology, proliferation indices, and apoptosis. In addition, molecular biomarkers relevant to endometrial carcinogenesis, including PTEN and microsatellite instability, are being examined. Finally, because of the well-known association of estrogen exposure with endometrial hyperplasia and cancer, a biomarker panel of genes that are regulated by estrogen and are involved in estrogen-dependent growth regulation of the endometrium will be examined by quantitative PCR.

Women with a body mass index of >32 kg/m² have a 4-fold increased risk and those with a body mass index of >35 kg/m² have a 6-fold increased risk for developing endometrial cancer (393). The presumed mechanism underlying the association of obesity and endometrial cancer is that obese women are in a hyperestrogenic state due to increased peripheral conversion of androstenedione to estrone in the adipose cells. This increase in circulating estrogens results in a hyperproliferative drive of the endometrium. In addition, premenopausal obese women are frequently anovulatory, resulting in irregular menstrual cycles. In the absence of ovulation, progesterone levels are low, and endometrial cells continue to proliferate without glandular differentiation. Weight loss for obese women may be the most direct strategy to decrease endometrial cancer risk. Chemopreventive strategies are also an option, and several agents have been proposed, including aromatase inhibitors, progestins, oral contraceptives, and a local intrauterine device, which contains progestin. Elevated endogenous estrogens may not fully account for the relationship between obesity and endometrial cancer (394). Hyperinsulinemia is being investigated as a cofactor, and insulin resistance and hyperinsulinemia are possible targets for chemoprevention strategies.

One of the scientific challenges is determining rational end points. The majority of endometrial cancers arise through a stepwise progression from normal epithelium to complex atypical hyperplasia (CAH) to endometrial cancer. CAH remains a reasonable surrogate histologic end point for chemoprevention studies and women with CAH represent an important cohort for endometrial cancer chemoprevention studies. However, preliminary results from a recent Gynecologic Oncology Group study has shown that CAH is a fairly unreliable and unreproducible histologic finding, as ~40% of CAH had concurrent endometrial cancers (395). Earlier, molecular end points, including PTEN mutational status and microsatellite instability as well as evaluating estrogen-regulated genes as modulatable biomarkers, are newer areas of investigation.

Accelerating the Development of Chemoprevention Drugs

Progress in chemoprevention drug development requires further elucidation of genetic progression models for each cancer target organ for both risk assessment and cohort selection (Fig. 3) as well as matching the genetic lesion with new molecularly targeted therapies and molecular imaging (Fig. 3). Because chemoprevention drug development is targeted to relatively healthy people, drugs must be safe when given chronically. Toxicogenomics and pharmacogenomics should be incorporated into drug development strategies. Regulatory policy must recognize that carcinogenesis, leading to symptomatic cancer, is a chronic disease and therefore, like cholesterol-lowering for heart disease, may merit acceptance of modest toxicity. Regulatory guidance for acceptable clinical end points and trial designs for accelerated approvals will help guide the process (Table 5).

Integrating the best science

Moving from histopathologic IEN to molecular IEN. The scientific basis of molecular carcinogenesis continues to be clarified. Molecular drug targeting is also advancing rapidly. The prior overview of IEN (1) defined the target histopathologic lesion providing a focus for rational chemoprevention. Histopathologic IEN, usually the best risk marker for later cancer development, is usually a precursor for invasive cancer and is sometimes recognized clinically as a disease process requiring intervention. Progress in developing the genetic progression models for many target organs has led to the concept of molecular IEN (the molecular lesions detectable in the target histopathologic IEN lesions) resulting in models with better predictive values. Numerous advances in microarrays, imaging science, proteomics, nanotechnology advances, etc., underlie this progress. Most importantly, molecular IEN data are helping to identify molecular targets for chemoprevention drug development in at least eight areas (Table 3).

Better risk models will allow better definition of cohorts who will benefit from intervention—these models will incorporate new molecular technologies. Until recently, cancer risk models have relied primarily on population-based statistics and usually have provided only relative risks for cancer development based on one or a very few risk factors. The best of these, the Gail Risk Model for breast cancer (231, 396), includes enough variables and is supported by a vast amount of data correlated to estrogen exposure so that it can be used to estimate absolute risk. However, even the Gail model is limited by reliance on data obtained by interview rather than direct measurement and, as noted earlier in this report, does not incorporate pathology or molecular markers. Better risk models will incorporate histopathologic and molecular differences between precancers or cancers and normal tissue that could be determined directly for a given subject and so would provide a more complete definition of risk (168). Some examples of molecular and histopathologic risk models already exist. The simplest are based on germ-line mutations in tumor suppressors (e.g., APC and p53) and DNA repair mechanisms (e.g., MLH and BRCA) along with occurrence of well-documented precancers, such as colorectal adenomas. However, newer models have incorporated acquired somatic lesions [e.g., the work by Fabian et al. (10) using perioareolar FNA to correlate high risk for

Table 5. Initiatives for accelerating development of cancer prevention drugs**Biomarker, end point, and technology**

- Exploration of chemoprevention strategies based on understanding of genetic progression and biological processes associated with carcinogenesis
- Efforts and policies to foster projects for development and validation of biomarkers in chemoprevention drug development and monitoring
- Efforts/investment in risk-model creation, including data from new molecular methods, such as gene microarrays, proteomics, and nanotechnologies
- Efforts/investment in precancer, IEN, both histologic and predysplastic as a significant part of the new NCI/NCGR Cancer Genome Project

Toxicology and regulatory guidance initiatives

- Efforts/investment to develop new methods to evaluate and monitor toxicity, especially toxicities occurring with chronic administration of chemoprevention drugs
- Development of regulatory guidance relevant to all aspects of chemoprevention drug development
- Efforts/policies to gather postmarketing surveillance data of approved drugs

Structural changes to improve clinical trials

- Augmentation of ongoing and future trials of molecularly targeted agents for cancer treatment by incorporating (nesting) studies to obtain data relevant to chemoprevention
- Development of chemoprevention strategies to prevent cancer recurrence (i.e., using adjuvant settings)
- Development of dedicated clinical research networks capable of translating knowledge of pathogenesis into meaningful clinical tests and interventions

Industry incentives

- Exploration of strategies that would reduce the costs and hurdles for commercial development of chemoprevention drugs, including fostering industry collaboration on common problems, such as biomarkers, postmarketing toxicity data, new regulations on chemoprevention drugs, patent, and data package exclusivity
- Encourage opportunities to accelerate progress in applied research and development of preventive vaccines

Multisector cooperation

- Efforts/investment to establish PPPs with multiple government agencies, such as NIH/NCI, FDA, Centers for Medicare and Medicaid Services, and the pharmaceutical and biotech industries, as well as other stakeholders to foster team science, phase III trial prioritization, regulatory review, and education

patterns (398)]. Both of these techniques have been applied in preliminary models to predict breast cancer risk.

Validation of risk models remains a major challenge. Pepe et al. (325) defined criteria for moving biomarkers for early detection and risk from early research to clinical utility. Particularly important is collection of comprehensive and relevant data from the subjects that include general demographics and baseline and serial samples from clinical trials with these subjects that allow correlations to be made between cancer incidence and precursor lesions/measurements. The molecular progression-based risk model described by Reid et al. (327) and shown in Fig. 4 was developed using this type of information.

Better preclinical and human toxicology. The lesson of COX-2 is that the safety of chronic administration of drugs is unpredictable and is a major challenge to chemoprevention. Methods for detecting, evaluating, and avoiding safety problems will be imperative.

Because they target biological processes also seen in cancer (e.g., inflammation), drugs marketed for treatment of other diseases of aging, such as arthritis, diabetes, cardiovascular disease and its precursors (e.g., hypertension and high cholesterol), and Alzheimer's, have potential utility for chemoprevention. Further, people who are likely to benefit from chemopreventive intervention may also be undergoing treatment for these other diseases of aging. Collaborations to ensure access to toxicity data developed for all indications of potential chemopreventive agents are important to guide dosing, delivery, and patient population. Additional efforts to mine data on promising chemopreventive agents (individually or by class) in these other indications, to identify potential safety issues from long-term dosing or to identify contraindications that would also apply in chemoprevention settings could contribute to chemopreventive drug development. Furthermore, the presence of other disease(s) as stratification factors in clinical chemoprevention studies could help reveal safety issues specific to the presence of other disease and could be useful in defining appropriate subjects for intervention. Pharmacogenomics and toxicogenomics include a wide array of new molecular mechanism-based methods that will help with this characterization both preclinically and clinically. For example, molecular signatures associated with toxicity of specific classes of compounds or specific toxic effects are under development. The FDA is encouraging these methods with the intent of using them in drug regulatory decision-making on safety and efficacy. An ability to identify individuals likely to be susceptible to toxic side effects using pharmacogenetic methods could allow use of drugs with rare but major side effects.

Defining the minimal effective dose and timing of dosing of an agent for a given chemoprevention setting is another possible way to reduce toxicity. This requires reliable drug-effect markers that can be measured noninvasively and possibly extensive preclinical pharmacokinetics and pharmacodynamics studies that evaluate agent levels and target modulation in tissue of interest. Other strategies include using agent combinations to lower agent doses and hence agent-specific toxicities while maintaining efficacy. In addition, intermittent dosing and local delivery of agent to the chemoprevention target (e.g., lung, skin, lung, and oral cavity) to lessen systemic exposure are being explored.

breast cancer with the presence of hyperplasia with cellular atypia and work by Reid et al. (23) that has implicated elevated levels of cyclin D1, p16 LOH, and p53 LOH and gene mutation in describing the risk of Barrett's metaplasia progressing to esophageal adenocarcinoma].

Improved imaging and molecular technologies will contribute to the development of risk models. Some promising methodologies are quantitative histopathology, such as provided by computer-assisted image analysis (397) and DNA microarray and proteomics analysis [e.g., using hierarchical clustering techniques for identifying relevant DNA expression

Another strategy involves establishing and incorporating biomarkers of chronic toxicity (e.g., inflammation, drug metabolism induction, and markers for cardiovascular, liver, central nervous system, and musculoskeletal effects) into preclinical and clinical studies. Data on a standardized set of markers across multiple studies would allow evaluation of the predictive value for clinical safety, which could be used to identify problems early in agent development or identify individuals at risk who could be removed from treatment, whereas toxicity was subclinical and more likely reversible. Implementing this strategy would require assurance that the toxicology variables used could be evaluated across the studies, for example, with standard collection variables and algorithms for normalizing or otherwise allowing data from different contributing laboratories to be compared (e.g., Cancer Bioinformatics Grid methodologies could be a model). In addition, a concerted effort to collect postmarketing surveillance data on agents could be established that would rely on a network of preidentified clinical centers rather than volunteer reporting. The FDA Medical Device Surveillance Network established in 2002 to capture adverse events associated with approved devices is a model.

Incorporating chemoprevention science into regulatory policy

FDA guidance on exploratory/pilot studies. The FDA has been increasing its efforts to help sponsors improve the efficiency of drug development programs and to encourage mechanism-based investigations. One of these efforts resulted in a guidance on exploratory investigational new drugs. This guidance describes, for example, the incorporation of pilot clinical studies that might include just a few subjects and a short time frame to examine specific variables needed to design larger studies. These exploratory studies will be useful to help establish drug-effect markers, determine target tissue distribution, and identify potential effective doses.

Accelerated marketing approvals. The accelerated pathway for gaining marketing approval as defined in 21 CFR Section 314.500 would be applied to chemopreventive drugs. This mechanism allows early marketing approval based on strongly supported surrogate end points for disease incidence in the setting of life-threatening disease, such as cancer. Accelerated approval would be followed by additional studies to ensure efficacy and safety after long-term administration. For chemoprevention, prevention or regression of IEN closely linked to cancer development could serve as the surrogate end point in many cancer settings, and postmarketing studies would be designed to correlate the effects used to gain accelerated approval with cancer incidence and/or to expand the data on modulation of the IEN as well as to evaluate chronic toxicity, optimal dose regimens, rebound and resistance. The approval of celecoxib for regression of colorectal adenomas (and therefore prevention of these adenomas from progressing to cancer) in patients with FAP is the first example of application of the accelerated approval mechanism to chemoprevention. The primary follow-up study focuses on prevention of adenomas in young patients bearing the FAP genetic lesion(s) (e.g., APC mutations) who have not yet expressed the phenotype. The FDA has also suggested the inclusion of interim analysis of surrogate end points in phase III trials with the intent of using the interim results to gain accelerated

approval that would be confirmed by completion of the phase III study.

Draft guidance for new chemoprevention end points. The definition of clinical end points sufficient for drug marketing approval is another important issue in mechanism-based drug development. The FDA is collaborating with oncology researchers to define end points for cancer treatment studies. Workshops and Oncology Drug Advisory Committee meetings have been held on end points for lung, colon, and prostate cancer studies. Other cancer settings will also be evaluated, and it is expected that the FDA will issue guidance documents on the results. Chemoprevention will benefit from similar consideration of end points. In fact, in March 2002, following on the work of the AACR IEN Task Force, the FDA Gastrointestinal Drugs Advisory Committee (including gastroenterologists, oncologists, basic scientists, and statisticians) considered criteria for obtaining drug marketing approval based on colorectal adenoma end points. This committee is a model for future panels to ensure that rapid progress is made in the development of sound evidence-based strategies for chemoprevention. These panels would consider and recommend clinical trial design, definition of end points and their measurement, and specific efficacy and safety criteria for obtaining approvals—both full new drug approvals and accelerated approvals. The Gastrointestinal Advisory Committee recognized that it was not feasible to conduct a prevention trial with cancer incidence as an efficacy end point. Instead, adenoma reduction would be the end point leading to an accelerated approval. Rebound would be evaluated after stopping treatment and agent exposure would be extended postapproval to evaluate chronic toxicity and development of resistance. Both the confirmation of efficacy and the evaluation of chronic safety provided by these postmarketing efforts will be critical strategies for all chemopreventive agents.

Provide incentives to industry and the research community for developing chemopreventive drugs

The success of chemoprevention relies on effective collaborations between academic researchers and the pharmaceutical and biotechnology industries. A primary incentive for industry to invest in chemoprevention research would be adequate intellectual property protection. Currently, development of cancer preventive drugs requires enormous investments in time and money, and these drugs have an even higher risk of failure than cancer treatment drugs. Compared with research for medicines to treat existing disease, chemoprevention research currently requires much more time to produce the evidence required by FDA to get marketing approval.

The concept of intellectual property is based on the premise that a limited period of exclusivity forms the basis for the incentive that society provides to encourage investment in medical innovations. This limited term is not currently adequate for chemoprevention drugs due to the longer time required to gain marketing approval. There are two kinds of intellectual property—patents and data package exclusivity (also known as regulatory exclusivity).

Patent extension. Patents provide a fixed period of exclusivity. In the United States, patents can be obtained to protect the active molecule itself, new ways of using the molecule (e.g., treatment or prevention), a new formulation, and a new way

to make the product. As a result of the trade agreement known as TRIPS, a period of 20 years from the filing date is granted for patents in all fields of technology. After the patent expires, third parties (generic drug manufacturers) may enter the market. The 20-year period may seem like fair incentive for innovation, allowing one to recoup the investment of research and development, but because patents only protect novel inventions a patent application cannot be submitted after the new invention has already been disclosed (e.g., by starting a clinical trial, publishing information about the drug in the scientific literature, or even submitting a new drug application to FDA). Thus, most of the patent life will have elapsed at the time of marketing approval even with an efficient drug development process.

Because it takes years to complete even treatment trials, there is little time to recoup the hundreds of millions of dollars it takes to bring a drug to market. This is why most research and development investment is directed at conducting shorter, less expensive clinical trials to support treatment claims. If a product is developed first for a treatment use, as many are, there is a significant disincentive to pursue a secondary claim for prevention, as there will be little patent protection left by the time that claim ever achieves FDA approval.

Data package exclusivity. A pharmaceutical innovator can obtain data package exclusivity protection for its products in the United States, Europe, Japan, and most major markets during which the regulatory agency will not allow a third party (generic company) to reference the highly valuable safety and efficacy data of the innovator company necessary to obtain marketing approval. These clinical trial data are produced at great expense and represent the single most costly part of drug research and development. Data package exclusivity allows the innovator a defined period of marketing exclusivity to protect the clinical trial data that the innovator produces and submits to the regulator to show the product is safe and effective. This type of intellectual property is triggered by the data that are generated in the course of the clinical trial process and is not the actual "invention" of the product itself.

Generally, this period of data package exclusivity runs parallel to the patent term; however, unlike patents, this protection begins at the time of marketing approval. Therefore, there is no advantage to conducting either treatment or chemoprevention research because the period of exclusivity is not dependent on how long it takes to gain approval.

Unfortunately, the period of data package exclusivity is inadequate in the United States, which is the primary market for most companies: 5 years of protection for new chemical entities and only 3 years for new uses. Exclusivity is longer in both Europe and Japan. In Europe, the drug sponsor is given 10 years of data protection for new chemical entities, and an additional year can be obtained for new uses.

Changing the U.S. patent law to the prior system of starting the clock at the time of patent issuance rather than when the patent was filed, extending the patent life for chemoprevention uses, or making the U.S. period for data package exclusivity conform with that of Europe would significantly spur investments in chemoprevention research and begin moving the nation away from a system in which 98% of all pharmaceutical discovery and development is directed to treatment claims.

It would also be beneficial to chemoprevention research to provide mechanisms so that the research community could access investigative agents from more than a single company for evaluation in clinical studies. Many effective chemoprevention strategies will depend on combinations of drugs with complementary mechanisms of action and nonoverlapping toxicities and different manufacturers. Possibly, such combinations could be evaluated in phase II studies, backed up by strong evidence from preclinical studies, with data given a "Safe Harbor" by the FDA.

Logistical framework for sample and data sharing

A key factor in accelerating chemoprevention drug development is well-annotated and high-quality human tissue and blood samples and tissue images to study molecular and histopathologic progression and to validate risk models. These tissue resources should allow cancer researchers to take advantage of advances being made in genomics, transcriptional profiling, and proteomics and quantitative pathology (e.g., computer-assisted image analysis; i.e., by analysis and comparison of data across multiple institutions and studies). Thus, the samples should be accompanied by detailed demographic and clinical data, and acquisition variables should be annotated and standardized. Samples acquired in most existing tissue banks do not meet these standards. In addition, many issues regarding ownership and distribution of the samples, ethics of using the samples beyond the original trial to which the subject's consent, and protection of the privacy of medical information associated with the samples are unresolved. An important step in moving these studies forward would be development of a national institutional review board for tissue samples or a commitment from the major centers to accept decisions from other institutional review boards.

The National Biospecimen Network was conceived to address the logistics as well as the technical and ethical issues surrounding tissue sample sharing. The promising model described in the National Biospecimen Network report has several essential requirements. (a) Standardized collection of many fresh/frozen cancer specimens derived primarily from academic medical centers and community hospitals. Potential users would include scientists and researchers at academic institutions, government agencies, and biotech and pharmaceutical companies. Contribution of samples would be encouraged by provision of access to the tissue bank and data to the contributors. (b) Accurate, highly standardized clinical, demographic, pathology, and social history annotation; collection of longitudinal data, including biomarker measurements. (c) Prompt and equitable specimen accessibility; data sharing would be done via the Internet. Data generated on National Biospecimen Network samples would be returned to the National Biospecimen Network to help expand the knowledge base. (d) Informatics platforms (e.g., Cancer Bioinformatics Grid) to facilitate sharing of data and results. (e) Protection of patient privacy; data would be deidentified in compliance with the Common Rule for protection of human research subjects and the Health Insurance Portability and Accountability Act.

Multisector cooperation

Public-private partnerships. The needs for access to promising agents and well-annotated tissue to develop the best

chemoprevention strategies as well as convergence of scientific, regulatory, and health policy thinking on the processes and outcomes highlights the value of multisector cooperation. Public-private partnerships (PPP) among NCI, FDA, Centers for Medicare and Medicaid Services, pharmaceutical and biotechnology companies, AACR, American Society of Clinical Oncology, the media, and cancer prevention advocates (e.g., the Cancer Prevention Research Foundation) provide venues in which pooling of resources and other modes of cooperation can take place. A critical aspect of a PPP is a Memo of Understanding, which defines the roles, responsibilities, privileges, and mutual benefits of the members. Master agreements, including standardized Material Transfer Agreements, recognition of institutional review board decisions across centers, and standardized intellectual property templates, are needed to facilitate these types of studies. Many PPPs relevant to chemopreventive drug development are planned or already exist. The National Biospecimen Network is an example of a PPP in planning. NCI has forged numerous partnerships with industry (e.g., via Clinical Trial Agreements) to carry out clinical development of promising chemopreventive agents. FDA has recognized the utility of PPPs to implement critical path initiatives, many of which will affect chemoprevention. For example, the formation of a consortium of six major pharmaceutical companies with C-Path and FDA to share internally developed laboratory methods in toxigenomics was announced recently (<http://www.fda.gov/bbs/topics/news/2006/NEW01337.html>). It is anticipated that this effort will lead to identification of more efficient methods for evaluating safety in preclinical and clinical studies and during postmarketing surveillance. A multisector Oncology Biomarker Qualification Initiative PPP led by NCI and FDA to develop and validate clinical and imaging-based biomarkers has also been launched. Other possibilities include agreements among manufacturers and the NCI, allowing the development of an agent combination composed of two or more investigational drugs from different companies or sharing of safety data from investigational studies on several drugs. Partnerships among NCI, FDA, Centers for Medicare and Medicaid Services, and academia may be convened to develop strategies for integrating chemoprevention studies into screening and cancer therapeutics settings and discuss strategies that would allow insurance reimbursement to subjects.

Team science will allow faster development of effective chemoprevention strategies. The translation of critical knowledge on molecular and genetic progression to prevention of cancer is a multidisciplinary process that requires participation of many difference disciplines.

This widely recognized need for team science to accelerate medical research has resulted in numerous public research plans, including the NIH Roadmap and the FDA Critical Path Initiative. Cancer research is already benefiting from NCI efforts to fund collaborative science involving networks of researchers across many disciplines and institutes. These include the Specialized Programs of Research Excellence, which are organ based and encourage interaction among the participating scientists, with NCI and other NIH programs, industry, and not-for-profit entities to design and implement clinical evaluations in cancer prevention, detection, and treatment. The Early Detection Research Network has a

mandate to develop and test promising biomarkers and technologies for early detection of cancers and encourages collaborations between academia and industry. Program Project Grants, Bioengineering Research Partnerships, and Imaging Response Assessment Teams are other examples NIH/NCI initiatives that support team research. In addition, the Cancer Bioinformatics Grid sponsored by the NCI is developing tools for data standardization and sharing. Some of the most significant challenges confronting these collaborations but are related to ownership of intellectual property and integration of well-established but disparate policies and procedures of the participating institutions.

A cooperative, interdisciplinary, multisector evaluation of issues will be important for ensuring safety of chemopreventive strategies and in defining subjects who will benefit from chemopreventive intervention. The Toxicology Forum (<http://www.toxforum.org>) encourages open, nonadversarial dialogue and research planning among government, industry, and academia on controversial subjects in public health. The Forum has addressed many subjects relevant to chemoprevention, including drug-induced cardiovascular toxicity, preclinical toxicity of peroxisome proliferator-activated receptor agonists, and estrogenic substances in soy. Another model is the new FDA/C-Path/pharmaceutical company collaboration on toxicogenomics cited above.

Collaborative review and reevaluation of mechanism-based regulatory policy. More communication among stakeholders is necessary to bring effective chemopreventive drugs to the market. The existing system was designed primarily to approve drugs for the treatment of clinically evident disease, which discourages pharmaceutical companies from embracing chemoprevention. This resulted in few new drug applications for chemoprevention drugs, and until this gap is recognized, and all affected parties are engaged in a discussion to correct it, the industry will continue to focus on the discovery and development of therapeutic rather than preventive drugs. Several collaborative efforts have begun to address the need for guidelines (1, 11, 399), and the recent priority given this by the FDA Critical Path initiative and the NCI/FDA Biomarker Qualification Initiative provides an opportunity for progress. Many of the topics requiring productive collaboration are summarized in Table 5.

Education of public on precancer. It is imperative that the public engage in this discussion. This will require increased awareness of the diseases, its origins, and the opportunities to reduce the nation's cancer burden. The general public's understanding of chemoprevention of cancer needs to be brought up to the level of that obtained in other chronic diseases, such as those prevented by lipid lowering and reduction of systemic blood pressure. The benefits could be enormous from lifestyle modification to support of public initiatives for research, including clinical trial participation and tissue specimen banking. Because the greatest potential to affect the course of cancer is in the earliest stages of disease, an individual must be willing to take action when disease is subclinical (i.e., before the emergence of signs and symptoms, when the individual is otherwise healthy). This represents an enormous challenge and can only be approached through comprehensive industry, federal, and state government-sponsored educational programs.

References

- O'Shaughnessy JA, Kelloff GJ, Gordon GB, et al. Treatment and prevention of intraepithelial neoplasia: an important target for accelerated new agent development. *Clin Cancer Res* 2002;8:314–46.
- Anderson WF, Umar A, Viner JL, Hawk ET. The role of cyclooxygenase inhibitors in cancer prevention. *Curr Pharm Des* 2002;8:1035–62.
- Kelloff GJ, Boone CW, Steele VE, et al. Mechanistic considerations in chemopreventive drug development. *J Cell Biochem Suppl* 1994;20:1–24.
- Dannenberg AJ, Subbaramaiah K. Targeting cyclooxygenase-2 in human neoplasia: rationale and promise. *Cancer Cell* 2003;4:431–6.
- Subbaramaiah K, Dannenberg AJ. Cyclooxygenase 2: a molecular target for cancer prevention and treatment. *Trends Pharmacol Sci* 2003;24:96–102.
- Park JW, Kerbel RS, Kelloff GJ, et al. Rationale for biomarkers and surrogate end points in mechanism-driven oncology drug development. *Clin Cancer Res* 2004;10:3885–96.
- MoothaVK, Lindgren CM, Eriksson KF, et al. PGC-1 α -responsive genes involved in oxidative phosphorylation are coordinately downregulated in human diabetes. *Nat Genet* 2003;34:267–73.
- Majumder PK, Febbo PG, Bikoff R, et al. mTOR inhibition reverses Akt-dependent prostate intraepithelial neoplasia through regulation of apoptotic and HIF-1-dependent pathways. *Nat Med* 2004;10:594–601.
- Troester M, Perou CM. Functional genomics for identifying surrogate endpoint biomarkers in breast cancer chemoprevention. In: Kelloff GJ, Hawk ET, Sigman CC, editors. *Cancer chemoprevention*. Vol. 2. Strategies for cancer chemoprevention. Totowa (NJ): Humana Press; 2005. p. 115–22.
- Fabian CJ, Kimler BF. Breast cancer chemoprevention: current challenges and a look toward the future. *Clin Breast Cancer* 2002;3:113–24.
- Kelloff GJ, Sigman CC, Johnson KM, et al. Perspectives on surrogate end points in the development of drugs that reduce the risk of cancer. *Cancer Epidemiol Biomarkers Prev* 2000;9:127–37.
- Kelloff GJ, Fay JR, Steele VE, et al. Epidermal growth factor receptor tyrosine kinase inhibitors as potential cancer chemopreventives. *Cancer Epidemiol Biomarkers Prev* 1996;5:657–66.
- Sansal I, Sellers WR. The biology and clinical relevance of the PTEN tumor suppressor pathway. *J Clin Oncol* 2004;22:2954–63.
- Sidransky D. Emerging molecular markers of cancer. *Nat Rev Cancer* 2002;2:210–9.
- Vogelstein B, Fearon ER, Hamilton SR, et al. Genetic alterations during colorectal tumor development. *N Engl J Med* 1988;319:525–32.
- Ilyas M, Straub J, Tomlinson IP, Bodmer WF. Genetic pathways in colorectal and other cancers. *Eur J Cancer* 1999;35:1986–2002.
- Le QT, Giaccia AJ. Therapeutic exploitation of the physiological and molecular genetic alterations in head and neck cancer. *Clin Cancer Res* 2003;9:4287–95.
- Fearon ER, Vogelstein B. A genetic model for colorectal tumorigenesis. *Cell Death Differ* 1990;6:759–67.
- Dillon DA, Howe CL, Bosari S, Costa J. The molecular biology of breast cancer: accelerating clinical applications. *Crit Rev Oncog* 1998;9:125–40.
- Parsons R. Human cancer, PTEN and the PI-3 kinase pathway. *Semin Cell Dev Biol* 2004;15:171–6.
- Leslie NR, Downes CP. PTEN function: how normal cells control it and tumour cells lose it. *Biochem J* 2004;382:1–11.
- VandeWoude GF, Kelloff GJ, Ruddon RW, et al. Reanalysis of cancer drugs: old drugs, new tricks. *Clin Cancer Res* 2004;10:3897–907.
- Reid BJ, Blount PL, Rabinovitch PS. Biomarkers in Barrett's esophagus. *Gastrointest Endosc Clin N Am* 2003;13:369–97.
- Maley CC, Galipeau PC, Li X, Sanchez CA, Paulson TG, Reid BJ. Selectively advantageous mutations and hitchhikers in neoplasms: p16 lesions are selected in Barrett's esophagus. *Cancer Res* 2004;64:3414–27.
- Barnes PJ, Karin M. Nuclear factor- κ B: a pivotal transcription factor in chronic inflammatory diseases. *N Engl J Med* 1997;336:1066–71.
- Hanahan D, Weinberg RA. The hallmarks of cancer. *Cell* 2000;100:57–70.
- Aggarwal BB. Nuclear factor- κ B: the enemy within. *Cancer Cell* 2004;6:203–8.
- Crowell JA, Page JG, Levine BS, Tomlinson MJ, Hebert CD. Indole-3-carbinol, but not its major digestive product 3,3'-diindolylmethane, induces reversible hepatocyte hypertrophy and cytochromes P450. *Toxicol Appl Pharmacol* 2005;211:115–23.
- Kelloff GJ, Bast RC, Jr., Coffey DS, et al. Biomarkers, surrogate end points, and the acceleration of drug development for cancer prevention and treatment: an update prologue. *Clin Cancer Res* 2004;10:3881–4.
- Baserga R. Controlling IGF-receptor function: a possible strategy for tumor therapy. *Trends Biotechnol* 1996;14:150–2.
- D'Ambrosio C, Ferber A, Resnicoff M, Baserga R. A soluble insulin-like growth factor I receptor that induces apoptosis of tumor cells *in vivo* and inhibits tumorigenesis. *Cancer Res* 1996;56:4013–20.
- Bol DK, Kiguchi K, Gimenez-Conti I, Rupp T, DiGiovanni J. Overexpression of insulin-like growth factor-1 induces hyperplasia, dermal abnormalities, and spontaneous tumor formation in transgenic mice. *Oncogene* 1997;14:1725–34.
- Kulik G, Klippel A, Weber MJ. Antiapoptotic signaling by the insulin-like growth factor I receptor, phosphatidylinositol 3-kinase, and Akt. *Mol Cell Biol* 1997;17:1595–606.
- Spitz MR, Barnett MJ, Goodman GE, Thornquist MD, Wu X, Pollak M. Serum insulin-like growth factor (IGF) and IGF-binding protein levels and risk of lung cancer: a case-control study nested in the β -Carotene and Retinol Efficacy Trial cohort. *Cancer Epidemiol Biomarkers Prev* 2002;11:1413–8.
- Chan JM, Stampfer MJ, Ma J, et al. Insulin-like growth factor-I (IGF-I) and IGF binding protein-3 as predictors of advanced-stage prostate cancer. *J Natl Cancer Inst* 2002;94:1099–106.
- Furstenberger G, Senn HJ. Insulin-like growth factors and cancer. *Lancet Oncol* 2002;3:298–302.
- Kirman I, Poltoratskaia N, Sylla P, Whelan RL. Insulin-like growth factor-binding protein 3 inhibits growth of experimental col carcinoma. *Surgery* 2004;136:205–9.
- Decensi A, Formelli F, Torrisi R, Costa A. Breast cancer chemoprevention: studies with 4-HPR alone and in combination with tamoxifen using circulating growth factors as potential surrogate endpoints. *J Cell Biochem Suppl* 1993;17G:226–33.
- Colletta AA, Benson JR, Baum M. Alternative mechanisms of action of antiestrogens. *Breast Cancer Res Treat* 1994;31:5–9.
- Favoni RE, de Cupis A, Bruno S, et al. Modulation of the insulin-like growth factor-I system by *N*-(4-hydroxyphenyl)-retinamide in human breast cancer cell lines. *Br J Cancer* 1998;77:2138–47.
- Decensi A, Robertson C, Ballardini B, et al. Effect of tamoxifen on lipoprotein(a) and insulin-like growth factor-I (IGF-I) in healthy women. *Eur J Cancer* 1999;35:596–600.
- Torrisi R, Decensi A, Formelli F, Camerini T, De Palo G. Chemoprevention of breast cancer with fenretinide. *Drugs* 2001;61:909–18.
- Weinstein IB. Disorders in cell circuitry during multistage carcinogenesis: the role of homeostasis. *Carcinogenesis* 2000;21:857–64.
- Weinstein IB. Cancer. Addiction to oncogenes—the Achilles heel of cancer. *Science* 2002;297:63–4.
- Mills GB, Lu Y, Kohn EC. Linking molecular therapeutics to molecular diagnostics: inhibition of the FRAP/RAFT/TOR component of the PI3K pathway preferentially blocks PTEN mutant cells *in vitro* and *in vivo*. *Proc Natl Acad Sci U S A* 2001;98:10031–3.
- Gazdar AF, Shigematsu H, Herz J, Minna JD. Mutations and addiction to EGFR: the Achilles "heel" of lung cancers? *Trends Mol Med* 2004;10:481–6.
- Thun MJ, Henley SJ, Gansler T. Inflammation and cancer: an epidemiological perspective. *Novartis Found Symp* 2004;256:6–21; discussion 2–8, 49–52, 266–9.
- Platz EA, De Marzo AM. Epidemiology of inflammation and prostate cancer. *J Urol* 2004;171:S36–40.
- Caruso C, Lio D, Cavallone L, Franceschi C. Aging, longevity, inflammation, and cancer. *Ann NY Acad Sci* 2004;1028:1–13.
- Schottenfeld D, Beebe-Dimmer JL. Advances in cancer epidemiology: understanding causal mechanisms and the evidence for implementing interventions. *Annu Rev Public Health* 2005;26:37–60.
- Dannenberg AJ, Altorki NK, Boyle JO, et al. Cyclooxygenase 2: a pharmacological target for the prevention of cancer. *Lancet Oncol* 2001;2:544–51.
- Philip M, Rowley DA, Schreiber H. Inflammation as a tumor promoter in cancer induction. *Semin Cancer Biol* 2004;14:433–9.
- Dannenberg AJ, Lippman SM, Mann JR, Subbaramaiah K, DuBois RN. Cyclooxygenase-2 and epidermal growth factor receptor: pharmacologic targets for chemoprevention. *J Clin Oncol* 2005;23:254–66.
- Bartsch H, Nair J. Oxidative stress and lipid peroxidation-derived DNA-lesions in inflammation driven carcinogenesis. *Cancer Detect Prev* 2004;28:385–91.
- Christensen ND. Emerging human papillomavirus vaccines. *Expert Opin Emerg Drugs* 2005;10:5–19.
- Khanna R, Moss D, Gandhi M. Technology insight: applications of emerging immunotherapeutic strategies for Epstein-Barr virus-associated malignancies. *Nat Clin Pract Oncol* 2005;2:138–49.
- Mohanna S, Macco V, Bravo F, Gutuzzo E. Epidemiology and clinical characteristics of classic Kaposi's sarcoma, seroprevalence, and variants of human herpesvirus 8 in South America: a critical review of an old disease. *Int J Infect Dis* 2005;9:239–50.
- McGlynn KA, London WT. Epidemiology and natural history of hepatocellular carcinoma. *Best Pract Res Clin Gastroenterol* 2005;19:3–23.
- Schistosomes, liver flukes and *Helicobacter pylori*. IARC Working Group on the Evaluation of Carcinogenic Risks to Humans. Lyon, 7-14 June 1994. IARC Monogr Eval Carcinog Risks Hum 1994;61:1–241.
- Moss SF, Blaser MJ. Mechanisms of disease: inflammation and the origins of cancer. *Nat Clin Pract Oncol* 2005;2:90–7; quiz 1 p. following 113.
- Fischbach W, Chan AO, Wong BC. *Helicobacter pylori* and gastric malignancy. *Helicobacter* 2005;10 Suppl 1:34–9.
- Duberg AS, Nordstrom M, Torner A, et al. Non-Hodgkin's lymphoma and other nonhepatic malignancies in Swedish patients with hepatitis C virus infection. *Hepatology* 2005;41:652–9.
- Young LS, Rickinson AB. Epstein-Barr virus: 40 years on. *Nat Rev Cancer* 2004;4:757–68.
- Werness BA, Levine AJ, Howley PM. Association of human papillomavirus types 16 and 18 E6 proteins with p53. *Science* 1990;248:76–9.
- Dyson N, Howley PM, Munger K, Harlow E. The human papilloma virus-16 E7 oncoprotein is able to bind to the retinoblastoma gene product. *Science* 1989;243:934–7.
- Mahdavi A, Monk BJ. Vaccines against human papillomavirus and cervical cancer: promises and challenges. *Oncologist* 2005;10:528–38.
- Gillison ML. Human papillomavirus-associated head and neck cancer is a distinct epidemiologic, clinical, and molecular entity. *Semin Oncol* 2004;31:744–54.
- Mao C, Koutsky LA, Ault KA, et al. Efficacy of human papillomavirus-16 vaccine to prevent cervical intraepithelial neoplasia: a randomized controlled trial. *Obstet Gynecol* 2006;107:18–27.
- Villa LL, Costa RL, Petta CA, et al. Prophylactic quadrivalent human papillomavirus (types 6, 11, 16,

- and 18) L1 virus-like particle vaccine in young women: a randomised double-blind placebo-controlled multicentre phase II efficacy trial. *Lancet Oncol* 2005;6:271–8.
70. Clevers H. At the crossroads of inflammation and cancer. *Cell* 2004;118:671–4.
71. Karin M. Inflammation and cancer: the long reach of Ras. *Nat Med* 2005;11:20–1.
72. Vakkila J, Lotze MT. Inflammation and necrosis promote tumour growth. *Nat Rev Immunol* 2004;4:641–8.
73. Balkwill F, Charles KA, Mantovani A. Smoldering and polarized inflammation in the initiation and promotion of malignant disease. *Cancer Cell* 2005;7:211–7.
74. Coussens LM, Werb Z. Inflammation and cancer. *Nature* 2002;420:860–7.
75. Mann JR, DuBois RN. Cyclooxygenase-2 and gastrointestinal cancer. *Cancer J* 2004;10:145–52.
76. Grubbs CJ, Lubet RA, Koki AT, et al. Celecoxib inhibits *N*-butyl-*N*-(4-hydroxybutyl)-nitrosamine-induced urinary bladder cancers in male B6D2F1 mice and female Fischer-344 rats. *Cancer Res* 2000;60:5599–602.
77. Fischer SM, Lo HH, Gordon GB, et al. Chemopreventive activity of celecoxib, a specific cyclooxygenase-2 inhibitor, and indomethacin against ultraviolet light-induced skin carcinogenesis. *Mol Carcinog* 1999;25:231–40.
78. Brown JR, DuBois RN. Cyclooxygenase as a target in lung cancer. *Clin Cancer Res* 2004;10:4266–9s.
79. Malkinson AM. Role of inflammation in mouse lung tumorigenesis: a review. *Exp Lung Res* 2005;31:57–82.
80. Mao JT, Cui X, Reckamp K, et al. Chemoprevention strategies with cyclooxygenase-2 inhibitors for lung cancer. *Clin Lung Cancer* 2005;7:30–9.
81. Lin DT, Subbaramaiah K, Shah JP, Dannenberg AJ, Boyle JO. Cyclooxygenase-2: a novel molecular target for the prevention and treatment of head and neck cancer. *Head Neck* 2002;24:792–9.
82. Lin EY, Pollard JW. Macrophages: modulators of breast cancer progression. *Novartis Found Symp* 2004;256:158–68; discussion 68–72, 259–69.
83. Chang SH, Ai Y, Breyer RM, Lane TF, Hla T. The prostaglandin E₂ receptor EP2 is required for cyclooxygenase 2-mediated mammary hyperplasia. *Cancer Res* 2005;65:4496–9.
84. Ross RK, Pike MC, Coetzee GA, et al. Androgen metabolism and prostate cancer: establishing a model of genetic susceptibility. *Cancer Res* 1998;58:4497–504.
85. Nelson WG, De Marzo AM, DeWeese TL, Isaacs WB. The role of inflammation in the pathogenesis of prostate cancer. *J Urol* 2004;172:S6–11; discussion S-2.
86. Kelloff GJ. Perspectives on cancer chemoprevention research and drug development. *Adv Cancer Res* 2000;78:199–334.
87. Crowell JA, Steele VE, Sigman CC, Fay JR. Is inducible nitric oxide synthase a target for chemoprevention? *Mol Cancer Ther* 2003;2:815–23.
88. Rigas B, Kashfi K. Nitric-oxide-donating NSAIDs as agents for cancer prevention. *Trends Mol Med* 2004;10:324–30.
89. Steele VE, Holmes CA, Hawk ET, et al. Potential use of lipoygenase inhibitors for cancer chemoprevention. *Expert Opin Investig Drugs* 2000;9:2121–38.
90. Shureiqi I, Chen D, Lee JJ, et al. 15-LOX-1: a novel molecular target of nonsteroidal anti-inflammatory drug-induced apoptosis in colorectal cancer cells. *J Natl Cancer Inst* 2000;92:1136–42.
91. Ghosh S, Karin M. Missing pieces in the NF- κ B puzzle. *Cell* 2002;109 Suppl:81–96.
92. Yang CS, Maliakal P, Meng X. Inhibition of carcinogenesis by tea. *Annu Rev Pharmacol Toxicol* 2002;42:25–54.
93. Yang CS, Landau JM, Huang MT, Newmark HL. Inhibition of carcinogenesis by dietary polyphenolic compounds. *Annu Rev Nutr* 2001;21:381–406.
94. Bettuzzi S, Brausi M, Rizzi F, Castagnetti G, Peracchia G, Corti A. Chemoprevention of human prostate cancer by oral administration of green tea catechins in volunteers with high-grade prostate intraepithelial neoplasia: a preliminary report from a one-year proof-of-principle study. *Cancer Res* 2006;66:1234–40.
95. Dhakshinamoorthy S, Long DJ II, Jaiswal AK. Antioxidant regulation of genes encoding enzymes that detoxify xenobiotics and carcinogens. *Curr Top Cell Regul* 2000;36:201–16.
96. Finley JW. The antioxidant responsive element (ARE) may explain the protective effects of cruciferous vegetables on cancer. *Nutr Rev* 2003;61:250–4.
97. Kwak MK, Wakabayashi N, Kensler TW. Chemoprevention through the Keap1-Nrf2 signaling pathway by phase 2 enzyme inducers. *Mutat Res* 2004;555:133–48.
98. Sporn MB, Liby KT. Cancer chemoprevention: scientific promise, clinical uncertainty. *Nat Clin Pract Oncol* 2005;2:518–25.
99. Kelloff GJ, Schilsky RL, Alberts DS, et al. Colorectal adenomas: a prototype for the use of surrogate end points in the development of cancer prevention drugs. *Clin Cancer Res* 2004;10:3908–18.
100. Feigelson HS, Henderson BE. Estrogens and breast cancer. *Carcinogenesis* 1996;17:2279–84.
101. Henderson BE, Ross RK, Shibata A, Paganini-Hill A, Yu MC. Environmental carcinogens and anticarcinogens. In: Wattenberg L, Lipkin M, Boone CW, Kelloff GJ, editors. *Cancer chemoprevention*. Boca Raton (FL): CRC Press; 1992. p. 3–17.
102. Kelloff GJ, Crowell JA, Hawk ET, et al. Strategy and planning for chemopreventive drug development: clinical development plans II: vitamin A. *J Cell Biochem Suppl* 1996;26:236–57.
103. Pollak MN, Schernhammer ES, Hankinson SE. Insulin-like growth factors and neoplasia. *Nat Rev Cancer* 2004;4:505–18.
104. Demierre MF, Higgins PD, Gruber SB, Hawk E, Lippman SM. Statins and cancer prevention. *Nat Rev Cancer* 2005;5:930–42.
105. Friis S, Poulsen AH, Johnsen SP, et al. Cancer risk among statin users: a population-based cohort study. *Int J Cancer* 2005;114:643–7.
106. Dale KM, Coleman CI, Henyan NN, Kluger J, White CM. Statins and cancer risk: a meta-analysis. *JAMA* 2006;295:74–80.
107. Jacobs EJ, Rodriguez C, Brady KA, Connell CJ, Thun MJ, Calle EE. Cholesterol-lowering drugs and colorectal cancer incidence in a large United States cohort. *J Natl Cancer Inst* 2006;98:69–72.
108. Eliassen AH, Colditz GA, Rosner B, Willett WC, Hankinson SE. Serum lipids, lipid-lowering drugs, and the risk of breast cancer. *Arch Intern Med* 2005;165:2264–71.
109. Bonovas S, Filioussi K, Tsavaris N, Sitaras NM. Use of statins and breast cancer: a meta-analysis of seven randomized clinical trials and nine observational studies. *J Clin Oncol* 2005;23:8606–12.
110. Chan TA, Morin PJ, Vogelstein B, Kinzler KW. Mechanisms underlying nonsteroidal anti-inflammatory drug-mediated apoptosis. *Proc Natl Acad Sci U S A* 1998;95:681–6.
111. Nanni S, Narducci M, Della Pietra L, et al. Signaling through estrogen receptors modulates telomerase activity in human prostate cancer. *J Clin Invest* 2002;110:219–27.
112. Krysan K, Reckamp KL, Dalwadi H, et al. Prostaglandin E₂ activates mitogen-activated protein kinase/Erk pathway signaling and cell proliferation in non-small cell lung cancer cells in an epidermal growth factor receptor-independent manner. *Cancer Res* 2005;65:6275–81.
113. Torrance CJ, Jackson PE, Montgomery E, et al. Combinatorial chemoprevention of intestinal neoplasia. *Nat Med* 2000;6:1024–8.
114. Zhang X, Chen ZG, Choe MS, et al. Tumor growth inhibition by simultaneously blocking epidermal growth factor receptor and cyclooxygenase-2 in a xenograft model. *Clin Cancer Res* 2005;11:6261–9.
115. Abbruzzese JL, Lippman SM. The convergence of cancer prevention and therapy in early-phase clinical drug development. *Cancer Cell* 2004;6:321–6.
116. Lee HY, Moon H, Chun KH, et al. Effects of insulin-like growth factor binding protein-3 and farnesyltransferase inhibitor SCH66336 on Akt expression and apoptosis in non-small-cell lung cancer cells. *J Natl Cancer Inst* 2004;96:1536–48.
117. Chang TH, Szabo E. Enhanced growth inhibition by combination differentiation therapy with ligands of peroxisome proliferator-activated receptor- γ and inhibitors of histone deacetylase in adenocarcinoma of the lung. *Clin Cancer Res* 2002;8:1206–12.
118. O'Reilly K, Rojo F, She Q-B, et al. mTOR inhibition induces upstream receptor tyrosine kinase signaling and activates Akt. *Cancer Res* 2006;66:1500–8.
119. Belinsky SA, Klinge DM, Stidley CA, et al. Inhibition of DNA methylation and histone deacetylation prevents murine lung cancer. *Cancer Res* 2003;63:7089–93.
120. Jemal A, Murray T, Ward E, et al. Cancer statistics, 2005. *CA Cancer J Clin* 2005;55:10–30.
121. Guileyardo JM, Johnson WD, Welsh RA, Akazaki K, Correa P. Prevalence of latent prostate carcinoma in two U.S. populations. *J Natl Cancer Inst* 1980;65:311–6.
122. Franks LM. Latent carcinoma of the prostate. *J Pathol Bacteriol* 1954;68:603–16.
123. Hankey BF, Feuer EJ, Clegg LX, et al. Cancer surveillance series: interpreting trends in prostate cancer. Part I. Evidence of the effects of screening in recent prostate cancer incidence, mortality, and survival rates. *J Natl Cancer Inst* 1999;91:1017–24.
124. Early detection, diagnosis, and prognosis [accessed 2005 Jun 7]. Available from: <http://prg.nci.nih.gov/prostate/prgdetect.html>.
125. Ouyang X, DeWeese TL, Nelson WG, Abate-Shen C. Loss-of-function of Nkx3.1 promotes increased oxidative damage in prostate carcinogenesis. *Cancer Res* 2005;65:6773–9.
126. Shen MM, Abate-Shen C. Roles of the Nkx3.1 homeobox gene in prostate organogenesis and carcinogenesis. *Dev Dyn* 2003;228:767–78.
127. Sheehan KM, Calvert VS, Kay EW, et al. Use of reverse phase protein microarrays and reference standard development for molecular network analysis of metastatic ovarian carcinoma. *Mol Cell Proteomics* 2005;4:346–55.
128. Ma X, Ziel-van der Made AC, Autar B, et al. Targeted biallelic inactivation of Pten in the mouse prostate leads to prostate cancer accompanied by increased epithelial cell proliferation but not by reduced apoptosis. *Cancer Res* 2005;65:5730–9.
129. Abate-Shen C, Banach-Petrosky WA, Sun X, et al. Nkx3.1: Pten mutant mice develop invasive prostate adenocarcinoma and lymph node metastases. *Cancer Res* 2003;63:3886–90.
130. Ayoub J, Jean-Francois R, Cormier Y, et al. Placebo-controlled trial of 13-*cis*-retinoic acid activity on retinoic acid receptor- β expression in a population at high risk: implications for chemoprevention of lung cancer. *J Clin Oncol* 1999;17:3546–52.
131. Kang GH, Lee S, Lee HJ, Hwang KS. Aberrant CpG island hypermethylation of multiple genes in prostate cancer and prostatic intraepithelial neoplasia. *J Pathol* 2004;202:233–40.
132. Nelson WG, De Marzo AM, DeWeese TL, et al. Preneoplastic prostate lesions: an opportunity for prostate cancer prevention. *Ann N Y Acad Sci* 2001;952:135–44.
133. Nelson WG, De Marzo AM, Isaacs WB. Prostate cancer. *N Engl J Med* 2003;349:366–81.
134. Thompson IM, Goodman PJ, Tangen CM, et al. The influence of finasteride on the development of prostate cancer. *N Engl J Med* 2003;349:215–24.
135. Ishikawa S, Soloway MS, Van der Zwaag R, Todd B. Prognostic factors in survival free of progression after androgen deprivation therapy for treatment of prostate cancer. *J Urol* 1989;141:1139–42.
136. Schatzl G, Madersbacher S, Haitel A, et al. Associations of serum testosterone with microvessel density, androgen receptor density and androgen receptor gene polymorphism in prostate cancer. *J Urol* 2003;169:1312–5.

137. Jewett MA, Fleshner N, Klotz LH, Nam RK, Trachtenberg J. Radical prostatectomy as treatment for prostate cancer. *CMAJ* 2003;168:44–5.
138. Prehn RT. On the prevention and therapy of prostate cancer by androgen administration. *Cancer Res* 1999;59:4161–4.
139. Bostwick DG, Qian J, Civantos F, Roehrborn CG, Montironi R. Does finasteride alter the pathology of the prostate and cancer grading? *Clin Prostate Cancer* 2004;2:228–35.
140. Parnes HL, Thompson IM, Ford LG. Prevention of hormone-related cancers: prostate cancer. *J Clin Oncol* 2005;23:368–77.
141. Lippman SM, Goodman PJ, Klein EA, et al. Designing the Selenium and Vitamin E Cancer Prevention Trial (SELECT). *J Natl Cancer Inst* 2005;97:94–102.
142. Platz EA, Leitzman MF, Visvanathan K, et al. Cholesterol-lowering drugs including statins and the risk of prostate cancer in a large prospective cohort study [abstract 4374]. *Proc Am Assoc Cancer Res* 2005;46.
143. Shannon J, Tewoderos S, Garzotto M, et al. Statins and prostate cancer risk: a case-control study. *Am J Epidemiol* 2005;162:318–25.
144. Singal R, Khurana V, Caldito G, Fort C. Statins and prostate cancer risk: a large case control study in veterans [abstract 1004]. *Proc Am Soc Clin Oncol* 2005;23.
145. Shibata MA, Kavanaugh C, Shibata E, et al. Comparative effects of lovastatin on mammary and prostate oncogenesis in transgenic mouse models. *Carcinogenesis* 2003;24:453–9.
146. Steiner MS, Raghov S. Antiestrogens and selective estrogen receptor modulators reduce prostate cancer risk. *World J Urol* 2003;21:31–6.
147. Steiner MS, Pound RC. Phase IIA clinical trial to test the efficacy and safety of toremifene in men with high-grade prostatic intraepithelial neoplasia. *Clin Prostate Cancer* 2003;2:24–31.
148. Raghov S, Hooshdaran MZ, Katiyar S, Steiner MS. Toremifene prevents prostate cancer in the transgenic adenocarcinoma of mouse prostate model. *Cancer Res* 2002;62:1370–6.
149. Andriole G, Bostwick D, Brawley O, et al. Chemoprevention of prostate cancer in men at high risk: rationale and design of the Reduction by Dutasteride of Prostate Cancer Events (REDUCE) trial. *J Urol* 2004;172:1314–7.
150. Gomella LG. Chemoprevention using dutasteride: the REDUCE trial. *Curr Opin Urol* 2005;15:29–32.
151. Bostwick DG, Burke HB, Wheeler TM, et al. The most promising surrogate endpoint biomarkers for screening candidate chemopreventive compounds for prostatic adenocarcinoma in short-term phase II clinical trials. *J Cell Biochem Suppl* 1994;19:283–9.
152. Kelloff GJ, Lieberman R, Brawer MK, Crawford ED, Labrie F, Miller GJ. Strategies for chemoprevention of prostate cancer. *Prostate Cancer Prostatic Dis* 1999;2:27–33.
153. Holmberg L, Bill-Axelsson A, Helgesen F, et al. A randomized trial comparing radical prostatectomy with watchful waiting in early prostate cancer. *N Engl J Med* 2002;347:781–9.
154. Chapple A, Ziebland S, Herxheimer A, McPherson A, Shepperd S, Miller R. Is "watchful waiting" a real choice for men with prostate cancer? A qualitative study. *BJU Int* 2002;90:257–64.
155. Albertsen PC, Hanley JA, Gleason DF, Barry MJ. Competing risk analysis of men aged 55 to 74 years at diagnosis managed conservatively for clinically localized prostate cancer. *JAMA* 1998;280:975–80.
156. Bill-Axelsson A, Holmberg L, Ruutu M, et al. Radical prostatectomy versus watchful waiting in early prostate cancer. *N Engl J Med* 2005;352:1977–84.
157. Wilt TJ, Brawer MK. The Prostate Cancer Intervention versus Observation Trial (PIVOT). *Oncology (Huntingt)* 1997;11:1133–9; discussion 9–40, 43.
158. Surgery versus watchful waiting in early prostate cancer; 2005 [accessed 2005 Jul 12]. Available from: <http://www.nci.nih.gov/clinicaltrials/results/surgery-vs-watchful-waiting0902>.
159. Leiva IM, Emmert-Buck MR, Gillespie JW. Handling of clinical tissue specimens for molecular profiling studies. *Curr Issues Mol Biol* 2003;5:27–35.
160. Kim J, Sun P, Lam YW, et al. Changes in serum proteomic patterns by presurgical α -tocopherol and L-selenomethionine supplementation in prostate cancer. *Cancer Epidemiol Biomarkers Prev* 2005;14:1697–702.
161. GLOBOCAN 2002: cancer incidence, mortality and prevalence worldwide. Lyon: IARC Press; 2004 [accessed 2004].
162. Pignone M, Rich M, Teutsch SM, Berg AO, Lohr KN. Screening for colorectal cancer in adults at average risk: a summary of the evidence for the U.S. Preventive Services Task Force. *Ann Intern Med* 2002;137:132–41.
163. Winawer S, Fletcher R, Rex D, et al. Colorectal cancer screening and surveillance: clinical guidelines and rationale—update based on new evidence. *Gastroenterology* 2003;124:544–60.
164. Pickhardt PJ, Choi JR, Hwang I, et al. Computed tomographic virtual colonoscopy to screen for colorectal neoplasia in asymptomatic adults. *N Engl J Med* 2003;349:2191–200.
165. Cotton PB, Durkalski VL, Pineau BC, et al. Computed tomographic colonography (virtual colonoscopy): a multicenter comparison with standard colonoscopy for detection of colorectal neoplasia. *JAMA* 2004;291:1713–9.
166. Davies RJ, Miller R, Coleman N. Colorectal cancer screening: prospects for molecular stool analysis. *Nat Rev Cancer* 2005;5:199–209.
167. Imperiale TF, Ransohoff DF, Itzkowitz SH, Turnbull BA, Ross ME. Fecal DNA versus fecal occult blood for colorectal-cancer screening in an average-risk population. *N Engl J Med* 2004;351:2704–14.
168. Freedman AN, Seminara D, Gail MH, et al. Cancer risk prediction models: a workshop on development, evaluation, and application. *J Natl Cancer Inst* 2005;97:715–23.
169. Lengauer C, Kinzler KW, Vogelstein B. Genetic instabilities in human cancers. *Nature* 1998;396:643–9.
170. Grady WM. Genomic instability and colon cancer. *Cancer Metastasis Rev* 2004;23:11–27.
171. Herman JG, Baylin SB. Gene silencing in cancer in association with promoter hypermethylation. *N Engl J Med* 2003;349:2042–54.
172. Schatzkin A, Lanza E, Corle D, et al. Lack of effect of a low-fat, high-fiber diet on the recurrence of colorectal adenomas. *Polyp Prevention Trial Study Group*. *N Engl J Med* 2000;342:1149–55.
173. Baron JA, Beach M, Mandel JS, et al. Calcium supplements for the prevention of colorectal adenomas. *Calcium Polyp Prevention Study Group*. *N Engl J Med* 1999;340:101–7.
174. Steinbach G, Lynch PM, Phillips RK, et al. The effect of celecoxib, a cyclooxygenase-2 inhibitor, in familial adenomatous polyposis. *N Engl J Med* 2000;342:1946–52.
175. Baron JA, Cole BF, Sandler RS, et al. A randomized trial of aspirin to prevent colorectal adenomas. *N Engl J Med* 2003;348:891–9.
176. Bertagnolli MM, Eagle CJ, Hawk ET; Group APwCS. Celecoxib reduces sporadic colorectal adenomas: results from the Adenoma Prevention with Celecoxib Trial [abstract CP-3]. *Proc Am Assoc Cancer Res* 2006;47.
177. Arber N. Chemoprevention of colorectal adenomas with celecoxib in an international randomized, placebo-controlled, double-blind trial [abstract CP-4]. *Proc Am Assoc Cancer Res* 2006;47.
178. Bresalier RS, Sandler RS, Quan H, et al. Cardiovascular events associated with rofecoxib in a colorectal adenoma chemoprevention trial. *N Engl J Med* 2005;352:1092–102.
179. Solomon SD, McMurray JJ, Pfeffer MA, et al. Cardiovascular risk associated with celecoxib in a clinical trial for colorectal adenoma prevention. *N Engl J Med* 2005;352:1071–80.
180. Williams JL, Kashfi K, Ouyang N, del Soldato P, Kopelovich L, Rigas B. NO-donating aspirin inhibits intestinal carcinogenesis in Min (APC(Min/+)) mice. *Biochem Biophys Res Commun* 2004;313:784–8.
181. Shureiqi I, Jiang W, Zuo X, et al. The 15-lipoxygenase-1 product 13-S-hydroxyoctadecadienoic acid down-regulates PPAR- δ to induce apoptosis in colorectal cancer cells. *Proc Natl Acad Sci U S A* 2003;100:9968–73.
182. Reddy BS, Rao CV. Chemoprophylaxis of colon cancer. *Curr Gastroenterol Rep* 2005;7:389–95.
183. Hawk ET, Umar A, Viner JL. Colorectal cancer chemoprevention—an overview of the science. *Gastroenterology* 2004;126:1423–47.
184. Fabian CJ, Kimler BF, Brady DA, et al. A phase II breast cancer chemoprevention trial of oral α -difluoromethylornithine: breast tissue, imaging, and serum and urine biomarkers. *Clin Cancer Res* 2002;8:3105–17.
185. Fabian CJ, Kimler BF, Anderson J, et al. Breast cancer chemoprevention phase I evaluation of biomarker modulation by arzoxifene, a third generation selective estrogen receptor modulator. *Clin Cancer Res* 2004;10:5403–17.
186. Buzdar A, O'Shaughnessy JA, Booser DJ, et al. Phase II, randomized, double-blind study of two dose levels of arzoxifene in patients with locally advanced or metastatic breast cancer. *J Clin Oncol* 2003;21:1007–14.
187. Bundred NJ, Anderson EA, Gee J, et al. Randomized, double-blind, placebo-controlled trial of short-term effects of gefitinib ("Iressa", ZD1839) on ductal carcinoma *in situ* [abstract 14]. *Breast Cancer Res Treat* 2003;82:510.
188. Chan KC, Knox WF, Gee JM, et al. Effect of epidermal growth factor receptor tyrosine kinase inhibition on epithelial proliferation in normal and premalignant breast. *Cancer Res* 2002;62:122–8.
189. Guix M, Kelley MS, Reyzer ML, et al. Short course of EGF receptor tyrosine kinase inhibitor ("Tarceva") reduces tumor cell proliferation and activation in untreated operable breast cancers: a strategy for phase II trials with signaling inhibitors [abstract 3008]. *Proc Am Soc Clin Oncol* 2005;23:194s.
190. Fabian CJ, Kimler BF, Simonsen M, Metheny T, Zalles CM, Hall M. Reduction in breast epithelial cell proliferation after six months of letrozole in high risk women on hormone replacement therapy with random periareolar fine needle aspiration evidence of atypia [abstract 5]. *Breast Cancer Res Treat* 2004;88:58.
191. Jensen EV, Jordan VC. The estrogen receptor: a model for molecular medicine. *Clin Cancer Res* 2003;9:1980–9.
192. 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;90:1371–88.
193. Fisher B, Costantino JP, Wickerham DL, et al. Tamoxifen for the prevention of breast cancer: current status of the National Surgical Adjuvant Breast and Bowel Project P-1 study. *J Natl Cancer Inst* 2005;97:1652–62.
194. Wickerham DL, Fisher B, Wolmark N, et al. Association of tamoxifen and uterine sarcoma. *J Clin Oncol* 2002;20:2758–60.
195. King MC, Wieand S, Hale K, et al. Tamoxifen and breast cancer incidence among women with inherited mutations in BRCA1 and BRCA2: National Surgical Adjuvant Breast and Bowel Project (NSABP-P1) Breast Cancer Prevention Trial. *JAMA* 2001;286:2251–6.
196. Day R, Ganz PA, Costantino JP, Cronin WM, Wickerham DL, Fisher B. Health-related quality of life and tamoxifen in breast cancer prevention: a report from the National Surgical Adjuvant Breast and Bowel Project P-1 Study. *J Clin Oncol* 1999;17:2659–69.
197. Chalas E, Costantino JP, Wickerham DL, et al. Benign gynecologic conditions among participants in the Breast Cancer Prevention Trial. *Am J Obstet Gynecol* 2005;192:1230–7; discussion 7–9.
198. Tchou J, Hou N, Rademaker A, Jordan VC, Morrow M. Acceptance of tamoxifen chemoprevention by

- physicians and women at risk. *Cancer* 2004;100:1800–6.
199. Ettinger B, Black DM, Mitlak BH, et al. Reduction of vertebral fracture risk in postmenopausal women with osteoporosis treated with raloxifene: results from a 3-year randomized clinical trial. Multiple Outcomes of Raloxifene Evaluation (MORE) investigators. *JAMA* 1999;282:637–45.
200. Kanis JA, Johnell O, Black DM, et al. Effect of raloxifene on the risk of new vertebral fracture in postmenopausal women with osteopenia or osteoporosis: a reanalysis of the Multiple Outcomes of Raloxifene Evaluation trial. *Bone* 2003;33:293–300.
201. Cummings SR, Eckert S, Krueger KA, et al. The effect of raloxifene on risk of breast cancer in postmenopausal women: results from the MORE randomized trial. Multiple Outcomes of Raloxifene Evaluation. *JAMA* 1999;281:2189–97.
202. Cauley JA, Norton L, Lippman ME, et al. Continued breast cancer risk reduction in postmenopausal women treated with raloxifene: 4-year results from the MORE trial. Multiple Outcomes of Raloxifene Evaluation. *Breast Cancer Res Treat* 2001;65:125–34.
203. Siris ES, Harris ST, Eastell R, et al. Skeletal effects of raloxifene after 8 years: results from the Continuing Outcomes Relevant to Evista (CORE) study. *J Bone Miner Res* 2005;20:1514–24.
204. Martino S, Cauley JA, Barrett-Connor E, et al. Continuing outcomes relevant to Evista: breast cancer incidence in postmenopausal osteoporotic women in a randomized trial of raloxifene. *J Natl Cancer Inst* 2004;96:1751–61.
205. Rosati RL, Da Silva Jardine P, Cameron KO, et al. Discovery and preclinical pharmacology of a novel, potent, nonsteroidal estrogen receptor agonist/antagonist, CP-336156, a diaryltetrahydronaphthalene. *J Med Chem* 1998;41:2928–31.
206. Ke HZ, Foley GL, Simmons HA, Shen V, Thompson DD. Long-term treatment of lasofoxifene preserves bone mass and bone strength and does not adversely affect the uterus in ovariectomized rats. *Endocrinology* 2004;145:1996–2005.
207. Komm BS, Kharode YP, Bodine PV, Harris HA, Miller CP, Lyttle CR. Bazedoxifene acetate: a selective estrogen receptor modulator with improved selectivity. *Endocrinology* 2005;146:3999–4008.
208. Cuzick J, Powles T, Veronesi U, et al. Overview of the main outcomes in breast-cancer prevention trials. *Lancet* 2003;361:296–300.
209. Cuzick J, Forbes J, Edwards R, et al. First results from the International Breast Cancer Intervention Study (IBIS-I): a randomized prevention trial. *Lancet* 2002;360:817–24.
210. Baum M, Budzar AU, Cuzick J, et al. ATAC Trialists' Group. Anastrozole alone or in combination with tamoxifen versus tamoxifen alone for adjuvant treatment of postmenopausal women with early breast cancer: first results of the ATAC randomized trial. *Lancet* 2002;359:2131–9.
211. Howell A, Cuzick J, Baum M, et al. Results of the ATAC (Arimidex, Tamoxifen, Alone or in Combination) trial after completion of 5 years' adjuvant treatment for breast cancer. *Lancet* 2005;365:60–2.
212. Goss PE, Ingle JN, Martino S, et al. A randomized trial of letrozole in postmenopausal women after five years of tamoxifen therapy for early-stage breast cancer. *N Engl J Med* 2003;349:1793–802.
213. Coombes RC, Hall E, Gibson LJ, et al. A randomized trial of exemestane after two to three years of tamoxifen therapy in postmenopausal women with primary breast cancer. *N Engl J Med* 2004;350:1081–92.
214. Boccardo F, Rubagotta A, Puntoni M, et al. Switching to anastrozole versus continued tamoxifen treatment of early breast cancer: preliminary results of the Italian Tamoxifen Anastrozole Trial. *J Clin Oncol* 2005;23:5138–47.
215. Goss PE, Strasser-Weippl K. Aromatase inhibitors for chemoprevention. *Best Pract Res Clin Endocrinol Metab* 2004;18:113–30.
216. Arun B, Goss P. The role of COX-2 inhibition in breast cancer treatment and prevention. *Semin Oncol* 2004;31:22–9.
217. Schreinemachers DM, Everson RB. Aspirin use and lung, colon, and breast cancer incidence in a prospective study. *Epidemiology* 1994;5:138–46.
218. Harris RE, Namboodiri KK, Farrar WB. Nonsteroidal antiinflammatory drugs and breast cancer. *Epidemiology* 1996;7:203–5.
219. Sharpe CR, Collet JP, McNutt M, Belzile E, Boivin JF, Hanley JA. Nested case-control study of the effects of non-steroidal anti-inflammatory drugs on breast cancer risk and stage. *Br J Cancer* 2000;83:112–20.
220. Pedersen TR, Kjekshus J, Berg K, et al. Scandinavian Simvastatin Survival Study Group. Randomised trial of cholesterol lowering in 4444 patients with coronary heart disease: the Scandinavian Simvastatin Survival Study (4S). *Lancet* 1994;344:1383–9.
221. Prevention of cardiovascular events and death with pravastatin in patients with coronary heart disease and a broad range of initial cholesterol levels. The Long-term Intervention with Pravastatin in Ischaemic Disease (LIPID) study group. *N Engl J Med* 1998;339:1349–57.
222. Blais L, Desgagne A, LeLorier J. 3-Hydroxy-3-methylglutaryl coenzyme A reductase inhibitors and the risk of cancer: a nested case-control study. *Arch Intern Med* 2000;160:2363–8.
223. Cauley JA, Zmuda JM, Lui LY, et al. Lipid-lowering drug use and breast cancer in older women: a prospective study. *J Womens Health (Larchmt)* 2003;12:749–56.
224. Graaf MR, Beiderbeck AB, Egberts AC, Richel DJ, Guchelaar HJ. The risk of cancer in users of statins. *J Clin Oncol* 2004;22:2388–94.
225. Veronesi U, De Palo G, Marubini E, et al. Randomized trial of fenretinide to prevent second breast malignancy in women with early breast cancer. *J Natl Cancer Inst* 1999;91:1847–56.
226. Arun B, Mohsin S, Miller A, et al. Acquisition of breast tissue in a biomarker modulation study using bexarotene in women at high risk of breast cancer [abstract 1002]. *Proc Am Soc Clin Oncol* 2005;23.
227. Fabian CJ, Kimler BF, Elledge RM, Grizzle WE, Beenken SW, Ward JH. Models for early chemoprevention trials in breast cancer. *Hematol Oncol Clin North Am* 1998;12:993–1017.
228. Arun B, Lammey J, Broglio K, et al. Downregulation of estrogen receptor expression with celecoxib in breast tissue of women at increased risk for developing breast cancer [abstract 4026]. Presented at the San Antonio Breast Cancer Symposium; 2004.
229. Fabian CJ, Kimler BF, Brady D, et al. Phase II chemoprevention trial of DFMO using the random FNA model. Presented at the 3rd Annual San Antonio Breast Cancer Symposium; 2000 Dec 6–9.
230. Arun B. Ductal lavage and risk assessment of breast cancer. *Oncologist* 2004;9:599–605.
231. Gail M, Brinton L, Byar D, Corel D, Green S, Schairer C. Projecting individualized probabilities of developing breast cancer for White females who are being examined annually. *J Natl Cancer Inst* 1989;81:1879–86.
232. Berry DA, Iversen ES, Jr., Gudbjartsson DF, et al. BRCA1/BRCA2, and prevalence of other breast cancer susceptibility genes. *J Clin Oncol* 2002;20:2701–12.
233. Claus EB, Schildkraut J, Iversen ES, Jr., Berry D, Parmigiani G. Effect of BRCA1 and BRCA2 on the association between breast cancer risk and family history. *J Natl Cancer Inst* 1998;90:1824–9.
234. Parmigiani G, Berry D, Aguilar O. Determining carrier probabilities for breast cancer-susceptibility genes BRCA1 and BRCA2. *Am J Hum Genet* 1998;62:145–58.
235. Chen Y, Thompson W, Semenciw R, Mao Y. Epidemiology of contralateral breast cancer. *Cancer Epidemiol Biomarkers Prev* 1999;8:855–61.
236. Listgarten J, Damaraju S, Poulin B, et al. Predictive models for breast cancer susceptibility from multiple single nucleotide polymorphisms. *Clin Cancer Res* 2004;10:2725–37.
237. Dunning AM, Dowsett M, Healey CS, et al. Polymorphisms associated with circulating sex hormone levels in postmenopausal women. *J Natl Cancer Inst* 2004;96:936–45.
238. Lo YL, Yu JC, Chen ST, et al. Breast cancer risk associated with genotypic polymorphism of the mitosis-regulating gene Aurora-A/STK15/BTAK. *Int J Cancer* 2005;115:276–83.
239. Cancer facts and figures 2005. American Cancer Society; 2005 [accessed 2006 Mar 15]. Available from: <http://www.cancer.org/downloads/STT/CAFF2005f4PWSecured.pdf>.
240. Cigarette smoking among adults—United States, 2003. *MMWR Morb Mortal Wkly Rep* 2005;54:509–13.
241. Peto R, Darby S, Deo H, Silcocks P, Whitley E, Doll R. Smoking, smoking cessation, and lung cancer in the UK since 1950: combination of national statistics with two case-control studies. *BMJ* 2000;321:323–9.
242. Anthonisen NR, Skeans MA, Wise RA, Manfreda J, Kanner RE, Connert JE. The effects of a smoking cessation intervention on 14.5-year mortality: a randomized clinical trial. *Ann Intern Med* 2005;142:233–9.
243. SEER cancer statistics review 1975–2002; 2005.
244. Ginsberg RJ, Rubinstein LV. Randomized trial of lobectomy versus limited resection for T₁N₀ non-small cell lung cancer. Lung Cancer Study Group. *Ann Thorac Surg* 1995;60:615–22; discussion 22–3.
245. Ashton RW, Jett JR. Screening for non-small cell lung cancer. *Semin Oncol* 2005;32:253–8.
246. Oken MM, Marcus PM, Hu P, et al. Baseline chest radiograph for lung cancer detection in the randomized Prostate, Lung, Colorectal and Ovarian Cancer Screening Trial. *J Natl Cancer Inst* 2005;97:1832–9.
247. Jett JR. Limitations of screening for lung cancer with low-dose spiral computed tomography. *Clin Cancer Res* 2005;11:4988–92s.
248. Hohagan JK, Marcus PM, Fagerstrom RM, et al. Final results of the Lung Screening Study, a randomized feasibility study of spiral CT versus chest X-ray screening for lung cancer. *Lung Cancer* 2005;47:9–15.
249. McWilliams A, Mayo J, MacDonald S, et al. Lung cancer screening: a different paradigm. *Am J Respir Crit Care Med* 2003;168:1167–73.
250. Saccomanno G, Archer VE, Auerbach O, Saunders RP, Brennan LM. Development of carcinoma of the lung as reflected in exfoliated cells. *Cancer* 1974;33:256–70.
251. Kerr K. Adenomatous hyperplasia and the origin of peripheral adenocarcinoma of the lung. In: Corrin B, editor. Pathology of lung tumors. New York: Churchill Livingstone; 1997. p. 119–33.
252. Prindiville SA, Byers T, Hirsch FR, et al. Sputum cytological atypia as a predictor of incident lung cancer in a cohort of heavy smokers with airflow obstruction. *Cancer Epidemiol Biomarkers Prev* 2003;12:987–93.
253. Risse EK, Vooijs GP, van't Hof MA. Diagnostic significance of "severe dysplasia" in sputum cytology. *Acta Cytol* 1988;32:629–34.
254. Frost JK, Ball WC, Jr., Levin ML, et al. Sputum cytopathology: use and potential in monitoring the workplace environment by screening for biological effects of exposure. *J Occup Med* 1986;28:692–703.
255. Tockman MS, Gupta PK, Myers JD, et al. Sensitive and specific monoclonal antibody recognition of human lung cancer antigen on preserved sputum cells: a new approach to early lung cancer detection. *J Clin Oncol* 1988;6:1685–93.
256. Bach PB, Kattan MW, Thornquist MD, et al. Variations in lung cancer risk among smokers. *J Natl Cancer Inst* 2003;95:470–8.
257. Lam S, MacAulay C, leRiche JC, Palcic B. Detection and localization of early lung cancer by fluorescence bronchoscopy. *Cancer* 2000;89:2468–73.
258. Venmans BJ, van Boxem TJ, Smit EF, Postmus PE, Sutedja TG. Outcome of bronchial carcinoma *in situ*. *Chest* 2000;117:1572–6.
259. Bota S, Auliac JB, Paris C, et al. Follow-up of bronchial precancerous lesions and carcinoma *in situ* using fluorescence endoscopy. *Am J Respir Crit Care Med* 2001;164:1688–93.

260. Massion PP, Carbone DP. The molecular basis of lung cancer: molecular abnormalities and therapeutic implications. *Respir Res* 2003;4:12.
261. Mao L, Lee JS, Kurie JM, et al. Clonal genetic alterations in the lungs of current and former smokers. *J Natl Cancer Inst* 1997;89:857–62.
262. Wistuba II, Lam S, Behrens C, et al. Molecular damage in the bronchial epithelium of current and former smokers. *J Natl Cancer Inst* 1997;89:1366–73.
263. Wattenberg LW, Wiedmann TS, Estensen RD, et al. Chemoprevention of pulmonary carcinogenesis by brief exposures to aerosolized budesonide or beclomethasone dipropionate and by the combination of aerosolized budesonide and dietary myo-inositol. *Carcinogenesis* 2000;21:179–82.
264. Pereira MA, Li Y, Gunning WT, et al. Prevention of mouse lung tumors by budesonide and its modulation of biomarkers. *Carcinogenesis* 2002;23:1185–92.
265. Lam S, leRiche JC, McWilliams A, et al. A randomized phase IIb trial of pulmicort turbuhaler (budesonide) in people with dysplasia of the bronchial epithelium. *Clin Cancer Res* 2004;10:6502–11.
266. Gunning WT, Kramer PM, Steele VE, Pereira MA. Chemoprevention by lipoxxygenase and leukotriene pathway inhibitors of vinyl carbamate-induced lung tumors in mice. *Cancer Res* 2002;62:4199–201.
267. Pepin P, Bouchard L, Nicole P, Castonguay A. Effects of sulindac and oltipraz on the tumorigenicity of 4-(methylnitrosamino)-1-(3-pyridyl)-1-butanone in A/J mouse lung. *Carcinogenesis* 1992;13:341–8.
268. Shepherd FA, Rodrigues Pereira J, Ciuleanu T, et al. Erlotinib in previously treated non-small-cell lung cancer. *N Engl J Med* 2005;353:123–32.
269. Keith RL, Miller YE, Hudish TM, et al. Pulmonary prostacyclin synthase overexpression chemoprevents tobacco smoke lung carcinogenesis in mice. *Cancer Res* 2004;64:5897–904.
270. Zhang Z, Liu Q, Lantry LE, et al. A germ-line p53 mutation accelerates pulmonary tumorigenesis: p53-independent efficacy of chemopreventive agents green tea or dexamethasone/myo-inositol and chemotherapeutic agents Taxol or Adriamycin. *Cancer Res* 2000;60:901–7.
271. Zhang Z, Wang Y, Yao R, et al. Cancer chemopreventive activity of a mixture of Chinese herbs (antitumor B) in mouse lung tumor models. *Oncogene* 2004;23:3841–50.
272. Clark LC, Combs GF, Jr., Turnbull BW, et al. Effects of selenium supplementation for cancer prevention in patients with carcinoma of the skin. A randomized controlled trial. Nutritional Prevention of Cancer Study Group. *JAMA* 1996;276:1957–63.
273. Weinberg OK, Marquez-Garban DC, Fishbein MC, et al. Aromatase inhibitors in human lung cancer therapy. *Cancer Res* 2005;65:11287–91.
274. Zhang Z, Wang Y, Lantry LE, et al. Farnesyltransferase inhibitors are potent lung cancer chemopreventive agents in A/J mice with a dominant-negative p53 and/or heterozygous deletion of Ink4a/Arf. *Oncogene* 2003;22:6257–65.
275. The effect of vitamin E and β carotene on the incidence of lung cancer and other cancers in male smokers. The α -Tocopherol, β -Carotene Cancer Prevention Study Group. *N Engl J Med* 1994;330:1029–35.
276. Omenn GS, Goodman GE, Thornquist MD, et al. Effects of a combination of β carotene and vitamin A on lung cancer and cardiovascular disease. *N Engl J Med* 1996;334:1150–5.
277. van Zandwijk N, Dalesio O, Pastorino U, de Vries N, van Tinteren H. EUROSCAN, a randomized trial of vitamin A and *N*-acetylcysteine in patients with head and neck cancer or lung cancer. For the European Organization for Research and Treatment of Cancer Head and Neck and Lung Cancer Cooperative Groups. *J Natl Cancer Inst* 2000;92:977–86.
278. Lippman SM, Lee JJ, Karp DD, et al. Randomized phase III intergroup trial of isotretinoin to prevent second primary tumors in stage I non-small-cell lung cancer. *J Natl Cancer Inst* 2001;93:605–18.
279. Kurie JM, Lotan R, Lee JJ, et al. Treatment of former smokers with 9-*cis*-retinoic acid reverses loss of retinoic acid receptor- β expression in the bronchial epithelium: results from a randomized placebo-controlled trial. *J Natl Cancer Inst* 2003;95:206–14.
280. Forastiere A, Koch W, Trotti A, Sidransky D. Head and neck cancer. *N Engl J Med* 2001;345:1890–900.
281. Vokes EE, Weichselbaum RR, Lippman SM, Hong WK. Head and neck cancer. *N Engl J Med* 1993;328:184–94.
282. Khuri FR, Kim ES, Lee JJ, et al. The impact of smoking status, disease stage, and index tumor site on second primary tumor incidence and tumor recurrence in the head and neck retinoid chemoprevention trial. *Cancer Epidemiol Biomarkers Prev* 2001;10:823–9.
283. Almadori G, Bussu F, Cadoni G, et al. Multistep laryngeal carcinogenesis helps our understanding of the field cancerisation phenomenon: a review. *Eur J Cancer* 2004;40:2383–8.
284. Matthias C, Jahnke V, Jones PW, et al. Cyclin D1, glutathione *S*-transferase, and cytochrome *P*450 genotypes and outcome in patients with upper aerodigestive tract cancers: assessment of the importance of individual genes using multivariate analysis. *Cancer Epidemiol Biomarkers Prev* 1999;8:815–23.
285. Gallo O, Sardi I, Pepe G, et al. Multiple primary tumors of the upper aerodigestive tract: is there a role for constitutional mutations in the p53 gene? *Int J Cancer* 1999;82:180–6.
286. Shin DM, Lee JS, Lippman SM, et al. p53 expressions: predicting recurrence and second primary tumors in head and neck squamous cell carcinoma. *J Natl Cancer Inst* 1996;88:519–29.
287. Hong WK, Sporn MB. Recent advances in chemoprevention of cancer. *Science* 1997;278:1073–7.
288. Silverman S, Jr., Gorsky M, Lozada F. Oral leukoplakia and malignant transformation. A follow-up study of 257 patients. *Cancer* 1984;53:563–8.
289. Lippman SM, Lee JJ. Reducing the “risk” of chemoprevention: defining and targeting high risk. 2005 AACR Cancer Research and Prevention Foundation Award Lecture. *Cancer Res* 2006;66:2893–903.
290. Partridge M, Pateromichelakis S, Phillips E, Emilion GG, A'Hern RP, Langdon JD. A case-control study confirms that microsatellite assay can identify patients at risk of developing oral squamous cell carcinoma within a field of cancerization. *Cancer Res* 2000;60:3893–8.
291. Rosin MP, Cheng X, Poh C, et al. Use of allelic loss to predict malignant risk for low-grade oral epithelial dysplasia. *Clin Cancer Res* 2000;6:357–62.
292. Braakhuis BJ, Leemans CR, Brakenhoff RH. Correspondence re: Rosin M.P. et al. 3p14 and 9p21 Loss is a simple tool for predicting second oral malignancy at previously treated oral cancer sites. *Cancer Res* 2002;62:6447–50. *Cancer Res* 2003;63:5167–8; author reply 8–9.
293. 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;56:2488–92.
294. Tabor MP, Brakenhoff RH, van Houten VM, et al. Persistence of genetically altered fields in head and neck cancer patients: biological and clinical implications. *Clin Cancer Res* 2001;7:1523–32.
295. Izzo JG, Papadimitrakopoulou VA, Liu DD, et al. Cyclin D1 genotype, response to biochemoprevention, and progression rate to upper aerodigestive tract cancer. *J Natl Cancer Inst* 2003;95:198–205.
296. Michalides R, van Veelen N, Hart A, Loftus B, Wientjens E, Balm A. Overexpression of cyclin D1 correlates with recurrence in a group of forty-seven operable squamous cell carcinomas of the head and neck. *Cancer Res* 1995;55:975–8.
297. Boyle JO, Langenfeld J, Lonardo F, et al. Cyclin D1 proteolysis: a retinoid chemoprevention signal in normal, immortalized, and transformed human bronchial epithelial cells. *J Natl Cancer Inst* 1999;91:373–9.
298. Lango M, Wentzel AL, Song JI, et al. Responsiveness to the retinoic acid receptor-selective retinoid LGD1550 correlates with abrogation of transforming growth factor α /epidermal growth factor receptor autocrine signaling in head and neck squamous carcinoma cells. *Clin Cancer Res* 2003;9:4205–13.
299. Moraitis D, Du B, De Lorenzo MS, et al. Levels of cyclooxygenase-2 are increased in the oral mucosa of smokers: evidence for the role of epidermal growth factor receptor and its ligands. *Cancer Res* 2005;65:664–70.
300. Hong WK, Endicott J, Itri LM, et al. 13-*cis*-Retinoic acid in the treatment of oral leukoplakia. *N Engl J Med* 1986;315:1501–5.
301. Chiesa F, Tradati N, Marazza M, et al. Fenretinone (4-HPR) in chemoprevention of oral leukoplakia. *J Cell Biochem Suppl* 1993;17F:255–61.
302. Stich HF, Hornby AP, Mathew B, Sankaranarayanan R, Nair MK. Response of oral leukoplakias to the administration of vitamin A. *Cancer Lett* 1988;40:93–101.
303. 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;90:1545–51.
304. Hong WK, Lippman SM, Itri LM, et al. Prevention of second primary tumors with isotretinoin in squamous-cell carcinoma of the head and neck. *N Engl J Med* 1990;323:795–801.
305. Khuri FR, Lee JJ, Lippman SM, et al. Randomized phase-III trial of low-dose isotretinoin for prevention of second primary tumors in stage I and II head and neck cancer patients. *J Natl Cancer Inst* 2006;98:441–50.
306. Shin DM, Khuri FR, Murphy B, et al. Combined interferon- α , 13-*cis*-retinoic acid, and α -tocopherol in locally advanced head and neck squamous cell carcinoma: novel bioadjuvant phase II trial. *J Clin Oncol* 2001;19:3010–7.
307. Seixas-Silva JA, Jr., Richards T, Khuri FR, et al. Phase 2 bioadjuvant study of interferon α -2a, isotretinoin, and vitamin E in locally advanced squamous cell carcinoma of the head and neck: long-term follow-up. *Arch Otolaryngol Head Neck Surg* 2005;131:304–7.
308. Choe MS, Zhang X, Shin HJ, Shin DM, Chen ZG. Interaction between epidermal growth factor receptor- and cyclooxygenase 2-mediated pathways and its implications for the chemoprevention of head and neck cancer. *Mol Cancer Ther* 2005;4:1448–55.
309. Brown LM, Devesa SS. Epidemiologic trends in esophageal and gastric cancer in the United States. *Surg Oncol Clin N Am* 2002;11:235–56.
310. Engel LS, Chow WH, Vaughan TL, et al. Population attributable risks of esophageal and gastric cancers. *J Natl Cancer Inst* 2003;95:1404–13.
311. Chow WH, Blot WJ, Vaughan TL, et al. Body mass index and risk of adenocarcinomas of the esophagus and gastric cardia. *J Natl Cancer Inst* 1998;90:150–5.
312. Lagergren J, Bergstrom R, Nyren O. Association between body mass and adenocarcinoma of the esophagus and gastric cardia. *Ann Intern Med* 1999;130:883–90.
313. Vaughan TL, Kristal AR, Blount PL, et al. Nonsteroidal anti-inflammatory drug use, body mass index, and anthropometry in relation to genetic and flow cytometric abnormalities in Barrett's esophagus. *Cancer Epidemiol Biomarkers Prev* 2002;11:745–52.
314. Wild CP, Hardie LJ. Reflux, Barrett's oesophagus and adenocarcinoma: burning questions. *Nat Rev Cancer* 2003;3:676–84.
315. de Martel C, Llosa AE, Farr SM, et al. *Helicobacter pylori* infection and the risk of development of esophageal adenocarcinoma. *J Infect Dis* 2005;191:761–7.
316. Drewitz DJ, Sampliner RE, Garewal HS. The incidence of adenocarcinoma in Barrett's esophagus: a prospective study of 170 patients followed 4.8 years. *Am J Gastroenterol* 1997;92:212–5.
317. Jankowski JA, Provenzale D, Moayyedi P. Esophageal adenocarcinoma arising from Barrett's metaplasia has regional variations in the west. *Gastroenterology* 2002;122:588–90.
318. Conio M, Bianchi S, Lapertosa G, et al. Long-term endoscopic surveillance of patients with Barrett's esophagus. Incidence of dysplasia and adenocarcinoma: a prospective study. *Am J Gastroenterol* 2003;98:1931–9.

319. Buttar NS, Wang KK, Sebo TJ, et al. Extent of high-grade dysplasia in Barrett's esophagus correlates with risk of adenocarcinoma. *Gastroenterology* 2001;120:1630-9.
320. Reid BJ, Levine DS, Longton G, Blount PL, Rabinovitch PS. Predictors of progression to cancer in Barrett's esophagus: baseline histology and flow cytometry identify low- and high-risk patient subsets. *Am J Gastroenterol* 2000;95:1669-76.
321. Schnell TG, Sontag SJ, Chejfec G, et al. Long-term nonsurgical management of Barrett's esophagus with high-grade dysplasia. *Gastroenterology* 2001;120:1607-19.
322. Weston AP, Banerjee SK, Sharma P, Tran TM, Richards R, Cherian R. p53 protein overexpression in low grade dysplasia (LGD) in Barrett's esophagus: immunohistochemical marker predictive of progression. *Am J Gastroenterol* 2001;96:1355-62.
323. Overholt BF, Lightdale CJ, Wang KK, et al. Photodynamic therapy with porfimer sodium for ablation of high-grade dysplasia in Barrett's esophagus: international, partially blinded, randomized phase III trial. *Gastrointest Endosc* 2005;62:488-98.
324. Weston AP, Sharma P, Topalovski M, Richards R, Cherian R, Dixon A. Long-term follow-up of Barrett's high-grade dysplasia. *Am J Gastroenterol* 2000;95:1888-93.
325. Pepe MS, Etzioni R, Feng Z, et al. Phases of biomarker development for early detection of cancer. *J Natl Cancer Inst* 2001;93:1054-61.
326. Rabinovitch PS, Longton G, Blount PL, Levine DS, Reid BJ. Predictors of progression in Barrett's esophagus III: baseline flow cytometric variables. *Am J Gastroenterol* 2001;96:3071-83.
327. Reid BJ, Prevo LJ, Galipeau PC, et al. Predictors of progression in Barrett's esophagus II: baseline 17p (p53) loss of heterozygosity identifies a patient subset at increased risk for neoplastic progression. *Am J Gastroenterol* 2001;96:2839-48.
328. Teodori L, Gohde W, Persiani M, et al. DNA/protein flow cytometry as a predictive marker of malignancy in dysplasia-free Barrett's esophagus: thirteen-year follow-up study on a cohort of patients. *Cytometry* 1998;34:257-63.
329. Galipeau PC, Li X, Longton G, et al. A panel of molecular and cytometric markers in Barrett's esophagus predicts progression to esophageal adenocarcinoma [abstract 404]. *Gastroenterology* 2004;126:A114.
330. Lee JJ, Hong WK, Hittelman WN, et al. Predicting cancer development in oral leukoplakia: ten years of translational research. *Clin Cancer Res* 2000;6:1702-10.
331. Schulmann K, Sterian A, Berki A, et al. Inactivation of p16, RUNX3, and HPP1 occurs early in Barrett's-associated neoplastic progression and predicts progression risk. *Oncogene* 2005;24:4138-48.
332. Rubio CA. Antitumoral activity of indomethacin on experimental esophageal tumors. *J Natl Cancer Inst* 1984;72:705-7.
333. Rubio CA. Further studies on the therapeutic effect of indomethacin on esophageal tumors. *Cancer* 1986;58:1029-31.
334. Tanaka T, Kojima T, Yoshimi N, Sugie S, Mori H. Inhibitory effect of the nonsteroidal anti-inflammatory drug, indomethacin on the naturally occurring carcinogen, 1-hydroxyanthraquinone in male ACl/N rats. *Carcinogenesis* 1991;12:1949-52.
335. Siglin JC, Barch DH, Stoner GD. Effects of dietary phenethyl isothiocyanate, ellagic acid, sulindac and calcium on the induction and progression of *N*-nitrosomethylbenzylamine-induced esophageal carcinogenesis in rats. *Carcinogenesis* 1995;16:1101-6.
336. Funkhouser EM, Sharp GB. Aspirin and reduced risk of esophageal carcinoma. *Cancer* 1995;76:1116-9.
337. Raj A, Jankowski J. Acid suppression and chemoprevention in Barrett's oesophagus. *Dig Dis* 2004;22:171-80.
338. Heath EI, Canto MI, Wu TT, et al. Chemoprevention for Barrett's esophagus trial. Design and outcome measures. *Dis Esophagus* 2003;16:177-86.
339. Garewal HS, Gerner EW, Sampliner RE, Roe D. Ornithine decarboxylase and polyamine levels in colonic upper gastrointestinal mucosae in patients with Barrett's esophagus. *Cancer Res* 1988;48:3288-91.
340. Fong LY, Pegg AE, Magee PN. α -Difluoromethylornithine inhibits *N*-nitrosomethylbenzylamine-induced esophageal carcinogenesis in zinc-deficient rats: effects on esophageal cell proliferation and apoptosis. *Cancer Res* 1998;58:5380-8.
341. Kresty LA, Stoner GD, Normelle D. Analysis of cyclin D1 and EGFR in Barrett's esophagus patients. *Proc Am Assoc Cancer Res* 2001;42:867.
342. Nijhawan PK, Wang KK. Endoscopic mucosal resection for lesions with endoscopic features suggestive of malignancy and high-grade dysplasia within Barrett's esophagus. *Gastrointest Endosc* 2000;52:328-32.
343. May A, Gossner L, Pech O, et al. Local endoscopic therapy for intraepithelial high-grade neoplasia and early adenocarcinoma in Barrett's oesophagus: acute-phase and intermediate results of a new treatment approach. *Eur J Gastroenterol Hepatol* 2002;14:1085-91.
344. Collard JM. High-grade dysplasia in Barrett's esophagus. The case for esophagectomy. *Chest Surg Clin N Am* 2002;12:77-92.
345. Weston AP, Sharma P, Topalovski M, Cherian R, Dixon A. Prospective long-term follow-up of Barrett's high-grade dysplasia: risky business. *Gastroenterology* 1999;116:A352-3.
346. Birkmeyer JD, Siewiers AE, Finlayson EV, et al. Hospital volume and surgical mortality in the United States. *N Engl J Med* 2002;346:1128-37.
347. El-Serag HB. Hepatocellular carcinoma: recent trends in the United States. *Gastroenterology* 2004;127:S27-34.
348. Yamamoto J, Kosuge T, Takayama T, et al. Recurrence of hepatocellular carcinoma after surgery. *Br J Surg* 1996;83:1219-22.
349. Llovet JM, Burroughs A, Bruix J. Hepatocellular carcinoma. *Lancet* 2003;362:1907-17.
350. Simonetti RG, Liberati A, Angiolini C, Pagliaro L. Treatment of hepatocellular carcinoma: a systematic review of randomized controlled trials. *Ann Oncol* 1997;8:117-36.
351. Thorgerisson SS, Grisham JW. Molecular pathogenesis of human hepatocellular carcinoma. *Nat Genet* 2002;31:339-46.
352. Bruix J, Boix L, Sala M, Llovet JM. Focus on hepatocellular carcinoma. *Cancer Cell* 2004;5:215-9.
353. Chang MH, Chen CJ, Lai MS, et al. Universal hepatitis B vaccination in Taiwan and the incidence of hepatocellular carcinoma in children. Taiwan Childhood Hepatoma Study Group. *N Engl J Med* 1997;336:1855-9.
354. Chang MH, Shau WY, Chen CJ, et al. Hepatitis B vaccination and hepatocellular carcinoma rates in boys and girls. *JAMA* 2000;284:3040-2.
355. Tsen YJ, Chang MH, Hsu HY, Lee CY, Sung JL, Chen DS. Seroprevalence of hepatitis B virus infection in children in Taipei, 1989: five years after a mass hepatitis B vaccination program. *J Med Virol* 1991;34:96-9.
356. Kane MA, Brooks A. New immunization initiatives and progress toward the global control of hepatitis B. *Curr Opin Infect Dis* 2002;15:465-9.
357. Niederau C, Heintges T, Lange S, et al. Long-term follow-up of HBeAg-positive patients treated with interferon α for chronic hepatitis B. *N Engl J Med* 1996;334:1422-7.
358. Liaw YF, Sung JJ, Chow WC, et al. Lamivudine for patients with chronic hepatitis B and advanced liver disease. *N Engl J Med* 2004;351:1521-31.
359. Jaeckel E, Comberg M, Wedemeyer H, et al. Treatment of acute hepatitis C with interferon α -2b. *N Engl J Med* 2001;345:1452-7.
360. Yoshida H, Shiratori Y, Moriyama M, et al. Interferon therapy reduces the risk for hepatocellular carcinoma: national surveillance program of cirrhotic and noncirrhotic patients with chronic hepatitis C in Japan. IJIT Study Group. *Ann Intern Med* 1999;131:174-81.
361. Camma C, Giunta M, Andreone P, Craxi A. Interferon and prevention of hepatocellular carcinoma in viral cirrhosis: an evidence-based approach. *J Hepatol* 2001;34:593-602.
362. Manns MP, McHutchison JG, Gordon SC, et al. Peginterferon α -2b plus ribavirin compared with interferon α -2b plus ribavirin for initial treatment of chronic hepatitis C: a randomised trial. *Lancet* 2001;358:958-65.
363. Fried MW, Shiffman ML, Reddy KR, et al. Peginterferon α -2a plus ribavirin for chronic hepatitis C virus infection. *N Engl J Med* 2002;347:975-82.
364. Pockros PJ. Developments in the treatment of chronic hepatitis C. *Expert Opin Investig Drugs* 2002;11:515-28.
365. Hugle T, Cerny A. Current therapy and new molecular approaches to antiviral treatment and prevention of hepatitis C. *Rev Med Virol* 2003;13:361-71.
366. Muto Y, Moriwaki H, Ninomiya M, et al. Prevention of second primary tumors by an acyclic retinoid, polypropenoic acid, in patients with hepatocellular carcinoma. Hepatoma Prevention Study Group. *N Engl J Med* 1996;334:1561-7.
367. Qiu DK, Ma X, Peng YS, Chen XY. Significance of cyclooxygenase-2 expression in human primary hepatocellular carcinoma. *World J Gastroenterol* 2002;8:815-7.
368. Koga H. Hepatocellular carcinoma: is there a potential for chemoprevention using cyclooxygenase-2 inhibitors? *Cancer* 2003;98:661-7.
369. Lara-Pezzi E, Gomez-Gavero MV, Galvez BG, et al. The hepatitis B virus X protein promotes tumor cell invasion by inducing membrane-type matrix metalloproteinase-1 and cyclooxygenase-2 expression. *J Clin Invest* 2002;110:1831-8.
370. Yamamoto H, Kondo M, Nakamori S, et al. JTE-522, a cyclooxygenase-2 inhibitor, is an effective chemopreventive agent against rat experimental liver fibrosis. *Gastroenterology* 2003;125:556-71.
371. Maclean J, Rybicki EP, Williamson AL. Vaccination strategies for the prevention of cervical cancer. *Expert Rev Anticancer Ther* 2005;5:97-107.
372. Tjalma WA, Arbyn M, Paavonen J, van Waes TR, Bogers JJ. Prophylactic human papillomavirus vaccines: the beginning of the end of cervical cancer. *Int J Gynecol Cancer* 2004;14:751-61.
373. Oltersdorf T, Elmore SW, Shoemaker AR, et al. An inhibitor of Bcl-2 family proteins induces regression of solid tumours. *Nature* 2005;435:677-81.
374. Bell MC, Crowley-Nowick P, Bradlow HL, et al. Placebo-controlled trial of indole-3-carbinol in the treatment of CIN. *Gynecol Oncol* 2000;78:123-9.
375. Richards-Kortum R, Mitchell MF, Ramanujam N, Mahadevan A, Thomsen S. *In vivo* fluorescence spectroscopy: potential for non-invasive, automated diagnosis of cervical intraepithelial neoplasia and use as a surrogate endpoint biomarker. *J Cell Biochem Suppl* 1994;19:111-9.
376. Powell MA, Pfeifer JD, Buttin BS. A pilot study of celecoxib in the treatment of patients with cervical and vaginal intraepithelial neoplasia [abstract 1830]. *Proc Am Soc Clin Oncol* 2003;21.
377. Harro CD, Pang YY, Roden RB, et al. Safety and immunogenicity trial in adult volunteers of a human papillomavirus 16 L1 virus-like particle vaccine. *J Natl Cancer Inst* 2001;93:284-92.
378. Koutsky LA, Ault KA, Wheeler CM, et al. A controlled trial of a human papillomavirus type 16 vaccine. *N Engl J Med* 2002;347:1645-51.
379. Suzich JA. Selection of vaccine candidate. In: 21st International Conference of the International Papillomavirus Society; Mexico City; 2004.
380. Boursarhin L, Combita A, Touze A. Immunization with HPV L1 VLPs induced cross-neutralizing antibodies. In: 20th International Papillomavirus Conference; Paris, France; 2002.
381. Deligdisch L, Gil J, Kerner H, Wu HS, Beck D, Gershoni-Baruch R. Ovarian dysplasia in prophylactic oophorectomy specimens: cytogenetic and morphometric correlations. *Cancer* 1999;86:1544-50.
382. Salazar H, Godwin AK, Daly MB, et al. Microscopic benign and invasive malignant neoplasms and a cancer-prone phenotype in prophylactic oophorectomies. *J Natl Cancer Inst* 1996;88:1810-20.

383. Brewer MA, Ranger-Moore J, Baruche A, et al. Exploratory study of ovarian intraepithelial neoplasia. *Cancer Epidemiol Biomarkers Prev* 2005;14:299–305.
384. Whittemore AS, Harris R, Itnyre J. Characteristics relating to ovarian cancer risk: collaborative analysis of 12 US case-control studies. II. Invasive epithelial ovarian cancers in White women. Collaborative Ovarian Cancer Group. *Am J Epidemiol* 1992;136:1184–203.
385. Rodriguez GC, Nagarsheth NP, Lee KL, et al. Progesterin-induced apoptosis in the *Macaque* ovarian epithelium: differential regulation of transforming growth factor- β . *J Natl Cancer Inst* 2002;94:50–60.
386. Schildkraut JM, Calingaert B, Marchbanks PA, Moorman PG, Rodriguez GC. Impact of progestin and estrogen potency in oral contraceptives on ovarian cancer risk. *J Natl Cancer Inst* 2002;94:32–8.
387. Taylor DD, Taylor CG, Black PH, Jiang CG, Chou IN. Alterations of cellular characteristics of a human ovarian teratocarcinoma cell line after *in vitro* treatment with retinoids. *Differentiation* 1990;43:123–30.
388. De Palo G, Veronesi U, Camerini T, et al. Can fenretinide protect women against ovarian cancer? *J Natl Cancer Inst* 1995;87:146–7.
389. Lazo JS, Schwartz PE, MacLusky NJ, Labaree DC, Eisenfeld AJ. Antiproliferative actions of tamoxifen to human ovarian carcinomas *in vitro*. *Cancer Res* 1984;44:2265–71.
390. Landen CN, Jr., Mathur SP, Richardson MS, Creasman WT. Expression of cyclooxygenase-2 in cervical, endometrial, and ovarian malignancies. *Am J Obstet Gynecol* 2003;188:1174–6.
391. Harlozinska A, Bar JK, Sobanska E, Goluda M. Epidermal growth factor receptor and c-erbB-2 oncoproteins in tissue and tumor effusion cells of histopathologically different ovarian neoplasms. *Tumour Biol* 1998;19:364–73.
392. Aarnio M, Sankila R, Pukkala E, et al. Cancer risk in mutation carriers of DNA-mismatch-repair genes. *Int J Cancer* 1999;81:214–8.
393. Brinton LA, Berman ML, Mortel R, et al. Reproductive, menstrual, and medical risk factors for endometrial cancer: results from a case-control study. *Am J Obstet Gynecol* 1992;167:1317–25.
394. Poticshman N, Hoover RN, Brinton LA, et al. Case-control study of endogenous steroid hormones and endometrial cancer. *J Natl Cancer Inst* 1996;88:1127–35.
395. Trimble CL, Kauderer J, Silverberg S, et al. Concurrent endometrial carcinoma (ED) in women with biopsy diagnosis of atypical endometrial hyperplasia (AEH): a Gynecologic Oncology Group (GOG) study. *Gynecol Oncol* 2004;92:393–511.
396. Gail MH, Greene MH. Gail model and breast cancer. *Lancet* 2000;355:1017.
397. Hoque A, Lippman SM, Boiko IV, et al. Quantitative nuclear morphometry by image analysis for prediction of recurrence of ductal carcinoma *in situ* of the breast. *Cancer Epidemiol Biomarkers Prev* 2001;10:249–59.
398. Perou CM, Sorlie T, Eisen MB, et al. Molecular portraits of human breast tumours. *Nature* 2000;406:747–52.
399. Kelloff GJ, Johnson JR, Crowell JA, et al. Approaches to the development and marketing approval of drugs that prevent cancer. *Cancer Epidemiol Biomarkers Prev* 1995;4:1–10.
400. Backlund MG, Mann JR, Holla VR, et al. 15-Hydroxyprostaglandin dehydrogenase is down-regulated in colorectal cancer. *J Biol Chem* 2005;280:3217–23.
401. Ding Y, Tong M, Liu S, Moscow JA, Tai HH. NAD⁺-linked 15-hydroxyprostaglandin dehydrogenase (15-PGDH) behaves as a tumor suppressor in lung cancer. *Carcinogenesis* 2005;26:65–72.
402. Gee JR, Montoya RG, Khaled HM, Sabichi AL, Grossman HB. Cytokeratin 20, AN43, PGDH, and COX-2 expression in transitional and squamous cell carcinoma of the bladder. *Urol Oncol* 2003;21:266–70.
403. Shao J, Lee SB, Guo H, Evers BM, Sheng H. Prostaglandin E₂ stimulates the growth of colon cancer cells via induction of amphiregulin. *Cancer Res* 2003;63:5218–23.
404. Pai R, Soreghan B, Szabo IL, Pavelka M, Baatar D, Tarnawski AS. Prostaglandin E₂ transactivates EGF receptor: a novel mechanism for promoting colon cancer growth and gastrointestinal hypertrophy. *Nat Med* 2002;8:289–93.
405. Buchanan FG, Wang D, Bargiacchi F, DuBois RN. Prostaglandin E₂ regulates cell migration via the intracellular activation of the epidermal growth factor receptor. *J Biol Chem* 2003;278:35451–7.
406. Dohadwala M, Yang S-C, Luo J, et al. Cyclooxygenase-2-dependent regulation of E-cadherin: prostaglandin E₂ induces transcriptional repressors ZEB1 and Snail in non-small cell lung cancer. *Cancer Res* 2006;66:5338–45.
407. Lippman SM, Gibson N, Subbaramaiah K, Dannenberg AJ. Combined targeting of the epidermal growth factor receptor and cyclooxygenase-2 pathways. *Clin Cancer Res* 2005;11:6097–9.
408. von Knobloch R, Konrad L, Barth PJ, et al. Genetic pathways and new progression markers for prostate cancer suggested by microsatellite allelotyping. *Clin Cancer Res* 2004;10:1064–73.
409. Palapattu GS, Sutcliffe S, Bastian PJ, et al. Prostate carcinogenesis and inflammation: emerging insights. *Carcinogenesis* 2005;26:1170–81.
410. O'Shaughnessy JA. Chemoprevention of breast cancer 1. *JAMA* 1996;275:1349–53.