Recent Results in Cancer Research

H.-J. Senn R. Morant (Eds.)

Tumor Prevention and Genetics III

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Tumor Prevention and Genetics III

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Professor Dr. Hans-Jörg Senn
Dr. Rudolf Morant
Zentrum für Tumordiagnostik und Prävention
Rorschacherstrasse 150
9006 St. Gallen, Switzerland

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Lifestyle and Medical Approaches to Cancer Prevention

Peter Greenwald

Division of Cancer Prevention, National Cancer Institute, National Institutes of Health, 6130 Executive Boulevard, Suite 2040, Bethesda, MD 20892-7309, USA
pg37g@nih.gov

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Abstract Cancer risk can be reduced by adopting a healthy lifestyle and by medical means. Tobacco control is central to public policies for cancer prevention. Overweight and obesity in the United States may contribute to 20% of cancer deaths in women and 14% in men. Cancer prevention strategies have progressed from a predominant lifestyle approach to a model that combines clinical investigations in a medical setting with public health interventions. This change stems from advances in identifying, developing, and testing agents with the potential either to prevent cancer initiation, or to inhibit or reverse the progression of premalignant lesions to invasive cancer. Encouraging laboratory and epidemiologic studies, along with secondary endpoints in treatment trials, have provided a strong scientific rationale for the hypothesis that a pharmacologic approach—chemoprevention—can reduce cancer risk. Numerous chemopreventive agents, including naturally occurring vitamins, minerals, phytochemicals, and synthetic compounds, have proved to be safe and effective in preclinical and clinical studies. Promising results have been reported for cancers of the prostate, breast, colon, lung, bladder, cervix, oral cavity, esophagus, skin, and liver. The use of emerging technologies, identification of biomarkers of risk, and advances in genetic research are being applied to chemoprevention research. An interdisciplinary research approach to investigate molecular and genetic markers—as well as chemoprevention and lifestyle strategies—that affect cancer risk is being applied to the most common types of cancer in the United States in women (breast) and men (prostate).

1 Introduction

Identification of cancer risk factors and potential prevention strategies have been some of the most important medical and research contributions to the improvement of public health in the past half-century (Steele 2003). Understanding the role of lifestyle, exposure to endogenous factors and exogenous environmental factors, and individual genetic and epigenetic variability have contributed significantly to this effort. Cancer prevention strategies have been developed based on results of epidemiologic, preclinical, and clinical studies that have generated clues for identifying risk factors that may be modulated by changes in lifestyle, such as smoking cessation or dietary modification (Greenwald 2002a). In addition, significant progress in medical interventions involving chemoprevention—a pharmacological approach to intervention that aims to prevent, arrest, or reverse either the initiation phase of carcinogenesis or the progression of premalignant cells—is beginning to pay dividends in reducing risks associated with cancer. Emerging technologies, identification of biomarkers of risk, and advances in genetics research also are finding applications in chemoprevention research that promise to speed the acquisition of knowledge on the molecular and cellular effects of chemopreventive agents.

2 Lifestyle Approaches

Population studies from the 1950s through the early 1980s provided compelling evidence that modifiable lifestyle choices can either increase or decrease cancer risk. For example, several landmark epidemiologic studies in the 1950s showed a clear association between smoking and lung cancer (Wynder and Graham 1950; Levin et al. 1950). In 1964, the U.S. Surgeon General's Report on Smoking and Lung Cancer was published, providing a comprehensive review of the evidence that established a strong link between smoking and lung cancer (U.S. Department of Health and Human Services 1964). A historically important contribution to cancer prevention research was made in 1981, when Doll and Peto published one of the first meta-analyses estimating the contribution of risk factors for cancer (Doll and Peto 1981). In their wide-ranging analysis, they estimated that 30% of cancer deaths were related to smoking, roughly 35% to diet, and possibly 35% to other causes (viruses, hormones, radiation, industrial carcinogens, etc.). This analysis, combined with the earlier studies on tobacco use, provided a stimulus to the cancer research community that lifestyle intervention research was a promising avenue for identifying approaches to prevent cancer and to reduce disease burden on society.

The focus of early efforts to reduce cancer risk was on carcinogens in the workplace and worker exposure, which led to systematic surveillance and a regulatory approach to limit industrial exposures (Higginson 1988). By the 1960s, based on the accumulating experimental and epidemiologic data, cancer prevention added a focus on smoking tobacco, diet, and other lifestyle choices, such as physical inactivity, that appeared to increase cancer risk. One of the first large intervention research efforts regarding lifestyle factors was on smoking and included increased surveillance efforts, development of behavioral interventions, and a continued effort of epidemiologic and clinical studies to better define the scope and health consequences of tobacco use.

Tobacco prevention and control efforts are central to public policies for cancer prevention because of the strong association between tobacco use and lung cancer, which accounts for 13% of cancer deaths in the world (18% for men; 7% for women) (GLOBOCAN 2000). Tobacco use also substantially increases the risk for cancers of the larynx, oral cavity, and esophagus and contributes to risk for cancers of the pancreas, uterine cervix, kidney, and bladder (Thun et al. 2002). In the United States and other developed countries, approximately one-fourth of adults smoke; in developing countries, tobacco use is rising and poses a significant public health challenge (Pandey et al. 1999). In the United States, smoking prevalence has declined slowly from highs in the 1960s. However, for some groups, such as African Americans and young people, there has been less progress in reducing the number of smokers (U.S. Department of Health and Human Services 1998). Interventions to reduce tobacco use encompass a wide range of strategies. Early efforts at the U.S. National Cancer Institute (NCI) stressed behavioral interventions, such as the Community Intervention Trial for Smoking Cessation (COMMIT), a large, community-based smoking control project that sought to translate knowledge about tobacco control interventions to public health applications (COMMIT 1995). The COMMIT intervention was proved effective in decreasing the prevalence of cigarette use among light-to-moderate smokers, although there was no overall reduction in smoking prevalence. A second large, state-based, community smoking control project, the American Stop Smoking Intervention Study for Cancer Prevention (ASSIST), was conducted in 17 states; its goal was to change the states' social, cultural, economic, and environmental factors that influence smoking behavior (Manley et al. 1997a). Results from ASSIST indicated that this multifaceted intervention model was responsible for a 7% decline in tobacco use in ASSIST states compared with non-ASSIST states (Manley et al. 1997b). A recent evaluation of the ASSIST program found that states with stronger tobacco control policies and greater ability to implement tobacco control programs had larger reductions in smoking (Stillman et al. 2003).

Overweight and obesity have long been associated with significant risk for cardiovascular disease mortality; the magnitude of the significance for

cancer mortality, however, has not been well quantified (Calle et al. 1999). A prospective cohort study of more than one million adults in the United States assessed cardiovascular disease mortality and body mass index (BMI), and found a significantly increased risk of death among men at a BMI exceeding 26.5 and women at a BMI greater than 25.0 (Calle et al. 1999). Among men, the relative risk at the highest level of BMI was 2.90. In a subsequent study, the authors assessed cancer mortality in the same cohort and found that the effect of overweight and obesity may contribute to 20% of cancer deaths in women and 14% in men (Calle et al. 2003). For specific cancer sites, there was a linear trend of increasing mortality from lower BMI to higher BMI for cancers of the stomach [relative risk (RR)=1.9] and prostate (RR=1.3) in men, and cancers of the breast (RR=2.1), uterus (RR=6.3), cervix (RR=7.8), and ovary (RR=1.5) in women (Calle et al. 2003). According to this analysis, more than 90,000 cancer deaths in the United States each year could be prevented if men and women maintained normal weight. Development of effective interventions to reduce the prevalence of overweight and obesity is essential. Recent research in experimental carcinogenesis models indicates that a regimen of caloric restriction (usually 20%–40% relative to ad libitum controls), which reduces obesity, may be one of the best broad-based interventions to reduce cancer risk (Hursting et al. 2003), although little consistent data exist in humans. Caloric restriction has a beneficial impact on mechanisms regulated by insulin-like growth factor (IGF)-1, including cell proliferation, apoptosis, and cell cycle regulation. To illustrate, caloric restriction increases the rate of apoptosis by reducing the DNA synthesis needed to increase the number and volume of preneoplastic lesions (Hursting et al. 2003). Achieving a greater understanding of the relationship between obesity and increased cancer risk will require a concerted effort using an interdisciplinary approach of basic and clinical research.

3 Medical Approaches

As epidemiologic studies and lifestyle intervention research continued in the 1980s, an earnest effort began in the medical community to design and conduct preclinical and clinical studies to better understand the biological basis of the carcinogenic process and how to influence cancer risk using a medical approach. The medical approach to cancer prevention focused on identifying, developing, and testing chemoprevention agents with the potential either to prevent cancer initiation, or to inhibit or reverse the progression of premalignant lesions to invasive cancer. Laboratory and epidemiologic studies provided the scientific rationale for investigating such agents (Greenwald et al. 1990). For example, epidemiologic studies supported an inverse relationship between the intake of vegetables and fruits and cancer risk, and

clinical studies identified possible phytochemical components of these foods—as well as interactions among the components—that might contribute to their ability to reduce cancer risk (Negri et al. 1991; Chemoprevention Working Group 1999). Animal and in vitro studies have identified hundreds of phytochemicals and micronutrients with potential chemopreventive effects. To illustrate, diallyl sulfide, a phytochemical found in *Allium*

Table 1. Selected NCI-sponsored phase I, II, and III cancer prevention trials in progress

Cancer site	Phase I	Phase II	Phase III
Breast	l-Perillyl alcohol SERM-3 (2 trials) Soy isoflavones	DFMO (2 trials) Exemestane l-Perillyl alcohol SERM-3 (2 trials) Tamoxifen (2 trials) Tamoxifen/4-HPR (2 trials) Targretin	Raloxifene/ tamoxifen
Colon	Curcumin Ursodiol	Celecoxib (2 trials) Celecoxib/DFMO DFMO/Sulindac Folic acid Vitamin D/calcium	Celecoxib
Lung	PEITC l-Selenomethionine/ vitamin E	Anetholetrithione Budesonide	
Prostate	Lycopene (3 trials) Soy isoflavones	Celecoxib DFMO DFMO/Casodex Flutamide Flutamide/leuprolide Flutamide/toremifene 4-HPR Selenized yeast Soy (dietary) Soy isoflavones Vitamin D analog	Finasteride Selenomethionine Selenium/vitamin E
Cervix		9-*cis*-Retinoic acid DFMO 4-HPR	
Ovary		4-HPR/oral contraceptive	
Endometrium		Medroxyprogesterone vs Depo-Provera	
Anogenital warts+HPV/HIV		Indole-3-carbinol	
Bladder		Celecoxib	DFMO 4-HPR
Skin	EGCG/ polyphenon E	Celecoxib Polyphenon E (green tea extract) Sulindac	DFMO 4-HPR

4-HPR, *N*-(4-hydroxyphenyl)retinamide (fenretinide); DFMO, difluoromethylornithine; EGCG, epigallocatechin gallate; PEITC, phenethyl isothiocyanate; SERM, selective estrogen receptor modulator.

vegetables such as garlic and onion, suppresses cell division in human colon tumor cells by interfering with the cell cycle (Huang et al. 1994). Using this type of knowledge in a medical approach to interventions for cancer prevention remains a significant challenge, but much progress has been achieved in recent decades.

The intensive chemoprevention effort at the NCI includes a stepwise process of clinical investigations to determine agent safety and pharmacokinetic profiles (phase I), identify intermediate-endpoint biomarkers that can serve as surrogate endpoints for clinical disease (phase II), and conduct large-scale, randomized, controlled trials (phase III) (Greenwald et al. 1995). In the past two decades, the NCI has evaluated more than 140 potential chemopreventive agents, including antiestrogens, antiinflammatories, antioxidants, arachidonic acid metabolism inhibitors, glutathione S-transferase (GST) and glutathione (GSH) enhancers, ornithine decarboxylase (ODC) inhibitors, protein kinase C inhibitors, retinoids, carotenoids, organosulfur compounds, calcium compounds, vitamin D_3 and analogs, and phenolic compounds (Greenwald 2001). These agents were assayed for efficacy in animal models representative of high-incidence human cancers, and approximately 90 were found to reduce tumor activity, incidence, or multiplicity (Steele et al. 1994). The most promising agents—including naturally occurring vitamins, minerals, phytochemicals, and synthetic compounds—have been selected for clinical investigations. To date, promising results have been reported for cancers of the prostate, breast, colon, lung, bladder, cervix, oral cavity, esophagus, skin, and liver, and the NCI is sponsoring approximately 65 of these in phase I, II, or III clinical trials (Greenwald 2001, 2002b). Table 1 lists selected chemoprevention agents being studied in NCI-sponsored clinical trials.

4 Integrating Lifestyle and Medical Approaches

Translating research results for the public remains a significant challenge as cancer prevention strategies progress from a predominant lifestyle approach to a model that combines clinical investigations in a medical setting with public health interventions (Greenwald 2002b). This developing paradigm (see Box 1) at the NCI is described within the context of the "seamless three-D" approach to cancer research: discovery, development, and delivery (von Eschenbach 2003). The focus of the three-D approach will be on creating a research environment to integrate disparate research communities in an effort to eliminate real and perceived bottlenecks that can slow down progress against cancer. Cancer prevention is integral to the strategic initiatives planned for this approach. For example, an interdisciplinary research approach to investigate molecular and genetic markers, as well as chemoprevention and lifestyle strategies that affect cancer risk, is being applied to the

most common types of cancer in the United States in women (breast) and men (prostate).

5 Box 1. The NCI "Seamless Three-D" Approach

- Discovery
  The process that generates new knowledge about fundamental cancer-related processes at the genetic, molecular, cellular, organ, person, and population levels.
- Development
  The process of creating and evaluating tools and interventions to reduce cancer burden including the prevention, detection, diagnosis, and treatment of cancer and its sequelae.
- Delivery
  The process of disseminating, facilitating and promoting evidence-based prevention, detection, diagnosis, and treatment practices and policies to reduce the burden of cancer in all segments of the population. Focus of these efforts will be on populations who bear the greatest burden of disease.

6 Breast Cancer

Most risk factors for breast cancer, other than genetic risk factors, are associated with exposure to estrogens. Early prevention efforts focused on selective estrogen receptor modulators (SERMS), such as tamoxifen, to reduce breast cancer risk (Anthony et al. 2001). These efforts were somewhat successful in women with diagnosed breast cancer, through reduction of cancer recurrence in the original breast and subsequent occurrence in the contralateral breast. Analyses of population and clinical studies have shown that recurrence and survival differences may be related to breast tumor characteristics, such as hormone receptor status (Li et al. 2002). Tamoxifen reduced breast cancer risk approximately 50% for some women at high risk, but increased the risk of uterine cancer, bone loss, and menstrual abnormalities (Fisher et al. 1998). Second- and third-generation SERMS have been developed that may improve the efficacy and safety of this promising class of agents. For example, the third-generation SERM arzoxifene was investigated in a phase II trial that indicated it may be effective in tamoxifen-sensitive and tamoxifen-refractory patients, without the high level of negative side effects seen in tamoxifen studies (Anthony et al. 2001; Buzdar et al. 2003).

New chemoprevention agents for breast cancer include aromatase inhibitors (AIs), a different class of agent from the SERMS, and food constituents. AIs are being investigated for their ability to lower peripheral and breast-tissue estrogen levels and have been shown to be equal or superior to tamoxifen in metastatic disease and in adjuvant studies (Anthony et al. 2001; Fabian 2001). Their mechanism of action may be the inhibition of cytochrome P450 in the final step of estrogen biosynthesis in peripheral tissues; they also have been shown to have a potential role in therapy for estrogen receptor-positive (ER^+) advanced breast cancer in postmenopausal women (Mokbel 2002). In addition, researchers are investigating other potential chemopreventive agents for their ability to prevent ER^+ tumors and estrogen receptor-negative (ER^-) tumors that are not responsive to SERMS or aromatase inhibitors. For example, tyrosine kinase inhibitors, retinoids, cyclo-oxygenase (COX)-2 inhibitors, farnesyl transferase inhibitors, and statins currently are being tested in NCI-sponsored phase I and II chemoprevention trials for breast cancer.

An important strategy for integrating lifestyle and medical approaches to cancer prevention is the identification of biomarkers of risk, which can help identify persons at high risk for disease and potentially identify those individuals who may respond to different chemopreventive agents, therapies, and lifestyle changes. For breast cancer, ER and progesterone sensitivity status are examples of biomarkers being used for these purposes. Other promising biomarkers include the human epidermal growth factor 2 (HER-2)/*neu* (c-*erb*B-2), *Ki67*, *p53*, IGF-1, and aberrant methylation and histone deacetylation of the promoter region of many tumor suppressor genes (Fabian and Kimler 2002; Krishnamurthy and Sneige 2002). Ross and colleagues (2003) recently reviewed 22 published studies on more than 4,000 patients and reported that circulating HER-2/*neu* antigen levels may be a predictive tumor marker for the presence and progression of HER-2/*neu*-positive breast cancer. Fabian and Kimler (2002) reviewed promising breast cancer risk biomarkers, including IGF-1 and IGF binding protein 3 (IGFBP-3) for premenopausal women; serum estradiol levels for postmenopausal women; and mammographic density, nipple aspirate fluid production, and breast intraepithelial neoplasia for premenopausal and postmenopausal women. New technologies and multidisciplinary approaches for screening and diagnosis are likely to identify other risk biomarkers that may allow identification of women at higher risk for breast cancer and those who may benefit from specific chemoprevention agents or other interventions. A vital component of an interdisciplinary approach to reduce breast cancer risk will be inclusion of lifestyle interventions, such as weight loss, increased physical activity, and reduction of alcohol intake, in clinical studies. Plausible biological mechanisms exist that suggest hormone-related breast cancer risk can be modulated by lifestyle components (McTiernan 2003).

7
Prostate Cancer

Prostate cancer represents another example of disease prevention by combining lifestyle and medical approaches. Epidemiologic and experimental studies have identified modifiable (diet, obesity, hormones, and screening history) and nonmodifiable (age, race, family history, and the presence of certain genetic polymorphisms) risk factors for prostate cancer (Brawley et al. 1998). Addressing prostate cancer prevention in a population-based clinical trial based on the hypothesis that chemoprevention strategies could modify risk factors, the Prostate Cancer Prevention Trial (PCPT) was conducted in a cohort of approximately 18,000 men 55 years of age or older with a prostate-specific antigen (PSA) below 3 ng/ml and a negative digital rectal examination (DRE) (Feigl et al. 1995). The PCPT compared finasteride with placebo; finasteride blocks the enzyme 5-alpha-reductase that is necessary to convert testosterone to dihydrotestosterone and thus blocks increased growth in the prostate. Administration of finasteride (5 mg/day for 7 years) resulted in a 25% reduction in the prevalence of prostate cancer (Thompson et al. 2003). Although tumors of Gleason grade 7–10 were more common in the finasteride group, the PCPT demonstrated that prostate cancer, at least in part, is preventable.

About the same time as the PCPT was confirming the value of chemoprevention for prostate cancer, three population-based clinical trials were reporting encouraging results on other agents that decreased the risk of prostate cancer. Secondary analysis of the Alpha-Tocopherol, Beta-Carotene Cancer Prevention Study (ATBC Study) indicated that men receiving vitamin E had a decrease in prostate cancer mortality (41%) and incidence (36%) (Heinonen et al. 1998); secondary analysis of the Health Professionals Follow-Up Study found that daily use of vitamin E (100 μg/day) decreased the risk of metastatic or fatal prostate cancer 44% compared with nonusers (Chan et al. 1999); and a secondary endpoint analyses from a multicenter, double-blind, randomized, placebo-controlled cancer prevention trial indicated that supplemental dietary selenium (200 μg/day) significantly reduced the risk of total cancer mortality (RR=0.50) (Clark et al. 1996), and prostate cancer incidence (RR=0.37) (Clark et al. 1998).

Based on these encouraging results, the NCI sponsored a randomized, prospective, double-blind study to determine whether a 7- to 12-year regime of daily supplementation of selenium and vitamin E will decrease the risk of prostate cancer in healthy men. This trial, the Selenium and Vitamin E Cancer Prevention Trial (SELECT), is a four-arm intervention trial comparing vitamin E alone (400 mg of racemic α-tocopherol), selenium alone (200 μg of 1-selenomethionine), combined vitamin E and selenium, and placebo; the trial design includes an optional multivitamin that does not contain selenium or vitamin E (Klein et al. 2001). Routine clinical evaluations will include

a yearly DRE and PSA test. SELECT is the largest prostate prevention trial ever conducted, and as of January 2004, approximately 90% of the targeted goal of 32,400 men had been enrolled. The primary endpoint is diagnosed prostate cancer; secondary endpoints will be the incidence of and survival from lung and colon cancer.

SELECT is an example of the application of all aspects of the "seamless three-D" approach to cancer prevention. The "discovery" and "development" process are addressed by an investigation of biomarker research and analysis that will be included in a nested case-control study within SELECT. This study will assess genetic polymorphisms of four genes—androgen receptor (*AR*), 5α-reductase type II (*SRD5A2*), cytochrome P450c 17α (*CYP17*), and β-hydroxysteroid dehydrogenase (*HSD3β2*)—on prostate cancer incidence (Hoque et al. 2001). These biomarkers of risk have been selected because of epidemiologic and experimental studies that suggest they may affect susceptibility to prostate cancer (Haiman et al. 2001). For example, polymorphisms in *CYP17* A1/A1 genotype may confer a significantly higher serum androgen level, which is associated with higher risk of prostate cancer, than is found in men with either the A1/A2 or A2/A2 genotype (Hoque et al. 2001). An additional benefit to this nested case-control study will be the possibility of identifying subpopulation groups, such as African Americans, within the SELECT participants that may have higher or lower risk associated with specific genetic polymorphisms.

SELECT also serves as an example of integration of the "delivery" component of the seamless three-D approach. The study is coordinated by the Southwest Oncology Group (SWOG), but includes more than 400 sites throughout the United States, Puerto Rico, and Canada. SWOG is one of many SELECT sites that belong to the NCI Community Clinical Oncology Program (CCOP), which is a creative mechanism designed not just to improve the accrual of patients to NCI phase III clinical trials and encourage community-based oncologists to participate in clinical research, but also is one of the most practical means to disseminate new information on state-of-the-art cancer treatment outside the traditional cancer centers and research-oriented medical centers (Kaluzny et al. 1989). By having local researchers and facilities participating in research translation efforts at the community level, patients and the public will benefit by having immediate access to the prevention and treatment strategies that are most relevant to their communities. For example, rates of prostate cancer are higher among African American men and in those in lower socioeconomic stratum, with each being an independent predictor of stage at diagnosis (Schwartz et al. 2003). Prevention and treatment strategies can be integrated and delivered in those CCOP communities that have been found to be effective for these specific groups.

8
New Technologies in Prevention Research

The use of new and emerging technologies in the past decade is responsible for much of the increase in the rate of discovery in chemoprevention research using medical approaches. Because carcinogenesis involves a myriad of molecular and cellular processes, progress in the future will depend on gaining a full understanding of the changes taking place in the cell and extracellular environment. Of particular interest for cancer prevention researchers are technologies that are being developed for the identification of biomarkers. For example, protein microarray technology is used in cancer biomarker discovery to capture posttranslational modifications. This method can be used with liquid phase protein separations of tumor lysates to allow direct identification of proteins for use for a variety of probes (Shin et al. 2003). Surface-enhanced laser desorption ionization (SELDI) allows minute amounts of patient sera to be applied to the surface of a protein-binding plate and analyzed by time-of-flight mass spectrometry (TOF MS) to create a molecular map of proteins from healthy tissue, precancerous tissue, and cancer (Yip and Lomas 2002). High-throughput technologies, such as the microarray and SELDI-TOF MS, allow tens of thousands of samples to be assayed in minutes rather than days or weeks. In addition, gene expression profiling of thousands of genes simultaneously has become possible with the introduction of serial analysis of gene expression (SAGE) technology (Ye et al. 2002). SAGE allows identification of expressed genes without first having to sequence the gene and hypothesize about which genes are thought to be expressed.

Not to be overlooked in the interdisciplinary approach that combines lifestyle and medical interventions is the need for development of technologies for computational and statistical analyses. As more cancer research depends on analysis of genetic and proteomic data, the integration of mathematical models with clinical results is crucial. Using microarray technology to identify gene expression of hundreds of thousands of genes can only be useful if researchers have access to computer programs and algorithms that make sense of the massive amounts of data produced through such efforts. One such effort is the collaboration between the NCI's Early Detection Research Network and the National Aeronautics and Space Administration's Jet Propulsion Laboratory. The collaborators hope to develop informatic architectures that (1) allow the sharing of results from the discovery process on biomarkers, (2) translate the results into clinical practice, and (3) enable data-sharing through development of database-sharing methods using the Web (Verma and Srivastava 2003).

9 Conclusions

Cancer prevention for the twenty-first century is creating a new paradigm based on incorporation of lifestyle and medical approaches developed in the past 50 years. This evolving approach will integrate knowledge and methods from many fields, and will be predominantly interdisciplinary in nature. Using clinical investigations in a medical approach with new methods of public health interventions is possible now by creating a research environment that considers all aspects of discovery, development, and delivery. Integrating lifestyle and medical approaches is the logical outcome of the progression of cancer prevention research.

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Application of Genetics to the Prevention of Colorectal Cancer

John L. Hopper

Centre for Genetic Epidemiology, Department of Public Health,
The University of Melbourne, 723 Swanston Street, Carlton, Victoria 3053, Australia
j.hopper@unimelb.edu.au

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Abstract A first-degree relative of an individual with colorectal cancer is on average at about a twofold increased risk. This could not occur without there being strong underlying risk factors that are correlated in relatives. About 90% of colorectal cases occur in people who are above median familial/genetic risk, so there is great potential to use genetics to prevent colorectal cancer. Two rare inherited syndromes have been identified: familial adenomatous polyposis (FAP) and hereditary non-polyposis colorectal cancer (HNPCC). The former appears to be mostly due to mutations in the *APC* gene, and the latter to mutations in mismatch repair (MMR) genes, so it would be better named as hereditary mismatch repair deficiency (HMRDS). By fully characterising a population-based series of early-onset cases, we have shown that MMR gene mutation carriers and their relatives can be more efficiently identified by characterising the tumours of early-onset cases, independently of their cancer family history, using immunohistochemistry (IHC)—not microsatellite instability (MSI) testing. This identifies the specific MMR gene likely to be involved, reducing the costs of mutation testing. Identification of genetically susceptible individuals using the tumour phenotype of affecteds, rather than family can-

cer history, could become the standard approach of cancer genetic services in the twenty-first century, and could lead to cancer prevention in individuals who are at a high genetic risk when young. There is an urgent need for research on the efficacy and optimisation of surveillance procedures in these high-risk individuals, and identification of the environmental, lifestyle and other genetic factors that exacerbate, or ameliorate, risk in mutation carriers.

1
Familial Aggregation of Colorectal Cancer

One of the best-established risk factors for colorectal cancer is having a family history of the disease. There is substantial confusion about what this means, what are its causes, and what can be made of it in terms of prevention of this important common disease. In the era of the Human Genome Project, the challenge is how to use genetic and molecular technologies to improve the health of individuals.

1.1
What Is Familial Aggregation?

It is important to differentiate between the clinical sense of 'familial clustering'—the existence of extended families with multiple-cases—and the epidemiological sense of 'familial aggregation', as recognised by on average an increased risk to close relatives of individuals with the disease compared to relatives of individuals without the disease.

Because colorectal cancer is a common disease, familial clusters of two or more cases would occur frequently even if there were no familial or genetic risk factors. For example, if an individual with colorectal cancer has two parents and four grandparents who have each lived their three score and ten years, then if their risks were not correlated, and based on a lifetime risk of 5%, the probability that at least one of these ancestors would have contracted colorectal cancer is greater than 1 in 4. If one takes into consideration another half dozen or so aunts and uncles, this probability is getting close to one-half. That is, a large proportion of cases with a 'family history' will not have any familial cause, let alone be due to inheritance of a high-risk gene mutation. Furthermore, so-called 'sporadic' cases (individuals with colorectal cancer but no known family history) can have an inherited high risk gene mutation—either due to having a de novo germline mutation or as a consequence of the less than perfect correlation between mutation carrier and disease, even in old relatives (referred to in the genetics literature as 'less than complete penetrance'). There is also a tendency for the scientific and clinical literature to blur the distinction between 'familial' and 'hereditary' cancer.

1.2
How Strong Is Familial Aggregation of Colorectal Cancer?

Familial aggregation of colorectal cancer on a population basis has been studied extensively. A meta-analysis (Johns and Houlston 2001) estimated that the increased risk associated with having an affected first-degree relative is about on average twofold. The magnitude of this increased risk is greater (1) the younger the age at diagnosis of the affected relative, (2) the younger the age of the unaffected individual at familial risk, (3) the closer the genetic relationship between individuals, and (4) the number of other affected blood relatives.

1.3
Why Is Familial Aggregation of Colorectal Cancer Important?

This seemingly modest risk could not occur without there being strong underlying risk factors that are correlated in relatives (Peto 1980; Hopper and Carlin 1992). If these 'familial' risk factors are genetic (correlation between first-degree relatives=0.5), mathematical models have shown that individuals in the top 25% of familial/genetic risk are at least 20 times more likely to de-

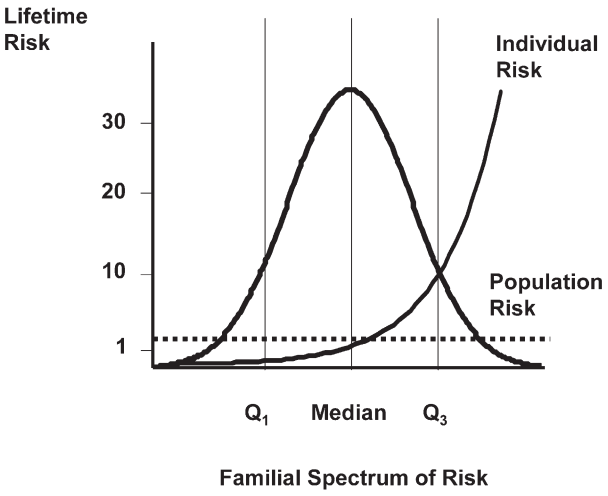

Fig. 1. Schematic representation of an underlying, normally distributed, familial spectrum of risk across which individual risk is assumed to increase exponentially. In order to explain a twofold increased risk associated with having an affected first-degree relative, if the familial risk has a correlation of 0.5 in first-degree relatives (e.g. as it would if it represented polygenic effects) the lifetime risk averaged over individuals in the upper quartile (i.e. to the right of Q_3) would need to be at least 20 times the risk averaged over individuals in the lower quartile (i.e. to the left of Q_1). The risk for individuals at the median of the familial spectrum of risk (*median*) lies below the population risk

velop colorectal cancer than those in the lower 25%. Consequently, and extrapolating from breast cancer (Pharaoah et al. 2002), it could be anticipated that 90% of colorectal cases will occur in people in the upper half of the wide distribution of familial/genetic risk. Figure 1 illustrates these points.

Uncovering all the sources of familial aggregation will be a major step in understanding the causes of the disease. Therefore, there is great potential to use genetics to prevent colorectal cancer, provided individuals who are at higher, if not highest, familial/genetic risk can be identified. We shall see below that the classic approach of using cancer family history may no longer be the way forward.

1.4 How Much Familial Aggregation, on a Population-Basis, Is Explained by Environmental or Lifestyle Risk Factors?

Epidemiological studies using questions that can be reasonably easily answered have identified some factors associated with an increased risk of colorectal cancer, such as body mass index (BMI), exercise and some aspects of diet, although the strengths of association with any one measured factor are generally rather modest. Some of these factors may themselves be familial, in that relatives are more alike than unrelated individuals of the same age. However, the strength of their familial aggregation is also modest; e.g., the correlation (r) between first-degree relatives is typically less than 0.4. Their effect on disease risk is not strong, and in theory these 'epidemiologically measured' risk factors each result in an odds ratio (OR) for disease in relatives of about 1.01 (Hopper and Carlin 1992). Under this theoretical model, the effects of multiple independent factors appears to be approximately additive, so that five such risk factors would give at most a 1.05-fold increased risk of disease and therefore explain only a small proportion of the average twofold familial risk of disease in first-degree relatives (see Sect. 3).

This should not be misinterpreted as proving that environmental or lifestyle risk factors explain little familial aggregation. There may be strong underlying risk factors for colorectal cancer, such as specific aspects of diet and particular hormonal factors, and the questionnaires used in epidemiological studies are only recording convenient surrogate measures. Measurement error (i.e. the random variation from asking people to categorise broadly their life experiences) results in attenuation of both the strength of that risk factor and of its correlation between relatives. These effects compound to greatly attenuate the proportion of familial aggregation attributable to an imprecisely measured familial risk factor (Hopper and Carlin 1992). Arguments about partitioning the genetic and environmental causes of diseases are becoming historic; the challenge is to measure these risk factors better.

2
What Are the Known Major Genetic Causes of Colorectal Cancer?

To date, two rare familial syndromes have been identified—familial adenomatous polyposis (FAP) and hereditary non-polyposis colorectal cancer (HNPCC). The majority of cases fulfilling these familial definitions have deleterious mutations in recently identified genes. There is confusion, however, because the words describing the familial syndromes, defined in terms of phenotypic characteristics of sets of relatives, are often interchangeably used with these underlying genetic syndromes. This is particularly problematic when it comes to HNPCC. As will be seen below, there is a case for the underlying (often implicitly assumed) genetic syndrome which describes the specific autosomal-dominant condition which predisposes to cancer caused by mutations in mismatch repair (MMR) genes being referred to as hereditary MMR deficiency syndrome (HMRDS) (Jass 1998). The expression 'HNPCC' should be taken out of the literature.

FAP and HNPCC/HMRDS explain only a fraction of the broad spectrum of familial risk across the population. For example, a population-based study of familial risks for early-onset colorectal cancer found that the average increased risk to first-degree relatives of cases with early-onset colorectal cancer was reduced from 3.2-fold to 2.7-fold after removing known 'HNPCC' cases (Jenkins et al. 2002). Much remains to be learnt about the familial and genetic causes of colorectal cancer.

2.1
Familial Adenomatous Polyposis

FAP is a rare familial syndrome. The phenotype of multiple adenomatous polyps at young age is implicated in a very high risk of early-onset colorectal cancer. Mutations in the adenomatous polyposis coli (*APC*) gene appear to be the cause of a high proportion of—but not all—clinically defined cases. There is a high prevalence of de novo mutations (i.e. 'sporadic' cases exist).

2.1.1
Application to Prevention of Colorectal Cancer

Testing for *APC* mutations in individuals clinically diagnosed with FAP, and their relatives, has had a profound impact on the prevention of colorectal cancer in individuals with an inherited high predisposition. The typical process involves symptomatic identification of individuals with multiple polyps, especially at a young age, or following investigations after a diagnosis of very early-onset colorectal cancer, followed by mutation testing for the *APC* gene and cascade testing in the relatives of identified mutation carriers. Identified mutation carriers are offered intensive surveillance, prophylactic

surgery, and/or chemoprevention. This has led to savings in mortality and unnecessary screening in non-carriers in these families. Note that identification of individuals with an inherited predisposition to FAP has historically been based on phenotype, not family history.

2.2 Hereditary Non-polyposis Colorectal Cancer

The expression 'hereditary non-polyposis colorectal cancer' (HNPCC) has been used extensively in the literature in multiple and confusing ways. Taken literally, it would appear to refer to colorectal cancer not caused by a pathway involving multiple polyps (as in FAP), occurring in individuals with a high genetic predisposition (i.e. hereditary).

In general practice, it is widely used to refer to either: (1) a specific familial syndrome (also known as Lynch II syndrome) based on the pattern and type of cancers occurring in families; (2) early-onset colorectal cancers in individuals with a strong family history of colorectal cancer, and/or other cancers, that exhibit microsatellite instability; and (3) colorectal cancer, and sometimes other cancers, occurring in individuals with a deleterious mutation in a DNA MMR gene. Furthermore, the adjective 'HNPCC' is used to describe families, colorectal cancers and even non-colorectal cancers (often referred to as 'extra-colonic cancers').

This historic confusion has arisen through: (1) clinical observation over a number of years that led to the identification of a familial syndrome of colorectal and other cancers; (2) linkage studies that eventually led to the identification of a family of DNA MMR genes, mutations which appear to predispose to cancers in the families identified by (1); and (3) tumour characteristics (see below) that were almost always evident in affected individuals with a germline mutation in a MMR gene.

The definition of this syndrome has changed in time. A meeting in 1990 of the International Collaborative Group on Hereditary Nonpolyposis Colorectal Cancer (ICG-HNPCC) in Amsterdam described, for research purposes, an HNPCC family as having: at least three relatives diagnosed with colorectal cancer, at least one affected member being a first-degree relative of at least two other affected relatives, at least two affected relatives in successive generations, and at least one affected member diagnosed before age 50 years (Vasen et al. 1991). These were referred to as the Amsterdam criteria. They were later modified by various groups, mostly for clinical reasons. In particular, the Amsterdam II criteria extend the age at diagnosis threshold to 55 years, allow for two or more affecteds in very small families, and include 'extra-colonic' cancers such as those of the endometrium, small bowel, ureter and renal pelvis (Vasen et al. 1999). This criterion has recently been readdressed (Umar et al. 2004)

2.2.1 DNA Mismatch Repair Genes

The connection between the familial HNPCC syndrome and the genes involved with DNA MMR arose from the observation that the majority of tumours from affected individuals in families meeting the Amsterdam criteria exhibit a replication error phenotype (Mitchell et al. 2002). This feature results from instability of microsatellite repeats during replication or microsatellite instability (MSI) (see Sect. 11). It is found in only a minority of colorectal cancers in individuals with no known family history (who are often referred to as 'sporadic' cases). Previous molecular studies in the yeast *Saccharomyces cerevisiae* had led to the identification of a group of genes, known as DNA MMR genes, which were involved in maintaining the fidelity of DNA replication. Defects in yeast MMR genes led to MSI, prompting formulation of the hypothesis that human homologues of these genes were involved in the HNPCC syndrome. Subsequently, several such homologues were identified, and two of them (*hMLH1* and *hMSH2*) were shown to reside on chromosomes 3p-21–23 and 2p21. Further supportive evidence came from the observation that pathogenic mutations in *hMLH1* or *hMSH2* could be identified and shown to segregate with the disease in a high proportion of kindreds that had shown linkage to the corresponding chromosomes. At present, five genes have been identified as having a role in susceptibility to colorectal cancer: *hMLH1*, *hMSH2*, *hMSH6*, *hPMS1*, and *hPMS2*.

The cancer risks for mutation carriers appear to be high, even at a young age, and their risk of colorectal cancer is likely to be about 20 or more times the population risk (Mitchell et al. 2002). Unfortunately, almost all the information on average risk in mutation carriers (referred to as 'penetrance' in the genetics literature) has come from studies of mutation-carrying families that were tested *because* they had multiple-cases, and the methods of statistical analysis have not necessarily adjusted correctly for this ascertainment. Consequently those estimates of penetrance are on average inflated. There is one small population-based study published (Dunlop et al. 1997). Nevertheless, it is clear that there are mutations in the MMR genes that are associated with a high risk of cancer, and that risk is likely to be higher in males than females (Mitchell et al. 2002).

A large number of families meeting the Amsterdam or similar criteria have been tested in one of more of the DNA MMR genes, usually just *hMLH1* and *hMSH2*. The general finding is that a deleterious germline MMR gene mutation can be detected in about 50% of more of these multiple-case families.

Mutations in *hMSH6* are found in cases who do not necessarily meet the Amsterdam criteria, and even in some with no known family history of colorectal cancer. Mutations in *PMS2* have been identified primarily in cases associated with Turcot's syndrome.

Mutation testing is expensive and complicated. Sequencing alone does not identify all deleterious genetic variants, and techniques are needed to test for large deletions and insertions.

To date, almost all mutation testing has been focussed on testing affected individuals from families meeting the Amsterdam criteria or other families with a strong history of colorectal cancer—or individuals with 'early-onset' disease. Furthermore, these tested individuals have almost all been ascertained through cancer family clinics or hospital series. There has been little mutation detection reported on population-based samples, and then mutation detection has usually been focussed on selected cases (e.g. Peel et al. 2000). One important exception (Farrington et al. 1998) found, by testing for germline intronic mutations in *hMLH1* and *hMSH2* in cases diagnosed before age 30 years sampled irrespective of family history, that 28% had a pathogenic mutation.

2.2.2 Microsatellite Instability

DNA MMR genes play a crucial in DNA-replication fidelity. Dysfunction in these genes has been associated with an increased mutation rate, and with widespread genome instability. This instability is responsible for a rapid accumulation of somatic mutations in different oncogenes and tumour suppressor genes which play a crucial role in tumour initiation and progression (Kinzler and Vogelstein 1996).

Genetic instability manifests as different numbers of simple repeated sequences (microsatellites) in tumour DNA compared with control DNA from the same patient. This resulting tumour phenotype was referred to as 'replication error', and is now more commonly referred to as MSI.

MSI is measured by screening panels of microsatellites including mono, di- and even tetranucleotide repeats. There has been debate about which and how many repeats should be tested, what the categories are and where the thresholds should be drawn. In 1997, the National Cancer Institute Workshop on Microsatellite Instability proposed five markers, known as the Bethesda panel (Boland et al. 1998). If two or more loci are abnormal, the tissue is classified as having high instability (MSI-H). If no alterations are found it is classified as microsatellite stable (MSS). If a single abnormality is found, testing of a further five markers is recommended, and if one or none of these are abnormal it is classified as having low instability (MSI-L). If two or more of the second set of markers are abnormal it is classified as MSI-H.

MSI (i.e. MSI-H or MSI-L) is almost always detected in tumours of cases known by testing to carry a germline MMR gene mutation. These mutation carriers have typically been identified from testing affecteds in families meeting the Amsterdam or similar criteria.

MSI is also detected in more than 10% of tumours from colorectal cases without a family history (so-called 'sporadic' cases, although this term is often confusingly used to imply such cases do not have a genetic susceptibility). This proportion increases as the age at diagnosis increases, and is associated with methylation of promoter and silencing of *hMLH1*.

MSI, especially MSI-H, predicts mutation carriers well in early-onset cases and/or cases with a strong family history. This does not appear to occur outside these settings, although there is little published data.

2.2.3 Immunohistochemistry

Under expression of a MMR gene protein, detected by immunohistochemistry (IHC), would imply the existence of a germline or somatic mutation in that specific gene. A high correlation has been observed between tumours which are immunohistochemistry negative (IHC −ve) and MSI, in Amsterdam families, and between IHC −ve tumours and the pathology and molecular features typical of early-onset cases from families meeting the Amsterdam criteria (Lindor et al. 2002).

3 MMR Gene Mutations, MSI and IHC

The inter-relationships, among individuals diagnosed with colorectal cancer, between (1) tumour MSI, (2) tumour IHC and (3) germline mutation status for the MMR genes has not been comprehensively studied. Most investigators have considered only pairs of these three aspects, or only tested for mutations in cases a priori thought likely to be mutation carriers, or tested only for the two major MMR genes, *hMLH1* and *hMSH2*. Furthermore, the cases have been ascertained through clinics, either on an ad hoc basis or less often through hospital-based series of early-onset disease, not through population-based sampling. An exception is the Victorian Colorectal Cancer Family Study (VCCFS).

3.1 Population-Based Characterisation of Early-Onset Colorectal Cancer

A population-based study of unselected early-onset cases was conducted in Melbourne, Australia from 1993–1997 (Jenkins et al. 2002). A total of 131 incident cases of histologically verified primary adenocarcinoma of the colon or rectum were identified through the Victorian Cancer Registry. All cases were under the age of 45 years at diagnosis. Attempts were made to interview, and to obtain a personal and family cancer history from, all first- and

second-degree adult relatives. Blood samples were sought from all affecteds, and from unaffected parents and siblings.

Tumour material was sought from all cases and 105 were tested for MSI using the Bethesda panel. IHC testing was conducted for MSH1, MSH2, PMS2 and MSH6. Mutation testing was conducted on case probands. This comprised manual sequencing of *hMLH1*, *hMSH2*, *hMSH6* and *PMS2* and testing for large deletions, in (1) all cases whose family met the Amsterdam II criteria, a random sample of 23 cases with no MSI or lack of protein expression and (2) all cases who were found to have MSI tumours or who were IHC −ve for any of the tested proteins (M.C. Southey et al., submitted). For the purpose of the discussion below we consider a variant to be a mutation if it is predicted to result in a truncated protein.

A total of 12 cases (9%) were from families that satisfied the Amsterdam criteria (including the case diagnosis), and of these 5 were *hMLH1* mutation carriers, 2 were *hMSH2* mutation carriers, 2 were *hMSH6* mutation carriers and one was a *PMS2* carrier. Of the 30 cases with a family history not fulfilling those criteria, the numbers of mutation carriers were 2, 1, 0 and 0, respectively, for those four genes. In the 61 cases with no known family history of colorectal cancer, the numbers of mutation carriers were 2, 1, 2 and 1, respectively.

That is, about one third of cases who would have been defined as 'HNPCC' by family history do not appear to carry a germline mutation in a MMR gene, so are not classified as HMRDS. Furthermore, 1 in 10 cases outside the family history definition of HNPCC carried a MMR gene mutation, and therefore are classified as HMRDS. That is, half of HMRDS cases did not fulfil the family history definition of HNPCC. This is illustrated in Fig. 2a.

All *hMLH1* and *hMSH2* mutation-carrying cases had MSI-H tumours, while all *hMSH6* mutation carriers had MSI-Stable tumours. Nevertheless, no predisposing mutation was detected in 28% of cases with MSI-H tumours, or in any MSI-Stable tumour. This is illustrated in Fig. 2b.

All *hMLH1* mutation-carrying case tumours lacked expression of the PMS2 protein, and all but one also lacked expression of the MLH1 protein. All *hMSH2* mutation-carrying case tumours lacked expression of both the MSH2 and MSH6 proteins. All *hMSH6* mutation-carrying case tumours lacked expression of the MSH6 protein, but not the corresponding heterodimer. No predisposing mutation was detected in cases with IHC tumours showing protein expression. This is illustrated in Fig. 2c.

That is, while the MSI testing would have led to the identification of all mutation carriers, to do so one would have had to test for germline mutations in both *hMLH1* and *hMSH2* in cases with MSI-H tumours. Using IHC to guide mutation testing would also have led to the identification of all mutation carriers, with approximately the same sensitivity, but it has the ad-

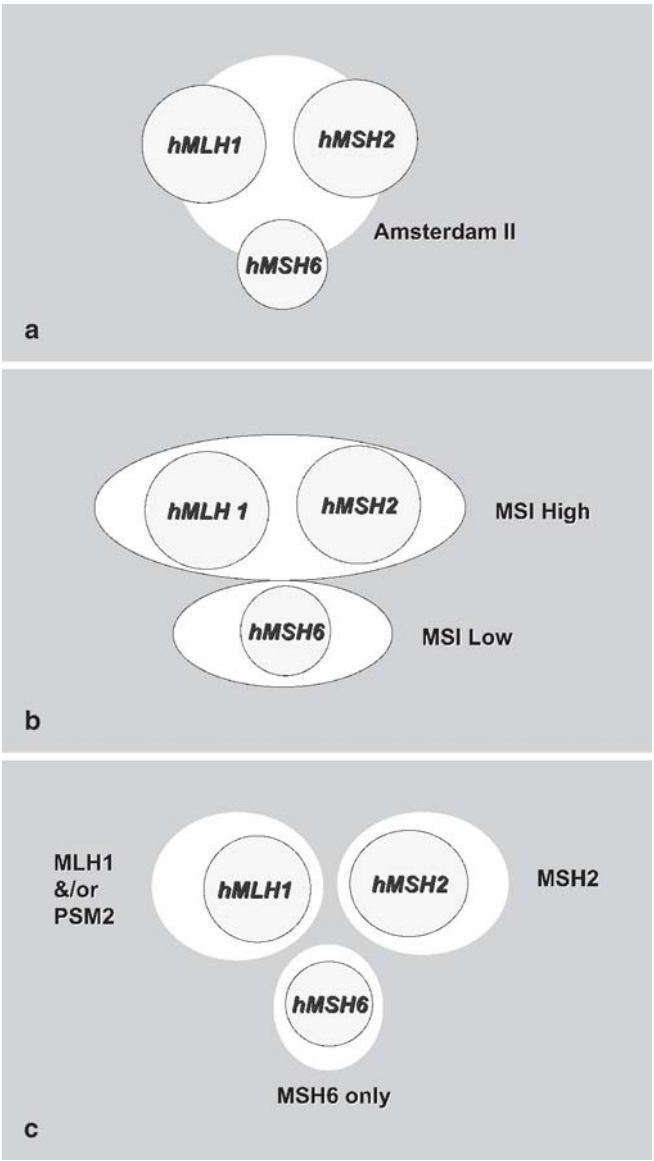

Fig. 2a–c. The inter-relationship between (**a**) Amsterdam II family history, (**b**) MSI-H and MSI-L status, and (**c**) IHC status of the tumour against germline mutation status, based on the findings of the population-based Victorian Colorectal Cancer Family Study. (Southey et al. submitted)

vantage that—by testing for all four proteins—the observed pattern would have correctly identified the specific gene to be tested.

3.2 Suggested Clinical Genetics Triage Regime

Figure 3 shows a suggested regime for identifying MMR gene mutation carriers. Unlike the traditional approach focused on multiple-case families identified opportunistically, this regime starts with a systematic assessment of early-onset cases identified through normal clinical practice.

Prior to surgery, it would seem essential that appropriate informed consent be obtained for access to the patient's tumour material for MSI and/or IHC testing, and collection of a blood sample for genetic testing. This consent would also need to address the possible consequences, and explain the possible outcomes. Given that only a small proportion of cases would be found to be mutation carriers, it would seem inappropriate at *this* stage to offer detailed genetic counselling—to do so would likely cripple the process. As is always the case with ethical issues, determination of what is appropriate is a local issue.

Given that consent is obtained, the tumour tissue would be subjected to MSI and/or IHC testing. Based on the Victorian population-based study (see Sect. 14), IHC testing, possibly followed by MSI testing in IHC −ve tumours for confirmation, would be most appropriate—and likely more cost-effective

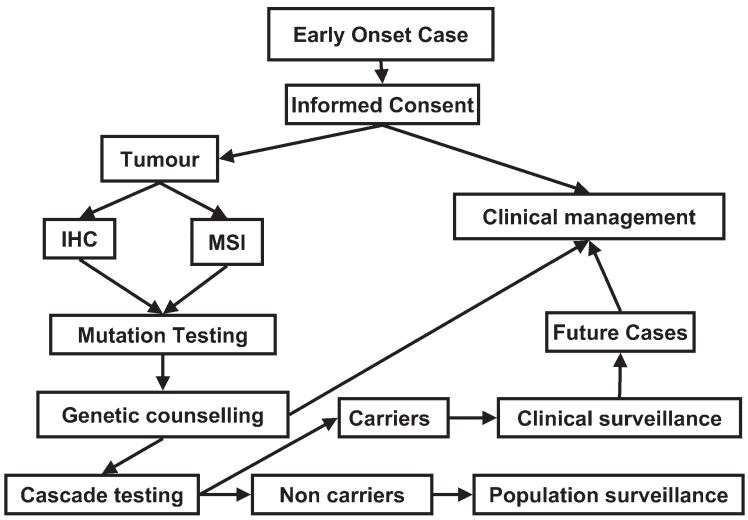

Fig. 3. Suggested triaging regime for identifying mismatch repair gene mutation carriers by systematically studying early-onset cases of colorectal cancer

(see Sect. 16). Again, issues and local resources may influence the laboratory protocols to be followed.

The molecular testing would then indicate, with a high degree of specificity, the set of cases that contains the MMR gene mutation carriers. Genetic testing could then be undertaken. If IHC results were available, mutation testing would be targeted to a specific gene, again with high specificity. The sensitivity of these approaches in the current setting, based on the population-based data (Sect. 14), is likely to be at least 50% for cases diagnosed before the age of 45 years. Before this regime could be extended to older ages at onset, it would be essential that appropriate and similar population-based data be assessed.

Once mutations are detected, the patients could be informed that some information on the cause of their cancer had been obtained, and if they wished to avail themselves of that information they would need to go through formal genetic counselling at a recognised cancer family genetics service. It might also be considered appropriate that genetic counselling be carried out earlier in the process, such as when the tumour IHC and/or MSI test were suggestive that the case had a germline mutation in a MMR gene. Genetic counselling would involve explaining the consequences of having, or not having, a mutated MMR gene, and the implications for their own clinical management, as well as all the possible implications for their relatives. Should the individual wish to proceed, a new blood sample would need to be drawn—in line with the local national accredited genetic testing protocols—and tested for the putative mutation. The individual could then be informed, and offered referral to appropriate clinical management services, which might include surveillance for colorectal and extra-colonic cancers. Cascade testing of relatives could be offered and conducted according to the local cancer family genetics service's protocols. Relatives found to carry the family mutation could also be offered referral to appropriate clinical management services. Relatives found not to carry the family mutation could be informed about the local recommended population screening policies and services. Those who had previously been concerned about having a genetic risk based on their family cancer history, especially those who had been under increased surveillance, could be reassured.

This approach will lead to the identification of individuals at truly high genetic risk of cancer. It is important to recognise that the details of how this regime be implemented would depend a great deal on local issues. Appropriate surveillance procedures would need to be implemented to prevent cancers. To achieve this there is an urgent need for research on the efficacy and optimisation of surveillance procedures in these genetically at-risk individuals, and identification of the environmental, lifestyle and genetic factors that exacerbate, or ameliorate, risk in mutation carriers. This is one of the major questions being addressed by the NIH-funded Colon Cancer Family Registry (http://www.cfr.epi.uci.edu).

3.3 Efficiency of Different Approaches to MMR Gene Mutation Detection

Table 1 shows, based on the results of the VCCFS population-based study of colorectal cancer cases diagnosed before age 45 years, the relative efficiencies of detecting mutation carriers from testing based alone on Amsterdam family history criteria, MSI testing, and IHC testing. The traditional approach to defining HNPCC would have led to testing of 12% of cases, and for every 100 cases there would be 36 mutation screens (if all three MMR genes are to be tested) detecting 7 carriers. The MSI-alone approach would detect twice as many carriers, but would involve more cases being tested. The IHC-alone protocol would involve less mutation testing, and also detect all mutation carriers. That is, the success of genetic testing to find mutation carriers would increase from 20% to 30% to 50% across the three regimes.

The costs for these three approaches will vary substantially depending on resources, skills, infrastructure, and so on. For example, obtaining an accurate and comprehensive family history can take considerable time and effort on behalf of the enquirer and the family members themselves. Given that one has access to tumour material, the laboratory costs for MSI testing currently is more expensive than for IHC testing. The latter should involve the input of a pathologist, at least in selecting tumour material for staining (the interpretation can be performed by a well-trained technician), and the costs for that professional service could vary greatly from setting to setting. If the relative costs for the three regimes are in the ratio of 4:2:1, and excluding testing for *PMS2*, which would be appropriate for an Australian clinical service, an approach built around IHC testing alone as the first step would be ten times more efficient for detecting mutation carriers than one based on family history alone, and less than three times more efficient than one based on MSI testing alone. The relative cost-efficiencies would need to be determined for each local situation.

Table 1. Relative efficiency of detecting mutation-carrying cases of colorectal cancer (for 100 cases under 45 years at diagnosis)

	Basis for assessment		
	Family history alone	MSI testing alone	IHC testing alone
Number of cases gene tested	12	>30	<30
Number of gene tests conducted	36	50	35
Number of case carriers detected	9	15	18
Success of genetic testing	25%	30%	50%
Relative cost	4	2	1
Relative efficiency	1	<3	10

IHC, immunohistochemistry; MSI, microsatellite instability.

4 Summary

It is well established that, on average, a first-degree relative of an individual with colorectal cancer is at about a twofold increased risk. This increased risk is greater the younger the age at diagnosis, and the younger the age of the at-risk relative. This seemingly modest risk could not occur without there being strong underlying risk factors that are correlated in relatives. If these 'familial' risk factors were of a genetic origin, in which case the correlation between first-degree relatives would be 0.5, individuals in the top 25% of genetic risk would be at least 20 times more likely to develop colorectal cancer than those in the lower 25%. Consequently, in theory 90% of colorectal cases would occur in people in the upper half of the wide distribution of familial/genetic risk. Therefore, there is great potential to use genetics to prevent colorectal cancer, provided individuals who are at higher, if not highest, genetic risk can be identified.

To date, two rare genetic syndromes have been identified: familial adenomatous polyposis (FAP) and hereditary non-polyposis colorectal cancer (HNPCC). The former appears to be mostly due to mutations in the *APC* gene. The latter appears to be mostly due to mutations in the MMR genes, and it would be better named hereditary mismatch repair deficiency (HMRDS), and the expression HNPCC should be taken out of the literature. These two genetic syndromes explain only a small fraction of the broad spectrum of genetic risk.

Identification of individuals with an inherited predisposition to FAP has historically been based on phenotype, not family history, with recruitment through affecteds followed by expansion to family members. On the other hand, identification of 'HNPCC' was originally based on cancer family history, and recruitment often through unaffected relatives of cases. Microsatellite instability (MSI) and immunohistochemistry (IHC) testing of cases, combined with mutation testing in MMR genes, has the potential to reverse the process for HNPCC to make it more like the traditional FAP scenario.

By fully characterising a population-based series of early-onset cases, a new regime for triaging has been proposed. It would appear that MMR gene carriers and their relatives can be much more efficiently identified by characterising the tumours of early-onset cases, independently of their cancer family history, using IHC—not MSI—as the key first step in triaging the mutation testing. This is likely to be more efficient because it is cheaper and identifies the specific MMR gene likely to be involved, reducing the costs of mutation testing. That is, identification of genetically susceptible individuals using the tumour phenotype of affecteds, rather than using family cancer history, could become the standard approach of cancer genetics and associated clinical services in the twenty-first century. The protocols for such a service will depend on local resources and issues. It is likely that this ap-

proach will lead to the prevention of cancers in individuals who are at a high genetic risk of cancer when young. To achieve this, there is an urgent need for research on the efficacy and optimisation of surveillance procedures in these genetically high-risk individuals, and identification of the environmental, lifestyle and genetic factors that exacerbate, or ameliorate, risk in mutation carriers.

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Genetics and Prevention of Oesophageal Adenocarcinoma

Rebecca C. Fitzgerald

MRC Cancer Cell Unit, Hutchison-MRC Research Centre, Cambridge, CB2 2XZ, UK
rcf@hutchison-mrc.cam.ac.uk

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Abstract Gastric cancer has been declining for more than half a century, whereas the incidence of oesophageal cancer is increasing rapidly. The histopathological subtype is also changing with a predominance of oesophageal adenocarcinoma compared with squamous carcinoma. The reasons for these epidemiological changes are not clear, although population-based data have implicated gastro-oesophageal reflux disease as a risk factor. In susceptible individuals reflux of duodeno-gastric contents can lead to the development of a columnar-lined oesophagus, commonly called Barrett's oesophagus. This can then progress to adenocarcinoma via a metaplasia-dysplasia-carcinoma sequence. At the current time, the mortality from oesophageal adenocarcinoma exceeds 80% at 5 years. Therefore, endoscopic surveillance programmes have been generally recommended for patients with Barrett's oesophagus in an attempt to detect early, curable lesions. Unfortunately these programmes are cumbersome and costly and have not yet been proved to reduce population mortality. In order to improve patient outcomes we need to be able to identify patients at high risk and to understand the triggers for disease progression. There is mounting evidence that there is an underlying genetic susceptibility to Barrett's oesophagus and oesophageal adenocarcinoma. However, this is likely to be as a result of multiple low penetrance susceptibility genes which have yet to be identified. Once patients are identified as having Barrett's oesophagus their chance for developing adenocarcinoma is in the order of 0.5%–1% per year. The histological assessment of dysplasia as a predictor of cancer development is highly subjective. Therefore multiple, specific somatic mutations in the tissue have been investigated as potential biomarkers. The most promising markers to date are the presence of aneuploidy, loss of heterozygosity of p53 and cyclin D1 overexpression. However, a study of evolutionary relationships suggest that mutations occur in no obligate order. Combinatorial approaches are therefore being advocated which include genomic profiling or the use of a panel of molecular markers in order to define the common molecular signatures that can then be used to predict malignant progression. An alternative approach would be to use markers for the final common

pathway following genetic instability, which is the loss of proliferative control. We have demonstrated an increase in the expression of a novel proliferation marker, Mcm2, which occurs during the malignant progression of Barrett's oesophagus. These Mcm2-expressing cells are detectable on the surface, and hence a cytological approach may be applicable. In view of the role of reflux components in the pathogenesis of Barrett's oesophagus the effect of acid and bile on the cell phenotype have been studied. These studies have demonstrated that pulsatile acid and bile exposure induce cell proliferation. The mechanism for the hyperproliferative response appears to involve p38 mitogen activated protein kinase (MAPK) pathways as well as protein kinase C (PKC) and cyclo-oxygenases. A clinical implication of the laboratory studies is that suppression of acid and bile may need to be profound in order to suppress cell proliferation and, by inference, ultimately prevent the development of dysplasia. There is some support for this concept from short-term clinical studies, and a large randomised chemoprevention trial is being instigated which will evaluate the effect of proton pump inhibitors with or without aspirin. Given the epidemic increase in oesophageal adenocarcinoma and the dismal 5-year mortality rate, a radical approach is necessary to prevent cancer development in individuals with pre-malignant lesions.

1 Introduction

There has been a dramatic change in the epidemiology of upper gastrointestinal cancer in the Western world over the past 30 years. In contrast to the substantial decline in the mortality from gastric cancer, there has been an increase in mortality from oesophageal cancer, particularly in white males [1]. This rise in oesophageal cancer mortality is accounted for by the increase in oesophageal adenocarcinoma, which is now more common than oesophageal squamous carcinoma [2]. Despite aggressive neo-adjuvant chemotherapy and surgery regimes, patients with oesophageal adenocarcinoma have a less than 80% survival rate at 5 years due to the advanced stage at presentation [3]. Therefore a radically different approach is required, and one possibility is earlier intervention. This is feasible given that there is a well-defined metaplasa–dysplasia–carcinoma sequence commonly referred to as Barrett's oesophagus (reviewed in [4, 5]).

Barrett's oesophagus occurs when the normal squamous oesophageal epithelium is transformed to a columnar lined epithelium characterised by an intestinal phenotype with goblet cells, sometimes referred to as specialised intestinal metaplasia [6]. This metaplastic phenotype develops as a consequence of reflux disease, and epidemiological data suggest that the odds ratio for developing oesophageal adenocarcinoma increases by as much as 40-fold in patients with chronic and severe reflux symptoms [7]. However, although this metaplasia–dysplasia sequence is well defined there are some major clinical challenges. First, only an estimated 5% of patients with Barrett's oesophagus are diagnosed [8, 9]. Second, only 0.5% per year of patients with Barrett's oesophagus will actually go on to develop adenocarci-

noma [10]. This incidence, which is lower than previously suggested, has now been confirmed in several recent studies [11, 12].

In current clinical practice a diagnosis of Barrett's oesophagus requires an endoscopy and histopathological confirmation. Once Barrett's oesophagus is diagnosed the patient may be offered regular endoscopic surveillance, depending on the specific hospital practice. In the UK there are currently no national surveillance guidelines, and although 75% of British gastroenterologists favour surveillance in principle, over 50% do not perform the quadrantic, 2-cm biopsy regime recommended by the American College of Gastroenterology due to practical constraints [13]. Apart form surveillance, there is no specific medical intervention apart form symptomatic control of reflux symptoms until the patient develops significant dysplasia (usually high-grade dysplasia) [14]. Once high-grade dysplasia or early cancer has developed, surgical intervention remains the gold-standard for fit patients, although experience with endoscopic therapies such as ablation therapy and endoscopic mucosal resection is rapidly accumulating. So far there is no good evidence that surveillance of Barrett's oesophagus reduces population-based mortality from oesophageal adenocarcinoma [15, 16]. However, numerous retrospective series and case-control studies suggest that patients whose cancers are detected during surveillance are at an earlier stage, and this has recently been shown to confer a survival advantage in a large population-based study [17], although these studies are subject to both lead-time and length-time bias. In order to make surveillance more practical and effective at the population level, alternative strategies need to be considered such as screening, better predictors of progression to cancer and chemoprevention.

2 Screening

Screening has been advocated in order to try and increase the population benefits of surveillance or chemoprevention strategies. The likelihood of detecting Barrett's oesophagus in the population increases significantly with the duration of reflux symptoms [18]. Hence, the American College of Gastroenterology has suggested endoscopic screening for patients with chronic gastro-oesophageal symptoms, especially in persons over the age of 50 years who have had reflux symptoms for greater than 5 years [14]. The compliance with this recommendation and the diagnostic yield is not yet known. Concern has been expressed about the high cost of a mass endoscopic screening programme when the absolute cancer risk is low, although on the other hand, the risks of endoscopy are low, patients are increasingly aware of the association between heartburn and cancer and there are medico-legal considerations [19].

In an attempt to reduce the burden of mass endoscopic screening, there has been an effort to develop a scoring system to predict the presence of Barrett's oesophagus using knowledge of patient demographics and symptoms. One such nomogram had a specificity of 63% and a sensitivity of 77% [20]. A subsequent questionnaire and endoscopy study recruited over 1,000 consecutive adult patients with reflux, and in this study Barrett's oesophagus was associated with duration of acid regurgitation, but not with frequency or severity. At a sensitivity of 80% the model had a specificity of 57% for Barrett's oesophagus [21]. Hence, both these studies suggest that symptoms are only modestly predictive of findings at endoscopy.

Further doubt has been cast over the symptom-based approach following a study of veterans attending for a screening colonoscopy that suggested that up to 25% of patients with Barrett's oesophagus may be asymptomatic [22], although a subsequent study suggests that this figure may be nearer to 6% [23].

The modest correlation between symptoms and an endoscopic diagnosis of Barrett's oesophagus suggests that there must be other factors, such as genetic susceptibility, affecting pathogenesis. There are several case reports of families with multiple affected persons with heartburn, Barrett's oesophagus and sometimes adenocarcinoma in up to four generations [24]. Analysis of the pedigrees in these studies suggests an autosomal-dominant pattern of inheritance with incomplete penetrance [25]. In another study, the first degree relatives of Barrett's patients were twice as likely to have heartburn symptoms than their spouse controls [26]. A recent study has shown an increased concordance for reflux in monozygotic pairs, compared with dizygotic pairs. Heritability accounted for approximately 30% of the liability to reflux disease in this population [27]. Linkage analysis of large pedigrees with severe paediatric reflux disease, inherited in an autosomal-dominant fashion, has identified a region on chromosome 13q14 as a susceptibility locus [28].

These studies provide collective evidence that genetic factors may play a role in reflux disease and Barrett's oesophagus. Although in some families there appears to be an autosomal-dominant pattern of inheritance, it is likely that multiple lower-penetrance genes account for familial clustering in the majority of cases of Barrett's oesophagus in keeping with other common polygenic diseases.

In the future, population-based screening methods based on some combination of genetic susceptibility testing, symptom nomograms and endoscopy may enable a greater proportion of affected individuals to have their Barrett's oesophagus diagnosed. This will only be worthwhile if effective surveillance or treatment regimes are available.

3
Predictors of Progression in Patients Undergoing Endoscopic Surveillance

The low incidence of Barrett's adenocarcinoma in patients undergoing surveillance has led to a search for putative risk factors at the demographic, clinical and molecular level.

In a population-based study in Northern Ireland, the malignancy rate in men was 2.5 times that in women and the only group with an incidence greater than 1% per year was men over the age of 70 years who had Barrett's oesophagus characterised by the presence of specialised intestinal metaplasia [12]. A multi-centre consortium has examined the relationship between patient age, segment length and the prevalence of dysplasia in over 300 patients. The diagnosis of dysplasia increased by 3% per year of age and 14% per centimetre of increased length. Multivariate analysis suggested that these were independent risk factors [29]. A similar-sized Australian cohort study of patients found that the presence of severe oesophagitis, ulceration, nodularity or stricture were predictive of malignant progression. One or two of these risk factors increased the chance of progression 7 or 14 times, respectively [30].

As a result of these studies it has been suggested that for surveillance to be cost-effective it should be restricted to patients with the highest risk profile such as age greater than 70 years, long segments, endoscopically visible lesions and the presence of specialised intestinal metaplasia.

However, as Murray and colleagues noted, limiting surveillance to these people would miss two-thirds of cancers [12].

4
Molecular Markers

In view of the high level of subjectivity for the histopathological grading of dysplasia, there has been much research examining the possibility of using the molecular profile of the Barrett's mucosa to predict the risk for progression.

It is known that there is an accumulation of a large number of alterations in oncogenes and tumour suppressor genes in the metaplasia–dysplasia–adenocarcinoma sequence [5]. The best studied of these is p53 [31, 32] and one large-scale phase IV study using high-throughput genotyping, which may be applicable to routine clinical use, suggested that 17p (p53P) loss of heterozygosity (LOH) is a strong and significant predictor of progression to cancer [33, 34]. Dolan et al. have recently suggested that p53 mutational analysis can identify patients at greater risk for progression in a retrospective and prospective study design [35]. The other markers which have progressed furthest in clinical studies are those using baseline flow cytometry

(tetraploidy and aneuploidy). In a phase IV study, the 5-year cumulative incidence for cancer was 43% and 57% for aneuploidy and tetraploidy compared with a 5% incidence for patients without either of these cytological abnormalities [36]. There is a need for further large prospective studies of this kind in order for these data to be able to influence clinical practice.

In contrast to studies examining a single gene or protein at a time there is a move towards a combinatorial approach. For example, a recent study suggested that insulin-like growth factor (IGF)1-R, c-Src and Bcl-X(L) are co-ordinately elevated in Barrett's-associated neoplasia [37]. Such an approach is logical, since a study of evolutionary relationships suggests that mutations occur in no obligate order, and clonal expansion of genetic instability leads to cancer extending over a period of months and years [38]. The ultimate panel of markers could be obtained with the advent of microarray technology. For example, it has been demonstrated that cDNA microarrays can differentiate between premalignant Barrett's samples and invasive oesophageal adenocarcinoma, and this algorithm could then be used to determine the characteristics of an 'unknown sample' using artificial neural networks. It is hoped that this global gene profiling approach will enable predictions to be made about which patients in the Barrett's population are likely to progress slowly versus quickly [39, 40] and ultimately to identify specific genes capable of predicting outcome (likelihood for progression). cDNA microarrays are useful for measuring genome-wide increases or decreases in DNA copy number. However, tumour-suppressor gene inactivation may arise as a result of a mutation of one allele and loss of heterozygosity (an example of allelic imbalance) of the other allele that do not lead to changes in DNA copy number. These alterations can be detected by use of genetic polymorphisms such as single nucleotide polymorphisms (SNPs). The validity of this approach has been demonstrated in a study in which genome-wide analysis was performed in SNP arrays (600 biallelic markers) on 10 patients with either high-grade dysplasia or oesophageal adenocarcinoma using normal DNA from control gastric tissues [41]. The SNP array yielded informative loci, and it is hoped that the advent of higher density SNP arrays will allow efficient, genome-wide, high-resolution searches for chromosomal changes associated with tumour initiation and progression, which could be used in parallel with cDNA microarray-based methods.

An alternative approach is to examine the final common end-point of these mutations, which is the loss of proliferative control. Hence, it has been demonstrated that expression of a novel proliferation marker—mini-chromosome maintenance protein, or Mcm-2— increases during Barrett's oesophagus carcinogenesis with a shift in the proliferative compartment towards the surface [42], confirming previous data [43]. Furthermore, in a retrospective longitudinal case-control study these changes predated the development of dysplasia, and the value of surface brushing samples stained for Mcm2 was also evaluated with promising results [42]. Hence, in contrast to

the previous disappointing studies evaluating cytology alone, the combination of cytology with a molecular marker may have the potential for use as a clinical tool [44].

5 Chemoprevention

Since endoscopic surveillance is unproven and expensive, chemopreventive agents are also being explored as a more cost-effective strategy. For example, a computer model evaluating treatments for patients with high-grade dysplasia (HGD) suggested that chemoprevention is cost-effective, although this strategy may be less favourable for the general population of Barrett's patients [45]. Chemopreventive trials are just starting to be applied in the context of Barrett's carcinogenesis. There has been concern about the length of studies required to demonstrate a beneficial effect of a chemopreventive agent if the outcome measure is restricted to cancer incidence. However, a recent cross-sectional study has shown that molecular markers can effectively demonstrate differences between patients with BE that correlated with serum selenium levels [46]. However, caution must be exercised when choosing a biomarker or surrogate endpoint [47].

Since acid reflux is an important aetiological factor, there has been interest in determining whether anti-reflux strategies might prevent cancer incidence in patients with BE. This is based partly on in vitro and immunohistochemical studies [48–50] which suggest that complete acid-suppression may be important in order to reduce proliferative indices. However, translating these research findings to the clinical situation is complex. Several groups have examined whether a surgical anti-reflux procedure or medical acid-suppression is an effective chemopreventive method. Interpreting these data are problematic, since many of the studies were retrospective cohort studies and the degree of acid-suppression is frequently not known. Corey et al. have performed a meta-analysis with 4,678 patient-years of follow-up in the surgical antireflux procedure group compared with the 4,906 patient-years in the medical group. The cancer incidence was 3.8/1,000 patient-years of follow-up in surgical group compared with 5.3 in the medical group. Hence, the risk of adenocarcinoma in subjects with BE was very low and was not significantly decreased by a surgical antireflux procedure [51].

On the other hand, if high-dose proton pump inhibitor (PPI) therapy is necessary to completely abolish acid-reflux for chemoprevention to be effective, then concern has been raised about the secondary increase in serum gastrin which is known to be mitogenic. In support of this hypothesis in vitro evidence that gastrin can induce proliferation via the cholecystokinin (CCK2) receptor [52]; although interestingly, none of the patients in this cohort actually had hyper-gastrinaemia, despite being on high dose PPI [53].

A randomised control trial is needed to evaluate the role of PPI therapy as a chemoprevention agent with documentation of the degree of acid suppression.

The other main chemopreventive agents that have been advocated are nonsteroidal anti-inflammatory drugs (NSAIDs) or the more specific cyclooxygenase (COX)-2 inhibitors. The rationale for this approach stems from the increase in COX-2 expression seen early in the Barrett's–dysplasia–carcinoma sequence and the chemopreventive effect of a COX-2 inhibitor in an animal model [54, 55]. There has been concern about the cardiovascular side-effect of COX-2 inhibitors, and a review of three case control trials suggests that aspirin may also be an effective chemopreventive [56]. A meta-analysis of observational studies evaluating both aspirin and NSAIDs suggests a protective effect for any use of these drugs against oesophageal cancer (squamous and adenocarcinoma). However, aspirin was more effective than NSAIDs [odds ratio (OR)=0.5; confidence interval (CI), 0.38–0.66; and OR=0.75; CI, 0.54–1.0, respectively] and there was a dose and frequency-of-use effect [57].

Two chemopreventive trials in BE are currently in progress. The 'Chemoprevention for BE Trial' (CBET) is a phase IIb, multicenter, randomized, double-blind, placebo-controlled study of the selective COX-2 inhibitor celecoxib in patients with Barrett's dysplasia [58]. The AspECT trial is a randomised, controlled trial with a two-by-two design to determine the effects of high- and low-dose proton pump inhibitor therapy with and without low-dose aspirin [59]. These should provide important data about the efficacy of these agents for the chemoprevention of oesophageal adenocarcinoma.

6 Conclusion

Oesophageal adenocarcinoma is increasing in incidence in the Western world and the mortality remains high despite advances in neo-adjuvant chemotherapy and surgery regimes. There is the opportunity to improve the outcome for patients as a result of the metaplasia–dysplasia–adenocarcinoma sequence. However, there are many clinical challenges in view of the number of patients with Barrett's oesophagus who are undiagnosed, the low absolute cancer risk and the cumbersome endoscopic methods for detection and surveillance. Detecting high-risk groups, through molecular profiling for example, may be one way forward in order that interventions can be highly targeted. Alternatively, mass screening and chemoprevention strategies may have a greater impact on reducing population mortality. More research is needed in this important area.

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Preclinical Models Relevant to Diet, Exercise, and Cancer Risk

R. James Barnard (✉) · William J. Aronson

Departments of Physiological Science and Urology, University of California, Los Angeles, Los Angeles, CA 90095-1606, USA
jbarnard@physci.ucla.edu

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Abstract Metabolic syndrome was initially described as an aggregation of risk factors for the development of coronary artery disease with insulin resistance and compensatory hyperinsulinemia as the underlying factor. In an earlier review, we suggested that hyperinsulinemia may also lead to prostate cancer (PCa), the most common male cancer in industrialized nations. Furthermore, we suggested that diet and exercise, known to be important in the development of insulin resistance, may also be important in the development of PCa. When we placed men from the United States on a low-fat diet and/or exercise program, serum levels of insulin, free testosterone, estradiol and insulin-like growth factor (IGF)-1 were reduced while sex hormone-binding globulin (SHBG) and insulin-like growth factor binding protein (IGFBP)-1 were elevated. These in vivo serum changes directly impacted on androgen-dependent prostate cancer cell lines in vitro to reduce cell growth and induce apoptosis. The reduction in serum IGF-1 and increase in IGFBP-1 with diet and exercise appear to be the most significant, as these changes lead to an increase in tumor cell p53 protein and its down-stream effector p21, which are responsible for the reduction in cell growth and induced apoptosis. Preliminary results from a clinical study with men on "watchful waiting" indicate that the observed in vitro effects of diet and exercise on prostate cancer cell growth also occur in vivo.

1 Introduction

Metabolic syndrome is now recognized as a major risk factor for the development of atherosclerotic disease, the number one killer in industrialized nations and rapidly increasing in developing countries. The National Cholesterol Education Program in the United States and the World Health Orga-

nization have emphasized this fact and have provided definitions and guidelines for identifying individuals with the syndrome [15, 58]. The syndrome was first described in 1988 by Gerald Reaven as the aggregation of coronary artery disease risk factors, including insulin resistance/hyperinsulinemia, hypertension, hypertriglyceridemia, and depressed high density lipoprotein (HDL) cholesterol; he called it "Syndrome X" [42]. The traditional feeling at the time was that obesity was the underlying cause of the abnormalities, a concept still held by many today [24]. In fact, abdominal obesity is used to help define the syndrome. However, Reaven had suggested that insulin resistance/hyperinsulinemia was the common feature and the other characteristics were secondary to this basic abnormality. Additional abnormalities including enhanced blood clotting and small dense very low density lipoprotein (VLDL) and low density lipoprotein (LDL) have been added to the list [4]. De Fronzo and Ferrannini [14] also suggested that insulin resistance is the underlying factor for the syndrome, and once it developed those with a genetic predisposition would develop the other aspects. However, they pointed out that environmental factors such as diet, body weight or physical exercise could modify insulin resistance, indicating that the final phenotypic expression involves both genetic and acquired influences. Haffner et al. [18] renamed the syndrome "insulin resistance syndrome" to stress the underlying importance of insulin resistance, as they had shown in a prospective study that insulin resistance preceded the other aspects of the syndrome. Most scientists now refer to the syndrome as "metabolic syndrome."

We [3, 5] subsequently reported that the syndrome could be induced in Fischer rats by feeding a high-fat, refined-carbohydrate diet and that insulin resistance/hyperinsulinemia preceded the other aspects (i.e., before any change in body fat or fat cell size). Switching the animals back to a low-fat, complex-carbohydrate diet reversed the abnormalities without caloric restriction [45]. We also showed in humans that aspects of the syndrome including hyperinsulinemia, hypertriglyceridemia, and hypertension could be controlled in as little as 3 weeks by placing individuals on a low-fat, high-complex-carbohydrate diet with daily aerobic exercise [2]. These changes occurred in individuals who remained obese [body mass index (BMI)>30], supporting our conclusion that obesity is not the underlying cause of metabolic or insulin resistance syndrome.

While doing these initial investigations, we read several studies reporting an inverse relationship between serum insulin and sex hormone-binding globulin (SHBG) [25, 37, 40, 49]. In human hepatoma cells, insulin was found to inhibit SHBG production [39]. A reduction in SHBG would result in increased free hormone to interact with tissue receptors. SHBG binds both testosterone and estradiol [1]. Thus, a reduction in SHBG and rise in free sex hormones should increase the risk for hormone-related cancer, i.e., prostate, breast, and endometrial. In fact, Gann et al. [16] reported that high testosterone and reduced SHBG were risk factors for prostate cancer, and

more recently Hsing et al. [22, 23] reported that elevated serum insulin is a risk factor for prostate cancer, and Lehrer et al. [28] reported that it is a risk factor for recurrence of prostate cancer (PCa) following radiation treatment. Based on these findings we suggested that cancer, especially the hormone-related cancers, may also be a part of metabolic syndrome [6]. Hammarsten et al. [19] have also suggested that insulin resistance syndrome is associated with the development of benign prostatic hyperplasia (BPH), thought to be a precursor of PCa. They found that components of the syndrome including type 2 diabetes, hypertension, abdominal obesity, depressed HDL-cholesterol and elevated fasting insulin were all significantly correlated with BPH.

2 Diet, Exercise, and Prostate Cancer

We decided to focus on prostate cancer (PCa) by placing men on a low-fat diet consisting primarily of grains, fruits and vegetables, and regular supervised exercise to examine serum changes in vivo and ultimately their effect on prostate cancer cell growth in vitro. The subjects were participants or employees from the Pritikin Longevity Center Residential Program. During their stay at the Center, food was prepared and served buffet style to the participants and consisted of 10%–15% of calories from fat (polyunsaturated/saturated fatty acid ratio=1.24), 15%–20% of calories from protein, and 65%–75% of calories from carbohydrates, primarily unrefined. Carbohydrates were in the form of high-fiber whole grains ($\geq$5 servings/day), vegetables ($\geq$4 servings/day), and fruits ($\geq$3 servings/day). Protein was primarily derived from plant sources, with nonfat dairy allowed for up to 2 servings/day. Fish or fowl was served in 100-g (3.5 U.S. oz.) portions 1 day/week and in soups or casseroles 2 days/week. The diet contained less than 100 mg/day of cholesterol and alcohol, tobacco, and caffeinated beverages were not allowed during the program. Before starting the exercise training, subjects underwent a graded treadmill stress test according to a modified Bruce protocol to determine the appropriate individual level of exercise intensity. Based on the results, the subjects were provided with an appropriate training heart rate value and given an individualized walking program. The exercise regimen consisted of daily treadmill walking at the training heart rate for at least 45–60 min. The training heart rate was defined as 70%–85% of the maximal heart rate attained during the treadmill test. After 3 weeks of the diet-and-exercise program, serum insulin was reduced by 43% while SHBG increased by 39% [52]. In a similar study with postmenopausal women, we also found that the diet-and-exercise program lowered serum insulin and increased SHBG [53]. Reed et al. [43] reported that placing men on a high-fat diet for 2 weeks lowered SHBG. Several studies have reported an inverse relationship between BMI and SHBG. However, we observed no cor-

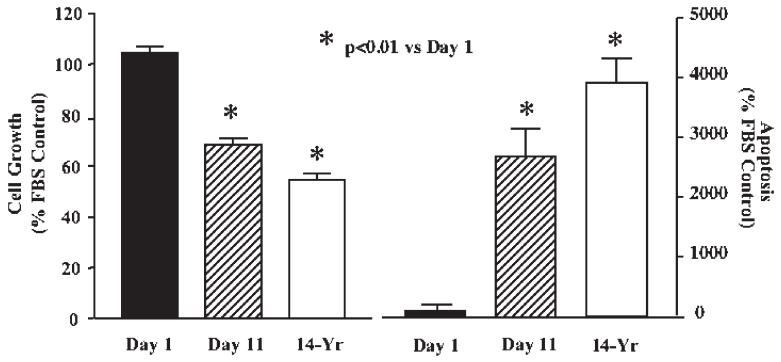

Fig. 1. Effect of serum changes following diet and exercise on LNCaP prostate cancer cell growth and apoptosis in vitro. (Data from Tymchuk et al. [54] and Ngo et al. [33])

relation between the change in BMI and the reduction in SHBG in our studies, supporting our contention that obesity is not the real cause of many of the abnormalities observed in obese individuals. We also recently showed that the diet-and-exercise program does reduce serum-free testosterone [54].

A low-fat diet has been shown to reduce the growth of LNCaP prostate tumor cells in nude mice [56]. LNCaP is a well-established cell line developed from a patient with prostate cancer. We used a cell culture assay to study tumor cell growth in response to changes in serum factors when men were placed on the low-fat, complex-carbohydrate diet and aerobic exercise program [54]. After just 11 days of the program, serum-stimulated growth of androgen-dependent LNCaP prostate tumor cells was reduced by 30%. We then obtained blood samples from men who had been following a low-fat diet-and-exercise program for 14±2 years and found an additional 15% reduction in LNCaP cell growth (Fig. 1). Serum changes resulting from either 11 days or 14 years of the low-fat diet and exercise had no impact on the growth of androgen-independent PC-3 tumor cells. In a group of control men with no change in diet or exercise over 11 days there was no change in LNCaP cell growth [100±4% vs 102±5% fetal bovine serum control (FBS)]. Serum samples were obtained from a group of men with diagnosed PCa before and 3 months after androgen deprivation therapy with leuprolide acetate. LNCaP cell growth was reduced from 85±8% to 56±6% FBS, similar to the level achieved with long-term diet and exercise. We used the serum from the 11-day study and showed that the diet-and-exercise program also reduced the growth of serum-stimulated LAPC-4 cells, another androgen-dependent prostate cancer cell line [34].

In an attempt to elucidate possible changes in serum following diet and exercise that might explain the reduction in androgen-dependent prostate cancer cell growth we first focused on three hormones: testosterone, estradi-

ol, and insulin [55]. Testosterone was chosen because LNCaP, like all early-stage prostate cancer, is androgen dependent, and we had previously reported that the diet-and-exercise program reduced serum-free testosterone [12, 54]. Estradiol was chosen because LNCaP cells have both estrogen receptors and a mutated androgen receptor that binds estrogen, and the cells are stimulated by estradiol [8]. In addition, we had previously reported that the diet-and-exercise program reduced serum estradiol in men by 50% [47]. Insulin was chosen because it is a known mitogen that activates cellular RNA via the mitogen-activated protein Kinase (MAPK) pathway, has been reported to stimulate the growth of a rat prostate cancer cell line in vitro, and is reduced with diet and exercise [2, 26, 41, 52]. We added each of these hormones to the LNCaP incubation medium and demonstrated that they were capable of stimulating LNCaP cell growth in our cell culture assay [55]. We then added each hormone individually and in combination to the serum obtained from the long-term diet and exercise subjects and could account for less than half of the reduction in LNCaP cell growth [55].

Next we turned to the insulin-like growth factor (IGF) family. Based on review articles by Yu and Rohan [60] and by Thissen et al. [50] on the insulin-like growth factor axis and an article by Cohen et al. [13] on the impact of the IGF axis on the prostate, we developed a model and started to investigate IGF-1 and its binding proteins [6]. The IGF axis consists of IGF-I, IGF-II, six different binding proteins (IGFBP 1–6), and two receptors, IGF-IR and IGF-IIR. Some investigators also include insulin based on the similarities between it and IGF-I, as well as the similarities between the insulin receptor and the IGF-I receptor. IGF-I is a peptide growth factor produced by the liver and other tissues, and is known to play a pivotal role in regulating cell growth, differentiation, and apoptosis (programmed cell death) [13, 30, 50, 60]. It is a potent mitogen for most tissues including the prostate [31, 44]. According to Yarak et al. [59], 75% of the circulating IGF-I comes from the liver. During growth and development, the amount of IGF-I is determined primarily by growth hormone. After maturation, growth hormone levels decline, and at this point insulin may become more important in determining serum levels of IGF-I. The activity of IGF-I is regulated by the six different binding proteins. IGFBP-3 is the most abundant and binds 90% of the circulating IGF-I but is not affected by different metabolic states. Conversely, IGFBP-1 and to a lesser extent IGFBP-2 are regulated by the metabolic state, being highest following fasting. According to our model (Fig. 2), the lifestyle of most industrialized countries, consisting of a high-fat, refined-sugar diet combined with physical inactivity, leads to insulin resistance and compensatory hyperinsulinemia. Elevated levels of serum insulin impact on the liver to decrease the production of SHBG, IGFBP-1, and IGFBP-2, while increasing the production of IGF-I. The elevated serum insulin might also stimulate estradiol production by fat cells, which would stimulate an increase in IGF-I re-

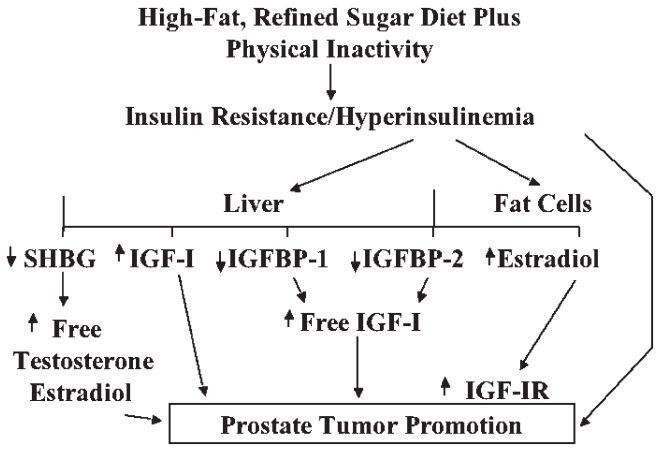

Fig. 2. Proposed model to explain the relationship of diet and exercise to insulin resistance and the development of prostate cancer. (Adapted from Barnard et al. [6])

ceptors in the tumor cells (this needs to be confirmed). The result would be enhanced prostate cancer cell promotion.

Serum levels of IGF-1 and IGFBP-1 are important as IGF-1 has been reported to be a biomarker predicting prostate cancer in prospective studies [9, 20, 48] and has also been reported to be a risk factor in case-control studies [10, 32, 57]. One of the case-control studies also reported that elevated serum IGFBP-1 reduced the risk for prostate cancer [10]. We measured insulin, IGF-I, IGFBP-1, and IGFBP-3 in serum from the diet-and-exercise subjects [33]. Insulin was reduced from 17±4.6 to 12.7±3.3 μIU/ml after just 11 days and was even lower in the long-term diet-and-exercise subjects (5.4±0.46 μIU/ml. IGF-I was reduced from 315±31 to 252±28 ng/ml and was 143±13 ng/ml in the long-term diet-and-exercise subjects. Conversely, IGFBP-1 was increased from 27±7 to 39±9 ng/ml in 11 days and was 69±12 in the long-term subjects. IGFBP-3 was unchanged by the diet-and-exercise program. We then studied apoptosis following serum stimulation of the LNCaP cells using two different methods, TUNEL and Annexin V, and found that the post 11-day diet-and-exercise serum induced apoptosis of LNCaP cells, which was further increased when the cells were grown in the serum from the long-term diet-and-exercise subjects (Fig. 1).

3
Exercise and Prostate Cancer

In an attempt to separate out the individual effects of diet vs exercise observed in our earlier studies with the androgen-dependent prostate cancer

Table 1. Effect of a very low-fat diet and/or intensive exercise on the IGF axis

	Control	Diet and exercise	Exercise
	n=14	*n*=8	*n*=12
Insulin (μlU/ml)	17±4.6	5.4±0.5	6.9±1.0
IGF-I (ng/ml)	315±31	143±13	128±12
IGFBP-I (ng/ml)	22±4	69±12	42±8
IGFBP-3 (ng/ml)	2,606±243	2,662±201	2,610±238

All diet-and-exercise as well as exercise data, except for IGFBP-3, were significantly different ($p<0.05$) from control. IGFBP-1 was significantly higher in the diet-and-exercise compared to the exercise group. Data from Barnard et al. [7]

cell lines, we obtained serum samples from men who had been involved in the Adult Fitness Program at the University of Nevada, Los Vegas (UNLV). The men were matched in age with a control group of men on no diet or exercise program, and for age and duration of participation to the long-term diet-and-exercise subjects previously studied [7]. Volunteers were requested who had participated in the program for at least 10 years; the average was 14.7 years. The program was held 5 days per week for 1 h and consisted of warm-up and flexibility activities followed by 45–50 min of continuous, strenuous exercise including calisthenics and swimming laps in the pool. There was no dietary intervention in the UNLV Adult Fitness Program. Table 1 shows data obtained from the three groups of subjects. Serum insulin and IGF-I were lower in the exercise and diet-and-exercise groups compared to controls, but were not significantly different from each other. IGFBP-1 was higher in both the exercise and the diet-and-exercise groups and was significantly higher in the diet-and-exercise group compared to the exercise group.

When the serum was used to stimulate the LNCaP cells in culture over 2 days, growth was reduced in both the exercise (65% FBS control) and diet-and-exercise (55% FBS control) compared to the Control group, where the growth was 99% of the FBS control (Fig. 3). When IGF-I was added to the exercise and to the diet-and-exercise serum to match the level in the control subjects, the reduction in LNCaP cell growth was eliminated. We then examined apoptosis in the cell cultures after 2 days using Annexin-V and TUNEL. The staining results from the two methods demonstrate that exercise as well as diet-and-exercise intervention increase apoptosis in the LNCaP cells. When the slides from the TUNEL staining were quantitatively analyzed via Adobe Photoshop, we found almost twice as much apoptosis in the diet-and-exercise samples compared to the exercise-only samples (Fig. 3). The fact that apoptosis was higher in the diet-and-exercise samples is in agreement with the significantly higher IGFBP-1 levels compared to the exercise-only group.

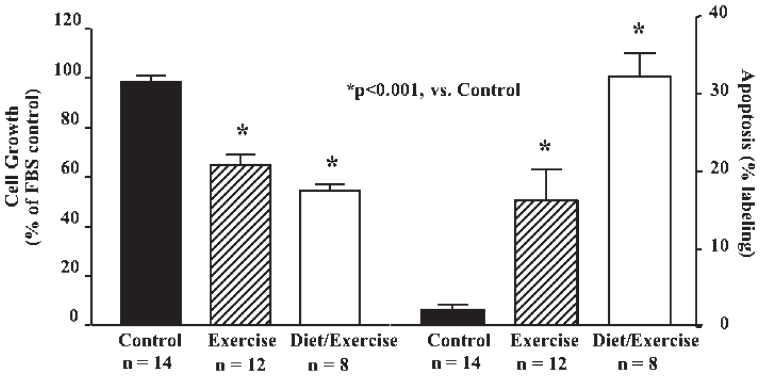

Fig. 3. Effect of a low-fat, high-fiber diet and/or regular exercise on serum-stimulated LNCaP cell growth and apoptosis. (Data from Barnard et al. [7])

In an attempt to elucidate possible mechanisms involved in the reduction in LNCaP cell growth and increased apoptosis observed in the exercise-serum-stimulated samples, we turned our attention to the action of the p53 gene. Several studies have shown that IGF-1 suppresses the action of p53, which normally responds to defects in DNA. With DNA damage, the p53 protein is phosphorylated and stabilized, which activates other genes or factors to cause cell cycle arrest, DNA repair, or apoptosis [17]. LNCaP cells were plated and allowed to attach and stabilize for 24 h. After the stabilization period, fresh serum from the control or exercise groups was added and the cells were allowed to grow for 2 days. The cells were lysed, centrifuged and the supernatant analyzed for p53 protein and for one of its down stream effectors, p21 protein. Both p53 and p21 proteins were significantly increased in the lysates from the exercise-serum-stimulated LNCaP cells compared to controls (Fig. 4). The p53 protein is known to induce an increase in

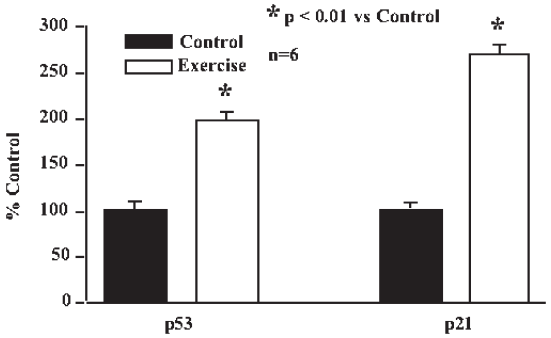

Fig. 4. Effect of regular exercise on serum-stimulated LNCaP cell p53 and p21 protein concentration. (Data from Leung et al. [29])

p21 protein to cause cell cycle arrest. Thus, the increase in p21 is consistent with the reduced cell growth observed in the exercise-serum-stimulated cells. Furthermore, we measured PCNA protein, a marker for cell cycling, and found that it was reduced by 33% in the exercise-serum-stimulated LNCaP cells, which is in good agreement with the 27% reduction in cell growth [29].

The lower serum IGF-I in the exercise group and the increase in p53 protein and apoptosis in the exercise-serum-stimulated LNCaP cells are in agreement with the report of Heron-Milhavet and LeRoith [21] in studies with UV-mimetic-damaged cells. These investigators reported that IGF-I prevented apoptosis through activation of the p38 MAPK pathway in cells following DNA damage. The suppression of apoptosis by IGF-I was associated with a decrease in cellular p53 protein content with no change in p53 mRNA. The decrease in p53 protein was associated with an increase in murine double minute 2 (MDM 2) protein and mRNA. MDM 2 is a RING finger ubiquitin ligase protein that binds to p53 and shuttles it to the cytoplasm for degradation. Our results showing that serum from exercise subjects with very low levels of IGF-I was associated with higher p53 and p21 protein and with a significantly higher amount of apoptosis in LNCaP cells suggests the possible involvement of the p38 pathway. These results also demonstrate that the LNCaP cells have an intact p53 pathway that is suppressed with serum from control subjects. According to Gurumurthy [17], an intact p53 pathway is characteristic of all early stage prostate cancer.

To further investigate the involvement of the p53 pathway in the exercise-serum-stimulated LNCaP cell growth reduction and induction of apoptosis, we utilized another cell line, LN-56. LN-56 is a LNCaP-derived cell line in which p53 was rendered nonfunctional by expression of a dominant-negative fragment of p53, known as genetic suppressor element 56 [46]. The results from the growth assay showed no significant difference between the control and exercise groups when the serum was used to stimulate the cells. The exercise-serum-stimulated growth in the LN-56 cells was 91% of the FBS control compared to 65% of FBS control for the LNCaP cells. When we examined apoptosis in the LN-56 cells, the exercise-serum-stimulated cells showed half the apoptosis observed with the control-serum-stimulated cells. This was opposite to the response observed in the LNCaP cells, where apoptosis was greatly increased in the exercise-serum-stimulated cells [29].

We also did experiments with LNCaP cells using two different types of IGF-I receptor blockers [29, 34]. One was an antibody to the IGF-I receptor and the other a kinase inhibitor, as the IGF-I receptor is a tyrosine kinase receptor like the insulin receptor. When the blockers were added to the control serum, LNCaP cell growth was reduced and apoptosis increased similar to the levels observed with the diet-and-exercise or exercise-alone groups. When the kinase blocker was added to the exercise serum, no additional effect was noted for the LNCaP cell-growth or apoptosis assays. Collectively,

the results from these experiments indicate that the reduction in serum IGF-I and increase in IGFBP-1, resulting from adopting a very low-fat diet and/or regular exercise, allows the prostate tumor cells to stabilize p53 protein and activate downstream effectors such as p21 to reduce cell growth and induce apoptosis. In addition to reducing the risk for PCa, Platz et al. [38] recently reported that regular exercise reduced the risk for BPH, thought to be a precursor of PCa. Unfortunately, they did not measure IGF-I. However, Chokkalingam et al. [11] did report that elevated plasma levels of IGF-I were associated with BPH.

4 Diet-and-Exercise Treatment for PCa

If the observations of reduced cell growth and the induction of apoptosis reported for our cell culture studies with androgen-dependent prostate cancer cell lines also occur in vivo, then a very low-fat diet and exercise program might be of value for the treatment of PCa patients, especially with early-stage cancer. In order to investigate the possible effectiveness of a very low-fat diet and exercise program on prostate cancer patients, Ornish and colleagues [35, 36] randomized a group of men on "watchful waiting" to control or to diet-and-exercise intervention. Both groups received standard medical care from their personal physicians. The patients all had biopsy-documented PCa, prostate-specific antigen (PSA) ranging from 4 to 10 ng/ml, and a Gleason score less than 7 for prostate adenocarcinoma. The men in the diet-and-exercise intervention group were prescribed a vegan diet with 10% of calories from fat supplemented with soy, 3 gm/day fish oil (omega-3 fatty acid) and 400 IU/day vitamin E. The exercise was to be aerobic, walking, jogging, etc. for 30–60 min 6 days/week. They were also encouraged to practice stress management techniques including yoga, breathing, imagery, etc. for 1 h/day.

At the end of 1 year, changes in serum PSA were small but statistically significant, with the control group showing an increase and the diet-and-exercise group showing a drop. Also at the end of 1 year, 6 of 43 men in the control group had gone on for conventional treatment due to rising PSA, while none of the 41 in the diet-and-exercise group had treatment (D. Ornish et al., submitted). There was very high adherence to the diet and exercise program, and even some in the control group had made significant lifestyle changes. We obtained serum samples from these patients to study in our bioassay using LNCaP cells. Compared to baseline, cell growth was reduced by 9% in the control group and by 60% in the diet-and-exercise group at the end of 1 year. The growth rate in the baseline samples was not significantly different from what we had previously observed with serum from men without PCa. The study is progressing with a plan to follow the patients for 5 years.

5 Summary

In summary, the typical lifestyle of most industrialized nations, consisting of a high-fat, refined-carbohydrate diet combined with a lack of physical activity, leads to insulin resistance and compensatory hyperinsulinemia. Insulin itself can be a factor in BPH and PCa, as it is a known mitogen and we demonstrated that it can increase the growth of LNCaP cells in culture. However, the effects of insulin on the liver may be far more important. Insulin suppresses the production of SHBG, resulting in more free testosterone. At the same time, it stimulates the liver to produce more IGF-1, a mitogen for prostate cancer. IGF-1 also protects tumor cells from apoptosis. While stimulating liver production of IGF-1, insulin also suppresses the production of IGFBP-1/2 that should increase the level of free IGF-1 as well. When men from the U.S. who are at high risk for PCa adopt a low-fat diet consisting primarily of whole grains, vegetables, and fruits, with limited amounts of meat, and start regular aerobic exercise for 45–60 min daily, serum factors change that directly impact on prostate cancer cells. The serum changes include reductions in insulin, IGF-I, estradiol, and free testosterone as well as increases in SHBG and IGFBP-1. These serum changes in vivo influence tumor cells in culture to reduce growth and induce apoptosis and are associated with increases in p53 and p21 protein in the tumor cells. The normal function of p53 is to respond to mutations in DNA by activating downstream factors, including p21, to arrest the cell cycle, repair DNA, or induce apoptosis. It is apparent that serum from U.S. men at high risk for PCa suppresses the p53 system. This appears to be the result of IGF-I-stimulating degradation of the p53 protein. With diet and exercise the p53 protein is stabilized to cause cell cycle arrest and induce apoptosis in the prostate tumor cells. This, of course, requires an intact p53 system which, according to Gurumurthy [17], is characteristic of early-stage PCa. Late-stage PCa is associated with mutations in the p53 gene itself and may explain why we observed no effect of diet and exercise on the growth of the PC-3 cell line. Exercise alone induces similar serum changes but to a lesser extent than is observed when exercise is combined with a very low-fat diet. There is also less of an effect on the LNCaP cell growth and apoptosis. These observations with exercise provide a mechanism to explain the epidemiological data reporting a reduction in prostate cancer risk in men who are physically active [27, 51]. Initial studies with men on "watchful waiting" indicate that the changes in prostate cancer cell growth observed in vitro also occur in vivo and may delay the need for aggressive treatment of the disease.

These results with the effects of diet and exercise on insulin and the IGF axis have important implications for not only prostate cancer but also for breast, colorectal, and lung cancer, since IGF-I has also been reported to be a risk factor for these cancers [60]. It does appear as though these cancers are also an aspect of metabolic syndrome and that the typical lifestyle of

most industrialized countries, consisting of a high-fat, refined-sugar diet combined with physical inactivity, may be a major underlying factor for cancer and many other health problems.

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Individualizing Interventions for Cancer Prevention

Michael Pollak

Cancer Prevention Centre, McGill University, 3755 Cote Sainte Catherine Road, Montreal, QC, H3T 1E2, Canada
michael.pollak@mcgill.ca

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Abstract Many cancer prevention strategies are unlikely to provide equal risk reduction in all subjects, but instead are predicted to be particularly useful for specific individuals. An important research challenge is to devise methods for individualization of cancer prevention recommendations, such that particular interventions are assigned to those who will gain the most. Research in this area is at an early stage, but progress that allows rational assignment of specific prevention strategies to particular individuals who will benefit would decrease the cost, minimize the toxicity, and increase the efficacy of interventions intended to prevent cancer.

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1 Introduction

Certain interventions to reduce cancer risk, such as smoking cessation, are universally applicable and do not need to be individualized. However, it is likely that many pharmacological approaches to cancer prevention will ultimately best be employed on an individualized basis, rather than being widely offered to heterogeneous groups of at-risk individuals.

Individualization of therapy must be based on understanding mechanisms of risk and mechanisms of risk-reducing agents. As we are in an early phase of exploring the use of drugs to prevent cancer, knowledge of mecha-

nisms is generally rudimentary, and individualization remains more a research topic than a clinical reality.

This situation differs markedly from other areas of medicine, where pharmacotherapy for prevention is more developed. Consider cardiology, for example. A standard prevention intervention is pharmacotherapy to lower elevated blood pressure. This intervention has clear, demonstrable efficacy, but it is axiomatic that it is applied only to the subpopulation of individuals with hypertension. A clinical trial of use of an antihypertensive applied universally to a population, with a goal of reducing risk of stroke or myocardial infarction, would likely reveal considerable toxicity and limited or no efficacy. The key is to apply the pharmacotherapy specifically to those who will benefit, rather than universally.

In general, major clinical trials for cancer prevention (for example, the finasteride prostate cancer prevention trial [1]) have been applied to large, heterogeneous groups of at-risk individuals, rather than to subpopulations for whom there is reason to believe that a particular drug will have efficacy. In some cases, such as the National Surgical Adjuvant Breast and Bowel Project (NSABP) tamoxifen prevention trial [2], there was an effort to include only women at or above a certain minimum threshold of absolute risk; but this is distinct from selecting subjects for whom there is reason to believe that the intervention will have particular efficacy. The obvious barrier to targeting prevention therapies to particular subgroups where most benefit will be achieved is the current absence of information regarding criteria that would predict benefit.

2 Possible Criteria for Selecting a Prevention Strategy for a Particular Individual

2.1 Absolute Level of Risk

Selecting a prevention strategy for individuals known to be at very high risk is commonplace. An obvious example concerns women with BRCA1 mutations. The precise pathophysiology underlying risk in carriers of this mutation is unclear, and there are no prevention strategies based on reversal of molecular mechanisms that increase risk. Yet we can identify a group of women with a very high lifetime breast cancer risk by mutation detection.

If we consider prophylactic mastectomy as a breast cancer prevention strategy that is unjustified for most women based on adverse aspects vs benefit considerations, but consider the presence of a BRCA1 mutation as evidence of very high probability of cancer, we recognize that in practice it is commonplace to use a prevention strategy unsuitable for most women for a

particular subset of women where the risk–benefit situation is distinct. BRCA1 mutation status may also identify a subset of women for whom tamoxifen is relatively ineffective at reducing risk [3].

At this point in time, research has provided a way to detect a subset of women at particularly high risk related to BRCA1 mutation, but has not yet provided a specific mechanism-based intervention to reduce the risk. This leaves us with a sophisticated molecular tool for assessing risk, but a relatively crude mechanism for risk reduction in individuals identified. The ultimate success would involve the discovery of a particular pharmacological (or gene therapy) intervention suitable for mutation carriers that could be offered specifically to this group, even if this intervention was ineffective for sporadic breast cancer. It is sobering to recognize the challenges involved: the efficacy of an intervention useful only for risk related to BRCA1 mutations might be difficult to detect in a prevention trial of unselected women.

Another example of selection of a prevention method based on absolute level of risk is the use of colectomy for those with certain polyposis syndromes predisposing to colorectal cancer.

The converse situation is rarely considered, but would represent enormous progress: if future research allows us to identify subsets of the population at particularly low risk of cancer (perhaps, for example on the basis of particularly robust DNA repair), then such individuals could be excluded from unneeded prevention interventions.

2.2 Probability of Efficacy

The level of enthusiasm for a specified intervention to prevent cancer for a particular at-risk individual does not relate only to the individual's absolute level of risk. Another obvious factor is efficacy. Returning to breast cancer as an example, a woman, even if at average rather than increased risk, would be more inclined to use tamoxifen if it were known that in her particular case, this agent would achieve a 90% risk reduction. It is likely that benefit of selective estrogen receptor modulators (SERMs) as chemochemopreventives varies between women based on varying mechanisms of risk operating in different women, and possibly also on pharmacodynamic differences.

Recent work in this area shows some promise [4]. A subset of women identified by clinical criteria (including criteria such as height, age at first birth, and age at menarche) as being at particular risk for estrogen receptor-positive breast cancer were found to enjoy increased risk reduction when given tamoxifen as a chemochemopreventive, as compared to unselected women. If confirmed, this represents major progress towards the rational use of SERMs in breast cancer chemoprevention.

If relatively crude clinical criteria are able to predict subsets of women who experience more or less benefit of SERMs in breast cancer chemopre-

vention, more quantitative biological markers might achieve even better discrimination. Examples include breast density [5–7], bone density [8, 9], and circulating estrogen levels [10]. All of these have been associated with breast cancer risk, but have not been rigorously evaluated as candidate predictors of efficacy of breast cancer risk reduction by SERMs. Various genetic polymorphisms (for example [11, 12]) also deserve study as candidate predictors of the benefit of particular prevention interventions.

Since many chemochemopreventives influence insulin-like growth factors [13, 14], research is underway to determine if changes in circulating analytes related to this area of physiology induced by prevention interventions might predict efficacy. It would obviously be useful if any biomarker that serves as a surrogate for successful chemoprevention could be used to restrict long-term administration of an agent to the subset of the population who will benefit. Where mechanisms of action are incompletely understood, it is possible that predictors of efficacy may be impossible to identify, but that an intervention could be widely offered, and then continued only in those individuals where the change in biomarkers predictive of benefit is achieved.

The finasteride prostate cancer prevention trial [1] may yield information that will allow definition of criteria for targeting of this agent to specific men who would experience particular benefit. This trial assembled a large biorepository that will facilitate such investigations. One example in this context concerns investigation of the significance of polymorphisms of genes encoding proteins involved in finasteride action. 5-Alpha reductase converts testosterone to dihydrotesterone and is inhibited by finasteride. It is possible that polymorphic variants of this enzyme are more or less susceptible to finasteride inhibition, and this would be related to efficacy of the agent as a chemochemopreventive agent, quite apart from the separate issue that such variation may also influence the baseline risk of prostate cancer [12, 15].

2.3 Probability of Toxicity

Another important issue to consider when recommending a particular prevention strategy to an at-risk individual relates to heterogeneity within populations with respect to tolerability of agents. If the risk of adverse effects is stochastic or idiosyncratic, this cannot easily be used as a guideline. On the other hand, some risks (such as the risk of thromboembolic events among tamoxifen users) may be estimated by clinical or laboratory assessments. In view of the need to carefully balance risks and benefits of long-term treatments given to healthy individuals, probability of adverse effects could be an important basis for decision-making. It is noteworthy in this regard that the Italian tamoxifen prevention trial [4] was designed only for hysterectomized women. This represents a certain way to exclude uterine adverse effects, but

narrows the proportion of women who might benefit from whatever risk reduction tamoxifen can achieve. If a molecular marker associated with the risk of tamoxifen-induced endometrial cancer existed, it certainly would be justified as part of the decision-making process in weighing pros and cons of use of this agent.

In practice, decision-making sometimes involves extremes in risk or in risk of adverse effects (e.g., mastectomy for BRCA1 mutation carriers, or tamoxifen for a woman with a recent pulmonary embolism), but more often involves a more subtle blend of potential health benefits and risks [16]. Integration of considerations related to absolute risk, expected efficacy, and risk of adverse effects will be the key to making rational decisions for individuals.

3 Preclinical Models

Little work has been done to attempt to model heterogeneity of benefit of cancer risk-reduction strategies in the laboratory. In order to explore in an experimental setting the hypothesis that a chemochemopreventive strategy may vary in its efficacy between individuals, we are now employing an experimental system based on two different mouse strains, known to vary with respect to their bone density and their circulating insulin-like growth factor (IGF)-I concentration [17].

C3H mice were observed to have circulating IGF-I levels of 560 ng/ml, while C57BL mice have levels of 380 ng/ml. Interestingly, the mice with the higher IGF-I had higher bone density [17]. Each of these has been associated increased breast cancer risk in women [9, 18].

We employed a chemical carcinogen challenge (dimethylbenzanthracene, DMBA), using an experimental protocol similar to that we previously described [19]. We used tamoxifen as a chemochemopreventive agent. We obtained results suggesting that the benefits of tamoxifen do indeed vary according to mouse strain. Mice with higher levels of IGF-I showed a modestly higher incidence of cancer following DMBA exposure. The reduction in tumor incidence achieved by tamoxifen was greater in low IGF-I mice (84% reduction) than high IGF-I mice (30% reduction, p=.05). If confirmed, this early result suggests that efficacy of tamoxifen as a chemochemopreventive may vary with circulating IGF-I concentration and bone density.

The preliminary results from the model system suggest that risk reduction may be greatest where baseline risk is already relatively low. This is plausible biologically, but cannot be extrapolated to humans, due to complexities that are inaccurately modeled in murine models. For example, in humans, tamoxifen use appears to lower IGF-I levels [13]. Obviously, a prevention intervention that is particularly effective for those at higher risk would be most useful.

4 Conclusion

Large clinical trials of pharmacological strategies to reduce cancer risk should not be designed simply to measure the average risk reduction among all treated subjects. A result formulated as "reduction of relative risk by 30%" clearly is an important achievement. Yet such a result in a chemoprevention trial does not necessarily imply that all participants experienced equal risk reduction—a significant proportion of subjects may experience no benefit whatsoever from a specific chemoprevention agent, while another subset may have experienced very substantial risk reduction. It is likely that chemoprevention benefit would be maximized and adverse effects minimized by using these trials to identify predictors of benefit or lack of benefit among subjects.

In order to address this ambitious objective, it will be necessary to collect DNA, serum, and/or results of ancillary information such as bone density from all subjects. On the completion of a trial, this will allow testing various hypotheses regarding hormonal, genetic, or other criteria proposed to define subgroups who stand to gain the most (or the least) from the specific therapy under study. While it is possible that a chemoprevention strategy may be found that reduces risk among all subjects, it is perhaps more realistic to recognize that benefits may vary among subjects, and that many interventions should ultimately be offered to subsets of at-risk individuals, rather than universally [20].

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Can Animal Models Help Us Select Specific Compounds for Cancer Prevention Trials?

Ernest T. Hawk (✉) · Asad Umar · Ronald A. Lubet · Levy Kopelovich · Jaye L. Viner

GI and Other Cancers Research Group, National Cancer Institute, Suite 2141, 6130 Executive Boulevard, Bethesda, MD 20892-7317, USA
hawke@mail.nih.gov

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Abstract Animal models provide unparalleled mechanistic insights into cancer development and potential opportunity for cancer prevention. Nevertheless, species differ markedly with regard to dietary exposures, cancer development, drug effects, and toxicity thresholds; therefore, testing in a single animal system may not predict human responses. Although replication of human cancer in animal models remains inexact, more than two decades of research have clearly yielded significant gains, as is evident in agents tested—and in certain cases, approved—for the prevention of epithelial cancers. Research efficiencies achievable through preliminary testing in genetically engineered and carcinogen-induced animal models enable us to probe genetic and signaling pathways that drive normal and neoplastic processes, and thereby figure prominently in decision trees for agent development.

1 Introduction

Progress in molecular cancer prevention, or cancer chemoprevention, is founded upon our understanding of the prolonged process of carcinogenesis that occurs prior to cellular invasion across the basement membrane, which pathologists regard as the defining event for cancer. Although technologic in-

novations in biologic imaging and molecular analyses are providing insights into the character of preclinical carcinogenesis; analysis, interpretation, and integration of these data into actionable information remain challenging. In particular, the clinical utility of these data must be efficiently tested and transformed into preventive strategies that halt, reverse, or retard cancer development. Human trials are a major rate-limiting step in transforming scientific premises into clinical tools, typically requiring vast investments of time, participants, and fiscal resources. As a result, the National Cancer Institute (NCI) has developed guidelines to optimize agent identification and prioritization for clinical testing. These criteria weigh the relative merits of data derived from preclinical model systems and various clinical contexts, that might provide objective guidance for the identification and prioritization of promising candidate agents. Because of the relative simplicity of in vitro, in vivo and ex vivo studies, these models are often used to gain preliminary insight into novel targets and as mechanistic probes for agent screening. Promising dose–response and dosing data from animal carcinogenesis models—testing single agents or agent combinations—is an important, yet too-often-overlooked, step in drug development schemes for cancer prevention. Consistent correlations between preclinical and clinical data have supported the utility of certain models—including carcinogen-induced models—in identifying and prioritizing promising compounds for cancer prevention.

Of course, no single animal model of cancer is an exact surrogate for human disease and all its multiple patterns. For example, human lung cancer has many different histologies (i.e., adenocarcinoma, squamous and small cell carcinoma). Obviously, an animal model that exhibits only one of these histologies cannot reflect all the variations and nuances of human carcinogenesis. Similarly, breast cancer gene array analysis has identified four or five distinct profiles that are strongly associated with estrogen receptor (ER) status. Again, no single model of breast cancer accurately reflects this pathophysiologic array of human disease [72].

The ideal animal model of human carcinogenesis is often debated and progressively approached, but never fully realized. First and foremost, such a model would be expected to mimic human carcinogenesis in terms of its genesis and biologic progression (Table 1). In addition, it would be immunologically intact, easily bred, inexpensive, and reproducible. To extend the model's usefulness in predicting the chemopreventive potential of promising agents, several additional subtle yet significant criteria must be satisfied (Table 2). The most important criterion would be its responsiveness to a spectrum of agents (e.g., hormonal/trophic agents, small molecules, vaccines, etc.) with known clinical preventive efficacy, as reflected by demonstration of high positive predictive value across various models. Beyond this, the animal model would be considered more useful and predictive if it also reproducibly recapitulates human carcinogenesis across the neoplastic spectrum (e.g., from the molecular to organ levels) following similar environmental

Table 1. Features of an ideal animal model of human carcinogenesis

Human Carcinogenesis	Animal Models
Environmental influences	Environmental influences
Genetic defects (mutations/pathways)	Genetic defects (mutations/pathways)
Epigenetic changes	Epigenetic changes
Changes in mRNA	Changes in mRNA
Changes in proteins	Changes in proteins
Biochemical expression	Biochemical expression
Histopathologic changes	Histopathologic changes
Organ level expression	Organ level expression
Precancer/cancer/metastasis	Precancer/cancer/metastasis
	Intact immune system
	Simple breeding
	Reproducible with low variance
	Quickly analyzable
	Continuous readout
	Inexpensive
	Limited patents/royalties

Table 2. Features of an ideal animal model for predicting chemopreventive agent efficacy

Animal Models for Chemopreventive Efficacy
Similar exposure to administered agent
Delivery
Absorption
Distribution
Serum/plasma concentrations
Metabolism
Excretion
Unintended effects (toxicities)
Spectrum
Severity
Incidence
Duration
High positive and negative predictive values (i.e., responsive to known positive and resistant to known negative agents at each step of neoplastic development)
Hormonal/trophic influences
Vaccines
Small molecules
Gene modifiers
Dose responsive
Reproducible with low variance
Quantifiable

exposures and in a reasonably quantitative and dose-responsive manner. Finally, a well-validated model would be associated with a high negative predictive value, reflecting its ability to distinguish agents likely to be ineffective in humans.

Data from cancer prevention in vivo studies and clinical trials are mutually informative, but are still somewhat limited owing to the relative immaturity of both disciplines. Animal model development for chemopreventive agent identification, in particular, is significantly hampered by the paucity of clinical cancer prevention data against which such models might be evaluated. Nevertheless, over the last decade more than ten chemopreventives have been shown to have clinical efficacy, although some are marketed as treatments for intraepithelial neoplastic lesions or treatments to reduce cancer risk, rather than as agents to prevent cancer per se [81]. As expected, relatively accessible organs—such as the skin, esophagus, colorectum, and bladder—have distinct advantages in terms of the time and resources required to demonstrate agent efficacy. Although most chemopreventive agents approved thus far have been developed and approved in these organs, recently progress has been accelerating in more challenging organs, such as the breast and prostate. Despite these promising advances, as noted above, no single animal model yet mimics the full range of human carcinogenesis and/or agent responsiveness. Back-validation of animal models using clinically effective as well as ineffective agents will guide us in optimizing and selecting appropriate models in which to test promising chemopreventives.

2 Lung Cancer Models

The first chemoprevention trials were initiated in the 1980s. At that time, chemoprevention—in fact, cancer prevention itself—was a somewhat novel concept. Agent selection/prioritization in these "first generation" cancer chemoprevention studies was rather crude, and the lung cancer trials testing beta-carotene have provided cautionary lessons.

Early observational studies identified inverse associations between dietary intake of fruits and vegetables and the development of lung cancer, in certain cases implicating beta-carotene as the most active dietary component [29]. Owing to these promising epidemiologic leads, two large randomized, placebo-controlled, phase III trials tested the chemopreventive efficacy of beta-carotene supplementation in patients at elevated risk for lung cancer [1, 48]. Contrary to the prevailing hypothesis, patients randomized to beta-carotene had 18%–28% increased risk of developing lung cancer and 15%–46% increased risk of lung cancer-associated mortality. These data prompted concerns that there may have been insufficient preclinical and/or early phase clinical data to support these large and costly trials [84]. In fact, there had been a few preclinical studies. For example, all-*trans*-retinyl acetate was shown to induce moderate reductions in metaplastic lung nodules in mice administered intra-tracheal 3-methylcholanthrene [11, 44, 68]. Although these results potentially corroborated human observational data, the links

were tenuous—involving as they did fundamentally different agents (i.e., fruits and vegetables vs a retinoid vs beta-carotene) and endpoints (i.e., cancer incidence vs metaplasia vs regression). Nevertheless, phase III demonstration of increased lung cancer incidence in two clinical trials inevitably trumped the hypothesis-generating preclinical work by Saffiotti and Nettesheim, and cast doubt on the validity of the 3-methylcholanthrene tracheal model as predictive of the chemopreventive efficacy of test agents against human lung cancer.

Preclinical data more consistent with study results emerged only after these large beta-carotene trials had been initiated. In 1991, Castonguay and colleagues reported null effects of beta-carotene and retinol against 4-(*N*-nitrosomethylamino)-1-butanone (NNK)-induced lung tumors in A/J mice [8]. Similarly, Murakoshi demonstrated reductions in tumor multiplicity in 4-nitroquinoline-1-oxide (4NQO)-treated mice with alpha– but not beta–carotene [38]. Finally, studies published in 1998 and 2002 showed that beta-carotene failed to modulate NNK-induced or tobacco smoke-induced lung tumors in A/J mice [10, 47]. Viewed retrospectively, preclinical data on beta-carotene derived from carcinogen-treated rodents neither supported its testing in definitive lung cancer prevention trials nor predicted its harms. In lung cancer chemoprevention, definitive evaluation of the A/J mouse or other animal models still awaits the identification of clinically effective agents. To that end, a series of preclinical experiments evaluating cancer incidence in the context of tobacco exposure followed by cessation using a widely published model [47] may be a useful first step in validating current animal models, given that smoking cessation clearly reduces lung cancer risk in humans over the long term [52].

3 Breast Cancer Models

Several rodent models of breast cancer have been developed—including early carcinogen (i.e., *N*-methyl-*N*-nitrosourea, MNU; 7,12-dimethylbenzanthracene, DMBA; ethyl methanesulphonate, EMS)-induced rat models, radiation-induced rat models, and genetically driven murine models [65]. Comparing human breast and MNU-induced mammary carcinogenesis models, the rodent system provides a reasonable approximation of ER-positive, but not ER-negative disease (Table 3). The predictiveness of this animal model for agent efficacy in humans is somewhat limited by the scarcity of clinically proven agents. Even so, the preventive efficacy of anti-estrogens (e.g., selective estrogen receptor modulators [SERMs]) has been consistently and broadly documented—most conclusively in five large, randomized, placebo-controlled clinical trials involving women at average or elevated risk for breast cancer [13, 14, 17, 54, 80]. Two of these five trials demonstrated statis-

Table 3. MNU-induced rat mammary tumors vs human breast cancers

Characteristic	Human ER+	Human ER−	Rat MNU	Reference
ER positive	Yes	No	Yes	[67]
Site of origin	TDLU	?	TEB	
Invasive	Yes	Yes	Minimally	
Pregnancy	Decreases	No effect	Decreases	
Response to hormonal therapies				
SERMs	+++	−	+++	[66]
Ovariectomy	+++	−	+++	
Aromatase inhibitors	+++	−	+++	
Ras mutation	Rare	Rare	50%	[36]
p53 mutation	30%–40%	60%	Rare	

SERM, selective estrogen receptor modulator; TEB, terminal end buds; TDLU, terminal duct lobular units; ? unknown.

tically significant 32%–49% reductions in breast cancer incidence with tamoxifen [14, 17]; and another suggested up to 65% reductions with raloxifene [13].

Striking correlations emerge from cross-model comparisons of the predictability of these human data for murine responses. As early as 1976, Jordan demonstrated that tamoxifen significantly reduced tumor initiation and growth in a DMBA-induced rat mammary model [25]. Following this initial demonstration, tamoxifen's efficacy was confirmed in DMBA [23, 26], NMU [20, 57], EMS [79], gamma-irradiation [85], and spontaneous [37] rodent mammary cancer models, validating their relevance to human responsiveness in studies of this chemopreventive agent or agent class. In addition, these models suggest other aspects of chemopreventive efficacy associated with tamoxifen. First, tamoxifen has been shown to have preventive as well as therapeutic efficacy [49, 62]. Second, the effects of tamoxifen wane upon agent cessation (although recent data suggest that raloxifene effects may be relatively more durable) [9, 64]. Third, these models provide insights into tamoxifen's activity against the entire spectrum of breast carcinogenesis [31]. Finally, these models demonstrate that tamoxifen reduces both tumor incidence and multiplicity [26]. Because tamoxifen inhibits the trophic influence of estrogenic hormones, this strategy may explain its efficacy against breast carcinogenesis—at least in the context of ER-positive disease (and possibly other organs as well).

More recently, aromatase inhibitors have been shown to improve disease-free survival among post-menopausal women treated in the adjuvant setting for breast cancer [19]. Importantly, DMBA- [7] and MNU-induced [34, 35] mammary models have also been shown to respond to aromatase inhibitors.

4
Prostate Cancer Models

Several animal models of prostate cancer have emerged over the last two decades or so [6]. These models largely involve carcinogen exposure or genetic manipulation and, unlike other cancers, often require promotion by testosterone. Recent data on finasteride lay a foundation for evaluating the predictiveness of animal models for human prostate carcinogenesis. The Prostate Cancer Prevention Trial randomized 18,882 men with a normal digital rectal exam and PSA level to finasteride 5 mg/day or placebo for 7 years [75]. Men randomized to finasteride were shown to have a 24.8% reduction in clinically evident prostate cancer over this period, but they also had a statistically significant increase in their Gleason scores. Although the clinical implications are uncertain, these results invite back-validation of finasteride in various animal models of prostate cancer in order to identify systems that may have predicted this human response.

Four preclinical studies have evaluated the chemopreventive potential of 5-alpha reductase inhibitors [15, 24, 77, 78]. In the spontaneous prostate cancer rat model (ACI/Seg rats), FK143 demonstrated mild preventive activity at low doses, but no dose response per se [24]. Tsukamoto reported dose-dependent reductions in macroscopic cancers in F344 rats administered DMBA and testosterone [77, 78], and Esmat demonstrated that finasteride induced up to 80% reductions in cancer incidence among male Wistar rats exposed to MNU and testosterone [15]. These carcinogen-driven animal models have served reasonably well as preclinical screens for certain prostate cancer chemopreventives, they have limited capacity to mimic histopathologic details of prognostic importance [70]. Although models of prostate carcinogenesis have improved over recent years, they still do not spontaneously develop prostate cancer. Nevertheless, newly developed models display early androgen-sensitive and late androgen-insensitive traits consistent with human neoplasia [53].

5
Colorectal Cancer Models

Animal models of colorectal cancer have followed two major developmental pathways. Since the 1970s, chemical carcinogens such as azoxymethane, 1,2-dimethylhydrazine, *N*-methylnitrosourea, and methylazoxymethanol acetate [58] have been used to induce cancers, often in more than 90% of exposed rodents. The other dominant cancer model has involved genetic manipulation. While disruptions of certain genes (e.g., *SMAD*, *MSH*-2, *MLH*-1, etc.) in mice may be sufficient to induce neoplasia, the most commonly used models target the *APC* gene, which controls colonic epithelial proliferation

and is compromised in more than 90% of human colorectal cancers. Examples include the Min mouse and other related models that harbor an $Apc^{\Delta 850}$ (Apc^{MIN}) mutation; or Apc^{1638} or $Apc^{\Delta 716}$ mice [28].

Interestingly, another model has been shown to induce spontaneous colorectal tumors in mice administered a "Western diet" that is low in calcium and fiber, and high in phosphorous and fat [33]. Corroborative data from multiple clinical trials show that calcium and aspirin reduce the recurrence of colorectal adenomas in patients at elevated risk due to prior colorectal neoplasia [21]. As early as 1988, Pence and Buddingh reported that F344 rats exposed to dimethylhydrazine were significantly protected by supplemental calcium or vitamin D administered in the context of high-fat diets, but not low-fat diets [50]. Following this, several other investigators confirmed protective effects of calcium supplements in carcinogen-treated rats [4, 27, 71, 83].

With regard to aspirin, sulindac, or other cyclooxygenase (COX) inhibitors, abundant clinical data suggest preventive effects against colorectal neoplasia [22]. Sulindac and celecoxib have been shown to regress prevalent colorectal neoplasia in patients at high risk for colorectal cancer in four randomized placebo-controlled trials [18, 30, 46, 73]. In addition, three randomized placebo-controlled trials have demonstrated aspirin's efficacy in preventing recurrent adenomas in patients at moderately high risk of colorectal neoplasia [3, 5, 69]. The chemopreventive efficacy of COX inhibitors in animal models of colorectal cancer was first demonstrated by Narisawa in 1981 [42]. Subsequently, more than 100 confirmatory studies have been published using different models (i.e., carcinogen driven, genetically induced, Western diet induced), COX inhibitors (e.g., indomethacin, aspirin, sulindac, piroxicam, rofecoxib, celecoxib, NO-releasing nonsteroidal anti-inflammatory drugs NSAIDs, etc.), exposure times and/or endpoints. Strong and consistent data from more than 90% of these studies show significant protective effects that are highly concordant with effects observed in humans. As a result, colorectal animal models are arguably the most robust preclinical system developed to date for cancer prevention research. Corpet et al. recently published a detailed review of this topic, and generated a Web site cataloguing animal data ranked by agent potency [12]. Resources such as this provide a systematic inventory of animal models that might be used for chemopreventive agent development for colorectal and other cancers.

6
Developments and Future Directions

6.1
Combination Approaches

Research has consistently shown the superiority of combination regimens over monotherapy for the management of chronic illness (e.g., cardiovascular disease, AIDS, tuberculosis and cancer). Combination approaches targeting molecular alterations that initiate or sustain carcinogenesis are expected to halt, reverse, or hinder progression to cancer, potentially using lower doses and with a lower risk of adverse effects. Striking additive or synergistic efficacy has been demonstrated in various animal models using agents with demonstrated chemopreventive properties. Strategies to efficiently prioritize promising agents include weighing data from mechanistic (i.e., in vitro and in vivo testing), toxicologic, observational, pharmacokinetic, and human studies. Co-administration of chemopreventive agents with synergistic or additive efficacy may improve a regimen's therapeutic index by allowing dose reductions of one or more agents. This principle has been amply demonstrated using combinations of agents with different mechanisms of action, such as NSAIDs co-administered with difluoromethylornithine (DFMO) [45, 56, 60], an epidermal growth factor receptor (EGFR) inhibitor [76], a matrix metalloproteinase (MMP) inhibitor [82], or statins [55], all of which markedly reduced intestinal tumors in mouse and rat models.

Impressive reductions have also been achieved in rat mammary carcinogenesis with retinoid/anti-estrogen combinations [2], and in skin carcinogenesis with an NSAID/DFMO combination [16]. On the strength of data such as these, combination approaches are currently being tested in several phase II cancer chemoprevention trials (e.g., DFMO plus the NSAID sulindac in persons at risk for colorectal cancer, and fenretinide plus tamoxifen in women at risk for breast cancer). Preliminary testing in animal models has yielded promising data on innovative approaches that combine pharmacologic and nutritional (i.e., high- and low-risk diets) regimens [59]. Finally, regional and/or low-dose therapies applied with preventive intent or chemopreventives applied as "cellular sensitizers" for traditional agents may also improve the therapeutic index of promising therapies [59].

6.2
Microarrays and Proteomics

Advances in genomics, proteomics, and metabolomics are expected to usher in high-throughput technologies for preclinical research and vice versa. This experimentation, although still in the developmental stages, is likely to expose key factors that define gene–environment interactions at the molecular,

individual, and population levels. Furthermore, this research is likely to define cancer subtypes that are more likely to respond to particular treatments or harbor significantly different prognostic properties [51, 72].

Properly applied, genomic and proteomic technologies will enable us to decipher the molecular profiles of animals likely to respond to test agents and/or serve as surrogate endpoint biomarkers (e.g., as drug targets or markers of drug response) in chemoprevention trials. Expression microarrays, for example, might expose molecular patterns predictive of disease, agent response, and toxicity from thousands of data points acquired from a single experiment. Molecular expression that is modulated by a certain intervention may unmask agent effects on signal transduction pathways (both good and bad), providing critical insights into potential agent toxicities and efficacy in humans [74]. Proteomic approaches may also help us identify potential biomarkers of cancer risk or surrogate endpoint biomarkers for cancer development. Although the applications of high-throughput genomic and proteomic technologies are still more theoretical than practical, in the near future they are expected to improve the design and accelerate the pace of preclinical and clinical research in the field of cancer prevention [32, 86].

Translational researchers anticipate developing regimens customized to each patient's genetic and molecular susceptibilities. Genetically ablated animal models may facilitate this effort, by exposing the genetic basis of carcinogenesis and susceptibility to toxicities, thereby expediting rational drug development. Refinements and selections in animal modeling will allow for a better comparison with human disease and responses to intervention [63]. Whether or not animal models will be able to predict for human cancer subtypes and susceptibilities prior to the advent of invasive disease remains a compelling research question.

7 Conclusion

Animal models provide important mechanistic insights into neoplastic transformation and carcinogenesis, and have already proved highly informative and feasible—technically as well as financially. The challenges of animal modeling are illustrated by the often-cited example of thalidomide, a tranquillizer marketed in the 1950s and early 1960s for pregnancy-induced morning sickness. Thalidomide-associated teratogenicity was only recognized in the 1960s after the drug had already caused an epidemic of congenital malformations worldwide. These severe birth defects had only been predicted by one rabbit strain (i.e., the New Zealand white rabbit—and only at doses 25 times higher than those given to humans) and a few primates (at doses 10 times higher than those given to humans). Three decades later, thalidomide received U.S. FDA restricted approval for the treatment of erythe-

ma nodosum leprosum, a debilitating skin complication of Hansen's disease (i.e., leprosy), and in 2003 it received landmark approval in Australia for the treatment of relapsed, refractory multiple myeloma. Wider applications of thalidomide are currently being explored (e.g., angiogenesis-dependent neoplasias, inflammatory diseases, AIDS cachexia, and Behcet's disease) [61]. The thalidomide tragedy of the mid twentieth century—and the drug's rehabilitation in the twenty-first—can be interpreted in different ways. At one illogical extreme, it demands researchers know what they're looking for before they find it. Alternatively, it illustrates the complexity and challenges involved in selecting models predictive of specific outcomes of interest.

Work by early colorectal chemoprevention researchers such as Narisawa and Reddy provides historical perspective for the developmental interplay between animal and clinical studies. In the 1980s they tested the chemopreventive efficacy of oral indomethacin [42, 43] and regional 5-fluorouracil (5-FU; intrarectal) [40] in carcinogen-exposed rats, documenting reductions in large bowel cancer incidence and burden. They later proved the preliminary efficacy of statins [41] and agent combinations [39, 60] in animal models. The 10- to 15-year latency between these promising leads and the initiation of clinical testing underscores the extent to which human trials have been the rate-limiting step in agent development. Limitations of animal models do not negate their critical role in agent development, nor should they hamper their incremental development and improvement. As increasing numbers of therapeutic and preventive technologies emerge, representative animal models are increasingly needed to prioritize among the many agents eligible for clinical testing. Accelerating advances in drug research make innovations in animal modeling both mandatory and inevitable.

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Problems with Using Biomarkers as Surrogate End Points for Cancer: A Cautionary Tale

Arthur Schatzkin

Nutritional Epidemiology Branch, Division of Cancer Epidemiology and Genetics, National Cancer Institute, 6120 Executive Blvd., Suite 320, Room 3040, Rockville, MD 20592-7232, USA
as17h@nih.gov

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Abstract Investigations employing surrogate cancer end points are especially attractive because they may be smaller, shorter, and cheaper than comparable studies with explicit cancer outcomes. For many potential surrogate end points—epithelial cell proliferation will be taken as an example—inferences are problematic because of the existence of alternative causal pathways to cancer that bypass the surrogate end point. Evaluating potential surrogates requires information on the following three questions: (1) What is the relation of the surrogate end point to cancer? (2) What is the relation of the intervention (or exposure) to the surrogate? (3) To what extent does the surrogate end point mediate the relation between intervention (exposure) and cancer? Data for these questions may derive from animal experiments, human metabolic studies, observational epidemiologic investigations (including ecologic studies), and randomized trials. Inferences to cancer from such downstream markers as colorectal adenomatous polyps and persistent human papillomavirus infection of the cervix are strong, though not absolutely unassailable. For all but these very-close-to-cancer markers, considerable caution is warranted in extrapolating from surrogate effects or associations to cancer.

Biomarkers can serve three valuable roles in cancer prevention research:

1. They can enhance the biologic plausibility of a particular hypothesis. The recent findings that alcohol intake raises estrogen levels in women [1] provide a plausible physiologic process that could account for the alcohol–breast cancer association observed in many epidemiologic studies [2].
2. Biologic markers of genetic susceptibility may "sharpen" or augment the credibility of a hypothesis.

a. *Sharpening relative risk*. Suppose a given exposure operates only among those with a particular allelic variant of a gene encoding a metabolizing enzyme. Elucidation of the exposure–gene interaction may be critical to observing the relation between the exposure and disease. Stratifying a study population, for example, among those with and without the pertinent metabolizing gene allelotype may reveal a relative risk that would otherwise be obscured in an analysis of the population as a whole.

b. *Enhancing exposure specificity*.

i. *Unraveling mixtures*. Suppose an association exists between a mixture (of foods, industrial agents, etc.) and cancer. If an interaction is observed between the mixture and the gene for a (known) specific metabolizing enzyme, this provides etiologic plausibility for the specific chemical component metabolized by that enzyme.

ii. *Ruling out confounding factors*. For many "lifestyle" exposures the relative risks for cancer are weak to moderate, and confounding is difficult to rule out. Alcohol and breast cancer is an example: the relative risk for moderate alcohol consumption is in the neighborhood of 1.2–1.3 and it is entirely possible that women who drink differ from those who do not in one or more factors that are truly causal for breast cancer. If there were an interaction between alcohol intake and a gene (like ADH3) involved in ethanol metabolism, such that, for example, those who metabolize ethanol more slowly have a qualitatively higher risk of breast cancer compared to more rapid metabolizers, this suggests that it is the alcohol itself, not some confounder, that is a cause of disease (unless the confounding agent is also metabolized by that gene—a not particularly likely scenario).

c. *Mendelian randomization—an antidote for confounding and measurement error* [3]. Certain genetic variants can be viewed as mimicking low or high exposure. In that vein, for example, MTHFR 677TT [4] or HFE [5], respectively, can be viewed as mimicking low folate or high iron exposure. If one of these genetic variants is associated with cancer, this provides independent evidence that the exposure is related to cancer. Because of the random assortment of alleles, this association is not likely due to confounding (though this cannot be ruled out entirely). Moreover, although dietary factors may be assessed with considerable error [6], measurement error is a minimal issue in the genetic association.

3. Finally, biomarkers may serve as surrogates for cancer in epidemiologic studies and clinical trials. This third role is discussed in this article.

1 Why Surrogate End Points?

The occurrence of cancer in humans is a relatively rare event. In the United States, for example, the annual age-adjusted incidence of breast cancer among women is about 100 per 100,000, or 0.1%; the incidence of colorectal cancer among men and women combined is around 50 per 100,000, or 0.05%. Because the incidence of cancer is relatively low, clinical trials and epidemiologic studies must be very large and lengthy (and therefore expensive) in order to accumulate enough cancer cases for meaningful analysis. Studies with surrogate end points are attractive because they can be smaller, faster, and cheaper than those with explicit cancer outcomes.

2 Examples of Potential Surrogate End Points for Cancer

The following are different types of potential surrogate end points for cancer, with specific examples:

- *Tissue*: adenomas; intra-epithelial neoplasia (IEN)
- *Cell*: proliferation, apoptosis
- *Molecular*: DNA adducts, DNA strand breaks
- *Clinical*: imaging end points (e.g., ovarian ultra-sound, mammographic densities)
- *Infection*: human papilloma virus (HPV) infection
- *Blood or urine analytes*: serum or urinary estrogen, prostate-specific antigen (PSA)

3 What Constitutes Surrogate End-Point Validity?

A useful definition of a surrogate end point appears in a preamble to a proposed accelerated approval rule for drugs, from the United States Food and Drug Administration: "A surrogate end point, or 'marker,' is a laboratory measurement or physical sign that is used in therapeutic trials as a substitute for a clinically meaningful end point that is a direct measure of how a patient feels, functions, or survives, and is expected to predict the effect of the therapy" [7]. The essential point, for this article, is that a study of a given intervention (or exposure) in relation to a surrogate end point gives the right answer about the relation of the intervention (exposure) to cancer.

For a surrogate end point to be valid, three conditions must be met:

1. The surrogate is associated with cancer. [The relative risk (RR) is a standard epidemiologic measure of this association.]
2. The exposure is associated with the surrogate. [Relative risk or correlation coefficient (RR, *r*) can be used to reflect this association.]
3. The surrogate mediates the association between exposure and cancer. (The attributable proportion [8] is an indicator of mediation.)

Two examples of the mediation criterion follow [9]:

1. HPV and number of reproductive partners and cervical cancer (Table 1).

Table 1. Number of sexual partners and the risk of cervical dysplasia

	Number of sexual partners				
	1	2	3–5	6–9	>10
Odds ratio					
Unadjusted	1.0	1.7	3.1*	4.7*	4.4*
Adjusted for HPV status	1.0	1.0	1.1	1.5	1.6

HPV, human papillomavirus.
*$p<0.05$.

2. Estradiol and body mass index (BMI) vs breast cancer [10].

Adjusted for free estradiol	RR [95% confidence interval (CI)] for BMI increase of 5 kg/m^2
No	1.19 (1.05–1.34)
Yes	1.02 (0.89–1.17)

Validity is pretty assured for surrogates both necessary for and relatively close, developmentally, to cancer (e.g., CIN3). For other potential surrogates, uncertainty reigns: it is possible to be misled by the existence of alternative pathways to cancer that bypass the surrogate marker (e.g., cell proliferation, as Fig. 1 illustrates).

In the case of hyperproliferation as a potential surrogate end-point biomarker, because an exposure may operate through an alternative pathway (apoptosis) that offsets the pathway through hyperproliferation, hyperproliferation may give the wrong answer about an intervention agent's effect on colorectal cancer in two ways. First, if an intervention reduces (or is associated with lower) proliferation but at the same time reduces apoptosis, then it could have no true effect on colorectal cancer. Second, an intervention

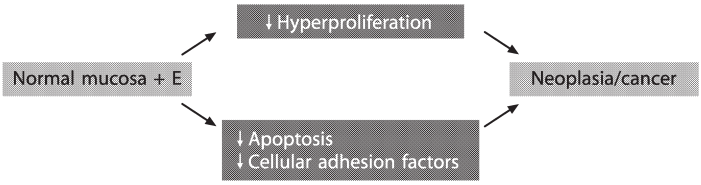

Fig. 1. Alternative pathways from normal colorectal epithelial tissue to neoplasia (adenoma or cancer)

could have no effect on proliferation but could enhance apoptosis, thereby truly lowering the incidence of colorectal cancer. In both these instances, the putative hyperproliferation surrogate marker would give the incorrect answer with respect to colorectal cancer.

Fleming and DeMets showed several examples, from clinical trials comprising intervention, putative surrogate end point, and major clinical end point (e.g., mortality), where the surrogate marker gave a misleading indication of the true clinical end point (mortality, for example) [11].

4 Is Surrogate Marker Validity Generalizable from One Exposure to Another?

A surrogate end point valid for one exposure or intervention vs a cancer is not necessarily valid for a second exposure or intervention. Why? Again, because an alternative pathway to cancer may exist, as Fig. 2 illustrates.

Suppose exposure 1 (*E1*) operates only through the marker *S*. Suppose exposure 2 (*E2*) also operates only through *S*. Then *S* is a valid surrogate for

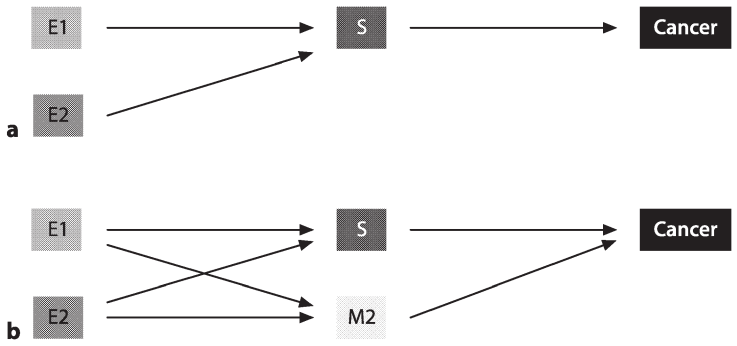

Fig. 2a, b. Surrogate validity for different interventions. In **a** the second intervention (*E2*) operates through the same marker as the first intervention (*E1*); in **b** the second intervention operates through a different marker

both *E1* and *E2*. Now suppose that a second marker (*M2*) exists. If E1 operates primarily through *S*, and only relatively minimally through *M2*, then *S* could still be a valid biomarker for *E1*. But we cannot be certain that *E2* operates similarly through *S* and *M2*—the *M2* pathway may be relatively stronger for *E2*, compared to *E1*, and that *M2* pathway may offset the pathway through *S*.

Thus, we cannot easily be certain that two different intervention agents have pathophysiologic effects so similar that if a given biomarker is a valid cancer surrogate for one agent it must be for the other. That is, we cannot avoid worrying that the second agent has some unanticipated effect on an (unknown?) alternative pathway?

5 Incomplete Validation: The Two-Stage Strategy

In the earlier discussion of validating surrogate end points, three criteria were mentioned: (1) The surrogate is associated with cancer, (2) the exposure is associated with the surrogate, and (3) the surrogate mediates the association between exposure and cancer.

Suppose, for a specific hypothesis, good evidence suggests that criteria (1) and (2) are true. Does this "two-stage" approach ensure that the exposure truly alters (or is associated with) the end point? For example, there are now data indicating that physical activity can lower estradiol levels in women [12]. There are also substantial data now that estradiol levels are directly associated with breast cancer [13]. These two facts, however, do not prove that physical activity necessarily reduces breast cancer risk. A counter-example is instructive: Hormone replacement therapy in women is associated with raised high-density lipoprotein (HDL) levels; HDL is clearly inversely related to cardiovascular disease (CVD) risk. But recent data from the Women's Health Initiative show that hormone replacement therapy (HRT) does not protect against CVD; if anything, HRT use increases CVD risk [14]. Thus, the "two-stage" strategy does not necessarily give the right answer. Why? In this case, it may be that alternative pathways mediating the relation between HRT and CVD offset the pathway through HDL.

6 Colorectal Adenomas as Surrogate End Points for Cancer

Over the last decade, adenoma recurrence trials have become a popular tool for investigating colorectal cancer hypotheses, with interventions ranging from drugs to dietary factors. The rationale for using adenoma recurrence

(defined as the growth of one or more new adenomas after prior detection and removal of one or more earlier adenomas) is:

- Relatively high prevalence in the middle-aged population, so that it is logistically feasible to find and recruit study participants recently diagnosed with a colorectal adenoma (though, in practice, only about 5%–10% of screenees get randomized).
- High recurrence rate (magnitude greater than cancer)—thus, an adenoma recurrence trial can be substantially smaller, faster, and cheaper than a trial with frank cancer end points.
- End-point assessment via standard clinical practice. The investigation can be integrated into standard endoscopic surveillance programs, thereby ensuring reasonably accurate and timely end-point assessment.
- Adenoma–carcinoma sequence. This is the fundamental biologic rationale for adenoma recurrence trials: most colorectal carcinomas develop from adenomas (that are large enough to be detectable at endoscopy).

Nevertheless, adenoma recurrence is not an absolutely conclusive surrogate for colorectal cancer. First, in polyp trials, all participants have had one or more adenomas already. Therefore, there is no information on how the intervention affects early (pre-adenoma) events that could be critical in carcinogenesis. At the other end of the carcinogenesis spectrum, because most recurrent adenomas are small, there is little information provided on later events, either (1) growth of small, non-advanced to large or advanced adenomas or (2) transformation of large or advanced adenomas to carcinoma. The intervention could have a critical impact on these early or late events, but the adenoma recurrence end point in the trial will not permit this impact to be evaluated.

Second, adenomas may be heterogeneous lesions, such that only a small subset eventually go on to cancer. An intervention can have different effects on the "innocent" and "bad" adenomas. If the intervention affects only innocent adenomas, because such lesions constitute the very large majority of recurrent adenomas, it would appear that the intervention lowers adenoma recurrence and, by inference, would reduce colorectal cancer incidence, when in fact the intervention has no effect on cancer. Alternatively, suppose an intervention affects only bad adenomas, not the innocent ones (Fig. 3).

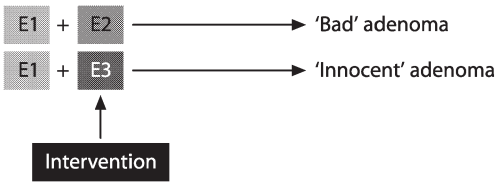

Fig. 3. Limitation of colorectal adenoma recurrence as a surrogate for colorectal cancer

Then it would appear that the intervention has only a minimal impact on adenoma recurrence (and, therefore, cancer), when the intervention really does reduce cancer incidence.

Alternative approaches to adenoma recurrence trials have been proposed, e.g., only persons with a prior large or advanced adenoma could be eligible for the trial. The rationale is that such participants are more likely than those with only a small, non-advanced lesion to have some sort of pre-malignant "field defect." There are serious cost implications to this strategy, however. Because only about one-third of participants in polyp trials have had a large/advanced lesion, three times as many individuals have to be screened to achieve the target sample size for the study. A second alternative strategy is to have as the end point only advanced adenomas (those 1+ cm, or with villous elements or high-grade dysplasia). The rationale is that the heterogeneity of recurrent adenomas is reduced: a much larger proportion of such end-point lesions is likely to progress to invasive cancer. But, because only some 1/6 of recurrent lesions in standard polyp trials are advanced, the sample size of the trials will have to be increased substantially—leading to a much more expensive investigation.

In summary, results of adenoma recurrence trials constitute strong—but not absolute—evidence on colorectal cancer. In addition, such trials are not inexpensive. Costs rise with alternative designs intended to strengthen inference.

7 Statistical Considerations

There is substantial variability ("noise") in biomarker measurements, with several sources of within-participant variation (e.g., over time, between specimen collections, reading-to-reading). The "signal-to-noise" ratio may be problematic: If within-person variation is large, it may not be possible to discriminate among participants. It may be possible to decrease within-participant variation by taking repeat samples (e.g., more biopsies, multiple blood samples). Information on variance components is critical. Such data are sparse (estradiol, proliferation). Measurement error will attenuate associations between exposure and marker, on the one hand, and marker vs cancer, on the other. Moreover, measurement error can lead to underestimation of extent to which surrogate mediates the effect of exposure on cancer

8 Conclusions

There are at least two ironies accompanying the surrogate end-point problem. First, the large, long, costly studies needed for evaluation are precisely the studies surrogates were designed to replace. Second, inferential certainty is directly associated with study cost—that is, you get what you pay for. Nevertheless, surrogate end points may be particularly valuable in phase II studies. And, in conjunction with other types of investigations (e.g., polyp trials plus cohort studies of colorectal cancer), such surrogate end-point markers may markedly enhance the "probability of being right" about the etiology and prevention of cancer.

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Can a Marker Be a Surrogate for Development of Cancer, and Would We Know It if It Exists?

William B. Armstrong[1] (✉) · Thomas H. Taylor[2] · Frank L. Meyskens[2]

[1]Department of Otolaryngology, University of California, Irvine, 101 The City Drive South, Bldg. 25, Ste. 191, Orange, CA 92868, USA
wbarmstr@uci.edu
[2]Department of Medicine, Chao Family Comprehensive Cancer Center, University of California, Irvine, 101 The City Drive South, Orange, CA 92868, USA

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Abstract Carcinogenesis proceeds through a very long preclinical period. Our collective hope is that multiple opportunities exist for chemoprevention to arrest or reverse progression towards malignancy. In the hope of faster progress with fewer subjects and lower total cost, much effort is being expended on the search for reliable biomarkers to predict the likelihood of developing cancer and/or to signal the effectiveness of chemopreventive therapy. Considerable attention is paid to identifying those markers that can act as surrogate markers for cancer development, since favorable modulation of the surrogate endpoint biomarker (SEBM) may demonstrate effectiveness of a putative preventive treatment. However, the complexity of the biology challenges our ability to measure the effectiveness of attempts to arrest or reverse carcinogenesis, other than through costly and time-consuming prospective trials with disease state as the endpoint. Despite much work, to date no prehistologic biological or molecular intermediate marker has been validated for sporadic cancers. Several factors accounting for the difficulties encountered in SEBM development are reviewed. Discussion is focused on the common thread of the complexity of the underlying biological changes in carcinogenesis limiting the effectiveness of any single biomarker. Additionally, the incidence of sporadic cancers is also low, further limiting the positive predictive value of any putative prognostic marker. Recent successes in development of chemopreventive agents show the concept is valid and worth pursuing, but the current strategies to develop biochemical and genetic markers to identify surrogate biomarkers is flawed, and need to be reassessed in light of the difficulties faced over the last 20 years.

1 Introduction

The old saying, "An ounce of prevention is worth a pound of cure," would seem especially true for cancers, and indeed that belief has motivated a great deal of research and other activities in the "fight" against cancers. Clearly there has been some success in cancer prevention, resulting from efforts in smoking cessation, weight reduction, cervical screening, and other lifestyle modifications. However, success from chemoprevention has been much more elusive. The path from identifying a likely chemoprevention agent through demonstrating that the drug is safe and reduces cancer risk in a large population is full of pitfalls. Basically, our understanding of the biology of cancers is still insufficient to make effective chemoprevention mechanisms obvious. We must proceed empirically at each step, at notable cost in time, effort, and money. Many believe appropriate use of surrogate endpoints could improve the efficiency of our work.

At the present level of understanding, cancer is not one disease but many disease entities. The histology and biology of tumors differ widely among organ sites. For tumors of the same histology in different organs, the genetic events leading to cancer are often different, and there seems to be variability in etiologic mechanism even within a cancer type. Thus, it is said there are multiple pathways to malignancy, and so a chemoprevention agent that successfully guards against one chain of biochemical events may be defeated by the redundancy of carcinogenic mechanisms. With improved understanding of all the relevant carcinogenic mechanisms, we might some day find an exploitable early common event to develop a chemoprevention agent analogous to the broad-spectrum antibiotic, but that is far beyond us at present.

To date, over one thousand candidate chemopreventive agents have been identified, making selection of the most promising compounds for detailed investigation a difficult task [26]. Selecting promising compounds for further study should be as rational as possible, since a great deal of effort is involved in confirming the usefulness of a putative chemopreventive agent. Trials must be long-term because the disease takes many years to develop, and long-term commitments from study participants with corresponding maintenance of staffing and infrastructure are necessary. Evaluation of putative preventive agents in trials where malignancy is the endpoint is expensive and cumbersome.

A valid surrogate holds the potential to place fewer subjects at risk and to answer important questions in a more economical fashion, while moving the field forward faster [40]. Attempting to improve efficiency, methods to identify markers of disease that can act as surrogate endpoints have been aggressively pursued, both to screen out ineffective chemopreventive agents and to make clinical evaluation of promising agents faster—using smaller numbers of subjects—and therefore cheaper.

Surrogate endpoints have seen some success in cardiology and other areas of medicine. However, in oncology, the same biologic complexity and pathway redundancy that challenges putative prevention agents challenges the identification of surrogate endpoint biomarkers. Unfortunately, despite much work, to date there are no validated prehistologic biological or molecular surrogate endpoint biomarkers for sporadic cancers. As long-time proponents of chemoprevention and the development of biomarkers, we now question if attempts to identify and validate surrogate endpoints to measure effectiveness of chemopreventive agents is a viable strategy, given the biological realities of carcinogenesis and the difficulties encountered.

2 Biomarkers and Surrogate Endpoints

By definition, a biological marker (biomarker) is "a characteristic that is objectively measured and evaluated as an indicator of normal biological processes, pathogenic processes, or pharmacological responses to a therapeutic intervention" [10]. A small subset of biomarkers demonstrates a strong correlation with the desired clinical endpoint and can serve as a substitute for the clinical endpoint. These surrogate endpoints are expected to be reasonably likely to predict clinical benefit or harm (or lack thereof) based on epidemiologic, therapeutic, pathophysiologic, or other scientific evidence [10].

What is required for a biomarker to be considered a surrogate endpoint? The United States Food and Drug Administration (FDA) has an expedited drug approval pathway for serious and life-threatening conditions based on use of surrogate endpoints, specified in the Food and Drug Modernization Act of 1997 [8]. According to FDA regulations, the standard is not rigidly defined. A "reasonably likely" standard was adopted in the regulations to accept study results utilizing a surrogate endpoint for granting expedited approval of therapeutic agents. Recognizing that this standard represents a compromise that could affect safety, additional requirements for further post hoc study following approval to describe clinical benefit and safety were included in the regulations.

The (American) FDA criteria for accepting a surrogate endpoint result are less rigorous than the criteria espoused by experts to validate a surrogate endpoint [10, 37]. For a biomarker to be a valid surrogate endpoint, it must meet two fundamental criteria [17, 37]. First, it must closely correlate with the target clinical endpoint. One expert has suggested 2.5%–10% false-positive and false-negative results as minimally acceptable levels for candidate surrogate endpoints [17]. This is a necessary, but not sufficient condition. For example, CD4 count and HIV viral load correlate with subsequent mortality from AIDS [18]. However, this does not mean that changes in these biomarkers will reliably measure effectiveness of a new drug to treat HIV in-

fection. For example, these markers do not adequately reflect toxic effects or interactions between agents in a multi-drug regimen [29].

The second requirement for validation is that the surrogate endpoint must fully capture the net effect of the treatment on clinical outcome [37]. Even a strong statistical association of biomarker levels with clinical outcome is no guarantee that a drug that modulates the biomarker will affect the target endpoint. A statistical association between the biomarker and clinical outcome does not indicate a unique or sufficient causative relationship. Clearly, the likelihood a surrogate will be effective is enhanced by choosing biomarkers integrally tied to the causal pathway(s) leading to the target endpoint.

To be useful, a surrogate endpoint's predictive abilities must hold across different treatment populations and with different therapeutic agents. A biomarker that faithfully predicts the clinical endpoint in one population must also demonstrate the same relationship in different treatment populations. In addition, the relationship of the biomarker to the clinical endpoint must hold up across treatments. If treatment with drug A demonstrates a favorable effect on the surrogate endpoint, which is verified by favorable modulation of the target clinical endpoint, then changes in the surrogate endpoint by treatment with drug B must also correlate with changes in the clinical endpoint in a corresponding fashion.

A valid surrogate endpoint captures the net effect of the drug on all the pathways affecting the clinical endpoint, accounts for toxicity, and shows little variability across populations. These requirements are extremely rigid, and in practice, no surrogate endpoint to date perfectly correlates with the true endpoint. In complex systems with multiple pathways and redundancy, the existence of a biomarker that faithfully reflects changes along all the important pathways becomes highly improbable.

Finding a single surrogate marker that serves well across all populations and treatments seems unlikely. One fallback position for the strategy is to concede this point and determine the "performance envelope" of candidate markers. Multiple studies across treatments and populations will be required to characterize the biomarker and demonstrate its characteristics as a surrogate endpoint. This modified strategy requires no less work and offers less in terms of overall efficiency of the discovery process. The ultimate judgment of surrogate endpoint utility will vary by disease process and intervention, and the standards required for judgment will differ correspondingly. For chemoprevention of cancer, the burden of proof is very high to be able to determine that a compound has clinical effectiveness and minimal to no toxicity, as any successful compound will be taken for many years by asymptomatic individuals.

3
Experience with Surrogate Endpoints in Drug Development

To provide a framework to better understand difficulties encountered using surrogate endpoints in cancer prevention trials, review of experience with surrogate endpoints in drug development for other disorders is instructive. In an earlier commentary [12] we indicated that surrogate endpoint development has been relatively successful for cardiovascular disease and AIDS. However, close analysis demonstrates that successful employment of surrogate endpoints has not been easily accomplished, has not been uniformly successful, and has been associated with some spectacular and instructive failures along the way.

The reliance on surrogate endpoints can lead to patient harm [17]. A sampling of studies where surrogate endpoints in clinical trials of a variety of drugs demonstrated favorable effects, but failed to demonstrate clinical benefit, or showed increased mortality is displayed in Table 1. A striking example of the potential risk of relying on surrogate endpoints is the experience with several antiarrhythmic agent trials to decrease premature ventricular contractions (PVC) when administered after myocardial infarction. Although the drugs did decrease PVCs, there was a significant increase in mortality with drug use. Increased mortality was also found in trials of promising agents shown to demonstrate increased exercise tolerance and cardiac output when used to treat congestive heart failure [35, 36].

The experience with drugs to treat hypertension has been more favorable. Two large prospective trials have demonstrated decreased total mortality with pharmacological management of hypertension [2, 4]. Control of blood pressure is now accepted as a surrogate endpoint for antihypertensive agents based on extensive experience. More recently, drugs including angiotensin-converting enzyme inhibitors and calcium channel blockers have been approved based on the surrogate endpoint of efficacy at decreasing blood pressure and perceived improved side-effect profile. There is concern by some that these drugs have not been compared directly with previously approved drugs, indicating a lack of faith in the surrogate-endpoint strategy, and long-term mortality studies have not been completed. However, as pointed out by Temple [43], these drugs have undergone extensive study in related diseases, and their side-effect profiles are well understood; so it seems a reasonable bet they will safely predict lack of toxicity for hypertension.

A number of cholesterol-lowering drugs have been developed based on the observed correlation of favorable levels of cholesterol, HDL, and LDL levels with lowered mortality [25]. Clofibrate and niacin were early drugs used to decrease cholesterol. The drugs effectively lowered cholesterol levels, but overall mortality was increased [1]. Early meta-analyses of randomized controlled trials of cholesterol-lowering interventions demonstrated decreased cholesterol and cardiac mortality, but overall the interventions were

Table 1. Surrogate endpoint experience in cardiology and other disciplines

Clinical Problem	Drug	Surrogate	Clinical endpoint	Outcome	Reference
Cardiovascular					
Arrhythmias	Encainide Flecainide	Arrhythmias	Survival	Increased mortality	[15]
	Moricizine	Arrhythmias	Survival	Increased mortality	[5]
Atrial fibrillation	Quinidine	Atrial fibrillation	Survival	Increased mortality	[14]
	Lidocaine	Atrial fibrillation	Survival	Increased mortality	[23]
Congestive heart failure	Milrinone	Cardiac output Ejection fraction	Survival	Increased morality	[35]
	Flosequinan	Cardiac output Ejection fraction	Survival	Increased mortality	[36]
Abnormal lipid/lipoproteins	Clofibrate/Niacin	Cholesterol	Survival	No decreased mortality	[1]
	Simvastatin	Cholesterol	Survival	Decreased mortality	[7]
Hypertension	High-dose diuretics	Blood pressure	Survival	No improvement in survival	[41]
	Calcium channel blockers	Blood pressure	MI/survival	Increased mortality in meta analysis	[21]
Osteoporosis	Sodium fluoride (post menopausal)	Bone density	Fractures	Increased fractures	[38]
HIV and AIDS	Antiretroviral agents	CD4	AIDS events survival	Failure to predict clinical outcome	[39]
	Antiretroviral agents	HIV mRNA	AIDS events survival	Failure to reflect treatment on clinical outcome	[9]
Chronic granulomatous disease	Interferon	Superoxide production, bacterial killing	Serious infections	Endpoint improved, surrogate did not	[3]
Critical care	Growth hormone	Nitrogen balance	Survival	Increased mortality	[42]
Hormone replacement therapy	Estrogen/progestin	Cholesterol/LDL/HDL	CHD, death, nonfatal MI	No decreased coronary heart disease	[31]
	Estrogen/progestin	Coronary artery diameter	CHD, death, nonfatal MI	No effect on coronary stenosis	[24]

CHD, coronary heart disease; HDL, high-density lipoprotein; LDL, low-density lipoprotein; MI, myocardial infarction.

associated with increased mortality from noncardiac causes, and a slight increase in overall mortality [19]. More recently, 3-hydroxy-3-methylgluaryl coenzyme A (HMG Co-A) reductase inhibitors have demonstrated improvements in mortality in a large, well conducted phase III trial [11]. The trial also discovered the threshold for benefit from these agents was much lower than previously thought, and persons characterized as being at low risk for cardiovascular disease could benefit from the lipid-lowering drug [11]. This information would not have been known without the large prospective trial measuring the true clinical endpoint.

Promising candidate surrogate endpoints have failed to predict clinical outcome in several other diseases. Counts of CD4 cells or viral DNA levels correlate with disease prognosis, but changes in these markers with drug treatment have not been as useful as hoped, especially in the settings of multi-drug regimens with significant toxicities and development of drug resistance [17, 18]. Sodium fluoride treatment was believed to be helpful for prevention of pathological fractures in persons with osteoporosis. Bone mineral density was proposed as a logical surrogate endpoint based on correlations of fractures and bone density [38]. Unfortunately, although bone density was increased by treatment, so were fractures, and it was learned that the bones became more brittle with treatment [38]. Most recently, hormone replacement therapy in postmenopausal women predicted to decrease cardiac risk instead failed to slow disease progression, and may have increased cardiovascular mortality [24, 31]. It was accepted almost as gospel that postmenopausal hormone therapy had a favorable effect on cardiovascular risk [22]. Two large studies failed to show benefit [24, 31], and one study suggests combination estrogen plus progestin increases the risk of coronary heart disease [31].

Discarding a useful agent is also a risk of using imperfect surrogate endpoints. The experience with interferon-gamma treatment for chronic granulomatous disease is instructive. Interferon-gamma was evaluated in a clinical trial in patients with chronic granulomatous disease [3]. The surrogate endpoints measured were bacterial killing and superoxide production. Drug treatment failed to modulate the surrogate endpoint, but interferon-gamma effectively decreased the number of serious infections in treated subjects [3]. This is an important reminder that reliance on an ineffective or inappropriate surrogate endpoint can result in discarding an effective agent.

Analyzing the experiences with developing surrogate endpoints in other fields reveals at least two lessons. First, many of the early studies evaluating surrogate endpoints failed because of inadequate knowledge of the drug's effects on the biological pathways and incomplete knowledge of the biochemical pathways (e.g., clofibrate and niacin for hyperlipidemia). Second, only after effective agents that demonstrably improved the clinical outcome were identified were surrogate endpoints accepted for drug approvals [e.g., angiotensin-converting enzyme (ACE) inhibitors for hypertension]. Identification

of promising biomarkers and promising drugs to treat a disease relies in large part on understanding the underlying disease pathophysiology. Determining the effectiveness of the biomarker as a surrogate endpoint in turn depends on having clinically effective drugs. The chemoprevention field is many years away from this point. Developmentally, the current status resembles cardiovascular disease research of the 1970s. Thus, perhaps the chemoprevention community should "bite the bullet" and concentrate on the identification of effective agents, delaying attempts at validation of surrogate markers until theoretical frameworks are in place to support such efforts. Such markers would serve to improve the efficiency of identifying second- or later-generation agents.

4 Challenges to Using Surrogate Endpoints in Chemoprevention Drug Development

There are two broad risks associated with use of surrogate endpoints. The first risk is the surrogate fails to adequately predict the true endpoint. The second risk is failure to identify competing or adverse effects on related or unrelated pathways. Competing drug effects on alternate pathways not captured by the surrogate can cancel out favorable drug effects, resulting in favorable modulation of the surrogate, but less-than-predicted or no favorable effect on the clinical endpoint. Unrecognized toxic effects can also exert an adverse impact on the clinical endpoint.

A very basic mathematical fact makes the use of surrogates to evaluate effectiveness of cancer chemopreventive agents very difficult outside of special populations. The ability of a given imperfect surrogate to predict disease is intimately tied to the prevalence of predisposing conditions in the population studied. As the prevalence of predisposing conditions decreases, the positive predictive value of an imperfect marker declines. Because sporadic cancers in the general population are rare, the discriminating ability of imperfect surrogates will be of limited clinical use, at best.

There are several reasons why surrogate endpoints fail to faithfully predict clinical endpoints. The surrogate endpoint may measure effects on a distinct parallel pathophysiological pathway, it may measure effects on only one of multiple important pathways, there may be unknown mechanisms that block clinical effect, or there may be toxic effects that have an adverse effect on the true endpoint. There may also be population differences that limit the applicability of the marker to populations not involved in "validating" it. Sporadic cancers are generated in a multi-step, multi-year, multi-pathway process, and selection of a single or group of markers along single or multiple pathways will not capture a high enough proportion of the risk of transformation to cancer to be useful [12, 13, 20]. Further limitations oc-

cur because we have not completely worked out all of the relevant carcinogenic pathways to cancer and identified all the critical checkpoints [12].

In a previous commentary we discussed how the mathematics of combinations illuminates the size of the problem inherent in monitoring changes on multiple carcinogenic pathways [12]. When multiple pathways contributing to cancer development can be disrupted, and when disruption of several (but not all) of these pathways is necessary to induce cancer, the number of possible combinations of distinct biomarker patterns that lead to cancer becomes very large, and the task of identifying and verifying the utility of each biomarker pattern is daunting. The number of subjects required to check and characterize each of the possible combinations of biomarkers could exceed the number of subjects in a phase II trial [12, 16].

Since single biomarkers are likely to be defeated by the redundancy of biochemical pathways to cancer, perhaps sets of biomarkers adequate to the task may be identified. A logical extension leads to the analyses of profiles of gene or protein expression. It is very seductive to hope that the ability to simultaneously measure genetic changes in thousands of genes using gene chip arrays will transform our ability to detect precancerous changes and monitor the effectiveness of chemopreventive treatments. An incredible amount of information is generated that must be analyzed to identify patterns of changes that predict cancer. Because of the multiple pathways and multiple points where disruption can occur, a very large number of samples will be required to identify all the patterns that predict the development of sporadic cancers, even of a particular type. In addition, the changes detected need to be early enough along the chain of carcinogenic events to be amenable to arrest or reversal by candidate chemopreventive agents. The same requirements for determining utility of the biomarker(s) derived from gene array studies apply, and the effect of alterations on the biomarker must be verified by determining the effect on cancer incidence. This does not mean the importance of gene chip technology in chemoprevention should in any way be discounted. To the contrary, the technology provides a powerful tool to better understand the pathophysiology leading to cancer, and the knowledge gained will stimulate new avenues of investigation that may lead to new candidate preventive and therapeutic agents.

An important issue confronting researchers using surrogate endpoints is the applicability of the surrogate endpoint. To be effective, the surrogate must be applicable not only to all members of the group tested but also to subsequent populations receiving the same treatment. This can only be determined by multiple studies on diverse groups of subjects. A second major problem is applicability across interventions. Because of the heterogeneity of the mechanisms of carcinogenesis across tumor types, it is doubtful that a nonhistological marker will either reliably measure effectiveness of different classes of chemopreventive agents against the same tumor, or pre-

dictably measure effectiveness of a single drug across multiple tumor histologies and locations.

The second broad risk of relying on surrogates is failure to identify counteractive effects on the clinical endpoint that are not reflected by the surrogate, or effects that produce unacceptable toxicity. Several examples in cardiovascular drug development have already been discussed. The experience with beta-carotene as a chemopreventive agent exemplifies the problems that can be faced even with seemingly innocuous compounds. It was not until phase III trials were conducted that a procarcinogenic effect of beta-carotene in persons who smoked while taking the drug was discovered [6, 33]. This paradoxical effect in the subgroup at highest risk for developing lung cancer was a sobering experience for the field, and likely would not have been detected in smaller trials using surrogate endpoints as the basis for approval.

5 Intraepithelial Neoplasia as a Surrogate Endpoint

Comparison of lessons learned from cardiovascular and other pharmacological drug development trials using surrogates with recent publications by leaders in chemoprevention indicates there is incomplete recognition or acceptance of the limitations of surrogate endpoints [28, 34]. O'Shaugnessy et al. [34] acknowledge limitations of surrogate endpoints, but assume that eradication of intraepithelial neoplasia (IEN) in itself will be of clinical benefit and predict decreased mortality for a number of cancer sites. The assumption that elimination of the IEN by chemopreventive agents will decrease cancer incidence seems logical, but needs to be proved. Given the number of failures of surrogate endpoints in other disciplines, IEN eradication by a chemoprevention drug cannot be assumed to predict decreased cancer incidence or mortality. The argument that a tangible clinical benefit is attained simply by eradication of the IEN (independent of cancer prevention) has weaknesses. For many sites, the presence of the IEN is not the problem, as most lesions at most sites are asymptomatic. It is the prevention of what the lesion may become (cancer) that is of clinical benefit, representing a change and not a static event.

Eradication of visible and histological evidence of the disease does not mean elimination of the genetic changes that can produce cancer, but an ineffective or partially effective chemopreventive agent could change a visible lesion that would develop into cancer to an invisible lesion that still develops into cancer. Unless genetic changes in the tissues can be conclusively reversed or managed, reliance on clinical regression of IEN as a surrogate is risky and needs to be eventually verified in definitive phase III trials with cancer incidence as the clinical endpoint.

In the oral cavity, it is estimated that over half of cancers do not have a preexisting clinically recognizable lesion before development of cancer. Clinical regression of the lesion does not mean the risk of transformation also disappears. There is a risk of converting a visible lesion to an invisible lesion that will still develop cancer. A similar problem is seen with nonsteroidal anti-inflammatory agents and colon cancer. Celecoxib was approved for cancer prevention for familial adenomatous polyposis (FAP) based on decreased numbers of polyps. Studies of sulindac for FAP found that polyps were more difficult to screen because they were flattened in appearance [32, 45]. The recently reported prostate cancer prevention trial (PCPT) [44] reported delay or prevention of low-grade prostate cancer and an increased proportion of high-grade prostate cancer. The question remains; are the subjects receiving benefit?

That clinical and histological regression of IEN can serve as a valid surrogate for cancer development has not been proved. There may be clinical benefit to treatment of IEN, but there are potential risks in using this approach, and these risks are too great to warrant approval of a chemopreventive agent based solely on its effect on IEN. For these reasons, we need to first focus on the true endpoints, cancer incidence and mortality, and then determine if changes in IEN do in fact reliably predict decreased cancer incidence and mortality.

Kelloff [27, 28] provides a theoretical construct using clinical and genetic changes in IEN as a surrogate endpoint. He acknowledges that clinical regression does not guarantee clinical response, and thus advocates use of molecular testing to demonstrate arrest or reversal of carcinogenesis at the molecular level. Here again, the theory is very logical. Unfortunately, we have not yet worked out all the relevant pathways to cancer, so we do not know all the genes and proteins that need to be monitored. Confirmation will require old fashioned, time-consuming, phase III clinical trials to answer the key question of whether cancer has been suppressed or eliminated sufficiently to warrant a lifetime consumption of a drug.

In the invited commentary accompanying O'Shaughnessy's article, Lippman et al. [30] suggest that while complete eradication of premalignant clones may not be possible, delaying the onset of cancer would convey real clinical benefit. This is a sensible position to take given the current state of knowledge about oncogenesis, but it does not remove the obligation to demonstrate decreased incidence of cancer or cancer mortality. If a treatment is effective, and it does in fact delay onset of cancer, this will be borne out in time-to-event analysis where the event is frank malignancy. Again, one cannot assume that delay in development of IEN or an intermediate endpoint will translate to actual clinical benefit.

6 Conclusion

Surrogate endpoints are not a "Holy Grail" that can guide us to effective chemoprevention agents. Biomarkers, however, can be useful in the early stages of chemoprevention drug development if used appropriately. The quest to demonstrate a marker that is a useful signal of both agent activity and risk of malignancy may consume more resources than it is intended to save. Future efforts should focus on identification of biomarkers that are mechanistically related to carcinogenic pathways affected by the drug, and are modulated by the drug. Thoughtful investigation of biomarkers in chemopreventive drug development can provide valuable knowledge about important carcinogenic pathways and the interactions of therapeutic agents with these pathways. If and only if an early common carcinogenic event (or limited number of early events) is identified, and only if that event can be exploited will efforts at developing surrogate endpoint biomarkers have a chance of being successfully employed. In the meantime, we should instead be using biomarkers as "shovels" to dig for the answers about the mechanisms of carcinogenesis and to select promising chemopreventive agents for further study.

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How Should We Move the Field of Chemopreventive Agent Development Forward in a Productive Manner?

Frank Louis Meyskens[1] (✉) · Eva Szabo[2]

[1] Department of Internal Medicine (Hematology/Oncology) and Chao Family Comprehensive Cancer Center, University of California, Irvine, Orange, CA 92868, USA
flmeyske@msx.ndc.mc.uci.edu
[2] Division of Cancer Prevention, National Cancer Institute, Bethesda, MD, USA

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Abstract Epidemiologic observations and preclinical experimental investigations suggest that the prevention or reversal of precancers should be an effective strategy in humans to control cancer. Although "proof of principle" has been established in humans, the results of randomized trials have not been confirmatory in most cases. Toxicity in normal or near-normal populations has also been greater than anticipated. We examine the problems associated with testing chemoprevention agents in humans and offer a process and guidelines that may better inform the logical development of this relatively young clinical field.

1 Introduction

The word "chemoprevention," with reference to cancer, was coined in 1976 and has evolved to encompass the suppression or reversal of cancer using natural or synthetic compounds (Sporn et al. 1976; Meyskens 1992a,b). Both prior to 1976 and subsequently, a considerable amount of epidemiologic and preclinical experimental evidence has accumulated suggesting that human cancer should either be preventable or else reversible or suppressible in its early stages. However, definitive large randomized trials in humans based on epidemiologic observations have generally yielded disappointing results (review cervix, Follen et al. 2001; colorectal, Viner et al. 2002), with adverse results (more lung cancers) produced by β-carotene supplementation in smokers (Omenn et al. 1996) and no effect of fiber supplementation on ade-

nomatous polyp recurrence in patients with one or more prior adenoma (Alberts et al. 2000; Schatzkin et al. 2000).

Randomized trials based on experimental data have yielded somewhat more encouraging results, with retinoids being demonstrated to clearly suppress the development of second cancers in head and neck cancer patients (Hong et al. 1990), and tamoxifen given at standard doses being effective in substantially decreasing the incidence of breast cancers in women at high risk for this event (Fisher et al. 1998). However, both studies demonstrated a sufficiently high level of toxicity (for putatively healthy individuals) such that neither compound has entered widespread usage, despite FDA approval for the latter indication. Attempts to use a lower non-toxic dose of retinoid were unsuccessful and ineffective in preventing secondary lung cancers in a well-defined cohort (Lippman et al. 2001). Similarly, the attempt to prevent prostate cancer with finasteride, a specific inhibitor of the conversion of testosterone to its active form dihydrotestosterone, has produced mixed results. The total incidence of prostate cancer was significantly decreased in a large randomized placebo-controlled trial; however, the number of advanced ($\geq$Gleason 7) tumors was significantly increased and a slightly increased incidence of urogenital side effects was noted (Thompson et al. 2003). Whether or not finasteride is approved by the FDA, these findings suggest that this agent may not be widely adopted.

One of us (F.M.) has posed a number of questions in this series of conferences (Meyskens 1998; Meyskens 2000a,b,c). The two broad questions we must now ask ourselves are: (1) Why have we been so unsuccessful in translating positive epidemiologic and experimental findings to clinical benefit? (2) How should we move the field of chemopreventive agent development forward in a manner that is more productive?

2 Through the Retrospectroscope

Based on the results of randomized studies done to date, a series of questions that need to be addressed, discussed, and debated has emerged:

1. *Are the results of epidemiologic observations alone ever enough to embark on a phase III trial?*

   Studies of non-oncological diseases have suggested that a very substantial effect must be evident in epidemiologic observations if a significant result is to be demonstrated in a randomized clinical trial (Ioannidis et al. 2001). In general, the effect demonstrated in a randomized trial is 40%–50% less than would be anticipated from observation studies. This caveat therefore

indicates that relative risks or odds ratios that are much greater than 0.5 or 0.6 will require a very large sample size in a randomized trial to demonstrate the 25%–30% reduction that might occur with a highly effective agent. In general, the effects of dietary compounds as measured by observation trials has been modest, so it is entirely likely that most cancer clinical chemoprevention trials involving dietary compounds have been underpowered to demonstrate a clinical effect, notwithstanding the possibility that the negative trials could also reflect assessment of the incorrect nutrient or dietary compound as well as incorrect doses of such compounds.
Our answer to this first question is:

No, epidemiologic observations alone are rarely, if ever, enough.

2. *What level of toxicity precludes further development of preventive agents?*

From the experience to date, it is clear that the presence of efficacy and excessive toxicity have about equal weight in determining whether an agent is developed or adopted, with potential effectiveness driving development and toxicity inhibiting both development and adoption. Several aspects about the assessment of toxicity in a chemoprevention setting are worth reviewing. Given that cancer prevention aims to prevent an event (cancer) that has not yet occurred and may never occur in a significant portion of the at-risk population, the toxicity of chemopreventive compounds must be considerably lower than the toxicity of agents used in cancer treatment trials, and the risk–benefit ratio must be considerably lower as well.
As an example (with the clarity provided by hindsight), first generation retinoids were used beyond the point at which it should have been evident that they were too toxic for most preventive indications; also, there was failure to recognize that effectiveness and toxicity were too closely linked to allow separation of these two features by simple dose reduction. Retinoid drug development would have been better served by the conduct of careful phase II dose–response preliminary efficacy studies before proceeding to large randomized trials. In contradistinction, tamoxifen had been used in the treatment setting for over 20 years by the time that the P-1 breast cancer prevention trial was begun and the side-effect profile was well known. However, tamoxifen had not been studied systematically in a randomized trial of the size of the P-1 trial, with the same attention to long-term toxicity monitoring as was provided by the P-1 trial. Hence, it should not have been a surprise that side effects were seen; what was surprising was that despite a very efficacious result, tamoxifen has not been widely adopted for risk reduction of breast cancer due to the perception by both patients and physicians alike that the drug is "too toxic."
On the other hand, toxicity of agents that have been used for other indications may be exaggerated and a potentially effective compound may be dismissed. An instructive case in point has been our experience in developing the polyamine synthesis inhibitor difluoromethylornithine (review,

Meyskens and Gerner 1999). Originally developed to treat leukemia using massive doses, the uncommon side effect of ototoxicity was uncovered. Once preclinical and mechanistic studies suggested that difluoromethylornithine may be a potent chemopreventive agent, its development was markedly hampered by the perception of ototoxicity. However, careful and systematic placebo-controlled studies have subsequently shown that polyamine-lowering in tissues can be achieved using doses that are 1/100th of those used for treatment, and that at these doses hearing loss in placebo and treated patients is equivalent (Croghan et al. 1991; Meyskens et al. 1994, 1998, 2001).

Our answer to the second question is:

Toxicity of chemoprevention agents in humans has, in general, not been well-delineated in the phase II setting, and careful placebo-controlled trials should be mandatory before proceeding to definitive phase III randomized studies. Toxicity has been both underestimated and overestimated from failure to critically assess this parameter in relation to the modulation of the relevant biologic/biochemical/molecular endpoint.

3. *How much can animal models tell us?*

In general, animal models have not adequately simulated the human disease being studied. The use of high single (or a few) doses of carcinogen in most animal models does not represent the manner in which humans are exposed to carcinogens. Transgenic animals that are highly engineered to produce a certain result have similar limitations. Nevertheless, demonstration that a particular compound reduces the incidence of tumors across a spectrum of animal models may suggest efficacy and provide important insights into mechanisms of carcinogenesis and cancer prevention. One must keep in mind, however, that the dose of the chemopreventive compound employed in animal studies may be unrealistically high for human use, thereby producing a toxic effect that cannot be detected in animal studies; however, this explanation has been rarely invoked for failure of a compound active in the preclinical setting that was ineffective clinically.

Of greater importance is the failure of animal models to develop the field of intermediate markers (Meyskens 1992, 2001). Although the measured endpoint is almost always tumor (adenoma and/or carcinoma) incidence or multiplicity in animal models, the relationship of the true endpoint of cancer to the intermediate markers has not been systematically assessed. However, in the human setting, where the development of cancer as an endpoint requires lengthy studies in very large numbers of participants, large numbers of potential markers are being advocated without the possibility of correlation with the true endpoint, which is rarely measured. A critical set of information that animal models could contribute to the database would be systematic studies of intermediate markers and their correlation to the true endpoint in models that represent the disease process in humans as closely as possible.

Our answer to the third question is:

Animal studies can provide valuable information aiding the decision-making process for chemopreventive agent development, both in agent identification and in validation of intermediate endpoints. However, the value of such studies in agent identification is frequently overestimated, while the value in validating intermediate endpoints has been underutilized.

4. The final and most critical question in chemopreventive agent development is:

   What level of evidence will lead to adoption of a chemopreventive compound for general usage?

   On the one hand, several compounds have been approved for chemoprevention (Table 1, broadly defined) and are in general use, including topical BCG for bladder carcinoma in situ and topical 5-fluorouracil and diclofenac (a COX-1 inhibitor) for actinic keratoses. Both aspirin and calcium have been shown to reduce adenomatous polyps in large randomized trials (Baron et al. 2003; Sandler et al. 2003), but their usage has thus far not been widely adopted—perhaps because the risk reduction was relatively small (about 20%). However, tamoxifen produced a substantial (50%) reduction in the P-1 breast cancer prevention study, but has not been widely adopted because "toxicity" in this cancer-free group of women has been deemed excessive (despite FDA approval). More surprising is the fact that tamoxifen does not seem to be widely used even in high-risk women who show a genetic predisposition to breast cancer. In contrast, the photosensitizer Photofrin has demonstrated a modest effect in Barrett's esophagus in a non-randomized trial, but its usage, at least in the U.S., appears to be substantial (review, Wang and Kim 2003). Although, these usages and approvals seem to undermine a call for the systematic development of chemoprevention

Table 1. How much/how often are chemopreventive agents used for approved indications

Condition	Agent	Use
Bladder CIS	BCG/topical	High
	Chemotherapy (several)	High
AK	5FU	High
	Diclofenac	?
Adenomas (FAP)	Celebrex	?
Adenoma (sporadic)	Aspirin	?
	Calcium	?
Barrett's esophagus	Photofrin	Often
Breast	Tamoxifen	Low (sporadic)
		? BRCA
Stomach	Antioxidants	?

AK, adenylate kinase; BCG, bacillus Calmette-Guérin; CIS, carcinoma in situ; FAP, familial adenomatous polyposis.

agents (Kelloff et al. 1995, 2000), the high cost, risk–benefit considerations, and potentially broad impact of chemopreventive agents mandate that agents be developed carefully. A proposed algorithm for the process by which candidate chemopreventive compounds enter definitive randomized trials (phase III and potentially phase IIb) is discussed below.
Our answer to the fourth question is:

Chemopreventive agent usage is dictated by risk–benefit assessments, both real and perceived. High efficacy and low toxicity are required. To ensure that both criteria are met, agent development guidelines, incorporating an assessment of all existing information and calling for ascertainment of missing information, are proposed.

3 Some Further Caveats

Other critical issues which are discussed in more detail elsewhere (Baker 2000; Armstrong et al. 2003) and in this volume (see the chapter by Armstrong et al.) include:

1. The multiple pathways to cancer and the limiting effect this may have on the development of biomarkers as surrogates for the true endpoint.
2. The common assumption is that modulation of a biomarker equates to a change in the incidence of the true endpoint and therefore is predictive; hence the biomarker is a surrogate. But this assumption is incorrect. This is a particularly common mistake when a marker seems to have good prognostic ability; that is, the presence of the marker is a good estimator of the disease endpoint. Simply put: prognostic is not predictive (also see Fleming and DeMets 1996 and Herrington and Howard 2003).
3. The term "surrogate endpoint biomarker" (SEBM) has been used in a rather cavalier fashion, and imprecision in language has resulted in much confu-

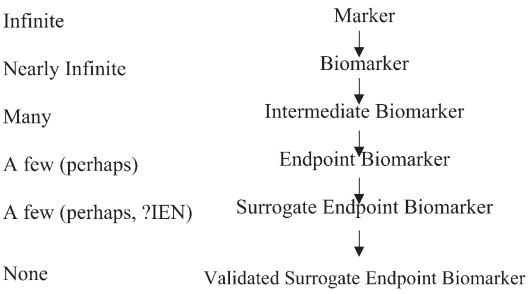

Fig. 1. The terminology of markers. In assessing the carcinogenesis process, representation ranges from a nearly infinite number of markers to the rare (currently none) validated surrogate endpoint

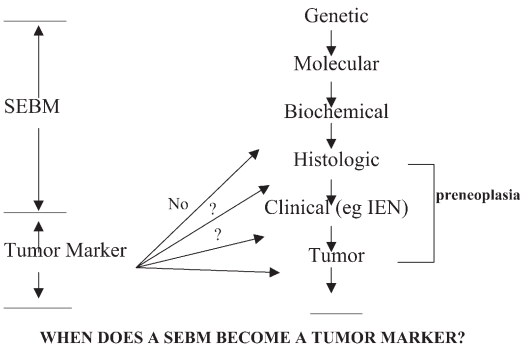

Fig. 2. A tumor marker is not a surrogate endpoint biomarker. The carcinogenesis process is a continuum, but once a marker has evolved from a potential surrogate (*SEBM*) to an actual tumor marker, the process of assessment and the implications changes

sion. A hierarchy of marker terminology is shown in Fig. 1. Accurate use of these terms is critical to avoid over- or underestimating progress.

4. Another serious mistake in cataloging is the equating of biomarker, especially SEBM, with tumor marker (Fig. 2). Notwithstanding the difficult issue of knowing when a cell becomes cancer, a tumor marker implies (and represents) something entirely different than an intermediate marker, and the two should not be confused if we are going to be successful in moving the field of chemoprevention ahead intelligently.

4 Guidelines

A major challenge facing those dedicated to bringing promising preclinical agents to clinical fruition is their systematic development. Although the process by which agents are advanced from preclinical to clinical studies and the systematic development of early clinical activity (pilot, phase Ia/Ib, IIa) is extremely important—a topic which we and others have discussed at length (Goodman 1992; Meyskens 1992b, 2001; Kelloff et al. 2000)—the critical juncture in chemopreventive agent development (and in the development of most drugs) is the decision to proceed to a definitive randomized phase IIb or phase III trial. The process by which this occurs in medicine in general has not always been systematic, and this is even more true for chemoprevention.

We propose a set of guidelines by which decision-making can be better informed (Table 2). The overall goal is to require the decision-maker to evaluate all available evidence that can be informative and to identify missing information before embarking on phase III trials, so that the final decision to proceed with lengthy and costly definitive studies will take place after full

Table 2. Level of evidence and relative merit in moving a chemopreventive agent to large randomized trials

1. Experimental evidence	Maximum points	Low
Mechanism	Low	↓
In vitro	↓	↓
Animal	High	↓
2. Epidemiologic		↓
Case-control	Low	↓
Cohort/ecologic	↓	↓
Secondary analysis	High	↓
3. Clinical		↓
Biomarker	Low	↓
Preneoplasia	↓	↓
Neoplasia	High	↓
4. Trials		↓
Phase Ia/Ib	Low	↓
Phase IIa biomarker/dose-response	↓	↓
Phase IIb biomarker/dose-response	High	High

Other beneficial effects on health (e.g., prevention of CAD, osteoporosis, etc.): additional positive points. Toxicity: negative points.

consideration of all information. The guidelines identify various types of evidence (experimental, epidemiologic, clinical, and trials) that should be considered and assign point values for each category. Within each category we have established a hierarchy of evidence, with increasing value given to those elements that are regarded as more likely to translate to or be correlated with clinical outcome. An important feature of this algorithm is that a maximal number of points will be allowed for each subcategory and for each criterion within a subcategory, regardless of the number of observations, or studies. For example, within the category of experimental evidence, the maximal assignable value for mechanistic data might be 25 points and for animal studies the total value might be 75 points. For epidemiologic evidence, the maximum value assignable to case-control studies might be 25 points while a positive secondary analysis of a randomized trial might be worth 150 points. The result of having a maximal point value for each subcategory is that the evidence from multiple weak studies would not be able to overcome the evidence from one stronger and more informative study in providing the rationale for further chemopreventive agent development.

Using such an approach, we have scored several completed trials using the information available in the original protocol. Not surprisingly, the evidence for the CARET Carotene And Retinol Efficacy Trial) study was weak, and the trial probably would not have been started without new non-epidemiologic data, were the proposed guidelines in force at that time. As is well-known, this trial produced more lung cancers in the treatment arm (Omenn et al. 1996), a result that could not have been anticipated at the time

the study was begun. In contrast, the evidence underlying the basis for the use of tamoxifen in the P-1 breast cancer trial was strong, consistent across all categories of evidence, and produced a high score. Therefore, it is not surprising that a favorable reduction in the number of breast cancers in the treatment arm was demonstrated (Fisher et al. 1998). An important consideration in the design of future chemoprevention trials will include a more complete evaluation of toxicity and assignment of negative values based on the known side-effects profile, as well as a more careful evaluation of dose–response effects and toxicity in the run-up to the randomized trial. Similarly, if an agent has been shown to have other beneficial effects on health (e.g., aspirin and cardiovascular health), this needs to be considered during the decision-making process, and positive points up to a preset maximum will also be assigned.

The development of these guidelines involves an interactive iterative process based on evaluation of prior studies whose outcomes are known. We anticipate that this process will also allow us to score ongoing trials for which results are not currently known and trials which are being considered. With time, a database will emerge that may allow us to prospectively recommend whether the evidence is sufficient from a scientific viewpoint to proceed to definitive randomized trials, all of which are lengthy and expensive. However, we recognize that the implementation of large trials is also influenced by non-scientific considerations, including public pressure, competing priorities, importance of the question, and a likelihood that the result of a definitive trial will lead to a change in clinical practice or public usage. The guidelines that we propose are meant to offer a framework for informed decision-making based on evaluation of all known evidence and recognition of "missing pieces".

5 Conclusions

Before the next generation of clinical chemoprevention trials begins, the following four key issues should be taken into consideration.

1. Generation of data in animal models that links/correlates biomarkers and cancer should be a high priority.
2. Non-validated biomarkers should be used as guides to developing drugs rather than as surrogates to estimate reduction of the true endpoint.
3. Assessments of efficacy and safety are equally important in determining whether a drug should be evaluated in a phase III randomized trial. While demonstration of the former (efficacy) is an absolute requirement for definitive phase III testing, demonstration of the latter (safety) is merely a prerequisite and is insufficient alone to merit further drug development. The

balance of efficacy and safety shifts, based on the clinical situation, with higher-risk clinical scenarios tolerating greater toxicity from potential interventions.

4. The systematic development of chemopreventive agents is a long process. Shortcuts have not led to much progress as reflected by a change in medical practice. Prior studies have established the "proof of principle" that several different epithelial cancers can be prevented, or at least delayed. The next step is the development of studies that will identify safe efficacious drugs that can be integrated into routine medical care of individuals identified to be at high risk for specific cancers. As a research community, we need guidelines to inform that process in a useful way.

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The Problems with Risk Selection; Scientific and Psychosocial Aspects

Anne-Renée Hartman

Dana Farber Cancer Institute, 44 Binney Street, SM 203, Boston, MA 0211, USA
anne-renee_hartman@dfci.harvard.edu

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Abstract Between 9,000 and 18,000 new cases of breast cancer per year in the United States are associated with a genetically defined predisposition [1, 2]. Mutations in *BRCA1* and *2* account for greater than 60% of inherited breast cancer. Mutations in additional undiscovered high and low penetrance genes may account for the other 40% of inherited breast cancer cases and possibly a subset of familial breast cancer cases that lacks an autosomal-dominant pattern of inheritance. False-negative rates resulting from gene sequencing of *BRCA1* and *2* may be as high as 10%–15%, making the identification of high-risk individuals a complex and often futile process for both patient and physician. As a consequence of technical limitations in *BRCA1* and *2*, genetic testing and the lack of comprehensive breast cancer prediction models that take into account both genetic and environmental factors, we are unable to quantify future breast cancer risk for many patients. This uncertainty often leads to the exclusion of high-risk individuals in screening and prevention trials, which is perhaps most evident in breast cancer screening trials incorporating the use of magnetic resonance imaging (MRI) to identify early cancers [3–10]. These studies demonstrate that MRI increases the sensitivity of a screening protocol in mutation carriers and succeeds at detecting earlier stage cancers [3–10]. Eligibility criterion for most of these trials was documented mutations in *BRCA1* and *2* or future breast cancer risk predicted by family history or models, thereby possibly excluding women at significantly elevated risk that testing failed to identify or whose risk is not adequately reflected based on current models used in risk assessment. We may be turning very high-risk women away from screening trials, recommending yearly mammography and clinical breast exam, when neither will be adequate for detecting their cancers early. In addition, the impact of risk-reducing strategies including bilateral prophylactic oophorectomy (BSO) and tamoxifen has not been analyzed in these studies. For example, a

40-year-old *BRCA2* carrier may only have a 10% and 50% lifetime risk of ovarian and breast cancer, respectively, and interventions including tamoxifen and breast MRI screening may significantly reduce the risk of both getting breast cancer and dying from it, thereby obviating the need for early screening or prophylactic surgeries, permitting these women to defer the quality of life struggles until they are older. A larger sample size is needed to determine the degree to which different subgroups of high-risk patients will benefit from MRI screening, with particular attention to women who have undergone BSO or who are taking tamoxifen. The challenges in risk selection are numerous and produce more questions than answers with regard to screening and management of high-risk individuals. In the future, we hope that early detection tools, risk-reduction strategies, and risk assessment preclude the need for prophylactic surgeries, inappropriate selection of patients for screening, and the associated decisions that compromise our patients' quality of life.

1 Introduction

1.1 Genetic Syndromes Associated with Breast Cancer Susceptibility

It is estimated that about 5%–10% of all breast cancer is inherited, meaning that a mutated gene is passed down through the germline in an autosomal-dominant pattern and causes a high penetrance for the disease. There are a few known genes that when mutated, encode a faulty protein that confers an extremely elevated breast cancer risk. Table 1 describes a list of known genes that give a high penetrance for the development of breast and other cancers. In contrast, there are potentially hundreds of other genes that when mutated, or differentially expressed, bestow a high risk for developing breast cancer. Furthermore, certain genes can be triggered by endogenous or environmental exposures and subsequently undergo somatic mutations or expression changes to contribute to breast cancer risk. An example of this would be mutagenesis in promoter-hypermethylated DNA caused by oxidative DNA damage [11].

Table 1. High-penetrance breast cancer genes

Syndrome	Gene	Contribution
Breast/ovarian cancer	*BRCA1*, *BRCA2*	40%–60%
Li-Fraumeni	*P53*	<1%
Cowden's Disease	*PTEN*	<1%
Peutz-Jeghers	*STK11/LKB1*	<1%
Muir-Torre	*MLH1*, *MSH2*	<1%
Klinefelter's	*47 XXY*	<1%
Ataxia-telangiectasia	*ATM*	<1%

1.2 Identifying "At-Risk Patients"

The identification of the sequence of the *BRCA1* and *2* breast cancer susceptibility alleles has allowed us to identify woman who carry deleterious alleles. However, more often than not, even when the family history is very significant, a deleterious allele is not identified. The goal of breast cancer risk assessment is to accurately quantify individual breast cancer risk and then recommend a personalized mangagement protocol. Several models are in use that aid in predicting a woman's risk of both carrying a mutation in *BRCA1* and *2* and of developing breast cancer either within 5 years or during her lifetime (Table 2).

While these models can be extremely helpful, they do not apply to all circumstances (Table 2). The Couch, Shattuck-Ediens, Frank, and BRCAPro models are most common models in use [12–15]. The following tables will summarize the pros and cons of using these models and describe how they help us in the risk selection process.

In order to offer genetic testing for these syndromes, one would like to see manifestations of symptoms that are indicative of the disease. Frequently, low-risk patients present to our cancer risk and prevention clinic requesting genetic testing. We use non-directive counseling techniques to help patients make decisions about testing. Therefore, many patients whom we deem to have a low risk ultimately get tested. While most of these individuals test negative, we have identified *BRCA1* and *2* mutations in families that

Table 2. Probability models

	Couch	Shattuck-Eidens	Frank	BRCAPro
Gene	*BRCA1/2*	*BRCA1*	*BRCA1/2*	*BRCA1/2*
Sample	>600 families with brca or ovca	800 affected women with multiple cases of brca and ovca	238 women with brca<age 50, ovca, and >1 1st- or 2nd-degree relative with brca/ovca	Published mutation frequencies, penetrance, cancer status, and age
Strengths	Now updated to include a larger sample size and *BRCA2* families	Can be used on women with no Fhx	*BRCA1* and *2*	Bayesian model, provides information on affected and unaffected relatives
Limits	Cannot use on unaffected probands; initially based on small sample size	Cannot use on unaffected probands; only uses proband and 1 other affected relative	brca<age 50; higher estimates due to stringent criteria	Overestimates Ashkenazi heritage; not good for ethnically diverse families
When to use	1 or more cases of brca	?	?	The most commonly used model in our risk clinic

brca, breast cancer; Fhx, family history; ovca, ovarian cancer.

Table 3. Risk models

	Gail	Claus
Variables	Age, race, age of menarche/menopause/first live birth, No. of previous breast biopsies, ADH	FH
FH	Only mothers, sisters, and daughters	1st- or 2nd-degree relatives
Strengths	Uses risk factors other than FH	Maternal and paternal FH; age of onset
Limitations	No paternal history or age of onset, No. of breast biopsies	Only includes 1st- and 2nd-degree relatives
When to use	All non-carriers	All non-carriers

ADH, antidiuretic hormone; FH, family history.

have a low pre-test probability, making us ill at ease with reliance on our own experience and probability models for predicting the likelihood of carriership. In contrast, we see patients that have a very high pre-test probability that test negative for *BRCA1* and *2*. Furthermore, other breast cancer genetic syndromes such as Cowden's disease can be difficult to diagnose because pathognomonic criteria, including mucocutaneous lesions, breast, endometrial, or thyroid cancer, macrocephaly, and Lhermitte-Duclos disease are not always present, making it difficult to make the diagnosis in the absence of a documented mutation in PTEN [16]. Consequently, we are concerned that a patient could have an overlapping genetic syndrome that may cause breast cancer or other cancers, but we are unable to give the patient any valuable information about risk or management of risk that may help prevent any future cancers they may develop. Do we send these patients back to their primary care doctor, or do we follow them with a special screening protocol, looking for cancers that may or may not develop?

The difficulty then arises how to counsel the patient on breast cancer risk reduction and management. Patients, physicians, and counselors alike are relieved when a mutation is not found. However, if a mutation is not identified, this relief may be falsely reassuring. If a mutation is not found, we determine 5-year and lifetime risk for developing breast cancer using the Gail and Claus Models [17, 18]. Table 3 summarizes the features of each model and its strengths and limitations. These models allow us to determine which patients are appropriate for chemoprevention trials. At Dana Farber Cancer Institute, we currently offer three prevention and early detection protocols; The STAR trial, evaluating tamoxifen versus raloxifene for prevention, The Wise Trial assessing the use of letrozole to lower serum estradiol levels in postmenopausal women, and The MRI/Tamoxifen trial to determine if tamoxifen can change breast density and the appearance and number of lesions screen-detected by breast MRI. Our breast cancer early detection protocol, Project Cadence, is a comprehensive screening protocol for *BRCA1* and *2* mutation carriers and women at 50% risk of being a carrier and utilizes

high-quality breast MRI, mammography, ductal lavage (DL), and clinical breast exam (CBE) to determine the sensitivity, specificity, positive predictive value (PPV), and negative predictive value (NPV) of each modality for the detection of invasive breast cancer, ductal carcinoma in situ (DCIS), and premalignant breast lesions. Women who are not mutation carriers, but have a high 5-year and lifetime risk for developing breast cancer based on the Gail and Claus models may be followed off-protocol with screening MRIs and will be included in a prospective cohort in the future.

How to manage women when they test negative for *BRCA1* or *2* but have a family history of only ovarian cancer is an additional challenge for cancer risk clinics. The estimated lifetime risk of developing ovarian cancer in North America is 1.4%. The relative risk for first-degree relatives of probands with ovarian cancer is 3.1% [19]. The probability that an individual with a first-degree relative will carry a mutation in *BRCA1* and *2* is less than 5%. How should we counsel these individuals when the likelihood that we will identify a mutation is low? If they proceed with testing and a mutation is not identified, how shall we counsel them on risk reduction for ovarian cancer? These are all very difficult questions to answer, and ones that are constantly being addressed in our cancer risk and prevention clinic.

1.3 Breast Cancer Management of High-Risk Women

A female mutation carrier of *BRCA1* has a 50% risk of developing breast cancer by age 50, emphasizing the need for sensitive screening strategies that begin at an early age [20, 21]. The only known effective intervention for preventing breast cancer in women that carry mutations in *BRCA1* and *2* is prophylactic mastectomy (PM) [22, 23], although tamoxifen may provide some benefit [24, 25]. For female mutation carriers who choose surveillance, screening with conventional mammography and clinical examination is recommended, despite concerns that this strategy may not have sufficient sensitivity to reduce breast cancer mortality, and that ionizing radiation from mammography may promote *BRCA*-related breast carcinogenesis [9, 27]. These issues are particularly relevant to women under age 50, in whom the sensitivity of mammography has been estimated to be lower than for women ages 50–64. Furthermore, as more women under the age of 30 are identified with *BRCA1* and *2* mutations, the concern of yearly exposure to ionizing radiation from 4-view mammography becomes greater. Due to these concerns, cancer risk clinics around the world have begun to test the sensitivity of additional screening imaging modalities in high-risk women.

1.3.1 Screening Mammography

Screening mammography is recommended for women over 50 years old who are at average risk based on the mortality reduction demonstrated from eight randomized controlled trials which evaluated the ability of mammography to reduce breast cancer mortality, but is not for women under 50 [28]. Estimates of the sensitivity of mammography from these published trials, which included women of all ages, have ranged from 39% to 89%. After exclusion of women older than 50, the sensitivity of mammography ranges from 39%–66%. Many published studies have confirmed that the sensitivity of mammography increases with age [29, 30]. The sensitivity of mammography may be further decreased in women who carry a deleterious mutation in *BRCA1* or *2;* pilot studies evaluating mammography in *BRCA1* and *2* carriers have found a high rate of false negatives [6–10]. This may be attributable both to increased breast density in young women and to tumor phenotype, including features such as pushing margins and lymphocytic infiltration, which may contribute to a smooth rather than a spiculated appearance of a mass on mammography [31]. Nonetheless, women with a family history of breast cancer, some of which carry a mutation in *BRCA1* and *2*, benefit from surveillance with mammography [7]. In these studies, surveillance with mammography identified more T1N0-stage tumors than women who had a family history that did not undergo screening with a reduction in mortality, strongly suggesting that women who opt not to pursue PM should be enrolled in a screening program. Furthermore, data from MRI screening trials suggest that the addition of screening breast MRI can boost the sensitivity of a screening program [3–10, 31, 32]. These data do not support reliance on annual mammography as the sole mode for the detection of early breast cancer in *BRCA1* and *2* mutation carriers. We currently recommend that all women who carry a known deleterious mutation in *BRCA1* and *2* include screening breast MRIs as part of their screening program, and we urge all centers that offer screening breast MRI to high-risk women to prospectively collect outcomes data on breast MRI compared to mammography so we have the potential ability to understand the utility of each modality in different cohorts of women.

2 Methods and Results for Screening High-Risk Women: Breast MRI, MR-Galactography, and Psychosocial Assessment of Screening

2.1 Breast MRI

As a diagnostic tool, contrast-enhanced MRI has demonstrated high sensitivity for the detection of invasive breast cancer, but specificity lower than that of mammography [33–36]. Recent centers have reported specificity of breast MRI to be as high as 98%, presumably related to increasing experience with breast MRI at these centers [4].

Breast lesions on contrast-enhanced MRI are assessed for malignancy based on morphologic and pharmacokinetic patterns of enhancement. Morphologic features of malignancy include spiculated borders and rim enhancement. Pharmacokinetic features of malignancy include a rapid rate of contrast uptake and an early rate of contrast washout. The specificity of MRI for invasive disease can be low because benign and malignant breast disease can have similar enhancement patterns, and most breast MRI centers have not had enough experience to know what is more likely to be benign than malignant based on subtle morphologic and dynamic features. As we have seen—along with others—low specificity can lead to high rates of costly additional imaging and biopsy procedures and perhaps more anxiety. The risk/benefit ratio for financial costs, mortality benefit, and psychosocial benefits or harm needs to be evaluated before we can recommend breast MRI as a screening tool to high-risk individuals.

Six international published studies and three published abstracts have evaluated the benefit of breast MRI added to a screening protocol using mammography and physical exam in high-risk populations [3–10, 32]. Some of these studies included ultrasound as a screening modality [9, 10]. Collectively, these studies demonstrate that MRI increases the sensitivity of a screening protocol in mutation carriers and high-risk non-carriers, and succeeds at detecting earlier stage cancers. The two largest of these studies have been published in abstract form and have demonstrated that breast MRI was significantly more sensitive than mammography in identifying early-stage invasive cancers and that the specificity was as high or higher than mammography [4, 5]. Because these trials accrued more than 750 and 1,900 women respectively, presumably these centers gained considerable experience in reading and interpreting breast MRI, and therefore they recommended fewer biopsies, thus increasing their specificity. The potential compromise for high specificity is missed invasive and non-invasive cancers and precancerous breast lesions that could ultimately impact breast cancer mortality. Kriege et al. reported the incidence of DCIS, but neither abstract reported the incidence of premalignant breast lesions or the rate of cancers detected

differentially in pre- and postmenopausal subjects. Because of the high sensitivity and specificity of breast MRI in these trials, recommendations for the exclusive use of screening breast MRI were made. However, before a blanket recommendation for the singular use of breast MRI can be made, data on PPV and NPV in specific cohorts need to be addressed.

All studies confirmed breast MRI's ability to detect invasive breast cancer with PPV values ranging from 9% to 88% and NPV values of 100% (Table 4). To be confident of an NPV, one needs to have pathologic confirmation that no cancer is present. This can only be assessed when women enrolled in a screening protocol opt for prophylactic mastectomy. There are few data reported on rates of cancer found at PM, and thus caution is advised when interpreting NPV from non-randomized screening trials.

Each of these screening studies had a slightly different definition of risk. Of these studies, only two had reported over 50% of the tested cohort to be *BRCA1/2* mutation carriers (Table 4) (Warner and Hartman). These studies had much stricter eligibility criteria for study entry. Eligibility criteria for Kriege et al. was a lifetime risk of 15%, only slightly higher than the lifetime risk for an average-risk woman in the U.S. Morris et al. defined high risk as either a personal or family history of breast cancer, a history of lobular carcinoma in situ, or atypical ductal hyperplasia (ADH) whereas Kuhl et al. defined risk strictly based on personal or family history of breast, ovarian, and male breast cancer. Stoutjesdijk et al. defined specific risk categories; high risk were mutation carriers, moderate risk was 30%–50% based on Claus, and low risk was 15%–30% based on Claus, while Tilanus-Linthorst defined low risk as a lifetime risk of 15%–25% based on Claus and family history and moderate risk as exceeding 25% based on Claus and family history. These data suggest that even women with a moderate, lifetime risk of 15% may benefit from MRI screening (Table 5). Additional factors such as age, menopausal status, and subtype of cancer detected were not reported in all these trials; and while it may appear that all risk categories will benefit from MRI screening, lack of data on epidemiologic characteristics of patients enrolled and cancers identified make this conclusion potentially erroneous.

Our screening trial, initially started at Stanford University and now being continued at Dana Farber Cancer Institute, evaluated the addition of high-quality breast mammography, CBE, and DL to assess the detection of breast cancer or pre-cancerous breast lesions. Women with inherited *BRCA1* or *2* mutations, or women with a greater than 10% risk of developing breast cancer at 10 years as estimated by the Claus model, were eligible. Patients were accrued from September 2001 to December 2003. Enrolled patients underwent biannual clinical breast exam, and annual mammography, breast MRI, and DL. Table 6 is an update of our patient characteristics and Table 7 an update of our screening results with mammography and breast MRI.

Table 4. Breast MRI screening trials results

	Type of study	Number of cancers found by mammo/MRI	Sensitivity/specificity	PPV/NPV
Kuhl [4, 8]	Prospective, non-randomized	[1]3/9; 9/9	33%/93% mammo 100%/95% MRI	[1]30%/94% mammo 64%/100% MRI
Warner [9]	Prospective, non-randomized	2/7; 7/7 3/7 ultrasound	33%/99.5% mammo 100%/91% MRI 60%/93% ultrasound 33%/99.5% CBE	66%/97% mammo 26%/100% MRI 19%/99% ultrasound 66%/97% CBE
Tilanus-Linthorst [7]	[2]Prospective, non-randomized	16/26; subgroup of mammo got MRI 3/26 not detected by mammo	[3]100% sensitivity for MRI	N/A
Kriege (abstract) [5]	Prospective, non-randomized	41 cancers found total	26%/99.8% mammo 71%/98% MRI	53% mammo 30% MRI
[4]Stoutjesdijk (*n*=179) [32]	Retrospective	6/13; 13/13	42%/96% mammo 100%/93% MRI	33%/97% mammo 43%/100% MRI
Morris [45]	Retrospective	[5]14/14 MRI	N/A	24%/100%
Hartman [6]	Prospective, non-randomized	0/1	Not reported (numbers too small)	9%/100% MRI
Robson (abstract) [3]	Prospective, non-randomized	1/3; 3/3	100%/81%	14.3%/100% MRI
Podo [10]	Prospective, non-randomized	1/8; 8/8	Not reported	88%/100% MRI

CBE, clinical breast exam, mammo mammography; MRI, magnetic resonance imaging; N/A, not available; NPV, negative predictive value; PPV, positive predictive value.
[1]Abstract reported at ASCO 2003 with 51 cancers detected (34% sensitivity mammo, 95% sensitivity MRI/95% specificity MRI, reportedly better than mammo and ultrasound; PPV: 54% MRI; 26% mammo; 16% ultrasound).
[2]Compared to women with a family history who presented with symptoms (control=unscreened group).
[3]Only a subset of the surveillance group had MRIs; could not calculate specificity.
[4]Of 179 women, only 75 had both mammography and breast MRI within a 4-month period.
[5]Retrospective analyses only evaluated mammographically occult tumors.

Table 5. Breast MRI screening trials cohort characteristics

	Patient number	Risk (mean age)	Percentage BRCA carriers	Menopausal status	DCIS	Premalignant lesions reported	BSO/tam use reported
Kuhl [4]	[1]462/750 (abstract)	[2]Personal or Fhx of brca or ovca (39)	28% tested 64% positive (2000)	Not reported	3/9 cancers (2000)	1/5 false + MRIs (2000) (1 atypical ductal hyperplasia,)	No
Warner [6, 9]	196	Known mutation carrier or mutation in family; ≥3 relatives with brca<50 or ovca (43)	49%	123/73 pre- and postmenopausal	1/7 DCIS seen on mammo only	Yes, 1 premalignant lesion identified by mammo only	No
Tilanus-Linthorst [7]	384	[3]Moderate=294 (43.3), high=384 (42.9)	Not reported	Not reported	Not reported	No	No
[4]Kriege (abstract) [5]	1,911	≥=15% lifetime risk; no personal hx brca	16%	Not reported	Yes	No	No
Stoutdesdijk [32]	179	[5]>15% lifetime risk of mutation carriers	Not reported	Not reported	3/13	No	No
Morris [45]	367	Personal or Fhx brca; LCIS; ADH (50, median)	5% positive 95% no or unknown	52% pre 48% post	8/14 cancers DCIS	13/59 lesions biopsied	No
Hartman [6]	41	Mutation carriers or >[6]10% risk at 10 (41, median)	58.5%	59% risk reduced by BSO/tam	1/1 DCIS	4/12 lesions biopsied	Yes
[4]Robson (abstract) [3]	54	All mutation carriers (44)	100%	50% premenopausal	2/3	Not reported	No
Podo [10]	105	Mutation carriers or had a 50% risk of being a carrier (46)	Not reported	Not reported	3/8	Not reported	No

2000, means data was published in 2000; ADH, atypical ductal hyperplasia; brca, breast cancer; BSO, bilateral salpingo-oophorectomy; DCIS, ductal carcinoma in situ; Fhx, family history; hx, history; LCIS, locular carcinonma in situ; tam, tamoxifen.

[1] Over 750 patients reported only in abstract form.

[2] Personal or family history is defined as at least one person with brca<age 35, ovca<age 40, bilateral brca, both brca and ovca, or male brca.

[3] Moderate risk=15%–25%; high risk≥25% risk based on Claus and Houlston tables.

[4] Published in abstract form only, ASCO 2003 [3].

[5] High=15%–30% lifetime risk; very high=30%–50% based on Claus tables; very high=mutation carriers.

[6] Ten-year risk based on Claus.

Table 6. Patient characteristics (median age, 42.5 years)

	Number	%	[2]BSO	Tamoxifen	brca	ovca
BRCA1 carriers	22	51.2	12	3	6	2
BRCA2 carriers	6	7.3	2	1	1	0
[1] Non-mutation carriers	14	31.7	2	2	2	1
[3] Variant unknown significance	4	9.8	2	3	3	0
Total	46	100	18	9	12	3

BSO, bilateral salpingo-oophorectomy; brca, previous history of breast cancer; ovca, previous history of ovarian cancer.
[1] This figure includes 1 patient with Cowden's disease based on clinical features and patients who had a greater than 10% risk of breast cancer at 10 years, based on the Claus model.
[2] Of *BRCA1* carriers with a prior BSO, 3 of 7 had a history of ovarian cancer.
[3] These include mutations in *BRCA1* and *2*, which have not been definitively shown to be cancer-causing mutations.

Table 7. Sensitivity and specificity of mammography and breast MRI

	MRI-detected (n=13)	Mammographically detected (n=13)
Invasive cancer	0/13	0/13
DCIS	1/13	0/13
Premalignant lesions	4/13	1/13
Benign findings	7/13	0/13
PPV for cancer	9%	N/A
PPV for premalignant disease	44%	20%

Table 8. Dana Farber MRI-prompted biopsy results

Mutation	[1]BSO	[2]DCIS	[3]IDC	Abnormal mammo
BRCA1 n=21	14	1	1	1
BRCA2 n=8	7	0	0	0
Total n=29	21	1	1	1

IDC, infiltrating ductal carcinoma.
[1] One patient with a history of BSO was also taking tamoxifen.
[2] The patient with MRI screen-detected DCIS also had calcifications seen on her mammogram.
[3] The patient with MRI-screen detected infiltrating ductal carcinoma had a normal mammogram.

The comprehensive screening trial has been launched at Dana Farber Cancer Institute and will continue as a multicenter collaboration with Stanford University. Table 8 is an update of our results thus far.

Table 9. Pathology of screen-detected lesions

	Type of biopsy	Pathologic lesion	F/U MRI
Patient 1, *BRCA1*	MRI-guided wire-localized excisional	Radial scar with ductal hyperplasia and proliferative fibrocystic changes	Unchanged
Patient 2, *BRCA1*	MRI-guided wire-localized excisional	7-cm high-grade DCIS	Mastectomy
Patient 3, *BRCA1*	MRI-guided wire-localized excisional	Radial scar, proliferative fibrocystic changes, focal atypical lobular hyperplasia	Pending
Patient 4, *BRCA1*prior BSO	MRI guided wire-localized excisional	Benign breast tissue	New area of enhancement/ 6-month MRI recommended
Patient 5, *BRCA1*	MRI-guided wire-localized excisional	Proliferative and non-proliferative fibrocystic changes	Pending
Patient 6 *BRCA1*prior BSO	MRI-guided wire-localized excisional	Fibrous scar	Unchanged
Patient 7, *BRCA2*	MRI-guided wire-localized excisional	Non-specific changes	Unchanged
Patient 8, *BRCA2*prior BSO	MRI-guided 14-g core needle biopsy	Stromal fibrosis/ microcalcifications	Improvement in scattered foci
[1]Patient 9, 2 biopsies	MRI-guided wire-localized excisional/mammogram-guided wire-localized excisional	Both consistent w/atypical ductal hyperplasia with duct extension	Unchanged
Patient 10	MRI core-guided	Non-proliferative fibrocystic changes	No-F/U MRI
Patient 11, 2 biopsies	MRI-guided 14-g core needle biopsy	2 intraductal papillomas	Post-surgical changes/ enhancing nodule no longer present

F/U, follow-up.
[1] Patient 9 had a third biopsy from a palpable breast nodule 6 months later, which was consistent with atypical lobular hyperplasia with duct extension.

Our study showed a lower malignancy detection rate which may have resulted from differences in patient populations with regard to risk reduction. Table 7 depicts our screening results. BSO in carriers of *BRCA1* and *BRCA2* mutations has been shown to reduce the risk of developing breast cancer by approximately 50% in pre-menopausal *BRCA1* and *2* mutation carriers [37, 38]. And while the potential protective effect of tamoxifen in mutation carriers is less well studied, a benefit in the development of contralateral breast cancer has been observed in *BRCA1* and *2* carriers [39]. Fifty-eight percent of our cohort consisted of women who had been risk-reduced before their

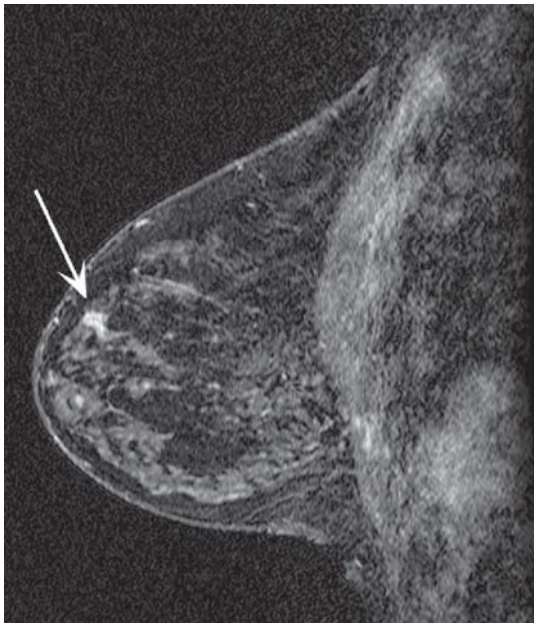

Fig. 1. Identification of atypical lobular hyperplasia by breast MRI. A *white arrow* points to the area of abnormal enhancement, which at biopsy was shown to be atypical lobular hyperplasia

initial screen. Eighteen out of 46 women had had a BSO, and nine out of 46 women were taking tamoxifen: two of these women had been risk reduced in both ways. None of the published studies has reported rates of risk reduction strategies in their cohorts, and because some of these studies accrued women in the early to mid-1990s, a lower rate of BSO, and thus a higher expected risk of breast cancer compared to our cohort would be expected. None of the patients in our series with malignant or high-risk results on biopsy had been risk reduced, and no patients with prior risk reduction had a malignant or high-risk result on biopsy. If this continues to be observed, it will confirm previous reports that BSO and tamoxifen are protective in patients at increased genetic risk of breast cancer [24, 40, 41]. In 46 patients, we identified one high-grade, extensive DCIS in a *BRCA1*-mutation carrier, and several other high-risk lesions, including atypical lobular hyperplasia (ALH) and radial scars (Table 9). All lesions except one case of ALH were screen-detected by MRI and missed by mammography. The one case of ALH was screen-detected by mammography alone.

Four studies have reported the identification of high-risk breast lesions including radial scars and atypical lobular and ductal hyperplasia in their screening cohort. In our study, four out of 41 patients, or 9.8%, were found to have high-risk findings, including DCIS, ALH, and radial scars (Fig. 1).

The potential prevention benefit and mortality benefit from the identification of these lesions has not yet been rigorously tested. It may be that high-risk lesions are predictive of future breast cancer in women at increased genetic risk, and that clinical benefit will follow from earlier detection of these lesions in such patients. The reporting of these lesions is crucial in the understanding of the clinical utility in identifying premalignant breast lesions in women at increased risk.

As of yet, none of these studies has demonstrated a survival benefit from breast MRI screening, and without a randomized trial we will be unlikely to cull this information from sub-optimally designed non-randomized trials. Our data indicate that women who have been risk reduced with either a BSO or tamoxifen may have a lower rate of cancer and premalignant findings on biopsy from screen-detected lesions. If additional data from non-randomized trials report low rates of cancer and premalignant lesions screen-detected by MRI in risk-reduced cohorts, a randomized controlled trial should be considered. The sensitivity of breast MRI lends itself to being a good screening test. However, the low specificity is problematic because it results in a large number of benign biopsies with attendant costs, time, and anxiety. We must really determine who is going to benefit from this technology.

2.2 Ductal Lavage and MR-Galactography

DL is a recently developed approach to identify high-risk and occult malignant lesions in breast epithelial cells in women at high risk for developing breast cancer. Two prospective studies with long-term follow-up have independently shown that women with cellular atypia detected in breast ductal cytology have an approximately fivefold increased relative risk of developing breast cancer [42, 43]. The clinical significance of identifying atypia in an increased-risk cohort is unclear, but could translate into a decrease in breast cancer incidence if appropriate interventions are developed and implemented in the future. However, significant inter-reader variability makes the reliable diagnosis of cytological atypia challenging, as can the use of different pathologic criteria in different series [1, 44]. Given time limitations, two to three ducts per breast, a fraction of the known total of five to eight ducts, are identified and lavaged on our current protocol. The volume that each duct represents is currently unknown and the sampling of only 50% or less of the breast volume may lead to bias: a finding of benign epithelial cells in three ducts may not be representative of the other ducts, which could be cytologically atypical. How then do we counsel women receiving DL with an abnormal result when other imaging tests including breast MRI are normal? Because mild atypia may suggest an unrecognized malignant lesion, or may represent a high-risk lesion with potential to develop into cancer, we choose to use DL in addition to CBE, mammography, and breast MRI as a screening

Table 10. DL results

	Number	Percent
First screen	30/38	79%
Second screen	15	39%
Ducts/patient (*n*=30)	1.37	
ICMD	3/30	10%
[1] Benign	18/30	60%
[2] Atypical	9/30	30%
Malignant	0/30	0%
BRCA carriers with atypia	4/9	49%
Atypical non-fluid yielding ducts	6/9	66%
[3] Atypia and BSO	3/9	33.3%
[4] Atypia and tamoxifen	1/9	11%

ICMD, insufficient cellular material for diagnosis.
[1] Some patients with multiple ducts lavaged and benign findings also had ICMD.
[2] Twelve atypical results were identified in 10 patients.
[3] Patients who had atypia and had a BSO prior to screening.
[4] Patients who had atypia and were on tamoxifen prior to screening.

tool for premalignant breast lesions. Our objectives are to determine if atypia detected on DL could increase the specificity of breast MRI and to determine lead-time, if any, for the development of breast cancer detected by imaging.

Table 10 shows our updated results. We identified seven women with atypical cytology—42.9% of whom were BRCA carriers—out of 30 women who underwent successful DL in our high-risk cohort. One patient with atypia had a high-risk lesion (ALH) involving the same breast. No patient risk-reduced by tamoxifen had atypia. There did not appear to be much difference in rate of atypia between patients who had been risk-reduced by prior BSO and patients who had not, although the small numbers and broad confidence intervals make it difficult to estimate the true difference in rates. Five of eight samples with atypia, or 62.5%, were from non-fluid yielding ducts. The finding of atypia identified in non-fluid yielding ducts has not previously been reported, and suggests that the identification and lavage of non-fluid yielding ducts should be investigated in clinical trials evaluating the utility of DL in risk assessment. Longer follow-up is required to determine whether the detection of atypia in lavage fluid can accurately predict the development of breast cancer.

Because of the high rate of atypia in our study in women who have a *BRCA1* and *2* mutation and normal findings on contrast-enhanced breast MRI, we are currently developing a protocol combining DL and breast MRI called MR-galactography to determine if we can increase the sensitivity of finding precancerous lesions. The purpose of MR-galactography is first to define the normal anatomy of the breast ductal system by better defining the

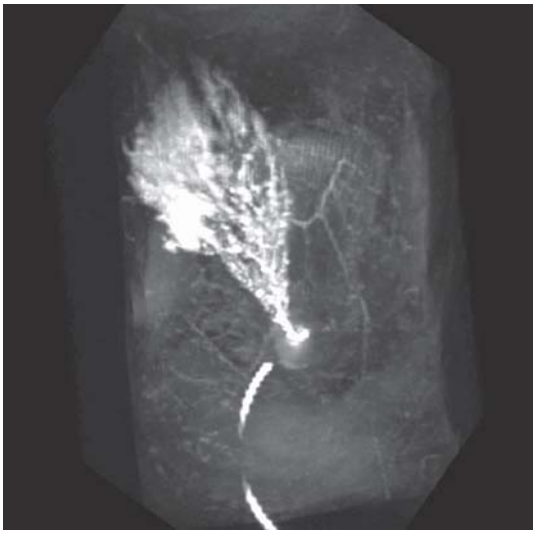

Fig. 2. MR-galactogram demonstrating the geographic distribution of a duct that had atypical cells when lavaged. The contrast-enhanced image did not show any parenchymal abnormalities

anatomical distribution and volume of the breast ductal system and determine the origin of atypical breast lavage specimens through MRI galactography. Very little is known about the anatomy of the ductal system and, when atypia is identified, how to localize the lesion when screening MRI and mammography are normal. One potential use for MR-galactography is to determine if atypia identified on ductal lavage seen in the context of a normal contrast-enhanced MRI can be localized using MRI galactography and if severe atypia may have a characteristic appearance on MRI. MR-galactography can be used to determine if subtle lesions on a contrast-enhanced image should be followed more closely or biopsied if they are geographically located in a duct that has atypia. To date, four of these procedures have been performed (Fig. 2). We have successfully been able to image three ducts per breast and not more due to technical limitations. The surface area of the nipple is not large enough to accommodate more than three catheters. We are currently working on developing smaller catheters to be able to assess more than five ducts and more than 75% of the breast volume.

In our small sample size thus far, we have not detected any abnormalities requiring biopsy in ducts where atypia has been identified on lavage. We plan to increase the sample size to 25 patients who have atypia.

3 Conclusions: Future Directions for Risk Selection and Breast Management

Our goals in risk selection and management for women at high risk for developing breast cancer are to test the hypothesis that breast MRI and DL can identify earlier stage breast cancers and high-risk lesions among women at increased genetic risk for breast cancer than those found by mammography and clinical breast exam alone. To test this hypothesis, we plan to accrue more than 500 mutation carriers in the next 3 years in this comprehensive screening protocol combining clinical breast exam, mammography, high-quality breast MRI, and DL to determine the rate at which both high-risk and malignant lesions are detected, and to optimize a protocol for further evaluation in a larger cohort. In addition, we plan to determine if women who have been risk-reduced with tamoxifen and BSO will benefit from the high sensitivity of breast MRI. Furthermore, other high-risk cohorts that do not have genetic risk, but are high risk based on other factors, may benefit from screening breast MRI as evidenced by data from the pilot trials assessing MRI as a screening tool in this population.

A trial of this magnitude is clearly needed to determine (1) the MRI interpretation criterion for screening high-risk women, (2) the trade-off between MRI sensitivity and specificity, and (3) the clinical utility of combining ductal lavage with MRI in a high-risk population. A collaborative effort between breast MRI centers is needed for rapid accrual of patients in order to answer these important questions in the management of women at high genetic risk for developing breast cancer.

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Chemoprevention of Lung Cancer

Stéphane Vignot[1] · Jean-Philippe Spano[2] · Sylvie Lantuejoul[3] · Fabrice André[1] · Thierry Le Chevalier[1] · Jean-Charles Soria[1] (✉)

[1]Department of Medicine, Institut Gustave Roussy, 39 rue Camille Desmoulins, 94805, Villejuif, France
soria@igr.fr
[2]Oncology Department, SOMPS, Hopital La Pitié-Salpetrière AP-HP, Paris, France
[3]Cellular Pathology, Lung Cancer Research Group INSERM 9924, CHU Michallon, Grenoble, France

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Abstract Lung cancer remains a major cause of mortality worldwide, despite advances in surgery, radiotherapy and chemotherapy. Most patients present with advanced disease, and early detection approaches are still experimental. Chemoprevention strategies are therefore essential. Chemoprevention can be defined as the use of specific natural or synthetic chemical agents to reverse, suppress or prevent progression to invasive cancer. The present review will provide an update on lung cancer clinical chemoprevention trials as well as the molecular basis of lung carcinogenesis. A better knowledge of lung carcinogenesis is obviously fundamental to improve chemoprevention strategies. Identification of molecular defects involved in premalignant lesions and/or invasive cancer could lead to clinical studies with new molecular-targeted agents (mainly tyrosine kinase inhibitors, farnesyl-transferase inhibitors and/or antiangiogenic molecules) and the development of surrogate biomarkers. Such biomarkers would be essential to detect high-risk patients,

select adequate chemoprevention strategies and monitor drug efficacy. New chemoprevention trials are planned with collaborative efforts of researchers involved in fundamental or clinical studies.

1 Introduction

Despite tobacco control campaigns, tobacco-related cancers remain a great concern. In particular, lung cancer is a major cause of mortality worldwide, especially in developing countries, where tobacco consumption is still rising. An estimated global annual incidence of over 1.2 million cases and an overall mortality of over 1.1 million cases are presumed [1]. Estimates of cancer incidence and mortality in Europe in 1995 were 377,000 new cases of lung cancer and 330,000 deaths from this disease [2]. In the United States, an estimated 171,900 new cases of lung cancer were expected annually in 2003, meaning an estimated 157,200 deaths per year from lung cancer [3]. The most effective treatment for non-small cell lung cancer (NSCLC) remains surgical resection, but at the time of diagnosis about 70% of NSCLC patients present with advanced diseases and/or visceral metastases. For these, no curative surgery is possible and treatment is based on chemotherapy with classical cytotoxics and/or new molecular-targeted therapies. Improving the survival rate of patients with lung cancer will not be achieved only by improving these strategies. Indeed, major efforts are currently being deployed to facilitate earlier detection of lung cancer in high-risk patients and to develop chemopreventive approaches. Chemoprevention is defined as the use of natural or synthetic agents to reverse, prevent or delay carcinogenic progression to invasive cancer. This strategy requires the understanding of molecular events leading to lung cancer in order to identify genetic factors involved in lung cancer progression. Modern chemopreventive medicine is thus tightly related to a better comprehension of the carcinogenic process. The understanding of the molecular and biological basis of lung cancer has significantly expanded over the last 20 years. The present review will provide an overview of the current genetic changes associated with lung carcinogenesis. This review will also summarize the outcome of the major lung clinical chemoprevention trials.

2
Lung Carcinogenesis

2.1
Basic Concepts

Two fundamental concepts should be considered because they underlie all chemoprevention strategies: multistep carcinogenesis and field cancerization.

Multistep Carcinogenesis. According to the multistep carcinogenesis concept (Fig. 1), cancer develops in a stepwise fashion, with an accumulation of molecular alterations progressing from preinvasive lesions to invasive disease

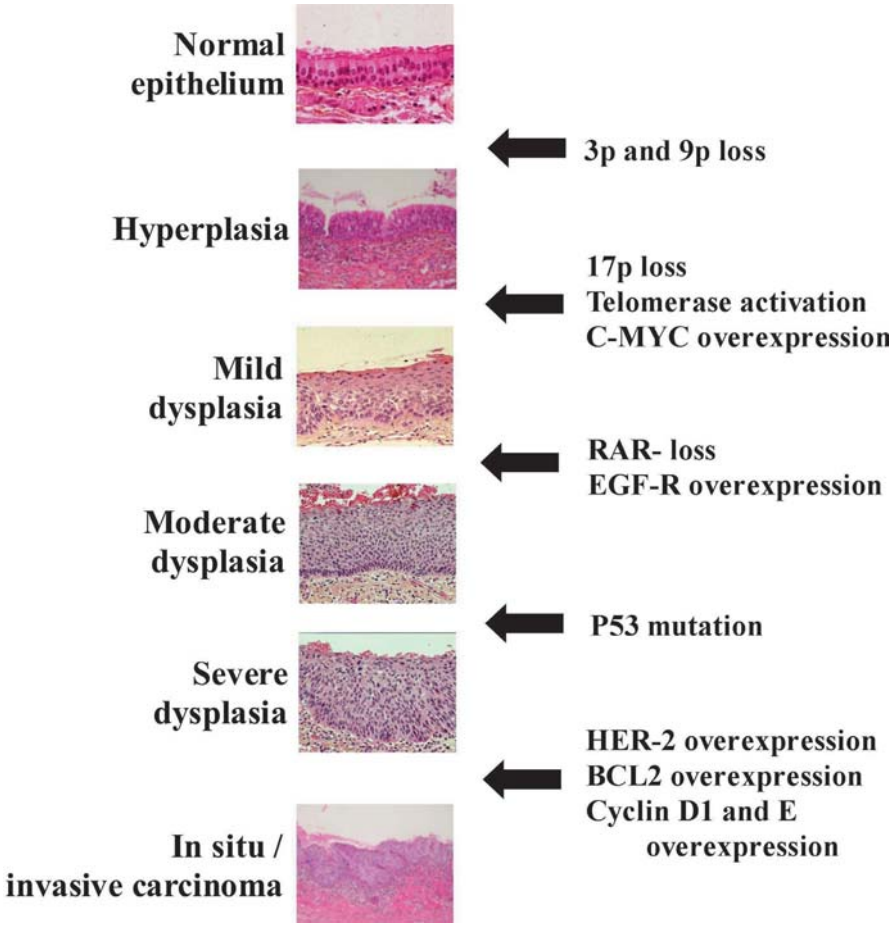

Fig. 1. The multistep carcinogenic process

[4]. The earliest events of this process are mutations, deletions or polysomy at the cellular genomic level. These genetic modifications are not initially translated into cellular morphologic alterations or tissular structural changes [5]. Additional events are necessary to induce phenotypic, then physiologic modifications at the tissular level (uncontrolled proliferation, invasion, metastasis, etc.). It has been suggested that 10–20 genetic events are necessary in the setting of lung cancer [6], the most relevant of these events will be described below.

Field Cancerization. Carcinogen exposure (e.g. cigarette smoke) to an entire epithelium (field) such as the lung will result in diffuse tissue damage. Thus, genetic changes and/or premalignant lesions in one area of the exposed field imply an increased risk of developing cancer in any other site within the same field [7]. Treatment or control of precancerous lesions is then a potential means to avoid invasive lesion development.

2.2 Molecular Basis of Lung Carcinogenesis

2.2.1 Genetic Susceptibility

Over 80% of lung cancers are attributed to tobacco and its carcinogenic products. However, epidemiological studies show that only 15% of smokers will ultimately develop lung cancer. The fact that 85% of smokers do not develop lung cancer indicates differences in susceptibility [8]. A study of genes implicated in activation or detoxification of tobacco carcinogens showed that enzymatic genetic polymorphisms may play a role in lung and head-and-neck cancer incidence. In this setting, it has been suggested that a high activity of cytochrome P450 could be a risk factor of lung cancer [6] and that specific mutations associated with cytochrome P450 genes could be implicated in lung cancer susceptibility [9]. Besides, the null genotype of detoxification enzyme glutathione S-transferase (GST) and M1 GST also seems to be a risk factor of lung and head-and-neck cancers [10–12]. Furthermore, recent case-control studies have shown that defective repair of genetic damage and increased sensitivity to mutagens have been associated with increased individual susceptibility to lung cancer [13]. In this setting, the DNA excision repair pathway might also be implicated [14].

2.2.2 Chromosomal Alterations

Fewer than 10% of lung cancers are diploid, and the large majority of patients with lung cancer present chromosomal abnormalities not only in tumour cells but also in histologically normal adjacent tissues [15]. The

amount of DNA (DNA index) has been correlated with the severity of dysplasia in precancerous bronchial lesions, and with greater tumour size, poor differentiation and node invasion in invasive lung lesions [16]. Various chromosomal imbalances were identified in lung cancers and in in vitro epithelial bronchial tumour cell lines. The most common chromosomal abnormalities in lung cancer are allelic deletions or LOH (loss of heterozygosity) at sites characterized by tumour suppressor genes which will be described below: 3p (*FHIT* and others), 9p (9p21 for $p16^{INK4}$, $p15^{INK4B}$ and $p19^{ARF}$), 17p (17p13 for *p53* gene and others), 13q (13q14 for *retinoblastoma* gene and others). 3p and 9p losses have been associated with smoking and are recognized as early events of lung carcinogenesis. They remain detectable many years after smoking cessation [17]. The loss of 17p13 is less common, suggesting that p53 alterations are rather a late event. The frequency and the number of chromosomal abnormalities parallel the phenotypic progression from premalignant lesions to invasive cancer [18]. Deletions affecting 3p, 5q, 8p, 9p, 17p and 18q chromosomal regions are also among common changes in lung cancer.

2.2.3 Oncogenes

Activation of oncogenes are related to genetic modifications including mutation, amplification or chromosomal rearrangement as well as to epigenetic changes such as hypermethylation. More than a 100 oncogenes have been identified to date; among them, several are implicated in lung carcinogenesis (Table 1). RAS, C-MYC, epidermal growth factor receptor (EGFR, also nammed HER1) and HER2/neu play an important role in lung cancer. Telomerase activity is also involved in this process.

– *RAS* mutations are detected more frequently in adenocarcinomas and large cell lung carcinomas or carcinoid tumours than in squamous cell carcinomas where mutation level is often lower [19]. The *RAS* family encodes 21-

Table 1. Main oncogenes implicated in lung carcinogenesis

Oncogene	Usual alterations
RAS	Mutations in adenocarcinoma, large cell lung cancer and SCLC
C-MYC	Genetic amplification both in SCLSC and NSCLC
EGFR	Overexpression in NSCLC (prognostic factor) and in SCLC
HER2	Overexpression in NSCLC (prognostic factor)
Cyclin E, D1, B1	Deregulation in premalignant lesions and in NSCLC
GRP/Bombesin	Overexpression in SCLC; higher level of expression in women
Telomerase	Overexpression in NSCLC (prognostic factor) and in almost all SCLC

NSCLC, non-small cell lung cancer; SCLC, small cell lung cancer.

kDa proteins able to bind guanosine triphosphate (GTP) to form RAS-GTP complex which transduces proliferation signals. This activation in RAS-GTP induces transcription factors C-FOS, C-JUN, C-MYC and DNA synthesis. Activating *RAS* mutations are mostly identified at codon 12 of the *K-RAS* gene induced by tobacco carcinogens like benzo[*a*]pyrene and nitrosamine, more rarely at codons 13 and 61, and infrequently in *N-* and *H-RAS* genes.

- Oncogenic activation of C-MYC occurs in 20% of small cell lung carcinoma (SCLC) and in 10% of NSCLC in relation with a genetic amplification. L- and N-MYC are also frequently overexpressed in NSCLC (35%) and this profile is very usual in aggressive neuroendocrine lung cancer [20]. Interestingly, patients with lung cancer present with a high C-MYC level in histologically normal or altered lung surgical margins [21]. This suggests that C-MYC expression is an early event in lung carcinogenesis.
- EGFR (HER1) and HER-2/neu are tyrosine kinase receptors both involved in lung cancer progression and overexpressed in NSCLC. EGFR overexpression has been associated with poor survival, an advanced stage, a poor differentiation, a high proliferation index and an increased risk of metastasis [22, 23]. HER-2/neu overexpression is also a pejorative prognostic factor, especially associated with a higher degree of chemoresistance [24]. EGFR and HER2/neu overexpression is mainly due to an increase of both transcription and translation, with only a low percentage of tumours presenting a gene amplification similar to the one observed in breast carcinomas with HER2/neu.
- More recently, the role of cyclins E, D1 and B1 as potential oncogenes in lung cancer has been highlighted [25–27]. Cyclin D1 and cyclin E overexpression is responsible for deregulation of RB phosphorylation in about 50% of lung carcinoma and is an early event (it can be detected by immunohistochemistry in half of dysplastic lesions) [28].
- Expression of neuroendocrine factors, including gastrin-releasing peptide/bombesin-like peptides (GRP/bombesin), and their receptors, has been reported in lung cancers. The GRP-receptor autocrine loop appears particularly important in SCLC. GRP mRNA expression was detected more frequently in females than in males, suggesting that this gene may be a factor in the increased susceptibility of women to tobacco-induced lung cancer [29].
- Telomerase is expressed in 80%–85% of NSCLC and in almost all SCLC [30, 31]. Telomerase is the key enzyme stabilizing the telomeres, which are highly complex terminal chromosome structures, whose correct function is crucial for normal cell survival. Telomerase is preferentially expressed in tumour cells with short telomeres and is not expressed in most somatic cells which usually have longer telomeres. The expression level is a prognostic factor in early-stage NSCLC [32] and its activity has been correlated with stage and node invasion [33]. Telomerase activity is detected in precancer-

ous lesions of the lung, reflecting the early involvement of the molecule in lung tumourigenesis [34].

2.2.4 Tumour Suppressor Genes

Tumour suppressor gene inactivation may be due to mutation, loss of chromosomal material (one or two alleles) or epigenetic changes such as methylation of the promoter regions.

The main tumour suppressor genes involved in lung carcinogenesis (Table 2) are those implicated in cell cycle control, apoptosis and differentiation.

- *p53*.This is a tumour-suppressor gene which has been called 'the guardian of genome'. It acts as a transcription factor implicated both in the G1 arrest control and in apoptosis. It reduces RB phosphorylation and induces a stop at the G1-S checkpoint to allow a DNA repair or to drive the cell to apoptosis mediated by BAX/BCL2. Its properties are abrogated as a result of mutations or pathway alterations [35, 36]. About 70% of lung cancers present a *p53* mutation which induces its abnormal stabilization. Mutations are detected in 70%–100% of SCLC and in 45%–75% of NCLC [37, 38]. In preinvasive lesions, *p53* aberrant expression was found from the level of mild dysplasia (25%) to that of CIS (75%), and with *RAS* mutation, *p53* mutation is in one of the most powerful tools for early lung cancer diagnosis and detection [39, 40]. The most common *p53* mutation is a GC to TA transversion. A strong correlation was observed between the frequency of these mutations and the global duration of tobacco exposure.
- *Cell cycle control*: RB protein is the main effector of G1 arrest mediated by *p53* in the context of DNA damage or oncogenic stress. RB protein expression is lost in 80% of SCLC but only in 15% of NSCLC and never in preinvasive lesions [41, 42]. In contrast, RB inactivation through deregulation of its

Table 2. Main tumour suppressor genes involved in lung carcinogenesis

Tumour suppressor gene	Usual alterations
p53	Mutations observed in NSCLC and SCLC
RB	Frequent loss of expression in SCLC (occasionally in NSCLC, almost never in preinvasive lesion)
p16	Inactivation by either mutations, deletions or promoter methylation
FHIT (3p14.2)	Frequent deletions LOH observed in premalignant lesions

LOH, loss of heterozygosity; NSCLC, non-small cell lung cancer; SCLC, small cell lung cancer.

phosphorylation is common in NSCLC. Two mechanisms are responsible for this deregulation: the loss of the CDK inhibitor $p16^{INK4}$, which negatively controls the cyclin-dependent kinase (CDK)–cyclin activity, and the overexpression of cyclin D1. Inactivation of $p16^{INK4}$ in NSCLC is mainly caused by exon 1 or 2 mutation (15%), homozygous deletions (30%–40%) or promoter methylation (30%–40%) [43], and there is a strict inverse relation between RB and p16 expression. Hypermethylation of *p16* can be detected in bronchial epithelium from chronic smokers with a high risk, suggesting inactivation of *p16* occurs early in lung tumourigenesis [44]. *p16* methylation status which can be also detected with a very high sensitivity (one allele methylated detected over 10^4 normal unmethylated alleles) in exfoliated cells, represents a promising tool for early detection of lung cancer [45, 46].

- *Apoptosis regulation*: *FHIT* is a tumour-suppressor gene implicated in the apoptotic process [47, 48] whose locus is 3p14-2, a fragile genomic region, frequently lost in lung cancers (more than 70%) [49]. It has been shown as a preferential target of tobacco smoke, since 80% of *FHIT* LOH were found in cancers and preinvasive lesions of smokers [50]. Alterations of the apoptotic pathway can also be related to two other genes: *BAX* and *BCL2* [51–53]. *BAX* is an apoptotic gene whose dimeric protein product formation induces apoptosis. *BCL2*, conversely, is a survival (antiapoptotic) gene, and the dimer BAX/BCL2 induces a neutralization of BAX and a loss of apoptosis. BAX/BCL2 deregulation (e.g. the inversion of the BAX/BCL2 ratio) has been studied on preneoplastic lesions [40]. A ratio below 1 indicates hyperexpression of BCL2 and loss of BAX as compared with normal bronchial epitheliums, and has been shown to increase with the severity of the preneoplastic lesions from low-grade to high-grade lesions.
- *Differentiation regulation by retinoids and their receptors*: Vitamin A and its analogs (retinoids) are differentiation and proliferation modulators of epithelial cells. They are able to invert airway cancerous progression by complex mechanisms. These mechanisms essentially consist of retinoids' capacity to regulate gene expression through nuclear transduction signal modulation mediated by nuclear retinoid receptors. These receptors act as ligand-activated transcription factors. It has been demonstrated that retinoid acid receptor (RAR)-β expression, one of these receptors, is lost in early stages of head-and-neck carcinogenesis (premalignant lesions of the oral cavity and tumours adjacent dysplastic tissues) and in lung carcinogenesis [54]. This receptor expression could be restored by 13-*cis* retinoic acid (13-cRA) administration. These results have been confirmed by in vivo studies [55].

Additionally, although a high proportion of loss of heterozygosity in 5q, near the APC (*adenomatous polyposis coli*) gene was established—and the loss of heterozygosity at APC locus occurs in 80% of dysplastic epithelia, 67% of in situ carcinomas and 50% of invasive cancers—the tumour-suppressor gene located at 5q has not been identified definitively [56].

2.2.5 Cyclooxygenase Activity and Carcinogenesis

Cyclooxygenase (COX-1 and -2) catalyses the synthesis of prostaglandins from arachidonic acid. Most tissues express COX-1 constitutively. On the other hand, COX-2 is inducible, and increased concentrations are observed in the context of inflammation and in the setting of invasive cancers such as NSCLC. The *COX2* gene is an immediate early response gene that is induced by growth factors, oncogenes, carcinogens and tumour-promoting phorbol esters, whereas the constitutive *COX1* is unaffected by these factors. COX-2 is upregulated in malignant tissue and seems to be important in carcinogenesis, as suggested by various experimental systems. For example, COX-2 expression and prostaglandin production have been shown as crucial for tumour growth and development in epithelial cancer such as colon cancer. COX-2 is frequently expressed in tissue samples from NSCLC and premalignant lesions [57] [expression evaluation by immunohistochemistry, RNA in situ hybridization or reverse transcriptase polymerase chain reaction (RT-PCR)]. Expression of COX-2 is associated with worse prognosis, at least in patients with early-stage disease [58]. In contrast, adjacent histologically normal epithelium and histologically normal epithelium from smokers without cancer show low levels of expression. Treatment with non-steroidal anti-inflammatory drugs (NSAIDs), which are COX inhibitors, reduces the growth of NSCLC cells in tissue culture and in xenograft studies with effects on proliferation, apoptosis, invasion, angiogenesis and tumour lymphocyte infiltration [59, 60]. Besides, expression of COX-2 in premalignant disease suggests it could be a good target for chemoprevention studies.

3 Chemoprevention

3.1 Definition

Chemoprevention, a term coined by Sporn in 1976, can be defined as the use of specific natural or synthetic chemical agents to reverse, suppress or prevent progression to invasive cancer [61]. The foundation of chemopreventive medicine is the translation of basic biological research into clinical chemical interventions, which attempt to halt the process of carcinogenesis. Its principles build on the concepts of field cancerization and multistep carcinogenesis. These basic principles also include the theory of the potential reversibility of some precancerous lesions, and the importance of the relationship between cancer cells and their environment (the concept of carcinogenic progress modulator genes) [4, 62].

Those concepts were validated by clinical trials studying the reversal of premalignant lesions such as leukoplakia using 13-cRA. Furthermore, this treatment was effective in preventing secondary tumours from occurring in patients who had been cured of head-and-neck cancer [63, 64]. In fact, vitamin A deficiency was first reported to be associated with changes in epithelial histology in 1925 and it was afterwards related with bronchial metaplasia and an increased incidence of cancer.

3.2 Interventional Strategies: Primary, Secondary and Tertiary Chemoprevention

Chemoprevention targets the carcinogenic process at earlier and potentially more reversible stages than those observed in the setting of invasive overt cancer. Chemopreventive strategies can be defined as follows. Primary chemoprevention's aim is to prevent the occurrence of cancer in healthy individuals at high risk: drugs are used to avoid cancerization of healthy epithelium submitted to carcinogenic agents such as tobacco. Secondary chemoprevention's aim is to prevent cancer in patients with premalignant lesions (intraepithelial neoplasia). Reversal of bronchial metaplastic lesions may prevent progression to lung cancer. Tertiary chemoprevention's aim is to prevent second primary tumours in patients cured from an initial cancer who have a very high risk of developing a secondary primary tumour according to the concept of field cancerization.

3.3 Lung Chemopreventive Agents

Nearly 2,000 natural and synthetic agents are presumed to have chemopreventive activity in experimental systems. Some of them have been studied in clinical trials: retinoids, *N*-acetyl-cysteine, β-carotene, calcium, α-tocopherol, selenium, tamoxifen, finasteride and NSAIDs [65–71]. The rationale for prevention of lung cancer is similar to that in head-and-neck cancer. In both diseases, chronic exposure to tobacco is the major risk factor and dysplastic epithelial lesions are thought to be a premalignant stage. As summarized in Table 3, all chemopreventive trials in current smokers are negative, and only a few are positive in former smokers.

3.3.1 Primary Chemoprevention

All lung primary chemoprevention trials are negative, or even show a deleterious effect of β-carotene in active smokers. Interestingly, a randomized phase II study with isotretinoin in heavy smokers suggested that smoking cessation was more important than the actual prevention with retinoids [67].

Table 3. Lung chemoprevention trials

Trial	Judgement criteria	(*n*)	Drugs	Results
Primary chemoprevention (general population, smokers)				
α-Tocopherol, β-Carotene Cancer Prevention Study Group 1994 [69]	Lung cancer	29,133	α-Tocopherol or β-Carotene	Negative Deleterious
CARET 1996 [70]	Lung cancer and cardiovascular pathologies	18,314	β-Carotene and retinol	Deleterious
Physicians Health Study 1996 [71]	Cancer and cardiovascular pathologies	22,071	β-Carotene	Negative
Secondary chemoprevention (precancerous lesions)				
Heimburger et al. 1988 [66]	Sputum atypia	73	Vitamin B12 and folic acid	Negative
Arnold et al. 1992 [76]	Sputum metaplasia	150	Etretinate	Negative
Van Poppel et al. 1992 [87]	Micronuclear (sputum)	114	β-Carotene	Positive
Lee et al. 1994 [67]	Metaplasia	87	Isotretinoin	Negative
McLarty et al. 1995 [88]	Sputum atypia	755	β-Carotene and Retinol	Negative
Kurie et al. 2000 [77]	Metaplasia/dysplasia	82	Fenretinide (200 mg/day)	Negative
Lam et al. 2002 [83]	Dysplasia	112	ADT (25 mgx3/day)	Positive
Tertiary chemoprevention (cured cancer)				
Pastorino et al. 1993 [68]	Second cancer	307	Retinyl palmitate	Positive
EUROSCAN 2000 [65]	Second cancer	1,023	Retinyl palmitate alone (300,000 UI/day 1st year, 150,000 UI/day 2nd year)	Negative
			N-acetylcysteine alone (600 mg/d 2 years)	Negative
			Both associated	Negative
U.S.-Intergroup NCI I91–0001 [80]	Second cancer	1,034	Isotretinoin (30 mg/day)	Negative (deleterious in smokers)

The ATBC trial (α-Tocopherol, β-Carotene Cancer Prevention Study Group) tested α-tocopherol and β-carotene in 29,233 50- to 69-year-old heavy smoker Finnish men. The subjacent rationale of this ATBC trial was the existence of epidemiological data showing inverted association between plasmatic or dietetic β-carotene levels and lung cancer incidence. Patients were randomized in four groups and received, for between 5 and 8 years, β-carotene (20 mg/day), α-tocopherol (50 mg/day), both, or placebo. Unexpectedly, both groups who received β-carotene supplementation showed an 18% increase in the incidence of lung cancer and an 8% excess in global mortality compared with placebo [69].

The CARET (β-Carotene And Retinol Efficacy Trial) [70] secondarily confirmed deleterious effect of the β-carotene combined with retinyl palmitate in chemoprevention of men and women at high risk for lung cancer. The patient population smoked at least 20 pack-years or had extensive occupation-

al exposure to asbestos. This trial was stopped after 21 months because of a 17% increase in mortality and a 28% increase in lung cancer incidence in the active treatment arm.

The Physician's Health Study group also evaluated the role of β-carotene versus placebo in prevention of lung cancer in 22,071 American physicians. Neither a benefit nor a deleterious effect on lung cancer incidence was identified in this study [71].

In China, a study evaluating β-carotene, α-tocopherol and selenium in the prevention of gastric and oesophageal cancer showed an insignificant decrease in the risk of lung cancer in a small cohort of patients [72].

In vitro and in vivo data provide some indications to understand the negative interaction between β-carotene and tobacco observed in the ATBC and CARET trials. It is possible that carcinogenesis mechanisms would be raised if elevated tissular β-carotene concentrations interact with highly oxidative tobacco smoke [73]. Other studies suggest a procarcinogenic effect of β-carotene implicating cytochrome P450 modifications in some circumstances [74, 75]. With these results it is admitted that the next primary chemoprevention trials should focus on former smokers. Indeed, the pursuit of tobacco consumption during a chemoprevention trial is not only deleterious at the level of the airway epithelium but could also lead to an inversion of the anticipated effect of the chemopreventive agent.

3.3.2 Secondary Chemoprevention

Randomized trials testing retinoids in precancerous lung lesions are negative in their vast majority. A randomized trial tested etretinate efficacy for 6 months versus placebo to decrease the number of metaplasia observed in sputum [76]. The reduction was of 32% in the etretinate arm versus 30% in the placebo arm. Isotretinoin or fenretinide use in this same setting did not provide satisfactory results [67, 77]. But it has been demonstrated that isotretinoin will decrease lung metaplasia index in the arm of patients who stopped smoking [67].

A randomized trial testing, in 755 workers exposed to asbestos, the use of β-carotene associated with retinol versus placebo did not demonstrate any improvement of cytological atypia observed in spittle, despite a 58-months follow-up. Many authors consider today that cytological atypia analysis in spittle or metaplasia analysis on bronchial biopsies is a not a very satisfactory modality to evaluate efficacy of chemopreventive agents on precancerous lesions of the bronchial tree. Indeed, spontaneous improvement of these indexes (mostly at smoking cessation) are frequent and make very difficult the evaluation of chemopreventive effect. To palliate this problem, biomarkers have been recently added to evaluate retinoid efficacy (at least at the molecular level if not clinical). Thus, two recent publications showed 13-*cis* retino-

ic acid efficacy on RAR-β receptor re-expression in lung metaplastic areas of smokers who received at least 6 months retinoid treatment [78, 79].

3.3.3 Tertiary Chemoprevention

In a randomized study, 307 patients with completely resected stage I NSCLC, received either 12 months of treatment with retinol palmitate or no treatment. At a median of 46 months of follow-up, patients who received retinol palmitate had a 35% lower incidence of second primary tumours than the control group (3.1% vs 4.8%) [68].

Nevertheless, the EUROSCAN trial did not confirm these initially encouraging results [65]. This double-blind, placebo-controlled, randomized trial tested for 2 years retinol palmitate, *N*-acetylcysteine or both in 1,023 patients treated for a lung cancer.

U.S.-Intergroup NCI I91-0001 was a randomized, double-blind, placebo-controlled study using low-dose 13-cRA after complete resection of stage I NSCLC (postoperative T1 or T2, N0). It included 1,304 patients who all had undergone surgery 6 weeks to 3 years prior to registering. After a median follow-up of 3.5 years, there were no statistically significant differences between the placebo and isotretinoin arms with respect to the time to second primary tumours, recurrence or mortality. Secondary multivariate and subset analyses suggested that isotretinoin was harmful in current smokers and beneficial in never smokers [80].

4 Future of Chemoprevention: Developing New Agents

The previous studies prove that strategies should be reassessed, and that new agents should be investigated. Recently, a new class of retinoids has been identified that seems to be more effective in growth inhibition and induction of apoptosis of lung cancer cell lines [81]. Such agents could be more efficient in lung chemoprevention than the retinoids that have been investigated so far. The way of administration should also be reconsidered: Is the oral administration the more relevant way? It is possible that other routes of administration (inhalational route in particular) may finally provide an effective way of prescribing retinoids [82].

Although retinoids are the most-frequently used pharmacological agents in chemoprevention trials, they are pretty toxic. Therefore, other molecules with a best-therapeutic index are currently in development to be used as chemopreventive agents.

Results of a randomized phase IIb study of anethole dithiolethione (ADT), an organosulphur compound originally developed as a radio-protec-

tant more than 30 years ago, have been recently published [83]. In total, 112 current and former smokers, with at least one site of bronchial dysplasia, were randomly assigned to receive placebo or ADT at 25 mg orally thrice daily for 6 months. Progression rate of pre-existing dysplastic lesions by two or more grades and/or the appearance of new lesions was statistically significantly lower at 8% in the ADT group than in the placebo group (17%). At the clinical level, the disease progression was statistically significantly lower in the ADT group (32%) than in the placebo group (59%). Adverse events were mostly gastrointestinal symptoms that resolved with dose reduction or discontinuation of the medication.

An epidemiological case-control study of chemoprevention of lung cancer among smokers found that daily intake of NSAID (aspirin or ibuprofen) for at least 2 years is associated with a 68% reduction of relative lung cancer (relative risk 0.32; $p<0.01$) [84]. Specific inhibitors of COX-2, one of two enzymes catalysing prostaglandin synthesis, and inducted by growth factors, oncogenes or carcinogens, are in study. Elevated levels of prostaglandin, whose proangiogenic effect was demonstrated, have been observed in head-and-neck cancers. COX-2 overexpression in epithelial cells inhibits apoptosis, favours genetic damage accumulation and allows the transformation of carcinogens into active metabolites. COX-2 mRNA levels are 150 times higher in head-and-neck cancers than normal oral mucous of healthy persons, and 50 times higher in normal epithelium adjacent to the tumour [85].

Development of molecular-targeted therapies is the next step in the therapy and prevention of cancer. As described before, EGFR is overexpressed in lung cancer and in premalignant lesions and seems therefore to be an accurate target for chemoprevention. Mutations in the *RAS* family are very frequently observed in lung carcinogenesis and targeting this pathway could also be attractive. However, the question is not only the identification of the best-targeted drug but also the adequate strategy to prove its efficiency. Chemopreventive studies are time-consuming studies that require many patients. In this setting, they would also become very expensive studies. Targeting high-risk populations and making use of potential intermediate biomarkers could significantly reduce the time and resources required for chemoprevention trials. Recent efforts have focused on the definition of these biomarkers of early carcinogenesis. The aim is to define at the biological level the epidemiological variable 'increased risk of cancer'. Defining such risk biomarkers has multiples advantages. These biomarkers would allow the follow-up of cancer at the molecular level and not merely at the phenotypic level. They could potentially be used as intermediate endpoints to evaluate the efficacy of chemopreventive strategies. Overall this approach has multiple advantages, such as shortening of the follow-up period and reducing the size of the cohort to treat [4]. However, there is still a need to definitively validate biomarkers with hard clinical criteria (secondary cancer appearing, cancer-related mortality, etc.).

Actually, new chemoprevention trials have been designed. They will focus on studying promising new biological agents in randomized phase II setting. Patients will have tissue and serum collected at specific points in the hope of developing a risk model for lung cancer development. These types of trials would be accrued within 3 years with endpoints assessed in 5–6 years. Any promising evidence would be applied into larger phase III trials for definitive testing. Members of the Lung Cancer Biomarkers Chemoprevention Consortium (an NCI-funded programme) propose to test two drugs: ZD1839, an EGFR inhibitor, and R115777, a farnesyl transferase inhibitor. Two randomized, placebo-controlled, double-blind, multi-institutional phase II trials are planned to investigate the reversal of premalignant bronchial lesions. The primary endpoint will be improvement in bronchial histology and the secondary endpoint will be Ki67 status, a proliferation indicator. Patients must have had a previous, definitively treated tobacco-related cancer (lung, head-and-neck, bladder, oesophagus), a 30-pack-year smoking history and confirmed sputum atypia. They will then be treated for 6 to 12 months, with serial bronchoscopies.

Finally, future chemoprevention studies will probably consider association of targeted agents to maximize preventive effect. Trials using tyrosine-kinase inhibitors (especially EGFR), farnesyl-transferase inhibitors and/or antiangiogenic molecules are expected.

In addition, it is obvious that one of the best preventive approaches is avoiding tobacco consumption. Health policy campaigns are essential in this area. They should be enriched by studies on pharmacological agents able to fight nicotine dependence in persons at risk. In that regard, analysis of the genetic polymorphism implicating D2 dopamine receptor and the enzyme cytochrome P2A6 [86], involved in nicotine dependence, are very promising.

5 Conclusion

The incidence and mortality associated with lung cancer has not been significantly modified over the last 25 years, despite the introduction of new cytotoxic drugs and development of multidisciplinary approaches combining surgery, chemotherapy and radiotherapy. Such considerations highlight the need to develop and reinforce chemopreventive approaches. Preliminary results demonstrating a retinoid efficacy to prevent cancer or revert premalignant lesions in the oral cavity and the larynx have not been confirmed in the setting of lung carcinogenesis. New agents have been identified through a better understanding of lung carcinogenesis and are currently being evaluated for chemoprevention: COX-2, EGFR and farnesyl transferase inhibitors. Complete characterization of molecular determinants of lung or head-and-

neck carcinogenesis is essential to enable rational and targeted development of chemopreventive agents. Modern chemoprevention trials should include an evaluation of biological markers of carcinogenesis in order to establish molecular risk models. This new approach of chemoprevention, based on a better comprehension of carcinogenic mechanisms and the use of targeted agents, is quite costly but very promising.

Search strategy and selection criteria: Data for this review were identified by searches of PubMed and references from relevant articles. Articles were found using the search terms 'lung cancer', 'chemoprevention', 'carcinogenesis', 'oncogenes' and 'retinoids'. Only papers published in English were included.

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Anti-nicotine Vaccination: Where Are We?

T. Cerny

Oncology/Hematology, Kantonsspital, 9000 St. Gallen, Switzerland
thomas.cerny@kssg.ch

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Abstract Nicotine is the main substance responsible for dependence on tobacco-containing products, which have a heavy impact on the public health of developed as well as non-developed countries by being a main etiologic factor for the induction of cardiovascular diseases and tobacco-related cancer. A vaccine against nicotine induces antibodies against the molecule, intercepting the nicotine on its way to its specific receptors. The binding of the antibody to nicotine in turn significantly diminishes the nicotine concentration in the brain shortly after smoking. This approach therefore interrupts the vicious circle between smoking and nicotine-related gratification. The preclinical data of our animal experiments are briefly summarized. At the end of 2003, three companies were in early clinical development of an anti-nicotine vaccine: Xenova (TA-NIC), Nabi (NicVAX) and Cytos (Nicotine-Qbeta). The carrier molecules are recombinant cholera toxin B (TA-NIC), an especially selected carrier protein (Nabi) and a virus-like particle VLP (Cytos). Another carrier is additionally used by Chilka in an advanced preclinical model, which showed superiority to cholera toxin B carrier. Cytos has successfully completed a phase I study with 40 healthy non-smoking volunteers. So far, results of a phase I trial by Cytos have shown no unexpected toxicities and phase II trials have now started in Switzerland (Cytos).

1 Introduction

The carcinogenic effect of tobacco was the most important discovery in the history of cancer epidemiology. In the decade 1990–2000 the estimated global tobacco-related deaths toll reached 3 million per year. In the period 2020–2030, global tobacco-related deaths could exceed 10 million annually. The WHO projects that one in ten people now alive will die of tobacco-related

disease if we cannot change this situation for the better. Morbidity and death due to chronic lung disease, cardiovascular disease and cancer mainly of the lung are well-documented events directly related to the total amount of tobacco use over one's lifetime. Women and adolescents are especially prone to develop tobacco-associated cancer early in life, with half as many pack-years compared to an average male smoker.

Modern tobacco control started in the United States with the mandatory printing of warnings against the health risks of smoking on all cigarette packages in 1965. Radio and television advertising for cigarettes has been banned in the USA since 1971, smoking has been forbidden on public transportation since 1990, and the tobacco industry as a whole has been legally challenged by federal and state governments since 1994.

Why are cigarettes so addictive? Over the years, the following, strongly simplified scheme of the mechanism of nicotine addiction has been developed: Nicotine, a compound naturally occurring in tobacco, is sterically very similar to the ubiquitous signaling molecule acetylcholine. It stimulates a heterogeneous group of nicotinic receptors of the adrenal glands, the neuromuscular gaps, and the brain. Like other dependence-inducing drugs, it increases the dopamine level in the nucleus accumbens of the brain (Fig. 1). It furthermore inhibits the enzymatic catabolism of dopamine [1, 2]. The nucleus accumbens itself is one of the main entrances to dependency. In order to make sure that fundamental activities for survival—such as eating, drinking, or sex—are performed, during evolution the brain has connected those activities with the sensation of satisfaction and pleasure. The so-called "highway of pleasure," conceived for this purpose, connects the nucleus accumbens with the hippocampus, where contextual information is stored, and the cerebral cortex, where pleasure enters consciousness [3]. The subjectively perceived difference between the pleasure of a cold beer after a hot day or an orgasm is apparently the consequence of a different activation of the same circuitry. Recent research demonstrates that mediators other than dopamine also play key roles in the network: glutamate

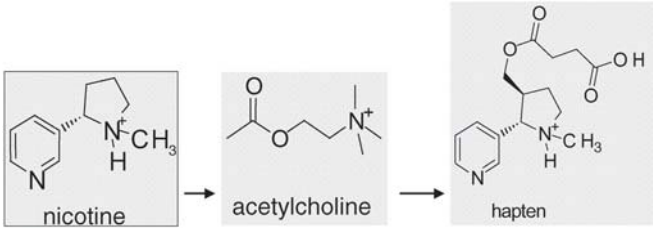

Fig. 1. Structural formula. Illustration of the close steric similarity as well as the similarity in electric change distribution between (−)nicotine and acetylcholine, which is responsible for their binding to the same groups of receptors. The functionalized nicotine hapten was used for coupling (*trans*-3-succinylmethylnicotine) and is shown to the *right*

receptors, for example, are essential in the development of cocaine dependency [4, 5].

2 The Anti-nicotine Vaccination Concept

Why could a vaccine be useful to combat nicotine dependency? In 1972, researchers at the University of Chicago immunized a rhesus monkey against morphine. The animal was shown to be partially protected against heroin (chemically almost identical to morphine), but the authors concluded in their last sentence that subsequent drug challenges could overcome the protective effect: "This blockade has been shown to be dose dependent and it can be overcome by high doses of drugs" [6]. The authors of the same group had published an earlier paper which demonstrates that the high doses of the same conjugate as used in their anti-heroin immunization experiment induces B-cell tolerance, a condition in which no new antibodies against the tolerance-inducing epitopes are produced by the B cells.

The concept of a prophylactic or therapeutic vaccine against drugs of abuse (including nicotine) interrupting the vicious circle between drug consumption and drug-induced stimulation was described for the first time in 1990 by E.H. Cerny [7]. Compared with other medications for smoking cessation, the vaccine concept has some unique advantages: The vaccine effect lasts for years, whereas receptor-antagonist-based medications with their typically short half-life may no longer be taken by the patient once withdrawal symptoms develop. Antibodies do not cross the blood–brain barrier and no secondary effects through interaction with brain receptors are expected. Moreover, having a different mechanism than any other therapeutic group used for smoking cessation, they could be an ideal complement to already established therapies and, as for other vaccines, the expected low or likely nonexistent toxicity as well as a low price may, under certain conditions, allow for a broad preventive application. Like other drugs of abuse, nicotine itself is too small a molecule to be immunogenic in humans and therefore has to be linked to a carrier protein. Useful coupling chemistries for the conjugation of nicotine to a functional group of the carrier protein had previously been developed in the course of the development of radioimmune assays (RIA), which are based on specific antibodies against nicotine [8–10].

3 Material and Methods

Full details can be found in the original publication of the year 2002 [11]. Therefore we only summarize some aspects of this methodological section.

Nicotine in cigarettes is present only as the (−)enantiomer. However, during the high temperature of cigarette combustion up to 11% of the nicotine is transformed into the (+)enantiomer, which has been shown to be pharmacologically active [12, 13]. Therefore, immunization with the racemic mixture is justified in order to maximize the vaccine effect. The strategy for the synthesis of the conjugate followed the derivatization procedure of nicotine as pioneered by Langone and van Vunakis [8, 9].

Immunization Protocols. Female Balb C (Harlan) mice, 7 weeks of age were used for all experiments. Immunizations were performed by subcutaneous (s.c.) injection at the base of the tail of 10, 30, or 100 μg of antigen (nicotine coupled to the carrier protein) in PBS (phosphate-buffered saline) together with 1 mg of Alum as (Alu-Gel S, Serva, Switzerland), in a total volume of 60–100 μl. Depending on the protocol, the animals were boosted at 2- to 4-week intervals with the same amount of antigen in adjuvant by the same route.

For intranasal (i.n.) immunizations, animals under light anesthesia were instilled in both nostrils with 5 μl of conjugate/nostril without adjuvant with the help of a micropipette.

Osmotic Pump. Miniature Alzet osmotic pumps (Alza corporation, USA) model 2004 were implanted into mice subcutaneously on the backs of the animals. The pump has a reservoir of 200 μl, a pumping rate of 0.25 μl per hour, and nicotine was delivered over 4 weeks. The administered dosage was 1.5 mg/kg/day for a mouse of 20 g, which is estimated to correspond to the nicotine-per-weight equivalent absorbed by a person weighting 70 kg, smoking 5 packages a day and absorbing 1 mg nicotine per cigarette.

Challenge with Radioactive Nicotine. The rationale for the calculation of the nicotine equivalent of 2 cigarettes in mice was as follows: a smoker of 75 kg smoking a cigarette absorbs about 1 mg of nicotine. A mouse weights about 20 g and the corresponding quantity per weight is therefore about 300 ng for a cigarette or 600 ng for 2 cigarettes. For practical purposes, 597 ng of non-labeled nicotine and 3 ng of 3H nicotine were injected into the tail vein.

Radioactivity measured in the brain was corrected for the amount of blood present in the brain, considering that 100 g of brain tissue contains approximately 3 ml of blood [14].

Enzyme-Linked Immunosorbent assay. A standard sandwich ELISA (enzyme-linked immunosorbent assay) was used to measure anti nicotine antibodies.

Significant IgA titers were found after vaccination, when given i.n. The IgA antibodies can be detected in the saliva as well as in the serum. The IgA antibodies could be detected in the saliva as well as in the serum.

4 Results of Preclinical Development

Results are best summarized in Figs. 2 and Fig. 3. Significant IgA titers have been found when the vaccine was given i.n.; the IgA antibodies can be detected in saliva as well as in the serum. Figure 2 shows the results of IgA and IgG measurements in both saliva and serum as determined by ELISA using a nicotine bovine serum albumin (BSA) conjugate coated to the solid phase. Each data point presents the result of pooled serum of 5 animals.

Most interesting are the results after nicotine challenge of immunized mice. Figure 3 demonstrates the distribution in the serum and the brain of a 3H-labeled nicotine bolus injected into the tail vein, which corresponds to the nicotine equivalent of 2 cigarettes in mice. The animals are sacrificed 5 min after injection. The mice of group IM1 were immunized 3×i.n. and the mice of group IM2 3×s.c.; serum of 5 animals was pooled for each data point.

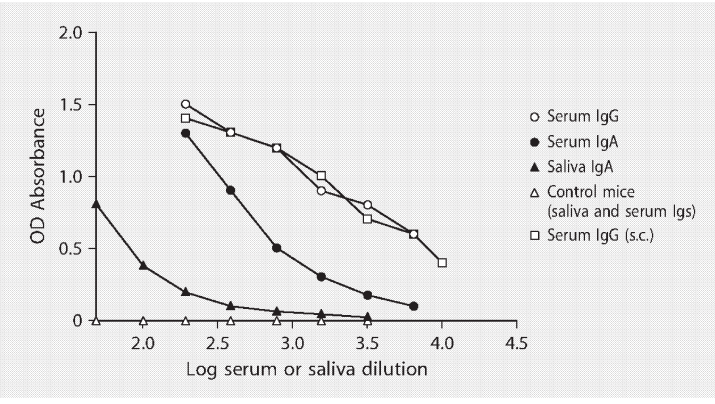

Fig. 2. Intranasal and subcutaneous immunizations. IgG and IgA ELISA results measuring anti-nicotine-specific antibodies in saliva and serum at day 30 after intranasal and subcutaneous immunization with nicotine cholera toxin B (CTB) Berna conjugate. Total doses of 3×30 μg of the nicotine CTB Berna conjugate were applied per mouse in PBS to both nostrils, whereas a control group received 3×30 μg of CTB Berna only. The plates for the ELISA assay were coated with nicotine-BSA conjugate. Two booster instillations were provided on days 7 and 15 post-immunization, and saliva was harvested on day 22 and blood on day 29

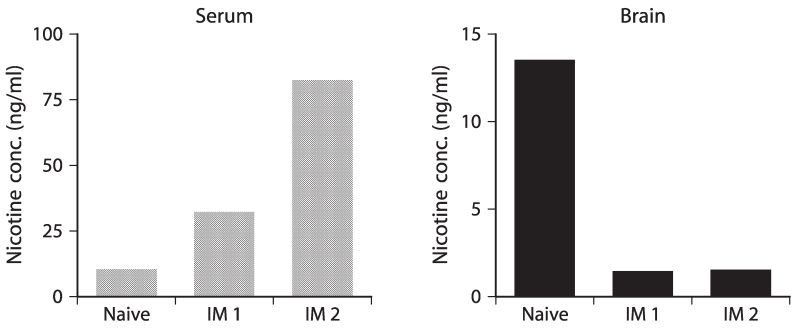

Fig. 3. Nicotine challenge. Distribution in the serum and the brain of tritium-labeled nicotine bolus injected into the tail vein corresponding to the equivalent of 2 cigarettes (600 ng in a mouse of 20 g) in mice sacrificed 5 min after injection. As one would expect, a significant amount of the nicotine is bound in the serum of the vaccinated animals as compared to the naïve animal, but less than 10% of the dose can be found in the brain. (IM1:3×i.n., IM2 3×s.c., serum of 5 animals is pooled for each data point

5 Discussion

Here we describe the preclinical development of an innovative anti-nicotine vaccine for s.c., intramuscular (i.m.), as well as i.n. application, which is in preparation for a phase I evaluation.

The described vaccination approach against nicotine leads to a significant and sustained level of neutralizing antibodies in the animal model and has no apparent toxicity. It leads to an important decrease of nicotine in the brain and therefore breaks the peak inflow of nicotine right after smoking, which is the prerequisite to establish or maintain a nicotine dependency. Intranasal immunization alone produces significant levels of IgA antibodies in saliva and serum as shown in Fig. 2. The efficiency of the intranasal immunization can be deduced from Fig. 3, where the protective effect after i.n. vaccination (IM1) is at least as good as after s.c. (IM2) vaccination. Typically, it takes about 5–6 weeks after the first immunization before high antibody levels are reached in the serum. One may ask if the continuous presence of nicotine in the body as expected in a heavy smoker may interfere with the development of the immune response. A comparison of the mice with and without an implanted nicotine pump, which dispensed the nicotine equivalent of 5 packages of cigarettes a day, answers this question: There is no significant difference in antibody titers obtained between the two groups (Fig. 3).

Figure 3 addresses the question of the reduced nicotine challenge of the brain after vaccination. As one would expect, after 5 min the majority of the radiolabeled nicotine equivalent of 2 cigarettes is bound in the serum of the vaccinated animals as compared to the naïve animals, but only less than

10% of the dose measured in non-vaccinated animals can be found in the brain of the vaccine-protected mice. The same experiment is performed with a challenge of 2.3 ng of radiolabeled nicotine and no signal is detected (less than 1% of control animal, data not shown).

In summary, the concept of neutralization of tobacco-associated nicotine through vaccination against a nicotine conjugate holds promise at the preclinical evaluation stage. Only clinical studies will show if this innovative strategy leads to a powerful tool to overcome and prevent tobacco-associated morbidity and mortality in the future.

6 Where Are We Regarding the Clinical Development?

There are severe ongoing clinical trials in Europe and the USA with the following competing companies:

- Cytos Switzerland with Nicotine-Qbeta (Immunodrug)
- Nabi Inc. USA with Nic-VAX
- Xenova Ltd. (GB) with TA-NIC

In Switzerland, the Cytos product Nicotine-Qbeta (Immunodrug)is now in broad phase II testing after a successful phase I was completed recently. The Cytos Nicotine Immunodrug is shown in Fig. 4 and because of the size of the VLP (virus-like particle) very many antigenic nicotine molecules can

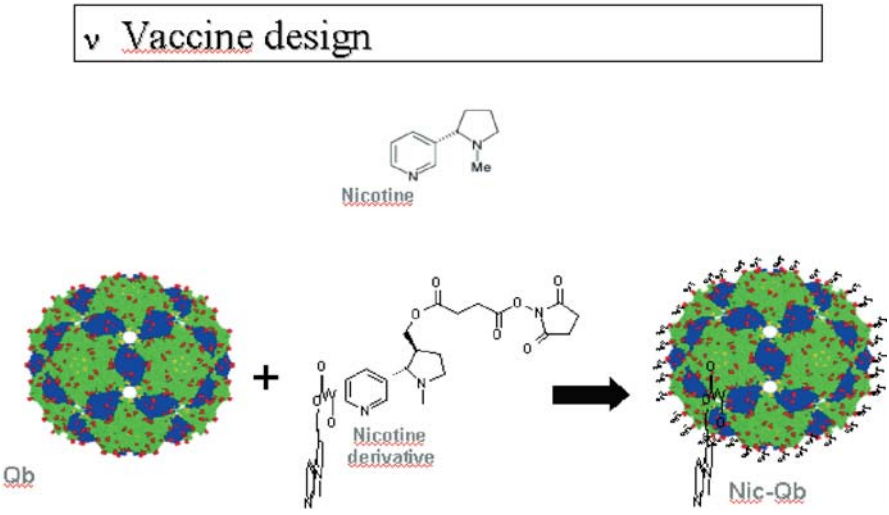

Fig. 4. Structure of the Cytos Nicotine Immunodrug

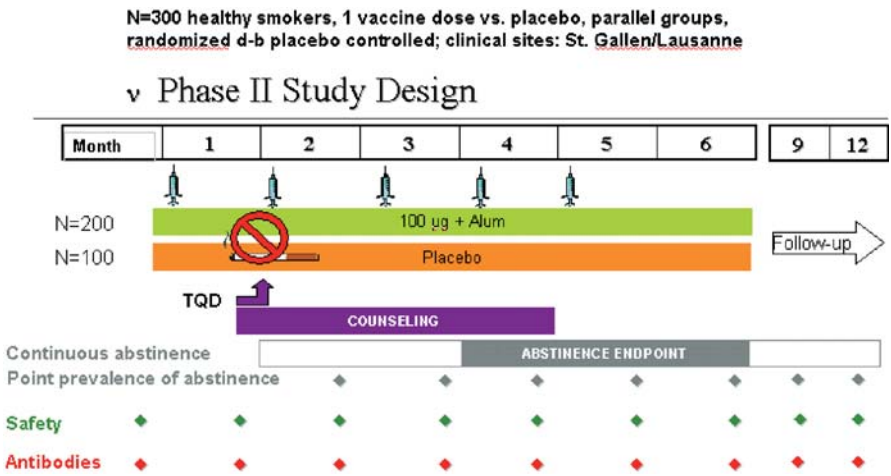

Fig. 5. Outline of the clinical trial with Cytos Nicotine Immunodrug

be bound to its surface. Figure 5 shows the ongoing study outline. Results are expected in early 2005 and the study is ongoing in St. Gallen and Lausanne/Switzerland. Results of the Nic-VAX and TA-NIC vaccination have not yet been published.

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Primary Prevention of Colorectal Cancer: Lifestyle, Nutrition, Exercise

María Elena Martínez

Arizona Cancer Center, Arizona College of Public Health,
University of Arizona, Tucson, AZ, USA

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Abstract The past two decades have provided a vast amount of literature related to the primary prevention of colorectal cancer. Large international variation in colorectal cancer incidence and mortality rates and the prominent increases in the incidence of colorectal cancer in groups that migrated from low- to high-incidence areas provided important evidence that lifestyle factors influence the development of this malignancy. Moreover, there is convincing evidence from epidemiological and experimental studies that dietary intake is an important etiological factor in colorectal neoplasia. Although the precise mechanisms have not been clarified, several lifestyle factors are likely to have a major impact on colorectal cancer development. Physical inactivity and to a lesser extent, excess body weight, are consistent risk factors for colon cancer. Exposure to tobacco products early in life is associated with a higher risk of developing colorectal neoplasia. Diet and nutritional factors are also clearly important. Diets high in red and processed meat increase risk. Excess alcohol consumption, probably in combination with a diet low in some micronutrients such as folate and methionine, appear to increase risk. There is also recent evidence supporting a protective effect of calcium and vitamin D in the etiology of colorectal neoplasia. The relationship between intake of dietary fiber and risk of

colon cancer has been studied for three decades but the results are still inconclusive. However, some micronutrients or phytochemicals in fiber-rich foods may be important; folic acid is one such micronutrient that has been shown to protect against the development of colorectal neoplasia and is currently being studied in intervention trials of adenoma recurrence. The overwhelming evidence indicates that primary prevention of colon cancer is feasible. Continued focus on primary prevention of colorectal cancer, in combination with efforts aimed at screening and surveillance, will be vital in attaining the greatest possible progress against this complex, yet highly preventable disease.

1 Introduction

Significant progress has been made over the last decade in identifying factors that modify risk of colorectal cancer. Large international variation in colorectal cancer incidence and mortality rates and the prominent increases in the incidence of colorectal cancer in groups that migrated from low- to high-incidence areas provided important evidence that lifestyle factors influence the development of this malignancy. These observations formed the basis for various hypotheses of lifestyle factors in the etiology of colorectal neoplasia. These and other hypotheses continue to be evaluated in a variety of study settings.

Data from epidemiological studies continue to advance our understanding of the role of numerous colorectal cancer risk factors. Lifestyle factors reviewed in this document include various dietary factors, alcohol consumption, tobacco, obesity, and physical activity. Since enhancement of our understanding of diet in colorectal cancer causality is likely to include incorporation of biological/molecular markers, the challenging and important area of gene-nutrient interactions is briefly discussed.

2 Primary Prevention of Colorectal Neoplasia

Primary prevention is defined as the identification and avoidance of environmental or lifestyle factors related to carcinogenesis. Approaches aimed at reduction of colorectal cancer risk by preventing its development or halting its process in early stages is considered an optimal strategy to reduce the overall cancer burden. Epidemiological and experimental evidence that dietary intake is an important etiological factor in colorectal neoplasia is convincing. Study designs used to test the existing hypotheses include: ecological studies, where patterns of consumption and cancer incidence or mortality rates are compared among different populations; case-control studies, where reported past diet as recalled by individuals with cancer is compared against recall among those without the disease; and, prospective studies

where diet is assessed among cancer-free individuals and correlated with subsequent cancer occurrence or mortality. The totality of the data suggests that in Western cultures, dietary factors may contribute to the causation of approximately 50% of colorectal cancer (Doll 1992; Kune and Vitetta 1992). However, precisely which specific nutrients, foods, or combinations of these are related to the development of colorectal cancer is not entirely clear.

2.1 Fat and Red Meat

Rates of colon cancer are strongly correlated with national per capita disappearance of animal fat and meat, with correlation coefficients ranging between 0.8 and 0.9 (Armstrong and Doll 1975; Rose et al. 1986). A sharp increase in colon cancer incidence rates in Japan in the decades following World War II coincided with a 2.5-fold increase in fat intake (Aoki et al. 1992). Intake of animal or saturated fat (Jain et al. 1980; Bristol et al. 1985; Potter and McMichael 1986; Kune et al. 1987c; Lyon et al. 1987; Graham et al. 1988; West et al. 1989; Whittemore et al. 1990; Peters et al. 1992) or red meat (Manousos et al. 1983; Miller et al. 1983; La Vecchia et al. 1988; Young and Wolf 1988; Lee et al. 1989; Benito et al. 1990; Gerhardsson de Verdier et al. 1991; Levi et al. 1999) has been shown to be associated with colon cancer risk; however, some studies do not support these associations (Berta et al. 1985; Macquart-Moulin et al. 1986; Tuyns et al. 1987; Meyer and White 1993). Data from earlier epidemiological studies provided some evidence for a positive association between dietary fat and increased risk of several cancers, including the colorectum. This in turn led to public health recommendations calling for a reduction in fat intake to 30% of calories (Food and Nutrition Board 1989; National Research Council—Committee on Diet and Health 1989). However, an overall review of studies recently published indicates that for colon cancer, there is no strong evidence for its association with high fat consumption per se and that the association may partly be explained by animal fat or red meat consumption (Willett 1998). In addition, whether this association is independent of total energy intake is also unclear. Results of combined data from 13 case-control studies of colon cancer (Howe 1993) showed a positive association between energy intake and no association for various fat components in the diet independent of energy intake.

Results of some prospective studies of colon cancer have shown positive associations between fat or red meat consumption, but the data are less compelling for total fat than for red meat (Bjelke 1980; Phillips and Snowdon 1983; Stemmermann et al. 1984; Garland et al. 1985; Hirayama 1986; Gerhardsson de Verdier et al. 1988; Hsing et al. 1998). Other cohort studies have shown statistically significant or suggestive positive associations for intake of processed meats and risk of colon cancer (Bostick et al. 1994;

Giovannucci et al. 1994b; Goldbohm et al. 1994). Reports from the World Cancer Research Fund (World Cancer Research Fund 1997), the Chief Medical Officer's Committee on Medical Aspects of Food (Chief Medical Officer's Committee on Medical Aspects of Food 1998), and a World Health Organization consensus statement (Scheppach et al. 1998) reached similar conclusions of a possible or probable increased risk for colorectal cancer associated with high intake of red meat or processed meat. However, recommendations from an expert workshop convened in Australia (Truswell 1999) noted that consumption of moderate amounts of meat, as part of an overall healthy diet rich in cereals and grain foods, fruit, and vegetables, is not associated with an increased risk of colorectal cancer, suggesting that this relationship remains controversial. A recent meta-analysis of 34 case-control and 14 cohort studies concluded that high intakes of red and of processed meat are associated with an increased risk of colorectal cancer (Norat et al. 2002). Summary relative risks (RRs) [95% confidence interval (CI)] for red meat intake were 1.35 (1.21–1.51) for all studies, 1.36 (1.17–1.59) for case-control studies, and 1.27 (1.11–1.45) for cohort studies; the corresponding figures for processed meat were 1.31 (1.13–1.51), 1.29 (1.09–1.52), and 1.39 (1.0901.76), respectively.

The mechanism responsible for the possible increased risk of colorectal cancer associated with red meat consumption is unclear. Research interest has recently focused on meat preparation methods. It is postulated that carcinogenic products formed by cooking meats at high temperatures may partly be responsible for the increased risk. When meat is fried, grilled, or broiled at high temperatures for substantial periods of time, mutagenic heterocyclic aromatic amines are formed from heating creatinine with amino acids (Sugimura and Sato 1983; Sugimura 1985; Wakabayashi et al. 1992). Results of some studies suggest that risk of colorectal cancer may be increased among meat eaters who consume meat with a heavily browned surface, but not increased among those who consume meat with a medium or lightly browned surface (Lee et al. 1989; Gerhardsson de Verdier et al. 1991). In a study of colorectal adenomas (Sinha et al. 1999), Sinha et al. showed a 29% increase in risk of having an adenoma for each 10 g/day of well-done or very well-done red meat consumed. Ongoing and future investigations should help determine whether levels of specific heterocyclic amines in the diet are carcinogenic in humans. Related to this area of research is the role of genetic variability in susceptibility to the adverse effects of specific risk factors, such as heterocyclic amines. If heterocyclic amines formed by cooking meat at high temperatures are involved in colorectal carcinogenesis, genetic polymorphisms in the various enzymes involved in metabolism of these carcinogens are likely to influence the risk of this malignancy. Individuals with this type of susceptibility may be at increased risk only if exposed to particular carcinogens. This type of gene-nutrient interaction is discussed in a later section.

2.2 Fiber, Fruit, and Vegetables

High consumption of fruit and vegetables has been shown to be associated with a decreased risk of colorectal neoplasia (World Cancer Research Fund 1997). Results of most published studies have shown an inverse association between intake of vegetables and colon cancer, while data for fruit consumption are less compelling (Modan et al. 1975; Phillips 1975; Bjelke 1980; Manousos et al. 1983; Miller et al. 1983; Macquart-Moulin et al. 1986; Kune et al. 1987c; Graham et al. 1988; Tuyns et al. 1988; Young and Wolf 1988; Lee et al. 1989; West et al. 1989; Benito et al. 1991; Peters et al. 1992; Mayne et al. 1994; Steinmetz et al. 1994; Levi et al. 1999). Results of an earlier pooled analysis of six case-control studies (Trock et al. 1990) showed a high intake of vegetables to be associated with an odds ratio (OR) for colon cancer of 0.48 (95% CI=0.41–0.57), and a weaker inverse association with fiber (OR=0.58 for upper versus lower categories). Foods high in fiber have also been shown to be inversely associated with colon cancer risk in most (Modan et al. 1975; Macquart-Moulin et al. 1986; Kune et al. 1987b; Graham et al. 1988; Slattery et al. 1988; Benito et al. 1990; Gerhardsson de Verdier et al. 1990a; Whittemore et al. 1990; Meyer and White 1993; Zaridze et al. 1993) but not all (Jain et al. 1980; Lyon et al. 1987; Tuyns et al. 1987; Willett et al. 1990; Peters et al. 1992) studies. Howe et al. (1992) reported a lower risk associated with higher fiber intake (OR=0.53 for upper versus lower quintile) based on pooled analyses of 13 case-control studies. Conversely, results of large prospective studies have shown weak or nonexistent inverse associations for fiber intake and risk of colon cancer (Willett et al. 1990; Bostick et al. 1994; Giovannucci et al. 1994b; Goldbohm et al. 1994; Fuchs et al. 1999). As with case-control studies, when sources of fiber were examined separately (Modan et al. 1975; Dales et al. 1979; Manousos et al. 1983; Miller et al. 1983; Zaridze 1983; Macquart-Moulin et al. 1986; Kune et al. 1987b; Lyon et al. 1987; Slattery et al. 1988; Heilbrun et al. 1989; Lee et al. 1989; Benito et al. 1990; Frudenheim et al. 1990; Gerhardsson de Verdier et al. 1990b, 1991; Willett et al. 1990; Hu et al. 1991; Bidoli et al. 1992; Iscovich et al. 1992; Peters et al. 1992; Thun et al. 1992; Meyer and White 1993; Giovannucci et al. 1994b), a reduced risk also appears to be stronger for vegetable sources than for other fiber components. In a recently published large prospective study of female nurses, which examined the role of fiber on risk of colorectal neoplasia, Fuchs et al. (Fuchs et al. 1999) found no association between fiber intake and risk of colorectal cancer or adenoma. The relative risk for women in the upper quintile of fiber intake (median of 24.5 g/day) was 0.95 (95% CI=0.73–1.25) compared to those in the lowest quintile (median of 9.8 g/day). No important associations were observed when analyses were conducted for cereal, fruit, or vegetable fiber. Also, no significant associations were shown for fiber intake and colorectal adenoma. In a later pub-

lication that focused on fruit and vegetable consumption (Michels et al. 2000), data from the Nurses' Health Study and the Health Professionals Follow-up Study cohorts were combined for a follow-up of over 1,700,000 person-years to yield 937 cases of colon and 244 of rectal cancer. No inverse association was shown for either men or women who reported consuming six or more servings per day of fruit and vegetables compared to those who consumed two or fewer servings per day.

Further controversy related to fiber and colon cancer emerged with the results of two recent publications. In the first, data from the Prostate, Lung, Colorectal, and Ovarian (PLCO) Cancer Screening Trial comprising 33,971 participants showed individuals in the highest quintile of dietary fiber intake to have a significant 27% lower risk of having prevalent adenomas in the distal colorectum (Peters et al. 2003). Perhaps more important are the findings of the European Prospective Investigation into Cancer and Nutrition (EPIC) study, where findings from 519,978 individuals showed that those in the highest quintile (mean intake of 31.9 g/day) had a 28% lower risk of developing colon cancer than those in the lower quintile (mean intake of 12.6 g/day) (Bingham et al. 2003).

Mechanisms responsible for the protective effect of fruit and vegetables include inhibition of nitrosamine formation, provision of substrate for formation of anti-neoplastic agents, dilution and binding of carcinogens, alteration of hormone metabolism, antioxidant effects, and the induction of detoxification enzymes by cruciferous vegetables (Steinmetz and Potter 1991b). Vegetables contain several compounds that possess a variety of anticarcinogenic properties (Steinmetz and Potter 1991b). Specifically, the anticarcinogenic properties of cruciferous vegetables have been mainly attributed to the degradation products of glucosinolates (e.g., isothiocyanates and indoles) which induce detoxification enzymes (Mehta et al. 1995). As discussed later, a possible mechanism of action of isothiocyanates is thought to be through the induction of glutathione S-transferases (GSTs) (Hecht 1995), enzymes involved in the detoxification of carcinogens.

Given the scientific and public health interest related to fruit and vegetable consumption and colorectal cancer risk, Schatzkin et al. (2000), conducted a multi-center trial testing a diet high in fiber, high in fruit and vegetables, and low in fat versus a usual diet. A total of 1,905 participants were followed for approximately 4 years for adenoma recurrence endpoints. Recurrence rates were essentially identical between the two intervention groups (approximately 40%), resulting in a RR of 1.00 (95% CI=0.90–1.12). Based on these results, the authors concluded that adopting a diet low in fat and high in fiber, fruit, and vegetables does not influence risk of adenoma recurrence. Likewise, results of a large chemoprevention trial testing the effects of a high versus low wheat bran fiber intervention on adenoma recurrence among 1,304 participants showed a lack of effect of the intervention on adenoma recurrence (Alberts et al. 2000). The OR for recurrent adenomas in

the high (13.5 g/day) versus the low (2 g/day) fiber group was 0.88 (95% CI=0.70–1.11). Furthermore, results of the 552-participant European Cancer Prevention Intervention Study showed individuals in the high-fiber group were at higher risk of adenoma recurrence compared to those in the low-fiber group (OR=1.67; 95% CI=1.01–2.76) (Bonithon-Kopp et al. 2000). Thus, the clinical trial data do not provide support to the protective effect(s) of fiber in the development of colorectal neoplasia. However, it must be stressed that intervention studies of adenoma recurrence do not address the hypothesis of whether fiber is associated with the risk of developing adenomas per se since these studies are conducted among individuals with a history of these lesions.

2.3 Calories

Total energy intake is correlated with nutrient and non-nutrient factors; correlations are generally highest for macronutrients. Therefore, the assessment of energy intake and colorectal cancer can be difficult, since the specific dietary factors themselves may be related to colon cancer risk. Variation in energy intake among individuals within a population is influenced largely by level of physical activity, metabolic efficiency, and body size (Willett and Stampfer 1986). Thus, when energy intake itself is assessed as a risk factor for colorectal cancer, the interpretation is not straightforward; caloric intake may simply be a surrogate for one or more of its influential factors (i.e., physical activity), which itself may be related to colon cancer risk. Given these issues, a strong argument has been made for taking total energy intake into account when assessing the etiological factors and colorectal cancer.

Results of most published case-control studies have shown a positive association between total energy intake and risk of colon cancer (Jain et al. 1980; Bristol et al. 1985; Potter and McMichael 1986; Kune et al. 1987b; Lyon et al. 1987; Graham et al. 1988; Slattery et al. 1988; West et al. 1989; Gerhardsson de Verdier et al. 1990a; Whittemore et al. 1990; Iscovich et al. 1992; Peters et al. 1992; Meyer and White 1993). As noted earlier, in the pooled analyses by Howe (1993), total energy intake was associated with a higher risk of colon cancer regardless of whether the energy source was fat, protein, or carbohydrate. Slattery et al. (1997b), reported similar findings based on three case-control studies, and suggested that total energy intake is more important than the specific energy sources (i.e., fat, protein, or carbohydrate). In contrast to the findings of case-control studies, cohort studies have shown no relationship or even a slight inverse association between total energy intake and risk of colon cancer (Stemmermann et al. 1984; Garland et al. 1985; Willett et al. 1990; Bostick et al. 1994; Giovannucci et al. 1994b; Goldbohm et al. 1994). In one of these studies (Bostick et al. 1994), a statistically significant relative risk of 0.62 was reported between high and low

quintiles of energy intake. The reason for the discrepancy between findings from cohort and case-control studies regarding energy intake and colon cancer is unclear. As a result of the inconsistency in the published findings, no firm conclusions can be drawn on the association between total caloric intake and risk of colorectal cancer.

2.4 Micronutrients

2.4.1 Calcium and Vitamin D

The role of calcium or vitamin D in colorectal neoplasia has been investigated in a variety of study settings including animal studies, international correlational studies, case-control and cohort studies, and intervention studies of adenoma recurrence. Additional settings include human intervention studies on the effect of calcium supplementation on cell proliferation (Lipkin and Newmark 1985; Buset et al. 1986; Gregoire et al. 1989; Rozen et al. 1989; Stern et al. 1990; Wargovich et al. 1992; Kleibeuker et al. 1993) and in vitro studies on human epithelial cells (Buset et al. 1986). It is hypothesized (Newmark et al. 1984; van der Meer 1985) that calcium might reduce colon cancer risk by binding secondary bile acids and ionized fatty acids to form insoluble soaps in the lumen of the colon, thus reducing the proliferative stimulus of these compounds on colon mucosa. Calcium can also directly influence the proliferative activity of the colon mucosa (Lipkin and Newmark 1985).

Results of analytic epidemiological studies that have examined the association between calcium as a risk factor for colorectal cancer have been inconsistent (Martínez and Willett 1998). Data from earlier large cohort studies show weak, non-significant inverse associations with no evidence of a dose-response relationship (Martínez and Willett 1998). Results from the Nurses' Health Study (Martínez et al. 1996), where data from three dietary questionnaires were collected prospectively over 6 years, did not support a major inverse association between calcium intake and risk of colorectal cancer over a 6-year period. However, more recent data from this nurses' cohort that included a longer follow-up period showed calcium to be associated with the development of distal but not proximal colon cancer (Wu et al. 2002); the authors further noted that maximum benefit occurs at intakes of 700–800 mg/day and no additional protection occurs at higher levels of intake. Support of the calcium–colorectal cancer hypothesis is also provided by the recent reported inverse association between dietary calcium and adenoma recurrence where the OR for individuals whose intake was above 1,068 mg/day versus those with intakes below 698 mg/day was 0.56 (95% CI=0.39–0.80) (Martínez et al. 2002).

The modest effect of calcium intake on risk of colorectal neoplasia observed in epidemiological studies is consistent with findings from the recent clinical trial results of adenoma recurrence intervention trials. Calcium supplementation (1,200 mg of elemental calcium vs placebo) among 913 participants who underwent adenoma removal was associated with a statistically significant reduction in risk of adenoma recurrence (Baron et al. 1999). The recurrence rate in the calcium group was 31% compared to that in the placebo group of 38% (RR=0.76; 95% CI=0.60–0.96). Similar results were observed in the European Calcium Fibre Polyp Prevention trial (Bonithon-Kopp et al. 2000), although the lower recurrence rate among the calcium group was not statistically significant in the smaller study of 552 participants.

Few epidemiological data have been published on the association between vitamin D and colorectal cancer (Martínez and Willett 1998). Four (Garland et al. 1985; Bostick et al. 1993; Kearney et al. 1996; Martínez et al. 1996) prospective studies have reported inverse associations for dietary vitamin D and colon or colorectal cancer, but this relationship was only significant in the Western Electric Study (Garland et al. 1985). Of the three published case-control studies of vitamin D and colon or colorectal cancer, two (Benito et al. 1991; Peters et al. 1992) show inconsistent, non-significant findings, and one (Ferraroni et al. 1994) reported a significant inverse association. In addition, stronger associations are generally shown when supplemental or supplemental plus dietary sources of vitamin D were assessed. Data from the prospective Nurses' Health Study (Martínez et al. 1996) show stronger inverse associations for vitamin D than for calcium: colorectal cancer rates were lower for women who remained in the upper tertile of total vitamin D intake on all three questionnaires as compared to those who were in the lower tertile (RR=0.33; 95% CI=0.16–0.70) and for women in the upper versus the lower category of average intake of total vitamin D (RR=0.42; 95% CI=0.19–0.91). However, since many of the women in the highest tertile were taking multivitamin supplements, a major source of vitamin D in this population, it is difficult to determine whether other vitamin or minerals in the supplements could also be important. A recently published study of adenoma recurrence showed dietary vitamin D to be associated with lower odds of recurrence, although results were not statistically significant (OR=0.78; 95% CI=0.54–1.13) (Martínez et al. 2002). Furthermore, secondary analyses of the Baron et al. trial revealed that higher serum 25-hydroxyvitamin D (25-OHD) levels were associated with a protective effect for those in the calcium-supplemented group, though not for those in the placebo group (Grau et al. 2003), lending further support to the importance of the biological intricacies of these two nutrients in colorectal neoplasia, as noted in the accompanying editorial comment by Jacobs et al. (2003).

2.4.2 Folate

In addition to animal data (Cravo et al. 1992), an increasing epidemiological body of evidence from prospective and retrospective observational studies supports the role of folic acid in reducing the risk of colorectal cancer. Eighteen studies of colon or colorectal cancer and seven of adenoma endpoints have been reported (Martínez et al. 2004). Overall, inverse associations with colon or colorectal cancer have been shown whether assessing folate from diet or in blood; studies of adenoma prevalence also support an inverse association.

Homocysteine, a sulfur amino acid formed by the adenylation and subsequent demethylation of methionine, has been shown to be a sensitive indicator of folate intake (Anonymous 1998; Selhub et al. 2000; Jacques et al. 2001), although it is influenced by additional factors (National Research Council 1998). Inclusion of homocysteine in the assessment of folate in carcinogenesis is important since it is possible that we are dealing with an issue of inadequate folate metabolism, indicated by reduced function of enzymes involved in homocysteine metabolism, rather than merely a state of folate deficiency. However, to our knowledge, only one study has been published on the association between serum homocysteine and colorectal cancer (Kato et al. 1999); results of this study showed a positive, non-significant association. Results of our own data from a chemoprevention trial (Martínez et al. 2004) show that individuals with plasma homocysteine levels lower than 7.84 μmol/l had an OR of 0.69 (95% CI=0.47,1.02; p-trend=0.02) compared to those with levels greater than 11.58 μmol/l. In this study, lower odds of recurrence were also shown for higher plasma folate (OR=0.66; 95% CI=0.46,0.97) and higher intake of total (dietary plus supplemental) folate (OR=0.61; 95% CI=0.42,0.89) and total vitamin B_6 (OR=0.65; 95% CI=0.45,0.94). Slightly weaker and non-significant associations were shown for dietary folate, methionine, and total vitamin B_{12}.

Additional evidence of a role for folate is that inherited variation in the activity of methylenetetrahydrofolate reductase (MTHFR), a critical enzyme in the production of the form of folate which supplies the methyl group for methionine synthesis (Kutzbach and Stokstad 1971), influences risk of colon cancer (Chen et al. 1996; Ma et al. 1997). This gene-nutrient interaction is discussed in more detail in a later section. In this proposed pathway, key nutrient and non-nutrient components are involved. Different endogenous forms of folate, 5-methyltetrahydrofolate and 5,10-methylenetetrahydrofolate, are essential for DNA methylation and DNA synthesis, respectively. When levels of 5,10-methylenetetrahydrofolate (which is required to convert deoxyuridylate to thymidylate) are low, misincorporation of uracil for thymidine may occur during DNA synthesis (Wickramasinghe and Fida 1994), possibly increasing spontaneous mutation rates (Weinberg et al. 1981), sen-

sitivity to DNA-damaging agents (Meuth 1981), frequency of chromosomal aberrations (Sutherland 1988; Fenech and Rinaldi 1994), or errors in DNA replication (Hunting and Dresler 1985; Fenech and Rinaldi 1994; James et al. 1994). Folate deficiency is related to massive incorporation of uracil into human DNA and to increased chromosomal breaks, and these abnormalities are reversed with folic acid supplementation (Blount et al. 1997). When methionine intake is low, levels of *S*-adenosylmethionine decrease, which stimulates MTHFR to convert 5,10-methylenetetrahydrofolate to 5-methyltetrahydrofolate. Homocysteine is methylated by 5-methyltetrahydrofolate to form methionine. Low production of methionine may occur by insufficient folate levels, which can in turn result in a low supply of methyl groups for DNA methylation. DNA hypomethylation is among the earliest events observed in colon carcinogenesis (Feinberg and Vogelstein 1983; Goelz et al. 1985; Feinberg et al. 1988; Makos et al. 1992; Issa et al. 1993; Cravo et al. 1994); however, it is unclear whether this process directly influences the carcinogenic process. Additional micronutrients involved in the DNA methylation process include vitamins B_6 and B_{12}. Furthermore, since alcohol is known to influence folate metabolism and methyl group availability (Finkelstein et al. 1974; Selhub and Miller 1992), its interaction with the key micronutrients needs to be considered in this process.

The findings from diverse study designs and populations, including animal data, along with evidence of a critical role of folate in DNA synthesis and methylation, indicate folic acid is a key nutrient in colorectal neoplasia etiology. However, much work needs to be done to identify specific key pathways of this complex area.

2.4.3 Other Micronutrients

Several additional micronutrients have been implicated in relation to colorectal neoplasia; however, the evidence for their specific role is unclear. Among the proposed micronutrients, those with antioxidant potential are thought to be important in protection against the development of colorectal cancer; these include β-carotene, selenium, and vitamins C and E, which have been shown to be inversely associated with colon cancer risk (Clark 1985; Byers and Perry 1992), perhaps through their effects on cell proliferation (Cahill et al. 1993).

Selenium, an essential trace mineral found in cereal grains and seafood has been shown to be inversely related to colorectal cancer (Nomura et al. 1987). Selenium is a cofactor for glutathione peroxidase, an enzyme involved in preventing free radical damage to tissues. However, given the wide variability of selenium concentration in soils and grains, assessment of intake is problematic, making epidemiological studies difficult to conduct. The correlations, although plausible, are mainly derived from ecological data; these

data show higher cancer mortality rates in low-selenium areas (Nelson 1987) compared to those of high regions. Perhaps the most provocative finding to date derives from secondary analyses of the Nutritional Prevention of Skin Cancer study (Clark et al. 1996) where a greater than 50% reduction in colorectal cancer incidence was shown for the selenium intervention compared with placebo. Since the results were based on secondary endpoint data among a population of selenium-deficient areas in the U.S., additional large trials will be needed.

Data on specific micronutrients such as vitamin C, β-carotene, or vitamin E are often derived indirectly from the observation of associations between high intake of rich sources of these nutrients and lower risk of colorectal cancer. To test the antioxidant hypothesis, Greenberg et al. (1994) conducted a randomized controlled trial of adenoma recurrence as the endpoint. Participants were randomized to one of four arms: 25 mg of β-carotene; 1 g of vitamin C and 400 mg of vitamin E; β-carotene, vitamin C, and vitamin E; or placebo. Recurrence rates after 4 years of follow-up were similar between the placebo group and the intervention groups, suggesting a lack of effect of chemopreventive properties by these antioxidant nutrients. Furthermore, results based on more recent epidemiological studies that have examined intake of these micronutrients indicate that there is insufficient evidence for a protective effect of these on risk of colorectal cancer (Albanes et al. 2000; Jacobs et al. 2001).

2.5 Alcohol

An association between alcohol intake and colon cancer risk has been observed in ecological (Kune and Vitetta 1992), cohort (Bjelke 1974; Williams and Horm 1977; Dean et al. 1979; Wu et al. 1987; Klatsky et al. 1988; Hirayama 1989; Carstensen et al. 1990; Stemmermann et al. 1990; Giovannucci et al. 1995a; Hsing et al. 1998), and population-based case-control studies (Tuyns et al. 1982; Ward et al. 1983; Kabat et al. 1986; Potter and McMichael 1986; Kune et al. 1987b; Freudenheim et al. 1990a; Longnecker 1990; Newcomb et al. 1993). Further, alcohol has been shown to be related to higher risk of colorectal adenoma (Giovannucci et al. 1993; Martínez et al. 1995). According to a review by Kroser et al. (1997), 3 of 4 cohort studies that investigated the association of alcohol and colon cancer among non-alcoholics showed significant results. In the same review, 8 of 16 retrospective studies also showed a positive significant association between alcohol and colorectal cancer. Kune and Vietta (1992) reported that a positive association between alcohol intake and colorectal cancer was found in 5 of 7 correlational studies.

The mechanism of action whereby alcohol increases the risk for colorectal cancer is unknown. One possibility is alcohol's role as an antagonist of folate and methionine metabolism (Finkelstein et al. 1974; Barak et al. 1987).

The alcohol breakdown product acetaldehyde may inactivate methyltetrahydrofolate, the form of folate required for methionine synthesis (Shaw et al. 1989). In rodents, the carcinogenicity of methyl-deficient diets is enhanced by ethanol (Porta et al. 1985). Based on these considerations, it was postulated that specific combinations of diet might be particularly deleterious (Giovannucci et al. 1995a). In a cohort study of men (Giovannucci et al. 1995a), alcohol, folate, and methionine intakes individually were moderately associated with risk of colon cancer, but combinations of high alcohol and low methionine and folate intakes yielded striking relative risks of 3.3 for total colon cancer and 7.4 for distal colon cancer. Further, among men with high intakes of folate or methionine, alcohol levels of greater than two drinks daily were not associated with risk of colon cancer. However, results of a large case-control study (Slattery et al. 1997d) did not support this mechanism. These findings suggest that the role of alcohol may depend on other dietary factors, particularly those related to methyl group metabolism.

2.6 Physical Activity and Obesity

In spite of the wide variation in physical assessment methodology among studies, including type of activity (leisure-time or occupational) and method of assessment, considerable consistency was found. Results of prospective (Gerhardsson de Verdier et al. 1986, 1988; Paffenbarger et al. 1987; Wu et al. 1987; Lynge and Thygesen 1988; Albanes et al. 1989; Marti and Minder 1989; Severson et al. 1989; Ballard-Barbash et al. 1990; Lee et al. 1991; Thun et al. 1992; Thune and Lung 1996; Martínez et al. 1997) and retrospective (Vlajinac et al. 1987; Gerhardsson de Verdier et al. 1988, 1990b; Slattery et al. 1988, 1990a; Brownson et al. 1989; Fredriksson et al. 1989; Peters et al. 1989; Benito et al. 1990; Kato et al. 1990a,b; Whittemore et al. 1990; Markowitz et al. 1992; Arbman et al. 1993; Chow et al. 1993; Dosemeci et al. 1993; Fraser and Pearce 1993; Vineis et al. 1993; Marcus et al. 1994; Longnecker et al. 1995; White et al. 1996) studies support an inverse association between physical activity and risk of colon, but not rectal cancer (Garabrant et al. 1984; Vena et al. 1985; Paffenbarger et al. 1987; Lynge and Thygesen 1988; Severson et al. 1989; Whittemore et al. 1990; Lee et al. 1991; Longnecker et al. 1995). The results are consistent whether assessing active versus non-active individuals or sedentary versus active. In a prospective study of female nurses (Martínez et al. 1997), leisure-time, physical activity, and body size were assessed in relation to the subsequent development of colon cancer. Women who were in the upper quintile of activity were at almost half the risk of developing colon cancer compared to non-active women (RR=0.54; 95% CI=0.33–0.90). The findings are supported by results of other published studies, including those of the Health Professionals Follow-up Study (Giovannucci et al. 1995b), a large prospective study of men. Although

published data indicate that higher levels of physical activity are not associated with lower risk of rectal cancer, data from a recent case-control study of 952 cases and 1,205 controls show that higher levels of long-term vigorous activity are associated with a 40% lower risk among men and 50% lower risk among women (Slattery et al. 2003).

Measures of body mass index (BMI) are not only good measures of obesity status but also provide a good estimate of long-term energy balance. Findings from numerous epidemiological studies show a positive association between BMI and risk of colon cancer (Lew and Garfinkel 1979; Waaler 1984; Garland et al. 1985; Phillips and Snowdon 1985; Wu et al. 1985; Klatsky et al. 1988; Chute et al. 1991; Lee et al. 1991; Le Marchand et al. 1992; Must et al. 1992; Bostick et al. 1994; Giovannucci et al. 1995b; Martínez et al. 1997). Furthermore, there is evidence suggesting that visceral or central adiposity is associated with higher risk of developing colon cancer, with a RR as high as 3.5 (Giovannucci et al. 1995b). Published studies also indicate that although risk of colon cancer is associated with a higher BMI among women, this effect appears to be stronger for men (Giovannucci 2002). Terry et al. (2001) recently published data indicating that the association between BMI and colon cancer in women is modified by menopausal status: a positive association was shown for pre-menopausal women but not among post-menopausal women. In an accompanying editorial, Giovannucci (2002) proposed that the effect of obesity on colorectal neoplasia in pre-menopausal women acts via the insulin/insulin growth factor pathway while in post-menopausal women, higher levels of oestrogens in obese women act in opposite directions to cancel out the effect of each other (i.e., BMI increases risk and oestrogens lower risk). In addition, when Slattery et al. assessed the joint effect of physical activity and BMI in a study of 2,073 cases and 2,466 controls, the highest risk of colon cancer occurred among those both physically inactive and with high BMI levels (Slattery et al. 1997b).

Several biological mechanisms have been proposed for the inverse association between physical activity and colon cancer (Bartram and Wynder 1989). In a cross-sectional analysis, Martínez et al. (1999) recently showed that a higher level of leisure-time activity was inversely related to the concentration of prostaglandin E_2 (PGE_2) in the rectal mucosa, suggesting a potential mechanism acting through PGE_2 synthesis. Prospective follow-up analyses of these data also show higher physical activity levels at baseline to be associated with lower PGE_2 levels from biopsies taken 8 to 26 months later (p=0.01). Hyperinsulinemia may also be an important mechanism through which physical activity exerts its protective effect. High insulin levels are related to physical inactivity, high body mass, and central deposition of adipose tissue; furthermore, insulin is a mitogen for normal and neoplastic colonic epithelial cells (Giovannucci 1995). Supporting a role for insulin, recent studies have found diabetes mellitus to be a risk factor (Hu et al.

1999), and a prospective analysis of insulin's influence found a direct association with colon cancer risk (Schoen et al. 1999).

2.7 Tobacco

Until recently, exposure to tobacco has not been implicated as an etiological factor in colorectal cancer; however, a higher risk of adenomatous polyps has been consistently observed among smokers in numerous studies with relative risks ranging from 1.4 to 3.6 (Giovannucci and Martínez 1996). An induction period of 30–40 years between smoking and risk of colorectal cancer has been proposed based on results from two large cohort studies (Giovannucci et al. 1994a,c). Subsequently, the vast majority of published studies have reported positive associations between cigarette smoking and colorectal cancer (Wu et al. 1985; Slattery et al. 1997c,d; Freedman et al. 1995; Heineman et al. 1995; Newcomb et al. 1995; Chyou et al. 1996; Le Marchand et al. 1997; Yamada et al. 1997; Hsing et al. 1998; Knekt et al. 1998), though several studies did not support an association (Baron et al. 1994; Nyrén et al. 1996; Nordlund et al. 1997; Tavani et al. 1997). Of note, three of the non-supportive studies (Baron et al. 1994; Nyrén et al. 1996; Nordlund et al. 1997) were conducted in Sweden, suggesting some factor, possibly genetic, in Swedes may counter the impact of smoking. In a review of the published data, Giovannucci and Martínez (1996) suggested that the evidence earlier in the decade tended not to support the hypothesis that smoking influenced colorectal carcinogenesis because a sufficient lag period had not elapsed between smoking and colorectal cancer risk. With the assumption that an increased risk emerges only about four decades after one begins smoking, a relatively consistent pattern materializes. The overall evidence supports the hypothesis that tobacco smoke is an initiator of colorectal carcinogenesis and the requirement for a very long induction period, possibly up to four decades, which suggests that studies assessing the role of tobacco exposure and colorectal cancer need to take into account this long induction period in their analyses. In the latest and largest published study to date, based on data from the Cancer Prevention Study II (CPS II, Chao et al. 2000), 312,332 men and 469,019 women were followed prospectively from 1982 to 1996. The relative risk for colorectal cancer mortality was 1.32 (95% CI=1.16–1.49) for women and 1.41 (95% CI=1.26–1.58) for men who reported being current smokers. Of interest, cigar or pipe smokers who smoked for 20 years or more were also at increased risk of dying from colorectal cancer (OR=1.34; 95% CI=1.11–1.62). A follow-up review by Giovannucci (2001) continues to support the adverse effect of tobacco on risk of colorectal cancer.

As rates of cigarette smoking continue to increase in developing countries, tracking colorectal cancer rates after the proposed induction period

will be extremely important. Based on estimates from the recently published results of the CPS II (Chao et al. 2000), if the association between tobacco exposure and colorectal cancer is causal, 12% of colorectal cancer deaths can be attributable to cigarette smoking in the U.S. Based on the accumulating data, the authors recommend that colorectal cancer be added to the list of tobacco-related cancers. From a public health prospective, the data to date suggest that continued efforts to prevent smoking among adolescents and young individuals are warranted.

3 Gene-Nutrient Interactions

Major advances in characterization of new genomes has provided tremendous excitement in the scientific community, and the area of cancer prevention is no exception. However, the availability of these new data poses an extremely challenging situation when trying to disentangle the role of several genes and their interaction with a variety of nutrients or other environmental factors. No longer is it conceivable to view a single function of one gene or one nutrient but rather how the interaction of the gene and the nutrient functions along with other molecules in the cell.

To better understand causality of colorectal cancer, it will be important to incorporate genetic and molecular markers of disease into epidemiological study settings. The merging of these two entities should enhance the understanding of biological mechanisms and disease causality. Furthermore, the integration of these markers into chemoprevention trials can also contribute to the understanding of the etiology of colorectal cancer. A current area of research involves the role of genetic variability in susceptibility to the adverse effects of specific risk factors. Within this context, an active research area has been to define the potential role of genetic polymorphisms in colorectal neoplasia. This line of inquiry aims at identifying and characterizing factors that may influence an individual's susceptibility to develop colorectal cancer or adenoma, given exposure to potentially harmful agents. Examples from recent reports discussed below illustrate the findings and challenges posed by this area of scientific research.

3.1 Folate and *MTHFR*

One example of a prominent gene-diet interaction in colorectal cancer involves the *MTHFR* gene and folic acid. As noted in an earlier section, diets low in folate and methionine and high in alcohol are associated with a higher risk of colorectal neoplasia. Such a dietary pattern results in a methyl-deficient diet, enhancing colorectal cancer risk by altering DNA methylation or

by influencing the production of thymidine, which is required for DNA synthesis (Blount and Ames 1995). MTHFR is an enzyme that regulates the metabolism of folate and methionine by converting 5,10-methylenetetrahydrofolate to 5-methyltetrahydrofolate, the form of folate required for methionine synthesis. A common mutation, identified as a C to T mutation at nucleotide 677, results in a substitution of valine for alanine. The homozygous mutant is present in 10%–15% of the population and has been shown to result in approximately 30% of the enzyme activity, compared with wild type (Frosst et al. 1995).

In an earlier study of men, Chen et al. (1996) observed a lower risk of colorectal cancer for individuals with the *MTHFR* variant homozygous genotype and who consumed more methionine (OR=0.27; 95% CI=0.06–1.20) or had a higher folate intake (OR=0.44; 95% CI=0.13–1.55) as compared with those with the variant heterozygous or the wild type homozygous with lower intakes of methionine or folate. Furthermore, among men with the variant homozygous genotype, those in the high alcohol category had a 15-fold risk of colorectal cancer compared to those in the low category, indicating that the inverse association with *MTHFR* is essentially eliminated by high alcohol consumption. Based on their findings, the authors suggested that the risk of colorectal cancer associated with the *MTHFR* homozygous variant genotype may differ depending on the folate and methionine content of the diet as well as alcohol intake. Similar findings were also reported by Ma et al. (1997) in a later publication. A case-control study by Slattery et al. (1999) found a weak association for the TT genotype in men (OR=0.8; CI=0.6–1.1) and women (OR=0.9; CI=0.6–1.2).

Studies of colorectal adenoma that have examined the interaction of folate and *MTHFR* show individuals with the TT homozygous mutant genotype who were also in the lowest category of folate intake (Ulrich et al. 1999) or RBC/plasma levels (Levine et al. 2000) have the highest risk of adenomas compared to the wild types with higher intakes. Although more data are clearly warranted, the studies support the relationship between nutrients involved in folate metabolism and methyl donor availability in earlier stages of carcinogenesis.

The mixed findings of the limited number of epidemiological studies suggest that the specific role of *MTHFR* and related nutrients (i.e., folate, methionine, vitamin B_{12}, etc.) is likely to be more complicated than originally proposed. The only consistent finding in the literature appears to be of a higher risk of neoplasia among individuals with low folate status and the TT homozygous mutant genotype. Future investigations must comprise a large number of participants and assess all key markers of folate status and the *MTHFR* polymorphism. In addition, the 1996 mandate by the United States Food and Drug Administration that all enriched cereal grain products be fortified with folic acid (Food and Drug Administration 1996) will need to

be taken into account when interpreting data from studies conducted after this mandate was implemented.

3.1.1
Red Meat and Genetic Polymorphisms

As noted earlier, heterocyclic amines—mutagenic products found in cigarette smoke condensate or those formed by cooking meat at high temperatures—are thought to be involved in colorectal carcinogenesis. Genetic polymorphisms in enzymes involved in metabolism of these carcinogens are likely to influence the risk colorectal neoplasia. If this hypothesis is correct, individuals with this type of susceptibility are at increased risk only if exposed to the relevant carcinogens. The metabolism of many of these compounds is mediated in part by cytochromes P450 1A1 (CYP1A1) and 1A2 (CYP1A2), which generate reactive metabolites that can produce DNA adducts. Enhanced metabolic activation of polycyclic aromatic hydrocarbons (PAHs) has been observed in homozygotes for an *Msp* I mutation in the 3′-end of *CYP1A1*. Some data show that the polymorphism associated with enhanced activation of *CYP1A1* is related to higher risk for colorectal carcinoma in situ and cancer (Sivaraman et al. 1994). A polymorphism that causes a highly inducible state of *CYP1A2* has also been described (Kadlubar 1994); however, since the exact locus of genetic variation remains to be identified, *CYP1A2* polymorphisms can only be determined by phenotypic assays.

Acetylation polymorphisms resulting from different forms of the *N*-acetyltransferase gene 1 (*NAT1*) and 2 (*NAT2*) lead to either fast or slow acetylation of xenobiotics. Rapid acetylation may only be important among individuals who consume a diet high in meats that are significant sources of heterocyclic amines. In studies that have found that rapid *NAT2* increased risk of colon cancer, the association was greatest among those in the higher quartiles of meat consumption (Wohlleb et al. 1990; Lang et al. 1994). It has been suggested that rapid NAT2 acetylation of heterocyclic amines formed in cooking of meat may be related to colon cancer risk (Lang et al. 1994). Furthermore, when the role of cooked meat preference and phenotype combinations of NAT2 and CYP1A2 in colorectal neoplasia were assessed (Lang et al. 1994), the combination of well-done meat cooking preference and rapid-rapid phenotypes was associated with an odds ratio of 6.45. However, these findings have not been replicated by more recent studies (Chen et al. 1998; Kampman et al. 1999).

3.2
Cruciferous Vegetables and Glutathione-S-Transferases

In contrast to enzymes that may activate carcinogens, glutathione S-transferases (GSTs) detoxify carcinogens, including smoking-related carcinogens

formed by CYP1A1, by conjugating them with glutathione. Cytosolic GSTs are a supergene family, are widely distributed in the mammalian species, and are grouped into five classes on the basis of subunit composition: α (A), μ (M), π (P), ϕ (T), and σ (Z). The *GSTM1*-null genotype, which is associated with enzyme inactivity of GSTμ, has been shown to be more frequent among patients with colorectal cancer (Strange et al. 1991; Zhong et al. 1993), although this finding has not been universal (Gertig et al. 1998). Vegetables contain several compounds that possess a variety of anti-carcinogenic properties (Steinmetz and Potter 1991b). Specifically, the anti-carcinogenic properties of cruciferous vegetables have been mainly attributed to the degradation products of glucosinolates (e.g., isothiocyanates and indoles), which induce detoxification enzymes (Mehta et al. 1995). A possible mechanism of action of isothiocyanates is thought to be through the induction of GSTs (Hecht 1995); in some study settings, cruciferous vegetables have been shown to induce GSTs (Fahey et al. 1997; Gerhauser et al. 1997). These enzymes catalyze the conjugation of glutathione with a large number of compounds bearing an electrophilic center, including carcinogens. There is some support in the literature for the role of GSTs in the metabolism of isothiocyanates in man (Hayes and Pulford 1995). In terms of individual isoenzymes, *GSTM1* and *GSTP1* appear to be the most efficient catalysts that rapidly conjugate isothiocyanates to glutathione, which is then excreted in the urine (Zhang et al. 1995). *GSTM1* is involved in the detoxification of tobacco-related carcinogens, such as epoxides and hydroxylated metabolites of benzo[*a*]pyrene. While there have been several studies of the impact of *GSTM1* deficiencies on susceptibility to a range of cancers, there have been no similar studies of *GSTP1*. Since these polymorphisms have only recently been identified, the literature regarding their role in carcinogenesis is unclear. The interaction between cruciferous vegetables, GST polymorphisms, and colorectal neoplasia has been assessed in two studies. Lin et al. (1998) found a lower prevalence of colorectal adenomas among people with the *GSTM1*-null genotype when comparing individuals in the highest versus lowest quartile of broccoli intake (OR=0.36; 95% CI=0.19–0.68). Results of the case-control study by Slattery et al. (2000) did not support an interaction between cruciferous vegetables as inducers of GSTs in colon cancer; however, there were suggestions of this effect among subsets of the population. Given the paucity published data, the hypothesis that cruciferous vegetable consumption might decrease risk of colorectal cancer through their increase in GST activity remains plausible and awaits results of additional studies.

3.2.1 Dietary and Lifestyle Guidelines

Recommendations from a panel of experts (Potter 1997) concluded that cancer is primarily caused by environmental factors. Furthermore, it was noted

Table 1. Public health goals and advice to individuals from the World Cancer Research Fund and the American Institute for Cancer Research

Environmental factor	Public health	Individual
Food supply and eating	Consume nutritionally adequate and varied foods, based primarily on foods of plant origin	Choose primarily plant-based diets rich in a variety of fruit and vegetables, legumes, and minimally processed foods
Body weight	Average BMI indices throughout adult life should be between 21 and 23, such that individual BMI be between 18.5 and 25	Avoid being overweight or underweight. Limit weight gain during adulthood to less than 5 kg (11 lbs)
Physical activity	Maintain an active lifestyle through adult life, with opportunities for vigorous activities	If occupational activity is low or moderate, take a 1-h brisk walk or similar exercise daily. Exercise vigorously for 1 h or more per week
Fruit and vegetables	Promote year-round consumption of a variety of fruit and vegetables, providing 7% or more of total calories	Eat 400–800 g (15–30 oz) or 5 or more servings a day of a variety of fruit and vegetables
Other plant foods	A variety of starchy or protein-rich foods of plant origin, preferably minimally processed, to provide 45%–60% of total calories. Refined sugar to provide less than 10% of total calories	Eat 600–800 g (20–30 oz) or more than 7 portions per day of a variety of cereals/grains, pulses/legumes, roots, tubers, and plantains. Prefer minimally processed foods. Limit consumption of refined sugar
Alcohol	Consumption of alcohol is not recommended. If consumed, restrict to less than 5% of total caloric intake for men and less than 2.5% for women	Alcohol consumption is not recommended. If consumed, limit to less than 2 drinks/day for men and 1 drink/day for women
Meat	If eaten at all, red meat should provide less than 10% of total calories	If eaten at all, limit red meat to less than 80 g (3 oz) per day. Choose fish, poultry, or meat from non-domesticated animals instead of red meat
Fats and oils	Total fats and oils should provide 15% but no more than 30% of total calories	Limit intake of fatty foods, particularly those of animal origin. Choose modest amounts of appropriate vegetable oils
Salt and salting	Salt from all sources should amount to less than 6 g/day (0.25 oz) for adults	Limit consumption of salted foods and use of cooking and table salt
Storage	Store perishable food in ways that minimize food contamination	Do not eat food which, as a result of long storage at ambient temperatures, is liable to contamination with mycotoxins
Preservation	Perishable food should be kept chilled or frozen if not consumed promptly	Use refrigeration and other appropriate methods to preserve perishable food as purchased and at home
Additives and residues	Establish and monitor the enforcement of safety limits for food additives, pesticides, and their residues, and other chemical contaminants in the food supply	When levels of additives, contaminants, and other residues are properly regulated, their presence in food and drink is not known to be harmful. However, unregulated or improper use can be a health hazard, particularly in economically developing countries
Preparation	When meat and fish are eaten, encourage relatively low-temperature cooking	Do not eat charred food. If eating meat, avoid burning of meat juices. Consume meat or fish that has been grilled in direct flame. Eat cured/smoked meats only occasionally

Table 1 (continued)

Environmental factor	Public health	Individual
Dietary supplements	Community dietary patterns to be consistent with reduction of cancer risk without use of dietary supplements	For those who follow recommendations, dietary supplements are probably unnecessary, and possibly unhelpful
Tobacco	Discouragement of production, promotion, and use of tobacco in any form	Do not smoke or chew tobacco

that evidence of dietary protection against a variety of cancers is strongest and most consistent for diets high in fruit and vegetables. Although the panel mainly focused in the areas of food and nutrition from a global perspective, 15 recommendations were provided that went beyond dietary factors (Table 1). The panel concluded that the principal causes of colorectal cancer are dietary. It also emphasized the strong consistent evidence for the protective effect of physical activity against colon cancer. Specifically, the expert panel noted that diets high in vegetables and fiber, low in meat, the avoidance of alcohol, and regular physical activity, may reduce the incidence of colorectal cancer by 66%–75%.

4 Summary and Future Challenges

The past decade has been filled with a plethora of literature related to the primary prevention of colorectal cancer. Although the precise mechanisms have not been clarified, several lifestyle factors are likely to have a major impact on colorectal cancer development. Physical inactivity and, to a lesser extent, excess body weight are consistent risk factors for colon cancer. Exposure to tobacco products early in life is associated with a higher risk of developing colorectal neoplasia. Diet and nutritional factors are also clearly important. Excess alcohol consumption, probably in combination with a diet low in some micronutrients such as folate and methionine appear to increase risk; diets high in red meat may also increase risk. Recent epidemiological and intervention studies have tended not to support an effect of fiber in colorectal neoplasia etiology. However, some micronutrients or phytochemicals in fiber-rich foods may be important; folic acid is one such micronutrient that has been shown to protect against the development of colorectal neoplasia and is currently being studied in intervention trials of adenoma recurrence.

A variety of future challenges lie ahead in the area of primary prevention of colorectal cancer. For example, repeated-measures analysis of dietary data in cohort studies has resulted in null associations for fiber, fruit, and vegeta-

bles with colorectal cancer. However, since only a limited number of cohort studies collect dietary data at different time points during the follow-up period, it will be difficult to assess consistency across studies. An additional yet unanswered question is whether lifestyle factors, including diet, play an important role only when the exposure occurs in childhood or early adulthood. While we can retrospectively assess exposure to tobacco prior to adulthood, assessment of diet is much more difficult. Therefore, assessment of lifestyle factors that may play a role very early in the colorectal carcinogenesis sequence may be particularly problematic.

The genetic events underlying colorectal cancer continue to be elucidated rapidly. The pre-malignant lesion, the adenoma, has been identified; the process by which this lesion progresses to cancer is well described. Since the carcinogenesis process for colorectal cancer spans several decades, there is ample opportunity to suppress the disease in its early stages, prior to the onset of malignancy. Furthermore, given that relatively few adenomas progress to malignant lesions, the identification of factors that predict the malignant progression of the adenoma will be an important task. As data from chemoprevention trials of adenoma recurrence become available, analyses nested within these trials will aid in identifying risk factors for overall recurrence as well as recurrence of advanced adenomas. Continued focus on the prevention of colorectal cancer, in combination with efforts aimed at screening and surveillance, will be vital in attaining the greatest possible progress against this complex, yet highly preventable disease.

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Chemoprevention of Colorectal Cancer: Ready for Routine Use?

Nadir Arber[1] (✉) · Bernard Levin[2]

[1] Department of Cancer Prevention, Tel-Aviv Medical Center, 6 Weizmann St., 64239 Tel-Aviv, Israel
[2] Division of Cancer Prevention, U.T. MD Anderson Cancer Center, Houston, TX, USA

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Abstract In the third millennium, preventive medicine is becoming a cornerstone in our concept of health. Colorectal cancer (CRC) prevention, in particular, has become an important goal for health providers, physicians and the general public. CRC fits the criteria of a disease suitable for chemopreventive interventions. It is a prevalent disease that is associated with considerable mortality and morbidity rates, with more than 1,000,000 new cases and 500,000 deaths expected, worldwide, in 2004. CRC has a natural history of transition from precursor to malignant lesion that spans, on average, 15–20 years, providing a window of opportunity for effective interventions and prevention. A pre-malignant precursor lesion (i.e. adenoma) usually precedes cancer, and helps to identify a subset of the population that is at increased risk of harbouring and developing cancer. Science and technology have evolved to a point where we are able to use our knowledge of cancer biology to identify individuals at risk and interrupt the process of malignant transformation at the level of the pre-cancerous lesion. Recent progress in molecular biology and pharmacology enhances the likelihood that cancer prevention will increasingly rely on chemoprevention. Chemoprevention, a new emerging science, means the use of agents to inhibit, delay or reverse carcinogenesis. Recent observations suggest a number

of potential targets for chemoprevention. Many agents have potential benefit but only modest chemopreventive efficacy in clinical trials. There is much evidence suggesting an inverse relationship between aspirin or non-steroidal anti-inflammatory drug (NSAID) consumption and CRC incidence and mortality. However, NSAID consumption is not problem-free; 1997 data show 107,000 hospitalisations and 16,500 deaths due to NSAID consumption in the U.S. alone. Therefore, although chemoprevention of CRC is already possible, drugs that have more acceptable side-effect profiles than the currently available NSAIDs are required. Cyclo-oxygenase (COX)-2-specific inhibitors, which have an improved safety profile compared to traditional NSAIDs that inhibit both the COX-1 and COX-2 enzymes, seem to be well-suited drug candidates for CRC prevention. The inhibition of the growth of pre-cancerous and cancerous cells without affecting normal cells is the ultimate aim of cancer treatment and is of particular importance in chemoprevention studies, which may be long term in nature, involving healthy subjects and minimal toxicity. Cancer prevention is certain to be a significant focus of research and intervention in the coming years, propelled by the realisation that we will be able to identify both individuals susceptible to specific cancers as well as the molecular targets that can alter or stop the carcinogenesis process. Pharmacology and genetics are collaborating to develop new chemoprevention agents designed to affect molecular targets linked to specific premalignant or predisposing conditions.

1 Introduction

The famed Viennese surgeon Theodor Billroth once said that cancer could be cured with the knife [1]. Dr. Billroth was later proved wrong as subsequent generations of physicians and scientists gained more knowledge about the nature and progression of cancer. Despite advances in surgery, radiation therapy and chemotherapy, cancer has surpassed heart disease and become the leading cause of death in the Western world [2]. Hence, at the dawn of this century, cancer prevention is the new frontier for cancer therapy.

Colorectal cancer (CRC) is a major health concern, with more than 1,000,000 new cases and 500,000 deaths expected in 2004. The lifetime risk of developing a colorectal adenoma is 40%, and about 10% of them will become malignant. The estimated lifetime risk for CRC is 5%–6% in the USA [3–5]. CRC is the leading cause of cancer-related deaths in the Western world. Recent data from the World Health Organization indicate that CRC has reached the highest incidence of all malignancies in Europe [6, 7]. Incidence rates increase sharply after the age of 50 years. Incidence and mortality are similar in both men and women [3]. In women, CRC ranks third after lung and breast cancer, and in men it ranks third after lung and prostate cancer. Despite diagnostic and therapeutic advances, long-term survival has not significantly improved over the last three decades, and nearly half of CRC patients will eventually die of their disease [3, 4]. The high prevalence and mortality associated with CRC render effective prevention an important public health and economic concern.

In recent years, cyclo-oxygenase (COX)-2-specific inhibitors have become commercially available for human use. They offer all of the well-known benefits of aspirin or non-selective NSAIDs (pain relief, fever and inflammation reduction) while sparing the gastrointestinal toxicity. Recent human studies assessing the influence of selective COX-2 inhibitors confirmed the particular relevance of these agents for sporadic CRC prevention. Their results might be noteworthy, since the degree of inhibition of colon carcinogenesis exceeded the inhibition seen with other commonly used NSAIDs.

2 Chemoprevention

Chemoprevention, a new science that has emerged during the last decade, presents an alternative approach to reducing mortality from CRC as well as other cancers. A proof of concept was demonstrated in a landmark study proving the importance of retinoids in the prevention of head and neck cancers [8]. In CRC, chemoprevention involves the long-term use of a variety of oral agents that can delay, prevent or even reverse the development of adenomas in the large bowel and interfere with the multi-step progression from adenoma to carcinoma. Chemopreventive approaches are especially important in patients who have a genetic predisposition, or who are particularly susceptible to environmental causes of CRC.

The ideal chemopreventive agent should fulfill the following criteria:

a. The drug must be efficacious.
b. It should have a convenient dosing schedule of not more than once a day.
c. It should be easily administered.
d. It should have no side-effects, or a very low profile of side-effects in high risk populations.
e. It should have a low cost.

Research on compounds for the prevention of CRC is now gaining momentum. Although there are several promising compounds with potential chemopreventive capabilities (Table 1) and agents with a proven efficacy (Table 2), these agents can reduce the incidence of CRC by no more than 20%. It appears that the most promising group of compounds is the non-steroidal anti-inflammatory drugs (NSAIDs). They can prevent CRC in about 50% of cases. The association between NSAIDs and CRC is intriguing and comprehensive. The discovery of their potential chemopreventive activity in sporadic human CRC, almost 20 years ago, represents an important example of chemoprevention.

Table 1. Dietary substances with potential chemopreventive properties

Green and black tea polyphenols
Resveratrol
Soy isoflavones
Curcumin
Phenethyl isothiocyanate
Sulforaphane
Lycopene
Perillyl alcohol
Vitamin D and derivatives

Table 2. Chemopreventive agents with proven efficacy

Hormone replacement therapy
Folate
Calcium
DFMO
Vitamins
Antioxidants
Fiber

DFMO, difluoromethylornithine.

3 Animal Studies: From Non-specific to Specific COX-2 Inhibition

Observations suggesting that NSAIDs reduce the incidence and mortality from CRC are supported by more than 100 well-conducted, randomised, double-blind, placebo-controlled animal studies. These experiments clearly demonstrated the consistent preventive effect of NSAIDs on carcinogen-induced colorectal tumourigenesis in rodents. The experiments have shown that the administration of various NSAIDs and a colon carcinogen results in fewer colorectal tumours per animal and fewer animals with tumours compared to the control group that was treated with the carcinogen alone (for reviews see [9,10]).

Min mice have been used widely as an experimental model for familial adenomatous polyposis (FAP). The Min mouse has a mutation in the *APC* (*adenomatous polyposis coli*) gene and develops intestinal adenomas similar to those in FAP patients. The administration of different NSAIDs to Min mice causes a dramatic reduction in tumour burden, and in some cases nearly abrogates the tumour burden completely [11–14]. In COX-2 knockout mice that have been crossed with Min mice, the progeny have demonstrated a marked reduction in the number of intestinal tumours [15]. Furthermore, in rats with chemically induced CRC, various NSAIDs can prevent tumourigenesis or dramatically decrease tumour load [16–18]. New studies have

shown that combinations of chemopreventive drugs may also hold exceptional promise for preventing the development of tumours. When Min mice are treated with sulindac or epithelial growth factor (EGF)-receptor inhibitors (such an EK-569) they develop approximately 50%–70% fewer tumours. Torrance and colleagues [19] showed that treatment with a combination of sulindac and EKI-569 resulted in a 97% reduction in tumour load. Moreover, when EKI-569 was combined with a dose of sulindac that would otherwise be too low to prevent disease progression on its own, the frequency of polyp formation was reduced by more than 95%. This study is particularly exciting because no adverse effects were reported. Further work to find effective chemopreventive combinations, and bring them into clinical use, will undoubtedly improve efforts to prevent and treat CRC.

4
Human Studies: From Observations to Expectations

The most compelling evidence for the role of NSAIDs in the prevention of colorectal tumours comes from clinical studies in FAP patients [20–22]. FAP is an autosomal-dominant inherited disease associated with a markedly increased risk of CRC and various other tumours at a young age. The genetic mutation responsible for this disease resides in the *APC* tumour-suppressor gene that also plays an important role in the sporadic adenoma-carcinoma sequence [23]. In the early 1980s, Waddell and Loughry were the first observant physicians to report the regression of rectal adenomatous polyps in FAP patients treated with sulindac and indomethacin as anti-inflammatory therapy [24]. These pioneering observations were followed by double-blind, placebo-controlled studies that have shown that sulindac reduces both the number and the size of colorectal adenomas in FAP patients [20–22].

These effects were recently confirmed by two trials. One study was an international, double-blind, placebo-controlled clinical study in 81 subjects with FAP. The patients received celecoxib (Pfizer, NYC, NY, USA) (200 or 400 mg b.i.d.) or placebo for 6 months. Patients treated with celecoxib had a 28% reduction in polyp number and 30% reduction in polyp burden, compared with patients who received placebo [25]. In a recent open labelled study, eight FAP patients received standard anti-inflammatory doses of rofecoxib (Merck, White House, NJ, USA) (25 mg qd) for up to 30 months. These patients experienced a significant suppression (~90%) of polyp growth [26].

5 Epidemiological Studies

Evidence has now accumulated from epidemiological studies and investigations with human subjects that NSAIDs hinder the development of CRC. Several epidemiological studies have shown that the use of aspirin and other NSAIDs can prevent adenoma formation (Fig. 1). They can also decrease the incidence of CRC (Fig. 2). Most importantly, their use is associated with a substantial decrease in the risk of death from CRC (Fig. 3). In these studies, patients treated routinely with one of several different types of NSAID had a decreased risk of mortality when compared with individuals who were not

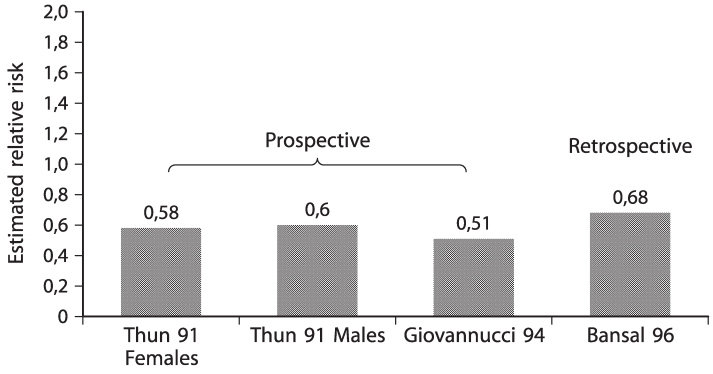

Fig. 1. Cancer-associated mortality (for details on the cited studies see [63, 64])

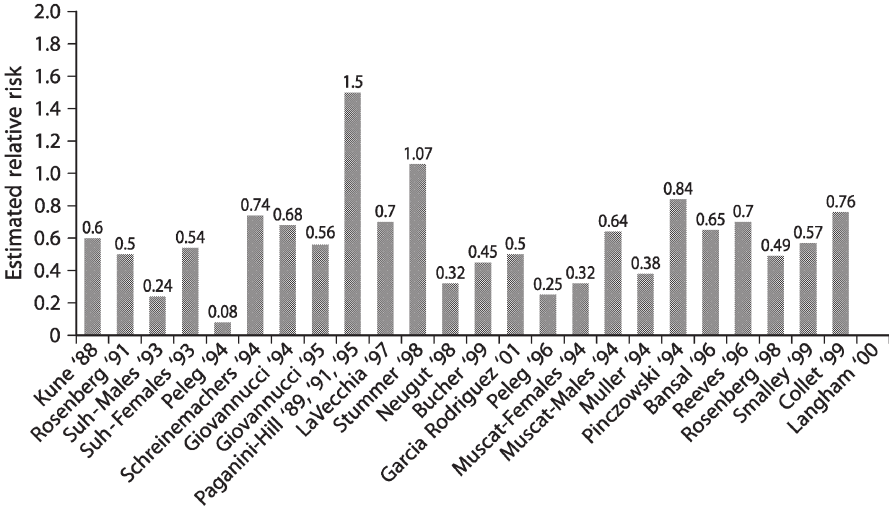

Fig. 2. Cancer incidence (for details on the cited studies see [63, 64])

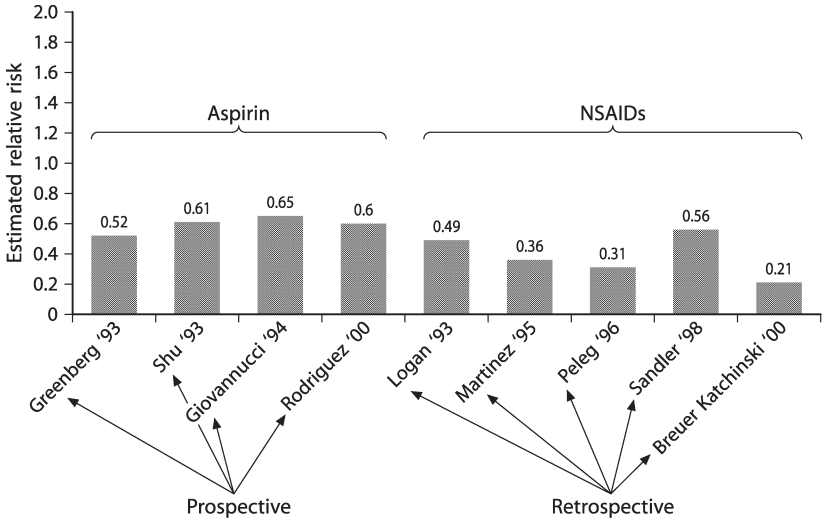

Fig. 3. Polyp/adenoma incidence (for details on the cited studies see [63, 64])

routinely treated with NSAIDs (for review see [27–30]). The protective effect of this class of agents has been shown in 32 out of 34 epidemiological studies (both case-control and cohort), which demonstrated a reduced risk in men and women for cancers of the colon and the rectum. The protective effect depends on the dose of the drug, but of greater importance is the duration of exposure.

6
From Non-specific to Specific COX-2 Inhibition

Unfortunately, the consumption of NSAIDs is not problem-free. The long-term use of NSAIDs is limited due to the high incidence of side-effects and the significant cost (of both the drugs and the treatment of their side-effects). The chronic use of aspirin, sulindac and other older NSAIDs can cause serious life-threatening gastrointestinal complications. Chronic intake of NSAIDs is associated with a high prevalence of gastroduodenal ulceration (in up to 20% of users) and with an estimated two- to fivefold increase in the relative risk for ulcer complications and mortality [31–33]. In 1997, in the U.S. alone, there were 107,000 hospitalisations and 16,500 deaths due to NSAID consumption, equalling the mortality from AIDS or leukaemia [34]. Therefore, although chemoprevention of CRC is already possible, it is essential that drugs that have more acceptable side-effect profiles than the currently available NSAIDs be developed (Table 3).

Table 3. Strategies for improving the therapeutic index of NSAIDs

Reduction of systemic exposure (dose titration, topical administration)
Combination with GI-protective drugs (PPI, H_2 receptor antagonists, misoprostol)
NSAID derivatives (NO-NSAIDs)
Selective COX-2 inhibitors
Combination therapy

COX, cyclo-oxygenase; GI, gastrointestinal; NSAID, non-steroidal anti-inflammatory drug; PPI, proton pump inhibitors.

There are at least two isoforms of the COX enzyme. COX-1 is found in the normal gastrointestinal mucosa and serves as a housekeeping protein. COX-1 is expressed constitutively and is required for physiological processes such as gastrointestinal mucosa maintenance and platelet aggregation. COX-2, on the other hand, is usually undetectable in the normal gastrointestinal mucosa. Its expression can be induced by inflammatory and neoplastic stimuli in endothelial cells, macrophages and intestinal epithelial cells [35, 36].

To diminish the side-effects of NSAIDs, while retaining their potency, a new class of NSAIDs was developed: the selective COX-2 inhibitors [37].

COX-2-specific inhibitors have an improved safety profile, compared to traditional NSAIDs that inhibit both the COX-1 and COX-2 enzymes. Two coxibs, celecoxib (Celebrex, Pfizer, NY, USA) and rofecoxib (Vioxx, Merck, NJ, USA), have been investigated in large randomised, double-blind controlled studies, CLASS [33] and VIGOR [39] trials to assess their clinical effectiveness and the risk of gastrointestinal toxicity. These studies showed that both coxibs were as effective in the treatment of symptoms of arthritis as non-specific NSAIDs and had significantly fewer gastrointestinal adverse events than conventional NSAIDs.

7
COX-2 Inhibitors Can Prevent CRC

The side-effects associated with the dual COX-inhibitory effect of the older NSAIDs meant that physicians were reluctant to adopt their widespread use for the prevention of CRC. A strong case can be made for COX-2 being an important target for cancer prevention. Two important arguments support the rationale for opting for NSAIDs which specifically inhibit COX-2 activity. First, as previously mentioned, COX-2 is usually found only at neoplastic or inflammatory sites. An increased level of COX-2, but not of COX-1, was detected in 40% of colorectal adenomas and in up to 85% of CRC [36]. Second, COX-1 is constitutively expressed in normal tissue and produces prostaglandins (PGs), maintaining the normal physiological homeostasis. By in-

hibiting COX-1, non-selective NSAIDs abrogate a series of key prostaglandin defence mechanisms, as a result of which the toxicity of non-selective NSAID is expressed in the gastrointestinal and renal mucosa. These side-effects are especially pronounced in the stomach and duodenum.

Reddy et al. [40] showed that celecoxib, a selective COX-2 inhibitor, had chemopreventive activity in the rat aberrant crypt focus model induced by azoxymethane. In a landmark study, Oshima et al. [15] demonstrated that crossing COX-2 knockout mice with *APC* mutant Min-mice resulted in a marked reduction in the number of intestinal adenomas. Both celecoxib and rofecoxib were successfully shown in this model to inhibit polyp number and multiplicity in a dose-dependent manner [41, 42].

One particularly interesting mechanism proposed for the anti-cancer effects of NSAIDs is that COX-2 is required for angiogenesis during the growth of a tumour and that NSAIDs work, in part, by blocking this neovascularization [43]. Using an in vitro co-culture system, researchers have shown that COX-2 can regulate the production of angiogenic factors produced by CRC cells. Inhibition of COX-2 by NSAIDs blocks the production of these factors and inhibits angiogenesis. Although these results do not account for the ability of NSAIDs to cause programmed cell death in vitro, they imply that inhibition of blood-vessel recruitment by NSAIDs may play a part in suppressing tumour growth in vivo.

We recently reported that unlike celecoxib, rofecoxib, up to its maximal concentration of 20 μM, did not inhibit cell growth or induce apoptosis in transformed cells in vitro [44, 45]. On the other hand, in vivo, rofecoxib-treated mice (13 mg/kg, a dose equal to the standard anti-inflammatory dose of 25 mg qd in humans) were significantly more tumour-free and had significantly smaller primary tumours compared to placebo mice. Rofecoxib also prevented tumour formation in the cecum, as well as the formation of liver metastasis. The latency period and the mortality rate were significantly better in the treatment mice compared to the mice receiving the control chow (unpublished data). These findings indicate that the beneficial effect of rofecoxib is more apparent in vivo and therefore there is a great incentive to explore gene expression profiles following treatment with rofecoxib in normal and malignant colonic tissues. A nested case-control study, which used data from a government insurance database on patients 65 years and older who underwent a diagnostic test for colorectal neoplasia, examined the effect of rofecoxib, celecoxib, aspirin and acetaminophen on colorectal neoplasia. Rofecoxib, celecoxib and NSAIDs were all protective against adenomas and CRC, but not acetaminophen [46].

International multi-centre trials are currently underway to evaluate the efficacy of celecoxib and rofecoxib in the secondary prevention of colorectal polyps. Each study recruited between 1,500 and 2,500 patients from over 100 sites. The primary endpoint in these studies is the number of patients with adenomatous polyps. Celecoxib is being evaluated in two of these studies.

Table 4. COX-2 inhibitors as adjuvant therapy in CRC

Study	Patient population	Treatment	Endpoint
PETACC V ACTION	Stage III	Adjuvant chemo±celecoxib×3 years	Disease-free and overall survival
VICTOR	Stage II or III	Adjuvant chemo+rofecoxib×2 years Placebo×2 years Rofecoxib×5 years Placebo×5 years	Survival
NSABP (Colon Polyp Prevention Trial)	Dukes' A or B_1	Celecoxib×3 years Placebo×3 years	Occurrence of new polyps

The NCI study is comparing two doses of celecoxib, 100 and 400 mg b.i.d., with placebo. In a second study, sponsored by Pfizer, 400 mg of celecoxib qd is being compared to placebo. In a third study, run by Merck, rofecoxib 25 mg qd is being evaluated. The results of these important studies will be available in the summer of 2005.

International multi-centre trials are also currently underway to evaluate the efficacy of celecoxib in the secondary prevention of other pre-malignant lesions such as Barrett's oesophagus, actinic keratosis, superficial bladder cancer and oral leukoplakia.

Finally, COX-2 inhibitors and celecoxib in particular are currently being evaluated as an adjunct therapy in more then 100 clinical trials. The three trials most important in CRC are summarised in Table 4. The outcome of patients with stage II and III CRC will be investigated in two phase III trials evaluating COX-2 inhibitors as adjuvant therapy. The first is the Pan-Europena Trials in Adjuvant Colon Cancer (PETACC) ACTION (Adjuvant Celecoxib Therapy in Oncology) which will evaluate 1,450 patients with stage III CRC. In this trial celecoxib will be administered simultaneously with adjuvant chemotherapy and during 3 years of follow-up. In addition to disease-free and overall survival, the ACTION trial will evaluate the interaction of adjuvant chemotherapy and COX-2 inhibition during simultaneous administration which may contribute to an anti-tumour effect in micrometastatic disease. The VICTOR (Vioxx in Colorectal Cancer Therapy: Definition of Optimal Regimen) trial will randomise 7,000 stage II and III CRC patients to receive Vioxx for either 2 or 5 years following completion of adjuvant chemotherapy. Again, the endpoint is survival. The National Surgical Adjuvant Breast Program (NSABP) is in the planning phases of a trial to evaluate the efficacy and safety of celecoxib in the prevention of adenomatous polyps in patients with previously resected Dukes' A or B1 colon cancer. Patients will receive celecoxib 400 mg b.i.d. or placebo for 3 years.

8
How Do NSAIDs Exert Their Chemopreventive Effects?

A growing body of work suggests that NSAIDs exert their effects through the modulation of the pathway for programmed cell death (apoptosis) in colon cells. After treatment with NSAIDs, CRC cells contract (chromatin condenses and nuclei fragment) and develop membrane blebs—all markers of apoptotic activation. These effects can be inhibited by drugs that block gene expression, suggesting that the cell death induced by NSAID treatment is a bona fide programmed cell death (a process that requires gene expression), and not necrotic cell death (caused by the general toxic effects of the drugs) [47].

Apoptosis is an important mechanism of colonocyte loss during crypt maturation [48], and during colonic carcinogenesis it is progressively inhibited [49]. Apoptosis is suppressed in sporadic adenomas, carcinomas of the colon and in the flat, rectal mucosa of FAP patients [48, 49]. Treating FAP patients with sulindac restored the frequency of apoptotic activation to normal and reduced the size and number of colorectal adenomas. Similarly, treatment of Min mice with sulindac restored the proportion of cells that underwent apoptosis [50]. Thus, NSAIDs may exert their chemopreventive effects by restoring a normal frequency of apoptosis in the colonic mucosa.

A substantial body of evidence now supports the idea that the induction of programmed cell death is one of the mechanisms underlying NSAIDs' cancer prevention. However, whether the induction of apoptosis is the primary method by which NSAIDs exert their anti-cancer effects, or whether other modes of action are important as well, remains to be seen. To date, physiological concentrations of NSAIDs in the enterohepatic circulation and in the mesenteric vasculature have not been well characterised. Moreover, studies using cultured cells are often done using higher NSAID concentrations than those that can be achieved in vivo. Therefore, conclusions drawn from these experiments may not properly correlate with the responses of the organism as a whole. Further experiments, which compare the in vivo and in vitro effects of NSAIDs at similar concentrations, need to be done to better characterise the molecular events that occur in the colon after treatment. For example, it would be useful to confirm that increased apoptosis occurs when tumours shrink in response to NSAID treatment. This could be done by treating Min mice with an NSAID, taking biopsy samples from their adenomas and comparing before-treatment results with those from several time points after treatment.

9
Mechanisms of NSAID-Mediated Apoptosis

9.1
COX-2-Dependent Mechanisms

One of the primary pharmacological properties of NSAIDs is their ability to inhibit the COX enzymes. Both COX-1 and COX-2 are involved in the pathway by which arachidonic acid molecules are converted into eicosanoids. The difference between the two COX isoforms lies in their distribution in the body and physiological function. COX-2 is the isoform most likely to be important in the pathogenesis of CRC. In fact, COX-2 deregulation occurs in all stages of the multi-step progression of CRC, from the first genetically altered cell, through hyperplasia, dysplasia, carcinoma and even metastasis formation.

Analysis of COX expression shows that COX-2 is increased in up to 90% of sporadic colon carcinomas and 40% of adenomas, but not in normal colonic mucosa [36]. In the adenomas of FAP patients, and in rats with experimentally induced colon tumours, higher than normal concentrations of COX-2, prostaglandins or both are seen [51–53]. These findings support the idea that the overexpression of COX-2 is important during CRC carcinogenesis. Moreover, the size of the tumours is directly related to an increase in the concentration of COX-2 [54]. Several studies have now provided strong evidence for the theory that NSAIDs cause apoptosis in CRC cells by blocking COX-2 activity. Tsujii and Dubois [55] found that rat intestinal epithelial cells, modified to increase expression of COX-2, were resistant to apoptosis. All of these changes, which suggest increased tumourigenic potential, support the notion that COX-2 over-expression alters the biology of intestinal cells and may play a major role in their transformation.

The COX-2-specific NSAIDs are structurally distinct from traditional NSAIDs, and were developed specifically for their ability to inhibit COX-2. The fact that these new COX-2 inhibitors also prevent CRC and induce apoptosis provides further mechanical evidence. Together, these findings support the involvement of COX-2 inhibition in NSAID-mediated apoptosis.

Direct genetic evidence for the role of COX-2 in colon cancer was provided by Oshima et al. [15] in a landmark study, using APC^{716} knockout mice. These mice harbour a truncation mutation in the *APC* gene, and develop hundreds of polyps in their intestinal tracts. Compared with control mice, the number of intestinal polyps was reduced by 34% when one COX-2 allele was knocked out, and there was an 86% reduction in polyp count when both alleles were deleted. The reduction in polyp number was accompanied by a reduction in average polyp size. This observation unambiguously demonstrates that COX-2 is involved in the process of tumourigenesis and that the inhibition of this enzyme can prevent CRC. However, Oshima and colleagues

noted that in mice, COX-2 is primarily expressed in the stroma of tumours, whereas in human CRC COX-2 is over-expressed in epithelial cells [56] or in both epithelial and interstitial cells. Thus, COX-2 may act by both paracrine and autocrine routes.

9.2 COX-2-Independent Mechanisms

Several recent observations cast doubt on the idea that COX is the sole target of NSAID action in the colon. For example, NSAID derivatives such as sulindac sulfone, which lack the ability to inhibit COX, can inhibit colon tumour growth [57]. Additionally, it appears that some NSAIDs can inhibit proliferation and induce cell death in cells that do not express COX-2 [58]. These findings suggest that other targets of NSAIDs that are common to some neoplastic cells may play a part. One potential mechanism involves the transcription factor NF-κB, which promotes cell survival and enhances proliferation. NSAIDs may promote apoptosis by inhibiting the activity of nuclear factor (NF)-κB [59]. This may occur by blocking the release of IκB from NF-κB, leading to a failure in NF-κB activation and the transcription of genes required for cancer cell growth and survival.

Another potential COX-independent mechanism of NSAID-mediated apoptosis involves the peroxisome-proliferator-activated receptor δ (PPARδ), a growth-promoting protein. PPARδ is over-expressed in CRCs [60] making the cells partially protected from NSAID-induced apoptosis. He and colleagues [61] reported that NSAIDs can bind to and inhibit PPARδ and cause it to dissociate from DNA. As a result, the cell is left unable to transcribe survival genes and they become apoptotic. It is particularly interesting that PPARδ is suppressed by *APC* [61].

10 Ready for Routine Use?

Chemoprevention should be used as an adjunct therapy in the very high risk population. Celecoxib should be used in FAP patients, and possibly in the setting of HNPCC. At the same time one must remember that, to date, the standard of care in these individuals is not chemoprevention alone, but surgery followed by meticulous endoscopic surveillance and polypectomy.

We predict that chemoprevention will have an important role in secondary prevention. Although we should wait for the results of the ongoing clinical trials, we predict that chemoprevention will play an important role in the treatment of CRC survivors, patients with advanced colonic neoplasia or in the setting of a very strong family history.

As evidence emerges of the efficacy of chemoprevention in individuals who are at high risk for CRC, it seems appropriate to consider a similar strategy in the general population. In light of the high prevalence of adenomatous polyps, which rises with age, especially after the age of 50, primary prevention should be employed [62]. In particular, aspirin can be effective in those at risk for CRC and cardiovascular disease, or calcium in postmenopausal women with adenomas.

It should be noted that since several environmental, dietary and lifestyle factors have a major influence on the risk of developing CRC, changing or avoiding such adverse factors can also lower the incidence of the disease in the average-risk population.

11 Summary

Inhibiting the growth of pre-cancerous and cancerous cells without affecting normal cells is generally the ultimate aim of cancer treatment, and is of particular importance in chemoprevention studies, which may be long term in nature, involve healthy subjects at the outset and have strict adverse-event requirements.

Cancer prevention is certain to be a significant focus of research and intervention in the coming years, propelled by the realisation that we will be able to identify both individuals susceptible to specific cancers, as well as the molecular targets that can alter or stop the carcinogenesis process.

Science and technology have evolved to a point where we are able to use our knowledge of cancer biology to identify individuals at risk and interrupt the process of malignant transformation at the level of the pre-cancerous lesion. Pharmacology and genetics are collaborating to develop new chemoprevention agents designed to affect molecular targets linked to specific premalignant or predisposing conditions. Recent progress in these fields increases the likelihood that cancer prevention will increasingly rely on chemoprevention. However, the value of such prophylactic strategies has yet to be confirmed in the current ongoing randomised, double-blind, placebo-controlled studies.

One of the most intriguing findings indicates that NSAID chemoprevention may occur through various distinct pathways, involving both COX-2-dependent, as well as COX-2-independent mechanisms. These data have led to the recent development of newer agents—the selective COX-2 inhibitors, which offer the benefits of cancer protection without the major disadvantages of gastrointestinal toxicity associated with the "old" NSAIDs. The presence of multiple potential biochemical targets offers a great potential in the future for possible combinations of potent inhibitors that may act more effectively than either agent alone.

In the intriguing jigsaw puzzle of chemoprevention, we now have a definite positive answer for the basic question "if", but several other parts of the equation (proper patient selection, ultimate drug, optimal dosage and duration) are missing. The most challenging task is to find the proper place for chemoprevention in the entire effort of cancer prevention, in subjects at risk for colorectal neoplasia as well as in those at risk for other tumours. The achievement of this important goal may contribute to the conversion of CRC into a truly preventable disease.

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Screening of Colorectal Cancer: Progress and Problems

Sidney J. Winawer

Memorial Sloan-Kettering Cancer Center, 1275 York Ave., New York, NY 10021, USA

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Abstract Considerable progress has been made in the past three decades in our understanding of the biology and prevention of colorectal cancer. The long natural history of colorectal cancer as it evolves from adenomatous polyps in the majority of cases provides opportunities for detection of early stage cancer and for prevention of cancer by removal of adenomas. Strong evidence of the effectiveness of screening has resulted in a worldwide consensus, as reported in evidence-based guidelines, that screening should be offered to all men and women age 50 and older, younger in the presence of factors that increase risk. Several options are now available for screening, and the emerging technology of stool DNA testing and virtual colonoscopy shows promise. However, many problems remain to be addressed. Screening rates are low. Successful strategies need to be implemented to overcome patient and system barriers. Resources, especially endoscopic capacity, may be inadequate to handle the burden of screening, diagnosis, and follow-up surveillances. There are quality-control issues at every step. Stratification of people by risk, a two-stage screening approach and less intensive surveillance following polypectomy can be helpful. Colorectal cancer screening is cost-effective and could save many lives each year if it were widely implemented.

1 Introduction

Colorectal cancer accounts for approximately 945,000 new cases and 500,000 deaths each year worldwide [1] (to put those numbers in context, see

Table 1. The burden of cancer (both sexes) in the more developed countries. Estimates in 2000 and expected cases in 2020 [1]

	Estimates in 2000			Expected cases in 2020 (*n*)
	Cases (*n*)	Deaths (*n*)	Ratio of cases:deaths	
Large bowel	610,591	301,648	2.02	806,176
Stomach	334,011	229,939	1.45	439,842
Pancreas	127,416	128,730	0.98	168,453
Liver	106,950	105,649	1.01	142,836
Esophagus	71,163	63,938	1.11	94,251
Total	1,250,131	829,904	1.50	1,651,558

Table 2. The burden of cancer (both sexes) in less developed countries. Estimates in 2000 and expected cases in 2020 [1]

	Estimates in 2000			Expected cases in 2020 (*n*)
	Cases (*n*)	Deaths (*n*)	Ratio of cases:deaths	
Stomach	543,026	416,614	1.30	983,414
Liver	457,406	442,916	1.03	801,685
Esophagus	341,163	273,560	1.24	624,574
Large bowel	334,123	190,761	1.75	162,401
Pancreas	88,969	84,734	1.05	162,401
Total	1,764,687	1,408,585	1.25	3,164,220

Tables 1 and 2). Considerable progress has been made in the past 25 years in our ability to reduce this burden, with the introduction of technology for screening, early diagnosis, and prevention by removal of the premalignant polyp [2]. During this period of time, greater insight into the adenoma–carcinoma sequence has provided us with an understanding of the progress made and the problems of screening. The risk for colorectal cancer in average-risk men and women, as well as in groups at increased risk, has also been clarified. Strong evidence has accumulated that screening is effective in reducing the incidence and mortality of colorectal cancer. Although tremendous progress has been made over the last 25 years in these areas, we now face many problems, including the challenging prospects of universal implementation of effective screening methods and integration of new technology and concepts as they develop [2].

2
Progress

2.1
Guidelines Consensus

There was considerable controversy as to whether screening was effective, but beginning in 1996 a consensus evolved reflected in positive guidelines by a number of evidence-based assessments by authoritative bodies including the U.S. Preventative Services Task Force, the Agency for Health Care Policy and Research,the American Cancer Society, World Health Organization, Ontario Expert Panel, Australian Task Force, and the European Screening Group, just to cite a few [2]. The U.S. Preventive Services Task Force upgraded its recommendation to Grade A [3]. The consensus was that all men and women should be screened for colorectal cancer and adenomatous polyps beginning at age 50, or younger in the presence of factors that increase risk.

The majority of patients who will develop colorectal cancer are considered to be at average risk (75%) with no special risk factors. People at increased risk include those with inflammatory bowel disease, which is associated with 1% of the colorectal cancers each year; familial adenomatous polyposis (FAP), 1%; hereditary non-polyposis colorectal cancer (HNPCC), 2%–5%; and those who have a family history consisting of one or two close relatives, 15%–20% [4]. We have a much better understanding today of the magnitude and clinical patterns in these increased risk groups. People having one first degree relative with either a colorectal cancer or an adenomatous polyp at a young age have approximately a twofold increased risk of colorectal cancer, and the risk begins about 10 years younger than in average-risk patients. The risk in close relatives increases inversely with the age in which the proband had an adenomatous polyp or colorectal cancer, the younger the age of the proband the greater the risk in the first-degree relatives [5].

2.2
Fecal Occult Blood Testing

The early evidence of the benefit of colorectal cancer screening with fecal occult blood testing demonstrated a shift in screen detected cancers to an earlier stage with fewer Dukes' D screen-detected cancers as compared to controls [6]. However, this stage shift was quickly challenged as possibly due to length bias and lead time bias. Length bias suggests that screening would pick up cancers that are slow growing more often than cancers that are more aggressive, and lead time bias suggests that screening merely detects the cancers earlier in their natural history, but with no change in their outcome [4]. However, more recently, results of three major randomized control trials

Table 3. Fecal occult blood test (FOBT) screening in randomized controlled trials [7–9]

Trial	Mortality reduction (%)		
	Biennial	Annual	Compliers
Minnesota[a] 47,000/18 years	21	33	45
Denmark 140,000/10 years	18	-	30
UK 153,000/7.8 years	15	-	-

[a] Sensitive slide test used.

demonstrated a mortality reduction in colorectal cancer of the entire screened cohort (Table 3). The largest reduction in mortality was in the University of Minnesota program, in which one group of patients was screened annually with a sensitive slide test. That group demonstrated a 33% reduction in mortality [7]. The compilers with the test demonstrated a 45% reduction in mortality.

The Minnesota Colon Cancer Control Study is a long-term, prospective, randomized, controlled trial of stool blood test efficacy in the detection of colorectal cancer [7]. In this trial, 46,551 participants between 50 and 80 years of age were assigned to either a study group that was offered annual stool blood testing, a second study group that was offered biennial stool blood testing, or a third control group that was not offered screening. The majority (approximately 83%) of stool slides used were rehydrated. The compliance rate for stool slide preparation was approximately 75%. The overall test positivity rate was initially 2.4% with nonrehydrated slides, and it subsequently increased to 9.8% with rehydrated slides. Nonrehydrated slide-test programmatic sensitivity was 80% and specificity was 98%, whereas rehydrated slide-test sensitivity increased to 92% and specificity decreased to 90%. The test's positive predictive value for colorectal adenomas and cancer was 31%. Patients with positive stool blood tests had a diagnostic evaluation with colonoscopy. During a 13-year follow-up period, this trial demonstrated that annual stool blood testing resulted in a significant 33% reduction in colorectal cancer mortality, an improved survival in those individuals diagnosed with colorectal cancer, and a shift towards detection of earlier-stage cancers. A recent update of this trial through year 18 demonstrated a 36% reduction in colorectal cancer mortality with annual stool blood testing [7].

A large United Kingdom population-based, randomized, controlled trial of screening with stool blood testing was performed in asymptomatic individuals between 50 and 74 years of age who were selected from general practitioner lists in the Nottingham area of England [8]. Of the 156,000 participants recruited, 52,258 individuals were randomized to the study group who were offered biennial screening with nonrehydrated stool blood testing. The initial compliance rate for stool slide preparation was 53%. The overall test

positivity rate was 2.3%. The slide-test sensitivity was 72% and specificity was 98%. The test's positive predictive value for colorectal adenomas and cancer was 53%. Patients with positive stool blood tests had a diagnostic evaluation with colonoscopy. An increased number of earlier-stage (Dukes' A or B) cancers were detected in the study group (90% versus 40%) as compared to the control group. The trial demonstrated a 15% reduction in colorectal cancer mortality, with a median follow-up of 7.8 years (odds ratio, 0.85) [8].

A large Danish population-based, randomized, controlled trial of stool blood test screening randomized 62,000 participants between 45 and 74 years of age to either biennial nonhydrated stool testing or a control group [9]. The initial compliance rate for stool slide preparation was 67%. The overall test positivity rate was 1.0%. Patients with positive stool blood tests had a diagnostic evaluation with colonoscopy. This trial, now with 10 years of follow-up data, reported an 18% reduction in colorectal cancer mortality, including deaths from complications related to colorectal cancer treatment, in the screened group (mortality ratio, 0.82). All of the trials in the United States and Europe are consistent with one another in terms of the magnitude of the mortality reduction as a function of the type of slide test used and the frequency of screening. This put to rest questions of screening bias, and for the first time convincingly demonstrated that screening for colorectal cancer with fecal occult blood testing is effective.

2.3 Sigmoidoscopy

Colorectal cancer mortality was also shown to be reduced by sigmoidoscopy in two-case control studies. A retrospective, case-control study from the Kaiser Permanente group has provided strong evidence that sigmoidoscopy can significantly reduce rectosigmoid cancer mortality [10]. This study compared the use of screening rigid sigmoidoscopy during the 10-year period prior to diagnosis in 261 individuals who died of rectosigmoid cancer with 868 matched controls. The results showed that only 8.8% of case patients had a screening sigmoidoscopy, as compared to 24.2% of the controls, with an adjusted odds ratio of 0.41, and that this negative association remained as strong with screening intervals from prior sigmoidoscopy to cancer diagnosis of as long as 9–10 years. This study demonstrated a 60% reduction in rectosigmoid cancer mortality.

A second smaller case-control study of screening sigmoidoscopy from the University of Wisconsin [11], which compared the records of 66 individuals of the Greater Marshfield Community Health Plan who died of colorectal cancer with 196 matched controls, found that case patients were less likely to have had a screening sigmoidoscopy than were controls (10% versus

30%). This study also demonstrated that a single screening flexible sigmoidoscopy resulted in a reduction in colorectal cancer mortality.

In 1993 the National Cancer Institute initiated a large, multicenter, long-term, randomized, controlled screening trial (PLCO trial: prostate, lung, colorectal, ovary) of 148,000 individuals that includes flexible sigmoidoscopy (60-cm sigmoidoscope) for colorectal cancer screening [12]. A second large, multicenter clinical trial to assess the efficacy of screening flexible sigmoidoscopy has been organized in England [13]. No long-term trials utilizing flexible sigmoidoscopy have yet reported incidence or mortality data. The combination of fecal occult blood testing added to sigmoidoscopy has also been associated with a mortality reduction in a study conducted in the U.S. [14]. As a result of these studies, colorectal cancer screening guidelines recommend that average-risk men and women age 50 or older (or age$\geq$40 with a family history) be offered options for screening which include either fecal occult blood testing annually or flexible sigmoidoscopy every 5 years, or a combination of the two [2, 3, 4].

2.4 Screening Colonoscopy

Although no studies in average-risk individuals have evaluated whether screening colonoscopy alone can reduce colorectal cancer mortality or incidence, some evidence suggests that this examination should be considered for colorectal cancer screening in this population. In comparison to fecal occult blood testing, which has a low sensitivity for the detection of adenomas, and to sigmoidoscopy, which examines at most only the distal third of the large bowel, colonoscopy (1) is very sensitive for the detection of cancer and both small and large adenomas, (2) completely examines the entire colon and rectum, and (3) provides the opportunity for the endoscopic removal of adenomas and biopsy of suspicious mass lesions.

There have been two major studies that have reported results of screening colonoscopy, one a U.S. Veterans Affairs Cooperative study [15], and another conducted in employees of Eli-Lilly. The total number of patients examined in both studies was more than 5,000, and the studies were similar in outcome, demonstrating that about 10% of asymptomatic patients over the age of 50, both men and women, have either a cancer or an advanced adenoma [15, 16].

2.5 Adenomas

The most frequent outcome of screening is not cancer, but an adenomatous polyp [17]. These polyps can be removed quite expeditiously by colonoscopy as an outpatient procedure—with the entire colon being examined—in

15–20 min. This was a major advance compared to the exploratory laparotomy and multiple colostomies that were done in the past. The feasibility of removing polyps through the colonoscope was reported in the mid-1970s [18], and shortly thereafter the National Polyp Study was organized as a randomized, multicenter, controlled trial to examine surveillance intervals and methods after polypectomy, to examine the potential incidence reduction of colorectal cancer following polypectomy, and to study the adenoma–carcinoma natural history and biology [19]. The National Polyp Study demonstrated that although many polyps were found at follow-up colonoscopy, the initial colonoscopy was successful in clearing out the colon of advanced adenomas [20]. Advanced adenomas are defined as those 1 cm in size or larger or with high-grade dysplasia or invasive cancer, and some investigators also include in the definition a high degree of villous component [21]. In the National Polyp Study, it was demonstrated that at a 3-year follow-up examination, only 3% of the patients had advanced adenomas after they had their colons cleared of all polyps at baseline. An examination at 1 year before the 3-year examination provided no additional benefit. This resulted in guidelines that now recommend omitting the 1-year examination after polypectomy and going to a 3-year examination for the first follow-up [2, 3, 4].

It was long believed that the adenoma was the precursor of colorectal cancer and its removal would result in the prevention of colorectal cancer [18]. This belief was substantiated by data from the National Polyp Study, which demonstrated a reduction (70%–90%) in observed cancers as compared to expected cancers in the National Polyp Study cohort following the baseline clearing colonoscopy [22]. This was later confirmed in an Italian multicenter study (66% incidence reduction) [23] (Table 4). The prevention of colorectal cancer by polypectomy is one of the best-kept secrets from the public. It is one of the most powerful prevention strategies that is available, and yet it is not widely known. In the National Polyp Study, it was further demonstrated that one could stratify patients into those at high risk for advanced adenomas in the future as compared to those at low risk for advanced adenomas. The low risk patients constituted 70% of the cohort and could have their first follow-up colonoscopy years after their clearing colonoscopy rather than at 3 years. It is reasonable to do the first follow-up colonoscopy in these patients at 5 years and reserve the three-year follow-up colonoscopy for only high-risk patients. This high-risk group is defined as those who have multiple adenomas at baseline or have a positive family history and are at age

Table 4. Effect of colonoscopic polypectomy on incidence of colorectal cancer [22, 23]

Study	↓ Incidence (%)
U.S. National Polyp Study	76–90
Italian Multicenter Study Group	66

over 60 at their first polypectomy. This stratification would reduce complications and costs, and conserve resources, which could be better directed towards the initial screening of patients [2].

3 Problems

3.1 Screening Rates

Screening rates for colorectal cancer are low and well below those for mammography. National Health Interview Surveys have consistently demonstrated this fact. In the U.S., only 20% of people over age 50 have had fecal occult blood test, and one out of three has had an endoscopic screening test in the previous 5 years [24]. One of the most frequent reasons for not getting screened (88%), is that "it was not recommended by my doctor," according to one survey [18]. The health care provider plays a very important role in motivating patients to be screened. Health care providers may be more motivated today because of the litigation that is now occurring in colorectal cancer with the most frequent reasons for litigation being failure to screen, failure to diagnose, and deviation from standard of care. Standard of care now is based on published guidelines rather than practice in the community. These goals have been enhanced recently by U.S. Congress legislation, which in the year 2000 provided Medicare reimbursement for fecal occult blood testing, flexible sigmoidoscopy, and, beginning in July 2001, Medicare reimbursement for screening colonoscopy. This legislation was based on the demonstration in recent years by many cost-effectiveness models, that colorectal cancer screening is cost-effective; costing less that US $20,000 per life-year saved, and is equivalent to screening mammography. Colorectal cancer screening has further been enhanced by the designation of March as National Colorectal Cancer Awareness Month, by former U.S. President Bill Clinton [18].

3.2 Implementation

Universal implementation of screening is a major challenge. In the U.S. the target population is 70 million men and women age 50 and over. The question is whether there are resources to accomplish screening in such a large population. There are several alternatives that can be considered. Direct population screening colonoscopy is one strategy. We can identify those people who have adenomas, who can then be further stratified into low and

high risk for subsequent advanced adenomas, with less intense or more intense follow-up surveillance, respectively.

Another approach is to try to identify those individuals who would be most likely to benefit from screening. One such approach is through genetic testing. However, genetic testing can identify only 5%–6% of those people destined to get colorectal cancer, and these individuals must first be identified by their family history of either FAP or HNPCC [25]. New genetic mutations in colorectal cancer have been demonstrated in the general population; two in Ashkenazi Jews and one in non-Jewish people. The genetic mutations in Ashkenazi Jews have a very low penetrance, and by themselves do not confer a striking increase in risk. They probably require additional polymorphisms to substantially increase the risk for colorectal cancer in those individuals [25]. There has been recent interest in studying genetic mutations in the stool, recent reports demonstrating that a panel of DNA mutations in stool could result in the detection of cancers and adenomas with a sensitivity of 50%–60% and specificity of over 90% [26–29]. The best stool marker that we have right now is the fecal occult blood test, which can reduce mortality if done on a regular basis annually. This can be done with either the guaiac-based tests or by an immunochemical test (Table 5) [30, 31]. In the two-stage strategy, screening sigmoidoscopy, a stool marker, or virtual colonoscopy would be used first. Only those individuals having a positive significant lesion found would be referred for fiberoptic colonoscopy with biopsy or polypectomy. The accuracy of virtual colonoscopy has not yet been well established, and it is not totally non-invasive [32, 33]. The sensitivity has ranged from 1/3 to over 90% for polyps over 1 cm in size. Patients require a preparation for 1 day before the examination, and have air instilled in the bowel. This is, however, promising and needs further study. There have been many advances recently in diagnostic endoscopy with magnification lenses, dye staining, spectroscopy, and optical coherence tomography, which would greatly enhance our ability to make tissue diagnosis more accurately and perhaps separate those patients endoscopically who do not need biopsy or polypectomy, especially in the case of very small polyps [18].

Table 5. Choice of fecal occult blood test (FOBT) [30]

Colonoscopy resources	Population compliance with diet and drug restrictions for guaiac test	Suggested FOBT
Limited	Reliable	High specificity guaiac test (e.g., Hemoccult)
Limited	Unreliable or uncertain	Immunochemical test
Readily available	Reliable	Sensitive guaiac test (e.g., Hemoccult Sensa)
Readily available	Unreliable or uncertain	Immunochemical test

The resource issue is critical. Endoscopy centers are being overwhelmed by the number of referred colonoscopies. These include screening colonoscopy, diagnostic colonoscopies in patients with a positive screening test, and colonoscopy in symptomatic patients. Endoscopic resources could be freed up for screening and diagnosis if the post-polypectomy surveillance interval could have reduced intensity. Patients can be stratified after initial polypectomy into those who are at low or high risk for advanced adenomas later. Those at low risk could have their first post-polypectomy examination deferred for 5 years rather than 3 years. Guidelines have been modified recently to make this change in recommendations [2].

3.3 Barriers

Many barriers exist that prevent widespread implementation of screening, including financial concerns, patient inconvenience, and patient perceptions of benefit, risk, and discomfort [34, 35]. For screening programs to be successful, a cascade of events must be negotiated from beginning to end [2]. Physicians must remember to offer screening, patients must accept this advice, insurers must pay for screening and follow-up testing, and patient care organizations must have systems to track whether screening has taken place and provide reminders if it has not. Screening examinations must be feasible for providers, which is a special problem for sigmoidoscopy, and the workforce to do examinations well must be in place, a problem for colonoscopy. If any one stage in this sequence is faulty, the screening program will fail. Therefore, those who care about effective screening programs must be concerned with all of these elements of success.

The number of places where breakdowns can occur is large. Some patients may not understand or carry out bowel preparation instructions. Providers must be able to perform tests correctly. Office staff, aided by information systems, must remember when screening tests are due and patients must accept part of this responsibility because they commonly change providers (because their health plan changes) or move out of the area, leaving new doctors unable to determine when 5–10 years have passed since the last endoscopy. Also, shared decision-making can be difficult to implement. Not all patients want to share decisions and many prefer doctors to make a recommendation. Physicians may lack time, skill, and resources to carry out shared decision-making correctly, and patients may not be able to digest the information and information presented. Many forms of patient information about colorectal cancer are available [2].

4 Concluding Remarks

Cost-effectiveness analyses have shown that the cost per year of life saved by screening with any of the tests recommended is reasonable by U.S. standards [2, 36–40]. Although the specific results vary among analyses, in general the marginal cost-effectiveness of this screening is less than US $25,000 per year of life saved. Screening for colorectal cancer was among the highest ranked services in an analysis of the value of preventive services based on the burden of disease prevented and cost effectiveness.

Though the up-front costs vary by screening modality, the long-term cost-effectiveness is similar across screening tests, so that decisions about which options to include, in the long run and from the perspective of society, do not need to be heavily affected by costs. Costs increase out of proportion to benefits with shorter intervals between screening examinations.

Screening has provided great opportunities. Screening can prevent colorectal cancer by polypectomy and find early-stage cancers for treatment with less morbidity. Screening can reduce the burden of treating advanced cancers, and can identify families at increased risk. Screening has also provided a better understanding of the biology of colorectal cancer [41].

Screening for colorectal cancer should be part of a complete prevention program, which includes a healthy lifestyle and familial risk assessment. Those individuals with increased familial risk require special screening approaches while those at average risk can have more standard screening. The average-risk individuals can be further stratified into those that require intensive follow-up and those who require less intensive or no follow-up at all. We are beginning to learn how to apply screening and surveillance approaches based on risk stratification for a more cost-effective approach in order to conserve resources and reduce complications and costs. Chemoprevention can be added to the program when substantial benefit of agents has been demonstrated. We now have a better understanding of the biology of colorectal cancer and the technology to intervene in that biology to make a difference in the lives of many people. We have the concepts and technology today to substantially reduce the mortality for colorectal cancer and even entirely prevent it [41].

Newer screening tests, or others yet to be developed, may with time replace the present options. Nevertheless, screening should take place with the tests available now and not wait until something better comes along. In this way, needless suffering and loss of life can be avoided for this leading cause of cancer death. Screening may become even more successful if the promise of new technologies is confirmed and they enter clinical practice. In the last analysis, the best test is the one that gets done, and gets done now.

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The Role of Endogenous Hormones in the Etiology and Prevention of Breast Cancer: The Epidemiological Evidence

Paola Muti

Department of Social and Preventive Medicine, University at Buffalo,
State University of New York, 270 Farber Hall, 3435 Main Street, Buffalo, NY 14214, USA
muti@buffalo.edu

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Abstract Breast cancer is the most common cause of cancer death in women worldwide. Rates vary about fivefold around the world, but they are increasing in regions that until recently had low rates of disease. Despite the numerous uncertainties surrounding the etiology of breast cancer, intensive epidemiological, clinical, and genetic studies have identified a number of biological and social traits as risk factors associated with breast cancer. Principal among them is the evidence of BRCA1 and BRCA2 susceptibility genes, familial history of breast cancer, age, higher socioeconomic status, ionizing radiation, tallness in adult life, alcohol consumption, and a variety of hormone and metabolic factors. Among the hormonal influences, a relevant etiological function has been ascribed to unopposed exposure to elevated levels of estrogens and androgens. In addition, new epidemiologic evidence has indicated that among the metabolic factors, glucose metabolism, hyperinsulinemic insulin resistance, and insulin-like growth factor bioavailability may also play a role in breast cancer. These endocrine and metabolic factors may represent future targets for breast cancer prevention.

Breast cancer is the most common cause of cancer death in women worldwide. Rates vary about fivefold around the world, but they are increasing in regions that until recently had low rates of disease [1–3]. Despite the numerous uncertainties surrounding the etiology of breast cancer, intensive epidemiological, clinical, and genetic studies have identified a number of biological and social traits as risk factors associated with breast cancer. Principal among them is the evidence of BRCA1 and BRCA2 susceptibility genes,

familial history of breast cancer, age, higher socioeconomic status, ionizing radiation, tallness in adult life, alcohol consumption, and a variety of hormone and metabolic factors [4, 5]. Among the hormonal influences, a relevant etiological function has been ascribed to unopposed exposure to elevated levels of estrogens and androgens [4–7]. In addition, new epidemiological evidence has indicated that among the metabolic factors, glucose metabolism, hyperinsulinemic insulin resistance, and insulin-like growth factor bioavailability may also play a role in breast cancer. These endocrine and metabolic factors may represent future targets for breast cancer prevention.

1 Introduction

In 1896, Beatson was the first to hypothesize the influence of ovarian activity on formation and progression of breast cancer [8]. At that time, these hormones were not known to be a unique class of substances. The first experimental proof of their presence in follicular liquids of a premenopausal ovary and their cancer promotion potential was shown more than 30 years later by Lacassagne [9]. In vitro and in vivo studies using natural, synthetic, or both kinds of sex steroid hormones demonstrated their potential in the formation and progression of benign and malignant tumors [10–11].

Epidemiological evidence of an association between sex steroid hormones and breast cancer risk based on retrospective study design, such as case-control studies, has been generally inconsistent. When the results were consistent across a few independent studies and supportive of the association of hormones and breast cancer, the findings were still compatible with the noncausal hypothesis that high hormone levels in breast cancer cases were due entirely or in part by the presence of the tumors or as consequence of the disease. Because of the disease-status effect on the endocrine or metabolic profile, this report describes only evidence from prospective cohort studies.

2 Sex Hormones and Breast Cancer in Postmenopausal Women

The hypothesis that cumulative exposure of breast tissue to ovarian hormones is one of the major determinants of breast cancer has existed for at least 30 years. Epidemiological evidence has been well corroborated the existence of the association in postmenopausal women. During the last 10 years, nine research groups have published results from prospective studies of endogenous hormones and breast cancer: Columbia, MO, USA [13, 14]; Guernsey, UK [15]; Nurses' Health Study, USA [16]; New York University Women's Health Study (NYU WHS), USA [17, 18]; Study of Hormones and Diet in the

Etiology of Breast Tumors (ORDET), Italy [19]; Rancho Bernardo, USA [20, 21]; Radiation Effects Research Foundation (RERF), Japan [22]; Study of Osteoporotic Fractures (SOF), USA [23]; and Washington County, USA [24, 25]. These studies, based on recruitment of thousands of healthy women and on their epidemiological surveillance, have indicated that high levels of estrogens and androgens precede the occurrence of breast cancer risk in postmenopausal women.

A recent pooled analysis of these nine large prospective cohort studies has then further supported the role of endogenous hormones in the etiology of breast cancer [26].

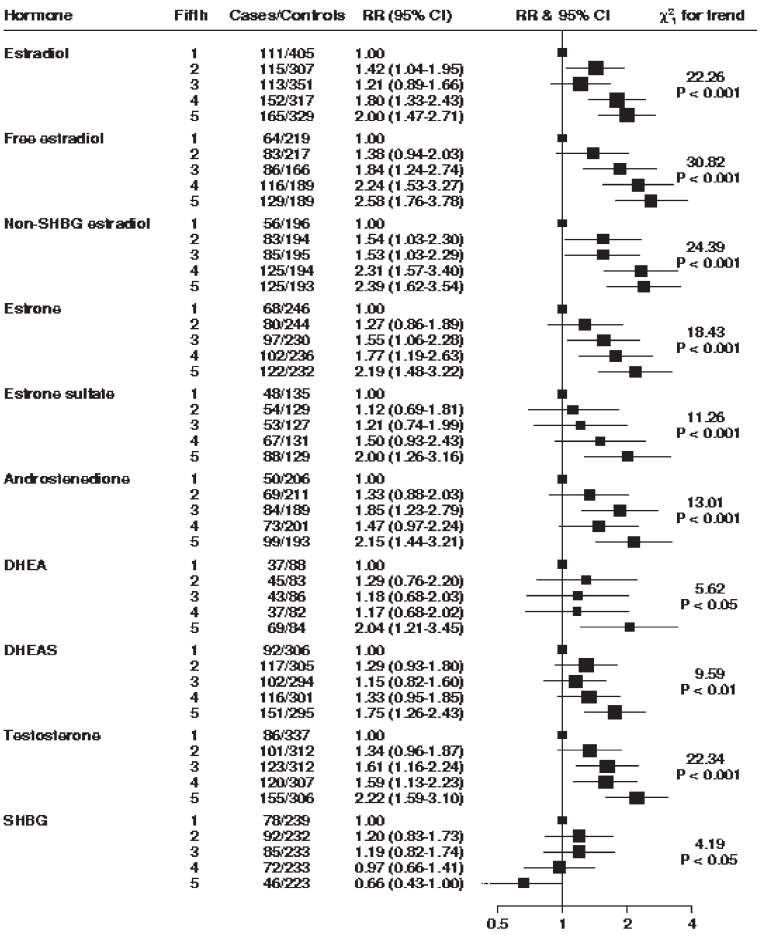

Fig. 1. Relative risk (*RR*) of breast cancer by fifth of hormone concentration. *CI* confidence interval; *DHEA* dehydroepiandrosterone; *DHEAS* dehydroepiandrosterone sulfate; *SHBG* sex hormone binding globulin. (From [26])

As reported in Fig. 1, in the pooled analysis of the prospective studies examining risk by quintiles of hormone serum concentration, both estrogens and androgens were significantly associated with an increase in breast cancer risk, with evidence of a dose-response relationship. The relative risk for breast cancer for women in the highest quintile for estradiol compared with women in the lowest quintile was 2.00 (95% confidence interval 1.47–2.71). The relative risks in the highest quintile compared with the lowest quintile for the other estrogens and the androgens were all approximately 2, and the highest relative risks were in the highest quintiles for free estradiol [relative risk 2.58 (1.76–3.78)] and non-sex hormone binding globulin (non-SHBG)-bound estradiol [relative risk 2.39 (1.62–3.54)]. For SHBG there was a significant inverse association with breast cancer risk [relative risk in top fifth 0.66 (0.43–1.00)].

Although the postmenopausal ovaries secrete a very small amount of estrogens, circulating estrogens in women after menopause are still produced through peripheral aromatization of the androgens, primarily androstenedione and testosterone. Thus, part of the etiological relation linking serum androgens to breast cancer could be explained by their aromatization into estrogens. In the pooled analysis, we separated by adjustment and stratification the effect of androgens on breast cancer risk from the effect of estrogens. We observed that the association between androgens and breast cancer held after adjustment for estrogens, indicating an independent effect of androgens on breast cancer risk.

Thus, results of this pooled analysis of the worldwide data from prospective studies has established not only that serum concentrations of endogenous sex hormones are precursors of breast cancer in postmenopausal women, but also that both estrogens and androgens are independently associated with the development of the disease through two possible independent pathways. While circulating estrogens may act directly on the breast tissue and breast cancer cells, the action of serum androgens may be mediated through their aromatization into estrogens within breast tissue and in breast cancer cells [27].

3 Sex Hormones and Breast Cancer in Premenopausal Women

The normal human ovaries produce all three classes of sex steroids: estrogens, progesterone, and androgens and all three have been considered in analytical studies on breast cancer etiology in premenopausal women.

Among the hormonal influences, a major role has been attributed to the unopposed exposure to elevated levels of estrogens. Various analytical studies on estrogens and breast cancer risk led to contradictory results irrespective of the type of estrogens they were analyzing [28]. Estradiol is by far the

most potent and the highest concentrated naturally occurring estrogen in premenopausal women. Thus, epidemiological studies conducted in premenopausal women have usually focused on estradiol in their analysis. Prospective studies with information from premenopausal women reported higher follicular but lower luteal estradiol in premenopausal women who subsequently developed breast cancer than in a sample of cohort members of the Washington County prospective study chosen as controls [25]. The opposite was previously found by Wysowski et al. [29], in the same cohort study. Rosenberg et al. [30] reported, in a case-control study nested in the New York University Women's Health Study, similar estradiol levels in cases and controls (although further adjustments for stage of menstrual cycle at blood drawing suggested that estradiol was on average non-significantly higher in cases). Kabuto et al. [22] found in the prospective cohort study conducted in Japan higher levels of bioavailable estradiol in breast cancer cases than in controls. Results from the prospective study conducted in the island of Guernsey (UK) by Key and colleagues showed that premenopausal breast cancer cases excreted less estrogen than controls when estrogens were determined in urine [31] and that estrogen levels were higher in cases than in controls when the hormones were determined in blood, although the difference was small and not statistically significant [32]. The number of breast cancer cases in those studies ranged between 22 [25] and 79 [30]. Several of those studies tried to control the ovarian phase variability using time interval between the date at specimen collection and the date at the subsequent menstrual period either as a matching variable or variable to adjust for in the analysis [30–32]. On the contrary, Wysowski et al. [29] and Helzlsouer et al. [25] used the time interval between date at the menstrual period preceding the blood collection and the date at blood drawing, while Kabuto et al. [22] did not control for menstrual phase. Only Helzlsouer et al. [25] controlled for hormone circadian rhythm, matching the set of cases and controls on time-of-the-day at blood drawing, although no specification on this matching criterion was given. All determinations performed in blood used radioimmunoassay methods, although none specified whether the determinations were done using direct or indirect methods and single or duplicate assays. This information may have influenced the technical variability of the hormone determination and thus the precision of the observed risk estimates.

Almost all prospective studies analyzing the relation of breast cancer with endogenous androgens in premenopausal women showed a positive association of testosterone levels with risk, with the only exception of Wysowski et al.[29] who did not find a difference in testosterone levels between breast cancer cases and controls in his nested case-control study. However, all observed risks were of low magnitude and not statistically significant [22, 25, 32].

During the menstrual cycle, progesterone, in conjunction with estrogens, regulates the functions of the sex organs. This hormone is important in preparing the uterus for implantation of the blastocyst and in maintaining pregnancy. In nonpregnant women, progesterone is secreted mainly during the luteal phase of the ovarian cycle by the corpus luteum, a yellow glandular mass in the ovary formed by an ovarian follicle following the discharge of its ovum.

Only a few prospective cohort studies have reported the association of luteal phase progesterone levels with subsequent breast cancer, but the number of cases was very small. Thomas et al. [32] reported a 9% lower mean serum concentration of progesterone, measured in early luteal phase, in cases than in controls (the study was based on 12 breast cancer cases). Wysowski et al. [29] found a 29% lower mean concentration of progesterone in cases than in control subjects after matching on time since last menstrual period (based on 17 breast cancer cases). Helzlsouer et al. [25], contrarily, reported a higher concentration of luteal phase progesterone in cases, but the study was based on nine breast cancer cases only. None of these differences was statistically significant.

In summary, evidence derived from prospective cohort studies is consistent to some extent, at least for the association of androgens with breast cancer. However, the small number of breast cancer cases in these studies and the difficulty in controlling hormone variability over the ovarian cycle may have weakened the strength of the observed association.

4 Hyperinsulinemic Insulin Resistance, Insulin-Growth Factor Bioavailability, Glucose Metabolism, and Breast Cancer Risk

In addition to the sex steroid hormones, there is some reason to believe that insulin and insulin-like growth hormone (IGF)-I and glucose metabolism may also play a role in breast cancer etiology.

Insulin is a powerful mitogenic agent [35], inducing a dose-dependent growth response in breast cancer cell lines acting via insulin receptor [36]. Moreover, insulin may also play a role in tumor promotion by up-regulation of ovarian steroid secretion [37]. Overall, insulin stimulates androgen production in ovarian tissue samples in in vitro studies [38–40].

IGF-I is a small peptide (about 7,500 Da) with a significant structural homology with proinsulin and insulin [41], which is highly regulated by growth hormone (GH) [42]. Despite their distinct immunological difference, IGFs and insulin share not only important similarities in their structure, their receptors, and their signaling pathways which determine their biological actions, but they also have a common ancestor, possibly an old serine protease [43]. The ancestor molecule may have stimulated cell and tissue

growth after food intake, and this function probably included some "insulin-like activity." The latter seems to have been refined by the emergence of proinsulin, whereas growth-promoting activity has been preserved mostly in the IGFs. Thus, despite the divergence of their biological functions and their refinement and adaptation to specific purposes, both insulin and IGFs share some common functions: IGFs respond to hyperglycemic stimulus and exert acute effects on metabolism, and insulin is able to stimulate growth [44, 45]. IGF-I stimulates multiple cellular responses that are related to growth, including synthesis of DNA, RNA, and cellular proteins [46]. IGF-I has well-documented effects on cell proliferation, and similarly to insulin, IGF-I has been shown to inhibit programmed cell death (apoptosis) [42–49]. Furthermore, in breast cancer cell lines, concentrations of insulin and IGF-I receptors are increased [50, 51]. The biological activity of IGF-I within tissues, including breast epithelium, is regulated by a family of major plasmatic binding proteins (IGFBPs), and partially also by the local production of IGF-I and IGFBPs within tissues [42, 52–53]. At least seven different IGFBPs have been identified so far, but only three of these (IGFBP-1, -2, and -3) are found at significant levels in blood. Over 90% of IGF-I is bound with IGFBP-3 plus another glycoprotein, called acid-labile subunit (ALS). Most of the remaining fraction is bound to the smaller binding proteins IGFBP-1 and IGFBP-2. A decrease in plasma IGFBP-3, with a transfer of IGF-I to IGFBP-1 or IGFBP-2, may result in greater IGF-I availability to its tissue receptors, since the large IGF-I/IGFBP-3/ALS complex cannot pass through the capillary barrier to target tissues, while the smaller complexes of IGF-I with IGFBP-1 or IGFBP-2 can [42, 53].

There is increasing evidence that IGF-I is also a direct modulator of the formation and biological availability of ovarian steroid hormones. IGF-I has been shown to share with insulin the function to up-regulate the secretion of sex steroid hormones and increase their bioactivity through the inhibition of sex hormone-binding globulin secretion in the liver [54–56].

There is consistent prospective epidemiological evidence of a close association between IGF-I and breast cancer risk, however more often in premenopausal women [57–60]. To date, three prospective studies have been conducted on serum insulin or C-peptide and breast cancer risk [58, 59, 61]. No evidence for a positive association between C-peptide and breast cancer was found by Jernström et al. in older postmenopausal women [61]; however, the study was limited by the small sample of breast cancer cases included in the analysis (45 breast cancer cases). Toniolo et al. [58] reported a positive association of C-peptide with premenopausal and postmenopausal breast cancer risk that was not statistically significant. Nonfasting condition at blood collection for these studies may, at least in part, explain the weakness of the observed association. In our recently published analysis [59], using a nested case-control study in the ORDET cohort prospective cohort, we observed a 70% relative risk increase for breast cancer in the two highest

quartiles of fasting insulin levels; however, all the confidence intervals included unity.

Glucose may play a direct role in the development of breast cancer by favoring the "selection" of malignant cell clones [62]. Neoplastic cells have been shown to extensively utilize glucose for proliferation [62]. Increased metabolism of glucose toward the pentose phosphate pathways is one of the central metabolic characteristics of malignant tissues [62].

In our above-mentioned study [59], we also analyzed the hypothesis that serum fasting glucose is associated with breast cancer. In premenopausal women, glucose was strongly and significantly associated with breast cancer risk: the age, body mass index (BMI), and reproductive variable adjusted relative risk for the highest quartile of serum glucose versus the lowest was 2.8 [95% confidence interval 1.2–6.5], p=0.02.

5 Conclusions

Breast cancer incidence rates are higher in Western countries than in Africa or Asia. Although both genetic and environmental factors may explain the large geographic variation in incidence rates, studies on migrants who moved from countries characterized by low incidence (i.e., Japan) to countries with higher incidence (i.e., the United States and Italy) showed a significant increase in breast cancer incidence in individuals that migrate in comparison with their peers in the countries of origin. This evidence suggests that environmental factors play a significant role in breast cancer development. In countries with high breast cancer incidence rates, lifestyle is characterized by an energy-dense diet rich in total and saturated fat and refined carbohydrates, and by low physical activity. A sedentary life and a high-fat, low-complex-carbohydrate diet have been associated with impaired glucose metabolism, hyperinsulinemic insulin resistance, and elevated serum levels of androgens and estrogens, the metabolic and endocrine patterns previously described to be associated to breast cancer risk. Hormones and metabolic factors therefore, might represent a possible etiological linkage between lifestyle characteristics and breast cancer.

Recent studies have observed the efficacy of changes in diet and in lifestyle in improving insulin sensitivity and reducing the availability of sex hormones [63–73]. These studies may indicate possible strategies for future breast cancer prevention.

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Innovative Agents in Cancer Prevention

Margaret M. Manson (✉) · Peter B. Farmer · Andreas Gescher · William P. Steward

Cancer Biomarkers and Prevention Group, Departments of Cancer Studies and Biochemistry, University of Leicester, LE1 7RH, UK
mmm2@le.ac.uk

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Abstract There are many facets to cancer prevention: a good diet, weight control and physical activity, a healthy environment, avoidance of carcinogens such as those in tobacco smoke, and screening of populations at risk to allow early detection. But there is also the possibility of using drugs or naturally occurring compounds to prevent initiation of, or to suppress, tumour growth. Only a few such agents have been used to date in the clinic with any success, and these include non-steroidal anti-inflammatory drugs for colon, finasteride for prostate and tamoxifen or raloxifene for breast tumours. An ideal chemopreventive agent would restore normal growth control to a preneoplastic or cancerous cell population by modifying aberrant signalling pathways or inducing apoptosis (or both) in cells beyond repair. Characteristics for such an agent include selectivity for damaged or transformed cells, good bioavailability and more than one mechanism of action to foil redundancy or crosstalk in signalling pathways. As more research effort is being targeted towards this area, the distinction between chemotherapeutic and chemopreventive agents is blurring. Chemotherapeutic drugs are now being designed to target over- or under-active signalling molecules within cancer cells, a philosophy which is just as relevant in chemoprevention. Development of dietary agents is particularly attractive because of our long-standing exposure to them, their relative lack of toxicity, and encouraging indications from epidemiology. The carcinogenic process relies on the cell's ability to proliferate abnormally, evade apoptosis, induce angiogenesis and metastasise to distant sites. In vitro studies with a number of different diet-derived compounds suggest

that there are molecules capable of modulating each of these aspects of tumour growth. However, on the negative side many of them have rather poor bioavailability. The challenge is to uncover their multiple mechanisms of action in order to predict their efficacy, to learn how to use them effectively in combination, and in some cases to redesign them to improve potency or bioavailability. These ideas are illustrated by dietary agents such as indole-3-carbinol (I3C), epigallocatechin gallate (EGCG), curcumin and resveratrol, all of which appear to have a number of different molecular targets, impinging on several signalling pathways. Ultimately it may be possible not only to suppress tumours and to extend quality of life by administering appropriate diet-derived molecules, but also to refine the definition of a cancer chemopreventive diet.

1 Introduction

The search for agents to treat established cancers has been long, intensive and often unrewarding. Initially the design was crude in biochemical terms, aimed at eliminating tumours by cytotoxic action, with little discrimination between cells in tumour and healthy tissue. Attempts were then made to target cytotoxic drugs specifically to tumour cells, for example by attaching them to monoclonal antibodies which would recognise the surface of malignant cells, or by using prodrugs to take advantage of activation systems specific to the tumour (Knox and Connors 1995; Connors 1995; Satchi, et al. 2001). More recently, armed with a far greater understanding at the molecular level of what drives carcinogenesis in different tissues, agents have been designed to target specific molecules believed to be pivotal to the tumourigenic process. And with the realisation that signalling pathways are complex and interactive, the use of combination therapies, which may well prove to be more efficacious, has also become popular.

Most of the research effort has been directed at established tumours, but increasingly the benefits of targeting earlier stages of the disease process are being appreciated. Indeed, the distinction between malignancy and preneoplasia, based largely in the past on histological findings, is often less apparent when molecular changes are recognised. Recently, the American Association for Cancer Research Task Force identified intraepithelial neoplasia as an important target for new agent development (O'Shaughnessy et al. 2002).

Where the primary cause of cancer is understood, as in the case of smoking and lung cancer, it is easy to suggest an effective preventive measure, although, as we know all too well from this example, implementation is another matter. When the cause is unknown, or total avoidance of the carcinogen(s) is not practical, then alternative prevention strategies are required. These may involve the use of molecules, naturally occurring or synthetic, alone or in combination, to thwart the carcinogenic process as early as possible and for as long as possible. A successful strategy is dependent on a detailed understanding of tumour development, the signalling pathways which

are deregulated and the phenotypic changes which are most diagnostic of the disease process. Key alterations to phenotype are not only definitive for tumour development, but provide biomarkers of efficacy of any potential chemopreventive agents.

Epidemiological studies have suggested that dietary habits could influence as many as 30% of cancers (World Cancer Research Fund/American Institute for Cancer Research 1997). While some components of diet have been blamed for inducing cancer, numerous studies suggest that there are also many protective agents, found particularly in vegetables, fruit, herbs and spices (e.g. Block et al. 1992). The exploitation of dietary molecules as chemopreventive agents is attractive for a variety of reasons, not least the long history of human exposure with little or no toxicity. Because the carcinogenic process involves many steps over a prolonged period of time, this presents many opportunities to intervene to slow down or halt the process. For a healthy cell to acquire full malignancy it must (1) develop self-sufficiency in growth signals, while becoming insensitive to inhibitory signals, (2) evade apoptosis, (3) be able to replicate indefinitely and (4) be capable of sustaining angiogenesis and metastasis (Hanahan and Weinberg 2000). With an increasing understanding of the signalling pathways which control these processes, and the ways in which they become deregulated in tumour cells, we can begin to understand how chemopreventive agents might act to prevent cancer. In fact, in vitro studies suggest there are dietary molecules capable of modulating each one of these malignant characteristics. Moreover, a number of these molecules cause growth arrest and apoptosis preferentially in tumour cells, by targeting components of various signalling pathways, such as those involving cell cycle control, mitogen activated protein kinases (MAPKs), phosphoinositide 3 kinase (PI3K) or nuclear factor (NF)-κB. By improving our understanding of the chemopreventive mode of action of dietary molecules and their primary target within the cell, it will become easier to recognise potentially useful new agents and predict the most effective combinations for treatment.

2 What Constitutes an Innovative Agent?

In identifying innovative agents for chemoprevention research, it is useful to consider several different parameters.

There is the challenge of designing and synthesising completely novel drugs for specific cellular targets, very much a chemotherapeutic approach, and one which will not be considered further here.

Existing biological resources can be exploited in the search for new molecules, because while there are many compounds already under investigation for their chemopreventive properties, we have only begun to scratch the sur-

face in terms of the number of possible efficacious agents in the plant kingdom. Sometimes it is a case of rediscovering molecules which have been used medicinally for centuries and beginning to define their properties scientifically, e.g. curcumin in the spice turmeric. In other cases one can start with a food source and systematically isolate the active components—e.g. tricin found in rice bran. These examples are discussed in more detail below.

The discovery of new mechanisms of action for existing (particularly naturally occurring) molecules is also likely to be a fruitful area, as this enables predictions to be made as to which tissue or cell type is most likely to be protected. It is worth noting that effects of agents can differ markedly from one cell type to another, even to the extent of inducing opposite effects. In this regard several of the large clinical trials conducted so far have produced unexpected results—either the agent showed no benefit or was detrimental to the assumed target organ, or in some cases benefit was found elsewhere in the body than was predicted. In most cases we still do not understand why a particular agent appears to affect so many different cellular components, as, with few exceptions, we have not identified the primary molecular targets.

Because of the high degree of overlap between different signalling pathways and the redundancy within a cell, it is unlikely that one compound with a very specific molecular site of action (if such a molecule exists) will be an effective preventive agent. Thus, agents with multiple mechanisms of action, and particularly the use of combination treatments, will become increasingly important. There are many examples of combination treatments where synergy has been observed, or where individual agents showed no effect, while the combination was active.

Finally, once efficacy for a molecule is established, it can provide a useful template for designing more effective analogues or derivatives with, for example, increased bioavailability or increased potency for a particular molecular target. Examples of such an approach can be found for the naturally occurring indoles, curcumin and epigallocatechin 3-gallate (EGCG).

Illustrations of each of these innovative aspects are given in the following sections.

3 Novel Dietary Agents

There are many flavonoids in the plant kingdom which may possess cancer chemopreventive efficacy. The isoflavone genistein from soya and the flavone quercetin, a constituent of onions, are examples of flavonoids which prevent cancer in rodent models of carcinogenesis. Genistein is currently in

clinical trial for the prevention of recurrent localized prostate cancer subsequent to radical prostatectomy.

However, it has been shown that both genistein and quercetin induce site-specific DNA cleavage in the breakpoint cluster region of the *mixed lineage leukaemia* (*MLL*) gene in vivo (Strick et al. 2000), which has raised concerns as to their suitability for widespread use in humans. This property is considered germane to the aetiology of childhood leukaemias, in which dietary soya as a source of genistein has been implicated. The assessment of risk versus benefit associated with these compounds demands the constant search for new agents which may have more advantageous pharmacological profiles. In such a search we focussed on the rice bran constituent tricin, which we showed to be a good inhibitor of the proliferation of human-derived breast cancer cells (Hudson et al. 2000), with the induction of G_2/M cell cycle arrest (Cai et al., 2004). In vivo studies in mice did not show any indication of toxicity to healthy tissue. Intriguingly, compared to genistein and quercetin, tricin lacks the ability to induce the *MLL* gene cleavage (Hudson et al. 2000), which suggests its further evaluation as a potential cancer chemopreventive agent would be worthwhile.

4 Screening New Agents

Before investing a lot of effort in a potential new agent, some form of screening is required to indicate efficacy. There are several problems with this approach: lack of good biomarkers, use of wrong biomarkers, and extrapolating in vitro findings to in vivo physiologically relevant mechanisms. Thus, useful new agents will only be reliably identified if the screening mechanism is robust. Most in vitro studies investigating a new dietary agent initially look for indications of growth inhibition, cell cycle arrest and induction of apoptosis in tumour cell lines. Where possible non-transformed cells are included to identify selectivity towards the tumour cell lines. To take things a stage further, a number of groups have combined a series of assays to screen large numbers of agents and provide a more detailed mechanistic profile of the potentially useful compounds. Sharma et al. (1994) screened 90 potential agents using six chemoprevention-associated biochemical endpoints. These included inhibition of 12-*O*-tetradecanoylphorbol-13-acetate (TPA)-induced tyrosine kinase activity in HL-60 cells, inhibition of ornithine decarboxylase (ODC) activity in rat tracheal epithelial cells; inhibition of poly (ADP-ribose) polymerase in propane sultone-treated primary human fibroblasts, inhibition of benzo[*a*]pyrene-DNA binding in human bronchial epithelial cells, induction of reduced glutathione in Buffalo rat liver cells, and inhibition of TPA-induced free radical formation in primary human fibroblasts or HL-60 cells. Eight compounds were found to be active in all six assays—

vitamin C, bismuththiol, esculetin, etoperidone, folic acid, hydrocortisone, indole-3-carbinol (I3C) and tocopherol succinate—and were regarded as highly promising preventive agents.

Gerhauser et al. (2003) also described a battery of cell- and enzyme-based in vitro assays which they felt were relevant for prevention of carcinogenesis in vivo. Their screen included modulation of drug metabolism [inhibition of cytochrome P450 (CYP)1A and induction of NAD(P)H:quinone reductase] activity in Hepa1c1c7 murine hepatoma cells, radical (DPPH) scavenging and antioxidant effects (scavenging of superoxide anion-, hydroxyl- and peroxyl-radicals), anti-inflammatory mechanisms [inhibition of lipopolysaccharide (LPS)-mediated NO generation by inducible NO synthase] in Raw 264.7 macrophages, cyclooxygenase-1 inhibition, and anti-tumour promoting activities [inhibition of phorbol ester-induced ornithine decarboxylase (ODC) activity in 308 murine keratinocytes]. This screen was used to test 22 known chemopreventive agents and identified curcumin (positive in 9/10 assays), quercitin (8/10) and resveratrol(7/10) as potent compounds. Xanthohumol, a prenylated chalone from hop, was identified as a promising novel agent, while several new mechanisms of action were identified for a known agent phenethylisothiocyanate (for example, NF-κB-mediated inhibition of NO production). Interestingly, I3C did not appear to be a useful chemopreventive agent in this screen.

The problems with screening new agents in the clinic particularly with regard to breast cancer have been highlighted by Fackler et al. (2003). In particular, they draw attention to the importance of good intermediate or endpoint biomarkers for efficacy, such as methylation markers and protein expression profiles. They discuss a range of novel agents such as retinoids, rexinoids, cyclooxygenase 2 (COX-2) inhibitors, histone deacetylase inhibitors, tyrosine kinase inhibitors, polyamine synthesis inhibitors, curcumin, soy and isoflavones with respect to prevention of breast cancer.

5 Novel Mechanisms

Curcumin has been widely studied because of its antioxidant and anti-inflammatory properties. It inhibits growth and induces apoptosis in a range of different cell types. Detailed mechanistic studies have revealed activity in a number of different signalling pathways, examples of which are listed in Table 1. One of its most studied properties is its ability to inhibit signalling through NF-κB and reduce expression of COX-2 (Singh and Aggarwal 1995; Plummer et al. 1999; Jobin et al. 1999). This is one reason why it is being investigated for prevention of colon cancer (Sharma et al. 2001; Ireson et al. 2001, 2002; Gescher et al. 2001; Perkins et al. 2002). However, new interactions with cell regulatory proteins continue to be identified. Curcumin was

Table 1. Possible chemopreventive agent mechanisms of action

Curcumin
Antioxidant and anti-inflammatory, induces HO-1
Induces growth arrest
Downregulates transcription factors AP-1, NF-κB, Egr-1
Downregulates growth factor receptors EGFR, HER2
Induces apoptosis, inhibits Bcl-2, Bcl-X_L, IAP, induces cyt-c release, caspase 3 activation
Downregulates expression of COX-2, LOX, NOS, MMP-9, uPA, TNF-α, cyclin D1, chemokines, cell surface adhesion molecules, c-myc, β-catenin, Pgp
Inhibits activity of protein kinases (EGFR, HER2, ERK, JNK, PKB/Akt, PKC, phosphorylase kinase, src, FAK, JAK/STAT)
Inhibits sulphotransferases, CYPs, GSTP1-1
Inhibits VEGF production
Andreadi et al. 2003; Anto et al. 2002; Anuchapreeda et al. 2002; Chen and Tan 1998; Duvoix et al. 2003; Han et al. 1999; Hong et al. 1999; Jaiswal et al. 2002; Jobin et al. 1999; Korutla et al. 1995; Leu et al. 2003; Liu et al. 1993; Motterlini et al. 2000; Mukhopadhyay et al. 2001, 2002; Plummer et al. 1999; Reddy and Aggarwal 1994; Shao et al. 2002; Singh and Aggarwal 1995; Squires et al. 2003; Surh et al. 2001; Woo et al. 2003
Epigallocatechin gallate
Induces G_0/G_1 arrest—decreases CDK2/4, cyclin D1, cdc2, increases p53, p21, p27
Downregulates signalling through Her2 neu, PDGFR
Downregulates transcription factors AP-1, NF-κB
Induces apoptosis
Induces GSTs
Inhibits p38
Inhibits TNF-α release
Inhibits urokinase, LOX, COX
Induces HO-1
Inhibits angiogenesis and VEGFR phosphorylation, inhibits VEGF induction
Agarwal 2000; Ahmad et al. 2000; Ahn et al. 1999; Andreadi et al. 2003; Atherfold and Manson 2002; Barthelman et al. 1998; Cao and Cao 1999; Chen et al. 1999; Chung et al. 1999; Dong et al. 1997; Hong et al. 2001; Lamy et al. 2002; Lin et al. 1999; Nomura et al. 2000; Okabe et al. 1999; Pianetti et al. 2002; Ren et al. 2000; Sartippour et al. 2002; Steele et al. 2000; Surh et al. 2001
Indole-3-carbinol
Induces drug metabolising enzymes
Alters oestrogen metabolism—2OH:16αOH ratio
Induces G_0/G_1 growth arrest—decreases CDK6, CDK2, Rb phosphorylation, cyclin activity; increases p16, p21, p27 expression
Induces apoptosis—increases Bax expression; decreases Bcl-2, Bcl-X_L, BAD, increases release of cyt c; induces TRAIL receptors DR4 and DR5
Decreases Akt and PI3K activities
Decreases NF-κB-DNA binding
Induces E-cadherin, catenins, BRCA1
Inhibits ER signalling
In vivo condensation products are active (see DIM)
Chinni et al. 2001; Chinni and Sakar 2002; Cover et al. 1998; Howells et al. 2002; Meng et al. 2000a,b,c; Rahman et al. 2000; Reviewed in International Agency for Research on Cancer 2004

Table 1 (continued)

Diindolylmethane (DIM)
Induces G_0/G_1 growth arrest
Upregulates GADD proteins, decreases CDK2 activity, increases p21, p27
Induces apoptosis—decreases Bcl-2, increases Bax
Inhibits phosphorylation of Akt
Inhibits ligand binding to p-glycoprotein
Inhibits ER and androgen signalling, activates ER function
Increases TNF-α
Decreases PSA expression
Anderton et al. 2003; Auborn et al. 2003; Chen et al. 2001; Firestone and Bjeldanes 2003; Hong et al. 2002a,b; Le et al. 2003; Leong et al. 2004; Riby et al. 2000; reviewed in IARC 2004 (Handbook of Chemoprevention)

AP, activator protein; BAD, BCL2-antagonist of cell death; COX, cyclo-oxygenase; CYP, cytochrome P450; EGFR, epidermal growth factor receptor; ER, oestrogen receptor; ERK, extracellular signal-regulated kinase; FAK, focal adhesion kinase; GADD, growth arrest and DNA-damage-inducible; GSTP1, glutathione S-transferase P1; HER2, v-erb-b2 erythroblastic leukaemia viral oncogene homologue 2; HO, heme oxygenase; IAP, inhibitor of apoptosis; JAK, Janus kinase; JNK, c-Jun N-terminal kinase; LOX, lipoxygenase; MMP, matrix metalloproteinase; NF, nuclear factor; NOS, nitric oxide synthase; PDGFR, platelet-derived growth factor receptor; Pgp, P-glycoprotein; PK, protein kinase; PSA, prostate specific antigen; STAT, signal transducer and activator of transcription; TNF, tumour necrosis factor; TRAIL, tumour necrosis factor–related apoptosis–inducing ligand; uPA, urokinase; VEGF, vascular endothelial growth factor; VEGFR, vascular endothelial growth factor receptor.

recently shown to be a direct inhibitor of v-Src, which led to a decrease in phosphorylation of Shc, cortactin and focal adhesion kinase (FAK). Curcumin also appeared to inhibit the activity of FAK directly. The result was loss of Src-mediated cell mobility (Leu et al. 2003), which could have important implications for invasion and metastasis.

EGCG, a polyphenol found in green tea, also affects growth, inducing G_0/G_1 cell cycle arrest and apoptosis in many cell types, and appears to modulate a range of signalling pathways and target molecules (Table 1). However, recently a potentially very useful property of this molecule was reported, which may partly explain why such an agent appears to affect the expression of many different molecules. Yang and co-workers (Fang et al. 2003) reported that EGCG inhibits the activity of 5-cytosine-DNA methyltransferase (DNMT). Hypermethylation of promoter regions of genes is an important mechanism to silence expression and occurs in many genes involved in cancer, such as tumour suppressor genes (Widschwendter and Jones 2002). DNMT is responsible for passing the hypermethylation state to daughter cells through methylation of the newly synthesised strand of DNA. Thus, its inhibition by EGCG was shown to reactivate silenced genes such as *$p16^{INK4\alpha}$*, *retinoic acid receptor β* and *O^6-methylguanine methyltransferase* in human oesophageal cancer cells (Fang et al. 2003). However, as always, the

situation is more complex, since hypomethylation can also contribute to oncogenesis, as described in human colon cancer cells (Feinberg and Vogelstein 1983). Data suggest that diets low in nutrients such as folate, required for methylation reactions and DNA biosynthesis, induce colon cancer in mice in the absence of a carcinogen and promote carcinogen-induced colon carcinogenesis in rats (reviewed by Lamprecht and Lipkin 2003).

6 Improved Formulation

Many dietary agents have extremely promising modes of action when investigated in cultured cells, but in vivo are much less effective. One reason for this is bioavailability. Many purified dietary compounds have limited solubility, making it difficult to attain plasma or tissue concentrations equivalent to those showing activity in vitro. On a cautionary note, there may be a very good reason why many of these compounds are poorly available to tissues, so any attempts to use them in humans at higher doses than are available from the diet should be assessed for possible toxic consequences.

Diindolylmethane (DIM), an in vivo condensation product of I3C, is more potent at inhibiting cell growth than the parent compound, while lacking some of the less desirable characteristics such as potent induction of CYPs (see Table 1 for mechanisms of action). Not only does administration of DIM to mice achieve higher plasma and tissue levels than can be attained from an equivalent dose of I3C, but DIM is more persistent than I3C, with detectable levels at 6 h after a single dose (Anderton et al., 2004a). In an effort to increase the bioavailability further, a patented formulation, BioResponse (BR) DIM, utilizing solubility enhancing micro-encapsulation technology, has been developed (Zeligs et al. 2002). We found that BR-DIM yielded higher drug concentrations than the crystalline form in all mouse tissues examined (bioavailability 2.6 versus 1.7%) (Anderton et al. 2004b). Thus increased benefit from poorly soluble agents may be gained simply by changing the way they are administered.

7 Analogues and Derivatives

In addition to improving bioavailability by changing formulation, considerable effort is being directed towards improving the physical properties of compounds such as curcumin, EGCG, I3C and DIM. Kumar et al. (2003) synthesised a derivative of curcumin, 4-OH-3-methoxybenzoic acid methyl ester (HMBME), which retained the polar functionality of curcumin, but demonstrated increased solubility and did not undergo reductive metabo-

lism. This derivative, like curcumin, caused apoptosis in human prostate cancer cells by targeting the PI3 K/Akt and NF-κB survival pathways. However, unlike curcumin, it did not cause growth arrest in non-tumourigenic fibroblasts. Green tea consumption has been linked to prevention of breast pancreatic, colon, oesophageal and lung cancer. But, while EGCG is more soluble than many potential chemopreventive agents, its bioavailability from tea is less than 1%. In an attempt to improve this parameter, Zaveri et al. (2003) chemically altered the structure of EGCG to improve cell permeability and found that the new analogues are more effective at inducing growth inhibition and G_1 arrest than the parent compound. One of these analogues, SR13193, showed equivalent potency at inhibiting the angiogenic factor, vascular endothelial growth factor (VEGF).

A similar strategy has been adopted for I3C and DIM. Jong et al. (2003) used computer modelling to design a drug, SR13668, based on the structures of I3C and its active oligomers, that would optimise I3C's anticancer activities, while minimising unwanted metabolic and oestrogenic effects. At concentrations 10- to 100-fold lower than DIM or I3C, this analogue induced cell cycle arrest and apoptosis, inhibited phosphorylation of Akt and inhibited cancer cell invasion, both in vitro and in vivo. It also exhibited greatly reduced ability to induce CYP, when compared to one of the oligomers, indolocarbazole, which is produced in vivo from I3C.

8
Combination Therapies

There are now a significant number of reports, both in vitro and in vivo, where a combination of two or more chemopreventive agents has been shown to be efficacious at doses where one agent alone was less effective or without effect (Khafif et al. 1998; Suganuma et al. 1999).

A number of studies indicate that retinoids or rexinoids in combination with selective oestrogen receptor modulators (SERMs) may be effective in preventing breast cancer development (Anzano et al. 1996; Bischoff et al. 1998; Suh et al. 2002). Wu et al. (2002) showed that the retinoid X receptor (RXR)-selective retinoid, LGD1069 (targretin), effectively suppressed oestrogen receptor (ER)-negative tumour development in a mouse mammary tumour virus ErbB2 transgenic mouse model, with minimal toxicity. They reported that this agent is now in clinical trial for women at high risk of breast cancer.

Using a Her-2/Neu transgenic mammary carcinoma model, Nanni et al. (2003) found that a combination of tamoxifen plus interleukin 12 (IL-12) was more effective at inhibiting carcinogenesis than either agent alone. Inhibition occurred through a reduction in estrogen receptor expression and an-

giogenesis. The latter was thought to be due to the crosstalk between tamoxifen and interferon-γ which is downstream of IL-12.

White or green tea, as well as the non-steroidal anti-inflammatory drug (NSAID), sulindac, were effective in reducing the intestinal tumours in Min mice (Orner et al. 2003). Mice treated with a combination of tea and NSAID had significantly fewer tumours than those treated with a single agent. Downregulation of the β-catenin/adenomatous polyposis coli (APC) pathway, either directly or indirectly, was suggested as the mechanism.

An ongoing chemoprevention trial (SELECT), using selenium and vitamin E, either individually or in combination, will evaluate the ability of these agents to prevent prostate cancer in healthy men over 55 years of age. Their effectiveness at inducing apoptosis was studied in androgen-unresponsive, p53-null, PC-3 human prostate cells. A combination of D-α-tocopherol succinate (VES) and methylseleninic acid (MSA) activated a greater range of caspases than either agent alone. This suggested that the mitochondrial pathway and the endoplasmic reticulum/cytokine signalling pathway might be involved in the induction of apoptosis by VES and MSA, respectively, and that the two pathways might act co-operatively to enhance the apoptotic effect following combination treatment (Zu and Ip 2003).

A study by Yasumaru et al. (2003), using mice injected with Colon 26 cells, or a range of colon cancer cell lines in vitro, suggested that combination therapy with COX-2 inhibitors and angiotensin-converting enzyme (ACE) inhibitors, which targeted the insulin growth factor-like receptor, might be a promising novel strategy for chemoprevention of colon cancer.

In a study using Madin-Darby canine kidney (MDCK)II and HT-29 cells, EGCG and its methyl metabolites were shown to be substrates for the multidrug resistance proteins MRP1 and MRP2, but not P-glycoprotein (Pgp). In the presence of MRP inhibitors, including indomethacin and curcumin, intracellular concentrations of EGCG were significantly increased (Hong et al. 2003).

The difficulties encountered in optimising cancer chemopreventive combinations have been illustrated in a study in which aspirin was combined with curcumin in the Min mouse model (Perkins et al. 2003). Aspirin delayed the development of adenomas in young mice only when given to the mothers, indicating the necessity for exposure in utero. In contrast, curcumin exerted its preventive effect only when administered over the lifetime of the animal from weaning. When combined, the efficacy of aspirin plus curcumin was not superior to that of the individual components, hinting at the possibility that both agents exert their efficacy via similar targets, but during different developmental stages.

9
Amelioration of Toxicity

The search is also on for novel cancer chemotherapeutic agents which lack the unwanted sequelae of traditional cytotoxicants, but thus far discovery programmes have not generated the breakthrough everybody is hoping for. However, there are a number of reports where agents which would be classed as chemopreventive have been used in combination with a chemotherapeutic regime to prevent the unwanted side-effects, while not compromising the efficacy against the tumour. The combination of I3C and ET-743 is such an example. ET-743 has been shown to possess promising activity against sarcomas and mammary carcinomas in early clinical evaluation (Delaloge et al. 2001). However, hepatotoxicity is a consistent unwanted side-effect of this agent. We have recently conducted experiments which suggest that I3C, given with the diet, is a potent hepatoprotectant in rats which have received an hepatotoxic dose of ET-743 (Donald et al., 2004). At the same time, I3C did not compromise the antitumour activity of ET-743 in a mammary carcinoma model; on the contrary, it enhanced it. DIM did not appear to be protective in this system. Thus I3C, like the thiol-containing nucleophile *N*-acetylcysteine, appears both to prevent cancer and to protect against specific unwanted effects of cytotoxic drugs.

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Conclusions

In searching for innovative strategies it is worth bearing in mind that to date some of the most convincing evidence of chemoprevention in action comes from epidemiological studies of the effect of diet, in particular exposure to sufficient levels of various vegetables and fruit.

Based on the examples described above, it is apparent that there are many exciting opportunities for developments in this field. In terms of the number of possible agents, only a very few have been characterised in any detail. Undoubtedly many more mechanisms of action will be revealed as we probe deeper and studies in basic cell biology reveal ever more complex signalling interactions. However, where multiple activities for a particular compound have been observed in vitro, it will be important to extrapolate the data to more complex three-dimensional models and to human tissue to determine which chemopreventive mechanisms are physiologically relevant.

Many studies suggest that a combination is more effective that a single agent, which is not surprising for a number of reasons. First, this is how the compounds are presented in the diet, where epidemiology has suggested efficacy. Second, the wiring of any cell and the alterations to phenotype in a cancer cell are so complex that a combination of agents, each tackling com-

plementary signalling cascades, should be much more effective. Third, since many of the dietary compounds are poorly bioavailable, making it difficult to achieve effective doses in vivo, exposure to a number of different compounds simultaneously may solve the problem.

Much of the science in this area is still descriptive in the sense that new mechanisms and signalling interactions are constantly being discovered. What is needed to advance the field is a better understanding of why a single compound can affect a range of target molecules and why its effects differ from one cell type to another. Is it an effect on cellular redox status or on the methylation pattern of gene promoters which elicits multiple downstream consequences? Identification of primary targets within the cell would help greatly in addressing such questions and allow predictions to be made as to the likely efficacy in any particular target tissue. This information would also facilitate the design of more potent analogues or effective combinations.

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The IARC Commitment to Cancer Prevention: The Example of Papillomavirus and Cervical Cancer

Silvia Franceschi

International Agency for Research on Cancer, 150 cours Albert Thomas, 69008 Lyon, France
franceschi@iarc.fr

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Abstract Every year approximately half a million women worldwide develop cervical cancer (CC) of whom 80% live in poor countries where population-based screening programmes are virtually non-existent. The role of sexually transmitted agents in the aetiology of cervical cancer has been suspected for more than a century, but knowledge in this field has rapidly expanded only in the last 20 years, after major improvements were made in detection methods for human papillomavirus (HPV). A dozen types of HPV have been identified in 99% of biopsy specimens from CC worldwide and the relative risk estimates for HPV in case-control studies of CC are in the 50 to 100 range. A meta-analysis done at the International Agency for Research on Cancer (IARC) included a total of 10,058 CC cases from 85 published studies. The most common HPV types identified in CC were, in order of decreasing prevalence, HPV 16, 18, 45, 31, 33, 58, 52, 35, 59, 56, 6, 51, 68, 39, 82, 73, 66 and 70. Over two-thirds of CC cases were associated with an infection of either HPV 16 (51.0%) or HPV 18 (16.2%). Despite the overwhelming importance of HPV, other factors contribute to the rare occurrence of CC after HPV infection. Nine case-control studies from the IARC have confirmed the adverse effect of long-term use of oral contraceptives, high parity, smoking and sexually transmitted infections (i.e. *Chlamydia trachomatis* and herpes simplex virus-2) after adjustment for, or stratification by, HPV infection. Ten surveys of HPV infection in population-based samples of approximately

15,000 women in four continents have shown that: (1) the prevalence of HPV infection varies greatly (between 2% and nearly 30%); and (2) the age distribution also varies widely, pointing to cohort effects. There is no effective medical treatment for HPV, but a prophylactic vaccine, based on late (L) 1 HPV 16 proteins, has been shown to be safe, highly immunogenic and efficacious in preventing persistent HPV infections. A multivalent vaccine against the most common oncogenic HPV types may thus ultimately represent the most effective way to prevent CC worldwide either alone, or in combination with screening. It may, however, take several years before this approach becomes a reality. Thus, early detection of CC precursor lesions by screening, and their treatment, will remain the most important measures for the control of CC for the foreseeable future.

1 Introduction

Every year approximately half a million women worldwide develop cervical cancer (CC) of whom 80% live in poor countries where population-based screening programmes are virtually non-existent. Screening with cervical cytology has greatly helped to reduce the incidence of, and death from, CC in developed countries through the detection and treatment of cervical precancerous lesions (many years before CC occurs), so that they do not progress to invasive cancer, and possibly death (Cuzick et al. 2000). However, the risk of CC remains high in many developing countries, mostly due to the lack or inadequacy of existing prevention programmes.

The role of sexually transmitted agents in the aetiology of CC has been suspected for more than a century, but knowledge in this field has rapidly expanded only in the last 20 years, after major improvements were made in detection methods for human papillomavirus (HPV) (Cuzick et al. 2000). A dozen types of HPV have been identified in 99% of biopsy specimens from CC worldwide (Walboomers et al. 1999) and the relative risk estimates for HPV in case-control studies of CC are generally greater than 100 (International Agency for Research on Cancer 1995).

There is no effective medical treatment for HPV, but a prophylactic vaccine, based on late (L) 1 HPV 16 proteins, has been shown to be safe and highly immunogenic (with anti-HPV IgG titres many times higher than those that follow natural infection, Villa et al. 2002). It has also proved to be efficacious in preventing persistent HPV infections in a trial of 1,523 HPV 16-naive young women in the United States (Koutsky et al. 2002). A multivalent vaccine against the most common oncogenic HPV types may thus ultimately represent the most effective way to prevent CC worldwide, either alone or in combination with screening.

It may, however, take several years before this approach becomes a reality. Thus, early detection of CC precursor lesions by screening and their treatment will remain the most important measures for the control of CC for the foreseeable future. However, cytology-based screening is cost intensive, and the

organisation of pap smear-based screening programmes in many high-risk developing countries is a major challenge in the face of limited health care resources and other competing health priorities. Thus, simple, effective, low-cost and low-technology alternatives to cervical cytology for CC prevention are urgently needed for high-risk countries. Visual inspection of the cervix uteri with acetic acid and with Lugol's iodine, which are based on the ability of the trained health personnel to detect acetowhite areas or yellow non-iodine intake areas in the cervical transformation zone, are currently being evaluated in experimental settings by the International Agency for Research on Cancer (IARC) as alternatives to cervical cytology (Sankaranarayanan et al. 2003; Sankaranarayanan and Wesley 2003).

The IARC has contributed substantially to progress in the HPV field through international collaborative studies, especially:

- Case-series investigations where the range of HPV types in cancer specimens can be identified
- Case-control studies, where relative risk for HPV and other risk factors can be computed
- Population-based surveys where the prevalence of, and risk factors for, HPV in women with different cytological findings can be studied
- Design of possible trials of new vaccines against HPV in order to accelerate the introduction of such vaccines in developing countries

Major recent achievements of IARC studies and plans for future studies will be reviewed, with a special focus on those which are essential to translate our knowledge on HPV into successful vaccination programmes.

2
Methods and Results

2.1
Distribution of Different HPV Types in Invasive and Pre-invasive CC Carcinomas

Prophylactic vaccines against particular HPV types hold great promise for reducing the global burden of CC. However, some 15 oncogenic HPV types have been suggested to be associated with CC, and the relative prevalence of these types may vary by region (Muñoz et al. 2003). We wanted, therefore, to identify worldwide and regional priorities for HPV types to be included in potential vaccines. Given that the final outcome in vaccine efficacy trials will be the prevention of pre-cancerous lesions (Plummer and Franceschi 2002), we have also tried to determine if the distribution of HPV types in high-grade squamous intra-epithelial lesions (HSIL) is representative of those that

go on to cause cancer, or if certain types are more likely to progress to malignancy.

All published studies presenting type-specific HPV prevalence data on CC and/or HSIL were identified and classified by geographical region (Clifford et al. 2003a). Worldwide and regional prevalence was estimated for each HPV type by performing a meta-analysis of all studies presenting data on each particular type. HPV type-specific prevalence was estimated independently for squamous cell (SCC), and adeno- and adenosquamous carcinoma (ADC). The relative risk for individual HPV types to progress from HSIL to malignancy was investigated by comparing HPV type distribution in HSIL and SCC.

The meta-analysis included a total of 10,058 CC, and 4,151 histologically verified HSIL cases drawn from 85 and 52 published studies, respectively. The most common HPV types identified in CC were, in order of decreasing prevalence, HPV 16, 18, 45, 31, 33, 58, 52, 35, 59, 56, 6, 51, 68, 39, 82, 73, 66 and 70 (Fig. 1). Over two-thirds of CC cases were associated with an infection of either HPV 16 (51.0%) or HPV 18 (16.2%). The next most prevalent types were HPV 45 (2%–8%), HPV 31 (2%–7%) and HPV 33 (3%–5%) in all regions except Asia where HPV types 58 (6%) and 52 (4%) were more prevalent than elsewhere. The HPV 16 family of viruses was more commonly found in SCC, whereas the HPV 18 family was more likely to be found in ADC. This study reinforces the view that HPV 16 and HPV 18 are the most important HPV types for vaccination in all regions. However, the relative priorities for these types vary somewhat by region.

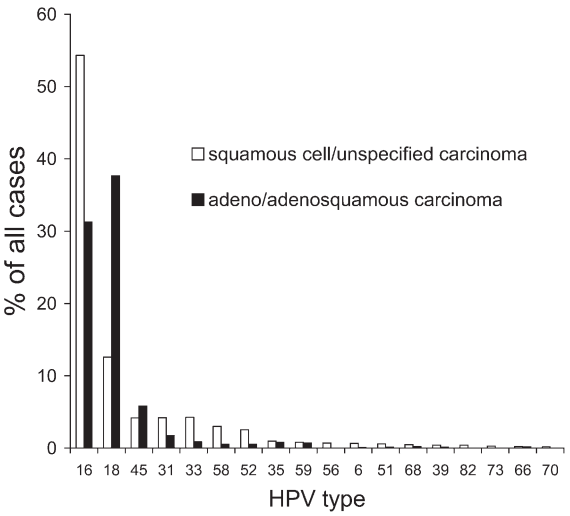

Fig. 1. Type-specific prevalence of human papillomavirus (*HPV*) in 10,058 worldwide cases of invasive cervical cancer by histological type. (Clifford et al. 2003a)

Table 1. Comparison of overall and type-specific HPV prevalence between SCC and HSIL cases (Clifford et al. 2003b)

HPV type	SCC		HSIL		SCC:HSIL prevalence ratio[a]	
	n	HPV %	*n*	HPV %		
All	8,550	87.6	4,191	84.0	1.04	(1.03,1.06)
16	8,594	54.3	4,191	45.6	1.19	(1.15,1.24)
18	8,502	12.6	4,191	7.2	1.74	(1.52,2.04)
33	8,449	4.3	4,155	7.3	0.59	(0.52,0.67)
45	5,174	4.2	1,835	2.5	1.70	(1.25,2.68)
31	7,204	4.2	3,889	9.1	0.46	(0.42,0.52)
58	5,646	3.0	2,084	6.6	0.45	(0.39,0.55)
52	5,304	2.5	2,062	4.8	0.53	(0.44,0.68)
35	6,223	1.0	2,704	4.4	0.22	(0.18,0.27)
59	4,488	0.8	1,489	1.5	0.54	(0.37,0.99)
56	4,493	0.7	1,872	3.2	0.22	(0.17,0.30)
51	4,580	0.6	1,981	3.2	0.19	(0.15,0.25)
68	4,148	0.5	1,437	1.1	0.43	(0.28,0.91)
39	3,899	0.4	1,841	1.0	0.39	(0.26,0.76)
66	4,799	0.2	1,670	2.2	0.10	(0.08,0.15)

HPV, human papillomavirus; HSIL, high-grade squamous intraepithelial lesion; SCC, squamous cell/unspecified carcinoma of the cervix.
[a] With 95% confidence intervals.

HPV type-specific prevalence data was summarised in a similar manner for HSIL, and the distribution of HPV types compared across SCC and HSIL (Clifford et al. 2003b). HPV 16 was the most common type in both SCC (54.3%) and HSIL (45.6%), but was more prevalent in SCC (SCC:HSIL ratio=1.19). HPV 18 and HPV 45 were also more prevalent in SCC than in HSIL, whereas the opposite was true for all other high-risk types (Table 1). Thus, HSILs infected with HPV 16, 18 or 45 appear to have greater potential for progression and any beneficial effect identified by randomised trials from the proportion of HSIL preventable by HPV 16 or HPV 16/18 vaccines may be an under-estimate of the beneficial effect of the vaccine on the prevention of invasive cervical cancer (ICC).

3 Major Risk Factors Other Than HPV

The role of cofactors that may influence the rare progression from HPV infection to CC retains a great importance. Such cofactors may be in the environment, the host or the virus itself. The identification of cofactors for HPV not only improves our understanding of the aetiology of CC, but may also be useful from a prevention standpoint.

Between 1985 and 1997, twelve case-control studies of CC were conducted by the IARC in 10 countries: Brazil (Eluf-Neto et al. 1994), Colombia and Spain (Muñoz et al. 1992; Bosch et al. 1993), Paraguay (Rolón et al. 2000), Peru (Santos et al. 2001), Mali (Bayo et al. 2002), Morocco (Chaouki et al. 1998), the Philippines (Ngelangel et al. 1998), Thailand (Chichareon et al. 1998) and India (Franceschi et al. 2003a; Rajkumar et al. 2003). These studies, which were published separately, have now been pooled in order to investigate the role of cofactors. Pooling of the data was facilitated by the common protocol used in all studies, which included a personal interview, collection of a blood sample and cervical scrapes for the identification of HPV DNA.

The following cofactors were investigated: oral contraceptive (OC) use, parity, smoking, HPV type, and the sexually transmitted infections herpes simplex virus-2 (HSV-2) and *Chlamydia trachomatis*. Many of these cofactors have been studied previously, and in some cases have been suspected for decades to be associated with CC risk. However, previous studies have not controlled for the strong confounding effect of HPV infection. The primary advantage of the pooled case-control study over previous studies is the use of accurate polymerase chain reaction (PCR)-based assays for the detection of HPV DNA from a wide range of types. The second advantage of the study is its size. In total, 2,506 women with CC and 2,491 control women were interviewed. Of these, 1,739 cases and 259 controls were HPV DNA positive. The large study size allows rarer cofactors to be studied that could not be adequately addressed in a single study. Finally, exposure to HSV-2 and *C. trachomatis* was assessed using gold standard assays.

Our current understanding of HPV as a necessary cause of CC implies that any cofactor must act in one of two ways: either by increasing the risk of acquiring HPV infection (and it would therefore be found to be associated with HPV infection among control women), or by increasing the risk of progression from infection to cancer. Risk factors for acquisition of HPV are discussed in Sect. 4. Risk factors for progression, which are reported here, were mainly evaluated by restricting the analysis to HPV-positive cases and HPV-positive controls.

Results are reported separately for each cofactor, but the analyses followed a common pattern. All analyses were adjusted for age and centre, which were frequency-matching variables, number of sexual partners, age at first intercourse and pap smear history, which are potential confounding factors. Confounding by the other cofactors listed here was also investigated.

3.1
Oral Contraceptive Use

In the multi-centric study, data were combined from 10 of the 11 studies to investigate the role of OCs. Among HPV-positive women, use of OCs was associated with an odds ratio (OR) of 1.42 [95% confidence interval (CI): 0.99–2.04] (Moreno et al. 2002). For duration of use, no increased risk was observed for users of less than 5 years compared with never-users (OR=0.73; 95% CI: 0.48–1.12) but a significantly increased risk was observed for use of 5 years or longer (OR=3.42; 95% CI: 2.00–5.84).

The role of OCs as a cofactor for CC was further investigated in a systematic review (meta-analysis) of published data, carried out in collaboration with the Cancer UK Epidemiology Unit, Oxford (Smith et al. 2003). The review included 28 studies and 12,531 women with ICC or in situ carcinomas of the cervix.

The excess risk increase for OC use of less than 5 years, 5–9 years, and 10 years or more were 10%, 60% and 120%, respectively. The results were broadly similar in developed and developing countries, for ICC and in situ CC, for SCC and ADC. In addition, they did not differ depending upon whether findings had been adjusted for HPV status, number of sexual partners, cervical screening, smoking and use of barrier contraceptives. The association with OC use was, however, consistently stronger in cohort than case-control studies. The limited available evidence (Fig. 2) suggests that the relative risk of CC may decrease after cessation of OC use (Smith et al. 2003).

Questions concerning the persistence of any effect of OC is critical when considering the absolute risk of CC among past users, hence the public

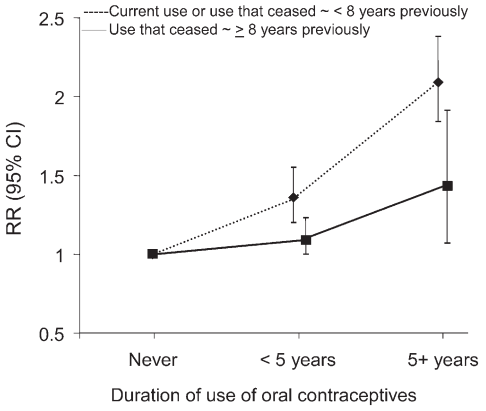

Fig. 2. Summary results on the relative risk (*RR*) with 95% confidence intervals (*CI*) of cervical cancer, according to time since last use and duration of use of oral contraceptives. (Smith et al. 2003)

health implications of our findings. Systematic reviews like ours are severely hampered by the lack of published data cross-classifying women by duration of use and time since last use. Henceforth, a decision has been made to promote a collaborative re-analysis of individual data from all relevant studies on CC, with the support of the WHO Human Reproduction Unit. All contributors have been contacted and asked to provide original data. Over 15,000 CC cases and 30,000 healthy women will be analysed.

3.2 Parity

Data from ten of the IARC case-control studies were used to examine the effect of parity (Muñoz et al. 2002). Among HPV-positive women, a direct association was found between the number of full-term pregnancies and risk of SCC. The OR for seven or more full-term pregnancies was 3.82 (95% CI: 1.90–7.67) compared with nulliparous women, and the trend was highly significant ($p<0.001$). No significant association was found between parity and ADC. Number of abortions and age at menarche and menopause were unrelated to the risk of CC of any histological type. It was concluded that high parity increases the risk of SCC among HPV-positive women and the decline in parity seen in most countries might partly explain the reduction in CC.

3.3 Smoking

Data from ten studies were combined to examine the effect of smoking (Plummer et al. 2003). Any degree of smoking was associated with an increased risk of SCC (OR=2.08; 95% CI: 1.33–3.27) compared with never smoking among HPV-positive women. There was no difference in risk between current and ex-smokers. The prevalence of smoking among women in the populations studied was low, and this precluded an investigation of dose-response effects by number of cigarettes per day or duration of smoking. We concluded that smoking increases the risk of SCC. No clear conclusions could be drawn for ADC due to small numbers. An important public health implication of this finding is that the widespread increase in smoking rates among young women may have an impact on CC incidence.

3.4 Herpes Simplex Virus-2

Data from seven studies were combined to examine the effect of infection with HSV-2 (Smith et al. 2002). In five studies, serum antibodies against herpes simplex virus-1 (HSV-1) and HSV-2 were tested by Western blot, which is considered the reference gold standard. In the other two studies, HSV-2 IgG antibodies were tested using a commercial kit (Gull/Pre-Meridian

HSV-2 ELISA). Among HPV-positive women, HSV-2 positivity was associated with increased risk of SCC (OR=2.19; 95% CI: 1.41–3.40) and ADC (OR=3.37; 95% CI: 1.47–7.74). Further adjustment for number of sexual partners, age at first sexual intercourse, infection with *C. trachomatis*, and use of OCs did not substantially reduce the OR for HSV-2. The principal advantage of this study is that the HSV assays used are type-specific, and so can distinguish between HSV-2 infections (which are almost exclusively genital) and HSV-1 infections (which are primarily non-genital).

3.5
Chlamydia trachomatis

Serum antibodies to *C. trachomatis* were tested in seven studies by a microimmunofluorescence assay, which is considered the gold standard measurement (Smith et al. 2004). Since antibodies against *C. trachomatis* are persistent, this assay measures cumulative exposure to past infections rather than current infection. The OR for the presence of *C. trachomatis* antibodies was 1.7 (95% CI: 1.1–2.5) in HPV-positive women. Additionally, a significant trend in risk ($p<0.001$) was observed with increasing *C. trachomatis* antibody titre. As with HSV-2, further adjustment for sexual variables did not eliminate the association with *C. trachomatis*.

3.6
The Male Role

Seven IARC case-control studies on CC also allowed the evaluation of HPV penile infection in the husbands of 445 women with ICC, 165 women with in situ carcinoma and 717 control women. The strongest variation in penile HPV infection was by country, with percentages among the husbands of control women ranging between 3% in Spain to 39% in Brazil. Having over 50 lifetime sexual partners (compared to only one) was associated with an OR of 2.3 (Franceschi et al. 2002). Male circumcision was associated with a reduced risk of penile HPV infection (OR=0.4) and of CC in monogamous women (OR=0.7) (Castellsagué et al. 2002).

Table 2 shows the comparison of the associations between cervical carcinoma and OC use, parity (among parous women only), smoking, HSV-2 and *C. trachomatis* according to three different models. Adjustment for HPV or restriction to HPV-positive women did not change most of the ORs in Table 2, with the possible exception of those for OC use. This highlights the difficulty of taking the strong effect of HPV infection on CC risk into account. The lack of substantial impact of adjustment for HPV or restriction to HPV-positive cases and controls suggest that either: (1) the currently available marker of HPV status (i.e. the presence of HPV DNA in cervical cells) is inadequate (e.g. because it has a different meaning in cases with CC and

Table 2. ORs[a] and 95% CIs[b] for squamous cell carcinoma of the cervix according to OC use, parity, smoking, HSV-2 and *Chlamydia trachomatis* serology, and by different models in the pooled analysis of IARC case-control studies

	All women	All women HPV-adjusted	HPV-positive women
	OR (95% CI)	OR (95% CI)	OR (95% CI)
OC use (years)			
Never	1.0 (0.9–1.1)	1.0 (0.8–1.2)	1.0 (0.8–1.3)
1–5	1.0 (0.9–1.2)	0.8 (0.6–1.1)	0.8 (0.6–1.0)
5–9	1.3 (1.1–1.6)	1.4 (0.9–2.0)	2.4 (1.3–4.5)
≥10	1.6 (1.3–1.9)	1.6 (1.1–2.3)	2.6 (1.4–4.6)
No. of full-term pregnancies			
1–2	1.0 (0.8–1.2)	1.0 (0.8–1.3)	1.0 (0.7–1.4)
3–4	1.5 (1.3–1.7)	1.5 (1.2–1.8)	1.4 (1.1–1.9)
5–6	1.8 (1.6–2.2)	1.8 (1.4–2.4)	1.6 (1.2–2.2)
≥7	2.1 (1.8–2.5)	1.7 (1.3–2.3)	2.2 (1.6–3.2)
Smoking			
Never	1	1	1
Ever	1.6 (1.3–1.9)	2.4 (1.8–3.2)	2.1 (1.3–3.3)
HSV-2			
Negative	1	1	1
Positive	1.8 (1.5–2.3)	1.5 (1.1–2.2)	1.9 (1.2–2.8)
C. trachomatis			
Negative	1	1	1
Positive	2.1 (1.7–2.6)	1.8 (1.3–2.5)	1.9 (1.3–2.8)

CI, confidence interval; HPV, human papillomavirus; HSV, herpes simplex virus; OC oral contraceptive; OR, odds ratio.
[a] Adjusted for age, centre, education, parity, age at first intercourse, sexual partners, pap smears, smoking, and OC use.
[b] Confidence intervals for OC use and number of full-term pregnancies are floating confidence intervals.

control women); or (2) HPV infection does not confound or modify the associations observed between CC and OC use, number of full-term pregnancies and smoking.

4 HPV Infection in Healthy Women

The incidence rates of CC vary more than ten-fold worldwide. Even after excluding countries where screening programmes have contributed to lowering rates, CC incidence ranges between less than 10/100,000 women in some parts of China, North Vietnam and Kuwait to more than 35/100,000 in Sub-Saharan Africa and some areas in India and Latin-America (Parkin et al. 2002). It is unclear to what extent such variation is attributable to differ-

ences in HPV prevalence at a population level. IARC has, therefore, promoted a series of population-based surveys of the prevalence of HPV DNA and serum IgG against HPV virus-like particles (anti-VLPs) in different parts of Latin America, Asia and Africa and in Spain and Italy. Type-specific HPV prevalence data have become even more important in the light of recent developments in prophylactic vaccines against specific types of HPV.

Similar prevalence surveys among random samples of women drawn from the general population have been completed and reported in South Korea (Busan, Shin et al. 2003), Thailand (Songkla in the South and Lampang in the North, Sukvirach et al. 2003), Vietnam (Hanoi and Ho Chi Minh City, Anh et al. 2003), Argentina (Cordoba, Matos et al. 2003), Colombia (Bogota, Molano et al. 2002a; Molano et al. 2002b; Molano et al. 2003a; Molano et al. 2003b), Mexico (Morellos State, Lazcano-Ponce et al. 2001), Nigeria (Thomas et al. 2004) and Spain (de Sanjosé et al. 2003). Additional surveys have been started in Turin, Italy; Santiago, Chile; Ambillikai, India; and Kampala, Uganda. Questionnaire information, and samples of exfoliated cervical cells and blood were collected. Type-specific prevalence of HPV DNA from cervical cells was analysed using GP5+/6+ primers, whereas anti-VLPs for HPV 16, 18, 31, 33 and 58 and HSV-2 were assessed using enzyme-linked immunosorbent assay. It is important to bear in mind that it turned out to be very difficult, notably in Asia, to perform pelvic examinations on unmarried/virgin women. The following findings are, therefore, truly representative of HPV DNA prevalence among married/sexually active women in the 15- to 65-year age range.

Korea. Overall HPV prevalence among 863 sexually active women was 10.4% for HPV DNA and 19.8% for anti-VLPs. The HPV types found most frequently were HPV 70, 16 and 33. The concordance between HPV DNA and anti-VLPs at an individual level was modest, but risk factors for the two HPV markers were similar. Risk factors for detection of HPV DNA or anti-VLPs were: number of lifetime sexual partners (OR for $\geq$4 vs 1=3.5 and 5.4 respectively), seropositivity for HSV-2 antibodies (OR=2.6 and 2.5, respectively), and being single or divorced. HPV DNA (but not anti-VLPs) was elevated among women whose husbands were thought by their wives to have extra-marital sexual relationships and those who had undergone a vasectomy.

Thailand. 1,035 women from Lampang, in the North, and 706 from Songkla, in the South were studied. HPV DNA and anti-VLPs were more common in Lampang (8.0% and 29.2%, respectively) than in Songkla (3.8% and 10.9%, respectively), in agreement with a North-South gradient in CC incidence in Thailand. The most common HPV types were HPV 16, 52, and 70. Risk factors for HPV infection were young age (<25 years, OR=2.5), HSV-2 seropositivity (OR=2.1), and a husband's extra-marital sexual relationships

(OR=2.1). Risk factors did not differ between high- and low-risk types and women below and above 45 years of age.
Vietnam. 922 women from Ho Chi Minh City and 994 from Hanoi were studied. HPV DNA prevalence was 10.9% and 2.0%, respectively. The most common types were HPV 16, 58 and 18. The major risk factors for HPV DNA detection were indicators of sexual habits, most notably the presence of HSV-2 antibodies (OR=2.4), nulliparity (OR=3.0) and current use of OCs (OR=3.2). Women in Hanoi showed the lowest HPV prevalence found so far in HPV surveys. In contrast to other populations, no HPV peak was detected in young women.

Argentina. In all, 987 women were studied. The prevalence of HPV DNA among sexually active women was 17.7%. The most common types were HPV 16, 35 and 18. Among women below age 45 the main risk factors for HPV detection were increasing lifetime number of sexual partners (OR=3.0; 95% CI: 1.9–4.8 for $\geq$3 vs 1), and severe vaginal discharge. OC use was associated with a significant reduction in HPV detection. None of these risk factors were associated with infections in women above age 45.

Colombia. In Bogota, 1,859 cytologically normal women were studied. The overall prevalence of HPV DNA was 14.8% and the commonest types were HPV 16, 58 and 56. There was a positive association between HPV detection and age less than 20 years (OR vs 35–44 years=9.6), three or more sexual partners (OR=2.1) and OC use (OR=1.4). In women below age 25, high education and intercourse with casual partners were associated with infection risk.

A subset of 227 women in Bogota with normal cytology, but positive for HPV DNA at study enrolment and at least one follow-up visit was studied (Molano et al. 2003a). The aim of the analysis was to search for determinants of HPV infection clearance. Results indicated that infections with HPV 16 (hazard ratio=0.6; 95% CI: 0.4–0.8), but not with high-risk HPV types other than HPV 16, had a significantly lower clearance rate than infections with low-risk types. Infections with a single type and multiple infections had similar clearance rates. There was an indication that parous women cleared HPV infections less efficiently than nulliparous women, but OC users may have less persistence of infection.

Nigeria. We interviewed and obtained a sample of cervical cells from 932 sexually active women aged 15 years or older from Idikan, an inner-city area of Ibadan, Nigeria. Thirty-one different HPV types were identified for an HPV prevalence of 26.3% overall. High-risk HPV types predominated, most notably HPV 16, 31, 35 and 58. One-third of infections involved more than one HPV type. Contrary to most populations studied so far, HPV prevalence was high not only among young women, but also in middle-aged and old

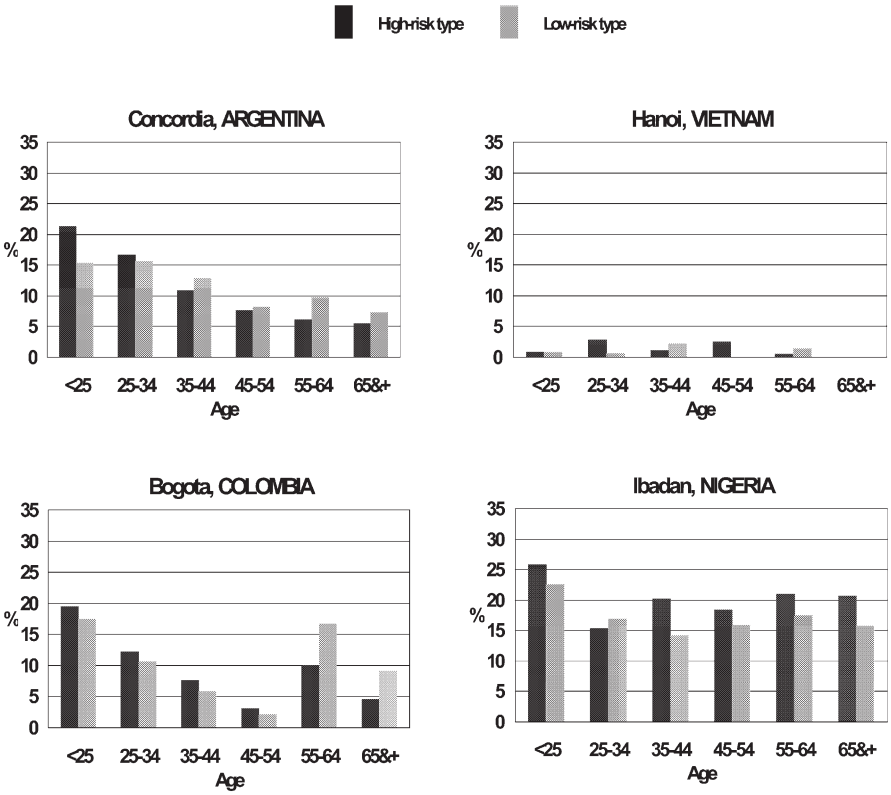

Fig. 3. Prevalence of cervical human papillomavirus HPV DNA in healthy women by age and HPV types: IARC, 1995–2002

women. Illiterate women (OR=1.7; 95% CI: 1.1–2.5) also showed increased HPV-positivity. Associations were found also with HSV-2 antibodies (OR=1.6; 95% CI: 1.1–2.1) and a husband's extra-marital relationships (OR=1.6; 95% CI: 1.0–2.6). High prevalence of HPV in all age groups may be a distinctive feature of populations where HPV transmission continues into middle age and CC incidence is very high.

Figure 3 shows the distribution of HPV infection (all types) in four locations of IARC surveys. They represent good examples of the four main age patterns that we and other investigators (Herrero et al. 2000) have identified worldwide:

1. The most common (e.g. in Argentina, Matos et al. 2003) includes a peak of HPV prevalence below age 25, when women start being exposed to HPV infection through first sexual intercourse.
2. In some countries, notably in Latin America, (e.g. in Colombia), a second increase in HPV prevalence is sometimes found among middle-aged women.

3. Steady low prevalence can be found in all age groups in some countries (e.g. North Vietnam) where HPV infection is rare.
4. Steady high prevalence can be detected in all age groups in the countries at highest risk for CC (e.g. Nigeria).

The extent to which differences in sexual habits, ability to clear HPV infection, and cohort effects account for this variation in the age curve of HPV prevalence in different world populations is not yet well understood.

University Students in Korea. In order to introduce a prophylactic vaccine against HPV, it is essential to understand the age at which women and men first acquire the infection, but very little is known about the prevalence of HPV infection among young adults in Asia. Therefore, we invited 900 female and 600 male students in Busan, South Korea to participate in a survey that included self-collection of vaginal cells or physician-performed collection of genital exfoliated cells in males (Shin et al. 2004). The prevalence of 25 different HPV types was evaluated, using a PCR-based detection and genotyping assay, among 672 female (median age=19) and 381 male students (median age=22).

HPV DNA was detected more frequently among female (15.2%) than among male (8.7%) students. High-risk types predominated in both genders. HPV prevalence was 38.8% among sexually active female students and 10.6% among sexually active male students. Being a current smoker (OR=3.8; 95% CI: 1.7–8.3) and reporting multiple sexual partners (OR for $\geq$4 vs 1 partner=6.9; 95% CI: 2.8–16.8) were the strongest risk factors for HPV detection in females. Among males, associations with sexual habits were in the same direction as in females, but they never attained statistical significance. Circumcision was frequently reported by males but did not seem to protect against HPV infection.

Young women in South Korea start sexual intercourse relatively late (median age=18) but HPV prevalence quickly rises to levels comparable to those found in college students in the United States (Winer et al. 2003) and Northern Europe (Woodman et al. 2001). The high participation of our study suggests that trials on new vaccines against HPV may be feasible among university students in South Korea (Shin et al. 2004).

5
HPV Vaccines in the Prevention of CC

Prophylactic vaccines to prevent HPV infection and therapeutic vaccines targeted at the HPV tumour antigens are in clinical trials (Galloway 2003). Early findings from a trial from Merck (Koutsky et al. 2002) have shown that prophylactic vaccines against HPV have a very high efficacy, at least in the

short term. It has provided added urgency to the evaluation and deployment of suitable HPV vaccines in areas of the world where CC is most common and particularly where screening programmes will be very difficult to setup and maintain (Franceschi et al. 2003b; Plummer and Franceschi 2002).

In principle, after HPV vaccines have become available for large-scale use, their effectiveness as a strategy for CC control can be measured either by monitoring secular trends in CC incidence or by conducting randomised trials. The former approach has greatly contributed to establishing the efficacy of the vaccine against hepatitis B virus (HBV) in the prevention of primary liver cancer (Huang and Lin 2000), but it is unlikely to provide convincing evidence of effectiveness. In fact, CC rates are subject to strong secular trends that are independent of intervention measures. A few phase III trials of HPV prophylactic vaccines are now being started by different pharmaceutical industries, but they are very expensive studies involving frequent and complicated investigations. It would be important, however, to start simpler trials designed to demonstrate the effectiveness of an HPV vaccine in field conditions, i.e. in developing or intermediate countries which suffer the major burden of mortality from CC, as soon as possible. Such trials may capture a difference in the most severe, and rarest, pre-invasive cervical lesions (i.e. the real target of any HPV vaccine) over a prolonged follow-up (20 years at least).

Relevant trials could be conducted in any country, but in order to accelerate the adoption of HPV vaccination in the populations that need it most, priority should be given to developing countries, most notably Asia, where 50% of worldwide CC cases occur. These trials should be large and of long duration (20 years at least) in order to capture a difference in the most severe pre-invasive cervical lesions, which take many years to develop. Consequently, the design must be simple and cost-effective. The trial design may be summarized as follows:

- Vaccination of young women before they become exposed to HPV (i.e. in conservative societies, before marriage).
- Long-term "opportunistic" monitoring of serious side effects (e.g. through monitoring of hospital admissions).
- No measurement of cervical outcomes until the subjects are of sufficient age to benefit from screening.

A fundamental difference between these trials and the phase III trials currently being conducted is that there are no plans for early gynaecological examination for the purposes of the trial only. The lack of early examination is beneficial to the participants since women under the age of 30 have a very low risk of CC and cervical intraepithelial neoplasia (CIN) III, but may undergo over-treatment of transient HPV infections which may manifest themselves clinically as CIN I. A corollary of the lack of early gynaecological ex-

aminations is that a population-based screening programme must be in place for the study participants in due time (e.g. when they reach the age of 30–35). This will ensure two things: first, that the control group receives an adequate standard of care, and second, that an outcome measurement, at the age when CIN III peaks, is taken for as many subjects as possible. A second requirement for this study is the ability to follow-up subjects over a long period, and in particular to accurately identify the treatment group decades after randomisation.

Different locations for such trials are conceivable, but it would be desirable that they include (and provide some information about) very different populations. We have considered the possibility of implementing this design in two areas: a rural area in Southern India and an urban one in South Korea, which are presented here in respect to their different strengths and weaknesses (Plummer and Franceschi 2002; Franceschi et al. 2003b).

Some areas of Southern India have a very high risk for CC. The age-standardised rate for Chennai (Madras) was 30.1 per 100,000 in the late 1990s (Parkin et al. 2002). This makes it an attractive location for CC prevention trials. Long-term follow-up of subjects is probably not feasible in an urban setting due to the very marked population movement in developing countries. In a rural setting, the most appropriate study design is a community intervention study with randomisation by village. This provides a simple mechanism for identifying the treatment group of a subject many years after randomisation, since it suffices to know a subject's place of birth. A cluster randomised trial also presents the only feasible opportunity to randomise males and thus to evaluate the usefulness of vaccinating both sexes. Men very rarely develop severe HPV-related diseases (e.g. cancer of the penis and the anus). They may therefore not respond to individual randomisation, but may agree to do so in the context of a community intervention. The efficacy of male vaccination will have to be evaluated in terms of its contribution to the decrease of pre-cancerous lesions in women.

To this extent, "discordant" couples (i.e. couples where only the husband or wife is vaccinated) will be most informative. The target population for a trial in southern India is unmarried women, i.e. women below age 19, as very early marriage is still common in rural India. The sample size of this trial should include approximately 80,000 women. These calculations are based on an assumption of vaccination at age 15 with a 10-year interval between vaccination and the first screening examination, with CIN III as an endpoint. The incidence rates for CIN are imputed from the incidence rates for ICC by assuming that CIN III occurs 5 years earlier and at a rate three times higher (hence 2/3 of CIN III will regress without progression to cancer). Loss to follow-up has not been factored into these calculations, since a realistic assessment of the rate of loss depends on the specific design of the study. However, in order to take into account loss to follow-up, a possible decline in CC incidence and the overwhelming difficulty of replicating com-

munity-based intervention trials, the target power of the study needs to be very high. These calculations use a target power of 99%, under the assumption that the chance to be able to replicate such huge trials is minimal.

South Korea is no longer considered a developing country but, on account of the recency of the economic and medical development, is still an intermediate-risk country for CC. The age-standardised rate for Busan county was 21.1 per 100,000 in the late 1990s, which is two- to four-fold higher than in most Western countries (Parkin et al. 2002). However, a few characteristics of the population and the health system in South Korea may be greatly beneficial to the implementation of a clinical trial. Recent IARC HPV surveys (Shin et al. 2003, 2004) have shown that infection with HPV occurs later in Busan than in Western countries, and it may thus be possible to offer HPV vaccines to women in the 18- to 22-year age range. A majority of young women in this age range in South Korea attend higher education and may thus be readily contacted, individually randomised, and offered the vaccine in university health facilities that have shown themselves to be open to collaboration in a study by IARC (Shin et al. 2004).

Most importantly, each person in South Korea has a unique national identity number, which will greatly facilitate long-term follow-up. Finally, the South Korean government has a strong commitment to implementing population-based screening programmes in the near future, including cytological screening for the prevention of CC in women aged 30 or older. Thus, the follow-up process in such a large trial of a prophylactic vaccine against HPV may benefit from the present development of national screening programmes.

6 Discussion

Many challenges remain in respect to the efficacy and efficiency of prophylactic vaccines against HPV (Galloway 2003).

Despite successful results in animal models and humans, it is not clear which elements of the human immune system are important in preventing or resolving HPV infections. High levels of circulating neutralizing antibodies induced by VLP vaccines have been shown to provide a high degree of protection against incident and persistent infection, but the duration of the protection is unknown. Ways to enhance mucosal immunity and cell-mediated immunity are being evaluated (e.g. intra-nasal or oral immunization, Galloway 2003).

Obviously, so-called chimaeric vaccines (i.e. vaccines able to prevent HPV infection and induce clearance of the infection at an early stage) would be a more preferable solution. They would substantially anticipate the benefits of vaccinations that, in the case of prophylactic vaccines, would take three or

four decades to become apparent. In fact, therapeutic vaccines may benefit not only sexually inexperienced women who have not yet been infected by HPV, but also older women who may be already harbouring HPV-related cervical lesions.

Furthermore, while safety and efficacy are essential for a vaccine, ways to reduce costs and increase vaccine coverage must also be considered. They will include formulating an oral vaccine, creating a stable vaccine that does not require an expensive cold-chain and/or one that can be produced in developing countries. Finally, it is worth bearing in mind that the sexually transmitted nature of HPV infection will probably enter into the public debate, as will the gender issue (i.e. the current restriction of current HPV vaccine to women as a target population). While the efficacy and opportunity of vaccinating boys as well as girls will have to be evaluated, ways to tackle an open discussion on HPV infection will have to be found in developing as well as developed countries.

The challenges above notwithstanding, HPV vaccine development holds great promise for reducing the mortality and morbidity of cervical neoplasia in the world's women.

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Health Economics in the Genomic Age

Thomas D. Szucs

Institute of Social and Preventive Medicine, Gloriastrasse 18a, 8006 Zurich, Switzerland
thomas.szucs@ifspm.unizh.ch

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Abstract Health economics has experienced a substantial rise within the healthcare industry over the past few years. Several disciplines have developed new techniques to evaluate the economic impact of pharmaceuticals in clinical care. Clinicians, pharmacists, economists, epidemiologists, and operations researchers have contributed to this field. Given the economic reality that resources are limited and needs and expectations are not infinite, medical economists try to find solutions on how these resources can be allocated optimally, to maximize the production of health or what society perceives as health. Health economists differentiate *allocation* efficiency and *production* efficiency. From the perspective of a health insurance plan allocation efficiency is reached when those drug classes or clinical programs are covered that will produce most health per expenditure. This requires a common monetary metric of health gains across the broad spectrum of diseases, conditions, and health outcomes. Once it is decided to cover a specific treatment or clinical program, economists try to identify the most cost-effective product within a class of comparable choices using cost-effectiveness and cost-utility analyses. Both allocation and production efficiency are two critically important concepts for the

economic success of biotech products. This article will provide a rationale why health economics is critically important for the future of healthcare and explains fundamental economic tools for evaluating products and services with special emphasis on gene-derived technologies.

1
Scarcity: The Driving Force for Rational Allocation of Resources

The fundamental aim of any healthcare system is to maximize the health and welfare of its population. Because resources will always be scarce in relation to the healthcare needs, a series of choices must be made. Decision-makers responsible for allocating resources need to prioritize between competing uses in order to maximize benefits (or health gains) under budgetary constraints [1, 2]. Prioritization takes place on different levels of the healthcare system. On the health authority level and senior health plan management level, planners decide on the specialty and service mix they wish to purchase for their beneficiaries with the goal to optimize resource allocation to health programs. This allocation process is often a mix of rational thinking and a political agenda [3]. In the increasingly privatized hospital market, decisions are made about the purchase of medicines and equipment with the goal to maximize profits. At the level of the individual physicians, prioritization is increasingly influenced by medical audits and other forms of peer review, with more clinical guidelines. These constraints usually impose the payers view on the economics of medicines upon individual physicians. This is not to say that it is generally bad to impose such constraints on the health delivery system, as long as such decisions are based on solid evidence. Pharmacoeconomics can help all parties to make informed choices.

1.1
Pharmacoeconomics and the Difference Between Biotech Products and Traditional Pharmaceuticals

Without simplifying the wide field of biotech products too much, there appear to be several critical differences between traditional small molecule pharmaceuticals and biotech products: (1) Biotech products are often more expensive than comparable traditional pharmaceuticals if they are available. (2) Many biotech products are targeting small- to moderate-sized patient populations for which they in some instances provide the only medication that substantially improves the underlying condition. (3) Although the knowledge in molecular biology and immunology is rapidly increasing, the physiologic and pathophysiologic effects of gene-derived products may not be completely understood, which may require additional resources to characterize and manage patient risks.

Table 1. Questions that an economic study can answer

Among many others, the following questions are most important for health economic research:
Which technology should be included in a limited list of services that can be covered/provided?
Which of several technologies is the most cost-effective even if it means higher upfront costs?
What are the relative costs and benefits of comparable technologies?
What is the cost per quality adjusted year of life saved by using a specific clinical strategy?
What effect will the results of a particular technology have on a patient's life expectancy and quality of life?

Because of their high price, payments of genomic-derived products will in most instances be through third-party payers. For the reasons described above, drug benefit plans—whether privately or governmentally funded—increasingly demand economic evaluations for coverage decisions [4].

Despite some methodologic challenges that will be described below, economic analyses are and will therefore be critical to the rational allocation of resources by manufacturers, providers and payers. Economic studies may provide answers to many but not all questions (Table 1). The force to rationally use new expensive technologies will require increased efforts in health technology assessment, a better appraisal of patient preferences, and more rigorous pharmacoeconomic analyses.

1.2 Cost Containment and the Evolution of Managed Care in Europe

All European countries have common objectives concerning health care, most importantly the provision of quality health care at an affordable cost. They also face similar problems: a dramatic demographic change (growth of the number and proportion of elderly people in their populations), a change in disease patterns (a shift towards more chronic and multifaceted illness), the continuing development of new and expensive health technologies and societal changes with increased expectations. These factors require new approaches by the healthcare industry to manage its cost. Because of their wide-ranging potentials but also because of their costs, biotech products will be granted particular scrutiny.

In the context of increasing cost containment, many European healthcare systems have adopted some form of "managed care" [5]. It is noteworthy that there has been a tremendous change in the organizational structure of managed care plans or networks over the past two decades. Additionally, there has also been considerable confusion and controversy over the definition of a managed care organization (MCO) by stakeholders and healthcare

Table 2. Factors contributing to the diffusion of managed care in selected European countries

	Germany	France	Italy	Spain	UK
Economic environment	✓	✓	✓	✓	✓
Socioeconomic factors	✓	✓	✓	✓	✓
Governmental regulatory framework	–	✖	✖	✖	✓
Health care structure	✓	✖	✖	✖	–
Consumer attitudes	✓	✖	✖	✖	✓

analysts. The Institute of Medicine has provided a good rough-and-ready definition whereby managed care should serve to: (1) control costs through improved efficiency and coordination; (2) reduce unnecessary or inappropriate utilization; (3) increase access to preventive care; (4) and maintain or improve the quality of health care [6].

Several factors contribute to the diffusion of managed care in Europe. The most important are (1) the overall economic environment, (2) socioeconomic factors, (3) the prevailing governmental framework, (4) the healthcare structure and (5) consumer expectations [7, 8]. The extent to which these contribute to managed care are displayed in Tables 2 and 3.

An important aspect to watch is the activities of the British National Centre of Clinical Excellence (NICE). The aim is that NICE should identify best practice and advise doctors and nurses on which treatments work best for patients and are cost-effective. The functions of NICE are (1) to appraise all new technologies for their clinical and cost-effectiveness and advise whether they

Table 3. Some factors contributing to the diffusion of managed care in selected European countries

	Who pays for health care?	Who is agent for the delivery of care?	What is the key consumer philosophy?
Germany	Government: 60% Patient/employer: 33%[a]	Health insurance Private insurers	Quality of care
France	Government: 75% Patient/employer: 25%	Government Mutual private insurers	Freedom of choice
Italy	Government: 75% Patient/employer: 25%	Public health authorities	Free health care provision
Spain	Government: 96% Patient/employer: 4%	Public health authorities Small input from private insurers	Free health care provision
UK	Government: 96% Patient/employer: 4%	Public health authorities Small input from private insurers	Free health care provision Strong loyalty to the NHS

NHS, National Health Service.
[a] Remaining 7% comes from other sources (e.g. charitable organizations, foundations).

should be in routine use in the National Health Service (NHS) or not, (2) to disseminate clinical guidelines based on clear scrutiny of the scientific literature in a form that is practical and useful to health professionals and (3) to develop and promote clinical audit. Interestingly, almost 5 years after inception most technologies have not been rejected due to unfavourable health economic data, but rather due to missing or insufficient clinical trial data. Among technologies so far rejected are the routine extraction of wisdom teeth, digital hearing aids (more data necessary), laparoscopic surgery of colon cancer, autologous cartilage transplantation, docetaxel as a first-line Rx for breast cancer and interferon beta and glatiramer acetate for multiple sclerosis.

Many European countries observe NICE very closely and have adopted much of its policy recommendations.

Based on experiences in the United States, it is often argued that MCOs can only survive if they (1) look beyond profits, (2) provide appropriate standards of care, (3) support teaching, (4) support research, (5) support care for the poor and (6) grant sufficient physician autonomy [9].

In conclusion, many aspects of managed care are here to stay and will further develop in European healthcare systems that are currently financed and run by governmental or quasi-governmental agencies. The economics of biotech products will be particularly scrutinized by MCOs, and negotiating reimbursement arrangements for expensive new biotech products will be critical for the growth of biotech industry [10]. However, in a competitive healthcare market the same organizations will increase their competitiveness when carefully investing in new technologies that may provide treatment for rare but grave diseases and sometimes save costs.

To fully understand the relations between payer and providers on one side and biotech industry on the other requires an understanding of the fundamentals of pharmacoeconomics as the basis for decision-making in modern managed care environments.

2 Some Health Economic Evaluation Techniques

Economic evaluation is a method to assess and evaluate costs of health interventions and the health outcomes associated with these interventions. Its central function is to show the relative value of alternative interventions for improving health. Analyses provide information that can help decision-makers in a variety of settings to weigh alternatives and decide which one serves their programmatic needs best. Such analyses are just one of the many factors on which the ranking of provided services is based. The role of economic evaluation is to supplement these qualitative factors by providing standardized, quantitative estimates of the likely increment in cost per unit of health benefit achieved.

A growing demand for cost-effectiveness and economic evaluation [11] is not a threat to patients: properly used it would help us to provide more cost-effective services to more beneficiaries, which ultimately will extend more lives and improve the quality of more lives. Nor should the application of its methods constitute a threat to practitioner's freedom to exercise their best professional judgement in individual cases or to the patients' rights to autonomy. But these freedoms and rights can best be exercised only in the presence of the sort of information required to develop a knowledge-based culture of critical evaluation in medicine.

2.1 The Major Components of an Economic Evaluation

All economic studies investigate the balance between inputs (the consumption of resources) and outcomes (improvements in the state of health of individuals and/or society).

2.1.1 Inputs (Costs)

Although the unit price of a drug is often a prime factor in decision-making, economic outcomes research provides a more comprehensive interpretation of cost. This is accomplished by determining the overall cost of a given diagnostic or therapeutic process from the initiation of diagnosis until a final outcome is achieved. The approach used by health economists is to consider costs as opportunity costs, i.e. they define a cost to be the consumption of a resource that could otherwise be used for another purpose. Once the resource has been used, the opportunity to use it for another purpose is lost. The various types of costs can be grouped under the following categories:

- Direct medical costs
- Direct non-medical costs
- Indirect costs

2.1.2 Direct Medical Costs

Interpretations of what belongs in each of these categories varies in the economic literature. Direct medical costs are defined as those resources used by the provider in the delivery of medical care. As an example, direct medical costs for a hospital include:

- Drugs
- Laboratory tests

- Medical supplies
- Use of diagnostic equipment—magnetic resonance imaging, computerized axial tomography (CAT) scans, and X-ray, for example
- Medical staff time for personnel such as physicians, nurses, pharmacists, physical therapists, and laboratory technicians
- Room and board—the cost of supplies, equipment, and personnel required for routine patient-related services such as food, laundry, and housekeeping

These are examples of costs that can be directly related to the care of patients. Other costs of operating a hospital include plant maintenance and repairs, utilities, telephone, accounting, legal fees, insurance, taxes, real estate costs, and interest expense. In general, most economic studies do not factor general operating costs into the dollar value assigned to the cost of resources expended for a given medicine.

Length of stay is an important cost factor from a hospital's perspective, especially when payment is determined by diagnosis related groups (DRGs). Hospital costs such as room and board are directly tied to increasing length of stay, regardless of the reason. The cost of laboratory tests, supplies, and medical staff time vary with the medical condition being treated, but are multiplied by length of stay.

2.1.3 Direct Non-medical Costs

Economic literature generally defines direct non-medical costs as out-of-pocket expenses paid by patients for items outside the healthcare sector. This category includes such costs as:

- Travel to and from the hospital, clinic, or doctor's office
- Travel and lodging for family members who live elsewhere
- Out-of-pocket contributions for domestic help or home nursing services
- Treatments that are not considered main stream and not covered by third-party payers

Although these costs are generally classified as "non-medical", they are directly related to the underlying condition, they must be paid by patients, and often constitute a substantial proportion of medical expenditures. What makes them "non-medical" is that they are not costs incurred by the healthcare provider, and are somewhat difficult to measure. For example:

- A patient's inability to afford competent follow-up care at home may result in poor compliance with drug therapies and eventual treatment failure. This may lead to additional hospital stays or office visits, which affect the provider's bottom line.

- High transportation costs may lead to missed appointments for necessary follow-up visits, which can result in deterioration of a patient's medical condition and increased treatment costs for the provider.
- Unpaid assistance by family members in providing home healthcare.

Even though these costs may not be directly incurred by the provider, they can be used in selling situations by making the provider aware of their potential economic impact. It may also be possible to use these costs to encourage payers (e.g. employers, insurance companies) to discuss the use of a more cost-effective test with the healthcare provider.

2.1.4 Indirect Costs

One definition of indirect costs is the overall economic impact of illness on the patient's life. These include:

- Loss of earnings due to temporary, partial, or permanent disability
- Loss of income to family members who forfeit paid employment in order to remain at home and care for the patient

Like direct non-medical costs, indirect costs are real to the patient, but abstract to the provider—but may impact the provider's direct medical costs. For example, patients who cannot earn income may not be able to pay their bills—including medical bills. Economic hardship may result in poor compliance with drug therapies as patients reduce doses or fail to refill prescriptions in order to save money. The medical provider may have to bear the additional costs of managing complications. Economic hardship may also result in missed follow-up appointments leading to the same types of problems for providers as described previously with direct non-medical costs.

2.2 Types of Formal Economic Evaluations

The most common methods employed by health economists are classical research designs such as cost-*benefit*, cost-*effectiveness* and cost-*utility* analyses.

2.2.1 Cost-Benefit Analyses

As applied to healthcare, cost-benefit analysis (CBA) measures all costs and benefits of competing therapies in terms of monetary units. For individual therapies net benefits can be calculated by simply subtracting the costs form

the benefits. If net benefits are positive, the intervention is worth undertaking from the economic perspective. Differences in net benefits of competing therapies or programs (e.g. intensive care unit versus new diagnostic equipment or preventive measures) can in theory be readily compared for an efficient allocation of resources. However, CBA requires assigning monetary values to life and to health improvements measured in a variety of dimensions including quality of life. This presents equal-benefit issues as well as substantial measurement problems. For these reasons, CBAs have not been widely used for evaluating drug therapies and the optimal allocation of resources [12].

2.2.2 Cost-Effectiveness Analyses

Cost-effectiveness studies measure changes in the cost of all relevant treatment alternatives, but measure the differences in outcomes in some natural unit such as actual lives saved, years of lives saved, events prevented or children immunized. Cost-effectiveness analysis (CEA) can also be applied equally to cases where the outcome is in terms of quality of life. CEA is useful in comparing alternative therapies which have the same outcome units, e.g. increase of life expectancy, but the treatments do not have the same effectiveness, in that one drug may lead to greater gains in life expectancy than an other. The measure compared is the cost of therapy divided by the units of effectiveness and, hence a lower number signifies a more cost-effective outcome.

This type of study has the advantage that it does not require the conversion of health outcomes to monetary units and thereby avoids equal benefit and other difficult issues of the valuation of benefits. It is therefore among the most frequently used tools to identify the most efficient strategy to reach a specific health target (production efficiency). It has the disadvantage of not permitting comparisons across programs (see CBA). In other words, the cost-effectiveness of a drug that aims to reduce infant mortality cannot be compared with a drug designed to improve functional status of senior citizens [13]. Rather, the value for money of an intervention is assessed by comparing the cost-effectiveness ratio with a threshold ratio, which corresponds to the decision-maker's willingness to pay for health gain. Moreover, it cannot compare outcomes measured in clinical units with quality of life measures.

2.2.3 Cost-Utility Analyses

Cost-utility analysis (CUA) compares the added costs of therapy with the number of *quality-adjusted life years* (QALY) gained. The quality adjustment

weight is a utility value which can be measured as part of clinical trials or independently. The advantage of cost-utility analysis is that therapies that produce improvement in different or multiple health outcomes can be more readily compared. The QALY measure is calculated by multiplying the length of time in a specific health state by the perceived utility of that health status (on a scale from 0 to 1). Many analysts are more comfortable with QALYs as a measure of the consequence of medical care than with the monetary units.

CUA is an improvement over CEA because it can measure the effects of multiple outcomes (such as the impact of vaccines on both morbidity and mortality or the impact on both pain and physical functional status). Cost per QALY can be computed and compared across alternative treatment scenarios. This is especially useful when only a limited and fixed budget is available and allocation among competing programs/therapies has to be optimized. A comprehensive overview of QALY estimates has been published by Tengs et al. [14].

2.3 Using Pharmacoeconomic Analyses for Decision-Making by Drug Benefit Plans

The use of economic evidence in decisions about medical technologies has become more widespread internationally. In countries such as Australia, the UK, Denmark, Finland, Norway, Portugal, Belgium, the Netherlands and some Canadian provinces, value for money is a consideration in purchasing and pricing decisions. Of these countries, Australia, Finland and Portugal have a national requirement for evidence on cost-effectiveness before reimbursement of prescription drugs or other health technologies [15].

An extremely important aspect is the fact that the quality of pharmacoeconomic studies is increasingly being scrutinized by policymakers and institutions [16]. Since pharmacoeconomics is a fairly new field, many aspects are still unstructured compared to the highly standardized guidelines for good clinical practice (GCP) for the conduct of randomized controlled trials.

Checklists have been developed in order to facilitate the appraisal of the quality of economic analyses and assist in minimizing possible bias [17]. These criteria are also being increasingly used in the peer review process by many biomedical journals and discussed accordingly.

2.4 Collecting Economic Data Alongside Phase III and IV Clinical Trials

It may be practical and cost-effective to gather certain data during a clinical trial, which is otherwise designed to measure the efficacy and adverse effects

of a compound under study. However, generating economic data in phase III is not without some controversy.

There are some researchers who point out that clinical trials measure efficacy—the performance of the drug in controlled circumstances. However, as the name cost-*effectiveness* suggests, such studies are aimed at determining the costs and benefits under routine clinical practice conditions. Whereas, drug regulatory authorities like the FDA and the European Medicines Evaluation Agency (EMEA) require the use of placebos as comparators in approval trials, this rarely provides useful information for economic analyses, particularly for the measurement of costs.

At the time phase III trials begin, a new compound may be compared against the existing "gold standard". However, by the time the new product gets to market, there may be other products which are more appropriate comparators but which were not on the market when the trials started. This situation is compounded by the economist's view that the comparator product should be the one which is most likely to be replaced in practice. Most drug benefit plans recognize this limitation and offer conditional reimbursement approval. The period of conditional approval should be used for updated economic assessment.

The process of collecting costs during clinical trials merits special attention. There are certain costs incurred on the patient's behalf as a result of procedures which would not normally accrue. These costs, called "protocol driven costs", must be isolated and not included in the analysis. One leading researcher states that, in her experience, this did not cause serious problems because these same added costs were being incurred in both arms of the trial and hence would cancel each other out.

2.5 Health Economic Studies in the Hospital Sector

In Europe the increasingly privatized hospital sector currently represents a market that is the least restrained by governmental agencies and subject to the most competition compared to other sectors providing health care. With the increased use of modern biotech products in hospitals it is advisable to conduct pharmacoeconomic studies for the hospital sector.

The economic perspective within hospitals might differ considerably from the perspective of a social security system or the societal perspective. Hospital decision-makers are held accountable for maximizing operational profits while providing optimal care and retaining referring physicians by optimally allocating internal resources. For example, a new biotech therapy may involve once-a-day dosing rather than continuous intravenous administration, thus freeing up nursing time to pursue other activities [18].

Before performing such pharmacoeconomic studies, researchers must decide on the best effectiveness measure depending on the expected magnitude

of treatment effects and its measurability. A pharmacoeconomic protocol designed to observe the reduction of the number of days spent in hospital would not be effective in measuring the decrease in alternative drug consumption. On the other hand, a pharmacoeconomic study with the intended objective of measuring the reduced preparation time of an antibiotic would be completely meaningless if a medical practitioner knows that the side-effects of the comparator drug are different in term of costs and consequences.

2.6 Randomized Clinical Trials Versus Observational Studies

There is much criticism of randomized clinical trials, especially over the fact that randomized clinical trials do not represent routine care. This is not an important issue when studying the efficacy of a new product for the purpose of regulatory approval. However, pharmacoeconomic studies aim to assess the economic consequences of new technologies in general practice (Table 4).

In pragmatic randomized trials, therefore, a new compound is not evaluated against a placebo or a reference drug (gold standard), but against any treatment used in real medical practice to treat the target condition (usual care). The evaluation is not made on the basis of one criterion—the efficacy—but on the basis of a whole set of items, as in routine care.

Pharmacoeconomic researchers and health policy decision-makers prefer pragmatic trials that focus on a drug's effectiveness over classical, randomized, placebo-controlled, trials with highly standardized protocols that focus on efficacy.

Table 4. Major differences: clinical vs economic trial

Clinical trial	Economic trial
Controlled	Real life
Registration[a]	Reimbursement[b]
Strict protocol orders	"Do what you normally do"
Protocol-induced resource use	Real resource use
Compared to placebo or to "gold standard"	Compared to relevant practice
Avoid co-morbidities	Include co-morbidities
Limited time	Time should include all relevant costs and effects
Drop-outs not analysed	Drop-outs crucial
High internal validity, low external validity	High external validity, low internal validity

[a] Denotes approval of medication in a country.
[b] Denotes coverage of medication by healthcare payers (e.g. sick funds).

2.7 Potential Financial Impact of Pharmacogenetics

Pharmacogenetics as a diagnostic tool has the potential to decrease cost for healthcare purchasers by improving effectiveness and drug safety [19]. Prescribers and pharmacists would prescribe and dose medications they know to be effective for an individual with a certain genotype. A perfect pharmacogenetic test would enable the selection of a drug that could provide significant cost savings, an increase in the effectiveness of the initially prescribed therapy, a reduced number of physician visits, eliminating the cost of prescribing ineffective pharmaceutical products and eliminating avoidable toxicity. Healthcare payers may also impose specific requirements for drug product payment requiring diagnostic tests as a form of "prior authorization" to payment. Identification of a patient with a genotype that reduces the success of preferred therapy could place the patient at risk for denial of future coverage—an expansion of the challenges of "pre-existing conditions" to include the likelihood of drug effectiveness [20].

2.8 Anticipated Benefits of Pharmacogenetics

The advances in genomic medicine will ultimately change the way we treat patients with modern pharmaceuticals. Several benefits of pharmacogenetics will play a major role in potentially containing healthcare costs.

- More powerful medicines
  - Pharmaceutical companies will be able to create drugs based on the proteins, enzymes and RNA molecules associated with genes and diseases. This will facilitate drug discovery and allow drug makers to produce a therapy more targeted to specific diseases. This accuracy not only will maximize therapeutic effects but also decrease damage to nearby healthy cells. *More powerful medicines are a prerequisite for higher efficiency.*
- Better, safer drugs the first time
  - Instead of the standard trial-and-error method of matching patients with the right drugs, doctors will be able to analyse a patient's genetic profile and prescribe the best available drug therapy from the beginning. Not only will this take the guesswork out of finding the right drug, it will speed recovery time and increase safety as the likelihood of adverse reactions is eliminated. *This will ultimately lower drug costs.*
- More accurate methods of determining appropriate drug dosages
  - Current methods of basing dosages on weight and age will be replaced with dosages based on a person's genetics—how well the body processes the medicine and the time it takes to metabolize it. This will maximize the therapy's value and decrease the likelihood of overdose. *This will reduce the costs of titrating patients up to the most efficacious dosage.*

- Advanced screening for disease
  - Knowing one's genetic code will allow a person to make adequate lifestyle and environmental changes at an early age so as to avoid or lessen the severity of a genetic disease. Likewise, advance knowledge of a particular disease susceptibility will allow careful monitoring, and treatments can be introduced at the most appropriate stage to maximize their therapy. *This will decrease overall healthcare costs, because most diseases are less costly to treat in early phases*.
- Improvements in the drug discovery and approval process
  - Pharmaceutical companies will be able to discover potential therapies more easily using genome targets. Previously failed drug candidates may be revived as they are matched with the niche population they serve. The drug approval process should be facilitated as trials are targeted for specific genetic population groups—providing greater degrees of success. *The cost and risk of clinical trials will be reduced by targeting only those persons capable of responding to a drug*.

Clarifying the economic [21] and ethical issues [19] of pharmacogenetic screening is critically important for future growth.

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Screening for Cancer: Are Resources Being Used Wisely?

Robert M. Kaplan

Department of Family and Preventive Medicine, University of California,
San Diego, Stein Clinical Sciences Building, Room 240, Mail Code 0628,
La Jolla, CA 92093-0628, USA
rkaplan@ucsd.edu

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Abstract Cancer screening is commonly offered in order to detect tumors at an early, treatable stage. These efforts are highly advocated and widely accepted by the general public. However, there is conflicting evidence about the benefits of screening for breast cancer in pre-menopausal women, prostate cancer in older men, and colorectal cancer for both sexes. This paper examines cancer screening in relation to a disease reservoir hypothesis. There is a reservoir of undetected disease that can be found with more aggressive screening. However, much of the disease that is detected may be classified as pseudodisease because it will have no effect of life expectancy or health-related quality of life. Pseudodisease is defined as detectable disease that will never be clinically significant. A second concern about screening is that randomized clinical trials often show benefits of cancer screening for disease-specific endpoints but no benefit for total mortality. Further, screening for some cancers may significantly increase healthcare costs without enhancing population health status. Improvements in biomarkers and in screening methodologies will significantly increase the number of cancers detected. Future research is necessary in order to determine which population-based screening programs are the best use of public health resources.

There are at least three important approaches to tumor prevention. One approach involves interventions to reduce cancer exposures or to manipulate genetic predispositions so that neoplastic changes never develop. A second approach requires screening so that disease can be detected and treated early. The third approach involves treatment of established disease to prevent it from progressing further. Among these three approaches, screening and early detection have received the most attention. The American Cancer Society (ACS) and other groups around the world have launched long-standing campaigns to persuade the public that an aggressive approach to screening and medical care saves a significant number of lives. But, are the assumptions behind this belief correct? The belief that screening saves lives is built on a foundation of conflicting evidence. Further, our faith in screening programs has allowed cancer-screening programs to gain an unusually large share of our healthcare resources. As a result, less money is available to spend on other important healthcare interventions. Ultimately, blind faith in cancer screening may harm public health by absorbing resources that might have been better used elsewhere. The purpose of this paper is to review these controversies. I begin by offering documentation supporting the public enthusiasm for screening.

1 Public Enthusiasm for Screening

Americans are enthusiastic about cancer screening. In one recent public opinion poll, Schwartz and colleagues interviewed a random sample of 500 adults selected from throughout the United States. Of the respondents, 87% reported that cancer screening is almost always a good idea and most (74%) endorsed the belief that cancer-screening saves lives [1]. Aronowitz documented the relentless campaign by the ACS to persuade the public not to delay in obtaining cancer tests. In fact, the campaign was prominent throughout the entire twentieth century, despite continuing questions about the efficacy of early detection [2]. Apparently, these messages have been effective. Schwartz and colleagues note that only 2% of the population feels that there are too many cancer-screening tests. Of the male respondents, 77% said they would continue trying to have the prostate-specific antigen (PSA) test even if their doctor had not recommended it, and 74% said that they would continue to have colonoscopy or sigmoidoscopy when it was not recommended [1].

Persuasive evidence suggests that pap smears done every year provide almost no new information beyond pap smears done on a three-year interval [3]. However, the survey results suggest that 58% of women would try to have pap smears on their current schedule even if their doctor recommended that there should be more time between tests. Several evidence-based re-

views suggest that mammography might provide little or no value for women older than age 75 [4, 5]. The United States is among a minority of countries that have no upper limit on recommended age to stop screening. In Finland, screening ends at age 59, while Australia, Canada, and Iceland stop at age 69. The UK stops at age 64 and Sweden only screens until age 69 [6]. Even in the U.S., the rate of screening for older women falls off after the age of about 75, suggesting that physicians intuitively know the diminished value of the test and stop ordering it [7]. Nevertheless, the Schwartz study found that 41% of the population would label an 80-year-old who declined a mammogram to be "irresponsible."

The public is not deterred by bad experiences with tests. Over a third of the survey participants had experienced at least one false-positive test. Yet in retrospect, 98% of these individuals were glad they had taken the screening test and most would do it again. In fact, 100% of those who had experienced a false-positive PSA test were still glad that the tests had been administered [1].

The public is clearly persuaded that cancer screening is a good idea. At the same time, professional organizations that have systematically reviewed the evidence have raised serious questions about the benefits of common tests such as mammography (particularly for the pre-menopausal woman) [8] and the PSA test [9–13].

In order to understand the controversy over screening, we have been developing a conceptual model known as the disease reservoir hypothesis [14].

2 The Disease Reservoir Hypothesis

The purpose of health care is to improve health. Health outcomes can be defined in terms of only two concepts: quantity and quality of life. A successful treatment is one that makes people live longer, improves quality of life, or both [15, 16]. If a treatment neither extends life expectancy nor improves life quality, we must challenge whether it has benefit. Biomarkers should only be considered important if they are correlated with either quality of quantity of life.

It is becoming increasingly clear that there are also huge reservoirs of undiagnosed disease in human populations. As diagnostic technology improves, the healthcare system will be challenged because these common problems will be identified in many individuals who may not benefit from treatment because their length of life or quality of life will never be affected. The problem has been fiercely debated in relation to cancer screening tests such as mammography and PSA [17, 18].

According to the ACS, screening and early detection of cancers save lives [19]. It is believed that the reservoir of undetected disease might be reduced

through more aggressive intervention. Screening guidelines have been proposed and compliance to guidelines is now used as evidence for high-quality medical care [20]. Further, test rates are increasing because there are now financial incentives for physicians of offer specific tests, such as mammography [21].

In order to better understand the problem, it is necessary to understand the natural history of disease. Public health campaigns assume that disease is binary: either a person has the "diagnosis," or they do not. However, most diseases are processes. It is likely that chronic disease begins long before it is diagnosed. For example, autopsy studies consistently show that most young adults who died early in life from non-cardiovascular causes have fatty streaks in their coronary arteries indicating the initiation of coronary disease [22]. Not all people who have a disease will ultimately suffer from the problem. With many diseases, most of those affected will never even know they are sick. For example, autopsy studies show that as many as 60% of men who die in their 70s or 80s have prostate cancer and that nearly 40% of older women have some evidence of breast cancer at the time they died. However, most of these people were never tested for these cancers and never knew of these problems. Diagnosis and treatment could have resulted in complications but are unlikely to have improved health [17].

Among those who do have problems, some may not benefit from treatment. For example, if smokers are screened for lung cancer, many cases can be identified [23]. However, clinical trials have shown that the course of the disease is likely to be the same for those who are screened and those not subjected to screening, even though screening leads to more diagnosis and treatment [24]. Very high proportions of elderly (older than age 75) women have ductile breast cancer in situ (DCIS) and nearly 40% of elderly men have prostate cancer [25]. The harder we look, the more likely it is that cases will be found. However, only about 3% of elderly men will die of prostate cancer and only about 3% of elderly women will die of breast cancer. A very sensitive test for prostate cancer may detect disease in ten men for each one man who will eventually die of this condition. These problems are not limited to cancer. Advanced magnetic resonance imaging (MRI) technology has revealed surprisingly high rates of undiagnosed stroke. One cross-sectional study of 3,502 men and women over age 65 found that 29% had evidence of mild stokes and that 75% had plaque in their carotid arteries [26].

Black and Welch make the distinction between disease and "pseudodisease" [27]. Pseudodisease is disease that will not affect life duration or quality of life at any point in a patient's lifetime. A diagnosis followed by surgical treatment may have consequences, often leaving the patient with new symptoms or problems. "Outcomes researchers," therefore, evaluate the benefits of screening and treatment from the patient's perspective [28]. Using information provided by patients, quality of life and mortality outcomes are combined into quality-adjusted life years (QALYs). QALYs are estimated to as-

sess whether patients are better off with or without screening and treatment [29].

3 Biases in the Interpretation of Screening Studies

In order to understand controversies surrounding screening, it is necessary to consider two biases: lead time bias, and length bias.

3.1 Lead Time Bias

Cancer screening may result in early detection of disease. Survival is typically calculated from the date that disease is documented until death. Since screening is associated with earlier disease detection, the interval between detection and death is longer for screened cases than for unscreened cases. Epidemiologists refer to this as lead time bias. Figure 1 illustrates this bias.

Imagine that two men each develop prostate cancer in 1990 and die in 2003. Hypothetically, the progression of the cancer is identical in these two men. The man illustrated on the top line of Fig. 1 was screened in 1990 and the cancer was detected. After this diagnosis, he lived 13 additional years before his death in 2003. The man shown on the lower line did not receive screening and developed symptoms of urinary retention in 2000. After this, he lived three additional years. Survival for the man on the top appears to be much longer than that for the man on the bottom, even though the interval between developing cancer and dying is exactly the same. Figure 1 shows changes in survival among those diagnosed with prostate cancer according to the ACS. These data prompted the conclusion, "Over the past 30 years, the survival rate for all stages combined has increased from 50% to 87%"

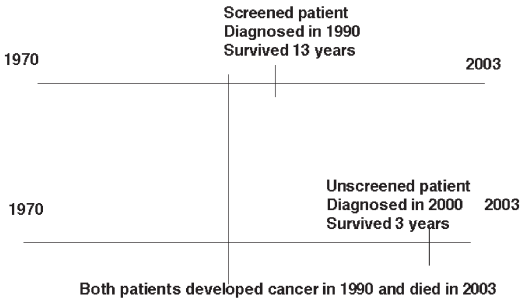

Fig. 1. Lead-time bias

[30] and that these changes are attributable to advances in cancer diagnosis and treatment.

Observational (nonrandomized) studies are unable to separate lead time bias from treatment effect and it has been suggested that increased survival associated with screening can be attributed to lead time and not to early detection and treatment [31, 32]. The only way to eliminate lead-time bias is to perform randomized clinical trials with long-term follow-up.

3.2 Length Bias

Tumors progress at different rates. Some cancers are very slow growing while other tumors progress very rapidly. Some cases may regress, remain stable, or progress so slowly that they never produce a clinical problem during an ordinary lifetime. These cases might be described as pseudodisease because they are not clinically important [33]. The probability that disease is detected through screening is inversely proportional to the rate of progression. For example, with rapidly progressing disease, early detection may not produce a clinical benefit because cases are detected too late. On the other hand, diseases with very long pre-clinical phases are more likely to be detected by screening. However, diseases that are progressing extremely slowly may never cause clinical problems.

4 Does Screening Find the Wrong Cases?

The rationale for screening is that disease can be caught in its earliest stages. This makes at least two important assumptions. First, cancer screening assumes that the test will find cancer at early stages and second it assumes that early treatment works better than late treatment. Welch challenged some of these basic assumptions [34]. First, Welch questioned whether screening identifies the most important cases of cancer. Imagine two people with cancer. In the first case, the disease is very slow growing and may take 30–40 years before it causes death. In the second case the interval between the beginning of the disease and death from cancer is only 6 months. Imagine now that individuals are screened every 5 years. For the slow-growing cancer, the test would identify disease each time it was administered. For a 50-year-old man with prostate cancer, for example, assume the disease was there at the first screening and it was there each time the test was re-administered at ages 55, 60, 65, 70, 75, and 80. Even though the disease was found on each test, it never affected the man's lifestyle and he died of another cause before he died of cancer.

The second patient had a very rapidly growing cancer that started at age 52. The test was unable to identify it at age 50, and when the man would have been 55 years of age he was no longer alive to be tested. Welch points out that the second man would have benefited from early detection while the first man may have been harmed. The man with the slow-growing cancer would never have been affected by the condition, but in fact is likely to have had treatment. The second man may have benefited from treatment, but his cancer would probably not be found by screening at a time when he could be helped.

5
What Do RCTs on Screening Tell Us?

The best scientific method for the evaluation of cancer screening is to conduct clinical trials (RCTs) in which patients are randomly assigned to screening or to usual care. Table 1 summarizes 12 randomized clinical trials, previously reviewed by Black and colleagues [35], in which total mortality was reported. The trials consider a wide variety of screening interventions including screening for breast cancer, screening for colorectal cancer (CRC), and screening for lung cancer. Follow-up ranged from 3 years to over 20 years. Among these, the study by Shapiro et al. was the most difficult to interpret because true denominators were hard to locate. Further, the study did not use the intention-to-treat principle. About one-third of the treatment group refused to be screened and was eliminated from the analyses. This group had a significantly higher mortality rate, and would have further shifted the result away from the benefits of screening. Table 2 summarizes the outcomes of these studies, excluding the difficult-to-interpret Health Insurance Plan (HIP) of New York study. The table provides information on disease-specific deaths and all-cause deaths in the intervention and control groups for all interventions. The table also summarizes the all-cause mortality and the proportion of all cases who died in the follow-up interval for the intervention and control groups. Finally, the table summarizes the absolute risk reduction (ARR) for disease-specific and for all-cause mortality. The ARR is the proportion of those who died in the control group minus the proportion who died in the intervention group. If the number is positive, there is a benefit of screening, while when the number is negative the benefits of screening are in the opposite direction. As the table shows, the absolute risk reduction for all-cause mortality is remarkably small. Among 11 trials, 5 had overall mortality in the opposite direction as expected and 6 had overall mortality in the expected direction. However, in nearly all trials the absolute risk reduction was near zero.

Two features of the tables are important. If cancer-screening tests really provide benefit, then we would expect those who were screened to have lon-

Table 1. Summary of clinical trials with reports of total mortality

Reference(s)	Problem	Intervention	Control	Follow-up	Disease-specific deaths	All causes deaths
Shapiro et al. 1998 [6]	Screening for breast cancer women age 40–64	Mammography plus clinical breast exam	Usual care	18 years	I=263 (1%)	I=73.7/10,000 person years
		n=30,131(20,128 completed test used in analysis)	*n*=30,565		C=307 (1%)	C=75.4/10,000 person years
Tabar et al. 1989 [57]	Screening for breast cancer	Mammography	Not invited for screening	7.9 years	I=160	I=6,899
		n=77,080	*n*=55,985		C=167	C=085
Andersson et al. 1998 [58]	Screening for breast cancer in women aged >45	Five rounds of Mammography at intervals of 18–24 months	Not invited for screening	8.8 years	I=63	I=1,777
		n=21,088	*n*=21,195		C=66	C=1,809
Bjurstam et al. 1997 [59]	Screening for breast cancer in women aged <50	2-view mammography 18 month interval	Not screened until 6–7 year	Until December 1994	I=18	I=409
		n=11,724	*n*=14,217		C=40	C=506
Roberts et al. 1990 [60]	Screening for breast cancer in women aged 45–64	Oblique and craniocaudal view mammography; Clinical examination	Not invited for screening	7 years	I=68	I=1,274
		n=23,226	*n*=21,904		C=76	C=1,490
Miller et al. 1992 [61], 2000 [62]	Screening for breast cancer in women aged 40–49	Mammography; physical examination of the breasts	Single physical examination of the breasts	11–16 years	(To end of 1996) I=105	(To end of 1993) I=413
		n=25,214	*n*=25,216		C=108	C=413
Miller et al. 2000 [62]	Screening for breast cancer in women aged 50–59	Annual two-view mammography (craniocaudal and mediolateral oblique views); Annual physical exam; taught self-exam	Physical exam only	13 years	I=88 (11.9%)	I=647 (88.1%)
		n=19,711	*n*=19,694		C=90 (12.9%)	C=600 (87.1%)
Mandel et al. 1999 [37]	Screening for colorectal cancer in people aged 50–80	Fecal occult blood test with rehydration	Not invited for screening	13 years	I_1=121	I_1=5,236
		Tested annually: I_1=15,570 Tested biennially: I_2=15,587	*n*=15,394		I_2=148 C=177	I_2=5,213 C=5,186

Table 1 (continued)

Reference(s)	Problem	Intervention	Control	Follow-up	Disease-specific deaths	All causes deaths
Hardcastle et al. 1996 [63]	Screening for colorectal cancer in people aged 45–76	Fecal occult blood screening *n*=76,466	Not invited for screening *n*=76,384	Until June 1995	I=360 C=420	I=12,624 C=12,515
Kronborg et al. 1996 [64]	Screening for colorectoral cancer in people aged 45–75	Fecal occult blood screening (Biennial Hemoccult II without rehydration) *n*=30,967	Not invited for screening *n*=30,966	10 years	I=205 C=249	I=6,228 C=6,303
Kubik et al. 1990 [65]	Screening for lung cancer in cigarette-smoking males aged 40–64	6-monthly screening by chest X-ray and sputum cytology *n*=3,171	No asymptomatic investigation *n*=3,174	5 years including 3 years of annual chest X-rays	I=85 C=67	I=341 C=293
Marcus et al. 2000 [24]	Screening for lung cancer	Chest X-ray; sputum cytology *n*=4,618	Usual Care *n*=4,593	20.5 years	I=337 C=303	I=2,493 C=2,445

C, control; I, intervention.

Table 2. Specific and all-cause mortality and AARs for RCTs of screening

Reference	Proportion dead from specific cause intervention	Proportion dead from specific cause control	ARR specific	Proportion dead from all causes intervention	Proportion dead from all causes control	ARR all causes
Tabar et al. 1989 [57]	0.002	0.003	0.001	0.092	0.094	0.002
Andersson et al. 1998 [58]	0.003	0.003	0.000	0.087	0.088	0.001
Bjurstam et al. 1997 [59]	0.002	0.003	0.001	0.036	0.037	0.000
Roberts et al. 1990 [60]	0.003	0.003	0.001	0.058	0.071	0.013
Miller et al. 1992 [61]	0.004	0.004	0.000	0.021	0.021	0.000
Miller et al. 2000 [62]	0.004	0.005	0.000	0.037	0.035	-0.002
Mandel et al. 2000 [37]	0.008	0.011	0.004	0.344	0.345	0.001
Harcastle et al. 1996 [63]	0.005	0.005	0.001	0.170	0.169	-0.001
Kronborg et al. 1996 [64]	0.007	0.008	0.001	0.208	0.210	0.002
Kubik et al. 1990 [65]	0.027	0.021	-0.006	0.134	0.119	-0.015
Marcus et al. 2000 [24]	0.073	0.066	-0.007	0.613	0.606	-0.007

ARR, absolute risk reduction; RCT, randomized controlled trial.

ger life expectancies than those who were not screened. As the tables show, it is quite remarkable that cancer screening trials have been unable to document that people who are screened live any longer than those who are not screened [35]. We call this the total mortality issue because it focuses on total life expectancy. Advocates for cancer screening recognize that there is no total mortality benefit but argue that there are benefits for deaths from specific types of cancer [36]. The center columns in Table 2 show the outcomes for specific types of cancer for screened and unscreened patients. For CRC, for example, there is a disease-specific benefit. This means that those screened are significantly less likely to die of CRC than those who are not screened.

The argument against considering all-cause mortality is that the benefits are diluted in studies of total mortality. There may be a benefit for a rare cause of death that will be washed out if this advantage is thrown into a large pool of noise. The Minnesota Colon Cancer Screening Trial offers perhaps the best example [37]. Patients were randomly assigned to occult blood screening annually (n=15,570), biennially (n=15,587), or to usual care (n=15,394). Over the 18-year period, the death rate from CRC was approximately 10 per 1,000, or 1%. Over the same period, the all-cause death rate was approximately 340 per 1,000, or 34%.

Suppose a reasonable expectation for screening is that it will reduce CRC deaths by 30%—that is, from 1% to 0.7%. Suppose, further, that screening is expected to have no effect on deaths from causes other than CRC—that is, that screening is expected to reduce the overall death rate from 34% to 33.7%.

To power a study to have an 80% chance of observing a statistically significant decline in CRC deaths if the true change is 0.3%, we need an n of approximately 15,000 in each of two arms, or a total n of 30,000. To get the same power to measure a 0.3% decline in total death rate, we would need an n of approximately 385,000 in each arm, or a total n of 770,000.

Now, if we are interested in determining whether to do a study of the effects of screening on all-cause mortality, we could take one of three positions:

- Because "only" 1% of persons will die of CRC over an 18-year period, we should not worry about reducing the CRC death rate. Even if screening has the hypothesized significant effect on CRC deaths, and a significant effect on all-cause deaths, we should not care about figuring it out, since the overall decline in death rate will be only 0.3%.
- A 0.3% decline in the all-cause death rate is a significant health benefit, but only if we can demonstrate conclusively that this decline actually occurs. If we can get enough money to do an RCT of screening that includes 770,000 people, we can be reasonably confident of determining whether screening has an effect on all-cause mortality. If it is demonstrated to have an effect,

and if the screening is determined to be "cost-effective," then we should be comfortable in recommending widespread screening programs.

- A 0.3% decline in the all-cause death rate is enough to get excited about, and we should be willing to suggest policy changes if we can demonstrate a significant decline in CRC deaths. We can conduct an RCT with 30,000 participants, and determine whether screening reduces CRC deaths. If it does, we should investigate plausible hypotheses that might link screening (or colonoscopies) to increases in death rates from other causes—e.g., perforations or infections stemming from the procedures. If we cannot demonstrate any plausible links between screening and increased death rates from other causes *and* if screening appears to be "cost-effective," then we should be comfortable in recommending widespread screening programs.

Does the first position makes sense? There are many diseases that result in death for a relatively small portion of the population. It does not make sense to say that because these diseases kill a relatively small number of people ("only" 1% of the population), that we should not care about figuring out how to prevent or treat them.

The second position also raises concerns. While it would be wonderful to have the resources to do an RCT with 770,000 people, we rarely will have this luxury (and if we did, we might well find better ways of using these resources). If we insist on being able to study the effects of screening on all-cause mortality as a condition of being willing to accept any evidence of the effects of screening on mortality, then we will almost certainly be stuck with no evidence at all. We will then not be able to have any evidence on the effects of screening for any disease that kills "only" 1% of the population.

6 Evidence-Based Medicine Approach

To push this debate one step further, a spreadsheet was developed (Table 3). The analysis assumes that 15,000 patients are assigned to each of the three conditions. We will focus on the annual screening and the control group. We will assume that 5,000 died in each group of causes unrelated to CRC. The second line in the spreadsheet shows this 5,000, plus the number of patients who died of CRC. Thus, the differences between groups in all-cause mortality assume that all of the other causes are constant except CRC. In this analysis there is a 32% reduction in deaths from CRC but only a 1% reduction in deaths from all causes. However, the absolute risk reduction (ARR) is identical in each case (.0037). Further, the number needed to treat (defined as 1/ARR) is identical for deaths from CRC and total mortality. In other words, if the likelihood of dying from all other causes remains unchanged and differences in mortality are attributable only to CRC deaths, then we need to

Table 3. Summary of benefits of annual and biennial screening for colon and rectal cancer

	Annual	Biennial	Control
n	15,000	15,000	15,000
Deaths all cause	5,121	5,148	5,177
Cumulative mortality [deaths/(*n*/1,000)]	341.4	343.2	345.133333
CRC deaths	121	148	177
Absolute RR for CRC (CRC deaths/*n*)	0.008066667	0.00986667	0.0118
Absolute RR for total (all deaths/*n*)	0.3414	0.3432	0.34513333
FOR CRC			
RRR	0.316384181		
ARR	0.003733333		
NNT	267.8571429		
For total			
RRR (D7-B7)/D7	0.010817076		
ARR D7-B7	0.003733333		
NNT 1/B15	267.8571429		

ARR, absolute risk reduction; CRC, colorectal cancer; NNT, number needed to treat; RCT, randomized controlled trial; RR, relative risk; RRR, relative risk reduction.

treat exactly the same number in order to reduce one CRC death as we need to treat to reduce one death from any cause. But the data from the Minnesota study do not support this picture. It appears that increases in deaths from other causes compensated for reductions in death from CRC [37].

7 Interpretation of Cancer Screening Evidence by Peer Panels

Professional groups that have reviewed the clinical evidence often disagree about the value of screening. For example, there are no studies that show a clear benefit from receiving a prostate specific antigen (PSA) test because completing the test does not appear to result in a reduction in overall mortality. However, treatment for prostate cancer (typically surgical removal of the tumor) carries with it a significant risk of impotence and incontinence [38]. Hence, receiving a PSA test may result in a reduction in quality of life [39]. Recognizing these uncertainties, most professional groups now recommend some sort of shared decision-making for PSA screening. Table 4 summarizes the recommendations from several groups.

Another example of differences of opinion between professional groups concerns the age for initiating screening for breast cancer using mammography [45–48]. Among women between the ages of 50 and 74, periodic screening results in significantly lower rates of death from breast cancer [49]. However, there is very little evidence that screening is of benefit to women younger than age 50. In February of 2002, the U.S. Department of

Table 4. Summary of recommendations for PSA screening

Group	Recommendation	Reference
U.S. Preventive Services Taskforce (USPSTF)	Do not screen	40
American College of Physicians-American Society of Internal Medicine (ACP-ASIM)	Shared decision-making	41
American Academy of Family Physicians (AAFP)	Shared decision-making	42
American Urological Association (AUA)	Offer screening if life expectancy >10 years	43
American Cancer Society	Screen except for men with short life expectancy	44

Health and Human Services endorsed mammography for women 40 years of age and older as evidence of commitment to preventive medicine. However, the public health benefit of promoting screening mammography for 40- to 50-year-old women may be somewhat limited. All clinical trials and meta-analyses have failed to show a population benefit of screening women in this age group [45-48, 50, 51].

In January of 1997 the National Institutes of Health (NIH) convened a panel to make recommendations about the use of screening mammography for women 40–50 years of age. Noting that no convincing evidence showed benefits of screening younger women, the committee recommended against screening pre-menopausal women. The conclusion of the panel review were rebuffed by the ACS. Richard Klausner, then the Director of the National Cancer Institute, decided to disregard the report of his own expert panel. Shortly thereafter, the ACS appointed a panel of experts chosen because each already believed that screening was valuable for 40- to 50-year-old women. The ACS panel recommended that 40- to 50-year-old women should be screened [51].

The controversy died down for a brief time but reemerged in 2001 when Olsen and Gotzsche reanalyzed earlier trials and classified studies by methodological quality [52]. In their analysis they noted that the only studies supporting screening mammography for women of any age were of low quality and that those studies not supporting screening mammography tended to have greater methodological rigor. The findings are summarized in Fig. 2. Remarkably, there appeared to be no benefit at all for screening—the relative risk ratio was nearly 1.0.

Even though many scholars agree with Olsen and Gotzsche, the controversy continues. For example, data from a key Swedish study have been reanalyzed and shown to support screening mammography [47]. However, all reviews of the data indicate that any benefits of screening mammography are very small and that screening offers little or no benefit in terms of increasing life expectancy when all causes of mortality are considered [35].

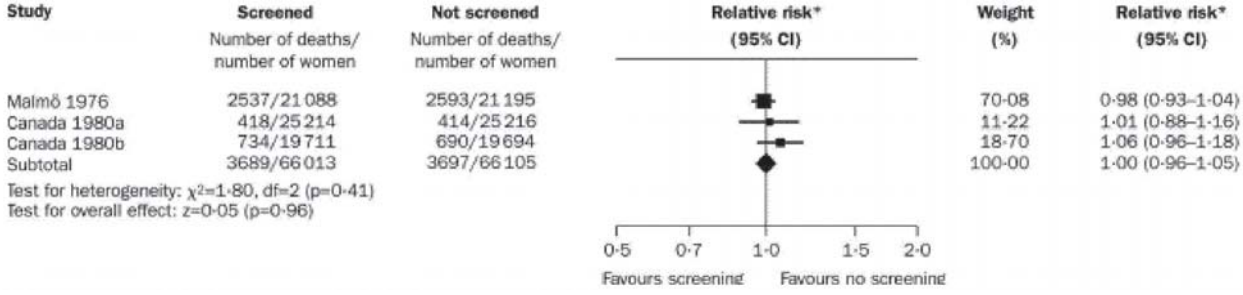

Fig. 2. Relative risk and confidence intervals for mammography using radionuclide computed tomography (RCT). *Malmo 1976* [58], *Canada 1980a* [61], and *Canada 1980b* [62]. (Used with permission from [52])

8
Variation in Screening and Impact on Healthcare Costs

The number of cancer screening tests offered is considered an indicator of quality health care. In fact, health plans are often evaluated by the proportion of patients who receive cancer-screening tests. We also know that rates of screening differ by community. For instance, cancer-screening rates are increasing and the number of cases identified early has gone up [54]. Communities with lower average income and education tend to get less screening than communities where most of the people are affluent. This has lead to calls to spend more money providing cancer screening for the medically underserved [54]. However, do we know that there is a health disadvantage for those living in communities where screening is less common?

There is substantial geographic variability in the rate of cancer screening. For example, analysis of Medicare claims shows that in Michigan and Florida mammograms are done routinely. In Lansing Michigan nearly 35% of all women had received mammograms and similarly high rates were observed in Fort Lauderdale and Sarasota, Florida. On the other hand, only 13% of the women in Oklahoma City had obtained mammograms and a variety of cities had similar rates. Salt Lake, for example, had a rate of 13.4% [53]. If screening provides benefit, outcomes should be better in areas that use more screening. In areas where screening is done more often, more cases of breast cancer are found. However, there appears to be no relationship between these screening rates and mortality rates from breast cancer [53]. Similarly, PSA screening is done much more commonly in the Pacific Northwest region of the U.S. than it is in New England communities, such as New Haven, Connecticut. Although many more cases are found and treated in the Pacific Northwest, there is no evidence of reduced mortality due to prostate cancer [53]. More screening finds more cases, but appears unrelated to better health outcomes.

9
Opportunity Costs

So far we have concentrated on controversies about the effects of screening on health outcome. Although some are skeptical about the benefits of testing, others have argued that the tests rarely harm patients. The PSA is a simple blood draw and, although unpleasant sigmoidoscopy and colonoscopy rarely result in perforated colons. Why not continue to test everyone? Perhaps the most important reason is that cancer screening adds significant cost to healthcare. There are subtle differences between how economists use the word cost and how it is used more generally. Most people think of cost in terms of dollars. However, economists try not to place value judgments

on decisions. Instead, they attempt to identify the alternatives and to spell out the consequences of various choices. Opportunity costs are the forgone opportunities that are surrendered when resources are used to support a particular decision. If we spend a lot of money on screening, for example, there will be less money to spend on other healthcare programs.

—A wide variety of analyses have evaluated cancer-screening tests using a metric of cost per QALY produced. Some programs, such as pap smears for older low-income women save both money and lives [55]. On the other hand, screening for prostate cancer with either digital rectal exams or PSA tests both drains resources and causes harm [56]. In many cases, cancer screening does produce some benefit. However, it is a very expensive use of resources in relation to many other alternatives. For example, lifelong screening with chest X-ray for skin thickness and local cutaneous melanoma may produce a benefit, but at a cost of $250,000 U.S. per QALY [41]. Programs such as smoking prevention produce a QALY for less that $1,000 U.S. In other words, 250 smoking prevention programs might be funded for the cost of one chest X-ray screening program. The population benefit of funding smoking prevention may be 250 times greater.

10 Conclusion

Cancer screening remains a controversial aspect of primary and cancer care. For nearly a century, charitable organizations have promoted early detection as the key to cancer prevention. However, evidence does not always support the value of early detection.

A disease reservoir hypothesis argues that disease is very common, particularly among older adults. However, much of this disease will never be clinically relevant because it will not affect life expectancy or reduce quality of life. True pathology that has no clinical impact might be described as pseudodisease. Many cases of cancer detected through screening might be pseudodisease because the affected patients would have never known about the problem during their lifetimes.

Two important problems keep the controversy about cancer screening alive. One problem is that randomized clinical trials fail to show the benefits of screening in terms of total mortality. Even trials that show disease-specific mortality benefits often do not show that screening extends life expectancy. A second problem is that the development of better biomarkers and improvements in screening methodology may make the problem more severe. Improved screening methodologies may identify more pseudodisease, and few new technologies clearly distinguish between true disease and clinically unimportant pseudodisease. It is unknown whether many current biomarkers are related to diseases that shorten life expectancy or reduce quality of

life. Systematic research is necessary to document the validity of these new outcome measures.

A final concern is that screening is expensive. If we devote healthcare resources to screening programs, fewer resources are available for other prevention programs. Current analyses suggest that most screening programs produce relatively few quality-adjusted years in relation to their cost. Promotion of screening programs remains controversial because some have argued in favor of using cancer prevention resources for alternatives other than screening.

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