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Surrogate end-point biomarkers in chemopreventive drug development

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Relevant and feasible surrogate end-points are needed for the evaluation of intervention strategies against cancer and other chronic, life-threatening diseases. Carcinogenesis can be viewed as a process of progressive disorganization. This process is characterized by the accumulation of genotypic lesions and corresponding tissue and cellular abnormalities, including loss of proliferation and apoptosis controls. Potential surrogate end-points for cancer incidence include both phenotypic and genotypic biomarkers of this progression. In the US National Cancer Institute chemoprevention programme, histological modulation of a precancer (intraepithelial neoplasia) has so far been the primary phenotypic surrogate end-point in chemoprevention trials. Additionally, high priority has been given to biomarkers measuring specific and general genotypic changes correlated with the carcinogenesis progression model for the targeted cancer (e.g., progressive genomic instability as measured by loss of heterozygosity or amplification at specific microsatellite loci). Other potential surrogate end-points include proliferation and differentiation indices, specific gene and general chromosome damage, cell growth regulatory molecules, and biochemical activities (e.g., enzyme inhibition). Serum biomarkers thought to be associated with cancer progression (e.g., prostate-specific antigen) are particularly appealing surrogate end-points because of accessibility. Potentially chemopreventive effects of the test agent may also be measured (e.g., tissue and serum estrogen levels in studies of steroid aromatase inhibitors). To establish chemopreventive efficacy, prevention of virtually all biomarker lesions, or of those lesions with particular propensity for progression, may be required. Ideally, the phenotype and genotype of any new or remaining precancers in the target tissue of chemopreventive agent-treated subjects would show less, and certainly no greater, potential for progression than those of placebo-treated subjects.

Introduction

Cancer chemoprevention can be defined as treatment of carcinogenesis — i.e., its prevention, inhibition or reversal (Hong & Sporn, 1997; Kelloff, 2000). In most epithelial tissues, accumulating mutations (i.e., genetic progression) and loss of cellular control functions are observed during the course of sequential histological changes that culminate in cancer. These changes are manifested as the transition from normal histology to early intraepithelial neoplasia, through increasingly severe intraepithelial neoplasia to superficial cancers and finally invasive disease. Although the carcinogenic process can be relatively aggressive (e.g., in the presence of a DNA-repair-deficient genotype or viral transformant such as human papillomavirus), these changes generally occur over a long time period (Table 1). Cancers generally develop over decades and intraepithelial neoplasia (e.g., prostatic intraepithelial neoplasia, colorectal adenomas) may also progress slowly.

The progressive nature of carcinogenesis underscores the advantage of chemoprevention — to intervene when the mutations are fewer, even before tissue-level phenotypic changes are evident. However, a major obstacle to chemopreventive drug development is the use of cancer incidence as the end-point for determining efficacy in clinical trials. Such studies entail huge sample sizes, lengthy follow-up periods and high cost (Hong & Sporn, 1997; Kelloff *et al.*, 1995, 2000; Kelloff, 2000). Typically, cancer incidence reduction trials have planned durations of 5–10 years with subject accrual in the tens of thousands. Surrogate end-

Table 1. Incidence and multi-year time course for progression of precancers in selected cancer targets

Target organ	Precancer (IEN)	Estimated incidence	Years for precancer formation	Years for progression from precancer to cancer	References
Prostate	PIN	40–50% of men aged 40–60 years	20	10 or more to latent cancer; 3–15 further years to cancer	Bostwick, 1992
Breast	DCIS	46 000 new cases in women in 2000	14–18 from atypical hyperplasia	6–10	Frykberg & Bland, 1993; Page *et al.*, 1985; Greenlee *et al.*, 2000
Colon	Adenoma	30–40% of the western population aged > 60 years	5–20	5–15	Bruzzi, 1995; Day & Morson, 1978; Zauber *et al.*, 1996
Bladder	Ta, T1, TIS	37 500 cases in USA for 1997	20	<5	Cotran *et al*, 1989; Scher *et al.*, 1997
Oesophagus	Barrett's metaplasia	0.4% of the western population	5–20	5–20 to severe dysplasia; 3–4 further years to cancer	Ovaska *et al*, 1989; Williamson *et al*, 1991; Miros *et al*, 1991; Cameron & Lomboy, 1992; Jankowski *et al*, 1993; Falk & Richter, 1996

DCIS, ductal carcinoma *in situ*; PIN, prostatic intraepithelial neoplasia; TIS, transitional-cell carcinoma *in situ*.

point biomarkers are an important aspect of the chemopreventive drug development process in that they provide a means for overcoming these obstacles (e.g., American Association for Cancer Research, 1999; Hong & Sporn, 1997; Kelloff *et al.*, 1995, 2000; Kelloff, 2000; Sporn & Suh, 2000). The use of phenotypic and genotypic biomarkers as surrogate end-points for cancer incidence would permit the evaluation of chemopreventive efficacy in most cancer targets in up to three years with no more than several hundred subjects. Use of surrogate end-points is possible only because of increasing knowledge of the genetic, histopathological and molecular basis of carcinogenesis. This expanding appreciation of the carcinogenic process will support the continuing efforts to identify, validate and apply biomarkers as surrogate end-points for cancer incidence.

Rationale for surrogate end-points of carcinogenesis: molecular progression models

Carcinogenesis is characterized by a progressive loss of proliferation and apoptosis controls and increasing disorganization, aneusomy and heterogeneity. The appearance of specific molecular and more general genotypic damage is associated with increasingly severe dysplastic phenotypes (Califano *et al.*, 1996, and other studies cited below). In many cases, critical early steps include inactivation of tumour-suppressor genes, such as those for adenomatous polyposis coli (*APC*) or breast cancer (*BRCA*) and activation of oncogenes such as *ras*. Carcinogenesis may follow multiple paths, and be multifocal; not all cancers in a given tissue nor all cells in a given cancer may ultimately contain the same lesions. Progression may also be influenced by factors specific to the host tissue's environment, such as the action of hormones produced in stroma around the developing epithelial tumour and changes in tissue and chromatin structure (Schipper *et al.*, 1996; Sporn, 1996; Bissell *et al.*, 1999; Sporn & Suh, 2000; Stein *et al.*, 2000). Genetic progression models have been established for many human cancers, including colon, brain, bladder, head and neck, non-small-cell lung cancer and cervical intraepithelial neoplasia (Fearon & Vogelstein, 1990; Sidransky & Messing, 1992; Sidransky *et al.*, 1992a,b; Simoneau & Jones, 1994; Kishimoto *et al.*, 1995; Rosin *et al.*, 1995; Thiberville *et al.*, 1995; Califano *et al.*, 1996; Mao *et al.*, 1996). These models indicate that the sequence of genetic damage leading to cancer can involve myriad combinations of targets in the array of pathways that govern proliferation and apoptosis. These genotypic lesions, and the corresponding tissue and cellular abnormalities, have high potential to serve as surrogate end-points when they are sufficiently stable to allow screening during carcinogenesis. Specific carcinogenesis-associated molecular lesions identified so far, while important, may not be the most informative among those that will be discovered as research continues. Most cancer is preceded by an abnormal histological precancer phenotype which integrates the progressive genetic and molecular changes. Thus, focusing on assessment of this abnormal phenotype and the accompanying genotypic changes within the target tissue appears at present to provide the best opportunity for validating surrogate end-points.

Phenotypic and genotypic surrogate end-points to establish chemopreventive efficacy

Intraepithelial neoplasia, the embodiment of the abnormal cancer phenotype, serves as a promising surrogate end-point for chemoprevention studies in epithelial tissues (Kelloff *et al.*, 1995, 2000; Kelloff, 2000). Although shorter than the period for developing cancer, the latency for progression of intraepithelial neoplasia can also be lengthy compared with the practical time frame for a chemopreventive intervention study. Importantly, the number of precancers may far exceed the number of cancers that subsequently develop in the target tissue. Additionally, behavioural (e.g., smoking history), environmental (e.g., hormonal status) and co-existing disease (e.g., immune system competence) factors may influence progression in individual subjects. Intraepithelial neoplastic lesions that will progress may also have particular characteristics predisposing them to develop into cancers. For example, the potential of colorectal adenomas to progress to cancer correlates with histological growth pattern, size and severity of dysplasia (Muto *et al.*, 1975; Hamilton, 1992, 1996).

For these reasons, histological determination of drug-induced prevention or regression of intraepithelial neoplasia alone may not be sufficient for assessing chemopreventive efficacy. The specific

and general genotypic effects comprising the progression models for carcinogenesis, and the underlying molecular pathology of the lesions, should also be considered in the evaluation. A reduced incidence of new precancers in the target tissue in agent-treated subjects would ideally be accompanied by a genotype reflecting decreased, and certainly no greater, carcinogenic potential. In particular, when regression of existing precancers is incomplete, the remaining lesions in the agent-treated subjects should have genotypes with equivalent or lower propensity for progression than placebo control subjects.

Potential surrogate end-points at major cancer target organs

Cancers in at least 12 organ systems have been evaluated as targets for chemopreventive agents: prostate, breast, colon, lung, head and neck, bladder, oesophagus, cervix, skin (non-melanoma and melanoma), liver, ovary and multiple myeloma (Kelloff, 2000; Kelloff *et al.*, 2000). Many classes of agent, including retinoids, antioxidants, anti-inflammatories, antiestrogens and antiandrogens, have shown promising chemopreventive activity in one or more of these organ systems (Hong & Sporn, 1997; Kelloff, 2000; Sporn & Suh, 2000; Kelloff *et al.*, 2000); more than 40 candidate chemoprevention drugs are currently under clinical development in studies sponsored by the US National Cancer Institute chemoprevention program (Kelloff, 2000; Kelloff *et al.*, 2000). Among the cellular mechanisms of chemopreventive action of these drugs are inhibition of angiogenesis, mutagenesis, proliferation and apoptosis, as well as modulation of hormone activity. Often single agents exhibit multiple interrelated and/or independent mechanisms that may each contribute to the overall chemopreventive effect. For example, in addition to modulating estrogen receptor binding, antiestrogens can inhibit insulin-like growth factor-I (IGF-I), while cyclooxygenase (COX)-2 inhibitors can modulate the peroxisome proliferator-activated receptors and the pathways controlled by these nuclear receptors. The selection of appropriate biomarkers to monitor the efficacy of these agents should consider their purported mechanisms of action in the target organ of interest. For all the biomarkers, it is highly desirable to measure modulation quantitatively as the difference between the biomarker value at baseline and the end of treatment. The change in the surrogate end-point measures on chemopreventive treatment should also be compared with that seen in appropriate controls. Thus, biopsies or other tissue measurements at baseline are essential.

Table 2 provides a target-organ based listing of the types of biomarker currently being used to study chemopreventive efficacy in clinical trials sponsored by the US National Cancer Institute. Many of these biomarkers were previously or are currently being evaluated, fully characterized and validated in animal models (Boone *et al.*, 2000) as well as in archival human tissues (e.g., Bacus *et al.*, 1999; Sneige *et al.*, 1999). As mentioned above, intraepithelial neoplasia are tissue-level phenotypic biomarkers that, because they are on the causal pathway to and are direct precursors of cancer, are generally considered suitable for following carcinogenesis. Cellular biomarkers such as nuclear and nucleolar morphology, mitotic index and DNA ploidy are also being evaluated; they may be useful in characterizing the progression potential of intraepithelial neoplasia (Kelloff, 2000; Kelloff *et al.*, 2000). Other possibly useful genotypic biomarkers include loss of heterozygosity and gene amplification, either at specific gene loci (e.g., those for tumour-suppressors such as *p53* or tumour growth accelerators such as c-*erb*B2) or at panels of microsatellite loci where mutations indicate increasing genomic instability (Califano *et al.*, 1996). These biomarkers appear to be particularly applicable as surrogate end-points for head and neck cancer, and may also prove useful in other tissues where microsatellite instability is a predominant feature of carcinogenesis, as in hereditary non-polyposis colorectal cancer (HNPCC) (Marra & Boland, 1995; Lynch & Smyrk, 1996).

Both phenotypic and genotypic changes during carcinogenesis may also be manifested by molecular biomarkers (Kelloff *et al.*, 2000). For example, excess proliferation may be seen in increased levels of cellular antigens such as proliferating cell nuclear antigen (PCNA) or Ki-67/MIB-1 or overexpression of growth factors such as epidermal growth factor (EGF), transforming growth factor (TGF)-α and IGF-I; reduced propensity to undergo apoptosis may be detected by increased expression of *bcl*-2. Aberrant differentiation may result in changes in G-actin, cytokeratins and blood-group

Table 2. Potential surrogate end-point biomarkers for chemoprevention trials in breast, colon and prostate

Type of biomarker	Breast	Colon	Prostate
Histological	DCIS, LCIS, atypical hyperplasia, mammographic density, nuclear and nucleolar morphometry	Adenomatous polyps, aberrant crypts, microadenomas, nuclear and nucleolar morphometry	PIN, nuclear and nucleolar morphometry
Genotypic	Gene amplification (*c-erb*B-2)	LOH, gene amplification	Chromosomal loss or gain (8p, 9q, (16q), gene amplification (*c-erb*B-2)
Proliferation/ growth control	Ki-67, *bcl-2*/*bax*, p53, cyclin D1, TGF-β, EGFR, VEGF, IGF-1 expression, S-phase fraction, apoptotic index	PCNA, Ki-67, *bcl-2*/*bax* expression, S-phase fraction, BrdU uptake, apoptotic index	PCNA, KI-67, p53, *bcl-2*/*bax*, pc-1, TGF-β,VEGF, IGF-1 expression, apoptotic index
Differentiation	Myoepithelial cell markers (S-100, keratin 17, vimentin), altered cytoplasmic glycoprotein expression, altered cell surface antigen expression	Altered blood group-related antigens, mucin core antigens (T, Tn, sialyl Tn antigens), apomucins (MUC 1,2,3 genes) cytokeratins, brush border membrane enzymes (sucrase, isomaltase)	Loss of high molecular weight cytokeratins (50–64 kDa), altered blood group related antigens, vimentin

BrdU, bromodeoxyuridine; DCIS, ductal carcinoma *in situ*; EGF, epidermal growth factor; EGFR, epidermal growth factor receptor; IGF, insulin-like growth factor; LCIS, lobular carcinoma *in situ*; LOH, loss of heterozygosity; PCNA, proliferating cell nuclear antigen; PIN, prostatic intraepithelial neoplasia; PSA, prostate-specific antigen; TGF, transforming growth factor; VEGF, vascular endothelial growth factor.

antigens. Other molecular biomarkers may reflect general changes in cell growth control. These include TGF-β, cyclins, p53 and other tumour suppressors, as well as mutations and overexpression of oncogenes associated with carcinogenesis such as *ras* and the transcription factors *myc*, *fos* and *jun*. Tissue- and drug-related biomarkers may also be useful. Examples of tissue-related biomarkers are the expression of estrogen receptors in breast and prostate-specific antigen (PSA) in prostate. Drug-related biomarkers associated with chemopreventive activity include inhibition of ornithine decarboxylase by 2-difluoromethylornithine and inhibition of prostaglandin biosynthesis by non-steroidal anti-inflammatory drugs (NSAIDs); while such biomarkers do not necessarily demonstrate a chemopreventive effect, they are useful in assessing whether a biologically active dose of the agent was present and in evaluating the chemopreventive mechanisms that are operating.

Cohorts for surrogate end-point chemoprevention studies

Another important challenge in chemoprevention research is the identification of appropriate cohorts for clinical trials. Patients at high risk for developing precancerous lesions and cancers often have the highest potential to benefit from chemopreventive interventions. In such cohorts where the time course of carcinogenesis is accelerated, shorter studies may be feasible. Additionally, these patients may afford the best opportunity to study intervention modalities, because the increased incidence and/or prevalence of disease permits the use of fewer subjects. Patients with previous cancers or precancers constitute one appropriate cohort for chemopreventive intervention, since they are at high risk for new primary cancers. For example, the lifetime risk for a second primary tumour of the aerodigestive tract following a squamous-cell cancer of the head or neck has been estimated at 20–40% (Benner *et al.*, 1992). Premalignant changes (surrogate end-points) can be followed at both phenotypic and genotypic levels in these subjects. Slaughter *et al.* (1953) coined the term "field cancerization" to describe the early evidence of carcinogenesis found in normal-appearing mucosa of patients with previous head and neck cancers. Many studies have confirmed this phenomenon (Hjermann *et al.*, 1981; Benner *et al.*, 1992; Hittelman *et al.*, 1996). In these studies, the degree of genetic change detected was correlated with histological progression of the lesion towards cancer. For example eight of 15 patients having high levels of genetic damage (3.5% or more of cells with three or more copies of chromosome 9) in premalignant lesions of the oral cavity subsequently developed aerodigestive tract cancer, compared with none among patients with lower levels. Similar results were found in relation to chromosome 9 in lung tissue from previous smokers (Hittelman *et al.*, 1996), chromosome 17 in breast tissue (Dhingra *et al.*, 1994) and chromosome 1 in cervical tissue from patients with various grades of cervical intraepithelial neoplasia (CIN) (Segers *et al.*, 1995). While none of these studies tracked the development of specific lesions into cancers, they all confirmed that carcinogenesis could be detected by genotypic changes in high-risk tissue.

The high incidence of new lesions in head and neck cancer patients suggests that a trial duration of up to three years would be appropriate for phase II and III studies using surrogate end-points; as little as three years may even be a feasible duration for detecting a reduction in the incidence of second primary cancers. Patients with superficial bladder cancer are also appropriate subjects for chemoprevention studies, because the recurrence rate is approximately 50% within 6–12 months (Soloway & Perito, 1992) and 60–75% within 2–5 years (Herr *et al.*, 1990; Harris & Neal, 1992). Similar high rates of recurrence or new lesions apply to colorectal adenomas (e.g., Winawer *et al.*, 1993). Studies in these settings would appear to be particularly promising for the validation of surrogate end-points, which may then be suitable for application in cohorts without previous precancers or cancers.

Germline mutations and other genetic and molecular evidence of susceptibility may also be used to define high-risk cohorts. For example, subjects with familial adenomatous polyposis (FAP), which is identified by loss of the *APC* tumour-suppressor gene, develop hundreds to thousands of colorectal adenomas (Burt, 1996). Fabian has described high-risk breast cancer subjects suitable for chemoprevention studies based on the presence of atypical hyperplasia, aneuploidy and overexpression of *p53* and EGF. These biomarkers

could potentially serve as surrogate end-points for breast cancer prevention trials (Fabian *et al.*, 1996). Patients scheduled for surgical treatment of precancer or early cancer also provide cohorts for obtaining early evidence of efficacy. Agents are administered to these patients during the weeks after diagnostic biopsy and before more definitive surgery, so that modulation of biomarkers in the precancer/cancerous, and, if possible, normal-appearing tissue in the target organ can be assessed. The National Cancer Institute is now using such protocols in phase I/early phase II studies of breast and prostate cancer prevention (Kelloff, 2000; Kelloff *et al.*, 2000).

Challenges in using surrogate end-points

Numerous issues must be addressed in both the preclinical and clinical phases of chemopreventive drug development efforts. For example, how long must treatment be continued (including whether treatment cessation results in recurrence of precancerous lesions)? Can chemoprevention be distinguished from regression of existing disease? Can lifestyle factors that may significantly influence trial outcomes (e.g., high-fat diets, total caloric consumption) be controlled? Are the results of trials in specific high-risk or undernourished populations applicable to other populations or the populace as a whole? Several additional philosophical and practical concerns specific to the application of surrogate end-points in the evaluation of chemopreventive efficacy must also be considered. Temple (1995, 1999) previously addressed many of these in the context of cardiovascular drug development. Particularly relevant to chemoprevention are issues of sampling, the clinical benefit of biomarker modulation, and whether adverse events prove limiting to long-term chemopreventive agent administration.

Sampling

A critical issue in the application and validation of surrogate end-points is the development of standardized, appropriate and quantitative techniques for sampling the target tissues. To date, the greatest progress has been made in tissues that can be directly observed: oral cavity, colon, larynx, bladder, oesophagus, cervix, bronchus, skin. In these tissues, the focal lesion can be identified and stained, and the area of cancerization can be defined and imaged (e.g., cervix). However, in more inaccessible tissues — prostate, ovary, breast, liver, pancreas—detection of the focal lesion is uncertain, and it is difficult to map and image the cancerization field. Advances in the basic sciences, particularly genomics and proteomics, and in biomedical technologies such as imaging, are providing tools for further growth in this area. New diagnostic methodologies such as gene-chip analyses, the confocal microscope, digital mammography, the LIFE scope for visualizing bronchial tissue and the magnifying endoscope for colorectal monitoring will enhance the possibilitites for monitoring of precancerous tissue. Such techniques can be used for the identification and evaluation of early molecular targets for intervention, as well as for quantitative assessment of cancer risks and tissue- and cell-based changes in these early stages of carcinogenesis. Brown and Botstein (1999) have reviewed the significant potential of functional genomics in biology — the utility ranges from identification of a mutant genotype by a single nucleotide polymorphism to subcellular localization of gene products to elucidation of gene expression patterns along signal transduction pathways. The sequencing and functional analysis efforts of the Cancer Genome Anatomy Project are a major contribution to this area. Gene-chip microarrays can be used to define and quantify contributors to risk once appropriate parameters for analyses have been defined. To this end, proven cases from archival specimens from properly designed tissue banks can be utilized to elucidate and validate relevant and useful end-points. The method for cluster analysis of genome-wide expression described by Eisen *et al.* (1998) could be applied to provide a generalized comparison of gene expression in baseline and post-treatment lesions, using known effective drugs and placebo.

Clinical benefit of surrogate end-point modulation

As described by Blue and Colburn (1996), surrogate end-points fall on a continuum from showing no particular clinical benefit but only correlation to the target disease end-point (e.g., drug effect markers), through demonstrating clinical benefit that is not a direct effect on the target disease (e.g., immunostimulation), to demonstrating clinical benefit directly related to the target disease (e.g.,

inhibiting colorectal adenomas). Initially, the criteria for selecting surrogate end-points support drugs with clinical benefit directly related to cancer incidence prevention. As understanding improves of the role of general genotypic and specific molecular changes in carcinogenesis, and with careful correlative studies, effects on surrogate end-points with antecedent impact on clinical outcome may also support chemopreventive drug efficacy.

There are several conditions in which treatment of precancerous lesions would appear to provide direct clinical benefit, irrespective of the potential for cancer prevention. These situations typically involve a change in standard of care based on regression or prevention of precancerous lesions that would engender reduced morbidity, enhanced quality of life, delayed surgery or reduced surveillance frequency. Subjects with genetic predisposition to cancer development (e.g., FAP) may achieve such benefits from chemopreventive interventions. FAP is characterized by germline mutations in the *APC* tumour-suppressor gene. Patients with FAP develop hundreds to thousands of colorectal adenomatous polyps beginning in their teen years, and in the absence of treatment will almost certainly develop colorectal cancer by the age of 50 years; they are also at risk for other lesions, particularly duodenal polyps and cancers, and desmoid tumours. Once adenomas begin to appear, these patients are monitored by periodic colonoscopy (at approximately six-month intervals), removal of existing polyps and cancer screening. When polyp burden becomes unmanageable, most patients have partial or total colectomies and undergo continued monitoring thereafter. Agents which prevent or slow the progression of the adenomas could benefit these patients by delaying or obviating the need for colectomy. A decrease in the frequency of surveillance colonoscopies and cancer screenings would also benefit patients with FAP, as it could those with sporadic colorectal adenomas. New adenomas occur within 1–3 years post-resection in approximately 30% of patients with sporadic colorectal adenomas or cancers (Hamilton, 1996). These patients routinely undergo colonoscopy with removal of new lesions at 1–5-year intervals. Preventive treatment could potentially increase the screening interval, thereby decreasing associated morbidity and lowering health care costs.

Other conditions in which organ removal or other major surgery with high morbidity is standard include Barrett's oesophagus and superficial bladder cancers. Barrett's oesophagus, a precursor of oesophageal cancer, is currently managed by endoscopy with biopsy of metaplastic and dysplastic lesions; severe dysplasia may mandate partial or total oesophagectomy (Roth *et al.*, 1997). Because of the high rate of their recurrence and potential for progression, treatment for superficial bladder cancers includes periodic surveillance (every three months) and removal of new lesions, and may include cystectomy (Linehan *et al.*, 1997). In both diseases, treatment has profound detrimental effects on quality of life. Both are examples of situations in which preventive agents could provide clinical benefit by reducing the frequency of surveillance and the need for surgery.

Quality of life

Chemopreventive drugs may ultimately be given to asymptomatic populations for years or decades. Therefore, minimal toxicity is essential. Determining standards in terms of allowable type and frequency of side-effects and impact on quality of life will be critical issues as chemopreventive drugs are introduced. It is possible that life-threatening toxicities compromising such long-term drug use would not be detected within the time-frame of surrogate end-point-based efficacy trials. In the meta-analysis of cholesterol-lowering interventions cited below, the investigators found that despite their cholesterol-lowering efficacy, fibrates such as gemfibrozil were associated with increases in non-coronary heart disease mortality by ~30% ($p<0.01$) and total mortality by ~17% ($p<0.01$) on long-term administration (Gould *et al.*, 1995). A different, but dramatic, example of unanticipated late toxicity is provided by the results of the Cardiac Arrhythmia Suppression Trial (Fleming & DeMets, 1996). This randomized, placebo-controlled trial of three type 1C antiarrhythmic drugs was designed to evaluate mortality reduction in patients experiencing ten ventricular premature beats per hour and few or no symptoms following a recent myocardial infarction. Entry into the trial required that the patients respond to antiarrhythmic therapy as measured by at least 70% reduction in ventricular premature beats as a surrogate for arrhythmia. This trial was stopped when it was found that drug treatment

was associated with increased mortality or cardiac arrest despite lowering ventricular premature beats (Echt *et al.*, 1991; Cardiac Arrhythmia Suppression Trial II Investigators, 1992).

Cardiovascular disease prevention: precedent for application of surrogate end-point biomarkers in drug development

Cancer chemoprevention shares the interest and need for surrogate end-points in drug development with other chronic diseases of ageing and life-threatening diseases. To date, the best characterized surrogate end-points in drug development have been for AIDS (Mellors *et al.*, 1996; Saag *et al.*, 1996) and cardiovascular drugs (Fleming & DeMets, 1996). In particular, the use of blood lipid-lowering as a surrogate end-point for cardiovascular disease provides a model for and insight into the issues surrounding the use of surrogates for cancer incidence in chemoprevention studies. In terms of the long time required for disease development, the multiple paths by which the disease progresses and the chronic administration of preventive drugs, the course of cardiovascular disease closely parallels carcinogenesis. In the cardiovascular setting, a well established surrogate end-point is cholesterol level, which is a validated predictor of coronary heart disease (Expert Panel on Detection, Evaluation and Treatment of High Blood Cholesterol in Adults, 1993). Modulation of cholesterol levels has been used to gain marketing approval for 3-hydroxy-3-methylglutaryl coenzyme A (HMGCoA) reductase inhibitors such as lovastatin (Sahni *et al.*, 1991; Fail *et al.*, 1992), simvastatin, pravastatin (Crouse *et al.*, 1992; Pitt *et al.*, 1993) and gemfibrozil (Frick *et al.*, 1987). HMGCoA reductase catalyses a critical step in cholesterol biosynthesis, the formation of mevalonate. Gould *et al.* (1995) carried out a meta-analysis of 35 randomized clinical trials that essentially summarized the evidence supporting cholesterol-lowering as a surrogate end-point for coronary heart disease. This review of all primary or secondary intervention studies of >2 years' duration included single-drug studies such as the Helsinki Heart Study of gemfibrozil (Frick *et al.*, 1987), as well as dietary (Dayton *et al.*, 1968; Burr *et al.*, 1989), surgical (Buchwald *et al.*, 1990) and multifactorial interventions (Miettinen *et al.*, 1982; Wilhelmsen *et al.*, 1986). The results show that cholesterol-lowering is correlated with coronary heart disease, non-coronary heart disease and overall mortality. Specifically, it was found that for every 10% lowering of cholesterol, coronary heart disease mortality was reduced by 13% ($p < 0.002$) and total mortality by 10% ($p < 0.03$), while no effect was found on non-coronary heart disease mortality. A caveat applies here, as to all studies with biomarkers — the relationship between lower coronary heart disease and lower cholesterol is derived from the average of individual responses, and the same correlation is not seen in each individual. The presence of confounding factors (e.g., smoking history and diabetes mellitus) may influence the proportion of disease attributable to any specific parameter in a multifactorial disease process.

Chemoprevention of colorectal adenomas

The data supporting validation of cholesterol levels as a surrogate end-point for coronary heart disease include an association with disease risk, in addition to the ability to predict activity of a given drug against that disease (Kelloff *et al.*, 2000). Analogous data might be applied to support the validation of a surrogate for cancer incidence. For example, it is well established that the presence of colorectal adenomas increases colorectal cancer risk (Hamilton, 1992; Winawer *et al.*, 1993) and that adenoma number, size and severity of dysplasia are predictive factors for cancer incidence. It has been estimated that 2–5% of all colorectal adenomas progress to adenocarcinomas if not removed or treated, with increasing rates for large and severely dysplastic polyps (Day & Morson, 1978; Hamilton, 1992; Bruzzi *et al.*, 1995). Cancer risk is reduced by polyp removal, and a strong correlation exists between the relative prevalence of adenomas and cancers across populations (Winawar, 1993). More than 20 epidemiological and intervention studies have demonstrated that regular NSAID use is associated with reduced adenoma incidence and that this decrease is correlated with declines in both cancer incidence and mortality (Greenberg & Baron, 1996). These data support the validation of adenomas as a surrogate end-point for colon cancer incidence.

A recently conducted clinical trial sponsored by the US National Cancer Institute and G.D. Searle examined the effect of the COX-2 inhibitor celecoxib at two doses against colorectal polyps in

subjects with FAP). Overexpression of prostaglandins and COX isoenzymes is observed in colorectal polyps and tumours from animals and humans with germline *APC* gene mutations. Early clinical evidence of polyp regression with the NSAID sulindac has been demonstrated in FAP patients. Additional support for the trial has come from preclinical efficacy studies with celecoxib, and substantial epidemiological evidence of a protective effect of NSAIDs against colorectal carcinogenesis. Preclinical and clinical studies demonstrating reduced gastro-intestinal toxicity of celecoxib compared with traditional NSAIDs support the use of a COX-2-specific inhibitor in a chemopreventive setting. In this randomized, double-blind, placebo-controlled study of 83 FAP patients, a six-month intervention with 800 mg celecoxib per day significantly reduced polyp number by 28%, with 53% of treated subjects showing a 25% or greater reduction. A blinded physicians' assessment indicated a qualitative improvement in the colon and rectum, and to a lesser extent in the duodenum, of treated subjects. This trial led to accelerated marketing approval of celecoxib by the US Food and Drug Administration, as an adjunct to standard care for the regression and reduction of adenomatous polyps in FAP subjects. Although it can be inferred from data supporting the correlation of polyp burden with colon cancer incidence, it remains to be demonstrated in a randomized, placebo-controlled clinical study that a reduction in cancer incidence will be engendered by a drug which prevents polyps. Nonetheless, this study was a landmark in chemoprevention research with surrogate end-points, demonstrating that polyp burden can serve as an appropriate end-point for quantitative and qualitative assessments of chemopreventive efficacy in FAP patients. Follow-up studies are planned to assess the relative effect of celecoxib on polyp regression and prevention, and to determine whether greater efficacy can be engendered by combination therapy of celecoxib with the antiproliferative agent 2-difluoromethylornithine.

Summary and perspectives on the use of surrogate end-points in gaining marketing approval for chemopreventive agents

The critical scientific aspects of developing surrogate end-points to characterize cancer chemopreventive efficacy should be applied to the design of clinical development strategies to gain marketing approval for chemopreventive drugs. The multi-path, multifocal and multi-year course of carcinogenesis suggests that, initially, the most successful strategies will use well defined precancers (intraepithelial neoplasia) as surrogate end-points for cancer incidence. Despite their close temporal and histological association with cancers, only a relatively small percentage of intraepithelial neoplastic lesions progress. Therefore, determination of chemopreventive efficacy will rely on assurance that the lesions most likely to progress are inhibited; the genotype of any post-treatment lesions should be indicative of an equivalent or lower progression potential than baseline lesions. The phenotypic changes seen in intraepithelial neoplasia during short-term studies are likely to be subtle, so that quantitative measurements such as computer-assisted image analysis are desirable. Similarly, the evaluation of genotypic changes requires sensitive, quantitative analysis of gene expression such as is afforded by the various DNA microarray techniques. Standardization to provide adequate sampling and handling of non-related biopsy effects (e.g., timing of breast cell proliferation assessment during the menstrual cycle) will be essential. The gold standard for validating surrogate end-points is correlation with cancer incidence reduction. However, the resources (e.g., time and number of subjects) required for this definitive validation are enormous. Continued discussion and research on alternative strategies among all interested parties are needed to ensure that surrogate end-point-based chemoprevention indications are feasible. Demonstration of the clinical benefit of prevention of intraepithelial neoplasia as described above for FAP, sporadic colorectal adenomas, superficial bladder cancers and Barrett's oesophagus is one possible strategy. A second approach would follow an accelerated pathway for gaining marketing approval, as defined in the United States Food & Drug Administration regulations based on strongly-supported surrogate end-points for disease incidence in the setting of life-threatening disease such as cancer.

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