PREFACE

Despite significant advances in cancer treatment and early detection, overall cancer incidence has increased, cancer-associated morbidity is considerable, and overall cancer survival has remained relatively flat over the past several decades (1,2). However, new technology allowing exploration of signal transduction pathways, identification of cancer-associated genes, and imaging of tissue architecture and molecular and cellular function is increasing our understanding of carcinogenesis and cancer progression. This knowledge is moving the focus of cancer therapeutics, including cancer preventive treatments, to drugs that take advantage of cellular control mechanisms to selectively suppress cancer progression.

Carcinogenesis is now visualized as a multifocal, multipath process of genetic progression occurring over a long time period and resulting in increasing loss of cellular controls. This process provides promising opportunities for chemoprevention, which involves using drugs, biologics, or nutrients to inhibit, delay, or reverse neoplastic progression at any time before the onset of invasive disease. Remarkable progress has been made in developing chemoprevention strategies, started by research on mechanisms of chemopreventive drugs and assays for evaluating these drugs in animal models (3–6), and led in the clinic by early studies on prevention of head and neck carcinogenesis (7,8).

Progressive disorganization provides a strong rationale for early intervention in carcinogenesis when mutations are fewer, even before tissue-level phenotypic changes are evident. However, the long latency also presents a significant challenge for prevention and treatment of early cancer (9,10). That is, cancer incidence reduction studies in subjects at relatively low risk may require thousands of subjects and many years to obtain significant and definitive results. The successful trial of tamoxifen as a chemopreventive for breast cancer illustrates the vast resources required for a primary prevention study, even when the cohort is well defined and the drug effect is already well characterized. This trial was carried out in women who at a minimum had a relative risk for breast cancer equivalent to a 60-year old (11). Six thousand six hundred (6600) treated women and an equivalent number of control subjects were required to achieve a significant ($p < 0.05$) treatment effect.

This inherent inefficiency in validating that chemopreventive treatment results in net clinical benefit for the patient (subject) has led to intensive research efforts to develop useful biomarkers. These biomarkers include measures of neoplastic progression, drug effect (or pharmacodynamic markers), and markers that measure prognosis as well as predict responses to specific therapy. All these biomarkers have the potential to greatly augment the development of successful chemoprevention therapies, but two specific types of biomarkers will have the most immediate impact on successful chemopreventive drug development—those that measure the risk of developing invasive life-threatening disease, and those whose modulation can "reasonably predict" clinical benefit and, therefore, serve as surrogate endpoints for later-occurring clinical disease. Thus far, the biomarker that best measures these two phenomena is intraepithelial neoplasia (IEN) because it is a near obligate precursor to cancer. As precancer, it is a very good risk marker for cancer development; and as a recognized disease that is being treated, it has been validated as a surrogate endpoint biomarker (12–14). Since IEN is discussed extensively in many chapters in this volume, the three important features that characterize IEN are presented in some detail below.

IEN is a near obligate precursor to cancer. IEN occurs in most epithelial tissue as moderate to severe dysplasia, is on the causal pathway leading from normal tissue to cancer, and is close in progression to cancer (invasive neoplasia). Genetic progression with loss of cellular control functions is observed as the phenotype gradually changes from normal histology to early dysplasia then to increasingly severe IEN, superficial cancers, and finally invasive disease. For example, in the breast it is estimated that progression from atypical hyperplasia through ductal carcinoma *in situ* (DCIS) to adenocarcinoma requires 10–20 years or more (15,16). Colorectal adenomas may form over a period as long as 5–20 years, and progression from adenoma to colorectal carcinoma usually requires another 5–15 years (17–20). Prostatic intraepithelial neoplasia (PIN) may develop over approximately 20 years. From PIN to early latent cancer may take 10 or more years, and clinically significant carcinoma may not occur until 3–15 years later (21). Progression is marked in target tissues by the appearance of specific molecular and more general genotypic damage associated with increasingly severe dysplastic histology. In many cases, critical early steps include inactivation of tumor suppressors such as APC in colon or BRCA in breast cancers, and activation of oncogenes such as *ras* in colon, lung, and pancreatic cancers. Progression is also influenced by factors specific to the host tissue's environment, such as the action of hormones and cytokines produced in stroma around

the developing epithelial tumor and changes in tissue structure. IEN shows these changes and provides a suitable target for treatment intervention because of its phenotypic and genotypic similarities and evolutionary proximity to invasive cancer.

IEN as precancer is a risk marker for cancer. Subjects with IEN, particularly severe IEN, are at significantly higher risk than unaffected populations for developing invasive cancer in the same tissues. Among measurable risk factors, only germline mutations that occur in genetic cancer syndromes confer higher risk. For example and as reviewed previously *(14)*, very strong evidence associates the presence of colorectal adenomas with subsequent development of invasive cancer; increasing risk correlates to type of histological growth pattern (villous > tubulovillous > tubular) and increasing size and severity of dysplasia. PIN as a risk marker for prostate cancer and the characteristics of PIN progression have also been described *(21–24)*. This evidence includes similar cellular morphology and atypia in high-grade PIN (HGPIN) and prostatic adenocarcinoma (cellular atypia observed in HGPIN is virtually indistinguishable from invasive cancer, except that in HGPIN no invasion has occurred). It also includes the spatial and temporal association of HGPIN to prostate cancer, with both being found primarily in the peripheral zone, and much more infrequently in the transition zone. As PIN progresses, the likelihood of damage to the basal cell layer and basement membrane increases. Certain cytoskeletal proteins, secreted proteins, and degree of glycosylation are shared by PIN and cancer, but not by benign prostatic hyperplasia or normal prostate epithelium. The most compelling data on the temporal relationship of PIN and cancer comes from studies showing that patients with HGPIN and no detectable cancer progressed to a 40% incidence of cancer in three years and to approximately 80% incidence in ten years.

IEN is precancer and in its own right is a disease; treatment provides clinical benefit. Because IEN is a near obligate precursor to invasive cancer, it is standard clinical practice to utilize invasive surgical interventions to reduce the burden of IEN, e.g., colon adenomas, oral leukoplakia, cervical IEN (CIN) 2/3, breast DCIS. Therefore, reducing IEN burden is an important and suitable goal for medical (noninvasive) intervention to reduce invasive cancer risk and to reduce surgical morbidity *(14)*. High-risk individuals with established IEN are cohorts for clinical trials to demonstrate the effectiveness of new chemopreventive agents for IEN treatment. Moreover, treatment is needed not only for clinically apparent IEN, but also the entire epithelial sheet at risk of developing IEN ("field cancerization," e.g., ref. *25*), to ensure reduced need for surgical removal of IEN.

These features of IEN explain why it is at present the best surrogate endpoint for invasive cancer, since no serious student of cell biology or pathology questions that the morphologic changes associated with IEN are part of and predict the cancer process, and that the lesions of genetic progression manifest themselves within IEN as cytological abnormalities of neoplasia—increased nuclear size; abnormal nuclear shape; increased nuclear stain uptake; variations in cellular size, shape and stain uptake; increased mitosis; abnormal mitosis; disordered maturation (differentiation) *(26)*. In summary, because of the probability that its presence will lead to cancer, IEN is already accepted as a validated endpoint for measurement of cancer risk reduction by both surgical and drug intervention.

Other promising surrogate endpoint biomarkers— genome/proteome expression profiles. Use (validation) of surrogate endpoint biomarkers that, unlike IEN, are not obviously intrinsic to neoplastic progression mostly fail because of the complexity of neoplasia as well as the need for surrogate endpoint biomarkers to "predict patient benefit with reasonable certainty" *(27)*. The failures result because the disease of cancer is tissue-based, and surrogate endpoint biomarker development has been constrained by naive approaches to modeling the disease and its multipath, multifocal development process with isolated molecular and cellular events. Further, for biomarkers to be useful, techniques to determine them need to be robust and exhaustively validated. When using biomarkers in studies, investigators need to comply strictly with validated methods to assure confidence that what is measured is consistent across studies. Achieving this objective may require extensive efforts such as those used to establish standards for the determination of cholesterol. Criteria for biomarker measurements have been the subject of many reviews (e.g., *12,14,28*), yet much of the lack of progress derives from faulty adherence to these methodologies. Nonetheless, a sound scientific basis now exists to characterize surrogate endpoint biomarkers for developing drugs *(12)*.

The multipath, multifactorial nature of carcinogenesis is predicted by the heterogeneity that can result from processing the human genome. The 30,000 or so human genes contain as many as several hundred thousand allelic variants from single nucleotide gene polymorphisms including splicing variants *(29)*. These variations are compounded another three- to fivefold by posttranslational protein modifications leading to a multitude ($>10^6$) of protein–protein interactions *(30)*. Even if only a small fraction of the genome is critical to cancer, the number of possible molecules and interactions involved is enormous. This level of complexity highlights the uncertainties of using isolated molecular and cellular biomarkers to measure carcinogenesis. Moreover, this complexity is heightened by expected intra-/intersubject and tissue variations.

Nonetheless, increasing understanding of genetic progression in cancer (e.g., *31,32*) and of signal transduction *(33)* in cancer target tissues, and observations of genotypic changes characteristic of selected cancers (e.g., *34,35*) combined with advances in technology for measuring and characterizing changes in gene and protein expression *(30)*, suggest that analyses of such patterns of gene expression have potential for development as surrogate endpoint biomarkers. For example, progress made in gene chip technology suggests that within a few years it will be trivial to measure 6–12 genes defining a genetic progression model *(36)*. As for all surrogate endpoint biomarkers, the feasibility of genome/proteome expression patterns as surrogate endpoint biomarkers will depend on careful evaluation in the context of carcinogenesis. Therefore, the characterization of molecular markers of carcinogenesis and their future development and validation as surrogate endpoint biomarkers will be most effectively done *in situ* within IEN. The further development of genetic progression models will also proceed in this context, as it has from inception. In the not-too-distant future, as understanding of the minimum number of disrupted pathways yielding malignancy grows, patterns of change representing carcinogenesis will be relatively easy to measure. This process will evolve with progress that is being made in understanding and analyzing systems biology. With this understanding will come surrogate endpoint biomarkers in predysplastic tissue (a normal morphologic phenotype); the predictive value of these data will begin to exceed the predictive value of abnormal morphology (IEN). This molecular pathology within IEN lesions, or even prior to appearance of these lesions, will also allow better identification of individuals at risk, improve study efficiency, and provide better quantitative estimation of drug efficacy than effects on IEN alone *(12,14,28)*. These advances in tissue-based biomarkers will be augmented by biomarkers that can be measured non-invasively by molecular imaging, and by functional genomic and proteomic research *(14,28)*.

Net clinical benefit is required. Drug approvals are based on clinical benefit, so the approval of drugs for chemoprevention will depend on some measure of clinical benefit—reduced morbidity, organ preservation, lower cost for surveillance—as well as efficacy against precancers. Because chemopreventive drugs will most likely be administered chronically, they will be expected to demonstrate long-term safety, duration of effect, and minimal drug resistance, or provide alternative strategies to minimize toxicity and maximize efficacy.

Promising Chemopreventive Agents, the companion volume, surveys ongoing efforts to identify drugs, natural products, and other agents that may have potential in cancer chemoprevention. The agents are grouped by pharmacological and/or mechanistic classes and vary widely in terms of stage of development as chemopreventives, ranging from extensively studied groups such as nonsteroidal antiinflammatory drugs (NSAIDs) and antiestrogens to drugs with recently identified potential based on mechanistic activity (e.g., protein kinase inhibitors, histone deacetylase inhibitors, and anti-angiogenesis agents), as well as agents yet to be evaluated in chemoprevention settings (e.g., proteasome and chaperone protein inhibitors). Attention is devoted to food-derived agents (such as tea, curcumin, and soy isoflavones), vitamins, and minerals because of their high promise for prevention in healthy populations.

Provided in this volume, *Strategies for Cancer Chemoprevention,* are guidelines for cancer chemopreventive drug development. Part I is devoted to general strategies and methods for drug discovery, preclinical efficacy, characterization of precancers, safety evaluation, clinical cohorts, and clinical trial design. Part II reviews strategies for and status of chemopreventive agent development at major cancer targets—prostate, breast, colon, lung, head and neck, esophagus, bladder, ovary, endometrium, cervix, skin, liver, and multiple myeloma. Both sections heavily document the characterization and application of reliable biomarkers in chemopreventive drug development.

The first several chapters of Part I consider discovery and preclinical evaluation of new agents. For example, an elegant approach to the challenges of identifying chemopreventive agents in natural products, particularly food plants (e.g., antioxidants, antiinflammatory compounds, and well-defined mixtures) is presented (Chapter 1); this approach addresses factors such as standardization of plant growth and extraction conditions, and considers co-development of a well-defined mixture and its likely active component. The development of preclinical models for evaluating potential chemopreventive agents is particularly important because of the potential for validating surrogate endpoints in animal models where an intermediate biomarker can be evaluated, along with subsequent effects on cancer incidence, and ultimately survival. Chapters in this volume describe well-established carcinogen-induced animal models of carcinogenesis in major cancer targets (Chapter 2), as well as newly defined transgenic and gene knock-in/knock-out mouse models of molecular targets for chemoprevention (Chapter 3), and animal models of genetically inherited cancer susceptibility (Chapter 4).

The importance of precancerous histopathology, particularly IEN, in chemoprevention has been stated. Characteristics and progression of this pathology in most cancer targets are comprehensively reviewed in Chapter 5. The use of computer-assisted image analysis to analyze precancerous tissue in the prostate is described as

an example of the potential application of new quantitative imaging techniques to evaluate chemopreventive efficacy in IEN (Chapter 6). As noted before, genome/proteome expression profiles have high potential as surrogate endpoints for carcinogenesis because they correlate with the clinical progression of carcinogenesis. Several chapters in the book assess potential applications of genomics and proteomics to chemoprevention. Uses of genomics databases in discovery of chemopreventive agents and in designing chemopreventive strategies are surveyed (Chapter 7). Surrogate endpoint biomarkers for breast cancer based on functional genomics are described (Chapter 8), as are applications of proteomics in clinical cancer settings (Chapter 9) and interpretation of genome-based data (Chapter 10).

Determining which populations will likely benefit from chemopreventive intervention, particularly those who are asymptomatic, is a significant challenge and an opportunity for chemoprevention. Two approaches are laid out in this volume. One is the construction of multi-factorial models of absolute risk, based primarily on epidemiological statistics (Chapter 11). The second explores the correlation of genetic polymorphisms to cancer susceptibility (Chapter 12). The remaining two chapters in Part I examine some practical aspects of clinical evaluation of chemopreventive agents—i.e., clinical trial design issues (Chapter 13) and subject recruitment (Chapter 14).

For each cancer target organ covered in Part II, one chapter provides an overview of carcinogenesis in the target organ, including cancer and precancer incidences, genetic progression, and risk factors, along with potential opportunities for chemoprevention. Known and promising chemopreventive agents, surrogate endpoints, and clinical trial designs are summarized. For a number of these targets, additional chapters address specific topics that contribute to chemoprevention strategies.

In addition to an overview of strategies for prostate cancer chemoprevention (Chapter 15), a second chapter addresses the controversial topic of using prostate-specific antigen for determining risk and monitoring the progression of prostate cancer (Chapter 16). The overview of breast cancer chemoprevention focuses on defining populations at risk based on evidence of early genetic progression (Chapter 17) and is accompanied by two supplemental chapters. One describes development of ductal lavage as a technique for sampling breast cells in assessment of early neoplasia (Chapter 18), and the second addresses the well-recognized need to control estrogenic activity in suppressing breast carcinogenesis (Chapter 19). Quite possibly, the most significant advances in clinical chemoprevention have been made against colorectal carcinogenesis (Chapter 20) where genetic and histopathological progression of early dysplasia to adenoma to

cancer has been well-studied. An additional important preventive strategy in colon is screening for and excision of adenomas (Chapter 21). Chapter 22 provides an overview of lung cancer chemoprevention accompanied by an article on topical delivery as a strategy to allow administration of drugs to lung that may be too toxic for systemic administration (Chapter 23); topical administration is also a promising strategy in other accessible targets such as skin, oral cavity, colon and cervix.

The review of bladder cancer chemoprevention (Chapter 24) focuses on the potential use of chemopreventive drugs to stop the recurrence of superficial bladder cancers; this cohort is at very high risk for recurrence and progression, and a successful chemopreventive intervention could be expected to provide clinical benefit from organ preservation (by delaying or reducing the need for cystectomy). Chapters on the esophagus discuss prevention and delay of progression of Barrett's esophagus, a precursor to adenocarcinoma and an increasing risk factor for esophageal cancer in western populations (Chapter 25), as well as prevention of squamous cell carcinoma (Chapter 27). A third chapter looks at sophisticated new techniques for imaging esophageal dysplasia (Chapter 26). The high rate of second primary tumor formation has been well-documented for the head and neck, which have been studied for more than 20 years as a site for chemoprevention (Chapter 28). Recently, aneuploidy and other biomarkers of genetic progression have been carefully documented as risk and prognostic indicators of head and neck carcinogenesis and potential endpoints for chemoprevention studies (Chapter 29). Incidences of non-melanoma skin cancer are higher by far than any other cancer, and melanoma incidence is increasing (Chapter 30). In addition to oral and topical small molecule drug treatments for prevention and treatment of non-melanoma precursor lesions (actinic keratoses, basal cell nevus syndrome), opportunities exist for novel immunotherapies in skin carcinogenesis and vaccination against melanoma (Chapter 31).

Screening for and surgical removal of suspect CIN is well established, and drugs have shown activity in reducing CIN severity (Chapter 32). Moreover, human papillomavirus infection is strongly associated with onset of cervical cancer, and immunoprevention strategies (both treatment and prophylactic) are under development for populations at risk (Chapter 33). Thus far, chemoprevention strategies in endometrium have not been established; however, remarkable advances have been made in documenting and quantifying carcinogenesis-associated changes in endometrial tissue that provide opportunities for preventive intervention (Chapter 34). In ovary (Chapter 35), pancreas (Chapter 36), liver (Chapter 37), and multiple myeloma (Chapter 38), precancerous lesions that may be targets for chemoprevention

are suggested, along with potentially effective chemo-preventive drugs.

The two volumes of *Cancer Chemoprevention* demonstrate that the science of chemoprevention research is solidly established, very active, and offers great promise for lessening the burden of human cancer. Progress in building and understanding genetic/molecular progression models of many human cancers based on seminal work described by Vogelstein and colleagues for the adenoma-carcinoma sequence in colon cancer *(37)* has been substantial and is being enhanced by newer and better animal models. Understanding molecular progression leads to synthesis and discovery of new molecularly targeted agents with high promise of efficacy that, once evaluated for safety, will have an impact on cancer incidence and mortality. The evaluation of drug effect and drug efficacy biomarkers along with better technologies for their measurement is progressing, and the science and utility of surrogate endpoint biomarkers in developing cancer chemopreventive agents against sporadic cancers are solidly established. The issue of validation is a relative one, and IEN is validated for most target organs sufficiently to establish that its prevention/removal provides clinical benefit. With rigorous attention to methodology and to emerging scientific data and new technologies, there is every expectation that new surrogate endpoint biomarkers will now be developed in the context of IEN. These new surrogate endpoint biomarkers will improve the efficiency of clinical chemopreventive agent development, better identify those patients (subjects) who are likely to benefit (or not to benefit), while also opening the door to even earlier identification of individuals at risk (e.g., those with predysplastic molecular lesions that occur prior to IEN). The rapid pace at which systems biology and new technologies are evolving will make surrogate endpoint biomarker science a very productive and exciting area, but will also evoke the need for careful validation of such markers in the context of clinical trials.

Prospects are bright that surrogate endpoint biomarkers will make cancer chemoprevention studies more efficient and informative; however, hard work and exceptional dedication to sound, standardized methods will be required to assure that the application of these efforts in developing chemopreventive drugs is fruitful. The eventual acceptance of surrogate endpoint biomarkers may entail more than scientific rationale. Scientific and regulatory policy changes may also be required (e.g., *38*). It is often observed that candidate surrogate endpoint biomarkers are expressed at higher incidences than the symptomatic clinical disease that they approximate. Such will always be the case when completely unrelated endpoints (other causes of death) do not allow carcinogenesis to go to completion. Based on existing disease models, it is likely that all high-grade IEN-carrying confirmed genetic lesions would end

in cancer if the host lived long enough *(12,32)*. Validation, like causality, is a relative term that only becomes absolute when all variables and elements of a process are known and can be studied quantitatively. It is undesirable and short-sighted to require any data more rigorous than validation based on probabilistic estimates that are consistent with current medical and regulatory practice. Based on existing knowledge, we can assume that IEN and the earlier biomarkers within IEN are on one or more of the possible causal pathways to carcinogenesis. Because they sometimes precede the cancer endpoint by several years, there is potential for interference, diversion, role in other biological processes, etc., that would keep these events from being ideal surrogate endpoint biomarkers. However, intervention to treat or prevent could be shown to provide clinical benefit, much like lipid-lowering in cardiovascular disease and viral load reduction in AIDS *(27,39)*. If interventions show compelling efficacy against surrogate endpoint biomarkers and can be administered safely to populations at risk, it would seem prudent to formulate scientific and regulatory policy changes allowing the use of these biomarkers in evaluating interventions that would lead to more efficient drug approvals to prevent this dread disease.

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REFERENCES

1. Sporn MB. The war on cancer. Lancet 1996;347:1377–1381.
2. Jemal A, Murray T, Samuels A, et al. Cancer statistics, 2003. CA Cancer J Clin 2003;53:5–26.
3. Sporn MB. Approaches to prevention of epithelial cancer during the preneoplastic period. Cancer Res 1976;36:2699–2702.
4. Wattenberg LW. Chemoprophylaxis of carcino-genesis: a review. Cancer Res 1966;26:1520–1526.
5. Wattenberg LW. Inhibition of chemical carcino-genesis. J Natl Cancer Inst 1978;60:11–18.
6. Wattenberg LW. Chemoprevention of cancer. Cancer Res 1985;45:1–8.
7. Hong WK, Endicott J, Itri LM, et al. 13-cis-Retinoic acid in the treatment of oral leukoplakia. N Engl J Med 1986;315:1501–1505.
8. 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.
9. 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.

10. Hong WK, Sporn MB. Recent advances in chemo-prevention of cancer. Science 1997;278:1073–1077.

11. Fisher B, Costantino JP, Wickerham DL, et al. Tamoxifen for prevention of breast cancer: report of the National Surgical Adjuvant Breast and Bowel Project P-1 Study. J Natl Cancer Inst 1998;90:1371–1388.

12. 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;9:127–137.

13. Kelloff GJ. Perspectives on cancer chemopreven-tion research and drug development. Adv Cancer Res 2000;78:199–334.

14. O'Shaughnessy JA, Kelloff GJ, Gordon GB, et al. Treat-ment and prevention of intraepithelial neoplasia: an im-portant target for accelerated new agent development. Clin Cancer Res 2002;8:314–346.

15. Frykberg ER, Bland KI. In situ breast carcinoma. Adv Surg 1993;26:29–72.

16. Page DL, Dupont WD, Rogers LW, Rados MS. Atypical hyperplastic lesions of the female breast. A long-term follow-up study. Cancer 1985;55:2698–2708.

17. Muto T, Bussey HJ, Morson BC. The evolution of cancer of the colon and rectum. Cancer 1975;36:2251–2270.

18. Day DW, Morson BC. The adenoma-carcinoma sequence. Major Probl Pathol 1978;10:58–71.

19. Hamilton SR. The adenoma-adenocarcinoma sequence in the large bowel: variations on a theme. J Cell Biochem 1992;16 Suppl G:41–46.

20. A multicenter study of colorectal adenomas. Rationale, objectives, methods and characteristics of the study co-hort. The Multicentric Study of Colorectal Adenomas (SMAC) Workgroup. Tumori 1995;81:157–163.

21. Bostwick DG, Brawer MK. Prostatic intra-epithelial neo-plasia and early invasion in prostate cancer. Cancer 1987;59:788–794.

22. Lipski BA, Garcia RL, Brawer MK. Prostatic intra-epithelial neoplasia: significance and management. Semin Urol Oncol 1996;14:149–155.

23. Sakr WA, Billis A, Ekman P, et al. Epidemiology of high-grade prostatic intraepithelial neoplasia. Scand J Urol Nephrol Suppl 2000;205:11–18.

24. Foster CS, Bostwick DG, Bonkhoff H, et al. Cellular and molecular pathology of prostate cancer precursors. Scand J Urol Nephrol Suppl 2000;205:19–43.

25. Slaughter DP, Southwick HW, Smekjal W. Field cancer-ization in oral stratified squamous epithe-lium; clinical implications of multicentric origin. Cancer 1953;6:963–968.

26. Foulds L. Multiple etiologic factors in neoplastic devel-opment. Cancer Res 1965;25:1339–1347.

27. Temple RJ. A regulatory authority's opinion about surro-gate endpoints. In: Nimmo WS, Tucker GT, eds. Clinical Measurement in Drug Evaluation. New York: John Wiley and Sons, Inc., 1995:1–22.

28. Kelloff GJ, O'Shaughnessy JA, Gordon GB, et al. Counterpoint: because some surrogate end point biomarkers measure the neoplastic process they will have high utility in the development of cancer chemopreventive agents against sporadic cancers. Cancer Epidemiol Biomarkers Prev 2003;12:593–596.

29. Black DL. Protein diversity from alternative splicing: a challenge for bioinformatics and post-genome biology. Cell 2000;103:367–370.

30. Petricoin EF, Zoon KC, Kohn EC, et al. Clinical prote-omics: translating benchside promise into bedside reality. Nat Rev Drug Discov 2002;1:683–695.

31. Califano J, van der Riet P, Westra W, et al. Genetic pro-gression model for head and neck cancer: implications for field cancerization. Cancer Res 1996;56:2488–2492.

32. Ilyas M, Straub J, Tomlinson IP, Bodmer WF. Genetic pathways in colorectal and other cancers. Eur J Cancer 1999;35:1986–2002.

33. Hanahan D, Weinberg RA. The hallmarks of cancer. Cell 2000;100:57–70.

34. Sudbo J, Kildal W, Johannessen AC, et al. Gross genomic aberrations in precancers: clinical implications of a long-term follow-up study in oral erythroplakias. J Clin Oncol 2002;20:456–462.

35. Sudbo J, Reith A. Which putatively pre-malignant oral lesions become oral cancers? J Oral Pathol Med 2003;32:63–70.

36. Chen Y, Yakhini Z, Ben Dor A, et al. Analysis of expres-sion patterns: the scope of the problem, the problem of scope. Dis Markers 2001;17:59–65.

37. Vogelstein B, Fearon ER, Hamilton SR, et al. Genetic altera-tions during colorectal-tumor development. N Engl J Med 1988;319:525–532.

38. Metz DC, Alberts DS. Gastrointestinal cancer prevention in the United States: the road ahead. Cancer Epidemiol Biomarkers Prev 2003;12:81–83.

39. Lesko LJ, Atkinson AJ, Jr. Use of biomarkers and surro-gate endpoints in drug development and regulatory deci-sion making: criteria, validation, strategies. Annu Rev Pharmacol Toxicol 2001;41:347–366.