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Cancer Prevention II

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Cancer Prevention II

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Preface

More than 180 participants and experts from 31 countries met for the fifth time in 10 years in St. Gallen, Switzerland for a 3-day conference to discuss important current issues of clinical cancer prevention. The meeting was again organized and co-sponsored by St. Gallen Oncology Conferences (SONK).

While SONK has been extremely successful in organizing large international congresses on "Primary Therapy of Early Breast Cancer" as well as "Supportive Care in Cancer" for more than 20 years, the idea of promoting interdisciplinary, clinically oriented meetings on cancer prevention is a more recent and not yet generally accepted and welcomed concept in modern oncology. Since today's medical expenses are soaring and medical research budgets are stagnating or even being cut, neither politicians nor industry is willing to risk an additional unpredictable channel of expenses, such as that demanded by clinical cancer prevention efforts!

In Switzerland—and we fear in many other parts of the globe—some 97%–98% or even a greater percentage of health budgets is spent for curative and palliative/rehabilitative medicine. Since a meager 2%–3% of national health budgets is for preventive medicine, even less than that proportion is specifically allocated for cancer prevention. When the money for "curing and caring" for the diseased populace runs short, there is likely not much left for partly controversial disease prevention in the (still) healthy part of the population. Although this might be an extremely short-sighted view, it is noticeably prevalent with health politicians and even with large parts of the medical profession, at least in Continental Europe, today.

Despite this ironic situation, we have decided to keep trying to promote the promising field of clinical cancer prevention by organizing biannual international conferences in view of the accumulating interactions between molecular genetics and biology, epidemiology and clinical cancer prevention. Together with a growing number of scientific and professional partners, we intend to periodically set the stage for a comprehensive scientific discussion forum critically analyzing the development of more efficient and more acceptable primary and secondary cancer prevention approaches for the future. It is rather unfortunate that the oncology-oriented pharmaceutical industry—especially in Europe—is not yet willing or prepared to support this fascinating field, especially chemoprevention, by more appropriate research involvement and educational funding.

It was our privilege to co-organize this meeting again on behalf of the International Society of Cancer Prevention (ISCaP, New York, NY, USA) together with the European School of Oncology (ESO, Milan, Italy) and the European Society of Medical Oncology (ESMO, Lugano). For this fifth prevention conference in March 2008 we were able to generate some new and greatly welcomed additional and "neutral" supporters or sponsors: Cancer Research UK (CRUK, London, UK), the Union Internationale Contre le Cancer (UICC, Geneva, Switzerland), the European Association of Cancer Research (EACR, Nottingham, UK), the American Cancer Society (ACS, Atlanta, GA, USA), and the Swiss Cancer League (Bern, Switzerland). Very little financial support was provided by industry. The local organizers were Prof. Hans-Jörg Senn, MD, Prof. Ursula Kapp, MD, and Prof. Florian Otto, all from the prevention-oriented Tumor Center ZeTuP in St. Gallen, Switzerland.

This 2008 St. Gallen International Cancer Prevention Conference—in contrast to the previous meetings in 2004 and 2006—was primarily targeted to primary prevention, and even more specifically at the chemoprevention of major cancer types such as breast, colorectal, cervical, and lung. Besides the traditional sessions on health politics and organ-site-oriented cancer prevention efforts, we tried for the first time to upgrade this 2008 conference with a well-prepared consensus session on the present state of the art of chemoprevention of colorectal cancer by aspirin and nonsteroidal antiinflammatory drugs (NSAIDs), chaired by Prof. Jack Cuzick, president of ISCaP and director of the Wolfson Institute of Preventive Medicine in London, UK, and by Dr. Peter Greenwald, the director of the prevention branch of the NCI in Bethesda, MD, USA.

This consensus of the use of aspirin and NSAIDs in chemoprevention of colorectal cancers will be published separately in a major oncology journal. As is the tradition, the majority of the invited expert contributions to the conference are published in this internationally well-known series, *Recent Results in Cancer Research*, by Springer. We hope you enjoy its multifaceted content.

Already the organizers invite dedicated scientists, epidemiologists, and clinicians interested in primary and secondary (clinical) cancer prevention to the next international cancer prevention conference, which will be held in St. Gallen, 18–20 March 2010.

Hans-Jörg Senn, Ursula Kapp, Florian Otto

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Part I

Cancer Prevention and Health Politics

Do We Make Optimal Use of the Potential of Cancer Prevention?

1

Peter Greenwald and Barbara K. Dunn

Abstract Three decades of intensive experimental and clinical research on cancer prevention have yielded an impressive body of scientific knowledge about cancer epidemiology, causation, and preventative measures. Despite our increased understanding in these critical areas, this knowledge is not being translated adequately into initiatives that will impact public health. The recent release of the World Cancer Research Fund/American Institute for Cancer Research report on diet and lifestyle strategies for cancer prevention—grounded in an evidence-based, systematic review of the published literature—is a strong acknowledgment of the benefits of a lifestyle approach to reduce cancer risk. The report also emphasizes the need to increase basic nutritional science research to make optimal use of the knowledge gained in the past three decades. Medical approaches—represented by chemoprevention clinical trials—also have become more focused based on results from basic science leads. The expansion of preclinical chemoprevention studies and greater attention to "first-in-human" prevention trials that safely shorten the timeline for new drug development are needed. The development of a prevention focus for what the U.S. Food and Drug Administration calls "exploratory investigational new drug studies" and what investigators at the National Cancer Institute are calling "phase 0" clinical trials will contribute to the decision-making involved in designing larger cancer prevention clinical trials. Past achievements in phase III prevention clinical trials—such as the Prostate Cancer Prevention Trial, the Breast Cancer Prevention Trial, and the Study of Tamoxifen and Raloxifene—have provided early successes as evidence of the potential for public benefit to be derived from this research. Nevertheless, the application of these findings to clinical practice and the design of future prevention trials remains a challenge. Current strategies include the refinement of risk assessment models for several major cancers. Additional initiatives, based on emerging basic and clinical research, involve the development of potential biomarkers for cancer risk and early detection by the National Cancer Institute's Early Detection Research Network. Although a recent progress report indicates that biomarkers of cancer susceptibility and exposure have been identified, continued work is needed to validate such markers for clinical use. Using this information optimally for prevention through lifestyle changes or medical interventions will

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demand commitments from public and private research institutions. Another area of emerging research is the development of a systems biology approach to cancer prevention. This will demand the creation of multidisciplinary teams of researchers from biological sciences, informatics and engineering scientists, and researchers from many fields not generally focused on disease prevention. To facilitate this and other new approaches, and to make effective use of information and strategies for cancer prevention, intensive training efforts must be implemented to develop the next generation of basic and clinical scientists—and physician researchers—capable of working in a cross- and multidisciplinary research environment. Training current researchers in new approaches will add efficiency to their combined research experiences.

1.1 Introduction

For most of the past 35 years, trends in the incidence and mortality rates of all major cancers in the United States showed steady increases. This pattern changed in the 1990s when decreases started to emerge (National Cancer Institute 2007), with mortality rates declining at approximately half that of incidence rates (Ries et al. 2007). While for some of the most common types of cancer in the United States—breast, prostate, colorectal, and lung—considerable progress has been made regarding mortality and incidence, in specific cancer types in some population groups (e.g., lung cancer in women and prostate cancer among African Americans) such progress is not evident.

The role of cancer prevention underlies much of this observed decrease in cancer incidence and mortality. For three decades, an impressive body of research has accumulated indicating that lifestyle and medical prevention strategies can have a major impact on cancer incidence and mortality. Nevertheless, doubt exists as to whether clinicians and other health professionals are making optimal use of existing knowledge regarding cancer prevention strategies. Cancer prevention offers a key opportunity to reduce the disease burden both on individuals and on the healthcare system. To achieve the maximum benefit from cancer reduction, major initiatives in prevention must include both lifestyle and chemoprevention approaches.

The following sections discuss current research on lifestyle and medical intervention studies—as well as selected molecular and genetic studies—in cancer prevention. In addition, a review is presented of progress in several areas: the translation of research findings into public benefit; new approaches for designing and developing clinical trials to target individuals most likely to benefit from trial findings; and suggestions for increased and novel approaches to training with a goal of producing the multidisciplinary researchers needed for working with emerging high-throughput and "-omic" (e.g., genomic, proteomic, transcriptomic, and metabolomic) technologies.

1.2 Lifestyle Interventions

Preventing cancer through lifestyle modifications and other interventions has received increased attention in the past decade as more is understood about the role of nutrition, weight gain/loss, and the level of physical activity and cancer risk. Since the Doll and Peto quantitative analysis of estimates of avoidable cancer risks in 1981 (Doll and Peto 1981), accumulating evidence suggests that lifestyle may contribute to as much as 70% of cancer cases; nutrition alone is a factor in at least 30%–40% of cancers. Adopting lifestyle modifications—in areas involving diet,

physical activity, use of tobacco, and weight control—offers a major approach to cancer prevention for most individuals. In the past, however, apart from the avoidance of tobacco, limited convincing evidence had been available to make recommendations regarding these lifestyle areas. This situation changed rapidly as findings from basic, epidemiological, and clinical research began to fill in gaps in our knowledge. For example, the recent release of *Food, Nutrition, Physical Activity, and the Prevention of Cancer: A Global Perspective* (World Cancer Research Fund 2007)—the 2007 expert report developed and published by the World Cancer Research Fund (WCRF) and the American Institute of Cancer Research (AICR)—highlighted the role of lifestyle on cancer prevention. The report is evidence-based and draws from a substantial body of cancer prevention literature published in the past decade.

What distinguishes this recent report from past documents is the utilization of increasingly available data from controlled clinical trials and large prospective studies on nutrition and cancer. Table 1.1 highlights the recommendations from the report, which incorporates government recommendations (U.S. Department of Health and Human Services 2005). Table 1.2 highlights the report's findings on lifestyle factors and decreased or increased risk of cancer by cancer site. The inclusion of a factor in Table 1.2 indicates that the authors of the report found the evidence to be either "probable" or "convincing" for its use in assessing the level of cancer risk. "Convincing" is the highest level of evidence for a recommendation, based on the judgment that the evidence will be unlikely to change over time and is based on congruent results from at least two independent cohorts. The underlying evidence has favorable attributes including: (1) no substantial heterogeneity in the data; (2) plausible dose responses; (3) consistent evidence from laboratory studies; and (4) accountability for error. Taken in totality, the evidence suggests that specific lifestyle changes could have a major impact on cancer prevention if optimal use of the information became part of physician practice and public policy recommendations.

Table 1.1 WCRF/AICR (2007) recommendations adapted from *Food, Nutrition, Physical Activity, and the Prevention of Cancer: A Global Perspective*, incorporating 2005 U.S. dietary guidelines

General recommendations for cancer prevention
1. Be as lean as possible without becoming underweight (goal: BMI 21–23)
2. Be physically active for at least 30 min every day
3. Avoid sugary drinks. Limit consumption of energy-dense foods (particularly processed foods high in added sugar, or low in fiber, or high in fat)
4. Eat more of a variety of vegetables, fruits, whole grains and legumes such as beans
5. Limit consumption of red meats (such as beef, pork, and lamb) and avoid processed meats
6. If consumed at all, limit alcoholic drinks to 2 for men and 1 for women a day
7. Limit consumption of salty foods and foods processed with salt (sodium). Avoid moldy cereals (grains) or legumes
8. Aim to meet nutritional needs through diet alone. Do not use supplements to protect against cancer
Special population recommendations
9. New mothers ideally should breastfeed exclusively for up to 6 months and then add other liquids and foods
10. Cancer survivors after treatment should follow the recommendations for cancer prevention

Table 1.2 Convincing evidence of decreased or increased risk of cancer by cancer site and lifestyle factor (*Food, Nutrition, Physical Activity, and the Prevention of Cancer: A Global Perspective*; WCRF/AICR 2007)

Lifestyle factors with probable and/or convincing decreased risk of cancer[a]	
Colorectum	Foods containing dietary fiber, garlic, milk, calcium supplements, increased physical activity (probable evidence)
Mouth, pharynx, larynx	Non-starchy vegetables, fruits, foods containing carotenoids (probable evidence)
Esophagus	Non-starchy vegetables, fruits, foods containing beta-carotene (probable evidence)
Stomach	Non-starchy vegetables, *Allium* vegetables, fruits (probable evidence)
Lung	Fruits, foods containing carotenoids (probable evidence)
Pancreas	Foods containing folate (probable evidence)
Prostate	Foods containing lycopene, foods containing selenium, selenium supplements (probable evidence)
Breast	Lactation
Lifestyle factors with probable and convincing increased risk of cancer[a]	
Liver	Aflatoxins
Colorectum	Red meat, processed meat, alcoholic drinks (men only), body fatness, abdominal fatness, adult-attained height
Lung	Arsenic in drinking water, beta-carotene supplements
Mouth, pharynx, larynx	Alcoholic drinks
Esophagus	Alcoholic drinks, body fatness
Breast, premenopausal	Alcoholic drinks (probable evidence)
Breast, post-menopausal	Alcoholic drinks, body fatness, adult-attained height
Pancreas	Body fatness
Endometrial	Body fatness
Kidney	Body fatness

[a] Evidence is convincing unless otherwise noted as probable

The potential for research opportunities geared toward improving the science of nutrition and cancer emerged directly from this report. These opportunities include integrating the recommendations on chronic diseases, and on promoting positive health and well-being. The relationship between causation and prevention should be elucidated and a revived look at descriptive studies, such as those on migrant populations, is needed.

Other important research gaps include studies on determinants of rapid growth and early puberty; dietary energy restriction in humans; food systems and dietary patterns; foods common in traditional diets; populations in parts of the world for which cancer is uncommon; and follow-up studies of exclusively breastfed children. There also is a need to develop standard definitions of physical activity and processed meat, and to determine when in the course of life specific preventative interventions are most effective. WCRF and AICR have committed to regularly updating the report as new evidence is published. (A summary and complete report can be found at http://www.wcrf.org/research/fnatpoc.lasso.)

Other important findings of the past decade relating lifestyle interventions to cancer prevention include the emerging recognition of obesity as a major factor in cancer etiology. Calle and

colleagues suggested that being overweight or obese contributes to 15%–20% of cancer deaths; given the increasing numbers of obese Americans, the promotion of weight control has potential as a broadly effective lifestyle approach to cancer prevention (Calle et al. 2003). Regular, moderate physical activity also has been associated with reduced risk of various cancers, including colon cancer (Samad et al. 2005).

A preventative approach of lifestyle modifications that targets diet, physical activity, and weight control is likely to impact morbidity and mortality due to cancer.

1.3 Medical Interventions

Unlike lifestyle interventions, which are generally designed to target cancer risk broadly in populations, medical interventions are more specific in that they focus on limited cancer types in individuals or subpopulation groups that are at increased risk of developing those cancers. Both types of intervention, however, are important for overall reductions in cancer morbidity and mortality. The field of study involving the medical intervention approach to cancer prevention is maturing as it incorporates knowledge generated from basic, epidemiological, and clinical research. In particular, the increased understanding of the molecular, genetic, and epigenetic processes that contribute to or prevent carcinogenesis feeds directly into the formulation of medical preventative interventions. New approaches for designing and implementing cancer prevention clinical trials will also directly affect investigators' ability to provide evidence of benefits (or lack of benefit) for medical interventions. The use of emerging technologies and the collaborative efforts of multidisciplinary research teams are expected to accelerate the pace of new discoveries.

1.4 The Changing Landscape of Clinical Studies

The use of lifestyle or medical interventions ideally depends on their evaluation in clinical trials—preferably testing each intervention in relation to a control group in a randomized controlled trial (RCT). Before cancer prevention agents—nutrient- and non-nutrient-based—can be tested in RCTs, however, they must undergo testing in a phased clinical trial regimen to guarantee the safety and efficacy of the agent. For cancer prevention clinical research, the U.S. National Cancer Institute (NCI) traditionally has used a three-phase approach for testing chemoprevention agents. These potential chemoprevention agents are tested for safety and pharmacokinetic profiles in a small number of individuals (phase I trial); intermediate-endpoint biomarkers that are modulated by the agent and have potential to serve as surrogates for clinical disease endpoints are identified and tracked in trials with as many as several hundred individuals (phase II trial or a combination of phase I and phase II trials); and a large-scale, randomized, controlled trial is conducted to determine if the agent reduces cancer risk, the critical clinical endpoint in cancer prevention research (phase III trial). NCI encourages extensive follow-up to further evaluate the long-term safety and efficacy of an intervention. More than 150 potential chemopreventative agents have been identified in preclinical studies sponsored by the NCI's Division of Cancer Prevention (DCP), and development continues on the more than 40 agents that have shown evidence of safety and chemopreventative efficacy. Figure 1.1 depicts the approach of chemoprevention research and the stages in the carcinogenic process that may be targeted by chemopreventative agents.

An effort is being made at NCI to shorten the time an agent spends in the phased system, and

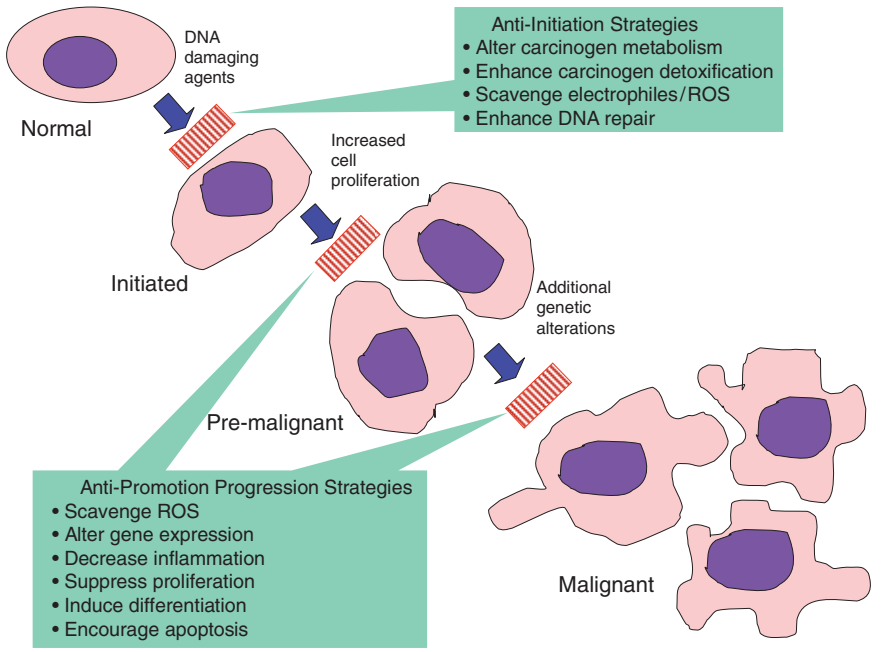

Fig. 1.1 Chemoprevention strategies in the carcinogenic process. (Adapted from Greenwald 2002, reproduced with permission from the BMJ Publishing Group)

to reduce the number of agents in need of testing in phase I and phase II trials prior to advancing to a more definitive, higher phase of clinical testing. In the past, one of the shortcomings of the phased system has been its inability to eliminate early in the process those agents that make their way to phase II trials but are deemed inappropriate for phase III trials because of unsatisfactory results from the phase II trials (e.g., lack of efficacy or safety). The recent implementation of "phase 0" trials makes use of advances in methodologies and technologies to study the pharmacokinetic and pharmacodynamic properties of an agent before introduction to the traditional phased system. Pharmacokinetic studies address the movement, distribution, and fate of an agent in the body over time. Pharmacodynamic testing, in contrast, elucidates the biochemical and physiological effects of an agent on the body, focusing on the drug's interaction with various molecular and cellular structures within target tissues. Considered together, data from pharmacokinetic and pharmacodynamic studies help researchers determine a rational dosage regimen for testing in the conventional phases (I, II, III) of clinical trials. A phase 0 trial utilizes much lower doses of drug, thereby minimizing risk, in fewer patients. This approach allows agents that are not producing the desired effects to be weeded out earlier, and in this manner may shorten by up to a year the time it takes to move a potential chemopreventative agent from the laboratory to actual clinical use.

New genomic, proteomic, and metabolomic investigational techniques, together with novel high-throughput and imaging technologies are having an impact on the time it takes for new chemopreventative agents (and drugs for disease treatment) to move from the laboratory to the clinic. The U.S. Food and Drug Administration (FDA) has developed the Critical Path Initiative for agent (drug) development that is meant to

speed the process used for moving promising agents forward to clinical development (Green 2007). The initiative was developed based on the observation that fewer investigational new drug (IND) applications were being submitted in the past few years. Thus, the Critical Path Initiative was developed to study the tools and strategies for "proof-of-principle" studies or, as they are sometimes known, "first-in-human" studies; these tools and strategies are critical for determining whether development of specific agents should move forward. In addition, the time and cost of moving to "first-in-human" studies is reduced. The FDA is optimistic that the Critical Path Initiative will improve the efficiency of the drug-testing process and encourage researchers, both private and public, to increase their commitment to the development of new agents for the prevention and treatment of cancer as well as other chronic diseases.

1.5 Prevention Clinical Trials

Clinical prevention trials have shown that specific agents can reduce the risk of different cancer types. Making optimal use of this evidence for clinical practice is critical for reducing the burden of cancer on society. Examples of clinical trials with impressive results for cancer prevention, with major potential for translation into clinical practice, are those for breast and prostate cancers. In addition, large screening trials are currently being conducted for prostate, colon, lung, and ovarian cancers, with the intent of evaluating modalities for early detection, allowing more effective clinical management and thereby reducing the resulting cancer burden.

The Prostate Cancer Prevention Trial (PCPT) was the first large-scale phase III trial for prevention of prostate cancer. PCPT tested the 5-alpha-reductase inhibitor finasteride, which inhibits the conversion of testosterone to dihydrotestosterone, a key promoter of prostate cancer. This placebo-controlled trial of 7 years duration included more than 18,000 men with normal digital rectal examination (DRE) and serum prostate-specific antigen (PSA) less than 3 ng/ml. In 2003, the PCPT findings showed that finasteride reduced the period prevalence of prostate cancer by approximately 25% compared with placebo (Thompson et al. 2003). Participants in the finasteride group who did develop prostate cancer, however, had a higher incidence of high-grade tumors, in the range of Gleason score 7–10, than those in the placebo arm. The concern precipitated by this observation led to a re-analysis of the PCPT data on high-grade tumors (Lucia et al. 2007). Results of the re-analysis suggested that the increase in high-grade cancer was due, at least in part, to increased detection. This, in turn, resulted from the normal part of the prostate having volumes that were lower in the finasteride than the placebo group, thus selectively facilitating detection of any cancerous tissue nested in prostate exposed to drug. Such detection bias appears to have been more important than any direct effects of the intervention on tumor morphology. As a result, high-grade cancer was detected at earlier stages and was less extensive in the finasteride group than in the placebo group.

Clinical trials addressing breast cancer prevention have yielded adequate knowledge to improve prevention efforts in the public arena. One of the best examples from three decades of clinical experience with breast cancer prevention is the knowledge gained from the Breast Cancer Prevention Trial (BCPT) together with that from subsequent trials. The BCPT began recruiting in 1992 for premenopausal and postmenopausal women at an increased risk of developing breast cancer. More than 13,300 women were accrued to the trial and randomized to a tamoxifen or placebo arm. Results of the trial indicated that women taking tamoxifen had 49% fewer diagnoses of invasive breast cancer and noninvasive breast cancer (e.g., ductal or

lobular carcinoma in situ) compared to women in the placebo arm of the trial (Fisher et al. 1998). Concerns, however, were raised in the BCPT about the increase in endometrial cancer and thromboembolic events among women taking tamoxifen. In search of an equally or more effective preventative agent that would be less toxic, the Study of Tamoxifen and Raloxifene (STAR) compared raloxifene, an approved osteoporosis drug, to the BCPT-established standard of care for breast cancer prevention, tamoxifen. Results of STAR indicated that raloxifene was as effective as tamoxifen in reducing the risk of invasive breast cancer, but did not increase the risk of endometrial cancer and thus had a better overall benefit:risk profile (Vogel et al. 2006).

Many challenges exist for reducing the incidence of breast cancer, especially in those cancers that are not hormonally mediated. Additional agents are being investigated for preventative efficacy in preclinical and clinical studies in selected populations; these include aromatase inhibitors, retinoids, and bioactive food components (BFCs; e.g., soy and fish oil). The roles of timing of exposure, dose, and presence of other risk factors are only now beginning to be understood. This is especially true for nutritional approaches to cancer prevention. Research on soy and breast cancer illuminates the complexity of the effects of dietary factors on cancer risk. Several epidemiological studies have suggested that higher intake of soy or soy products is associated with a reduction in breast cancer risk, at least in some populations (Wu et al. 1998). For certain women, however, soy appears to increase the risk of breast cancer, which suggests that soy may be a dietary factor that can be both beneficial and harmful, depending on the circumstances. Because isoflavones preferentially bind to and activate the estrogen receptor (ER) and have estrogen-like properties, this activity has been proposed as the mechanism by which soy isoflavones reduce the risk of ER+ breast cancer (Messina et al. 2006). In contrast, further in vitro and animal studies, and a few small human studies, indicate that soy isoflavones (i.e., genistein) can stimulate the growth of pre-existing ER+ tumors. More studies are planned to investigate whether timing of intake of soy or soy products has an impact on breast cancer risk in high-risk women or on survival of breast cancer patients. Polymorphisms in genes relevant to estrogen metabolism and activity also appear to have both positive and negative effects on breast cancer risk through the modulation of soy isoflavones. The negative correlation between breast cancer risk and urinary and serum isoflavone levels was especially strong for women with a particular polymorphism in the gene *ESR1*, which encodes the estrogen receptor, the critical mediator of signaling in cells in response to estrogen (Low et al. 2005a). For women with the variant ESR1 genotype, differences in mean plasma estradiol levels for the highest and lowest tertiles of serum isoflavones would translate into a more than 30% difference in breast cancer risk.

A similar situation occurs with soy and prostate cancer. Isoflavones may modulate circulating androgen and estrogen concentrations in men and affect the risk of prostate cancer, which, like breast cancer, may be hormone dependent. The soy metabolites enterolactone and equol affect plasma androgen concentrations, and this may be modified by *CYP19A1* (cytochrome P450, family 19, subfamily A, polypeptide 1) polymorphisms (Low et al. 2005b). Case-control studies have found that men with the ability to degrade the soybean isoflavone, daidzein, to equol have a lower incidence of prostate cancer than men lacking this ability (Akaza et al. 2004).

Screening trials evaluate interventions aimed at the early detection of pre-cancer and/or cancer, in the hope that this approach will translate into a decreased cancer burden. The Prostate, Lung, Colorectal and Ovarian (PLCO) Cancer Screening Trial, for example, is a large-scale clinical trial designed to determine whether certain cancer screening tests reduce deaths from prostate, lung, colorectal, and ovarian cancer. The premise of the trial is that cancers identified at earlier

stages lend themselves to more effective treatments. PLCO also is important because it is targeting four cancers that cumulatively account for approximately 42% of new cancers each year in the United States as well as 46% of deaths.

PLCO began accruing participants in 1992 and in 2001 completed the randomization into two study groups: (1) the comparison group comprising participants who receive routine healthcare from their health providers, and (2) the intervention group comprising participants who receive a series of designated, scheduled exams to screen for prostate, lung, colorectal, and ovarian cancers. The screening of participants ended in 2006, with follow-up exams to continue for up to 10 years to determine the benefits or harms of screening. Preliminary findings on follow-up testing for men with unusual DRE or PSA levels higher than 4.0 ng/ml have shown that 74.2% of men with positive screening tests underwent additional diagnostic testing; 31.5% underwent biopsy, with 1.4% of the men in the screening arm diagnosed with prostate cancer (Andriole et al. 2005). Similar compliance and follow-up testing was seen among participants undergoing screening for the other PLCO cancers. Whether enhanced compliance to screening will lead to reductions in mortality from these four cancers will not be known until well past 2010.

1.6 Biomarkers for Cancer Prevention

A major challenge for prevention clinical trials has been securing timely endpoints to provide adequate proof that cancer (or other chronic disease) has been prevented. This often takes a decade or more in cancer prevention clinical trials, as participants must be followed long enough for disease rates to be examined. Such long trial durations, compounded by the need for participant sample sizes large enough to achieve adequate event rates for statistical evaluation, can stress the limited resources available for cancer-related research. In an age when methodological, computational, and technological advances evolve in vastly shorter periods of time, cancer prevention researchers have begun looking for biomarkers to serve as surrogate markers to identify cancer risk, assist in early detection and progression of disease, and to assess the efficacy of preventative (i.e., lifestyle or medical) interventions. Measurement of biomarkers can provide empirical evidence of the effect of interventions in basic research or clinical trials by identifying their impact on molecular and cellular pathways related to disease initiation and progression. In addition, surrogate endpoint biomarkers (SEBs) offer the promise of reducing the time needed to determine if cancer prevention interventions have a benefit (or cause harm). However, at present, putative SEBs still have to be validated in the context of clinical trials.

Biomarkers can serve multiple purposes in clinical research; they may be used for early detection as well as to determine susceptibility, exposure, or effect of an intervention. For example, in cancer prevention clinical trials using lifestyle interventions, biomarkers of exposure to dietary factors are commonly assessed to document exposure and determine cancer risk (Milner 2003). The advent of emerging technological and methodological approaches in nutrition science has made possible more accurate measurements of dietary intakes and metabolic processes involved in interactions that may influence cancer risk. For example, basic research on dietary isothiocyanates (ITCs), found primarily in cruciferous vegetables, has elucidated mechanisms-of-action that suggest a role for these nutrients in preventing carcinogenesis. These mechanisms include inhibition of carcinogen-activating enzymes, induction of carcinogen-detoxifying enzymes, increase of apoptosis, and arrest of cell cycle progression (Zhang 2004). Preclinical and human studies have provided evidence that the intake of ITCs is inversely related to the risk of lung, breast, and colon cancers (Zhang 2004). Because ITCs are

metabolized and excreted in urine, their usefulness as a biomarker of exposure has been hypothesized and confirmed in clinical studies.

To address the need for identifying cancer prevention biomarkers, in 2000 the National Cancer Institute established the Early Detection Research Network (EDRN), an investigator-driven network designed to conduct translational research aimed at identifying biomarkers both for the early detection of cancer and for documentation of cancer risk (National Cancer Institute 2008). EDRN investigators have more than 120 biomarkers in development and have been instrumental in identifying and initiating validation studies of markers for major cancers, such as: prostate (protein profiling of serum for high-grade prostatic intraepithelial neoplasia and levels of PCA3, a noncoding RNA, in urine); colon (*K-ras* mutations in stool and urine); and breast (panels of autoantibodies in sera). Clinical validation studies are in progress for serum colon cancer-specific antigen (CCSA)-2 and CCSA-3 in colon cancer and serum des-gamma-carboxy prothrombin and alpha-fetoprotein-L3 in liver cancer. A signature accomplishment of the EDRN is its development of a process to validate biomarkers; validation confers the highest level of confidence that the biomarker is linked to the disease process and provides a "proof-of-principle" of its use in risk assessment, diagnosis, and/or treatment. EDRN is collaborating with investigators from large clinical studies to obtain biologic samples for use in validating specific biomarkers. Biomarkers that were validated in several of these preliminary studies will subsequently be tested in sera from cases and matched controls collected in the PLCO trial.

Samples from the PLCO trial also have been made available to biomarker investigators based on an application process open to all. For example, a recent biomarker study investigated the relationship of obesity-related hyperinsulinemia to increased risk of prostate cancer, a previously suggested association (Weiss et al. 2007). Insulin-like growth factor (IGF)-1 and IGF binding protein (IGFBP)-3 have been shown from experimental studies to influence cellular growth, metabolism, and apoptosis, with potential impacts on prostate cancer. A nested case-control study was conducted from prediagnostic serum samples collected for the PLCO trial; 727 incident cases of prostate cancer and 887 matched controls were assessed for levels of IGF-1 and IGFBP-3. Results of the study showed no overall association between IGF-1 and IGFBP-3 (independently) and prostate cancer risk. However, the molar ratio of IGF and IGFBP-3 was related to the risk for aggressive prostate cancer in obese men (Weiss et al. 2007). The design of the PLCO trial allowed the collection of samples at baseline and each year during the trial, up to the year cancer was diagnosed. This sample repository is a valuable resource that may be shared with investigators who are seeking to identify, develop, or validate biomarkers of risk or for early detection.

For making optimal use of our increasing knowledge of biomarkers in cancer prevention, there must be increased planning for collection of biomarker information in prevention clinical trials. This is occurring in the NCI-sponsored Selenium and Vitamin E Cancer Prevention Trial (SELECT), a randomized, double-blind, placebo-controlled, population-based trial investigating selenium and vitamin E in the prevention of prostate cancer (Klein et al. 2001). An important feature of SELECT is the collection and preservation of blood samples that will permit the evaluation of a wide variety of biomarkers associated with hormone-related genes that are prominent in prostate carcinogenesis, such as the androgen receptor, *CYP17A1*, *SRD5A2*, and *HSD3B2* (Hoque et al. 2001). NCI also is working with the Women's Health Initiative (WHI) to assess biomarkers. The WHI is a set of clinical trials and an observational study in approximately 160,000 healthy, postmenopausal women to test the effects of postmenopausal hormone therapy, diet modification, and calcium and vitamin D supplements on heart disease, fractures, and breast and colorectal cancer. The biomarker study includes a genome-wide single nucleotide polymorphism scan for markers

associated with breast cancer, cardiovascular disease, and stroke and should be completed by 2010 (Prentice and Qi 2006).

Aside from providing a fingerprint of a disease state, biomarkers also are needed to define "normal" so that change, for example, from a premalignant to malignant state, can be monitored. Identifying changes at the earliest possible stages in the carcinogenic process will be of great benefit, as will biomarkers that can distinguish between changes indicative of a general response versus changes indicative of a response that is specific to the cancer cell. Furthermore, researchers will need to understand the significance of changes in biomarkers in the context of carcinogenesis. Identifying and validating biomarkers that reflect changes occurring during the transition from a premalignant to a cancerous cell will be of great use in cancer prevention research, an area with the potential to impact favorably public cancer prevention measures and ultimately survival.

1.7 Systems Biology

Basic and clinical research during the last half of the twentieth century has yielded data reflecting the vast complexity of components and interactions of biological systems at multiple levels: molecular, cellular, and organ. To provide meaningful order and develop a systematic approach that addresses this complexity, a relatively new biological study field—systems biology—has emerged that focuses on complex interactions in biological systems. Systems biology may be viewed as a field of study or as a set of protocols, or approaches, which are used to conduct research. As a field of study, systems biology involves investigations into how interactions give rise to the function and behavior of a biological system. Examples include interactions of enzymes and metabolites in a metabolic pathway (Snoep and Westerhoff 2005). As a set of protocols for conducting research, systems biology is not limited to the integration of the complex observations making up a biological system. Rather, this incarnation of systems biology imbues it with a more global role: it includes the underlying theory driving the research, computational modeling to develop testable hypotheses about a biological system, and experimental validation; these protocols then use the knowledge gained to create a quantitative description of cells or cell processes to refine the computational model or theory (Kholodenko et al. 2005). This is the focus of systems biology most relevant to cancer prevention research, which is using techniques and methods in proteomics, metabolomics, transcriptomics, and high-throughput technology to collect quantitative data for the development and validation of disease and risk models.

Recently, whole genome approaches have led to the identification of genetic loci that are relevant to complex diseases in a way that the candidate gene approach could not do. In a somewhat analogous fashion, systems biology goes beyond the single molecule or pathway to understand (1) the higher level properties and dynamics of complex biosystems, including both the interactions among their parts and with other systems, (2) how these systems are established and perturbed, and (3) what happens to them when they are perturbed. Because cancer can arise from perturbation of a number of different yet interconnected pathways, a systems biology approach may prove useful in developing better prevention and treatment strategies (Hornberg et al. 2006). In the area of molecularly targeted therapies, for example, the promising results obtained with early efforts in this direction are often thwarted by resistance to the targeted interventions or by an extremely limited spectrum of candidate tumors. As data on the molecular interactions underlying cancer accumulate, analysis of multiple inputs or parameters at the same time will be critical to designing better targeted and individually tailored interventions that interfere simultaneously with collateral, or alternative, molecular paths to the cancer in question (Liu et al. 2006). In a well-established example, tamoxifen is a drug that targets a

specific molecule, the ER, and its use has yielded substantially increased survival among women with ER+ breast cancer. Yet tamoxifen fails to benefit some individuals, and some ER+ breast cancers never respond to this ER targeting agent. Analysis of multiple pathways that affect cell proliferation shows that ER+ breast cancers with high growth factor receptor expression [particularly epidermal growth factor receptor (EGFR) and human epidermal growth factor receptor 2 (HER2)] are more likely to be resistant to endocrine therapy. High levels of the protein known as ER coactivator amplified in breast cancer (AIB1) also appear to reduce the effectiveness of tamoxifen (Massarweh and Schiff 2006). Thus, a multipronged approach that targets multiple, growth-promoting molecules and pathways, namely those alternative pathways that bypass the originally intended target (in this example, the ER), may lessen the possibility of resistance and provide a more permanent cure.

1.8 Future Directions for Attaining Optimal Impact in Cancer Prevention

Cancer prevention research has expanded tremendously over the past three decades. Information on lifestyle and medical interventions has become more evidence-based as methodologies have become more rigorous. Yet the knowledge gained has not been optimally used in translation of findings to patients and the public, leaving major challenges to the implementation of effective cancer prevention strategies and interventions. For a few interventions for which acceptance and uptake in the clinical and community settings have occurred—tamoxifen use to prevent contralateral, or secondary primary, breast cancers and smoking cessation among men leading to decreases in lung cancer mortality—impressive progress has been made. These examples of success point to the potentially enormous public health benefits that would be experienced if other documented prevention interventions could be translated into widespread use.

Development of a multidisciplinary approach to cancer prevention investigations, using both lifestyle and medical strategies, is critical. The incorporation of "-omic" technologies and methodologies into such multidisciplinary investigations lends increasing credence to the possibility that an individual's risk level can be assessed, thus allowing individualized recommendations for cancer prevention. This may include recommendations for nutritional interventions, the ideal level and type of physical activity, and tailored chemoprevention regimens based on genetic and/or metabolic profiles. Such an approach has been suggested by nutrition researchers, as comprehensive DNA and metabolic profiles have come closer to reality (Arab 2004). An individual's DNA or metabolic profile would serve several important purposes for disease prevention and control. First, it would allow the design of clinical intervention studies to focus recruitment of those individuals with identified genomic or epigenomic profiles who are likely to benefit from a given intervention. A second potential benefit would be that by honing in on such very high-risk individuals in clinical prevention trials, the number of participants in such trials could be decreased, since the anticipated event rate (breast cancer occurrence) would be expected to increase. Hand in hand with increased event rate is the expectation that the time it takes to observe the effects of the intervention at a statistically significant level would shorten. Another anticipated benefit from establishing baseline metabolic profiles of trial participants is that these molecular entities will serve not only as markers of increased risk but may also function as biomarkers whose measurement and modulation in response to interventions has potential to serve as surrogates for clinical efficacy. Together, these modifications in trial design should lead to the need for fewer

human and economic resources in conducting cancer prevention trials.

Important challenges exist also in the training of a new generation of multidisciplinary investigators who are comfortable working with emerging technologies, but have expertise in cancer, to make optimal use of knowledge gained for cancer prevention. Training is supported through many types of NIH grants, but increased support of scientists early in their careers is necessary. Inclusion of training in the many medical, biological, physical, engineering, and other sciences important to cancer research, such as nutrigenomic, genomic, and metabolic research, should be made available. In addition, the training of individuals from multiple disciplines (e.g., basic, biomedical, clinical, engineering, and information sciences) who are interested in pursuing research on nanotechnology tools and/or applications for the prevention of cancer needs emphasis.

A challenge for making optimal use of current resources is how to collect, manage, and use the overwhelming amount of data generated by "-omic" technologies. For example, multidimensional protein identification technology (MudPIT) enables the analysis of as many as 60,000 proteins at a time (Chen et al. 2006). For useful analysis of these large proteomic datasets in cancer prevention, the proteome must be better defined related to its role in cancer promotion and progression, a better understanding of the differential expression of proteins in different cell compartments must be developed, and the proteome must be measured with a high degree of quantitative accuracy. Targeted arrays that address specific questions or biological pathways of interest also may be useful for resolving the difficulties attendant in handling large datasets. Ultimately, the integration of genomics, proteomics, and metabolomics will be essential for predictive interpretations in prevention research.

Even with enhanced information gathering provided by emerging technologies in concert with basic and experimental research, optimization of the potential of cancer prevention will not occur without a concerted effort to translate this information from the bench to the bedside and to the community.

Prevention is a major overall strategy for reducing the burden of cancer on society. We are not making optimal use of the potential of cancer prevention, but progress is encouraging, as shown by the continued decline in incidence of certain cancers. A comprehensive approach making use of new technologies, informatics, and nanotechnology, along with enhanced multidisciplinary training, will provide a sound base of experimental researchers to develop investigative strategies to address both lifestyle and medical strategies to prevent cancer.

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Predictors of Successful Cancer Prevention Programs

2

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Abstract Finding the optimal use of health-care resources requires the reliable estimation of costs and consequences. Acquiring these estimates may not be difficult for some common treatments. More difficult is the optimization of resources in the area of diagnostics. Only a few attempts have been made to optimize the use of resources in the area of prevention. Several aspects have to be considered when optimizing the resources for prevention: (1) participation rates in structured prevention programs are low, (2), acquiring data on follow-up and outcomes is difficult, (3) there are concerns about the quality of information available to public, and (4), the public is often unaware of scientific assessments of prevention programs. As prevention programs are costly long-term projects, a strategy to select these programs according to possible predictors of success might be useful. The few analyses of cancer prevention in the literature have been directed towards the most common malignant diseases (as assessed by incidence) such as cancer of the breast, colon, lung and prostate. We argue that incidence is a poor marker for selecting secondary prevention programs. Incidence may be a misleading indicator for two reasons: incidence of disease does not predict efficiency of management or good health outcomes, and incidence does not separate clinically significant from non-significant disease. The traditional strategy is based on the assumption that more screening increases the chance of cure. We propose an alternative outcomes model that suggests better disease management justifies new prevention programs. Indicators for better disease management are effective and efficient treatments as well as high-quality screening (sensitivity and specificity) techniques and possibly "side-effects of prevention programs," which provide early signs of success to motivate the patient's participation, to keep up with the program and finally to succeed.

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2.1 Introduction

Optimal use of health-care resources presumes the reliable estimation of the costs and the consequences of health-care services. These estimates are available for many treatments. More difficult is the optimization of resources in the area of diagnosis and prevention where these estimates are rare. Optimizing the resources for prevention requires consideration of several issues: first, the difficulty in acquiring follow-up and outcome

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data; second, the assessment of value of prevention programs from a scientific point of view; third, concerns about true information of the public and assessment of the value from the view of the public; and finally, the assessment of value of prevention programs by individual persons resulting in the participation rates in structured prevention programs. These aspects make it difficult to select the optimal among several possible prevention programs. The saying "an ounce of prevention is worth a pound of cure" may be not true (Gérvas Camacho et al. 2007; Sackett 2002; Schwartz et al. 2004; Welch 2004) unless there is some supporting evidence.

Most fields in health care require evidence before a new strategy is adapted for routine clinical practice. In preventive medicine the generation of such data is difficult for two reasons. Large and long-running studies have to confirm the successful prevention of the target problem, which is usually many years into the future. Long-term effects may attract less attention than immediate effects. In addition, professionals and patients tend to invest less energy to reach distant benefits unless there are early indicators which confirm that the intended long-term benefit will be achieved. Second, unlike in clinical trials on new treatments, it is not possible in prevention studies to start with a small pilot study followed by the main study because of the required long study periods. These decisions, which have to be made before initiation of a prevention program, will be easier if predictors of successful prevention programs can be identified. The identification of such predictors is the aim of this paper.

2.2 Methods

We performed a literature search on established and recommended programs to screen for and prevent malignant diseases in Europe and the United States. In order to assess the quality of prevention programs we looked for the markers' "structure" and "process". In addition we searched for data on effectiveness of prevention programs. Finally we tried to find selection criteria for preventive programs.

2.3 Results

2.3.1 Quality of Prevention Programs

2.3.1.1 Low Participation Rate in Structured Prevention Programs

The Zentralinstitut, the central institute of the major public health insurer in Germany, analysed 506,500 cases of colonoscopy screening data in addition to information from 544,000 colonoscopies performed over 3 years (Zentralinstitut 2007). The acceptance rate in those aged up to 74 years was 8.8% for men and 10.2% for women.

2.3.1.2 Difficulty in Acquiring Follow-Up and Outcome Data

The acquisition of follow-up and outcome data is often a problem in prevention studies. After about 18 months, both the trial staff and the members of the target groups lose interest in participation. Yet an 18-month observation period is insufficient for primary or secondary prevention studies. For this reason, the loss of interest and attention might be prevented if predictive indicators of late effects can be found.

2.3.1.3 Concerns About Correct Perception of Scientific Information by the Public

There is strong evidence that the public support screening programs (Schwartz et al. 2004). The propagation and measurement of tumour mark-

ers in blood samples was widely recommended in the 1970s and 1980s and was well accepted by the patients. Increasing scientific evidence and the corresponding publications (Hayes 1996; Jacobs and Haskell 1991) have led to a considerable reduction in their use as screening tool. This reduction was difficult to achieve because doctors as well as their patients had received different information in the years before. Scientists had published the advantages of tumour marker screening and practicing doctors as well as patients were consequently convinced of the predictive value of tumour markers and the need to include them in follow-up programs.

There is typically more enthusiasm for screening when no or only little harm of the screening is perceived by the target groups. This is true for blood tests and for mammography screening but only to a lesser extent for procedures which patients find more harmful or unpleasant such as endoscopies and biopsies. For a more detailed discussion the scientific information on secondary prevention will be presented for colon and breast cancer.

2.3.1.3.1 Effectiveness Using the Example of Screening for Breast Cancer

A review of the Cochrane collaboration on breast cancer screening (Goetzsche and Nielsen 2006) pointed out that 2,000 women of the age group 50–69 years have to be screened to prevent one additional death from breast cancer. There is general agreement on a small but true benefit from breast cancer screening although the estimates vary among authors. This gain in life-years has to be compared with three disadvantages associated with screening for breast cancer.

First, about 30% of the expected breast cancer cases will have a false-negative screening result (clinically detected cancer following a negative screening result). Second, about one-fifth of all screening tests will produce a false-positive result and will cause anxiety, concerns and costs of additional tests (Elmore et al. 1998). Third, in 10 of these 2,000 women, screening will lead to the diagnosis of breast cancer (and subsequent treatment) that would have neither influenced the life expectancy nor the quality of life of the patient if the cancer had not been detected by mammography. We use the term pseudodisease to describe identifiable pathology that has no clinical importance in terms of life expectancy or quality of life (Kaplan 2006; Shorter 1997; Woolf 2003). Pseudodisease has been discussed in relation to oncology but is also referenced in cardiovascular disease (Black and Czum 2007) and hypothyroidism (Woolf 2003). Patients have difficulty understanding this information because we do not know which individuals with positive results will develop clinical disease. At best, we can only offer proportions in the populations. In the case of breast cancer, we have to treat all women with a confirmed diagnosis although we know that 20%–30% of these patients will not benefit from the treatment they receive (Goetzsche and Nielsen 2006; Barrat et al. 2005).

2.3.1.3.2 Effectiveness Using the Example of Screening for Colon Cancer

Early studies (Mandel et al. 1993) reported in 1993 that a 33% reduction in the 13-year cumulative mortality can be achieved by faecal occult blood testing (FOBT). The study randomized over 45,000 adults to usual care, annual screening or biannual screening. A critical review of this study indicates that several of today's epidemiologic requirements, such as protocol adherence and avoiding a considerable variation in the applied diagnostic methods, would call such a conclusion into question. In addition, this conclusion was supported by the annually but not biannually tested study group. In an update of the study 6 years later, the authors (Mandel et al. 1999) described a 21% mortality reduction in the biannual screening group and presented slightly better results in an additional report 1 year later (Mandel et al. 2000). Hardcastle et al. (1996) claimed that the reduction in mortality

reported in several studies was observed not in unselected populations. They completed a randomized trial in which controls were not told about the study and received no intervention. Screening-group participants were sent a Hemoccult FOBT kit with instructions from their family doctor. In this randomized study a 15% reduction in mortality was confirmed. A similar reduction in mortality was reported for biennial screening by Kronborg et al. (1996). The result of this study was confirmed some years later in a 13-year follow-up evaluation (Jørgensen et al. 2002). It may be important to notice that only the screened patients but not controls received information about the study, like in the trial of Hardcastle et al. (1996). There is increasing evidence that the information itself can significantly influence the results of clinical trials (Porzsolt et al. 2004). This possibility makes it difficult to interpret the observed survival differences.

In the German survey, 85.1% of lesions with a size of 1–3 cm were completely removed. Advanced stages of adenomas including 'Tis' were found in 6.6% and cancer in 0.9% of the investigated persons. Most of the detected malignant lesions were at favourable cancer stages. Males with cancer were 2.5 years older, and women with cancer were 4 years older than other participants of the screening program (Zentralinstitut 2007).

Some years later, a re-analysis of published data reported that death due to colon cancer can be avoided in 1 of 862 screened persons (Moayyedi and Achkar 2006). This reduction in cancer-related mortality did not influence the overall mortality. A systematic review of the data on secondary colorectal prevention prepared by the Cochrane collaboration (Hewitson et al. 2007) confirmed a modest reduction in colorectal cancer mortality, a possible reduction in cancer incidence through the detection and removal of colorectal adenomas, and potentially, the less invasive surgery that earlier treatment of colorectal cancers may involve. Harmful effects of screening include the psycho-social consequences of receiving a false-positive result, the potentially significant complications of colonoscopy or a false-negative result, the possibility of overdiagnosis (leading to unpleasant and unnecessary investigations or treatment) and the complications associated with treatment.

A similar, rather reluctant interpretation of colorectal screening data was recently published (Kerr et al. 2007). This report confirmed the expected effect of FOBT screening but did not support the benefit of flexible sigmoidoscopy. These inconsistent reports demonstrate the difficulty of adequate interpretation of available scientific data. More advanced diagnostic techniques such as computed tomographic colonography are recommended by some groups (Kim et al. 2007) but may not yet be sufficiently standardized for use in large studies (Mulhall et al. 2005).

For the discussion of effectiveness it should be recalled that the concept of false-positive and false-negative results does not apply to colon cancer, as the therapeutic intervention (polypectomy) is integrated in two necessary tests (colonoscopy and histologic examination). Pseudodisease, however, may be a significant problem in colon cancer prevention but we are not aware of data that support reliable conclusions.

In the German study on colon cancer screening there were 2.7 complications per 1,000 investigations. Most complications (1.6/1,000) were due to bleeding, followed by cardiopulmonary complications (0.8/1,000) and perforations (0.3/1,000) (Zentralinstitut 2007). These complications have to be considered when assessing the value of colon cancer prevention.

An unsolved scientific problem concerns cases of spontaneous remission. According to our present understanding of the concept of malignant disease, such cases are difficult to investigate by a direct approach. Occasional cases of spontaneous remission are described in various types of cancer such as hepatocellular carcinoma (Ohtani et al. 2005), Hodgkin's disease (Bang et al. 2005), lymphoma (Abe et al. 2007), melanoma

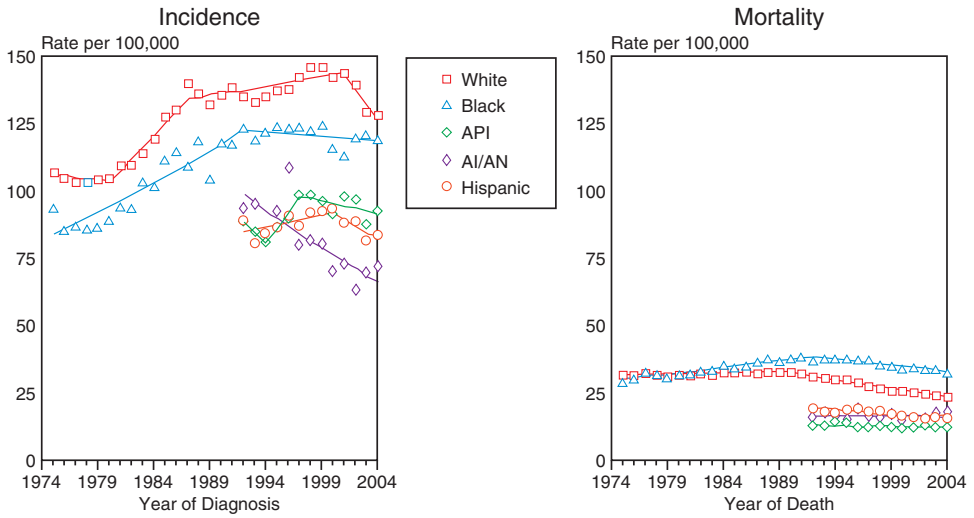

Fig. 2.1 SEER incidence and United States death rates for female breast cancer 1975–2004

(High et al. 2005), and small cell lung cancer (Horino et al. 2006). As there are some indicators that neoplastic lesions of gastric cancer may not progress or even regress after eradication of *Helicobacter pylori* eradication therapy, spontaneous remission is discussed even in patients with precancerous gastric disease (Malfertheiner et al. 2006). If these spontaneous remissions would indeed occur more often in the early stages of a disease than in more advanced stages then they would have to be considered in the interpretation and understanding of secondary prevention. As this question cannot be answered without additional data, spontaneous remission may add additional uncertainty to the consideration of secondary prevention.

2.3.1.4 Value of Prevention Programs from the Public and Scientific Point of View

Women have a 1 in 12 risk (in the UK) or a 1 in 8 risk (in the United States) of developing breast cancer but only if they manage to escape other threats to life and survive to the age of 80. Incidence is not equivalent to mortality: In England and Wales, only one woman in 26 will have died of breast cancer by the age of 80 (Bunker et al. 1998). In the United States, breast cancer mortality has remained roughly constant since 1940 while incidence has nearly doubled (Harris et al. 1992). The National Cancer Institute's Surveillance Epidemiology and End Results (SEER) data (Fig. 2.1) confirm these results.

These figures could indicate that therapy has effectively held back an epidemic of breast cancer. More likely these data indicate the problem of overdiagnosis and overtreatment. We are probably detecting some cases of breast cancer that existed before but which could not be detected by previous screening methods, nor did these cases of breast cancer affect their host. At present it is impossible to differentiate these inert cases of cancer (pseudodisease) from other cases of breast cancer that impair both their host's length and quality of life. Since these two types of cancer cannot be differentiated, the detection and subsequent treatment of these

cases of pseudodisease may result in overtreatment in up to 20% of breast cancer cases detected by mammography.

This risk of overtreatment as well as the risk of false-negative and false-positive diagnoses has to be compared with the advantages of breast cancer screening. This assessment of value is rather complicated as it will be different from the public and the scientific point of view. Scientists may consider the only advantage of mammography the proportion of breast cancer deaths that can be prevented by it. We know from studies by Schwartz et al. (2004), however, that people in the general population value mammography high enough to accept the described false-positive and false-negative diagnoses. As more than 950 of 1,000 women who undergo mammography will finally get the expected "good news" that no cancer could be detected, we presume that this positive information is of considerable value for the tested population. This value of "perceived safety" (Porzsolt et al. 2007) may explain the demand of mammography despite its small benefit and considerable disadvantages.

Politicians are convinced that prevention and health promotion will improve health, quality of life and power. They promote prevention as societal task but not only as a task of health-care politics (Apitz and Winter 2004). Since such recommendations, which are not specific for a particular country, are supported by scientific statements it is almost impossible to find out whether or not the public's perception of political information is justified by the original scientific data. Small changes in information introduced by the operator may considerably change its perception by the receiver. As all partners in the health-care system try to present their messages in the most positive frame, it can be expected that the information may not remain unchanged on its way from the place where it is generated, the scientific lab, to scientific publications and translation into a political statement, and then down to the final destination, i.e. the perception by the public.

2.3.2 Recommended Prevention Programs

2.3.2.1 European Union

The 2003 explanatory memorandum (Health-EU 2003) of the "Europe Against Cancer Programme," which was founded in 1985, includes three key elements. First, the partnership approach (bringing together all the national actors involved in all areas of cancer prevention); second, the code against cancer (10 rules for a healthy lifestyle, www.cancercode.org); and third, the long-term vision of lowering the cancer-specific mortality of the European population, originally set at 15% in the period of 1987–2000. The annual cancer-specific mortality in Europe actually fell by a total of 10%, equating to around 92,000 lives saved.

These key elements are based on the assumption that well-managed population screening is more effective than individual screening on demand. It follows that early detection of cancer by screening is one of the strategic areas of cancer prevention. It is also recognized that organized cancer screening should only be offered to healthy people if there is sufficient evidence that screening leads to a decrease in disease-specific mortality or the occurrence of advanced disease. Consequently, the following recommendations were released by the EU: mammography screening for breast cancer in women aged 50–69, faecal occult blood for colorectal cancer in men and women aged 50–74, and Pap smear screening for cervical abnormalities every 3–5 years, starting between the ages of 20 and 30. Other test may also be recommended once research shows that they meet the criteria for organized cancer screening.

2.3.2.2 United States

The Agency for Healthcare Research and Quality (AHRQ) published recommendations for prevention programs in the United States. Their rec-

ommendations include screening for breast cancer, cervical cancer and colorectal cancer (U.S. Preventive Services Task Force 2007).

The Unites States Preventive Services Task Force (USPSTF) found fair to good evidence that several screening methods are effective in reducing mortality from colorectal cancer. The USPSTF concluded that the benefits from screening substantially outweigh potential harms, but the quality of evidence, magnitude of benefit, and potential harms vary with each method (U.S. Preventive Services Task Force 2002).

Screening for the genetic risk of breast and ovarian cancer is not recommended. The USPSTF found fair evidence regarding important adverse ethical, legal, and social consequences that could result from routine referral and testing of these women. The USPSTF concluded that the potential harms of routine referral for genetic counselling or *BRCA* testing in these women outweigh the benefits (U.S. Preventive Services Task Force 2005).

Screening for testicular cancer is not recommended as the low incidence of testicular cancer and favourable outcomes in the absence of screening make it unlikely that clinical testicular examinations would provide important health benefits (U.S. Preventive Services Task Force 2004a).

Screening for lung cancer is not recommended because its benefit has not been established in any group. The mortality rate of screening (due to the necessary biopsies) range from 1.3% up to 11.6% and morbidity rates can be as high as 44% (U.S. Preventive Services Task Force 2004b).

2.3.3 Critical Appraisal of Traditional Selection Criteria

Cancer incidence and mortality is presented in the introduction of most (secondary) cancer prevention programs. Although not stated explicitly these presentations may induce the impression that justification of secondary prevention depends on incidence and mortality. The increasing information and knowledge about variables that influence incidence data may lead to a more detailed interpretation of high incidence and not necessarily justify a secondary prevention program.

It is well known that the course of malignant diseases may vary considerably. The morphologic diagnosis is a poor predictor of the course of disease. Prostate cancer is a well-known example. Most prostate tumours grow slowly, do not produce metastases and do not affect the life of their hosts. Some cases of prostate cancer grow fast, metastasize in several organs, and impair the patient's quality and length of life. Similar biologic variation has been shown for other types of cancer such as breast cancer (Güth et al. 2006), B cell malignancies (Dave 2006) and cancer of the urinary bladder (Brauers and Jakse 2000). About 10 years ago it was commonly believed that patients with untreated breast cancer will die of breast cancer. Today we know that a considerable proportion of breast cancer cases are not life threatening to their hosts. This rather benign type of cancer is another example of pseudodisease. Although the proportion of pseudodisease is much smaller in breast cancer than in prostate cancer, management strategies including secondary prevention must be re-examined. In some circumstances, health services consume resources without producing added value. Inappropriate use of services might harm population health by taking limited resources away from programs that would have produced more benefit.

Besides pseudodisease, the scientific evidence that supports the possibility of spontaneous remissions should be discussed. This evidence is limited to single reports and partial reviews. Spontaneous remissions have attracted very little attention.

Several investigators have suggested that some tumours spontaneously regress. Because breast cancer is rarely left untreated, we know surprisingly little about the natural history of the

disease. In one analysis, Zahl and Maehlen (Zahl et al. 2004; Zahl and Maehlen 2006) attempted to piece together the natural history of breast cancer. They used a creative method for comparing age-matched groups of women living in four Norwegian counties. In 1996, these areas of Norway began screening women with mammography every other year. In the analysis, they considered the group that was screened three different times between 1996 and 2001. Eligibility was defined as being between the ages of 50 and 64 in 1996. For a comparison, they used women who were between the ages of 50 and 64 in 1992. In other words, rather than going to another locale to compare results, they went to another segment of time to get their comparison group. These women would have been screened three times between 1992 and 1997 if there had been a program. The women in the comparison group were all invited to receive a one-time "prevalence" mammogram. The "prevalence" mammogram is used to get a snapshot of how many women have breast cancer at any particular point in time. In this case a sample of women was invited for a test once the larger screening program began. In summary, the screened group had three mammograms while the comparison group had only one mammogram. Because of the slight time overlap of the two groups, the mammogram for the control group was given at the same age as the third mammogram for the screened group. We would expect, then, that the prevalence of breast cancer should be the same in the two groups. However, that is not what happened. The analysis suggested that the incidence of invasive breast cancer was 22% higher in the screened group than in the comparison group.

2.3.4 Incidence in Primary and Secondary Prevention

Although incidence is used to describe the rates of newly diagnosed cases in primary as well as secondary prevention, the interpretation of incidence is different in primary and secondary prevention (Fig. 2.2). In primary prevention, incidence describes the rate of diagnosed cases at the end of the completed prevention program. These incident cases represent the failures of a primary prevention program.

In secondary prevention, incidence usually describes the rate of cases detected by screening at the beginning of the program. As large cancer statistics, e.g. the report on cancer incidence and mortality in Europe (Boyle and Ferlay 2005), use incident data from different sources there is some risk that incident data from different sources may describe different populations. Some of these data may include patients who participated in secondary preven-

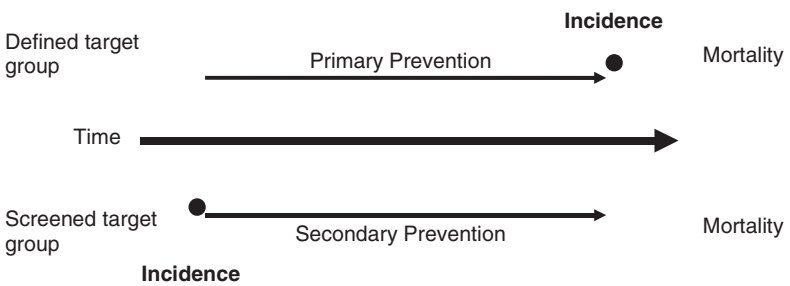

Fig. 2.2 Interpretation of 'incidence' in primary and secondary prevention. In primary prevention, incidence describes the rate of prevention failures at the end of the prevention program. In secondary prevention, incidence usually describes the rate of cases detected by screening at the beginning of the prevention (*dots*)

tion programs. These cases may include patients with pseudodisease and patients with spontaneous remissions. As incidence data based on screening are influenced by several additional variables such as the quality of the screening tools and the selected populations, it is rather difficult to interpret these data. In summary, incidence data have to be interpreted with caution and are not ideal predictors of successful prevention programs.

2.4 Discussion

There are several lessons we can learn from prevention studies. First, we need to understand the extent of our uncertainty about the benefits of prevention programs and share it with the public, patients and policymakers. No diagnosis/treatment process is free from risks. Application of treatment with no solid scientific evidence of benefit exposes patients to risk when there may be no potential gain. People invited to be screened for serious diseases must be told about the risks, benefits and limitations in a way that instils realistic expectations and ensures fully informed consent in those who participate (Gérvas Camacho 2002; Gérvas Camacho et al. 2007; Smith 1992). Public health policies in Europe focus on primary and secondary prevention and provide information on health factors. They claim that prevention and lifestyle can avoid some types of cancer and improve the health condition of the population (http://www.cancercode.org/). This view is derived from the somatic mutation theory of carcinogenesis, which includes several paradoxes (Baker and Kramer 2007) such as the presence of distinct precancerous lesions at the onset of promotion, the large number of genetic instabilities found in hyperplastic polyps that are not considered cancer, and spontaneous regression.

Second, "If we want more evidence-based practice, we need more practice-based evidence" (Green and Glasgow 2006). In other words, we need to conduct and carefully evaluate prevention studies to identify possible differences of expected efficacy derived from scientific reports and observed results in daily practice. It is not only the formal difference between a laboratory experiment and an ideal but artificial condition of a clinical trial and finally a real world situation which is perceived by scientists. Patient's perceptions may influence the outcome. Patients are enthusiastic about screening and prevention. Such programs will motivate the patient to achieve the expected effects and not expected "side-effects" of prevention programs, e.g. perceived safety, hope, and a positive perspective. We assume that these side-effects of prevention programs will support the achievement of favourable outcomes. If, however, the time required to achieve a perceptible success of a prevention program is too long, the participant's motivation and interest in the program may diminish. The effectiveness of prevention programs may be increased if we identify early success indicators and change the programs in a way that helps the patients to experience these early indicators of success, allowing us to reliably assess the achieved results.

Third, we should avoid repeating earlier mistakes by testing only hypotheses that are supported by mainstream assumptions. An example is the randomized German acupuncture study, which included more than 250,000 patients and 10,000 physicians. Several preliminary reports published between 2004 and 2006 indicated that acupuncture is being successfully applied in a variety of patient groups even though the underlying mechanism is not understood (Szczurko et al. 2007; Tournaire and Theau-Yonneau 2007).

The lessons discussed in this paper suggest that different types of predictors may be used to identify successful prevention programs (Table 2.1).

First and most importantly, epidemiologic indicators are needed to confirm at least some causal relationship of the planned prevention program and the expected outcome. These indi-

Table 2.1 Predictors of successful prevention programs. The success of prevention programs depends primarily on epidemiologic predictors which are different for primary and secondary prevention. Successful prevention programs have to meet social and individual predictors as well. These predictors are the same in primary and secondary prevention

Types of predictors	Primary prevention	Secondary prevention
Epidemiologic predictors	Causal relationship of expected outcome and of:	
	Lifestyle and behaviour	Screening and therapy
Social predictors	Public acceptance, political decisions, advice of health care professionals, recommendations of family and friends	
Individual predictors	Personal preferences and values, acceptance of scientific and social recommendations	

cators are different in primary and secondary prevention. In primary prevention these indicators are related to changes of lifestyle or behaviour (or both). In secondary prevention these indicators have to confirm the quality of disease management (Mayer and Mayer 2004; Stagmo et al. 2004; Campbell 2004) while confirming the effectiveness of the screening methods, as assessed by sensitivity and specificity as well as of the treatments. Treatments are adequately assessed by testing effects on survival and on quality of life. The efficiency of treatment in early stages of cancer cannot always be predicted from experience with advanced stages. Extending survival in prostate cancer (Antonarakis et al. 2007) will generate added value if it can be related to new treatment modalities rather than to new selection strategies. However, distinguishing these differences will be difficult. Lung cancer is not a good candidate for secondary prevention because screening does not lead to treatments that enhance health outcomes.

Depending on the point of view, confirmation of the quality may also include the efficiency of diagnostic tests and treatment methods. Efficiency may be related to monetary, i.e. tangible, costs as well as to intangible costs such as side-effects or invasiveness of the procedure.

The epidemiologic data are not sufficient to predict the success of a prevention program. Public perceptions of prevention programs and political decisions, the recommendations of traditional (Irvine 2001) health-care professionals and the support of the program by families and friends will contribute to its final success. Finally, individual indicators concerning members of the target group will also contribute to the success of a prevention program. Such predictors include “expected and unexpected side-effects of prevention”. Side-effects can be either perceived safety or anxiety of bad news, or the desire of patients to contribute to the process of a cure (Rozenberg et al. 2007). Such signs of success may be rather important to maintain the participants’ motivation and interest in the program.

The predictors listed in Table 2.1 summarize three issues which are discussed in this paper and might be considered when planning new cancer prevention programs. Epidemiologic criteria have to demonstrate efficiency and the high quality of the planned intervention. Social criteria can be used to assess the support of the program by the social environment; and finally, individual criteria will help to estimate the chances that a prevention program will succeed.

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Cancer Prevention in the Developing World: Mission Impossible?

3

Franco Cavalli

Abstract Cancer is set to become the newest epidemic in the developing world. This fact is still largely unknown. The UICC, therefore, has been acting in order to inform policymakers, with the aim of pushing them to act in order to tackle this looming disaster. The implementation of a cancer control plan, encompassing prevention, screening and treatment in each country, should therefore become the declared goal of all health policymakers worldwide.

Today cancer kills more people worldwide than human immunodeficiency virus (HIV), tuberculosis (TB) and malaria combined (see Fig. 3.1). This fact is largely unknown not only within the lay press but to some extent even in the scientific community. This might be one of the reasons—even while governments and international agencies give much attention to e.g. HIV and malaria—that cancer is seldom mentioned.

Neoplastic diseases are sometimes also thought to be a hallmark of the developed world, while on the contrary cancer is set to become the newest epidemic in the developing world.

Based on the most recently estimated incidence rates, the 11 million cancer cases diagnosed in 2002 will reach roughly 17 million in 2020 and 27 million by 2050 [1]. These estimates assume no change in the risk pattern of cancer incidence. As shown in Fig. 3.2, a yearly increase of 1% of the risk would add at least another million cases per year [2]. Close to two-thirds of the cancer cases expected for 2050 will most probably occur in low-income countries: since the cure rate there is much lower than in the high-income countries, the difference in mortality will be even more pronounced. In fact, it has been predicted that already by 2020 almost two-thirds of cancer deaths will occur in developing countries, and this proportion could reach three-quarters around 2050 [3].

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3.1 A Changing Pattern

Worldwide there are important cancer health disparities, which reflect differences in cancer incidence, mortality and prevalence among different populations. For the most common cancers, global disparities in incidence, mortality and prevalence are evident and likely due to complex interactions of non-modifiable (i.e. genetic susceptibility and ageing) and modifiable

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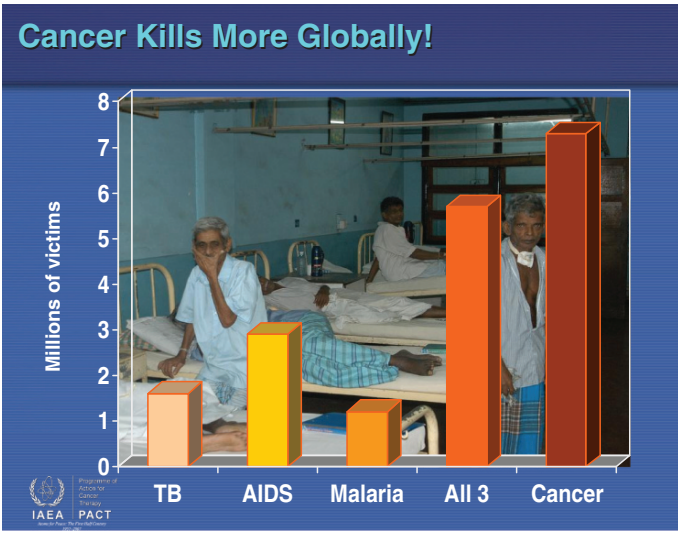

Fig. 3.1 Number of deaths related to tuberculosis, acquired immunodeficiency syndrome (AIDS), malaria and cancer

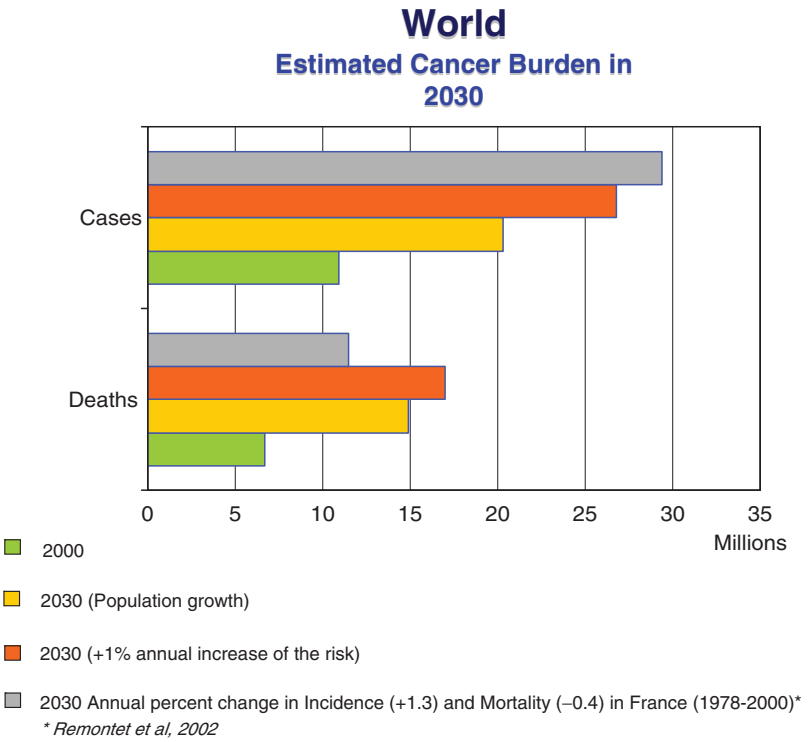

Fig. 3.2 Latest estimation of cancer burden for 2030

risk factors (i.e. tobacco, infectious agents, diet, and physical activity) [4]. Indeed, when risk factors among populations are intertwined with differences in individual behaviours, cultural beliefs and practices, socio-economic conditions and health-care systems, global cancer disparities are inevitable. Therefore it is important to realise, for example, that in Africa tobacco use is currently estimated to be related to only 10% of deaths, while infections remain the most important risk factor related to the pathogenesis of cancer in that continent, affecting 30% of cancer mortality [5].

The sharp increase in cancer incidence in the low- and medium-income countries is due to different factors. First, life expectancy is increasing in many of these countries, and some of them (e.g. China) will soon have a demographic structure similar to those of Western countries. Moreover, in developing countries tumours related to Western lifestyles (e.g. cancers of the breast, prostate and colon-rectal tract) are increasing in addition to poverty-linked tumours (e.g. cervical cancer, liver cancer) [3]. It is also possible that death attributable to modifiable behavioural and environmental factors are swiftly becoming more important in the developing world as compared with the more affluent regions [6]. However, the most relevant differences between the two parts of the world have to be sought in the field of prevention and even more so regarding early detection and treatment [7]. Prevention campaigns are very rare events in low-income countries, even in situations where they could be implemented relatively easily. Notwithstanding that many of them have signed the WHO Framework Convention on Tobacco Control (FCTC) treaty, the tobacco industry has, for example, successfully forestalled legislation in most of them [8].

Early detection remains elusive for the vast majority of the poor populations of the world, even in tumours such as cervical cancer, for which the efficacy and economic viability of early detection has been clearly demonstrated [9].

Differences in treatment outcomes are not very significant in tumours such as lung or oesophageal cancer, for which results are dismal even in the developing world. However, differences are sometimes very dramatic, e.g. in cervical cancer and even more so in paediatric oncology [10, 11].

We have to assume that these differences will increase further due to the crisis of health-care systems in many countries. Moreover, diagnosis and treatment for most tumours are becoming more sophisticated and concomitantly much more expensive [12].

Mainly in countries in which the health expenditure per capita is only a few dollars yearly, prevention and early detection must therefore become the cornerstones of a national control plan. The fact that such plans are the most effective weapon in organising the fight against cancer worldwide has been largely recognised in the last few years. Alas, only a minority of countries have so far implemented such national cancer control plans, and among them are few in the developing world [3]. A first positive step has, however, been accomplished by the World Health Assembly of the WHO in May 2005, when the fight against cancer was for the first time unanimously declared a priority for all governments [13]. Nevertheless, a huge international effort will be necessary in order to practically realise what remains, for the time being, only a declaration of intent of all members of the WHO.

3.2 A Possible Roadmap

We should not consider this bleak forecast as an unavoidable natural event. There are many ways in which the situation could be improved, but a worldwide co-ordination of huge effort is required. Even in very poor countries, some important preventive measures can be implemented [7].

This is particularly true regarding the fight against smoking, where not only the international tobacco lobby but often local economic and political interests are blocking such preventive efforts. The urgency of this issue is best demonstrated by the Chinese example: It has been calculated that if current trends in smoking continue, by 2030 3 million Chinese will die every year because of lung cancer [14]. The onus therefore rests on the oncology community to increase its pressure in order to oblige governments to comply with the rules they have accepted by ratifying the FCTC. This is also the main reason why the International Union Against Cancer (UICC), after its successful first worldwide campaign in paediatric oncology "My Child Matters" [15], has now started a 5-year-long worldwide campaign entitled "Today's Children, Tomorrow's World" [16]. This campaign is devoted to prevention and more specifically will focus on adolescents and their parents. During 2007 a worldwide inquiry has been carried out in order to understand what adolescents and their parents know about cancer prevention. Initial results of this investigation will be presented at the UICC World Cancer Congress, which will take place in Geneva at the end of August 2008. On World Cancer Day (February 4) of 2008 the first message ("Give children and young people a smoke-free environment") was launched with a wide array of activities in over 50 countries.

Over the next 3 years the campaign will concentrate each year on one of the following messages:

- Encourage an energy-balanced lifestyle based on healthy diet and physical activity [17].
- Learn about the possibilities to fight against infections, including the use of appropriate vaccines [5].
- Teach children and teenagers to avoid UV exposures by being "sun-smart" [18].

These World Cancer Campaigns are a part of the overall UICC strategy which is depicted in Fig. 3.3.

The roadmap is based on the World Cancer Declaration (WCD) [19], which was accepted for the first time at the World Cancer Congress in Washington (July 2006). This declaration, which defines priorities for the worldwide fight against cancer, will be updated in Geneva at the end of August 2008 and then in Beijing in 2010. The WCD sets the basis for the different campaigns, which should mobilise resources worldwide and eventually put pressure on governments, so that they are solicited to comply with the WHO resolution about the fight against cancer, which they accepted in May 2005. Moreover the WCD has been refined in continental versions (London Declaration on AfrOx) [20] or to apply it to different national situations (e.g. Tianjin Declaration for China): in the latter, e.g. the fight against tobacco was declared to be the absolute priority for this country, encompassing one-fifth of the world's population. Further pressure can be put on governments through the important Programme for Action on Cancer Treatment (PACT) launched by the International Agency for the Atomic Energy (IAEA) and to which many international agencies and organisations (including UICC) are participating [21]. In that programme, equipment for radiotherapy might be provided to governments, which should, however, demonstrate how they are including this treatment modality within the framework of a national cancer control plan, which in turn should help to establish which are the most important priorities for each country.

Another glimpse of hope is coming from the fact that at least the vaccination against hepatitis B virus is gaining momentum through different campaigns launched by the Global Alliance for Vaccines and Immunization (GAVI), which is particularly important in countries such as Mongolia, where almost 60% of all tumours are represented by liver cancer related to hepatitis

Fig. 3.3 Roadmap of UICC strategy

viruses [22]. The same could be true for human papilloma viruses, since two vaccines have now been developed against human papilloma virus (HPV) types 16 and 18, which cause almost 80% of cervical cancer cases [23]. It has been calculated that these vaccines could save at least half a million lives each year by reducing the incidence of liver and cervical cancer [6]. However, screening remains mandatory for cervical cancer, since the results of vaccination will take 20–30 years; furthermore, the currently available vaccines do not cover all strains of HPV. Some concern has even been voiced claiming that possibly those strains of HPV which are not affected by the current vaccines could become more oncogenic [25]. And, most important of all, the current prices of these vaccines against HPV are absolutely unaffordable for the vast majority of countries and for almost every country in the developing world. While the price policy of pharmaceutical companies, as has been the case for HIV, must be re-discussed in the near future, even in oncology, in order to find worldwide affordable solutions, screening for cervical cancer currently remains the only possible and applicable strategy in the developing world [24]. Besides a few examples, however, the old screening system based on Pap smears has largely failed to solve the problem in most countries, mainly because it is fraught with a lot of logistic and methodological problems. Currently, however, a new, simpler and less expensive methodology such as visual inspection of the cervix with acetic acid, as well as HPV DNA testing, are available, and therefore mass screening is gaining momentum again in most developing countries [9, 25].

3.3 A Provisional Conclusion

Objectively, the near future looks quite bleak if one considers the possibility of tackling the huge task of implementing cancer prevention in the developing world. One might therefore easily vconclude that it is really an impossible mission. However, we as scientists and human beings cannot take this assessment as being an unavoidable natural event. Besides our wishes to avoid a looming disaster in the developing world, there are some glimpses of hope, which should help us in forging a global alliance in order to have enough resources to tackle this immense challenge. The most important step would be of

course to include cancer on the world political agenda. A number of wealthy new players have recently reshaped how affluent countries confront infectious diseases in the developing world; however, cancer kills more people than tuberculosis, malaria and HIV put together. Improving the outcome in oncology will undoubtedly also have profound economic impact. Therefore, the implementation of a cancer control plan, encompassing prevention, screening and treatment in each country, should become a declared goal of health policymakers worldwide. A first step has been accomplished by the World Health Assembly of the WHO in May 2005, when the fight against cancer was for the first time declared a priority for all governments. In that resolution, prevention and early detection were considered to be cornerstones of the cancer control plans which would have to be established worldwide [13]. Nevertheless only a broad alliance including non-governmental organisations such as the UICC and the major health charities will be able to develop the necessary strength to mobilise enough resources to avoid the cancer disaster that is looming in the developing world.

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Is Cancer Prevention Ever Going to Be Profitable?

4

Thomas D. Szucs and Konstantin J. Dedes

Undoubtedly, the war on cancer is an expensive endeavour. It is estimated that, where data are available in Europe (e.g. Germany and France), cancer care accounts for a similar proportion of overall health-care expenditure to that in the USA, i.e. approximately 5%. Currently, no society can afford all of the potential cancer treatments for all the patients that could benefit from them. How the required resources should be provided is one of the great on-going debates, and different countries approach this problem in different ways. Additionally, the more we understand about the pathology, pathogenesis, diagnosis and treatment of cancer, the more options are created. Many of these options are new diagnostic tools and more effective treatments. Obviously these innovations, paired with an increasing patient pool, are leading to tremendous health-care expenditures, well surpassing current budgets. Hence, the question arises of how many resources should be devoted to the management of cancers, given that resources are scarce and many other fields and specialities are competing for these resources. This paper addresses the question of whether preventive measures in oncology eventually pay off.

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4.1 Introduction

The increasing cancer burden (incidence and prevalence such diseases), together with an increasing demand for and the promise of modern diagnostic and therapeutic technologies, implies that there will continue to be a dramatic increase in health expenditures for oncology. In several countries, national health services are no longer capable of sustaining the increasing costs of oncology.

Before addressing the main question of whether cancer prevention might be profitable, it is worthwhile to define profitability.

Profit generally is the making of gain in business activity for the benefit of the owners of the business. The word comes from Latin meaning "to make progress" and is defined in two different ways, one for economics and one for accounting.

Pure economic profit is the increase in wealth that an investor has from making an investment, taking into consideration all costs associated with that investment including the opportunity cost of capital. Accounting profit is the difference between price and the costs of bringing to market whatever it is that is accounted as an enterprise in terms of the component costs of delivered goods and/or

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services and any operating or other expenses. A key difficulty in measuring either definition of profit is in defining costs.

An economic profit arises when revenue exceeds the total (opportunity) cost of the inputs, noting that these costs include the cost of equity capital that is met by "normal profits" (for example, if the resources had been allocated elsewhere). A business is said to be making an accounting profit if its revenue exceeds the accounting cost the firm "pays" for those inputs. Economics treats the normal return on investment as a cost, so when that expected minimal expected return is deducted from total accounting profit, what is left is economic profit (or economic loss).

In preventive medicine, profitability will fluctuate with the balance between prevention costs and morbidity costs (Fig. 4.1).

In order to strive to answer the question whether cancer prevention ever will be profitable, it is necessary to answer to questions:

1. What will be the economic future of cancer care?
2. Does cancer prevention offer "value for money"?

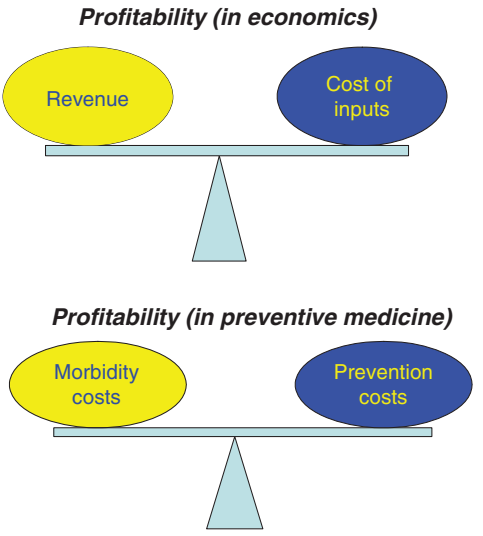

Fig. 4.1 Different definitions of profitability

4.2 What Will Be the Economic Future of Cancer Care?

It is estimated that, where data are available in Europe (e.g. Germany and France), cancer care accounts for a similar proportion of overall health-care expenditure to that in the United States, i.e. approximately 5%. Currently, no society can afford all of the potential cancer treatments for all the patients that could benefit from them. How the required resources should be provided is one of the great on-going debates, and different countries approach this problem in different ways. Additionally, the more we understand about the pathology, pathogenesis, diagnosis and treatment of cancer, the more options are created. Many of these options are new diagnostic tools and more effective treatments. Obviously these innovations, paired with an increasing patient pool, are leading to tremendous health-care expenditures, well surpassing current budgets. Hence, the question arises of how many resources should be devoted to the management of neoplasms, given that resources are scarce and many other fields and specialities are competing for these resources. To address this issue it is important to know how many resources are allocated to oncological care and how efficiently this care is being provided; hence, we must ask: What do we get for the money?

4.3 How Do We Determine "Value for Money"?

We can address the question of value for money from two points of view: one macroeconomic and the other microeconomic.

If we wish to assess the macroeconomic impact of cancer care, we have to determine the relative burden and the economic benefits of cancer care compared with other medical fields. Recently, Hartmann et al. [1] determined this elegantly with a simple analysis of the German health-care setting. For the years 2002 and 2004 they calculated the expenses related to cancer care at €13.4 billion and €15 billion, respectively. The largest three entities of these costs were neoplasms of the digestive and respiratory system in addition to breast cancer. These three cancers make up approximately 45% of total costs. In addition, over 12,000 life-years are lost due to malignancies (Table 4.1).

By dividing the incremental costs by the incremental benefits over 2 years it is also possible to obtain a cost-per-life-year estimate on the societal, macroeconomic level (Table 4.2). Hartmann and colleagues have determined that oncology yields a cost-effectiveness value of overall €140,750 per life-year gained. In comparison, the cost per life-year gained in psychic and behavioural disorders amounts to €6,395,000, in digestive disorders €223,500. The most cost-effective area is the prevention of injuries, which yields a macroeconomic cost-effectiveness ratio of €14,538 per life-year gained. Interestingly, the management of lip, mouth and pharyngeal tumours is most effective, at €39,000 per life-year gained, whereas the field of digestive organ tumours is least cost-effective (€126,000/life-year gained).

Table 4.1 Years of life gained in Germany per disease category

ICD 10 category	Years of life gained
Injuries	39,000
Malignant neoplasm	12,000
Circulatory system	11,000
Digestive system	8,000
Respiratory system	4,000
Psychic and behavioural disorders	2,000
Musculoskeletal system and connective tissue	0
Endocrine, nutritional and metabolism diseases	−2,000

For a microeconomic point of view it is necessary to determine the cost-effectiveness relationship of specific interventions (prevention or treatment) in oncology.

In this application, economic evaluation is a method to assess and valuate costs of specific 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 such economic evaluations is to supplement 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 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.

Economic evaluation is not only about alternatives and costs. It is also about consequences and especially about the good and the bad consequences for patients and society in general. Cost-effectiveness methods, when properly and responsibly applied, have a major contribution to make by enabling better-informed decisions.

Table 4.2 Cost-effectiveness of cancer screening programmes

Service	Cost per life-year saved (in USD[a])	Author
Breast cancer		
Quality-controlled mammography screening versus opportunistic screening	75,209	Neeser (2007) [7]
Annual mammography and breast exam vs just breast exam, women age 40–64	42,501	Christie (1977) [8]
Annual mammography and breast exam for women age 40–49	111,979	Eddy (1988) [9]
Annual mammography for women age 55–64	275,009	Kristein (1977) [10]
Colon cancer		
Flexible sigmoidoscopy every 5 years	19,561	Khandker (2000) [11]
Annual FOBT	43,273	Khandker (2000) [11]
Colonoscopy every 10 years	78,540	Khandker (2000) [11]
FOBT plus flexible sigmoidoscopy every 5 years versus no screening	42,799	Frazier (2000) [12]
Cervical cancer		
Cervical cancer screening every 3 years for women age 65+	80	Fahs (1992) [13]
Cervical cancer screening every 5 years for women age 65+	3,049	Fahs (1992) [13]
One-time cervical cancer screening for women age 65+	3,370	Fahs (1992) [13]
Annual versus every 3 years cervical cancer screening for women age 65+	78,631	Fahs (1992) [13]
Prostate cancer		
PSA screening from age 50 to 75	29,705	Doggett (2007) [14]
PSA in combination with DRE, compared to no screening in men, age 65	20,664–25,145	Kramer (1993) [15]
PSA in combination with DRE, compared to no screening in men, age 70	36,408–44,750	Kramer (1993) [15]
PSA in combination with DRE, compared to no screening in men, age 75	68,877–92,032	Kramer (1993) [15]
Skin cancer		
Screening one time	10,403	Losina (2007) [16]
Screening every 2 years	83,121	Losina (2007) [16]
Screening annually	604,404	Losina (2007) [16]

DRE, digital rectal exam; FOBT, faecal occult blood test; PSA, prostate-specific antigen; USD, United States dollars

[a]Adjusted to 2008 USD

4.3.1 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.

1. Cost-Benefit Analyses
   - As applied to health care, cost-benefit analysis (CBA) measures all costs and benefits of competing therapies in terms of monetary units. Generally, a ratio of the discounted monetary value of benefits to costs is calculated for each competing therapy. Differences in cost-benefit ratios of competing therapies or programmes (e.g. intensive care unit versus new diagnostic equipment or preventive measures) can be readily compared for an efficient allocation of resources. 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. 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 [2].

2. Cost-Effectiveness Analyses
   - Cost-effectiveness studies (CEA) 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. CEA can also be applied equally to cases where the outcome is in terms of quality of life. Cost-effectiveness analysis 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; that is, one drug may lead to greater gains in life expectancy than another. 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 tool to identify the most efficient strategy to reach a specific health target (production efficiency). It has the disadvantage of not permitting comparisons across programmes (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 [3]. Moreover, it cannot compare outcomes measured in clinical units with quality-of-life measures.

3. Cost-Utility Analyses
   - Cost-utility analysis 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 an 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.
   - Cost-utility analysis is an improvement over cost-effectiveness analysis 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 programmes/therapies has to be optimized. A comprehensive overview of QALY estimates has been published by Tengs et al. [4].

4.3.2 Applying Cost-Effectiveness Evaluations

Recently, Cohen et al. determined whether preventive measures or curative technologies are more cost-effective [5]. They discovered that the distributions of cost-effectiveness ratios for preventive measures and treatments are very similar.

Another way of looking at cost-effective ratios is to ask the question: How much health can money buy? Essentially, this is the reciprocal value of the classical cost-effectiveness ratio costs per life-year gained. Russell determined this metric for several preventive measures [6].

An important intervention in cancer prevention is screening for the most frequent cancer among women, namely breast cancer. The aim of screening mammography is to detect and treat precancerous and early cancerous lesions which are associated with very favourable prognosis. Mortality can be efficiently improved by reducing cases diagnosed in advanced stages. However, debate is on-going regarding the pros and cons of mammography screening programmes. Recently, Neeser et al. have analysed the cost associated with a nationwide mammography screening programme compared to opportunistic screening, as it is currently practiced in Switzerland [7].

The authors found that life expectancy could be best improved if screening is started at 40 years of age rather than at older ages. Cost per life-year gained amounted to US $73,000. In contrast, screening efforts for ovarian cancer using sonography in combination with blood tests (CA125) have shown promising results; however, implementation of this screening has not been achieved [17]. High intervention costs for screening a cancer type with low incidence resulted in an unfavourable cost-effectiveness, prohibiting its use as a recognized screening tool.

Recently, a breakthrough has been achieved in the field of primary prevention of cancer, as the first vaccine able to avoid invasive cancer has been adopted in most health-care systems worldwide. However, along with the clinical approval of the human papilloma virus (HPV) cancer vaccine, endless discussions have started regarding ethical, medical and particularly economic issues surrounding the nationwide implementation of this preventive intervention. The claims for economic evaluation of this intervention have been striking, as it targets the population of all 12- to 26-year-old healthy women, and thus the measure has a considerable impact on health-care budgeting.

For Switzerland, using modelling techniques, it has been estimated that a vaccination programme with a quadrivalent HPV vaccine against types 6, 11, 16 and 18 would prevent about 62% of cervical cancers. The cost for this prevention would result in SF 26,000 per quality-adjusted life-year gained [18].

4.4 So Will Cancer Prevention Ever Be Profitable?

There are several reasons why we might be able to answer this question with a clear affirmative. First and most obviously, the prevention programme has to demonstrate that it works, i.e. show clinical effectiveness. Second, the effectiveness should translate into meaningful economic benefits. This second task is certainly

more critical. The economic benefit will depend heavily on the magnitude of the clinical effectiveness and on the potential reduction of downstream costs. Such downstream costs are reflected in expenses for procedures and treatments. Given that many cancers are increasingly transforming themselves into chronic and hence expensive disease entities, we can expect that the costs of long-term treatments will increase tremendously. This development is enhanced by longer and more costly (targeted, often biological) therapies. In addition, cancer treatments are improving from year to year. On the other hand, diagnostic procedures are becoming more powerful, enabling the diagnosis of tumours at an earlier, less costly stage.

Hence, it is conceivable that, given these developments, cancer prevention will, in the long run, become increasingly profitable; it may, however, take some time.

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Part II

Cancer Prevention: The Scientific-Epidemiological Base

Energy Metabolism, Cancer Risk, and Cancer Prevention

5

Michael Pollak

Abstract Research concerning "cancer energetics" has become a popular area of investigation. This topic comprises two distinct fields: energetics at (1) the cellular level and (2) the whole organism level. Both of these have relevance to Cancer Risk and Cancer Prevention.

The field of cellular energetics includes studies of specific energy sources (glucose, fatty acids, etc.) utilized by various normal and neoplastic cell types, the various metabolic pathways used (glycolysis vs oxidative phosphorylation, etc.), and related issues. One of the key issues in this field (reviewed in [1]) relates to the Warburg hypothesis, which concerns the preferential use of glycolysis rather than oxidative phosphorylation by cancer cells. Recent studies have supported some of Warburg's classic observations in this area. At first glance, the preferential use of glycolysis by cancer cells seems paradoxical, because glycolysis yields substantially less energy per glucose molecule consumed than does oxidative phosphorylation. However, on closer examination, the explanation may relate to the fact that neoplastic cells require large supplies not only of energy, but also of the substrate molecules required for membrane synthesis and so on. Glycolysis yields these substrates as by-products, while oxidative phosphorylation does not. While glycolysis yields substantially less energy per glucose molecule consumed than oxidative phosphorylation, part of the neoplastic phenotype involves very efficient glucose transport into the cell (which is the basis for tumor imaging with labeled glucose in positron emission tomography scanning). Thus, while the energy yield per glucose molecule through glycolysis is relatively low, energy demands can be met as the supply of glucose is assured by the high levels of glucose transport, and the building blocks for macromolecular synthesis are also provided.

Whole organism energetics concerns the impact of the balance between caloric intake and energy consumption on carcinogenesis and cancer behavior. Large-scale population studies (for example [2]) have established that excess body weight is associated with increased risk of subsequent cancer mortality. Further work has shown that this is not simply attributable to a relationship between body size and cancer risk. Rather, it involves for many cancers a combined increase in risk with a worsening of prognosis, such that the effect of obesity on cancer-specific

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mortality is for many cancer types greater than the effect on risk. Given the magnitude of the global "obesity epidemic," this topic deserves our attention. In affluent countries, the proportion of the population considered overweight to a degree sufficient to influence cancer behavior has been rapidly increasing. In certain areas, more than a third of the population would now be estimated to have increased their risk of cancer mortality based on body mass index. This threatens to attenuate recent progress in cancer control. While fewer data are available concerning the specific influence of childhood obesity on subsequent cancer risk, this is another area of great concern.

What are the mechanisms by which obesity (energy intake in excess of energy expenditure) may influence neoplasia? Does excess food intake influence cellular energetics? Surprisingly little is known about the relationship between "whole organism" nutrition and cellular bioenergetics. Evolutionary pressure has resulted in mechanisms that preserve circulating levels of fuels such as glucose, even in the setting of starvation, almost until the time of death. There is only limited evidence that alterations in blood levels of glucose, lipids, or other blood constituents that are a consequence of excess energy intake have a direct effect on cellular energy metabolism, but this possibility requires further study. On the other hand, there is considerable evidence that it is the changes in the endocrine environment which arise as a consequence of excess energy intake that influence carcinogenesis and cancer progression. These changes include increased tissue and circulating levels of insulin, inflammatory cytokines, and alterations in adipokines such as leptin or adiponectin.

We have recently been extending earlier work concerning the relationship of insulin-like growth factors to cancer risk [3] by examining the role of insulin itself as a candidate mediator of the effect of obesity on cancer mortality. It is well known that obesity is associated with increased insulin resistance in classic "target tissues" for insulin action such as fat, muscle, and liver, which leads to elevation in circulating insulin levels. Recent results show that insulin receptors are perhaps unexpectedly commonly expressed on many neoplastic cell types [4]. Thus, insulin may directly stimulate cancer growth in obese, hyperinsulinemic subjects.

A review of older literature reveals that this is not a new concept. For example, more than 30 years ago, it was demonstrated that the growth of carcinogen-induced mammary cancer in rats was greatly curtailed, and in some cases tumors actually regressed, when insulin levels were lowered by administration of alloxan, which is a pancreatic beta cell poison that results in insulin deficiency [5]. This insulin deficiency in fact modeled type I (insulin-deficient) diabetes and was associated with hyperglycemia. The authors attributed the effect on the mammary gland tumor to the insulin deficiency. However, by current standards, this work is interesting but incomplete, and requires confirmation with endpoints including changes in signal transduction.

Our more recent collaborative work with Dr. Venkateswaran and colleagues [6] has yielded data consistent with the older data. In this study, our goal was to model variation of insulin level within a clinically relevant range, rather than to extremes, and determine if this would influence the behavior of a prostate cancer xenograft model. We used a high-fat, high-sucrose diet to induce a moderate elevation in insulin levels, and observed that this resulted in a significant acceleration of tumor growth. While the experiment did not demonstrate in a formal fashion a causal link between the rise in insulin level and the more aggressive proliferation, we did observe the presence of insulin receptors in the tumors, and documented increased activation of the signaling pathway downstream of the insulin receptor in the tumors of the animals on the diet that led to hyperinsulinemia.

Recent data from population studies provide further evidence for an association of high insulin levels with more aggressive behavior of breast,

prostate, and other cancers in subjects who are overweight. While this association may be causal, it must be recognized that other factors that potentially may influence tumor behavior, such as leptin, do vary with insulin levels—thus insulin may be acting as a surrogate for another mediator rather than being directly involved mechanistically. However, the simplest model to account for the association would postulate that insulin itself is indeed the mediator. In the case of breast cancer, we [7] and others [8] have observed an increased risk of disease relapse among women with higher levels of insulin or c-peptide, an insulin surrogate. In the case of prostate cancer, data from the Physicians' Health Study has shown a relationship between higher levels of c-peptide and the risk of fatal prostate cancer [9]. Similar studies are underway for colorectal and other cancers.

In terms of relevance to clinical cancer prevention, the relationship between body mass index and overall cancer mortality [2] implies that efforts to avoid excess energy consumption relative to utilization would be useful. In fact, it is unclear if avoiding obesity would act at the very earliest stages of carcinogenesis, or if (like many other cancer prevention strategies) it would actually act to prevent early cancer progression events.

At present, while there are many datasets that demonstrate a relationship between obesity and cancer mortality, there is a paucity of long-term intervention studies to demonstrate conclusively that interventions that improve energy balance, such as dieting and increasing exercise, reduce cancer risk. However, this would seem to be likely.

If further studies provide additional evidence that insulin is an important mediator of the effect of obesity on risk, the potential role of metformin in cancer prevention will deserve study. Metformin is widely used in type II diabetes, where it is known to act to reduce hyperglycemia by reducing hepatic glucose output [10]. This has a secondary effect of lowering insulin levels.

Metformin has other actions that may be relevant. There is in vitro evidence that it acts directly on cancer cells as an AMP kinase (AMPK)-dependent growth inhibitor, which could provide a further benefit [11–12]. This mechanism involves activation of the LKB1–AMPK pathway, which is a signaling system that normally serves to reduce cellular energy-consuming activities when there is cellular energy depletion. This involves, in part, inhibition of m-tor-dependent protein translation and inhibition of proliferation, which may complement the benefits of reduction of circulating insulin level. On the other hand, recent evidence suggests that some neoplastic cells may react to this "perceived cellular energy deficiency" by increasing secretion of vascular endothelial growth factor (VEGF), in an attempt to increase vascular supply, and this can have undesired effects [13]. It remains to be determined if this action of metformin will outweigh its potential utility, as metformin has beneficial effects in other in vivo models [14, 15].

Early population studies detected unexpectedly low cancer incidence and mortality among diabetics on metformin [16, 17], so this topic deserves further research. It remains possible that—particularly among metabolically defined subsets of individuals at increased risk for cancer, namely those who are obese and hyperinsulinemic, or those who have the so-called "normal weight, metabolically obese" phenotype [18]—metformin or other insulin-lowering approaches (including lifestyle modification) will be particularly important as risk-reduction strategies.

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Promises and Limitations of Biomarkers

6

David F. Ransohoff

Abstract In many "omics" fields, extraordinary promises have been made about the ability of biomarkers for detecting early cancer, for predicting prognosis, and for predicting response to therapy. Yet very few markers are brought to clinical practice, and many are not even found to be reproducible. This essay discusses chance and bias as threats to validity that can explain the huge disconnect between promise and product, along with approaches to address those threats.

6.1 Promise Versus Product

In many "omics" fields, extraordinary claims have been made about the accuracy of biomarkers for early detection of cancer, for predicting prognosis, and for predicting response to therapy. Yet such claims often turn out to be nonreproducible [1–12], and very few new markers have been brought out of the omics pipeline into clinical application [13–15].

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6.2 Threats to Validity from Chance and Bias

The disconnect between claims and reality can be explained in part by a lack of attention to fundamental threats to validity resulting from chance [16] and, more importantly, bias [17]. Bias refers to a systematic difference between the compared groups so that the answer is incorrect. These problems have been discussed before [18, 19]; the purpose of this essay is to identify possible approaches to address them. There is no quick fix or simple solution. Laboratory investigators may not appreciate that if bias has occurred before samples reach their laboratory, fatal bias may be "hardwired in" to such a degree that no amount of laboratory analysis or bioinformatics analysis can fix it. This means that laboratory investigators will need either to learn details about threats to validity and how to address them, or they will need to enlist the help of—and work closely with—colleagues in epidemiology and biostatistics who can.

In the meantime, laboratory investigators sometimes ask: Can using "guidelines" or following "phases" of research help solve current problems?

The role of guidelines is to help assure adequate reporting of what was done in a study, i.e.,

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to assure description of research design, so that design can be judged by a reviewer or reader [20–24]. The role of providing transparency is critically important, but guidelines do *not* directly prescribe details of research design, and they cannot indicate how an investigator should—in design—avoid important biases [18, 19]; they address only reporting. Similarly, the role of "phases" is to provide a rough idea about the order in which to formulate research questions in the process of marker development [25]; using "phases" does not directly address research design and problems of bias [18, 19]. Laboratory investigators must understand that there is simply no shortcut or checklist that can solve current problems in marker research. At the same time—and while this situation may seem frustrating—there may be new approaches, including what might be called shortcuts, to improve both the reliability and the efficiency of marker research [18, 19].

6.3 Addressing Current Problems

6.3.1 Every Study Should Be Reliable

Importantly, every study, even "early" ones, should be reliable in the sense that a study should not contain fatal flaws due to chance or bias. This idea may sound obvious, but it is worth emphasizing because many investigators seem to believe that addressing bias can be saved for some later stage of research, while early research can be done without great attention to design problems. This belief risks leading to wasted effort because, if one were to use a fatally flawed study(s) as the foundation for subsequent investment, that work and investment would turn out to be misdirected and wasteful. If, for example, a $100 million proteomics initiative is based on the premise that substantial "discrimination" was achieved in early studies using serum proteomics patterns, so that the purpose of the initiative is to refine the technology and platforms that did the initial discriminating, then if it turns out that there was no "real" discrimination in the initial studies—that results were due to chance or bias—then the subsequent work risks being fruitless [26, 27].

6.3.2 Drug Research Versus Marker Research

In studying drugs there is an orderly—and necessary—set of phases that drug research must follow after preclinical development. One important feature of a drug study is that it obviously has to be prospective in order for the drug to be administered. This feature of design allows powerful protections against bias—such as randomization and blinding—to be more easily employed than when a study is not prospective. Another reason that phases are used in drug research is that, since a drug may cause adverse events, the overall process of development must be done in an incremental and careful way that protects patients. Initial clinical studies are done to understand dose, then to assess toxicity, and then to assess efficacy. The net result of using prospective randomized and blinded design is that the fundamental comparison between treated and nontreated subjects may be very strong. For all these reasons, drug research tends to involve generally reliable studies. This does not mean that such studies necessarily lead to successful development and a useful product; nor does it mean that data and interpretations of earlier studies will always be supported in later ones. But it does mean that the fundamental building blocks—individual studies—tend to be strong in drug research.

In contrast, in a study of a marker for diagnosis or prognosis, the strength of the fundamental comparison is substantially more subject to bias, because protective measures such as randomization and blinding either cannot be used or are difficult to use. For example, there cannot be

randomized assignment of disease vs nondisease groups that are otherwise equal in order to achieve the baseline equality that randomization is used for in a clinical trial. Said another way, studies of markers are observational (meaning that no agent is administered). Because randomization cannot be used, and because blinding and other measures to prevent bias are often not used, studies of markers for diagnosis and prognosis are routinely threatened by serious bias. In one example of bias, cancer specimens were analyzed on a certain day by a mass spectrometry machine that is known to "wander" over time, while the control specimens were run on a different day. This difference in analysis likely accounted for the difference in results observed between cancers and noncancers [10, 11]. In another example, bias may have occurred in a study of prostate cancer when the cancer group consisted of 67-year-old men while the control group was 30 years younger and the majority were women [5, 18]. In the first example, the bias occurred after specimens reached the basic investigator's laboratory; in the second example, the bias occurred before specimens reached the laboratory. Numerous biases can occur before specimens reach the laboratory, and many may be fatal to a study's intended comparison. Bias that is hardwired into a study cannot be adjusted for by laboratory analysis or bioinformatics. For this reason, investigators must learn to understand sources of bias and how to approach them [17].

6.3.3 Role of Specimens

Specimens have an underappreciated role in helping to assure the strength of comparison and the reliability of a study's results. The central concept is that, after specimens are collected, a "study" has been done, regardless of whether the process was ever conceptualized as a study [18]. In other words, by the time specimens are collected, bias has—or has not—been hardwired into the study. It is the investigator's responsibility to describe (and the editor's responsibility to expect) enough details that a reader could judge whether a major bias might have occurred. Details include description of the source of subjects, how subjects were included and excluded, how and where specimens were collected from cases and from controls, and so on. Investigators may ask what sorts of details need to be reported. The guiding principle is that the details that need to be reported are those for which, if there were any systematic difference between cases and controls, a systematic difference in results might have occurred. Even though guidelines for reporting exist [20–24], there is no formula or checklist for exactly what to report. Indeed, deciding what is a potentially important source of bias and what details need to be considered in design (or in reporting) is one of the most sophisticated and difficult challenges in conducting clinical research [17, 18].

6.3.4 Shortcuts: By Using Already-Collected Specimens

Important "shortcuts" may be available in marker research that are totally unavailable in drug development research. It may be possible to conduct both discovery and validation on the same larger group of samples. It may be possible to simultaneously test multiple technologies using the same samples, in a way that could never be contemplated for drug development research. It may be possible for many research questions to be studied using banked specimens, and such studies may provide results strong enough to satisfy a regulatory agency. These proposed approaches, described in detail elsewhere [18, 19], are currently just "proposals on the table" and need to be discussed by methodologists and leaders in the field. In the meantime these proposals and ideas highlight important differences between drug development research

and marker development research, as well as the substantial opportunity for useful shortcuts to make marker research more efficient [18, 19].

6.4 Conclusion

While molecular markers hold great promise for use in diagnosis, prognosis, and predicting response to therapy, that promise cannot be realized until we appreciate and learn how to address the threats to validity of clinical research.

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Part III

Cancer Prevention, Tobacco and Nutrition

The EPIC Study: An Update

7

Paolo Vineis and Elio Riboli

7.1 The EPIC Study Design

The European Prospective Investigation into Cancer and Nutrition (EPIC) study is an ongoing prospective study aiming to investigate the relationship between diet, lifestyle and environmental factors and the incidence of cancer at various sites [1, 2]. The EPIC cohort was initiated in 1992 and has gradually grown into a multi-centre study recruiting 521,000 participants aged 35–70 years, among 23 centres in 10 European countries: Denmark, France, Germany, Greece, Italy, the Netherlands, Spain, Norway, Sweden and the United Kingdom. The study centres and target populations were selected from the general population of specific geographic areas, towns or provinces in order to have diversity in the levels of exposure and cancer frequency. Therefore, comparisons can be made between populations with heterogeneous dietary habits and lifestyles and with different rates of cancer occurrence.

After obtaining ethical approval from the International Agency for Research on Cancer (IARC) Ethical Review Committee and from the local ethical committees at the participating centres, participants were invited to take part in the study. After signing an informed consent agreement EPIC participants were administered two questionnaires.

The core component of the multiple-choice questionnaires was common to all participating centres, with some optional questions specific to some study centres only. The questionnaires aimed at gathering lifestyle and personal history data and included questions on education, socioeconomic status, employment, current and past occupation that might have led to carcinogen exposure, environmental tobacco smoke, contraceptive and reproductive history, use of hormone replacement therapy, physical activity, history of previous and/or current illnesses, any medical and surgical treatment and hospitalization. Work, recreational, household and vigorous physical activity were assessed in each centre at baseline as part of the standardized lifestyle questionnaire. The total physical activity level for the participants was ascertained using the Cambridge physical activity index, which combines all occupational, household and recreational activity.

In addition to lifestyle data, anthropometric measurements were carried out in all EPIC centres except France, Norway and the Oxford cohort (where information was collected from participants without actual measurements). Measurements included measuring the height, weight and hip circumference of all subjects based on similar protocols. Adjustments were

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made for between and within observer variability, and for reporting bias.

7.1.2 Exposure Variables

Detailed information on lifetime history of consumption of tobacco products was assessed by means of questions on smoking status (current, past, or never smoker), type of tobacco used (cigarettes, cigars, or pipe), number of cigarettes currently smoked, and age when participants started and, if applicable, quit smoking.

Dietary intake assessment was carried out by extensive country-specific dietary questionnaires, aiming to provide high compliance rates and to detect between and within country variations in dietary habits. Dietary questionnaires estimating average portion sizes systematically, containing up to 260 food items, were used in Italy, Germany, Greece and The Netherlands. Questionnaires similar in content but structured by meals were used in France, Spain and Ragusa (Italy). Semi-quantitative questionnaires using the same standard portion sizes assigned to all subjects were used in Denmark, Norway, Naples (Italy) and Umeå (Sweden). A semi-quantitative food frequency questionnaire and a 7-day food record were used in the United Kingdom, while in Malmö (Sweden) the combination of a short non-quantitative food-frequency questionnaire was combined with a 14-day record on hot meals (lunch and dinner).

The various dietary assessment methods used were tested and evaluated in a series of validation pilot studies conducted within the collaborating centres participating in EPIC, prior to the actual recruitment of the main study cohort [3], with the aim of assessing the extent to which the candidate dietary assessment methods would detect significant between person variation in true dietary intake level in the given study population. Furthermore, dietary measurements across cohorts were 'calibrated' in order to ensure the comparability of the dietary exposures across the participating centres [4]. This was achieved by collecting additional dietary intake data via face-to-face interviews using a well-standardized 24-h diet recall method common to all EPIC centres, administered to a representative sub-samples of 5%–12% of the whole EPIC cohort, depending on the centre (total 36,900 participants).

The baseline dietary assessments conducted on the whole cohort are used to rank the participants within centres and estimate the long-term usual dietary intake locally. The food intakes estimated from the 24-h dietary recall are transformed into a scale common to all centres (to improve the between cohort comparability of risk estimates) and used as a reference method to correct for random or systematic bias (i.e. over—or underestimations) of the average intake of baseline dietary assessments [5].

7.1.3 Follow-Up and Case Ascertainment

A total of 521,483 eligible participants (153,451 males and 368,032 females) aged 25–70 years were recruited. After enrolment, the study investigators contact the EPIC participants every 3–4 years. New cancer cases among the EPIC participants are identified through the cancer registries in seven of the participating countries (Denmark, Italy, The Netherlands, Norway, Spain, Sweden and the United Kingdom). In France, Germany and Greece, a combination of methods is used including cancer and pathology registries, health insurance records and active follow-up of study participants and their next-of-kin. Data on total and cause-specific mortality are obtained either through cancer mortality registries or via active follow-up. Incident

cancer cases are coded according to the International Classification of Diseases-Oncology 2nd Edition (ICD-O-2).

7.1.4 Biological Samples

Biological samples including plasma, serum, white blood cells and erythrocytes were collected in duplicates from around 400,000 EPIC participants at the time of enrolment and have been stored in liquid nitrogen at the IARC. All EPIC centres complied with the protocol for sample collection and storage. Time elapsed between blood collection and straw preparation was within 24 h (except for one centre), generally 2–5 h. Straws were kept at −80°C for 24–72 h (but in one centre for months) and then stored in liquid nitrogen.

7.2 Recent Findings on Diet and Biomarkers

7.2.1 Fibres, Meat Intake and Colorectal Cancer

One of the first analyses of the EPIC study data examined the effects of dietary fibre intake on colorectal cancer risk. A previous analysis of all studies worldwide completed up to the late 1990s indicated that fibre intake reduced colorectal cancer risk. However, this consensus view had been challenged by later studies that showed no protective effect of fibre intake in either prospective or intervention studies. During the first 5-year follow-up period, 1,056 individuals developed colorectal cancer among the EPIC cohort [6]. The study showed that dietary-fibre intake was significantly and inversely related to colon cancer incidence, but not to rectal cancer incidence [6]. After correction of the risk estimates with more detailed dietary data, the study showed that an approximate doubling of fibre intake was associated with a 40% reduction in colorectal cancer incidence. These findings indicate that doubling total fibre intake from current average levels in most populations (about 20 g/day) might halve the risk of colorectal cancer—particularly colon cancer.

Another early achievement of EPIC was to show that an elevated intake of red meat also increased the risk of colon cancer, acting in conjunction with low fibre intake (Fig. 7.1). A mechanistic explanation for the effect of red (and apparently not white) meat has been put forward in experiments that suggested that red meat leads to endogenous nitrosation and the production of carcinogenic nitrosamines [7].

7.2.2 Cancer, Hormones and BMI

EPIC has clearly confirmed previous findings on the role of estrogens in breast carcinogenesis (Fig. 7.2). What is new, however, at least in a large prospective study, is an association with testosterone; the biological meaning is not totally clear [8].

One of the main findings in a large number of studies on breast cancer is its relationship with weight and body mass index (BMI). The same has been observed in EPIC, where, in addition, we have been able to detect a clear relationship between estrogens in serum and BMI (Fig. 7.3) [9].

Anthropometry also plays an important role in other types of cancer, such as colon cancer. Table 7.1 shows the results from EPIC men, with a clear dose–response relationship with weight. A similar association has been found in women [10]. Increased weight/BMI is likely to act in colon carcinogenesis through the insulin axis, i.e. the mechanism involves increased peripheral resistance to insulin, and increased levels of C-peptide and (in some investigations)

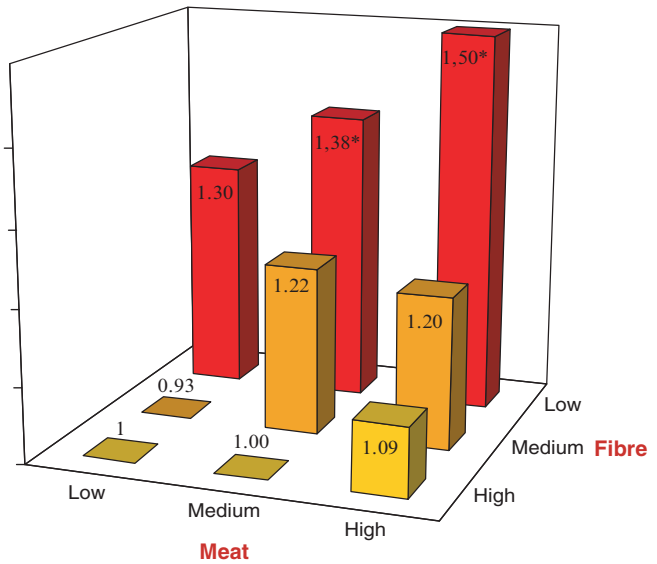

Fig. 7.1 The role of red meat and fibres in colon cancer. Red and processed meat increases the risk of colorectal cancer particularly in people who eat less than 17 g of fibre per day. (From Norat et al., *JNCI* 2005 [17])

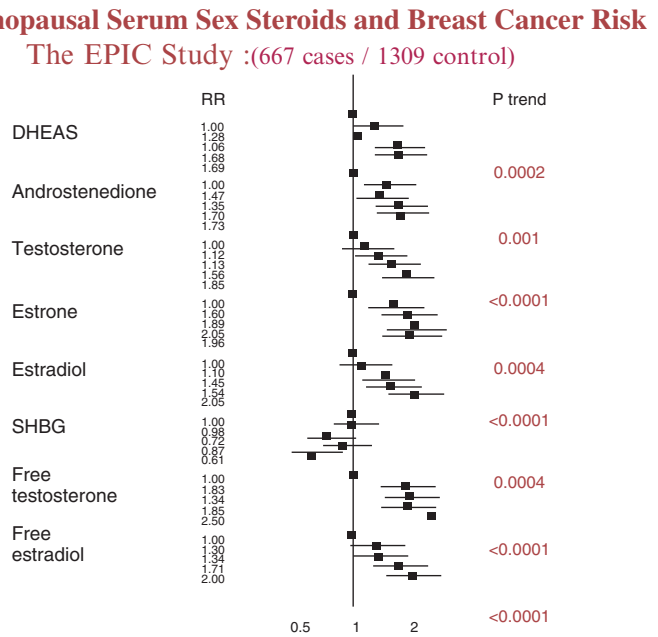

Fig. 7.2 Sex hormones in breast cancer [18]

of insulin-like growth factor (IGF). A previous prospective investigation in New York [11], in fact, has detected a dose–response relationship between C-peptide—a compound related to the insulin metabolism—and the risk of colon cancer (Fig. 7.4).

7.2.3 The Overall Picture: Diet, Hormones and Western Lifestyle

Table 7.1 Weight and colon cancer

Weight, kg	*N*	Adjusted HR
<71.0	72	1 (Referent)
71.0–76.9	68	0.91 (0.65 to 1.28)
77.0–82.7	79	1.06 (0.76 to 1.48)
82.8–89.9	93	1.24 (0.89 to 1.73)
90.0	109	1.43 (1.02 to 2.02)
p_{trend}		.007

Pischon et al. *JNCI* 2006 [10]. Anthropometry and colon cancer: men

As Table 7.2 summarizes, the aetiological associations that we have reported from EPIC can be subsumed under a common broad interpretation, i.e. Westernization of lifestyle including changes in dietary habits, reproductive habits and a sedentary life. These changes are clearly at the roots of the high frequency of breast and colon cancers in Western societies, and are likely also to shed light on the emerging epidemic of the same cancers in developing countries, particularly those with a rapidly growing economy like China and India.

7.2.4 The Future: GWA, New Biomarkers

Like other prospective studies, EPIC is extremely useful for the investigation of gene-environment interactions, and several papers have been already published ([12–14] are examples), including a Genome-wide Association study (GWA) on lung cancer [15]. The development of new effective, validated biomarkers is a key

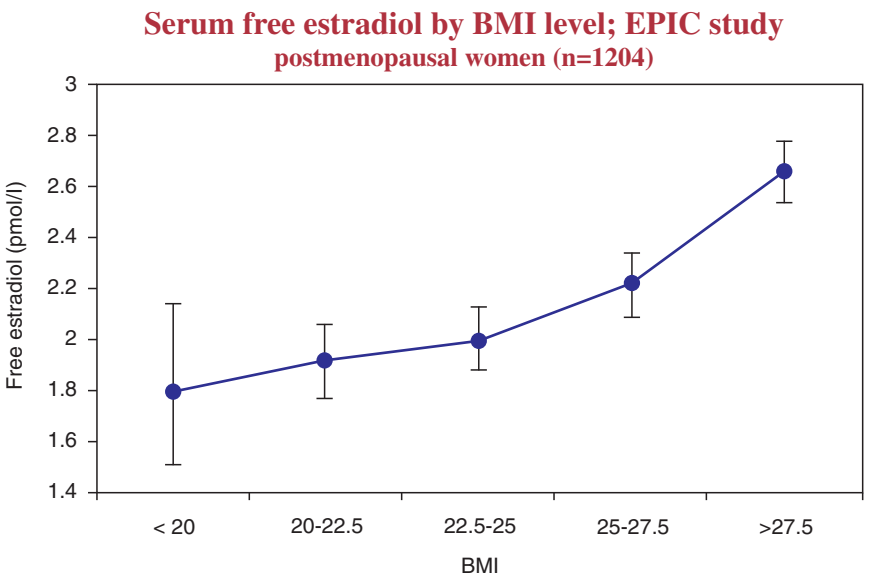

Fig. 7.3 Estradiol levels in relation to BMI

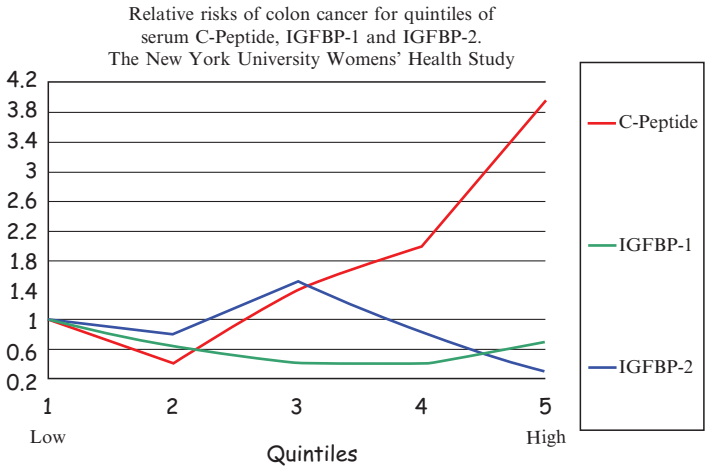

Fig. 7.4 Serum C-peptide, IGF binding protein (IGFBP)-1 and IGFBP-2 in the New York University Women's Health study [11]

Table 7.2 "Westernization" of lifestyle and cancer

Western lifestyle:
-Energy dense diet, rich in:
-Fat
-Refined carbohydrates
-Animal protein
-Low physical activity
-Smoking and drinking
Consequences:
-Greater adult body height
-Early menarche
-Obesity
-Diabetes
-Cardiovascular disease
-Hypertension ... and cancer

Table 7.3 Mutations in plasma DNA (CFDNA) in relation to bladder cancer in the EPIC study

	OR	95% CI
KRAS2	5.15	1.34–19.72
TP53	1.81	0.66–4.97
TP53 (MT1+Mt2)	2.00	0.66–6.06
TP53+KRAS2	2.08	0.90–4.76
TP53 (MT1+Mt2)+*KRAS2*	1.95	0.89–4.31

OR and 95% CI adjusted for age, sex, time and site of recruitment and smoking history (Gormally et al., *Cancer Res* 2006 [16])

issue for the success of future studies. An example is represented by the investigation of mutations in plasma DNA (Table 7.3) [16]. Other pilot studies have been started on the use of epigenetics, proteomics and metabonomics, which, however, require validation before clear inferences can be drawn.

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8 Anti-angiogenic Properties of Chemopreventive Drugs: Fenretinide as a Prototype

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Abstract Several cancer chemopreventive agents have been demonstrated to exert antiangiogenic effects. Blocking tumor angiogenesis, a process critical for tumor mass expansion and metastasis, represents an intriguing approach not only to cancer therapy, but also to cancer chemoprevention. We found that angiogenesis is a common and key target of many chemopreventive molecules, where they most likely suppress the angiogenic switch in premalignant tumors, a concept we termed "angioprevention." In this manuscript we use as an example the synthetic retinoid *N*-(4-hydroxyphenyl)retinamide (4HPR), a molecule with confirmed clinical applications in breast cancer adjuvant therapy to prevent cancer recurrence and under evaluation in neuroblastoma and glioblastoma treatment.

8.1 Molecular Regulators of Angiogenesis as Drug Targets for Chemoprevention

Angiogenesis is a key biological process through which new blood vessels are formed from preexisting ones. This process is rare under normal physiological conditions, except for specific processes such as wound healing and the female reproductive cycle, but it is a rate-limiting step in a number of pathological situations, including tumor growth and metastatic dissemination (Folkman 1971). Angiogenesis is a fundamental step in the transition of tumors from a dormant state, where limitations in oxygen and nutrient supply restrict tumor expansion, to a malignant state where the tumor forms new aberrant vessels that permit expansion and facilitate metastasis formation.

A dynamic balance between pro- and antiangiogenic factors regulates the process of tumor angiogenesis. The relative expression levels of these molecules determine whether vascular cells become angiogenic or remain quiescent. The acquisition of the angiogenic phenotype, the "angiogenic switch", appears to be a key step in early tumor progression, whereby the tumor transforms from a microscopic lesion with a limited malignant potential to a rapidly expanding mass that favors acquisition of malignancy (Hanahan and Folkman 1996). Tumor cells acquire an angiogenic phenotype when the expression of proangiogenic factors, such as vascular endothelial growth factor (VEGF), interleukin 8 (IL-8), or transforming growth factor-β (TGF-β) are upregulated, and/

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or the expression of inhibitors, for example thrombospondin, TIMPS, uPAR, angiostatin, endostatin, or interferons, are downregulated.

The multistep mechanism of angiogenesis closely parallels that of tumor cell extravasation: it involves degradation of the vascular basement membrane by cellular proteases, penetration and migration of endothelial cells into the extra-cellular matrix (ECM), and proliferation. A critical step in the metastatic process is the production of lytic enzymes, such as matrix metalloproteinases (MMP), that catalyze the proteolytic degradation of the ECM essential for cell invasion. Proteolytic enzymes can be subdivided on the basis of preferential extracellular matrix substrates into collagenases, gelatinases, stromelysins, and elastases. The MMPs are zinc-dependent enzymes secreted by both endothelial and tumor cells in inactive proenzymatic forms. Gelatinase A (MMP-2) and gelatinase B (MMP-9) have the ability to cleave type IV collagen, a major component of the basement membrane, thus these MMPs were suggested to be particularly important for tumor metastasis and angiogenesis (Kleinman et al. 2001). Experiments with MMP-2 knockout mice demonstrated that tumor angiogenesis is reduced, whereas MMP-9 knockout mice show a defect in bone formation associated with a lack of angiogenesis (Itoh et al. 1998; Vu et al. 1998). However, the function of the MMPs is known to extend well beyond that of matrix degradation (Egeblad and Werb 2002), possibly explaining the limited efficacy of MMP inhibitors in clinical trials.

Another important protease regulated by growth factors and oncogenes is urokinase (uPA) and the uPA receptor (uPAR) system and its inhibitors. Urokinase is a serine protease that catalyzes the conversion of inactive plasminogen into plasmin. uPA binds to specific cell surface receptor (uPAR), and it is directly controlled by specific inhibitors (PAIs). Overexpression of metalloproteases and uPA correlates with the invasive phenotype of endothelial cells and is associated with poor prognosis in cancer patients (Egeblad and Werb 2002; Gualandris et al. 1997). A complex signaling system associated with these proteases again results in pleiotropic effects in both physiological and pathological states.

Several antiangiogenic drugs are able to inhibit endothelial cell invasion by direct inhibition of protease activity. For example, we and others have demonstrated that the chemopreventive and therapeutic properties of *N*-acetyl cysteine (NAC) and epigallocatechin-gallate (EGCG), the main flavonol found in green tea extracts, are associated with antiinvasive and antiangiogenic effects through inhibition of uPA and MMP activity (Cai et al. 1999; Cao and Cao 1999; Garbisa et al. 2001; Benelli et al. 2002; Fassina et al. 2004). The AKT inhibitor and antitumor agent deguelin, a flavonoid from *Mundulea sericea*, can inhibit tumor dissemination by inhibiting both tumor necrosis factor (TNF)-induced cellular invasion (Nair et al. 2006) and endothelial cell migration, invasion, and metalloprotease production (Dell'Eva et al. 2007).

8.2 Angioprevention: When Cancer Chemoprevention Meets Angiogenesis

In cancer chemoprevention therapies, natural and synthetic substances are administrated for long periods to prevent the insurgence of primary tumors in subjects at risk, or to prevent tumor relapse after surgical removal. Obviously, such long-term administration requires compounds with low toxicity and limited side effects. We observed that several chemopreventive drugs, such as nonsteroidal antiinflammatory drugs (NSAIDs), protease inhibitors, steroids, and natural or synthetic retinoids, all show antiangiogenic activities as a common and key effect, and coined the term "angioprevention" (Tosetti et al. 2002) for this concept.

Chronic inhibition of angiogenesis represents an interesting approach beyond its clinical

application in chemoprevention. In principle, the antiangiogenic strategy has several advantages over traditional therapies, including less toxicity, limited to administration period, and a reduced risk to develop drug resistance, even in advanced cancer. Indeed, endothelial cells, the primary targets of antiangiogenic drugs, are genetically stable and show low mutagenicity, as compared to epithelial cells (Boehm et al. 1997). For example, we demonstrated that NAC, used in the clinic as a mucolytic, can prevent not only in vivo carcinogenesis (De Flora et al. 1996), but can also reduce tumor cell invasion through inhibition of metalloproteases (Albini et al. 1995), and angiogenesis and metastasis formation in vivo (Cai et al. 1999; Albini et al. 2001). Therefore, angiopreventive drugs might be usefully employed to interfere with different steps of the tumorigenic process. In a primary prevention setting they could block early tumor progression before tumor cells acquire an angiogenic phenotype. In secondary prevention they could inhibit neovascularization by blocking the progression from in situ to invasive cancer. Finally, angiopreventive drugs showing antiinvasive properties could limit metastatic dissemination in a tertiary prevention setting.

8.3 Retinoids and 4HPR as Angiopreventive Molecules

One of the most interesting compounds in chemoprevention is vitamin A. Vitamin A, or retinol, is a fat-soluble vitamin housed both in animal (principally liver, cod-liver oil, kidney, eggs, and dairy products) and vegetable food (mainly in yellow-orange fruits and vegetables, and in lower quantity in broccoli leaves and spinach) as retinol itself or as beta-carotene derivatives. This nutrient is very important to many biological processes: extended vitamin A deficiency leads to different levels of blindness, skin alterations, and gut or lung infections.

Several epidemiological studies point out that retinol concentration in blood inversely correlates with the risk of cancer. In animals it mainly controls epithelial differentiation and protects the epithelial tissue from the keratinization process. Even in the 1970s it was well-known that keratinized epithelia in vitamin A-deficient patients reverse into a normal phenotype after retinol administration. Since most cancers are epithelia-derived carcinomas, vitamin A was investigated for its potential antitumor activity.

Of retinol present in the body, 80% is stored in the hepatic stellate cells (HSCs) localized in the space of Disse (i.e., between sinusoidal endothelial cells and parenchymal cells) (Wake 1971), which also regulate vitamin A homeostasis (Senoo and Wake 1985). The question arises as to whether the more we ingest vitamin A, the better? As usual, the maxims about moderation (there is nothing that in excessive quantity has a good effect) apply even to vitamin A. Chronic ingestion, in fact, leads to liver parenchymal cell alterations and—in rodent models—loss of body weight and modification of bone structure (Hixson and Denine 1978).

In order to select for the beneficial effects of retinol, many analogs have been synthesized, and as expected synthetic retinoids cause less toxicity in normal cells (Sabichi et al. 2003), although high-dose toxicities are observed and some limitations in terms of bioavailability are found in vivo. Retinoid analogs are hydrophobic compounds not amenable to systemic administration, and their low bioavailability decreases the efficacy in vivo and generates a gap between in vitro and in vivo results that makes extrapolation of activities and mechanisms complex. Therefore, substantial effort has been made to develop synthetic analogs of vitamin A with high efficacy and limited side effects. One of the most promising retinoids at the clinical level is the synthetic derivative of all-*trans* retinoic acid, *N*-(4-hydroxyphenyl)retinamide (4HPR), also known as fenretinide (Fig. 8.1).

Early preclinical studies indicated that 4HPR is able to inhibit breast carcinogenesis (Moon

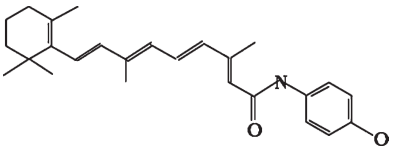

Fig. 8.1 Chemical structure of fenretinide

et al. 1979). In subsequent studies 4HPR has shown antitumor activity in vitro and in experimental models of ovarian, prostate, and lung cancer in vivo (Formelli and Cleris 1993; Pienta et al. 1993; Pollard et al. 1991; Conaway et al. 1998). Most ovarian cancer cell lines are resistant to tumor necrosis factor-related apoptosis-inducing ligand (TRAIL)-induced apoptosis (i.e., TNF apoptosis); however, fenretinide administration in combination with TRAIL results in a proapoptotic activity due to activation of multiple caspases (Cuello et al. 2004). 4HPR induces apoptosis rather than differentiation in many tumors (e.g. human neuroblastoma cell lines, androgen-independent prostatic cells, malignant hemopoietic cell lines and cervical carcinoma cells) by different mechanisms, one of which is an increase of ROS production. A small increase in ROS amount is sufficient to favor oncogene expression with a pro-mitogenic effect, whereas high ROS levels induce cell apoptosis. Fenretinide tested on melanoma cells activates the NADPH oxidase regulatory subunit Rac, leading to a ROS generation at a apoptosis inducing levels. Interestingly, Rac is more sensitive in metastatic cells than in primary tumor cells, such that 4HPR is more effective on the former (Kadhara et al. 2008).

Thus 4HPR is particularly being investigated as a promising combination with ABT-737 for acute lymphoblastic leukaemia (ALL). ABT-737 is not effective in cells presenting high levels of the antiapoptotic Mcl-1 protein; 4HPR, by enhancing ROS levels and activating JNK, induces Mcl-1 phosphorylation and its inhibition, favoring apoptosis (Kang et al. 2008).

Different neuroblastoma cell lines (FISK, NASS, SY5Y, IMR32, SJ8, SJNB10) and multicellular tumour spheroids treated with 4HPR show decreased levels of Ki-67 and increased levels of cleaved-PARP, markers of proliferation and apoptosis respectively (Cuperus et al. 2008).

Several studies have focused on the reformulation of the molecule to enable water-solubility. 4HPR bound to a synthetic polyamino acid poly (l-glutamic acid) (PG-4HPR) or to polyvinyl alcohol (PVA-4HPR) has been evaluated in ovarian cancer (Zou et al. 2007) and neuroblastoma tumor models (Orienti et al. 2007), respectively, and exhibit increased antitumor activity in vivo as compared to un-conjugated 4HPR.

Administration of 4HPR to premenopausal women in a large 5-year study of chemoprevention reduced the insurgence of a second breast tumor (Veronesi et al. 1999).

We have demonstrated in two different experimental models of human cancer that the antitumor activity of 4HPR is clearly related to inhibition of angiogenesis (Ferrari et al. 2003a, b, 2005; Pfeffer et al. 2003; Tosetti et al. 2003).

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Retinoids and Breast Cancer Prevention

9

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Abstract Preclinical models suggest that retinoids inhibit mammary carcinogenesis. Induction of apoptosis is a unique feature of fenretinide, the most studied retinoid in clinical trials of breast cancer chemoprevention due to its selective accumulation in breast tissue and its favorable toxicological profile. In a phase III breast cancer prevention trial, fenretinide showed a very strong trend of reduction of incidence of second breast malignancies in premenopausal women, which was confirmed by the 15-year follow-up. Interestingly, ovarian cancer incidence appeared reduced during treatment in the same trial. This warrants further research on fenretinide mechanisms of action and potential efficacy and provides the rationale for a phase III primary prevention trial in young women at high risk for breast cancer.

9.1 Introduction

Retinoids play a crucial role in cellular and tissue differentiation because of their capability to activate and/or repress specific genes and consequently to suppress tumor promotion and modify some properties of fully transformed malignant cells (Chambon 1996). Many activities of retinoids are initiated by ligand-induced dimerization of retinoic acid receptors (RARα, -β, and -γ) and retinoid X receptors (RXRα, -β, and -γ), followed by receptor binding to retinoid response elements on DNA and transactivation of retinoid response target genes (Rehman et al. 2004).

Both normal and malignant epithelial breast cells express retinoid receptors: they are involved in normal tissue development, and a direct/indirect effect on gene expression as consequence of multiple signal transduction pathways is one of the possible mechanisms underlying breast cell growth inhibition by retinoids.

Over the last decade breast cancer prevention has focused attention mainly on endocrine therapies using selective estrogen receptor modulators (SERMs) and aromatase inhibitors (AIs). The use of tamoxifen, the best-known selective estrogen receptor modulator, is able to significantly reduce breast cancer incidence in high-risk women. Both SERMs and AIs, however, have no effect in reducing the risk of estrogen receptor-negative breast cancer. For this reason, preventative therapies for estrogen receptor-negative breast cancer are needed. A number of novel chemopreventative agents such as tyrosine kinase inhibitors,

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selective cyclooxygenase-2 inhibitors, and rexinoids have recently shown in animal models the ability to prevent estrogen receptor-negative mammary tumorigenesis.

The synthetic RXR-selective retinoid bexarotene (LGD1069) suppressed ER-negative mammary tumorigenesis in several transgenic mouse models with minimal side effects, mostly cutaneous and in the high-dose group after many months of treatment (Wu et al. 2002).

The newer rexinoid LG100268 (268) seems to be even more potent than bexarotene and with a greater specificity for binding to RXRs (Boehm et al. 1995). This rexinoid and two new SERMs, arzoxifene and acolbifene, as individual drugs, delayed the development of estrogen receptor-negative tumors in animal models. Moreover, the combination of a SERM and 268 was strikingly synergistic, as no tumors appeared in any mouse fed the combination of 268 and a SERM (Liby et al. 2006). Furthermore, 268 seems to be a fascinating compound to study in chemoprevention trials because of its capability to act within the tumor microenvironment and inhibit the recruitment and the activation of endothelial cells: it has been recently shown that 268 is able to reduce vessel formation both in vitro and in vivo (Sogno et al. 2007).

Synthetic modification of the carboxyl end of retinoic acid with an *N*-4 hydroxyphenyl group results in the formation of *N*-4-(hydroxyphenyl)retinamide (4-HPR) or fenretinide. *N*-4-(Hydroxyphenyl)retinamide is more potent than all-*trans* retinoic acid (ATRA), both as an antiproliferative agent and inducer of apoptosis in the majority of cancer cell lines tested (Oridate et al. 1996; Zou et al. 1998).

Fenretinide was synthesized in the late 1960s. It is the synthetic amide of retinoic acid 4-HPR and is considered the less toxic retinoid studied in chemoprevention clinical trials. The studies on fenretinide's biological activity immediately showed the preferential accumulation of this drug in the breast instead of the liver (Sporn et al. 1976) and the growth inhibition by fenretinide of chemically induced mammary carcinoma in rats was described for the first time in 1979 (Moon et al. 1979). Even though 4-HPR can transactivate certain retinoid receptors and RAR antagonists can partially block 4-HPR-induced apoptosis compared with ATRA (Sun et al. 1999), more recent data demonstrated that 4-HPR binds with low affinity to RAR with a poor transactivation of RAR/RXR response elements in human breast cancer cells (Sheikh et al. 1995).

Because of the promising data in different experimental models and the favorable toxicity profile as compared with other retinoids, fenretinide began to be studied in chemoprevention trials, targeting different organs (Kelloff et al. 1994).

9.2 Clinical Trials

A phase I dose-ranging study of fenretinide selected the 200-mg daily dose as the safest one (Costa et al. 1989) and the same study also provided important information on its pharmacokinetics (Formelli et al. 1989). Visual impairment through diminished dark adaptation is the most frequent side effect related to a dose-linear decrease of plasma retinol induced by fenretinide administration, and in order to minimize this symptom, a monthly 3-day drug holiday was adopted (Formelli et al. 1989). A multicentric phase III randomized trial with fenretinide then started in 1987 (Veronesi et al. 1999): participants were stage I (T1–2 N0) breast cancer patients, aged 33–70 years, who had undergone surgery for breast cancer within the previous 10 years. As in the 1980s, these patients were not candidates for adjuvant systemic therapy, so they represented a suitable population to test fenretinide for the prevention of second breast cancer. Women were randomly assigned to receive either no treatment or 200 mg/day fenretinide orally for 5 years. No placebo control arm was included in the study design because of both the large size of the capsule containing the drug and the objective nature of the main outcome measure. A 3-day drug interval at the end of each month was recom-

mended. The main outcome measure was the occurrence of contralateral breast cancer as the first malignant event. The secondary endpoint was the incidence of ipsilateral breast cancer reappearance, defined as either local recurrence in the same quadrant or occurrence of a second breast malignancy in different quadrants from the primary tumor. Accrual started on March 1987 and was closed on July 1993. A total of 2,972 patients entered the study, 2,867 of whom were assessable, giving an 87% power to detect the expected difference. The two groups were well balanced for all patient and tumor characteristics.

After a median follow-up duration of 97 months (Veronesi et al. 1999), fenretinide showed no significant effect on overall contralateral breast cancer occurrence. However, when the analysis was stratified by menopausal status, a statistically significant beneficial trend in premenopausal women on both contralateral and ipsilateral breast cancer was found (hazard ratio (HR) 0.66, 95% confidence interval (CI), 0.41–1.07, and HR 0.65, 95% CI, 0.46–0.92, respectively), compared to an opposite trend in postmenopausal women (contralateral breast cancer HR 1.32, 95% CI, 0.82–2.15; ipsilateral breast cancer HR 1.19, 95% CI, 0.75–1.89). This phase III trial suggested a possible role of fenretinide as a preventative agent acting at different levels of breast carcinogenesis, but indicated its lack of efficacy on the progression to a more malignant phenotype, possibly as a result of the loss of retinoid receptor expression (Widschwendter et al. 1997). Importantly, the results of a recent late analysis (Veronesi et al. 2006) in the subgroup of 1,739 participants who were regularly followed-up for up to 15 years in a single center indicate that fenretinide induced an overall 17%, durable reduction of second breast cancer incidence, which approached statistical significance. Moreover, when the analysis was stratified by menopausal status, there was a 38%, statistically significant reduction of second breast cancers in premenopausal women. Remarkably, the protective effect persisted for up to 15 years, i.e., 10 years after retinoid cessation. Most notably, the younger were the women, the greater was the benefit of fenretinide, which was associated with a remarkable 50% risk reduction in women aged 40 years or younger, whereas the benefit disappeared after age 55.

These results are limited to a subject subgroup (premenopausal women) followed in a single center, representing 60% of the original cohort. Noticeably, completion and further update of the follow-up is currently ongoing in the original participating centers. The subgroup differed slightly from the original whole cohort, as proportionally more women underwent breast-conserving surgery and were enrolled within a year from surgery. However, these factors, which are associated with a higher rate of ipsilateral breast cancer and distant metastases, were evenly balanced between arms and were accounted for in the multivariate analysis. Moreover, randomization was stratified by center, and no significant heterogeneity across centers was evident in the initial results (Veronesi et al. 1999).

Finally, one strength of the study is that all women underwent a regular clinical follow-up with uniform procedures in a single center. This analysis confirms and further extends the notion that the protective effect of fenretinide occurs exclusively in premenopausal women or women aged 55 or younger.

Admittedly, this subgroup analysis had not been foreseen when the study was planned. While there are plausible biological explanations for this selective effect, our findings are hypothesis-generating and do not have immediate practical clinical implications, but they do provide the rationale for testing the drug's efficacy in premenopausal women. In fact, there are already plans to open a new phase III prevention trial with fenretinide in young women at high risk for breast cancer due to familial/genetic predisposition.

Initial clinical experience is ongoing with bexarotene as well. In a phase II double-blind randomized clinical trial, 87 women at high risk for breast cancer (≥10% chance of carrying *BRCA-1* or *-2* mutation) received 200 mg/m^2 bexarotene or placebo for 28 days. Breast core needle biopsies were taken on days 1 and 29 in 66

women. Assessed for Ki-67 and cyclin-D1 expression were 55 pre- and post-treatment samples. No significant reduction in ki-67 expression was found in women taking bexarotene but a reduction in cyclin-D1 expression was observed in a subgroup analysis of postmenopausal women. The treatment was well tolerated but bexarotene was associated with a more frequent hypertriglyceridemia (57%), subclinical hypothyroidism (49%), and mild skin reactions (34%) compared to placebo (6%, 0%, and 6% respectively) (Brown et al. 2007). These results could be used to plan future cancer prevention trials using this rexinoid.

9.3 Fenretinide and Ovarian Cancer

Studies in vitro have demonstrated that 4-HPR inhibits the growth of several human cancer cell lines, including ovarian cancer cells (Formelli et al. 1996). This agent has also shown antitumor activity in ovarian cancer animal models (Formelli and Cleris 1993). Furthermore, retinoid receptors have been associated recently with ovarian cancer prognosis, providing further evidence for their use in the clinic (Kaiser et al. 2005).

Interestingly, in the Italian phase III breast cancer prevention trial (Veronesi et al. 1999) the incidence of ovarian cancer during the 5-year intervention period was significantly lower in the fenretinide arm (0 cases versus 6 cases in the control group), whereas 3 cases of ovarian cancer occurred in the fenretinide group after treatment discontinuation (De Palo et al. 1995). An update of the effect of fenretinide on ovarian cancer has been provided subsequently (De Palo et al. 2002). After a median of 121 months, a total of 6 cases of ovarian cancer had occurred in the fenretinide arm as opposed to 10 cases in the control arm (p=n.s.). A protective effect was suggested in women with a high probability of carrying a *BRCA-1* mutation. Indeed, fenretinide was highly effective in inhibiting the growth of *BRCA-1*-mutated breast cancer cell lines (Simeone et al. 2005). When considering the protective activity of fenretinide on second breast cancer in young women and a similar trend on ovarian cancer, at least during intervention (De Palo et al. 2002), it appears that women with germline *BRCA-1* and *-2* mutations may be ideal candidates for further investigation of this retinoid.

9.4 Conclusions and Future Perspectives

SERMs and AIs directly involve the hormonal pathway of the pathogenesis of the disease, and their target is most likely limited to hormone-responsive tumors. The most important results of prevention clinical trials so far have shown that tamoxifen has great effect as a chemopreventative agent but it may have serious side effects, while raloxifene may have a better toxicity profile, but it seems unable to reduce the incidence of cancer precursors (as tamoxifen does) and it has been tested so far only during postmenopause.

Fenretinide has shown to possess several good properties both in preclinical models and clinical trials. In particular, the prolonged effect demonstrated in premenopausal breast cancer patients in the Italian phase III trial, together with an apparent protective effect on the ovaries, has been accompanied by a very low toxicity profile (mainly reversible skin dryness and rashes and dark adaptation difficulties, often overcome by a monthly weekend suspension of the drug). All these characteristics make fenretinide an excellent candidate for chemoprevention in a cohort of young healthy women with a high susceptibility to early onset breast and ovarian cancer, such as those who carry a germline mutation or have a significant family risk. Furthermore, since the drug's activities are probably not strictly influenced by hormonal respon-

siveness, it is possible that it may also have effects on hormone-nonresponsive cancers, and this may be very useful, especially in the case of *BRCA-1* mutation carriers.

Novel rexinoids such as bexarotene and LG100268, which have been shown in mice to prevent estrogen receptor-negative breast cancer, can be considered promising compounds to be used to plan future cancer prevention trials.

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Part IV

Cancer Prevention, Genetics and Vaccines

Cancer Prevention by Vaccination Against Hepatitis B

10

Mei-Hwei Chang

Abstract Chronic inflammation caused by persistent infection is closely related to a number of cancers; these include hepatitis B (HBV) or C and hepatoma, human papilloma virus and cervical cancer, and *Helicobacter pylori* and gastric cancer. The first evidence of cancer prevention by vaccination in humans was provided by HBV vaccination in infants. Chronic HBV is related to approximately 60%–90% of hepatocellular carcinomas (HCC) in adults and nearly 100% of childhood HCC in areas endemic for HBV infection. The first universal HBV vaccination program was launched in Taiwan and has continued for more than 20 years. Three or four doses of HBV vaccine were given to all infants starting from the first week of life. In addition, infants of high-risk mothers (with positive hepatitis B e antigen or high HBsAg titers) were given hepatitis B immunoglobulin within 24 h after birth. At 20 years after the launch of the HBV vaccination program in Taiwan, chronic HBV infection (HBsAg seropositive) rates in the general population below 20 years of age have revealed a remarkable reduction from 10%–17% before the vaccination program to 0.7%–1.7% after the program. HCC incidence rate in children 6–14 years old also fell from 0.52–0.54 to 0.13–0.20 per 100,000 (R.R.=0.25–0.36). HCC prevention failure is mainly related to vaccine failure to prevent chronic HBV infection. The causes of vaccine failure have included intrauterine infection, vaccine escape mutants, genetic hyporesponsiveness, and poor compliance. Future efforts to reduce vaccine failure will improve the efficacy of liver cancer prevention by HBV vaccination. The experience of HCC prevention by HBV immunization may be applied to the prevention of other infection-related cancers.

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10.1 Introduction

The etiology of cancer is multifactorial. Hosts persistently infected by a microorganism that induces chronic inflammation, tissue injury and regeneration may finally develop cancer (Hold and El-Omar 2008). Examples of infectious agents that are closely related to carcinogenesis are listed in Table 10.1.

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Table 10.1 Examples borne from the accumulation of plenty of evidence on infectious agents that are closely related to the cause of cancer

Infectious agent	Related cancer
Hepatitis B virus (HBV)	Hepatocellular carcinoma
Hepatitis C virus	Hepatocellular carcinoma
Human papillomavirus (HPV)	Cervical cancer
EB virus	Burkitt's lymphoma, lymphoproliferative disorder
Helicobacter pylori	Gastric cancer

Hepatocellular carcinoma (HCC) is one of the 10 most common cancers in the world (Parkin et al. 2001). It is a good example demonstrating the close relationship between an infectious agent and cancer, as well the effect of preventing its related cancer by vaccination. The peak age of HCC in adults is 40–60 years of age in most countries, although in hyperendemic areas for HCC it can develop early in childhood as demonstrated in Asia and Africa (Larouze et al. 1976; Ni et al. 1991). Hepatitis B virus (HBV) infection is closely related to the oncogenesis of HCC (Popper et al. 1982; Beasley et al. 1981). The seroprevalence rates of hepatitis B surface antigen (HBsAg) in adult HCC patients differs in different countries. In Asian adults it ranges from as low as 20% in Japan to as high as 60%–80% in most Asian countries (Tandon and Tandon 1997).

Although the role of hepatitis C virus (HCV) in HCC is increasing in adults in many countries, the importance of HBV in childhood HCC remains unchanged. HCV is not an important etiologic agent of HCC in children (Chang et al. 1993). Up to now in the world literature, HCV-related HCC has been reported in the explant liver of a child who had received transplants.

10.2 Cancer Prevention by Vaccination

Current therapies for most cancers are unsatisfactory. Vaccination is the best way to prevent both precancerous and cancerous lesions. The goal of cancer prevention by vaccination can be achieved when (1) a microorganism is the main etiologic agent of the cancer and (2) vaccination can effectively prevent infection of the microorganism. Furthermore, evidence should show that prevention of the microorganism's infection can prevent its related cancer.

10.2.1 Advantage of Cancer Prevention by Vaccination

Strategies for preventing cancer can be divided into three categories: primary, secondary, and tertiary. Primary prevention aims to prevent cancer development in healthy subjects, secondary prevention seeks to prevent cancer development in high-risk subjects and those with precancerous lesions, and tertiary prevention involves preventing cancer recurrence in successfully treated cancer patients.

Using liver cancer prevention as an example, primary prevention seeks to prevent HBV and HCV infection and thereby the related liver cancer. Primary preventative methods are vaccination, and the avoidance of risky behavior, especially alcohol abuse, IV drug abuse, and skin piercing, in the case of liver cancer.

Applying this to the broader principle of cancer prevention, vaccination to prevent cancer has the following advantages: it (1) has a low cost, (2) is safe, (3) saves time (4) has high efficacy, and (5) is easy to conduct in a large population—for example, by integration into the WHO's Expanded Program on Immunization (EPI) in infancy.

10.3 Chronic Hepatitis B Virus Infection and Hepatocellular Carcinoma

Worldwide, HBV is the most important cause of HCC. This is particularly true in areas where both HCC and HBV infection are prevalent (Ryder et al. 1992; Chen et al. 1987). In endemic areas, such as Asia and Africa, most primary HBV infections occur during early childhood, which leads to a high rate of persistent infection. The chronicity rate reaches a plateau in early childhood (Hsu et al. 1986). In low-prevalence areas, such as North America, Northern Europe, and Oceanic areas (prevalence rate around 0.1% in the general population), HBV infection occurs mainly in adolescents and adults.

10.4 Strategies to Prevent Viral Hepatitis B-Associated Hepatocellular Carcinoma

10.4.1 Primary Prevention by Immunoprophylaxis

Of the strategies aiming toward preventing viral HBV-associated HCC (Fig. 10.1), immunoprophylaxis against viral hepatitis and its related HCC is the most cost-effective.

10.4.2 Treatment of Viral Hepatitis/Cirrhosis

Although the effect of interferon therapy in preventing HCV-related HCC seems slightly favorable, more evidence from a larger study population and a longer duration follow-up is needed (Tanaka et al. 2000). The effect of HCC prevention by interferon therapy has been controversial in HBV-related HCC (Camma et al. 2001). Lamivudine therapy against HBV had borderline efficacy in preventing the progression of liver cirrhosis and hepatoma (Liaw et al. 2004).

10.4.3 Chemoprevention

Trials of chemoprevention against hepatoma in animals and one trial in humans were conducted using oltipraz or chlorophyllin to produce an alteration in metabolites or to form tight complexes with aflatoxin B1 (Kersler et al. 2004; Egner et al. 2001). Curcumin and retinoid were also found to possess some chemopreventative

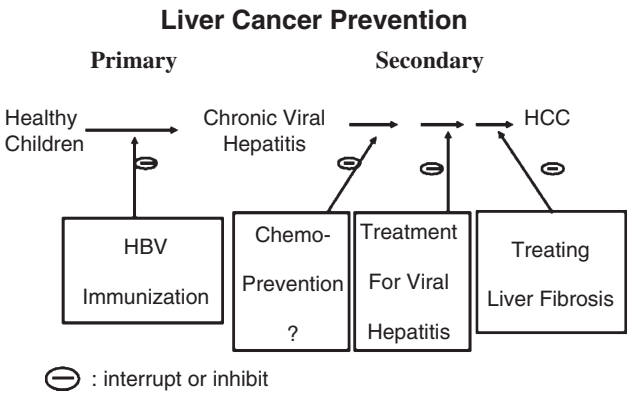

Fig. 10.1 Strategies of primary prevention in healthy subjects and secondary prevention subjects with chronic hepatitis virus infection for liver cancer

effect on human hepatocarcinogenesis (Chuang et al. 2000; Moriwaki et al. 2007). However, further confirmation of direct evidence on HCC reduction is needed.

10.4.4 Liver Transplantation for End-Stage Cirrhosis

The cost–benefit analysis of HCC prevention by liver transplantation for liver cirrhosis has not demonstrated its justification. But liver transplantation is indicated in end-stage liver cirrhotic patients and in liver cirrhotic patients with early cancerous lesions.

10.5 Cancer Prevention by Hepatitis B Vaccination

Since the peak age of HCC in adults is around 40–60 years, it will take approximately 40 years or longer to evaluate the effect of universal HBV vaccination in infancy on the reduction of HCC in adults. It is therefore reasonable to study whether childhood HCC can be prevented by HBV vaccination. The change of HCC incidence in children may reflect the future effect of HBV vaccination on HCC in adults. In comparison to most other parts of the world, Taiwan has a high prevalence of HBV infection and HCC in children. HCC in children is closely related to HBV and the characteristics are similar to HCC in adults (Chang et al. 1989). Children with HCC are nearly 100% HBsAg seropositive, and most (86%) of them are hepatitis 'e' antigen (HBeAg) negative. The HBV genome was demonstrated to be integrated into host genome of HCC children (Chang et al. 1991). HCC children are mostly (94%) maternal HBsAg positive (Chang et al. 1989). The histologic findings of the tumor portion are similar to that in adult HCC. Most (80%) of the nontumorous portion has liver cirrhosis.

10.6 Why Should the Hepatitis B Vaccine Be Given at Birth to Prevent Cancer?

The best timing for HBV immunization to prevent cancer is in infancy. This is because maternal transmission to infants is the main route of infection for both children and adults (Chang et al. 1989). The HBsAg seropositive rate in mothers of HCC children was 94%, which is significantly higher than that (36%) of the fathers of HCC children, and the rate (50%) in the mothers of control HBsAg carrier children (Chang et al. 1989).

Perinatal transmission from highly infectious mothers to their neonates is an important route for HBV infection in Asian countries and many other endemic areas (Stevens et al. 1975). It accounts for about 40%–50% of HBsAg carriers in hyperendemic areas. Without immunoprophylaxis, infants who are infected by their HBeAg-positive, HBsAg-positive mothers, more than 90% will develop chronic HBV infection during follow-up. The relatively high maternal viral load transmitting to the small neonate with a physiologic immature immune system during perinatal period may explain the high rate of persistent infection. In contrast, less than 5% infants of HBeAg-negative mothers become HBsAg carriers, and if infected, it is mostly with acute or fulminant hepatitis.

The age of HBV infection is an important factor affecting the outcome of HBV infection (Table 10.2). The younger the HBV infection occurs, the higher the rate of chronic infection will be. For those infected at a preschool age, the chronicity rate after HBV infection decreased to approximately one quarter (23%; Beasley et al. 1982). Infection occurring in young adults resulted in an even lower chronicity rate (<3%; Beasley et al. 1983a).

Comparing the strategies for HBV vaccination, a universal immunization program for infants is better than immunizing only high-risk groups. Integration of HBV into the WHO

Table 10.2 Age of infection and maternal HBV status influence the outcome of HBV infection in children (Beasley et al. 1982, 1983a, b)

Age of infection	Rate of persistent infection
Perinatal period	
Mother HBeAg(+), HBsAg(+)	>90%
Mother HBeAg(-), HBsAg(+)	<5%, with risk of FH, AH
Preschool age	23%
Young adults	2.7%–10%

AH, acute hepatitis; FH, fulminant hepatitis

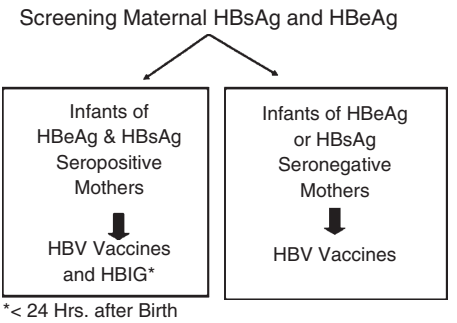

Fig. 10.2 The world's first universal hepatitis B vaccination program of infants in Taiwan

vaccination program (EPI) of infancy is the most effective approach and is having the highest compliance. The efficacy in preventing HBV infection and HCC is very high, while the cost of the required HBV vaccine is very low.

10.7 Hepatitis B Immunoprophylaxis Program

The world's first universal HBV vaccination program was launched in July 1984 in Taiwan (Chen et al. 1987). Prevention of HBV infection can be achieved by either passive or active immunization. Passive immunization using hepatitis B immunoglobulin (HBIG) provides temporary immunity. Pregnant women are screened for both serum HBsAg and HBeAg. Infants of mothers with HBeAg-negative serum, or with HBsAg-negative serum, received plasma-derived hepatitis B vaccine at 0, 1, 2, and 12 months or recombinant HBV vaccines at 0, 1, and 6 months. Infants of mothers with positive serum HBeAg and HBsAg receive HBIG within 2 h after birth in addition to three or four doses of HBV vaccine (Fig. 10.2). In infants of highly infectious mothers (HBeAg seropositive), three doses of HBV vaccine decreased the carrier rate to 24%. Injection of HBIG within 24 h after birth followed by three (0,1,6 months) or four (0,1,2,12 months) doses of HBV vaccine further reduced the carrier rate to 3% in pilot studies (Beasley et al. 1983b), and to 14% in the study of the general population (Hsu et al. 1988). The protective efficacy in infants of high-risk mothers was found to be 86% with the HBIG plus HBV vaccine, and 78% with three doses of the HBV vaccine (Beasley et al. 1983b). Active immunization with three or four doses of HBV vaccine without HBIG has proved to be immunogenic in more than 90% of neonates of non-carrier mothers or HBeAg-negative carrier mothers. The coverage rate of the HBV vaccine for neonates was around 84%–94%. The vaccination program was extended gradually to all preschool, primary, middle, and high school children, and finally to all the adults (Chen et al. 1987).

Different vaccination strategies have been used in different countries based on their basic epidemiologic features of HBV infection and HCC, and the resources of the supporting health systems. In many hyperendemic countries, HBV vaccination of all infants is performed, using three doses of HBV vaccine without HBIG. This same scheme is used for infants of HBsAg carrier mothers (Poovorawan et al. 1989). This proved to be satisfactory for prevention in Thailand, and was only 5%–10% less efficacious in infants of HBeAg-positive mothers than the combination of HBIG plus HBV vaccines. Whether those

5%–10% of children for whom prevention proved ineffective are at higher risk of HCC because of maternal transmission require further investigation. Using this HBV immunization program, the cost of maternal screening and HBIG can be reduced. In contrast, in addition to three doses of HBV vaccines for all infants, the United States program of universal HBV immunization provides screening of maternal HBsAg and HBIG within 24 h after birth for neonates of HBsAg-positive mothers, regardless of the maternal HBeAg status (Shepard et al. 2006).

10.8 Impact of Universal Hepatitis B Immunization on Chronic Infection

The seroprevalence of HBsAg (representing the chronic infection rate) and the HBV core antibody (anti-HBc, representing the total infection rate) have decreased in children in most parts of the world after the introduction of HBV vaccination programs. The HBsAg carrier population has been reduced to approximately one-tenth of the number before the universal HBV vaccination program. The seroprevalence of HBsAg in Taiwanese children fell from 10%–17% before, to 0.7%–1.7% after the HBV vaccination program. HBV immunization has proved to be effective in preventing both the total infection rate and the chronicity rate of HBV infection. The HBV vaccination program has reduced both the perinatal and horizontal transmission of HBV.

10.9 Demonstration of the Efficacy of Cancer Prevention by Hepatitis B Vaccination

After the implementation of the universal vaccination program of HBV in Taiwan, we have successfully demonstrated the decline of the incidence of HCC in children, which declined from 0.52–0.54 per 100,000 children for those born before the launch of the HBV vaccination program, to 0.13–0.20 per 100,000 children for those born after the program (Table 10.3; Chang MH et al. 1997; Chang et al. 2000, 2005).

10.10 Problems in Preventing Liver Cancer by Vaccination

After universal HBV vaccination, still around 10% of children of HBeAg-seropositive mothers became HBsAg carriers (Chen et al. 1996; Ni et al. 2001, 2007). The HCC prevention failure rate is approximately 30%–40%. The incidence of HCC in children only fell to around one-third of those born before the vaccination program (Table 10.3). This inconsistency of the efficacy may be explained by the successful prevention of almost all the horizontal transmission of HBV infection, while the maternal transmission route cannot be interrupted completely by the HBV immunization program.

Approximately 90% of the mothers of the HCC children with known serum HBsAg status were positive for HBsAg. This provides strong evidence of perinatal transmission of maternal HBV as the main route of HBV transmission in HCC children born after the immunization era; therefore, this route of transmission was not effectively eliminated by the HBV immunization program (Chang et al. 2005).

The relatively higher risk for HCC development in HBsAg carrier children born after the immunization era is due to the successful prevention of chronic HBV infection in children who acquired HBV infection by horizontal transmission and were at a lower risk for HCC. Those who failed to respond to HBV immunoprophylaxis were infected by highly infectious mothers, and were at a higher risk for developing HCC.

Table 10.3 Reduction of HCC incidence after HBV vaccination in children of 6 to 14 years old from July 1981 to June 2000 according to birth year (Chang et al. 1997, 2000, 2005)

Birth year[a]	Incidence (per 10^5)	RR	95% CI
1966–84	0.52–0.54	1	
1984–94	0.13–0.20	0.25–0.36	0.26–0.52

RR, risk ratio; CI, confidence interval
[a]Birth year was counted from July of one year to June of the next year

Failure to respond to HBV immunization or failure to conduct or follow the population-based HBV immunization protocol are the two most important causes of failure in HCC eradication in children after the implementation of the HBV immunization program. It is obvious that the lack of injection of HBIG in infants of highly infectious mothers would also be a possible cause of immunization failure in infants of high-risk mothers, in spite of the three or four doses of the HBV vaccine they received.

To eradicate HBV infection and its related cancer, we have to overcome the difficulties that hinder the success of universal HBV vaccination outlined in the following sections.

10.10.1 Inadequate Resources

How to reduce the cost of the vaccine and to increase funding for HBV vaccination to help children of hyperendemic areas with poor economic conditions are important issues to solve for the eradication of HBV infection and its related liver cancer.

10.10.2 Ignorance or Poor Compliance Due to Anxiety to the Safety of Vaccine

In low-prevalence areas, antivaccine perceptions should be reduced to the minimal degree by clarifying vaccine-related side effects. For instance, although lacking convincing evidence, the correlation between central nervous system demyelinating diseases and the HBV vaccine has been raised (Halsey et al. 1999). Education and propagation of the benefits of HBV vaccination will enhance the motivation of the public and governments to accept HBV vaccination. Ignorance of the government program or its implementers may also result in no vaccination program, or a refusal to participate in or ignorance of the vaccination program.

10.10.3 Vaccine Failure or Nonresponders

Further investigation of the mechanism of HBV vaccine failure or nonresponders will help to solve many problems. Interventions to prevent intrauterine infection, the development of an HBV vaccine covering the surface gene-mutants, and better vaccines for immunocompromised individuals, are examples of how to reduce the rate of vaccine failure or nonresponders.

Intrauterine infection of HBV, mostly due to high maternal viral load, is an important cause of HBV vaccine failure. Intrauterine infection occurs rarely, in approximately 5% of the infants of HBeAg- and HBsAg-positive mothers. In a study in Taiwan, 2.4% of the 665 infants of HBeAg- and HBsAg-positive mothers were seropositive for HBsAg at birth, suggesting intrauterine infection (Tang et al. 1998). All of them remained HBsAg-positive at 12 months of age.

A vaccine escape mutant is another cause of vaccine failure. Prevalence of HBV surface

gene *a* determinant mutants in children under age 15 with positive HBsAg or anti-HBc in 1984, 1989, 1994, and 1999 were 7.8%, 19.6%, 28.1%, and 23.1%, respectively (Hsu et al. 2004). Other causes of vaccine failure include genetic hyporesponsiveness, an immune-compromised host, etc.

10.10.4 No Effective Vaccine Available

With no vaccine available to effectively control HCV-related HCC, for example, vaccine development is an important future task.

10.11 Conclusions and Future Prospects

The results of treatments for HCC are not satisfactory. Prevention is a better way than therapy. Among the various prevention strategies, primary prevention by vaccination is the most cost-effective way. Universal HBV immunization in infancy has successfully reduced the prevalence of the chronic HBV infection rate to one-tenth of the pre-vaccination prevalence in children. The incidence of HCC in children was also reduced to approximately one-third to one-fourth after the HBV vaccination program.

10.11.1 Hepatoma Control

The success of the prevention of HCC in children is dependent on the success of the eradication of chronic HBV infection. Our study clearly iterates the importance of HBV immunization in the prevention of HCC, and thus it should be continued. The HBV vaccine should be given to all infancts worldwide.

Since the peak age of HCC in adults is around 40–60 years, theoretically we may expect a reduction of HCC in adults 40 years, or somewhat sooner, after the universal vaccination programs. Continuous efforts to promote the success of HCC prevention are needed. Methods to combat vaccine failure and improve the compliance of the HBV immunization program are very important for the future success of HBV-related HCC eradication.

Worldwide integration of HBV vaccination into the EPI program should be promoted further. We also need better strategies to prevent mother-to-infant transmission of HBV. Hopefully, control of HBV-related hepatoma will be reached several generations later. Furthermore, development of an HCV vaccine is needed to control HCV-related hepatoma.

10.11.2 Implication to the Control of Other Cancers

HBV vaccination to prevent hepatoma is a successful example of cancer prevention. We are anticipating that the impact of HBV vaccination on the control of HBV and its related diseases can be extended to other infectious diseases and their related cancers. This model can be applied in preventing HCV-related HCC, and other virus-related cancers, such as the Epstein-Barr virus (EBV) and nasopharyngeal cancer, papilloma virus and cervical cancer, *H. pylori* and gastric cancer, etc. The successful development and clinical application of papilloma virus vaccine is another exciting event of cancer prevention.

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Part V

Cancer Prevention and Target Organs I: Breast Cancer

Energy Restriction for Breast Cancer Prevention

11

Anthony Howell, Mary Chapman, and Michelle Harvie

Abstract Weight gain in adult life is an important risk factor for breast cancer. Observational studies indicate that pre- or postmeno-pausal weight loss is associated with a reduction in risk of postmenopausal breast cancer. Here we summarise lifestyle changes including continuous or intermittent energy restriction and/or exercise which may be beneficial for preventing breast cancer and also potential pharmacological approaches to prevention using energy restriction mimetic agents (ERMAs).

11.1 Introduction

Increased energy balance, either produced by increased energy intake or reduced expenditure (or both), may be responsible for approximately one-third of human mammary tumours (Vainio et al. 2002). Energy excess gives rise to alterations within the mammary cell (in the stroma and systemically) which alter epithelial cell metabolism and proliferation and promote carcinogenesis. Here we summarise the evidence for energy excess being a major factor in the development and progression of breast cancer and how this might be circumvented by the use of dietary or exercise energy restriction measures and the potential use of energy restriction mimetic agents (ERMAs). The future of this approach will depend upon the introduction of methods which make energy restriction acceptable on a population basis or by using simple non-toxic ERMAs.

Hypotheses to explain the beneficial effects of energy restriction have been summarised by Sinclair (2005) who suggested that the mild stress provided by energy restriction provides general protection from breast cancer and other chronic disease (hormesis). A related hypothesis suggests that during times of deprivation the body changes from growth and reproduction to dependence on somatic maintenance and repair (Shanley and Kirkwood 2000). Understanding the mechanism of energy restriction is not only important to develop optimal energy restriction approaches but also to determine targets for ERMAs. In turn, responsiveness to ERMAs can give insights into the key modulators of energy restriction.

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11.2 Risk Factors for Breast Cancer

Whilst survival from breast cancer is improving, the incidence of the disease continues to rise in most countries, indicating the need to determine the cause of the increase and to introduce preventive approaches in women most at risk (Bray et al. 2004). The rate of increase in incidence is illustrated by changes which have occurred in Iceland over the past century (Tryggvadottir et al. 2006). Not only has there been a fourfold increase in sporadic breast cancer, from 1.8% in 1920 to 7.5% in 2002, but there has been a similar increase in the penetrance of the *BRCA2* gene amongst mutation carriers, from 18.6% to 71.9%. Whilst screening and other factors may, in part, be responsible for the increase in incidence, it is likely that other factors such as population changes in reproduction and lifestyle have contributed to the increase. Reproductive changes which are likely to increase breast risk include the increased age of first pregnancy by about 5 years since 1970 (Soerjomataram et al. 2007) and the marked reduction in parity in many developing countries (Chia et al. 2005).

Energy intake above requirements (due to excess food intake) combined with reduced expenditure by exercise is also likely related to increased breast cancer risk (Harvie and Howell 2006). In the United States in 1980, 41.6% of women were estimated to be either overweight or obese, whereas this figure was 66.0% in 2004 (http://www.cdc.gov/nccdphp/dnpa/obesity/trend). In England, rates of overweight and obesity have increased from 31% in 1980 to 57% in 2004 (Zaninotto et al. 2006).

11.3 Effect of Weight and Weight Gain and Exercise Deficiency on Breast Cancer Risk

Body weight, body mass index (BMI), waist circumference and weight gain are risk factors for postmenopausal breast cancer (Reeves 2007; Harvie et al. 2003). Weight gain especially before the menopause is a particularly important risk factor (Eliassen et al. 2006; Han et al. 2006; Lahmann et al. 2005; Trentham-Dietz et al. 2000; Magnusson et al. 1998; Huang et al. 1997; Harvie et al. 2005) in both women with and without a family history of the disease, and mainly amongst women who have not taken postmenopausal hormone replacement therapy (HRT). In the Nurses Health Study, weight gain of 25 kg or more since age 18 increased the relative risk (RR) of postmenopausal breast cancer by 1.98 compared to those with stable weight (Eliassen et al. 2006). In this study estimated population attributable risk of postmenopausal breast cancer in women who have not taken postmenopausal hormone therapy was 16.4% for premenopausal weight gain and 7.6% for weight gain after the menopause. Weight gain in the 30s and 40s appears to be a particularly important risk factor for developing breast cancer after the menopause (Han et al. 2006; Harvie et al. 2005). This is the most common period for gain—it is often not appreciated that, on average, there is little gain in weight after the menopause (Health Survey of England: http://www.dh.gov.uk). The effect of reduced energy expenditure on breast cancer risk may be judged from studies relating risk to exercise. One-third or more risk reduction has been reported amongst women undertaking 4 h of exercise or more per week compared to sedentary counterparts. Risk is reduced amongst women with and without a family history and amongst both users and non-users of HRT (Monninkhof et al. 2007).

Studies estimating the interaction of exercise and weight suggest that the effects may be additive. Chang et al. (2006) estimated that women who were obese and undertook less than 4 h of moderate exercise per week were at double the risk of postmenopausal breast cancer compared with women of normal BMI who exercised more than 4 h per week. Weight gain and exercise may modify risk through different mechanisms, and it appears that weight gain is

associated with oestrogen and/or progesterone receptor-positive tumours, whereas exercise appears to be associated with both positive and negative receptor subtypes (Adams et al. 2006).

11.4 Mechanism of Weight Gain and Exercise Deficiency on Risk

It may be appropriate to view human breast cancer risk in the context of the two-stage initiation and promotion carcinogenesis model of Knudson (Moolgavkar 1986). In this report, Moolgavkar suggested that hormones promoted clonal expansion of cells that had been initiated earlier. This is consistent with the preventive effects of oophorectomy and tamoxifen with respect to premenopausal and postmenopausal breast cancer and tamoxifen, raloxifene and aromatase inhibitors with respect to postmenopausal breast cancer (Howell et al. 2007). It seems likely that energy excess may also have promotional effects and that excess energy and hormonal factors may act in concert to promote initiated mammary epithelial cells. Multiple animal models indicate that initiation can occur in the young mammary gland. In humans this may be in utero or during the teenage period of breast growth as judged by data derived from the follow-up of women exposed to radiation from atomic bomb explosions (Land et al. 2003) or mantle irradiation for Hodgkin's lymphoma (Horwich and Swerdlow 2004). Thus, hormonal stimulation and energy excess after the menarche may promote foetal initiation and during the 30s and 40s may promote initiation that had occurred during the teenage period. Weight gain has been linked to post- not premenopausal breast cancer. The development of postmenopausal breast cancer is known to occur in the premenopausal period, since premalignant lesions have been found in the majority of breasts thoroughly examined in the late premenopausal period (Nielsen et al. 1987; Wellings et al. 1975).

Premalignant and malignant lesions are associated with an increase in proliferation and loss of cell polarity (Liu et al. 2005). Several studies show that energy restriction reduces mammary cell proliferation (Klebanov 2007; Varady et al. 2007a; Stragand 1979; Jiang et al. 2003) and is likely to have a favourable effect on cell polarity. In the latter context it has recently been demonstrated that increased adenosine monophosphate related protein kinase (AMPK) is associated with increased cell polarity (Zheng and Cantley 2007; Hurov and Piwnica-Worms 2007). AMPK is an enzyme which senses the energy state of the cell and increases in activity when energy stores are low, when the ADP/ATP ratio is high. These and other recent studies are the first demonstrations of a relationship between epithelial function/morphology and cellular energy status.

11.5 Chronic Energy Restriction Reduces Cancer Risk

There are no prospective randomised trials of chronic energy restriction (CER) for breast cancer prevention (Harvie and Howell 2006). However, observational studies suggest weight loss reduces breast cancer risk (Harvie et al. 2005; Eliassen et al. 2006). In collaboration with the Iowa Women's Health Study, we assessed the effect of maintained weight loss (≥5% of body weight) from age 30 and also after the menopause in women who had gained weight up until these times (Harvie et al. 2005). Weight loss after age 30 resulted in a 38% reduction in postmenopausal breast cancer (RR 0.62; 95% CI, 0.47–0.82) compared with those who continued to gain weight, and after the menopause, weight loss resulted in a 22% reduction (RR 0.77; 95% CI, 0.65–0.94). In the Nurses Health Study, Eliassen et al. (2006), reported that women who had not taken HRT and lost 10 kg or more since the menopause were at lower risk than those who maintained weight (RR 0.43; 95% CI, 0.21–0.86). A small case control study linked weight loss in

BRCA1/2 mutation carriers to reduced risk (Kotsopoulos et al. 2005). Loss of at least 4.5 kg in the period from age 18 to 30 was associated with a decreased risk of breast cancer between age 30 and 49 (RR 0.47; 95% CI, 0.28–0.79).

Multiple studies have demonstrated that CER in rodents started at any time during life reduces breast cancer risk. Dirx et al. (2003) performed a meta-analysis of the reports of CER experiments in studies of spontaneous tumours in mice. The results of 14 studies showed an overall RR of 0.45 (95% CI, 0.39–0.59) indicating a 55% reduction in the incidence of mammary tumours. The results were similar regardless of the degree of CER, the time CER was initiated, whether there was restriction of fat, carbohydrate or protein or the duration of CER (the shortest period was 38 weeks). These experiments support a number of other experiments performed in carcinogen-induced tumours (Thompson et al. 2003) or xenotransplanted human tumour cell lines into nude deprived mice (Giovanella et al. 1982).

11.6 Intermittent Energy Restriction Also Reduces Breast Cancer Risk

Intermittent energy restriction (IER) to prevent breast cancer was tested in rodents after it was shown in the 1930s that this approach could increase rodent life span (Robertson et al. 1934). IER covers a wide range of experimental protocols from every other day (EOD) fasting (Varady and Hellerstein 2007), complete or partial energy restriction at less frequent intervals (Berrigan et al. 2002), or periods of up to 3 weeks of partial restriction and 3 weeks of ad lib feeding (Cleary et al. 2002; Pape-Ansorge et al. 2002). In general, these approaches reduce the risk of spontaneous and genetically engineered mammary tumours but are largely ineffective in carcinogen-induced tumour models.

For example, Carlson and Hoelzel (1945) studied the development of spontaneous mammary tumours in Wister rats. EOD fasting or fasting 1 day in 3 reduced the number of tumours and increased life span in animals who did develop mammary tumours. Another study used MMTV-TGF-α Lep± and MMTV-neu engineered mice and gave 3 weeks with 50% feeding followed by 3 weeks ad libitum feeding. Interestingly, IER mice had a greater tumour reduction than pair-fed CER mice (Cleary et al. 2002; Pape-Ansorge et al. 2002).

IER has been assessed in other diseases: The first suggestion of IER use in humans was reported by Vallejo (1956) who demonstrated that alternating days of ad lib food or a reduction to an estimated 700 calories for 2.5 years in members of a nursing home resulted in a significant reduction in admissions to the infirmary (123 vs 219 $p<0.001$) and a non-significant reduction in deaths (6 vs 13). Hill et al. (1989) randomised moderately obese women to have CER at 1,200 kcal/day, or an alternating diet providing an average of 1,200 kcal/day alternating between 600 to 1,800 kcal/day. The total weight loss for each regimen was about 8 kg over 3 months. However, the IER group experienced greater reductions in total cholesterol (14% vs 6% $p<0.05$). More recently Williams et al. (1998) compared a CER of 1,500–1,800 kcal/day with 5 days of a very low calorie diet (VLCD) of 400–800 kcal/day followed by a similar VLCD for 1 day in each of 15 weeks. The IER diet was associated with significantly improved glycaemic control. In rodents IER was shown to be superior to CER with respect to glucose tolerance (Anson et al. 2003).

11.7 Mechanism of the Effect of CER and IER

During proliferation of normal cells there is an alteration of metabolism so that glycolysis and lipid synthesis are increased and the tricarboxy-

lic acid (TCA) cycle is used to provide substrates for macromolecules (see DeBerardinis et al. 2008 for discussion). Experimentally progressive transformation of normal cells in vitro is associated with an increase in the cell's dependence on glycolysis and a reduced dependence on mitochondrial energy production (Ramanathan et al. 2005; Wu et al. 2006). Increases in glycolysis and lipid synthesis are seen in tumours (Warburg 1930; Medes et al. 1953) and are maintained by alteration in growth factor and signal transduction pathways. CER is associated with a number of changes within target cells. In general, there is a switch from anabolic processes such as cell division to catabolic processes directed towards cell maintenance. The switch results in inhibition of lipid synthesis and enhanced fatty acid oxidation (FAO) and increased mitochondrial activity. It is becoming clear that these changes are controlled by a number of cellular master regulatory molecules which include silent information regulator (SIRT)1 (Boily et al. 2008), AMPK and a co-factor, peroxisome-proliferator γ co-activator (PGC-1) α (Puigserver and Speigelman 2007) and several nuclear transcription factors including peroxisome proliferator-activated receptor (PPAR)-α, -δ and -γ (Fig. 11.1).

Studies of gene expression arrays in various tissues show that a large number of genes change during short-term CER, and they are also altered in the long-term (Dhahbi et al. 2004). These changes may provide clues with respect to the mechanism of the effectiveness of IER. Nearly all short-term fasting studies (24–48 h) have focussed on tissues other than epithelia and the results need confirmation in this tissue. Studies of the effects of short-term fasting on peripheral blood white cells (Bouwens et al. 2007), liver (Bauer et al. 2004), muscle (Spriet et al. 2004; Pilegaard et al. 2003) and fat (Nakai et al. 2008; Varady et al. 2007a, b) show, amongst many gene changes, a relatively consistent pattern of upregulation of carnitine palmitoyl transferase 1 (CPT1), the rate-limiting enzyme of FAO and PPAR-α and downregulation of the enzymes of fat synthesis and desaturation such as fatty acid synthase and stearoyl CoA desaturase 1 (SCD-1). Many studies also show upregulation of pyruvate dehydrogenase kinase 4 (PDK4), an enzyme that inhibits pyruvate dehydrogenase and thus entry of pyruvate into the TCA cycle, indicating an overall change from cell dependence on glycolysis to fat for energy, a phenomenon associated with increased mitochondrial biogenesis (Civitarese et al. 2007). Curiously, genes for enzymes of the glycolytic pathway in breast epithelial cells do not appear to be downregulated by energy restriction (Zhu et al. 2007).

A consistent feature of studies of CER and IER is the associated improvement in insulin sensitivity and the reduction of serum insulin and often, but not consistently, insulin-like growth factor (IGF)-1. Infusion of IGF-1 into animals with tumours controlled by CER showed reversal of the beneficial effects of CER in one study (Dunn et al. 1997) but not in the other (Zhu et al. 2005a, b).

11.8 Energy Restriction Mimetic Agents

Since CER and IER may prove to be difficult to introduce on a population basis to prevent breast and other cancers, there is interest in developing agents which mimic the potential benefits of energy restriction (ERMAs). In mammary epithelial cells, there are metabolic changes which accompany the development of malignancy which are potential targets for ERMAs (Young and Anderson 2008; Clapham and Arch 2007; Dilova et al. 2007). As outlined above, these targets include relative increases in glycolysis, lactate production and fat synthesis and relative decreases in mitochondrial activity and β oxidation of lipids (Ingram et al. 2006; Moreno-Sanchez et al. 2007). The first demonstration that an ERMA may be effective

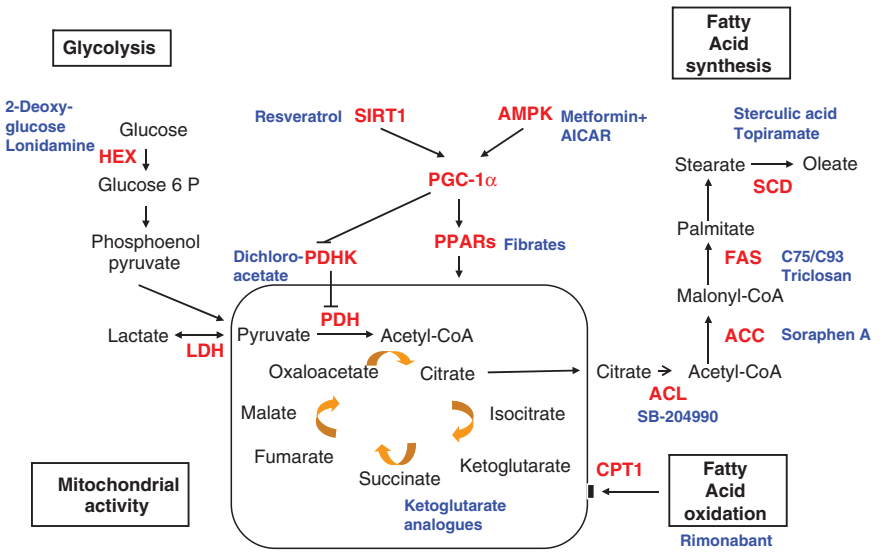

Fig. 11.1 Simplified view of some metabolic pathways which may be affected by CER, IER and ERMAs. In general ERMAs inhibit glycolysis and fatty acid synthesis and stimulate the other pathways shown. Key enzymes and co-factors in *red*. Drugs and processes which may affect the pathways are in *blue*. *HEX*, hexokinase; *LDH*, lactate dehydrogenase; *PDH*, pyruvate dehydrogenase; *PDHK*, pyruvate dehydrogenase kinase; *PGC-1alpha*, peroxisome proliferator receptor γ coactivator-1-α; *SIRT1*, silent information regulator 1; *AMPK*, adenosine monophosphate related kinase; *SCD*, stearoyl CoA reductase; *FAS*, fatty acid synthase; *ACC*, acetyl CoA carboxylase; *ACL*, ATP citrate lyase; *CPT1*, carnitine palmitate transferase 1

was by Lane et al. (1998) who treated rats with 2-deoxyglucose (2DG), which mimicked some of the effects of CER by inhibiting glycolysis. Since that time, 2DG has been shown to inhibit dimethylbenzanthracene (DMBA)-induced carcinomas in rats and the proliferation of tumours produced by the human mammary tumour cell line MCF7 in nude mice (Zhu et al. 2005a, b) and it improves functional and metabolic cardiovascular risk factors in rats (Wan et al. 2003).

In the following sections we examine the mechanism of action and activity of potential ERMAs. Few of these are likely to enter the prevention arena but they are mentioned as agents that indicate "proof-of-principle". We examine inhibitors of glycolysis and lipid synthesis, agents which stimulate activity of mitochondrial function and β oxidation of lipids and which activate the metabolic regulators AMPK, SIRT1 and PGC-1α.

11.9 Inhibitors of Glycolysis

Not only is glycolysis increased in many invasive tumours as first described by Warburg (1930) but there is also evidence of upregulation of enzyme activity in precursor lesions, which makes inhibition of this pathway an attractive approach (Isidoro et al. 2005) for prevention. Whilst there are a large number of molecules which have activity, most of these could not be used for prevention (for review see Chen et al. 2007). 2-Deoxyglucose—which is phosphorylated by hexokinase and cannot be metabolised further or

excreted from the cell, and therefore it, in turn, inhibits hexokinase—inhibits MCF-7 cell growth in vitro and in nude mice and elicits a 'starvation' response intracellularly, resulting in upregulation of AMPK and SIRT1 in MCF-7 cells (Jiang et al. 2008). Lonidamine is also an inhibitor of hexokinase and enhances mitochondrial function by preventing binding of hexokinase to the mitochondrial membrane. Lonidamine has been used to enhance the activity of various chemotherapeutic agents and is in clinical trial for the prevention of benign prostatic hyperplasia (Ditonno et al. 2005).

11.10 Inhibitors of Lipid Synthesis

Increased lipid synthesis in tumours was reported over 50 years ago (Medes et al. 1953). The activity of all four major enzymes of lipid synthesis is increased in tumours, making them targets for prevention and treatment of breast cancer (Swinnen et al. 2006; figure therein). It is likely that increased synthesis is related to the needs of proliferating cells to synthesise membrane lipid and is related to upregulation of lipogenic stimulatory molecules such as sterol regulatory binding protein-1 and SPOT 14 (Kinlaw et al. 2006). Expression of the four major genes for lipid synthesis is downregulated by CER in normal fat tissue in humans (Dahlman et al. 2005).

ATP citrate lyase (ACL) is the first enzyme of lipid synthesis and converts cytosolic citrate (a product of the TCA cycle) to acetyl CoA. The activity of ACL was reported to be 150 times higher in tumours than adjacent normal breast tissue (Szutowicz et al. 1979). RNAi knockdown and use of the ACL inhibitor SB-204990 reduces human tumour cell growth in nude mice (Hatzivassiliou et al. 2005) and decreases cholesterol and triglyceride, concentrations in serum in animal models (Pearce et al. 1998). Newly reported arylbenzenesulphonamide inhibitors of ACL also reduce cholesterol and limit weight gain (Li et al. 2007).

Acetyl-CoA carboxylase (ACC) catalyses the carboxylation of acetyl CoA to malonyl-CoA. There are two isoforms, ACC1 found in liver adipose tissue and the mammary gland and ACC2 in skeletal muscle and heart. *ACC2* knockout mice have a lean phenotype and increased rates of fatty acid and also glucose oxidation (Oh et al. 2005). Specific silencing of *ACC1* by RNAi reduced breast cancer cell survival (MCF7, MDA-MB-231 and HBL 100), but this inhibition was rescued by supplementation of the culture median by palmitate (Chajès et al. 2006). Recently the ACC inhibitor soraphen A was shown to inhibit the proliferation of prostate cancer cells but not cells from benign prostate hyperplasia (Beckers et al. 2007).

Fatty acid synthase (FAS) catalyses the condensation of acetyl-CoA and malonyl-CoA. It is not only expressed in invasive breast tumours but also preneoplastic lesions (Esslimani-Sahla et al. 2006). FAS inhibitors decrease cell proliferation and induce apoptosis in breast cancer cell lines (Pizer et al. 1996) and the FAS inhibitor C75 reduces the growth of MCF-7 xenografts in nude mice (Pizer et al. 2000) and may be particularly active when there is HER2 over-expression (Menendez and Lupu 2007). The antibiotic triclosan is also a FAS inhibitor and reduces nitrosomethylurea (NMU)-induced mammary tumours and preneoplastic lesions in rats (Lu and Archer 2005). Recently Brusselmans et al. (2005) reported that in a series of 18 naturally occurring phenolic compounds reduction of cell proliferation was strongly associated with their FAS inhibitory activity.

SCD-1 is the rate-limiting enzyme in the biosynthesis of monounsaturated fatty acids. It is a key controller in lipid partitioning between lipogenesis and oxidation. High SCD activity is associated with a wide range of disorders including diabetes, obesity and cancer (Dobrzyń and Dobrzyń 2006) *SCD-1* knockout is associated with an increase in FAO, increased AMPK

concentrations and leanness (Dobrzyń et al. 2004). Several inhibitors of SCD—including analogues of conjugated linoleic acid (Choi et al. 2002) and sterculic acid (Khoo et al. 1991)—inhibit the growth of mammary carcinomas in vitro and in vivo. Recently, potent selective orally bioavailable pridazinecarboxamide inhibitors have been reported (Liu G et al. 2007).

11.11 Activation of AMP-Activated Protein Kinase

AMPK is a regulator of the cellular response to low energy. AMPK concentrations increase in response to nutrient deprivation and pathological stresses and is upregulated by 2DG (Jiang et al. 2008), metformin (Zakikhani et al. 2006; Phoenix et al. 2008) and the cell-permeable nucleoside 5-aminoimidazole-4-carboxamide (AICAR) (Swinnen et al. 2005). 2DG and metformin reduce proliferation and growth of human mammary tumour cells in-vitro, tumour formation after carcinogenesis and human tumour cell growth in nude mice. Activation of AMPK results in inhibition of Akt and fat synthesis (by inhibition of acetyl-CoA carboxylase and HMG CoA reductase) and reduction of IGF-1 activity. It is unlikely that AICAR and 2DG can be used for prevention, but metformin treatment for diabetes is associated with reduced breast cancer risk and is being explored as a possible breast cancer preventive agent (Evans et al. 2005).

11.12 Stimulation of Mitochondrial Activity and Fat Oxidation

Tumour cell proliferation is reduced by diversion of pyruvate to the TCA cycle by inhibition of lactic dehydrogenase (LDH) (Fantin et al. 2006) or inhibition of PDK4 (which results in upregulation of pyruvate dehydrogenase) by 2-chloroacetate [in clinical use for the treatment of lactic acidosis (Bonnet et al. 2007)] thus increasing mitochondrial activity and reducing tumour cell proliferation. Several studies indicate that CER increases mitochondrial biogenesis probably related to upregulation of SIRT1 and PGC-1α, which in turn stimulates PPAR-α. PPAR-α agonists (e.g. fenofibrate, WY-14643) have been reported to suppress the growth of tumour cells (Panigrahy et al. 2008; Pozzi et al. 2007). It is of interest that 19% of genes regulated by CER are also regulated by PPAR-α including genes involved in FAO (Corton et al. 2004). FAO is also stimulated by the anti-obesity drug rimonabant, which has also been shown to have anti-tumour activity (Bifulco et al. 2006). Other approaches to mitochondrial stimulation include the use of cell-permeating α-ketoglutarate derivatives (MacKenzie et al. 2007).

11.13 Activation of SIRT1

SIRT1, an NAD+ dependent deacetylase, is known to activate a number of beneficial metabolic pathways including PGC-1α and AMPK and their downstream pathways (Lagouge et al. 2006). In turn, resveratrol and a number of other small molecules are known to activate SIRT1. Their CER mimetic effect upon activation of SIRT1 is demonstrated by improvement in health and survival in mice on a high-calorie diet (Baur et al. 2006) and the treatment of type 2 diabetes (Milne et al. 2007). Numerous studies show that resveratrol has anti-tumour activity. The clinical development of this promising agent has been summarised recently (Howells et al. 2007; Cucciolla et al. 2007).

Summary and Conclusions

The studies outlined above indicate that at least part of the increased incidence of breast cancer is related to energy excess. This conclusion is supported by the reduction of risk of breast cancer in women and in animal models by CER and IER. As yet, in women, the data are observational only, although there is evidence from a randomised trial that IER may be superior to CER (M. Harvie et al., in preparation). We also highlight the well-known phenomenon that carcinogenesis is associated with a change in cell metabolism. Inhibition of the induced anabolic changes or stimulation of the reduced catabolic changes can result in cessation of tumour growth. Some of the cellular metabolic changes seen in tumours are also present in pre-neoplastic lesions, suggesting that some ERMAs could be used for prevention; for example, resveratrol and other activators of SIRT1 and activators of AMPK. It seems likely that at least some of the new PPAR agonists and inhibitors of fat synthesis in development to treat diabetes and cardiovascular disease may ultimately be useful for the treatment and prevention of breast cancer (Harrington et al. 2007).

Although we have focussed on standard CER and IER paradigms, it is of interest that less well known phenomena may regulate energy balance. It appears that certain types of gut bacteria and alterations in biological clock signalling can be associated with leanness possibly acting via PGC-1α (Green et al. 2007; Bäckhed et al. 2007). Potentially, changes in risk related to migration and night shift working might be explained in these ways.

We have focussed on energy balance and risk to the exclusion of diet composition. However, it is important to realise the toxicity of the Western diet. A few weeks of a Western diet causes mammary epithelial proliferation and dysplasia in rodents (Xue et al. 1996) and it seems possible that ingestion of fructose and sucrose may be another mechanism behind increased lipid synthesis particularly via SCD (Miyazaki et al. 2004). Thus, prevention of breast cancer may ultimately be produced by eating a Mediterranean diet with periods of fasting. The question is how often and for what duration? Perhaps the easier alternative is to develop highly specific, non-toxic ERMAs.

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The Use of Tamoxifen and Raloxifene for the Prevention of Breast Cancer

12

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Abstract The NSABP Study of Tamoxifen and Raloxifene (STAR), launched in 1999, compared tamoxifen with raloxifene in a population of healthy postmenopausal women at increased risk for breast cancer to determine the relative effects on the risk of invasive breast cancer. To be eligible for participation, a woman had to be healthy with at least a 5-year predicted breast cancer risk of 1.66% based on the Gail model or a history of lobular carcinoma in situ (LCIS) treated by local excision alone. All participants were at least 35 years of age and postmenopausal. Between July 1999 and November 2004, 19,747 participants were randomized to receive either tamoxifen (20 mg, plus placebo) or raloxifene (60 mg, plus placebo) daily for a 5-year period. The mean age of the participants was 58.5 years; 93% were white and 51.6% had a hysterectomy prior to entering the study. Of the women, 71% had one or more first degree female relatives (mother, sister, daughter) with a history of breast cancer and 9.2% of the women had a personal history of LCIS. A history of atypical hyperplasia of the breast was noted in 22.7% of the participants. The mean predicted 5-year risk of developing breast cancer among the study population was 4.03% (SD, 2.17%) with a lifetime predicted risk of 16%. The mean time of follow-up is 3.9 years (SD, 1.6 years). There was no difference between the effect of tamoxifen and the effect of raloxifene on the incidence of invasive breast cancer; there were 163 cases of invasive breast cancer in the tamoxifen-treated group and 168 cases in those women assigned to raloxifene (incidence 4.30 per 1,000 vs 4.41 per 1,000; RR 1.02; 95% CI, 082–1.28). There were fewer cases of noninvasive breast cancer (LCIS and ductal carcinoma in situ [DCIS]) in the tamoxifen group (57 cases) than in the raloxifene group (80 cases), although the difference is not yet statistically significant (incidence 1.51 vs 2.11 per 1,000; RR, 1.40; 95% CI, 0.98–2.00). There were 36 cases of uterine cancer with tamoxifen and 23 cases with raloxifene (RR, 0.63; 95% CI, 0.35–1.08).

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12.1 Introduction

The history of medicine demonstrates that often the greatest medical advances are made through disease prevention rather than treatment, a truth that has special currency today with regard to breast cancer. The American Cancer Society has estimated that in 2007 there were 178,480 new cases of invasive breast cancer diagnosed in the United States and more than 1.3 million new cases worldwide (Jemal et al. 2007). Despite advances in both breast cancer screening and treatment, an estimated 465,000 women died as a result of breast cancer last year. Breast cancer is the most common cancer found in women in the United States and the second leading cause of cancer death in women. Finding a breast cancer prevention agent that is effective and acceptable, therefore, is a worthy goal.

In 1998 the National Surgical Adjuvant Breast and Bowel Project (NSABP) Breast Cancer Prevention Trial (P-1) demonstrated that the selective estrogen receptor modulator (SERM) tamoxifen could reduce the incidence of breast cancer by up to 50% in a population of otherwise healthy women at increased risk for the future development of the disease (Fisher et al. 1998; Fisher et al. 2005). However, tamoxifen has several well-documented toxicities, including uterine malignancy, thromboembolic disease, and cataracts. These risks and the perception that tamoxifen was an oncology drug have limited its use for breast cancer prevention.

Raloxifene hydrochloride is also a SERM. In 1998 the United States FDA approved it for the treatment and prevention of osteoporosis; one of the pivotal studies leading to that approval was the Multiple Outcomes of Raloxifene Evaluation (MORE) study, which included 7,705 postmenopausal women (Cummings et al. 1999). The primary endpoint of the MORE study was bone fracture, but breast cancer was a secondary endpoint, and MORE showed that 4 years of raloxifene treatment appeared to reduce the risk of receptor-positive breast cancer by 72%. Like tamoxifen, raloxifene did increase the risk of thromboembolic events, but there was no apparent increase in endometrial cancer. A direct comparison of raloxifene and tamoxifen in a group of women at increased risk for breast cancer was a logical next step.

12.2 The Study of Tamoxifen and Raloxifene

The NSABP's Study of Tamoxifen and Raloxifene (STAR) was a double-blinded, randomized clinical trial (Fig. 12.1) that began with 19,747 postmenopausal women who were at least 35 years of age and had a history of lobular carcinoma in situ (LCIS) treated by local excision alone or a modified Gail score demonstrating a 5-year risk for invasive breast cancer of at least 1.66% (Vogel et al. 2006; Land et al. 2006). Women in the study were assigned to receive either tamoxifen, 20 mg per day plus a placebo, or raloxifene, 60 mg per day plus a placebo, for a 5-year duration. The primary endpoint of the study was the development of invasive breast cancer. Secondary endpoints included noninvasive breast cancer, uterine malignancy, deep

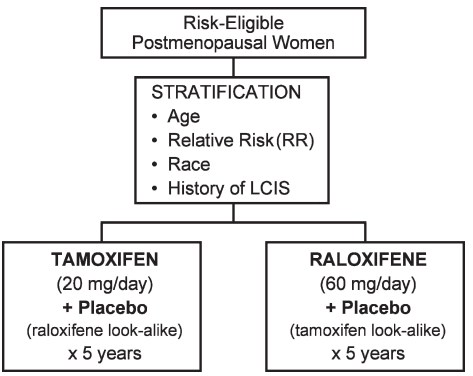

Fig. 12.1 Schema for the NSABP's STAR trial

vein thrombosis, pulmonary embolus, transient ischemic attack, cerebral vascular accident, cardiac disease, fractures, cataracts, quality of life, and death. To be eligible, candidates must not have taken tamoxifen, raloxifene, hormone therapy, oral contraceptives, or androgens for at least the previous 3 months. They could not be taking either warfarin or cholestyramine. To minimize the risk of stroke or thromboembolic events, women were not eligible if they had a history of stroke, transient ischemic attack (TIA), pulmonary embolus (PE), deep vein thrombosis (DVT), uncontrolled diabetes, uncontrolled hypertension, or uncontrolled atrial fibrillation.

A detailed description of the participant population has been published (Vogel et al. 2006). At the time of randomization, the mean age of this postmenopausal population of women was 58.5 years. The mean predicted 5-year risk of developing breast cancer in the study population was 4.03%, and their projected lifetime risk to 80 years of age was 16%. Over 70% of the women entering the trial had one or more first degree female relatives with a history of breast cancer. More than 9% reported a personal history of LCIS treated by local excision prior to enrollment in the study, and 22.7% had a breast biopsy prior to enrollment that demonstrated either atypical ductal or atypical lobular hyperplasia. More than half the participants reported having undergone a hysterectomy before randomization.

12.3 Results

12.3.1 Invasive Breast Cancer

With a mean follow-up time of 3.9 years, 163 of the women assigned to tamoxifen and 168 of those assigned to raloxifene had developed invasive breast cancer, demonstrating that there was no difference between the effect of tamoxifen and the effect of raloxifene on the incidence of invasive breast cancer. The rate per 1,000 was 4.30 in the tamoxifen group and 4.41 in the raloxifene group [risk ratio (RR), 1.02; 95% confidence interval (CI), 0.82–1.28]. There was no placebo-alone group in this trial. However, using the Gail model scores of the women who entered the trial, we can estimate the number of invasive breast cancers that would have occurred in an untreated group (Fig. 12.2) and demonstrate that there was about a 47% reduction in incidence from treatment in the trial (Costantino et al. 1999). The cumulative incidence of invasive breast cancer through 72 months for the two treatment groups was 25.1 for the tamoxifen group and 24.8 for the raloxifene group (p=0.83). When the treatment groups were compared by baseline characteristics of age, history of LCIS, or atypical hyperplasia, Gail score, and number of first degree female relatives with breast cancer, the pattern of no differential effect by treatment assignment remained consistent, and none of the RRs in these subsets were statistically significant. The characteristics of the invasive breast tumors, which were obtained from submitted pathology and laboratory reports, showed no significant differences between the treatment groups with regard to distribution by

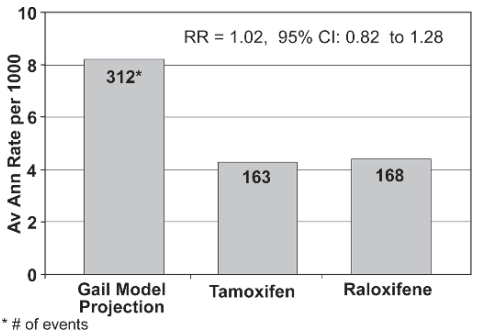

Fig. 12.2 Average annual rate and number of invasive breast cancers, including cancers that would have occurred in an untreated group in the STAR trial

tumor size, nodal status, or estrogen receptor level. A central pathology review of the tumors has not been performed.

12.3.2 Noninvasive Breast Cancer

Raloxifene did not appear to be as effective as tamoxifen in reducing the incidence of noninvasive breast cancer (LCIS or ductal carcinoma in situ [DCIS]), although the difference did not reach statistical significance. There were 57 cases of noninvasive breast cancer among the women assigned to tamoxifen and 80 among women who took raloxifene [1.51 per 1,000 women assigned to tamoxifen and 2.11 per 1,000 women assigned to raloxifene (RR, 1.40; 95% CI, 0.98–2.00) (Fig. 12.3)]. The cumulative incidence through 6 years was 8.1 per 1,000 in the tamoxifen group and 11.6 per 1,000 in the raloxifene group ($p = .052$).

12.3.3 Other Secondary Endpoints

More uterine malignancies occurred in the tamoxifen-treated women than in those treated with raloxifene, but the difference was not statistically significant. There were 36 cases in the tamoxifen group and 23 cases in the raloxifene group, with an annual incidence rate of 1.99 per 1,000 for tamoxifen and 1.25 per 1,000 for raloxifene (RR, 0.63; 95% CI, 0.35–1.08). Uterine hyperplasia (with and without atypia) was less common in the raloxifene-treated group (14 cases raloxifene; 84 cases tamoxifen [RR, 0.16; 95% CI, 0.09–0.29]). There were significantly fewer hysterectomies performed due to nonmalignant indications in the raloxifene group (221 tamoxifen; 87 raloxifene [RR, 0.39; 95% CI, 0.30–0.50]). There were no statistically significant differences between the treatment groups in regard to other malignancies.

No statistically significant differences were noted between the two treatment groups relative to the incidence of ischemic heart disease, TIA, stroke, or fractures (osteoporotic fractures or total fracture). Significantly fewer thromboembolic events (DVT or PE) occurred in the raloxifene group, 141 in the tamoxifen group, sand 100 in the raloxifene group, demonstrating a 30% reduction in favor of the raloxifene-treated women (RR, 0.70; 95% CI, 0.54–0.91). Fewer women on raloxifene developed cataracts during treatment, and fewer underwent surgical removal of their cataracts. After 6 years, the cumulative incidence of cataracts occurring during treatment was 77.9 per 1,000 in the tamoxifen group and 56.3 per 1,000 in the raloxifene group ($p = .002$); 260 in the tamoxifen group and 215 in the raloxifene group underwent cataract surgery (RR, 0.82; 95% CI, 0.68–0.99).

Mortality in the two groups was similar, with 101 deaths in those assigned to tamoxifen and 96 in those assigned to raloxifene, resulting in a rate of 2.64 per 1,000 and 2.49 per 1,000, respectively (RR, 0.94; 95% CI, 0.71–1.26). The distribution by cause of death did not differ by treatment.

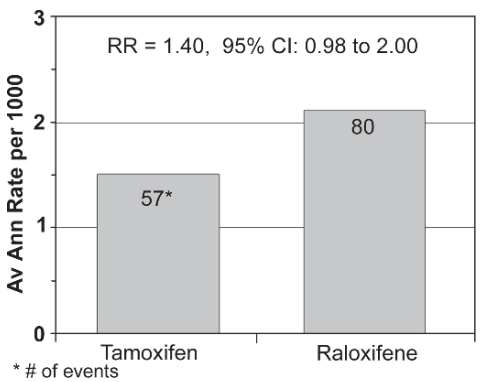

Fig. 12.3 Average annual rate and number of noninvasive (in situ) cancers in the STAR trial

12.4 Discussion

The results of the STAR trial demonstrate that raloxifene is an effective alternative to tamoxifen for reducing the risk of invasive breast cancer in healthy postmenopausal women at increased risk for the disease. Raloxifene is also an attractive choice for these women because it has fewer serious side effects. Although the difference in endometrial cancer has not yet reached statistical significance, the tamoxifen-treated women did have a significant increase in endometrial hyperplasia, a known risk factor for endometrial cancer. There were also more than twice as many hysterectomies for benign disease in the tamoxifen group. Participants in both groups continue to be followed.

Raloxifene does not appear to be as effective as tamoxifen in preventing the development of noninvasive breast cancer, DCIS, or LCIS. However, there is no suggestion that raloxifene is increasing the risk of noninvasive disease compared to tamoxifen; the difference in the average annual rate of noninvasive disease is only 0.6 per 1,000. In the NSABP P-1 prevention trial that compared tamoxifen to placebo, tamoxifen reduced noninvasive disease by 50%. That trial included both pre- and postmenopausal women, but the reduction in noninvasive disease was apparent regardless of menopausal status. The mechanism to explain the differing effect between these two SERMs is not clear. It is interesting to note that women who entered STAR with a previous breast biopsy that demonstrated either atypical hyperplasia or LCIS benefited equally from tamoxifen and raloxifene in the reduction of risk of invasive breast cancer (Fig. 12.4). This suggests that raloxifene may actually be as effective as tamoxifen in blocking the progression of premalignant or noninvasive disease to invasive breast cancer.

Whether the data on noninvasive disease prove to be a barrier to the use of raloxifene remains to be seen. The postmenopausal woman who decides to take a SERM to reduce her risk of breast cancer could select tamoxifen and avoid this concern about noninvasive disease. Unfortunately, at the present time, there are no risk profiles or other methods to identify who is at greater or lesser risk of developing noninvasive disease. In the STAR study, the incidence of invasive breast cancer, despite the reduction achieved with either SERM, was more than twice the rate of noninvasive disease in the

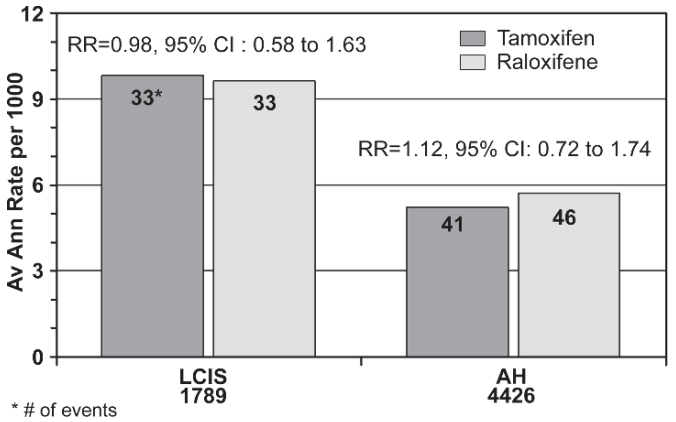

Fig. 12.4 Average annual rate and number of invasive breast cancers by atypical hyperplasia (AH) and (LCIS) and lobular carcinoma in situ (LCIS)

raloxifene group. The benefits of fewer endometrial cancers, fewer deep vein thromboses and pulmonary emboli, and fewer cataracts in the raloxifene-treated women may balance if not outweigh the noninvasive disease benefit currently known to be achievable with tamoxifen.

Follow-up recommendations for either raloxifene or tamoxifen are the same. Almost all of the noninvasive breast cancers in the STAR trial were identified as microcalcifications seen on annual mammograms. As a result, the tumors were small and most women had the option of breast-conserving procedures.

In summary, women at risk for breast cancer have the option of taking tamoxifen or raloxifene. However, tamoxifen, approved for this purpose in 1998, has been underutilized. Tamoxifen was well known to oncologists who had used it extensively to treat breast cancer patients with receptor-positive disease, but it was relatively unknown to primary care physicians who are the key providers of preventive healthcare. Tamoxifen was viewed as a "cancer drug," and media reports highlighting its toxicities proved to be a barrier to its use.

Raloxifene, on the other hand, has been utilized for more than a decade for the treatment and prevention of osteoporosis. Over 500,000 women in the United States are currently taking this drug for its benefits in bone, and on average, these women are older and have a lower breast cancer risk than do the women in the STAR trial. Most raloxifene prescriptions have been written by primary care providers. Thus, because these physicians are already familiar with this drug, barriers relative to its use for breast cancer chemoprevention may be lessened.

The ideal chemopreventive agent may still lie somewhere in the future, and significant work remains to be done before we arrive at that point. Tamoxifen and raloxifene reduce the risk of invasive breast cancer by 50%, an impressive benefit but one that leaves substantial room for improvement. The cancers that are prevented by SERMs are estrogen receptor positive. While these estrogen receptor-positive tumors make up the majority of breast cancers that occur, estrogen receptor-negative cancer is not rare. Although tamoxifen is approved for premenopausal women, raloxifene is not. Efforts already underway in the laboratory and the clinic should help us address these gaps. However, at this point in time, raloxifene may offer the best chance for breast cancer prevention for many women.

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Prevention of ER-Negative Breast Cancer

13

Yuxin Li and Powel H. Brown

Abstract The successful demonstration that the selective estrogen receptor modulators (SERMs) tamoxifen and raloxifene reduce the risk of breast cancer has stimulated great interest in using drugs to prevent breast cancer in high-risk women. In addition, recent results from breast cancer treatment trials suggest that aromatase inhibitors may be even more effective at preventing breast cancer than are SERMs. However, while SERMs and aromatase inhibitors do prevent the development of many estrogen-receptor (ER)-positive breast cancers, these drugs do not prevent the development of ER-negative breast cancer. Thus, there is an urgent need to identify agents that can prevent ER-negative breast cancer. We have studied the cancer preventative activity of several classes of drugs for their ability to prevent ER-negative breast cancer in preclinical models. Results from these studies demonstrate that rexinoids (analogs of retinoids that bind and activate RXR receptors), tyrosine kinase inhibitors (such as EGFR inhibitors and dual kinase inhibitors that block EGFR and HER2/neu signaling), and cyclo-oxygenase 2 (COX-2) inhibitors all prevent ER-negative breast cancer in transgenic mice that develop ER-negative breast cancer. Other promising agents now under investigation include vitamin D and vitamin D analogs, drugs that activate PPAR-gamma nuclear receptors, and statins. Many of these agents are now being tested in early phase cancer prevention clinical trials to determine whether they will show activity in breast tissue and whether they are safe for use in high-risk women without breast cancer. The current status of these studies will be reviewed. It is anticipated that in the future, drugs that effectively prevent ER-negative breast cancer will be used in combination with hormonal agents such SERMs or aromatase inhibitors to prevent all forms of breast cancer.

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Despite aggressive screening to detect early breast cancer and significant advances in treatment, breast cancer is still the most common cancer in women excluding skin cancer, and it remains the second leading cause of cancer death in women, exceeded only by lung cancer [1]. Recently, the incidence of breast cancer in the United States has declined. However, the decreased incidence was observed only in women aged 50 years or older and was more evident in estrogen receptor (ER)-positive than in ER-negative cancers. The incidence of ER-negative breast cancer, which has a poor prognosis and often occurs in premenopausal women, has not shown significant change. Therefore, there is an urgent need to prevent ER-negative breast cancer.

Primary prevention approaches of breast cancer can be categorized into prophylactic surgery,

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lifestyle changes, and chemoprevention. Prophylactic surgeries, which consist of bilateral oophorectomy and bilateral mastectomy, are highly invasive approaches that only apply to women with an extremely high risk of breast cancer, such as hereditary breast cancer. The invasive nature has limited their extensive clinical usage. Although lifestyle changes are considered as safe and natural processes, recent meta-analyses of clinical data failed to demonstrate consistent, strong, and statistically significant association between lifestyle changes and breast cancer incidence, except for regular alcohol consumption and weight gain [2, 3]. These interventions could reduce a women's risk of breast cancer by 5%–10%. Given the limitation of prophylactic surgeries and the modest effect of lifestyle changes, recent breast cancer prevention studies have focused on preventative therapy, which has been shown to be effective in reducing the risk of breast cancer in randomized clinical trials.

13.1 SERMs and Aromatase Inhibitors

Chemoprevention was first defined by Michael Sporn as "prevention of cancer by the use of pharmacological agents (natural or synthetic) to inhibit or reverse the process of carcinogenesis" [4]. A critical issue in the development of chemopreventative agents is to understand the carcinogenesis process and identify targets that are essential for carcinogenesis. The study of estrogen signaling and the identification of ER ultimately led to the design of drugs targeting ER. Selective estrogen receptor modulators (SERMs), which exert selective agonist or antagonist effects on ER depending on different target tissues, represent the major group of compounds that block the ER signaling. Tamoxifen is a SERM that has estrogen antagonist effect to breast, but remains as an estrogen agonist in bone and the uterus. Tamoxifen was found to reduce the incidence of contralateral breast cancer by nearly 50% as a secondary endpoint in several adjuvant studies [5]. These observations suggested that giving tamoxifen to healthy high-risk women would produce equivalent results, and ultimately led to a series of cancer prevention trials using tamoxifen [6–9].

Cuzick et al. performed a metaanalysis of the four tamoxifen prevention trials [10]. The overall reduction in breast cancer incidence caused by tamoxifen was 38% (95% CI, 28–46, $p<0.001$). More importantly, tamoxifen reduced the risk of ER-positive breast cancer by 48%, but had no effect in reducing the risk of ER-negative breast cancer. Recent updated data based on extended follow-up shows similar results (Table 13.1) [6–9]. Based on the results of the National Surgical Adjuvant Breast and Bowel Project (NSABP) P1 trial, the United States Food and Drug Administration (FDA) approved tamoxifen for cancer risk reduction in women at high risk of breast cancer. However, tamoxifen caused increased risk of a variety of side effects including increased risk of endometrial cancer, venous thromboembolic events, hot flushes, and vaginal discharge. Concerns about these side effects have limited the use of tamoxifen for breast cancer prevention.

Raloxifene (Evista) is a second-generation SERM that has estrogen antagonist effects on the breast and uterus, but has estrogen agonist effects in bone and on lipid metabolism. It also has been shown to increase the risk of thromboembolic events. To directly compare the effectiveness and toxicity profile of raloxifene and tamoxifen, the NSABP launched the Study of Tamoxifen and Raloxifene (STAR) trial in postmenopausal women at high risk of breast cancer [11]. The results of the STAR trial demonstrated that raloxifene and tamoxifen were equivalent in preventing breast cancer, but that raloxifene had less toxicity. Because of these results, the FDA approved raloxifene to prevent breast cancer in postmenopausal women who are at high risk of breast cancer or who have osteoporosis. Raloxifene now joins tamoxifen as the second

Table 13.1 Breast cancer prevention trials using tamoxifen

Trials	Population	Number randomized	Therapy	Median follow-up	IBC incidence	
					ER+	ER–
Royal Marsden [6]	Age 30–70 with family history of breast cancer	2,471	Tamoxifen 20 mg vs Placebo ×5–8 years	13 years	53 vs 86	24 vs 17
NSABP-P1 [7]	Pre- or postmenopausal women age ≥ 35 with a >1.66% 5-year risk or with LCIS	13,388	Tamoxifen 20 mg vs Placebo ×5 years	7 years	70 vs 182	42 vs 56
Italian [8]	Women aged 35–70 who had a total hysterectomy	5,408	Tamoxifen 20 mg vs Placebo ×5 years	135 months	40 vs 52[a]	19 vs 21[a]
ISBS–1 [9]	Women aged 35–70 with increased risk of breast cancer	7,154	Tamoxifen 20 mg vs Placebo ×5 years	96 months	87 vs 132	35 vs 35
Total		28,421			250 vs 452	120 vs 129

[a]Includes ductal carcinoma in situ (DCIS)

chemoprevention drug to be approved for breast cancer risk reduction. However, neither of these agents prevents the development of ER-negative breast cancer.

Aromatase inhibitors (AIs) offer an alternative approach to antagonize the estrogen signaling pathway by inhibiting the activity of aromatase, a rate-limiting enzyme catalyzing the last step in estrogen synthesis. Three third-generation AIs, anastrozole, letrozole, and exemestane, have shown superiority to tamoxifen in treating metastatic breast cancer, early stage breast cancer in the adjuvant setting, and in preventing the development of contralateral breast cancer in adjuvant studies [12]. These AIs are currently being tested in clinical trials in women with ductal carcinoma in situ (DCIS) breast cancer or in high-risk women without breast cancer to determine whether they can prevent the development of invasive breast cancer. However, in spite of the promising preventative effect of AIs, these agents are not expected to reduce the risk of ER-negative breast cancer. Thus, prevention of ER-negative breast cancer will rely on the development of novel chemopreventative agents that target nonestrogen signaling pathways.

13.2 Novel Agents for the Prevention of ER-Negative Breast Cancer

Mammary tumorigenesis is a complex process that involves aberrant regulation of multiple signaling pathways. To effectively prevent ER-negative breast cancer, identification of critical estrogen-independent signaling pathways will be necessary. Recently, molecular biology studies have revealed many signaling pathways that are involved in ER-negative mammary

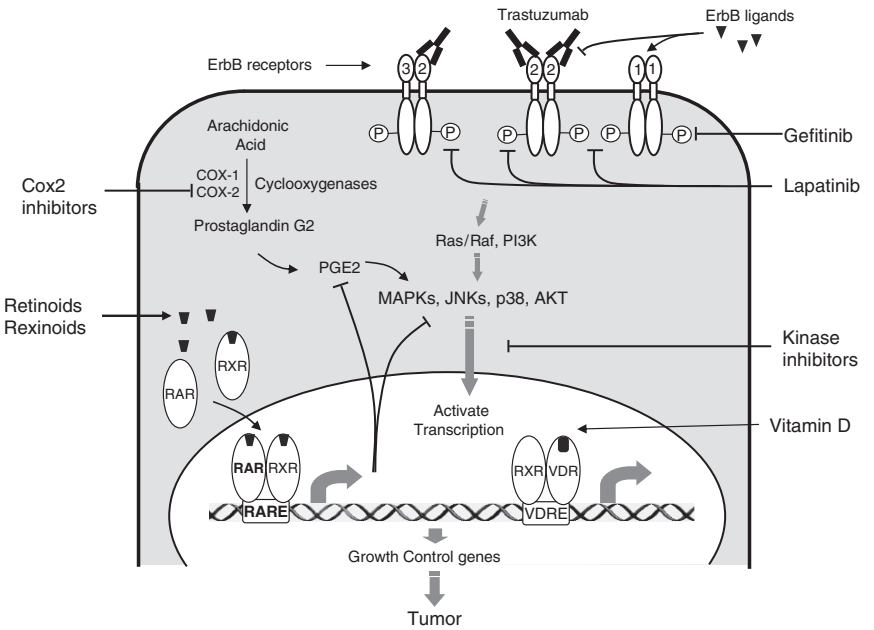

Fig. 13.1 Novel target for the prevention of ER-negative breast cancer. Novel agents targeting nonendocrine pathways include retinoids, COX-2 inhibitors, EFGR/tyrosine kinase inhibitors, transcription factor inhibitors, and others

tumorigenesis. Targeting these pathways using pharmacological inhibitors represents a promising strategy for ER-negative breast cancer prevention. Figure 13.1 illustrates some of the estrogen-independent signaling pathways that are critical for breast cell growth. Novel agents targeting these nonendocrine pathways have shown cancer preventative effects in animal models. Representative agents include tyrosine kinase inhibitors against erbB receptors, COX-2 inhibitors, and ligands of nuclear receptor families such as retinoids and vitamin D-related compounds.

13.3 Retinoids

Retinoids are natural and synthetic derivatives of vitamin A (retinol) that have profound effects on development, metabolism, differentiation, and cell growth. Retinoids exert their activity primarily through binding to two types of nuclear receptors, retinoic acid receptors (RARα, -β, and -γ) and retinoid X receptors (RXRα, -β, and -γ). The ligand-bound receptors then form dimeric complexes which bind DNA at specific retinoid responsive elements and regulate the transcription of genes controlling cellular proliferation, differentiation, and apoptosis (Fig. 13.2). Accumulating epidemiological investigations, experimental studies using animal models, and clinical trials have provided strong evidence for the use of retinoids in cancer prevention.

The cancer preventative activity of retinoids was first demonstrated by Waun Ki Hong in 1990, who showed that daily usage of isotretinoin (13-*cis*-retinoic acid) for 12 months prevented second primary tumors in patients with squamous-cell carcinoma of the head and neck [13]. Thereafter, naturally occurring retinoids 9cRA and retinyl acetate, and the synthetic

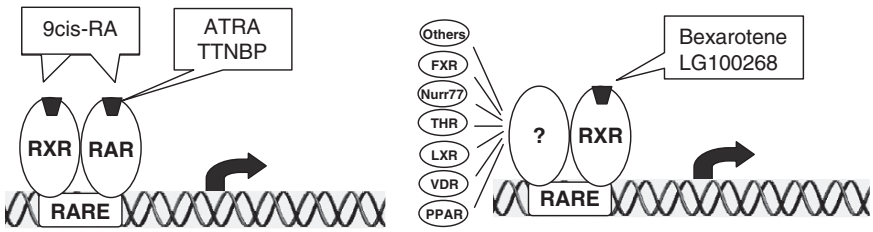

Fig. 13.2 Retinoids prevent cancer through different retinoid receptor pathways. Receptor-selective retinoids bind to either RAR or RXR. The ligand-bound receptors then form dimeric complexes that bind DNA at specific responsive elements and regulate the transcription of genes controlling cellular proliferation, differentiation, and apoptosis. RAR forms a dimer with RXR, while RXR is able to dimerize with many different partners

retinoid fenretinide (*N*-4-hydroxyphenyl, 4HPR), have been reported to prevent breast cancer development in mice and rats exposed to chemical carcinogens dimethylbenz(a)anthracene (DMBA) and methylnitrosourea (MNU) [14, 15]. Fenretinide is one of the most extensively studied retinoids in cancer prevention due to its favorable toxicological profile in humans. A multicenter phase III chemoprevention trial using fenretinide to reduce the incidence of secondary breast cancer was conducted in Italy [16]. A total of 2,972 women with stage I breast cancer were randomized to receive 200 mg/day of fenretinide or no drug for 5 years. After a median follow-up of 97 months, fenretinide showed no effect on contralateral breast cancer occurrence and a nonsignificant 17% reduction in ipsilateral breast tumor reappearance. However, when menopausal status was considered, fenretinide significantly reduced the occurrences of both contralateral and ipsilateral breast cancer incidence in premenopausal women (HR=0.66, 95% CI=0.41–1.07; and HR=0.65.95% CI=0.46–0.92, respectively). In postmenopausal women, an opposite trend was observed in which fenretinide slightly increased the incidence of contralateral and ipsilateral breast cancer. Recently, an updated analysis after 14.6 years of follow-up of 1,739 women demonstrated similar results [17], showing the continuous protective effect of fenretinide in the premenopausal women even 10 years after cessation. Thus, these results suggest a beneficial effect of fenretinide only in premenopausal women. More importantly, fenretinide was observed to reduce second tumors in premenopausal women irrespective of the hormone receptor status of the primary cancer, suggesting that retinoids have a chemopreventative effect on ER-negative as well as ER-positive breast cancer.

Recently, RXR-selective retinoids, commonly referred as rexinoids, have been studied as cancer preventative agents. Rexinoids bind primarily to RXR, a multifunctional nuclear receptor that can form heterodimers with many different nuclear receptors including RAR, vitamin D receptor (VDR), peroxisome proliferator-activated receptor (PPAR), liver X receptor (LXR), and Nurr77. Preclinical studies have demonstrated that rexinoids maintain the chemopreventative effect of naturally occurring retinoids, but have greatly reduced toxicity. Wu et al. found that 9cRA, a retinoid that binds both RAR and RXR, significantly delayed the ER-negative tumor development in SV40 Tag mice and *N*-methyl-*N*-nitrosourea (MNU)-treated rats [15, 18]. However, 9cRA induced significant cutaneous toxicity including hair loss and skin erythema [18]. In contrast, the RXR-selective rexinoid bexarotene (LGD1069) demonstrated a similar cancer preventative effect in SV40 Tag mice with no observed tox-

icity [19]. The RAR-selective retinoid TTNPB was found to be highly toxic and minimally efficacious in suppressing mammary tumorigenesis in the same animal model [19]. Thus, these results suggested that the toxicity of retinoids is primarily mediated by the RAR signaling pathway, while the anticancer effect of retinoids is mediated by RXR-dependent pathways. Due to their favorable toxicity profile, rexinoids are particular attractive cancer preventative agents. In addition to its effect in SV40 Tag mice, bexarotene has also been shown to delay mammary tumor development in Mouse mammary tumor virus (MMTV)-ErbB2 transgenic mice and *P53*-null mice, two animal models that develop ER-negative breast cancers [20]. In the MMTV-ErbB2 transgenic mice, which exclusively form ER-negative mammary tumors, median time to tumor development was reduced from 230 days in the vehicle group vs 416 days in the bexarotene-treated mice. At the time when all the vehicle-treated mice have developed tumors, only 24% of the high-dose bexarotene-treated mice had tumors. Cutaneous toxicity was mild and only observed in the high-dose group after many months of treatment (an average of 205 days). These promising findings led to the development of a phase II clinical trial at Baylor College of Medicine to test the preventative efficacy of bexarotene in women at high risk of breast cancer. The results of this study were recently presented at the San Antonio Breast Cancer Symposium [21]. Bexarotene was found to reduce cyclin D1 RNA expression in breast cells from postmenopausal women (but not premenopausal women). A similar, but nonsignificant reduction in breast cell proliferation was seen in these post-menopausal women. These results demonstrate that bexarotene has a biological effect on breast cells in women at high risk of breast cancer.

Although bexarotene has promise as a drug to prevent breast cancer development, previous clinical trials using bexarotene to treat cutaneous T-cell lymphoma demonstrated that it has some adverse effects including hyperlipidemia, cutaneous toxicity, and rare mild hypothyroidism [22]. The toxicity of bexarotene may be attributed to its weak RAR-binding activity. Recently, a more RXR-specific rexinoid, LG100268, has been developed. This rexinoid has no detectable RAR-binding activity, and thus it is likely to be less toxic than bexarotene. Recently, we found that LG100268 is more effective than bexarotene in preventing the ER-negative mammary tumor development in MMTV-ErbB2 mice [23]. High-dose LG100268 treatment has almost totally prevented tumor development in MMTV-ErbB2 mice. Most importantly, no skin toxicity was observed in LG100268-treated mice. We also found that LG100268 significantly prevents the developments of premalignant lesions including hyperplasia and DCIS, suggesting that rexinoids might prevent both the initiation and progression of mammary tumorigenesis [23].

Although significant progress has been made toward understanding the RAR/RXR-mediated signaling pathway, the mechanism by which retinoids suppress carcinogenesis is still poorly understood. It is well accepted that retinoids exert their anticancer effects through altering the expression of genes regulating cell proliferation and apoptosis. These alterations are achieved through activation or repression of key signaling pathways including RAR/RXR, AP-1, mitogen-activated protein (MAP) kinases, and PI3/Akt pathways. Our preclinical data indicated that bexarotene and LG100268 prevent mammary tumorigenesis primarily through an antiproliferation effect. Rexinoids can either upregulate growth-inhibiting proteins such as RARβ, IGFBP-3, TGFβ, and DEC2, or downregulate growth-promoting proteins such as cyclin D1 and COX-2 [24]. All these changes lead to cell cycle blockade and/or induction of apoptosis. Considering the promiscuous nature of RXR protein, which can bind to a variety of nuclear receptors, the chemopreventative activity of rexinoids is due to regulation of a complex of multiple signaling pathways, rather than a single, specific mechanism.

13.4 Vitamin D Receptor

VDR is a nuclear receptor that modulates gene expression when activated by its ligand 1,25-dihydroxyvitamin D3 (1,25$(OH)_2$D3), a biologically active form of vitamin D. Activated VDR then forms a dimer with RXR, binds to VDR response element, and regulates the transcription of target genes (Fig. 13.2). Therefore, downstream signaling pathways of VDR may share some of the same pathways activated by rexinoids. 1,25$(OH)_2$D3 has been shown to inhibit cell proliferation and promote apoptosis in breast cancers independent of ER status. Thus, vitamin D compounds that target VDRs are potential chemopreventative agents for ER-negative breast cancer. Consistent with these results, results from epidemiological studies suggest an inverse association exits between sunlight exposure (a major source of endogenous vitamin D production) and breast cancer incidence. Recent prospective cohort studies have analyzed the effect of vitamin D intake on breast cancer incidence regarding menopausal status. The four studies of vitamin D intake in postmenopausal women did not find significant correlation between vitamin D intake and breast cancer risk [25]. In contrast, the two prospective studies of premenopausal women demonstrated that vitamin D intake was associated with significant breast cancer risk reduction (35% reduction in the Women's Health Study [26], and 34% reduction in women in the Nurse Health Study [27]).

In preclinical studies, 1,25(OH)D3 was shown to inhibit the growth of breast cancer cells in an ER-independent manner and reduce the risk of mammary tumors in animal models [28, 29]. However, the use of 1,25(OH)D3 for cancer prevention is hindered because of its hypercalcemic toxicity. To overcome this problem, several less calcemic vitamin D analogs have been synthesized and evaluated for their chemopreventative effects. Among them, 1alpha-hydroxy-24-ethylcholecalciferol (1alpha(OH)D5) and 22-oxa-1,25-$(OH)_2$D3 (OCT) were found to inhibit the proliferation of both ER-positive and ER-negative breast cancer cells [30, 31]. Intratumor administration of OCT remarkably delayed the growth of human derived ER-negative breast cancer cell line (MX-1) implanted in athymic mice [29]. These results suggest that these analogs are promising agents to prevent the development of ER-negative breast cancers.

13.5 EGFR/Tyrosine Kinase Inhibitors

Estrogen, retinoids, and vitamin D regulate cell growth and differentiation through activation of nuclear receptors. Peptide growth factor receptors represent a different group of signaling molecules that are critical for the growth and differentiation of both normal and malignant tissues. Among these peptide growth factor receptors, the erbB family of type I tyrosine kinase receptors has been implicated in the development of breast cancer. ErbB receptors include epidermal growth factor receptor (EGFR; also termed HER-1 or ErbB1), ErbB2 (also termed HER-2 or neu), ErbB3 (HER-3), and ErbB4 (HER-4) (see Fig. 13.1). All four members have an extracellular ligand-binding domain, a single transmembrane domain, and an intracellular domain with tyrosine kinase activity. Ligand binding to the extracellular domain of ErbB receptors induces the auto- or heterodimerization of the ErbB family members and activates the intrinsic tyrosine kinase activity, resulting in phosphorylation of the specific tyrosine residue within the intracellular domain. Phosphorylated tyrosine residues then recruit effector proteins and activate downstream signal transduction cascades such as MAP kinase pathway, PI3K–AKT pathway, signal transducer and activator of transcription (STAT) pathway, and mammalian target of rapamycin (mTOR). Activation of these

effectors leads to cell proliferation and increased survival ability, which promote breast cancer development independent of ER status. Thus, agents that block the erbB signaling pathways are promising agents to treat and prevent breast cancer.

Two strategies have been used to inhibit ErbB activity: the first involves blockade with monoclonal antibodies and the second the use of small molecule kinase inhibitors to inhibit ErbB activity. Monoclonal antibodies directly block the peptide binding at the extracellular domain. The monoclonal antibodies strategy has been particularly effective. Trastuzumab, a monoclonal antibody against HER-2 receptor, is highly effective in treating HER2-positive breast cancers. However, monoclonal antibody treatment may be difficult in women without breast cancer. Therefore, most chemopreventative studies have been conducted with small molecule tyrosine kinase inhibitors (TKI) due to their favorable oral bioavailability, potentially less toxicity, ability to inhibit truncated forms of EGFR and HER2 receptors (EGFR VIII and p95), and their ability to target multiple ErbB receptors.

Lenferink et al. found that blockade of the EGFR with tyrosine kinase inhibitor AG-1487 significantly delayed breast tumorigenesis in MMTV/neu+MMTV/TGF-alpha bigenic mice [32]. The delay was associated with inhibition of EGFR and neu signaling, reduction of cyclin-dependent kinase 2 (Cdk2) and MAPK activities, downregulation of cyclin D1, and an increase in the levels of the cell cycle inhibitor p27. Recently, our laboratory has demonstrated that gefitinib (ZD1839 or Iressa), an EGFR tyrosine kinase inhibitor, suppressed ER-negative mammary tumor formation in MMTV-ErbB2 transgenic mice [33]. Median time to tumor development was significantly delayed from 140 days of vehicle treatment to 220 days of high-dose gefitinib treatment. Moreover, we also demonstrated a strong growth-inhibitory effect of gefitinib in normal human mammary epithelial cells, which supports its role as a chemopreventative agent. We further observed that gefitinib prevented the development of pre-neoplastic diseases including hyperplasia, mammary intraepithelial neoplasia (MIN), and invasive breast cancer after 4 month of treatment, suggesting that gefitinib prevents cancer development at its early stages [33]. However, the rare side effects of gefitinib have limited its clinical use. In patients with lung cancer, gefitinib use was observed to be associated with interstitial lung disease (overall incidence at about 1%) [34]. Concerns about this potentially serious side effect caused the FDA to halt clinical cancer prevention trials using gefitinib.

Since erbB receptors can form heterodimers with other erbB proteins, blocking a single erbB receptor might induce the activity of other erbB heterodimers and result in drug resistance. Preclinical studies demonstrated that dual inhibition of ErbB-1 and ErbB-2 tyrosine kinases exerted greater biological effects in inhibiting cell proliferation and survival than inhibition of either receptor alone [35]. To obtain better anticancer activity, dual kinase inhibitors or pan-ErbB inhibitors, which target more than one erbB receptor, have been developed.

Lapatinib (GW572016, Tykerb) is a dual kinase receptor that targets both EGFR and ErbB2 receptors. It has been shown to inhibit tumor cell growth in vitro and in xenograft models for a variety of human tumors. Several clinical studies demonstrated that lapatinib was effective to treat ErbB1 and/or ErbB2 overexpressing metastatic breast cancers and trastuzumab-resistant breast cancers [36]. Recently, the FDA approved lapatinib to be used in combination with capecitabine (Xeloda) for patients with advanced or metastatic breast cancer whose tumors overexpress HER2 [and who have received prior therapy including an anthracycline, a taxane, and trastuzumab (Herceptin)]. Our group has studied the cancer preventative activity of lapatinib. The results from these studies showed that lapatinib significantly delayed breast cancer development in MMTV-ErbB2 transgenic mice (T.E. Strecker and P.H. Brown, in preparation). Like gefitinib, lapatinib

also prevented the development of premalignant mammary lesions in these mice, suggesting that lapatinib inhibited both the initiation and progression of mammary carcinogenesis. The anticancer effect was associated with proliferation inhibition and apoptosis promotion, as well as reduced activation of downstream signaling effectors such as Erk1/2 and AKT.

Many other novel multitarget inhibitors have been developed. These include: HKI-272, BIBW-2992, and BMS-599626 targeting EGFR and ErbB2; CI-1033 targeting EGFR, ErbB2, and erbB4; and ZD6474 and AEE788 targeting EGFR, ErbB2, and VEGFR. All these agents are currently undergoing clinical trials for the treatment of solid tumors and breast cancers. Selection of appropriate candidate agents for prevention studies will depend heavily on the toxicity profiles of these agents.

13.6 COX-2 Inhibitors

Accumulating epidemiology data suggest that long-term usage of aspirin or nonsteroidal anti-inflammatory drugs (NSAIDs) is associated with reduced risk of cancer from various tissues, especially the digestive tract. Recently, more data have linked NSAID usage to breast cancer. A number of observation studies demonstrated that NSAID usage was associated with a 20% reduction in risk of breast cancer [38], suggesting the chemopreventative potential of NSAIDs on breast cancer. The main target of NSAIDs is cyclooxygenase (COX), which consists of two isoforms, COX-1 and COX-2. COX enzymes catalyze the conversion of arachidonic acid to prostaglandin G2 (PGG2), which is further catalyzed by the peroxidase activity of COX to PGH2, a common precursor for all other prostanoids including PGI2, PGE2, PGF2, PGD2, and TXA2. COX-1 and COX-2 have similar catalytic activities but distinct expression patterns. COX-1 is constitutively expressed whereas COX-2 is expressed only under certain stimuli including growth factors, tumor promoters, and cytokines. Moreover, COX-2 is activated by many oncogenes including v-*src*, v-*Ha-ras*, and *HER-2*/*neu*. Aberrant expression of COX-2 is a marker of poor prognosis in human breast cancer and correlates with increased tumor size, negative ER status, HER-2 overexpression, and the presence of metastatic lesions. This correlation between COX-2 expression and breast cancer prognosis, as well as the results of several prevention studies that showed that NSAIDs prevent the development of breast cancer in rats and mice, indicates that COX-2 may be a useful target for breast cancer prevention. Celecoxib, a selective COX-2 inhibitor, has been shown to reduce the incidence and multiplicity of DMBA-induced mammary tumors in rat models by 68% and 86%, respectively [39]. Nimesulide, another selective COX-2 inhibitor, significantly reduced the incidence and multiplicity of PhIP- (2-amino-1-methyl-6-phenylimidazo[4,5-b]pyridine) and NMU-induced rat mammary tumors [40]. In MMTV-ErbB2 transgenic mice, which develop ER-negative cancers, celecoxib at 500 ppm delayed the onset of mammary tumor development and decreased the PGE2 level by 50%, suggesting that COX-2 inhibitors might be useful to prevent ER-negative breast cancer [41].

Compared to NSAIDs, COX-2 inhibitors have less gastrointestinal toxicity, which is believed to be due to COX-1 inhibition. This led to extensive clinical testing of the chemopreventative effect of selective COX-2 inhibitors. However, due to their selective inhibition of PGI2 synthesis, COX-2 inhibitors were found to increase the risk of thrombotic cardiovascular incidents. These rare but serious side effects have essentially halted the development of COX-2 inhibitors as cancer prevention agents. Therefore, researchers are searching for alternative strategies to antagonize the COX-2 pathway. Downstream activation of the COX-2 product, PGE2, is an important mediator for tumorigenesis. Blocking PGE2

activity through targeting prostanoid receptors (EP receptors) is thought to be a promising strategy to prevent cancer development [42, 43]. New agents targeting alternative COX-2 pathways are expected to retain the anticancer activity of COX-2 inhibitors, but may have reduced side effects. These new strategies will be the focus of future studies targeting COX-2 pathways.

13.7 Statins

Statins are 3-hydroxy-3-methylglutaryl coenzyme A (HMG-CoA) reductase (rate-limiting enzyme for mevalonate synthesis) inhibitors that are widely used in United States to lower the plasma cholesterol level and reduce mortality from cardiovascular disease. Recently, numerous observational and clinical studies indicate that statin usage may have potential beneficial effects on breast cancer risk. Lipophilic statins, which can permeate the cell membranes and affect cell and tumor growth in vitro and in vivo, have been found to be associated with a 50% reduction of breast cancers incidence in large observation studies [44]. In contrast, Bonovas et al. performed a meta-analysis of seven large randomized controlled trials (RCT) and nine observational trials (four cohort and five case-control) showing that there is no association between statin usage and breast cancer risk [45]. However, these divergent results have been criticized due to their multiple limiting factors including small numbers of cases, multiple statins, doses, and treatment durations, making any conclusions less than convincing. Thus, the cancer preventative potential of statins remains unclear.

Although the beneficial effects of statins on breast cancer development remain controversial, there is strong preclinical evidence suggesting that lipophilic statins can inhibit ER-negative breast tumor growth. Atorvastatin, lovastatin, simvastatin, and fluvastatin have been found to significantly inhibit the in vitro proliferation of both ER-positive and ER-negative breast cancer cell lines. Inhibition was between 10% and 90%, with greater efficacy observed in ER-negative cancer cells [46]. In addition, statins have been shown to reduce tumor growth in mouse models of ER-negative breast cancer [47, 48]. Laboratory investigations imply that the anticancer effects of statins may involve reducing levels of mevalonate and its downstream products such as isoprenoid intermediates that provide lipid attachment sites for activated Ras, Rac, and Rho family members. All these cytoplasmic signaling molecules affect important cancer pathways including apoptosis, proliferation, angiogenesis, and immune response, and ultimately lead to inhibition of tumor initiation and growth. Statins are currently being widely used to reduce hyperlipidemia and have been found to be relatively safe and well tolerated. It is possible that statins could promote health by reducing the risk of heart disease as well as cancer. However, the wide use of statins has made it very difficult to design randomized clinical trials to assess the breast cancer preventative effect of statins.

In addition to the agents summarized above, there is a growing list of molecularly targeted agents that block critical signaling pathways in cancer cells. Promising agents include PPAR ligands, imatinib mesylate (Gleevec), demethylating agents, histone deacetylase inhibitors, polyamine synthesis inhibitors, metalloprotease inhibitors, angiogenesis inhibitors, and triterpenoids. Future preclinical and clinical studies are needed to determine the efficacy of these agents in preventing ER-negative breast cancers.

13.8 Combination Chemoprevention

It is well accepted that carcinogenesis is a multistep process that involves the activation of complex signal transduction pathways. Breast cancer has many different subtypes that have different responses to specific anticancer agents. Therefore,

many targeted agents are only effective in a specific subgroup of breast cancers. The ultimate aim of chemoprevention is to prevent all breast cancers. To achieve this goal, combination chemoprevention offers a promising approach.

Crosstalk between the ER pathway and the EGFR/ErbB2 pathway has been shown to contribute to tamoxifen resistance. Thus, coadministration of antiestrogens with EGFR or ErbB2 inhibitors may not only increase the efficacy of antiestrogens to prevent ER-positive cancer, but may also prevent the development of ER-negative cancer. In addition, preclinical studies have shown that combinations of SERMs with rexinoids effectively prevent breast cancer in transgenic mice when compared to either agent alone [49]. Results from Michael Sporn's lab demonstrate that arzoxifene and rexinoid LG100268 together prevent the development of both ER-positive and ER-negative breast cancers in animal models [49, 50].

The combination of other chemopreventative agents that target nonendocrine signaling pathways represent novel approaches to prevent both ER-positive and ER-negative breast cancer. Promising combinations include PPAR-gamma ligands and rexinoids; EGFR inhibitors and COX-2 inhibitors; and rexinoid and COX-2 inhibitors (P. Brown, unpublished observation). Besides improved effectiveness, a potential advantage of combination chemoprevention is through decreasing the dose of each individual agent, which would likely decrease the incidence of adverse effects. Considering the complex nature of cancer and the safety requirement for preventative agents, combination chemoprevention is likely to offer the greatest efficacy with the least toxicity.

13.9 Conclusion

Clinical cancer prevention studies have demonstrated that SERMs reduce the incidence of breast cancer and that chemoprevention is clinically feasible. Current chemoprevention studies are now testing the ability of AIs to prevent breast cancer. However, while SERMs are, and AIs may be, effective agents to prevent ER-positive breast cancer, they have no effect in reducing the incidence of ER-negative breast cancers. Through a better understanding of the estrogen-independent pathways that lead to mammary tumorigenesis, a growing number of chemopreventative agents have emerged that prevent ER-negative breast cancers in preclinical models. Rexinoids, COX-2 inhibitors, and EGFR tyrosine kinase inhibitors are the most promising agents that have been shown to prevent ER-negative tumorigenesis. Despite the promising effect of these novel agents, issues of safety and toxicity still hamper progress in the field. Clinically observed toxicity has adversely affected several ongoing chemoprevention trials including those of celecoxib and gefitinib. While many of these drugs are tolerated by cancer patients, the severity and frequency of side effects becomes a major concern when considering chronic preventative therapy in healthy women. Thus, future clinical studies of chemoprevention will depend heavily on the balance between efficacy and tolerability. With breast cancer risk assessment, it becomes critical to select the high-risk women who will be benefit most from chemoprevention. More recently, preclinical studies have shown that combination chemoprevention is a promising strategy that will greatly enhance the efficacy of cancer preventative effect. Thus, to ultimately prevent all forms of breast cancers, it will be necessary to combine safe and effective drugs targeting the ER as well as drugs inhibiting critical estrogen-independent pathways.

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14 Exogenous and Endogenous Hormones, Mammographic Density and Breast Cancer Risk: Can Mammographic Density Be Considered an Intermediate Marker of Risk?

Susen Becker and Rudolf Kaaks

Abstract Elevated mammographic density measures are a well-established, relatively strong risk factor for breast cancer development. A systematic review of prospective cohort studies and cross-sectional studies strikingly establishes parallels between the associations of combined postmenopausal estrogen and progestin replacement therapy with, on the one hand, mammographic densities and, on the other hand, breast cancer risk. Other parallel observations were the inverse associations of both mammographic density and breast cancer risk with the selective estrogen receptor modulator tamoxifen, and direct associations with prolactin. Paradoxically, however, high mammographic density has been found associated with higher risks of both estrogen- and progesterone-receptor positive (ER+/PR+) and negative (ER−/PR−) breast cancers, while hormone replacement therapy (HRT) use, but also circulating (blood) levels of androgens, estrogens, and prolactin appear to be associated more specifically to the risk of ER+ tumors. The effects of aromatase inhibitors and gonadotropin-releasing hormone agonists on breast density, as well as on breast cancer risk, still require further investigation. Regarding circulating levels of insulin-like growth factor (IGF)-I or IGFBP-3, studies did not show fully consistent relationships with mammographic density measures and breast cancer risk. In view of these various findings, it is impossible, at present, to propose mammographic density measures as an intermediate risk-related phenotype, integrating the effects of exogenous and/or endogenous hormones on the risk of developing breast cancer.

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14.1 Introduction

Human breast tissue is composed of epithelial tissue, collagen-containing stromal tissue, and adipose tissue, of which the proportions vary widely between women (Boyd et al. 1992). On

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mammographic (X-ray) images, the epithelial and stromal tissues appear as radio-dense, and adipose tissue as nondense parts (Oza and Boyd 1993). On the basis of such X-ray images, Wolfe in the 1970s proposed a classification system of mammographic tissue structures into four major parenchymal and fat tissue distribution patterns, referred to as "normal" (N1), prominent duct pattern occupying less than one-fourth (P1) or more than one-fourth (P2) of the breast volume, and "dysplastic" (DY) (Wolfe 1976). In the 1990s, more quantitative visual estimation methods were proposed for the classification of breast mammograms into six mammographic density categories (Boyd et al. 1995). Likewise, a Breast Imaging Reporting and Data System ("BI-RADS") was developed in the United States, for a visual and semiquantitative classification of breast densities into four categories of breast density. The latter system is used especially by physicians to evaluate mammograms in the context of mammographic screening for the early detection of breast tumors. In more recent years, computer-assisted, planimetric methods were developed for the quantitative determination of breast density, which nowadays is further eased by the digitization of mammographic images (Byng et al. 1998). These planimetric methods divide the total breast area on the mammogram into areas of either high or low density. Amounts of dense and nondense can then be expressed into either a relative mammographic density score, calculated as the ratio of dense tissue area divided by total breast tissue area and expressed either as a percentage (breast density%), or as the absolute area of dense mammary tissue (in cm^2).

More than 40 epidemiological studies—recently reviewed in a metaanalysis by McCormack and dos Santos Silva (2006)—have shown increases in breast cancer risk with increasing mammographic density, as assessed by Wolf's patterns, BI-RADS patterns, or planimetry. In the studies using the more quantitative, planimetric methods, relative risks of 4.50 or higher were observed for women having highly dense breasts (>75% of dense tissue) compared to women with nondense breasts (<5% dense tissue), independently of other major breast cancer risk factors, such as age, body mass index (BMI), age at first full-time pregnancy, or family history of breast cancer. In terms of the population-attributable fraction, about 40% of breast cancer occurrence can be associated with high breast densities in North American study populations (Boyd et al. 1998). In some prospective studies (Kerlikowske et al. 2007; van Gils et al. 1999), but not all (Vachon et al. 2007a), longitudinal changes in breast cancer density over periods up to 5 years have also been associated with parallel changes in breast cancer risk. Mammographic density thus appears to be one of the strongest risk factors for breast cancer, and increasingly is being proposed as an important factor in breast cancer risk prediction models (Chen et al. 2006; Vachon et al. 2007c). It is worth noting that in a number of studies (Kato et al. 1995; Maskarinec and Meng 2000) the absolute area of dense mammary tissue was found to be equally strongly associated with breast cancer risk measures as relative mammographic density measures. Measures of the absolute dense area may have the advantage of being less correlated with, or potentially confounded by, general adiposity (as discussed further in this review).

Increased density reflects increased volumes of stromal and epithelial tissues (Hawes et al. 2006), which are the mammary tissue types with the highest rates of cell proliferation. Cell proliferation rates are believed to be largely controlled by hormones (Albanes et al. 1988; Torres-Mejia et al. 2005; Trichopoulos and Lipman 1992). Furthermore, the epithelial compartment is thought to be the origin of most breast tumors, the development of which is also known to be hormone-dependent. It has thus been questioned whether mammographic density could be a useful intermediate surrogate marker for the effects of hormones on breast

cancer risk. In the present review, we summarize the results from epidemiological studies relating mammographic density measures to exogenous and endogenous sex steroid hormones, as well as circulating levels of prolactin (PRL) and insulin-like growth factor-I (IGF-I), and address the question about whether mammographic density can indeed be seen as an intermediate endpoint that would reflect influences of these hormones on breast cancer risk.

14.2 General Determinants and Correlates of Mammographic Density

Mean breast density declines with increasing age, but within a given age group shows wide between-subject variation. Age at mammography, late menarche, and late first full-term pregnancy are associated with increased mammographic densities, whereas the percentage of density decreases by about 2% with each successive pregnancy (Boyd et al. 2007). Furthermore, percent breast density decreases after menopause by 8% (Boyd et al. 2002a) with only 30% of women aged 75–79 showing mammographic densities above 50% (Stomper et al. 1996). Taken together, however, age, menopausal status, parity, and body weight jointly can account for no more than 20%–30% of the between-subject variance in mammographic density.

A larger overall proportion of between-subject variance, in fact, appears to be due to genetic factors. In studies of monozygous and heterozygous twins, the heritability was estimated to be around 60% for percent mammographic density (Boyd et al. 2002b), 65% for absolute dense area, and 66% for absolute nondense area (Stone et al. 2006). In premenopausal women, some studies (Ursin et al. 2001; White et al. 1998) but not all (Buist et al. 2006) have shown small variations in mammographic density during the menstrual cycle, with slightly increased densities during the luteal phase compared to the follicular phase (Soderqvist et al. 1997).

An early menarche, late age at first pregnancy, low parity, and late menopause are all also associated with increased risk of breast cancer. Intriguingly, however, breast cancer incidence rates do not decrease, but actually increase with advancing age, although with a higher slope before than after menopause. To resolve this apparent contradiction, the concept of breast tissue age, as opposed to chronological age, was coined (Pike et al. 1983). According to this concept, breast tissue aging starts at menarche, whereas the rate of breast tissue aging would decrease during each live pregnancy, slow further during the peri-menopausal period, and reach its lowest values after menopause (Pike et al. 1983). Adjusting for chronological age, mammographic density would reflect the degree of mammographic tissue aging that, cumulatively, a woman would have experienced, and the age-adjusted measures of mammographic tissue age would be directly related to breast cancer risk (Martin and Boyd 2008; Pike et al. 1983).

14.3 Sex Steroid Hormones and Breast Density

14.3.1 Postmenopausal Hormone Replacement Therapy

Two large-scale intervention studies—the Postmenopausal Estrogen/Progestin Interventions (PEPI) Trial and the Women's Health Initiative (WHI)—have shown increases in (percent) mammographic density among postmenopausal women who used hormonal replacement therapy (HRT) (Greendale et al. 2003; McTiernan et al. 2005). This increase was particularly clear for the use of estrogens combined with synthetic progestins (e.g., medroxyprogesterone acetate), while for estrogens alone no, or only very

moderate, increases in density were observed. These findings were fully in line with those from several large observational studies. For example, a study in Norway showed higher mammographic densities particularly among users of continuous regimens of estradiol combined with norethisterone acetate (E2/NETA) (Bremnes et al. 2007a), whereas a study in the United States showed prospective increases in density among women who started using HRT, compared to decreases in density among women that initially used HRT but then stopped its use (Rutter et al. 2001). Likewise, one study in the Netherlands showed a reduced rate of age-related reductions in percent mammographic density among women using combined (estrogen-plus-progestin) HRT use, but not among women using regimens based on estrogens alone, or users of tibolone, a 19-nortestosterone derivative with weak estrogenic, progestogenic, and androgenic activities (Van Duijnhoven et al. 2007).

Interestingly, a number of large prospective cohort studies in the United States and Europe (Bakken et al. 2004; Beral 2003; Greendale et al. 2003; Lee et al. 2005; Stahlberg et al. 2004) have also shown increases in breast cancer risk among postmenopausal women using HRT based on estrogens combined with (synthetic) progestins, but not among women using estrogens alone (Greendale et al. 2003; Lee et al. 2005), and these were confirmed in the WHI study trial (Chlebowski et al. 2003), which compared the effects of combined estrogen-plus-progestin HRT against those of HRT based on estrogens alone, and against a placebo.

14.3.2 Endogenous Sex Hormones

Prospective cohort studies have uniformly shown increased risks of breast cancer among postmenopausal women who have higher serum concentrations of androgens [dehydroepiandrosterone (DHEA), androstenedione, testosterone] and estrogens (estrone, estradiol), and lower concentrations of sex hormone-binding globulin (SHBG)—a plasmatic carrier protein that binds testosterone and estradiol with high specificity and reduces the bioavailability of these steroid hormones to their target tissues (Kaaks et al. 2005b; Key et al. 2002). In one prospective study, so far, these associations were shown to be strongest for the risk of breast tumors that express both estrogen and progesterone receptors (ER+/PR+ tumors). Furthermore, prospective studies have also shown increased breast cancer risks among premenopausal women who have higher blood levels of testosterone (Micheli et al. 2004; Kaaks et al. 2005a; Eliassen et al. 2006) and lower levels of progesterone (Micheli et al. 2004; Kaaks et al. 2005a), and one of these studies could also demonstrate an increase in risk especially of ER+/PR+ tumors in relation to more elevated serum levels of estradiol, measured during the follicular phase of the menstrual cycle (Eliassen et al. 2006).

In the light of these various relationships of endogenous sex hormones with breast cancer risk, which are particularly consistent among postmenopausal women, at least eight different research groups have also studied the cross-sectional relationships of circulating sex hormones with mammographic density measurements (Table 14.1). In statistical analyses that were unadjusted for BMI, four of these studies revealed inverse relationships of relative (percent) mammographic density with serum levels of estrone, estradiol, and free (or non-SHBG bound) estradiol (Boyd et al. 2002c; Tamimi et al. 2005; Verheus et al. 2007b; Warren et al. 2006). Furthermore, all of these studies also showed positive associations of percent mammographic density with serum levels of SHBG, and consequently, three of the studies showed negative associations with serum levels of free testosterone, unbound to SHBG. Mammographic density measures were also associated negatively with free estradiol, and positively with SHBG, in one study on premenopausal women (Boyd et al. 2002c).

Table 14.1 Summary of studies examining the correlation between endogenous sex hormones and breast density, in pre- and postmenopausal women

		% Breast density										Absolute dense breast area									
		Study size	Estrone	Estra-diol	Free estra-diol	DHEAS	Andro-stene-dione	Testo-sterone	Free testo-sterone	SHBG	Proge-sterone	Estrone	Estra-diol	Free estra-diol	DHEAS	Andro-stene-dione	Testo-sterone	Free testo-sterone	SHBG	Proge-sterone
Premenopausal																					
(Boyd et al. 2002c)	Unadjusted	193		o	–					+	+		o	–					+	o
	Adjusted for waist circumf.				o					o	o			o					o	o
Postmenopausal																					
(Aiello et al. 2005)	Unadjusted	45	Not stated																		
Never HT users	BMI adjusted		o	o	o	o	+*	o	o	o											
Past HT users	Unadjusted	43	Not stated																		
	BMI adjusted		–	–	–	–*	–	–	–	o											
(Boyd et al. 2002c)	Unadjusted	189		–	–					+	+		–*	–					+	+
Past HT users	Adjusted for waist circumf.				–					+	o			–*					+*	o
(Bremnes et al. 2007b)	Unadjusted	722	Not stated	Not stated																	
Past HT users	BMI adjusted		+*	o	o	o	o	o	o	+		+*	o	o	o	o	o	o	+		
(Greendale et al. 2005)	Unadjusted	404	o	o	o			o	–	+	o										
Past HT users	BMI adjusted		+	+	+			o	o	+*	+*										

(continued)

Table 14.1 (continued)

		% Breast density										Absolute dense breast area								
		Study size	Estrone	Estradiol	Free estradiol	DHEAS	Androstenedione	Testosterone	Free testosterone	SHBG	Progesterone	Estrone	Estradiol	Free estradiol	DHEAS	Androstenedione	Testosterone	Free testosterone	SHBG	Progesterone
(Johansson et al. 2008)	Unadjusted	226		+						+										
Never HT users	BMI adjusted			o						o										
(Tamimi et al. 2005)	Unadjusted	520	–	–	–	o	o	o	–	+	o									
Past HT users	BMI adjusted		o	o	o	o	o	o	o	o	o									
(Verheus et al. 2007b)	Unadjusted	775	–	–	–		o	–*	–	+		–*	o	o	o	o	o	o	o	
Past HT users	BMI adjusted		o	o	o	o	o	o	o	+*		No influence of BMI								
(Warren et al. 2006)	Unadjusted	1,413	–	–				–		+										
Past HT users	BMI adjusted		o	o				o		+*										

+, Positive significant correlation ($p<0.05$); –, negative significant correlation ($p<0.05$); o, no correlation; * weak correlation (p~0.1); HT, hormone therapy

The interpretation of these results is complicated by the fact that the relative mammographic density score is inherently confounded by adiposity. The denominator of this score—total breast area—is calculated as the sum of dense plus nondense breast tissue, where the area of nondense tissue predominantly reflects the amount of adipose tissue in the breast, which generally shows a strongly positive correlation (r>0.5 in many studies) with BMI or other measures of overall adiposity. Thus, measures of overall adiposity, such as BMI, but also other (e.g., metabolic and hormonal) variables that are strongly correlated with adiposity, tend to show reciprocal relationships with percent mammographic density. Among postmenopausal women, adipose tissue is the major site of synthesis of estrogens by peripheral aromatization of androgens, and BMI correlates strongly and positively with serum concentrations of both estrone and estradiol. In addition, in both pre- and postmenopausal women, increasing adiposity is associated with reduced insulin sensitivity and, due to an increase in circulating insulin levels, reduced serum levels of SHBG and increased fractions of free testosterone and estradiol unbound to SHBG. The observed direct associations of percent mammographic density with serum SHBG, and inverse associations with serum estrogens and free testosterone, could thus all be explained by the relationships of each of these variables with overall adiposity.

Statistical adjustments for BMI may remove some of the (negative) confounding of associations between serum sex hormones and SHBG with mammographic density, and indeed, in all studies that showed negative associations of percent mammographic density with serum estrogens and free testosterone and positive associations with SHBG, these associations were substantially weakened and often no longer statistically significant after statistical adjustments for BMI or waist circumference (as a measure of abdominal fat). Interestingly, in two of the studies (Bremnes et al. 2007b; Greendale et al. 2005), adjustment for BMI revealed weakly positive and statistically borderline significant associations between percent density with serum levels of estrone. Nevertheless, in these same two studies a weakly positive association of percent density with SHBG also remained, suggesting that there could have been residual negative confounding by adiposity, and that with a more complete adjustment for adiposity an even clearer positive correlation of percent density with estrone could have appeared.

Alternatively, some studies also related endogenous hormone levels to the absolute area of dense breast tissue, which does not have the inherent negative confounding by adiposity that affects the relative density measures. In these studies, no significant correlations were observed for absolute dense area with levels of either total estradiol or free estradiol (Bremnes et al. 2007b; Verheus et al. 2007b), but these two studies did suggest, respectively, either a weakly positive (Bremnes et al. 2007b) or negative (Verheus et al. 2007b) association of dense tissue area with estrone. Furthermore, a positive relationship between SHBG and absolute dense breast area was observed in two studies (Boyd et al. 2002c; Bremnes et al. 2007b), but in premenopausal women this association disappeared after adjustment for waist circumference. A third study (Verheus et al. 2007b) showed no association of SHBG with absolute dense area at all. Taken together, these studies do not suggest any clear association of total of bioavailable serum estrogens with absolute dense areas on mammographies.

Six of the studies also examined relationships of percent mammographic density with circulating levels of androgens, but globally showed no clear and consistent pattern of associations with serum concentrations of DHEA sulfate (DHEAS), androstenedione, or total testosterone, either before or after BMI adjustment (Aiello et al. 2005; Bremnes et al. 2007b; Greendale et al. 2005; Tamimi et al. 2005; Verheus et al. 2007b; Warren et al. 2006). Before adjustment for adiposity, negative correlations with percent

mammographic density were found for total testosterone (Verheus et al. 2007b; Warren et al. 2006) or free testosterone (Greendale et al. 2005; Tamimi et al. 2005; Verheus et al. 2007b), but these associations disappeared after adjustment for BMI, and also were not observed for absolute dense breast area in these same studies (Bremnes et al. 2007b; Verheus et al. 2007b).

Progesterone—the natural progestogen—was found to be positively associated with percent mammographic density only before adiposity adjustment, and unassociated with absolute dense breast area, in one study on premenopausal women (Boyd et al. 2002c). In postmenopausal women, adjusting for BMI, either a borderline positive correlation (Greendale et al. 2005) or no correlation (Tamimi et al. 2005; Boyd et al. 2002c) was observed between serum progesterone and percent mammographic density. Before adjustment for adiposity, one study showed a positive correlation (Boyd et al. 2002c), whereas two other studies showed no association (Greendale et al. 2005; Tamimi et al. 2005), between progesterone levels and percent mammographic density.

All of the above results were reported for women who were either never or former users of HRT at the time of blood donation and mammography. There were some data to suggest that between never users and former users the association of breast density with endogenous hormones could differ. Aiello et al. reported a weakly positive correlation of percent mammographic density with androstenedione, and no association with estrogens, among women who never used HRT; by contrast, this same study showed a negative correlation with androstenedione as well as with estrogens (after BMI adjustment) among past users of HRT (Aiello et al. 2005). Reasons for such possible heterogeneity between never users and past users of HRT are unclear. Users of HRT on average tend to be leaner than never users, and also the wash-out time for the effects of exogenous hormones on endogenous hormone metabolism is unclear. Further studies addressing this issue may be needed.

14.4 Selective Estrogen Receptor Modulators

Selective estrogen receptor modulators (SERMs) are substances that selectively block or modulate specific parts of intracellular signal transduction of estrogen receptors (ERs) (Jordan 2007). Two well-studied SERMs are tamoxifen and raloxifene. Tamoxifen has clear antiestrogenic actions in breast cells in vitro, and became the first drug for targeted treatment of patients with estrogen-receptor positive (ER+) breast tumors. Biologically the tamoxifen-ER complex, which is very similar to the natural estrogen-ER complex, acts as a transcription factor in the cellular nucleus. Contrary to the natural complex, however, the tamoxifen-ER complex is incapable of further recruiting certain transcriptional components, which leads to lack of expression of estrogen-responsive genes, and arrest of breast cancer cell proliferation (McDonnell et al. 1995; Metzger et al. 1988). Nevertheless, some of the estrogen responses may also be preserved, depending on tissue and cell types. Thus, tamoxifen retains estrogenic effects resulting in the reduction of serum lipid profiles (lower cholesterol levels) and in the preservation of bone density in postmenopausal women. In endometrial tissue tamoxifen also retains estrogenic responses and increases the risk of endometrial cancer. Raloxifene has biological actions that are similar to those of tamoxifen, but has additional antiestrogenic effects on the uterus, and lowers incidence rates of endometrial cancer.

Several randomized prevention trials have shown reduced risks of breast cancer after longer-term treatment with tamoxifen com-

pared with placebo, with an approximate 40% reduction in breast cancer incidence overall, no significant effect for ER-negative breast cancers, and a close to 50% reduction in ER-positive cancers (Cuzick et al. 2003). Likewise, randomized trials have also shown strongly reduced risks of breast cancer among initially cancer-free, post-menopausal women treated with raloxifene to prevent osteoporosis ("MORE" trial) (Cummings et al. 1999). Further results from this trial ("CORE" study) suggested that the benefit from raloxifene may depend on endogenous estradiol levels: among postmenopausal women whose baseline serum estradiol levels were above 10 pmol/l, 4 years of raloxifene treatment resulted in a 76% reduction in breast cancer incidence compared to the placebo group, whereas women who had undetectable estradiol levels had similar breast cancer risk whether or not they were treated with raloxifene (Cummings et al. 2002). In the Study of Tamoxifen and Raloxifene ("STAR") trial, performed among women that were estimated to be at increased risk of breast cancer, tamoxifen and raloxifene treatments resulted in equivalent reductions in breast cancer incidence rates, but raloxifene was associated with lower incidence rates of endometrial cancer and hyperplasia, cataracts, and thromboembolic events (Vogel et al. 2006).

Few studies have been published on the relationship between SERMs and mammographic density (Table 14.2). Tamoxifen caused a decrease of percent density, when administered to breast cancer patients (Atkinson et al. 1999) or to women who are at increased risk of developing breast cancer (Brisson et al. 2000; Chow et al. 2000; Son and Oh 1999). In the largest study ("IBIS-I" trial), among 388 women having a minimum initial breast density of 10% and an estimated twofold increased risk to develop breast cancer, a 5-year treatment with tamoxifen reduced breast density by 8% (Cuzick et al. 2004). Two-thirds of this overall breast density reduction was observed during the first 18 months of treatment, which made the investigators of this study speculate that breast density could be used as an early marker for prevention efficacy during tamoxifen treatment (Cuzick et al. 2004). Studies on raloxifene, however, have not shown so far any clear effects on mammographic density (Christodoulakos et al. 2002; Freedman et al. 2001; Jackson et al. 2003; Lasco et al. 2006; Table 14.2).

Taken together, these studies suggest that selective ER modulators, particularly tamoxifen, may reduce mammographic density. Unfortunately, it was not examined directly in these studies whether such reductions are indeed the result of diminished absolute dense tissue areas, although a priori this would seem likely. Although raloxifene showed less clear effects on mammographic density, data available from these first studies are still insufficient to draw definitive conclusions of an absence of effect on breast density. One major question, in this context, is whether the effects on breast density by raloxifene, but also tamoxifen, are modulated by blood concentrations of endogenous estrogens.

14.5 Aromatase Inhibitors

Aromatase inhibitors are effective alternatives to selective ER modulators for the treatment of estrogen-receptor positive breast cancer, and are also being studied as possible chemopreventative agents against breast cancer, among women at high risk for this disease. Letrozole and anastrozole belong to the group of reversible nonsteroidal imidazoles, and exemestane to the class of irreversible steroidal inhibitors. Aromatase inhibitors are being utilized as initial hormonal therapy on localized hormone receptor-positive breast cancer patients

Table 14.2 Summary of studies examining the correlation between tamoxifen, raloxifene, and relative breast density measurements

Author	Study population	Study size	Duration of treatment	Association
	Tamoxifen			
(Atkinson et al. 1999)	Cancer patients	94	Not stated	–14%
(Atkinson et al. 1999)	Cancer free	188	Not stated	o
(Brisson et al. 2000)	Cancer free, high risk	36	5 years	–9%
(Chow et al. 2000)	Cancer free, high risk	28	2 years	–4.3%/year
(Cuzick et al. 2004)	Cancer free, high risk	388	5 years	–8%
(Son and Oh 1999)	Cancer patients	102	22 months	– (More prevalent in pre than post)
			Raloxifene	
(Christodoulakos et al. 2002)	Cancer free, high risk for CVD or osteoporosis	48	12 months	o
(Freedman et al. 2001)	Cancer free, hysterectomy	87	2 years	–2%
(Jackson et al. 2003)	Postmenopausal women with osteopenia or osteoporosis	109	12 months	o
(Lasco et al. 2006)	Healthy	70	2 years	–

o, no association, –, negative association; CVD, cardiovascular disease

(Howell et al. 2005), as a switching reagent in the 5-year follow-up treatment after 2 to 3 years of taking tamoxifen (Coombes et al. 2004) and as extended adjuvant hormone therapy after 5 years treatment with tamoxifen (Goss et al. 2003). Compared to tamoxifen or a placebo, aromatase inhibitors were found to improve disease-free survival of breast cancer patients, with a 70%–80% reduction of new ER-positive breast cancer recurrences (Cuzick 2005).

So far, only very few studies have examined the effect of aromatase inhibitors on mammographic density. No significant mammographic changes were observed after 6 months of letrozole treatment in postmenopausal women at high risk of breast cancer who were on HRT, although this treatment did cause a 66% reduction in epithelial cell proliferation rates as measured by Ki-67 concentrations (Fabian et al. 2007). Comparable results were found for women with early-onset breast cancer that had first been treated with tamoxifen for 5 years (Vachon et al. 2007b).

14.6 Gonadotropin-Releasing Hormone Agonists

Based on the hypothesis that cyclic ovarian production of estradiol and progesterone accounts for the steep rise of breast cancer risk within increasing age among premenopausal

women, gonadotropin releasing hormone agonists (GnRHA) have been proposed as potentially chemopreventative agents against breast cancer (Pike et al. 1989). This type of agonist can drastically reduce ovarian sex steroid synthesis by blocking the pituitary release of luteinizing hormone. To prevent deleterious effects of estrogen deficiency, the addition of low-dose HRT to the GnRHA appears necessary. In a small randomized trial among 21 premenopausal women predisposed to familial breast cancer, aged 25–40 years, significant reductions in percent mammographic density were seen as a response to the reduced estrogen and progestogen exposures achieved after 12 months of treatment with a combined hormonal regimen consisting of GnRHA (leuprolide acetate depot by monthly intramuscular injections) combined with oral add-back administration of estrogens and progestins (Spicer et al. 1994). An extended follow-up of this study showed that the change in percent density persisted through 12 months of treatment (Gram et al. 2001). A similar, very small study, conducted by the same research group among eight premenopausal women carrying a *BRCA1* mutation, also showed a reduction in mammographic density in response to the GnRHA deslorelin, jointly administered with low-dose add-back steroids (estradiol, testosterone, intermittent medroxyprogesterone acetate) (Weitzel et al. 2007).

No studies have been conducted, so far, to examine whether GnRHA can reduce breast cancer occurrence among healthy women. Among premenopausal breast cancer patients, however, the addition of goserelin to standard adjuvant therapy was shown to be more effective than standard therapy alone, reducing breast tumor recurrence and improving survival (Baum et al. 2006).

These data show that the addition of goserelin to standard adjuvant therapy is more effective than standard therapy alone in premenopausal women with early breast cancer.

14.7 IGF-I and Its Binding Proteins

Serum levels of IGF-I have been associated with breast cancer risk in a number of prospective cohort studies, although observations are not entirely consistent (Allen et al. 2005; Gronbaek et al. 2004; Kaaks et al. 2002; Krajcik et al. 2002; Muti et al. 2002; Schernhammer et al. 2006; Toniolo et al. 2000). While initially such associations were reported particularly for breast cancer occurrence in premenopausal women (Allen et al. 2005; Krajcik et al. 2002; Muti et al. 2002; Toniolo et al. 2000), these reports were not uniformly confirmed by subsequent studies (Gronbaek et al. 2004; Kaaks et al. 2002; Schernhammer et al. 2006), and some studies also showed increased risks only among older women (Rinaldi et al. 2006). Nevertheless, the hypothesis that higher circulating levels of IGF-I could increase breast cancer risk remains plausible, as experiments in vitro have clearly demonstrated growth-promoting and antiapoptotic effects on mammary tumor cells (Ng et al. 1997). It has been hypothesized that elevated IGFBP-3 levels might reduce breast cancer risk, either by reducing the biological availability of IGF-I to cellular IGF-I receptors, or by independent pro-apoptotic effects through putative IGFBP-3 specific binding sites on cellular membranes (Pollak 2000; Yu and Rohan 2000). In a number of epidemiological studies IGF-I levels were associated with risk of cancer only when risk models included statistical adjustment terms for levels of IGFBP-3, or when the IGF-I/IGFBP-3 molar ratio was considered. Again, however, this observation has not been made uniformly across all studies, and there is some evidence that the relationship of IGFBP-3 with breast cancer risk, as well as the effects of statistical adjustments for IGFBP-3 on estimated relationships of risk with IGF-I, could be dependent on the type of immunoassay used for IGFBP-3 (Rinaldi et al. 2005). IGFBP-3 is

a complex molecule that occurs in the circulation in a variety of forms.

Among premenopausal women, cross-sectional studies showed either a positive association (Boyd et al. 2002c; Burshell et al. 2008; Diorio et al. 2005) or no association between IGF-I and relative mammographic density (Maskarinec et al. 2003; Verheus et al. 2007a) (Table 14.3), and in one of these studies IGF-I correlated positively also with absolute dense areas. IGFBP-3 levels, by contrast, were found to be negatively associated with mammographic density (Byrne et al. 2000a; Diorio et al. 2005; Maskarinec et al. 2003) after adjustment for adiposity variables, with the exception of one study (Boyd et al. 2002c). In three studies, the IGF-I/IGFBP-3 ratio showed a positive correlation with both relative breast density (Byrne et al. 2000a; Diorio et al. 2005; Maskarinec et al. 2003), and in one study this ratio showed only a weak and only borderline significant ($p < 0.1$) correlation with absolute dense breast area (Maskarinec et al. 2003; Verheus et al. 2007a).

Among postmenopausal women, mostly no correlations were found between IGF-I and percent mammographic density (Aiello et al. 2005; Byrne et al. 2000a; Diorio et al. 2005; Johansson et al. 2008). Five different studies also showed no relationship of IGFBP-3 with either percent density (Aiello et al. 2005; Boyd et al. 2002c; Bremnes et al. 2007c; Byrne et al. 2000b; Diorio et al. 2005) or absolute breast density (Boyd et al. 2002c; Bremnes et al. 2007c) with the exception of one study (Johansson et al. 2008). Divergent findings, showing positive [past HT users (Bremnes et al. 2007c)], negative [past HT users (Aiello et al. 2005)], and no correlation [never HT users (Aiello et al. 2005; Byrne et al. 2000a; Diorio et al. 2005; Johansson et al. 2008)], were published for the ratio of IGF-1/IGFBP-3 and percent breast density after BMI adjustment.

As in the case of SHBG and endogenous estrogens, adiposity is a potential confounder of the relationships of IGF-I, IGFBP-3, or their molar ratio, with percent breast density (Kaaks 2005). An elevated BMI is generally associated with a modest decrease in plasma IGF-I concentrations, with moderately increased levels of IGFBP-3, and thus with a reduced IGF-I/IGFBP-3 ratio. Since BMI also correlates inversely with relative mammographic density measures, one would expect a weak positive correlation of relative mammographic density measures with IGF-I, and especially the IGF-I / IGFBP-3 ratio. In the study by Maskarinec et al. (2003), a weakly positive correlation ($r = 0.13$) between the IGF-I/IGFBP-3 molar ratio and the percentage of mammographic density could be entirely accounted for by the direct correlation of IGFBP-3 ($r = 0.20$), and hence, the inverse correlation of the IGF-I/IGFBP-3 ratio ($r = -0.19$), with the nondense area.

14.8 Prolactin

PRL, a pituitary hormone, is important for mammary epithelial cell proliferation and differentiation and initiates lactation at higher concentration levels. It is further involved in the glandular breast development during pregnancy. Studies at the cellular level in vitro, and in vivo with multiple transgenic and knockout models have confirmed a role for PRL in breast cancer development (Clevenger et al. 2003; Harris et al. 2004). In parallel, prospective epidemiological studies have shown a positive association between PRL and breast cancer risk in both pre- and postmenopausal women, with a 30%–40% increases in risk comparing highest vs lowest quartile levels (Tworoger and Hankinson 2008). Other, smaller studies (Wang et al. 1992; Kabuto et al. 2000; Helzlsouer et al. 1994; Manjer et al. 2003) showed similar results, although findings were not always statistically significant. The association of PRL levels with

Table 14.3 Summary of studies examining the correlation between IGF-I, its binding protein 3, and prolactin with breast density measurements in pre- and postmenopausal women

			% Density				Absolute dense breast area				Absolute nondense breast area			
		Study size	Prolactin	IGF-I	IGFBP–3	IGF-I/ IGFBP–3	Pro-lactin	IGF-I	IGFBP–3	IGF-I/ IGFBP–3	Pro-lactin	IGF-I	IGFBP–3	IGF-I/ IGFBP–3
Premenopausal														
(Boyd et al. 2002c)	Unadjusted	193	o	+	–		o	+	–					
	Adjusted for waist circumf.		o	+	o		o		o					
(Byrne et al. 2000a)	Unadjusted	65		+*	o									
	BMI adjusted			+	–	+								
(Diorio et al. 2005)	Unadjusted	783		–	+									
	BMI adjusted			+	–	+*								
(Maskarinec et al. 2003)	Unadjusted	263		+*	–	+		o	o	+		o	+	–
	BMI adjusted			o	–*	+		o	o	+*		o	o	o
(Verheus et al. 2007a)	Unadjusted	684	Not stated			Not stated			Not stated					
	BMI adjusted			o				o				o		
Postmenopausal														
(Aiello et al. 2005)	Unadjusted	45	Not stated											
Never HT users	BMI adjusted			o	o	o								
Past HT users	Unadjusted	43	Not stated											
	BMI adjusted				o	–								
(Boyd et al. 2002c)	Unadjusted	189	+	+	o		+	+	o					
Past HT users	Adjusted for waist circumf.		+		o		+		o					
(Bremnes et al. 2007c)	Unadjusted	553	Not stated			Not stated								
Never HT users	BMI adjusted			+	o	+*		+	o	+*				
past HT users	Unadjusted	170	Not stated			Not stated								
	BMI adjusted			+	o	+		+	o	+				

(continued)

Table 14.3 (continued)

			% Density				Absolute dense breast area				Absolute nondense breast area			
		Study size	Prolactin	IGF-I	IGFBP–3	IGF-I/ IGFBP–3	Pro-lactin	IGF-I	IGFBP–3	IGF-I/ IGFBP–3	Pro-lactin	IGF-I	IGFBP–3	IGF-I/ IGFBP–3
(Bremnes et al. 2007b)	Unadjusted	722	Not stated			Not stated								
Past HT users	BMI adjusted		o				o							
(Byrne et al. 2000a)	Unadjusted	192		o	o	o								
Never HT users	BMI adjusted			o	o	o								
(Diorio et al. 2005)	Unadjusted	783		+	o	+								
Never HT users	BMI adjusted			o	o	o								
(Greendale et al. 2007)	Unadjusted	400	+											
Past HT users	BMI adjusted		+											
(Johansson et al. 2008)	Unadjusted	226	o	o	–	+*								
Never HT users	BMI adjusted		o	o	–*	o								
(Tamimi et al. 2005)	Unadjusted	520	+*											
Past HT users	BMI adjusted		+											

+, positive significant correlation ($p<0.05$); –, negative significant correlation ($p<0.05$); o, no correlation; * weak correlation ($p\sim0.1$); HT, hormone therapy

breast cancer risk appears to be specific for steroid hormone-sensitive tumors (Tworoger and Hankinson 2008).

Regarding mammographic densities, one study showed no correlation of serum PRL levels with either relative or absolute breast density measures among premenopausal women (Boyd et al. 2002c). However, in postmenopausal women not currently using exogenous HRT, positive relationships for relative breast density as well as absolute dense breast area before and after BMI adjustment were published in a number of studies (Boyd et al. 2002c; Greendale et al. 2007; Tamimi et al. 2005), although two further studies showed no such correlations (Bremnes et al. 2007b; Johansson et al. 2008).

Table 14.4 Hormonal exposures, mammographic density and breast cancer risk

	Breast cancer	Breast density
HRT (E+P)	↑	↑
Tamoxifen	↓	↓
Plasma prolactin	↑	↑
Plasma sex steroids	↑	o
Aromatase inhibitors	↓	–
GnRHA	–	Possibly ↓
IGF-I	↑	o

14.9 Discussion

We have reviewed observed relationships between hormonal exposures, measures of mammographic density, and breast cancer risk, with the aim to examine whether mammographic density measurements could be seen as a potential intermediate marker of hormonal influences on breast cancer risk. Key observations include some striking parallels between the associations of combined postmenopausal estrogen and progestin replacement therapy with, on the one hand, mammographic densities and, on the other hand, breast cancer risk. Further, equally interesting parallels are the inverse associations of both mammographic density and breast cancer risk with the selective ER modulator of tamoxifen, and direct associations with PRL (Table 14.4). The effects of aromatase inhibitors and gonadotropin-releasing hormone agonists on breast density, as well as on breast cancer risk, still require further investigation.

At first sight, the interesting parallel findings for HRT use may suggest that the combination of estrogens and progestins enhance breast tumor development through pathways—e.g., enhanced cell proliferation—that simultaneously are reflected by increased mammographic densities. However, several observations would seem to challenge this view. In the Nurses' Health Study cohort (Tamimi et al. 2007; Ziv et al. 2004), but also in the study of the San Francisco Mammography Registry (Tamimi et al. 2007; Ziv et al. 2004), increased mammographic density was associated with higher risks of both ER+/PR+ and ER−/PR− breast cancers. These findings are in stark contrast with observations that combined HRT use (Chen et al. 2006; Fournier et al. 2008; Kumar et al. 2007; Li et al. 2003), but also circulating (blood) levels of estrogens (Eliassen et al. 2006; Missmer et al. 2004; Tamimi et al. 2007) and PRL (Tworoger et al. 2004; Tworoger et al. 2007) among both pre- and postmenopausal women, are related specifically to the risk of ER+ tumors.

The findings from tamoxifen intervention studies, showing reductions in both breast cancer occurrence and mammographic density in the tamoxifen intervention groups, strongly suggest a role for estrogens in the regulation of breast epithelial and/or stromal proliferation patterns, as well as in breast tumor promotion. Again, however, this reduction in tumor occurrence appears to be specific to ER+ tumors (Fisher et al. 2005). A further contrasting finding is that in cross-sectional studies there is no

clear evidence for a positive association of circulating estrogens with mammographic density, although observations from a few studies suggested that this lack of association could have been due to (residual) confounding by adiposity. Besides the estrogens, there is a total absence of association between circulating total or bioavailable androgens and mammographic density measures, again in stark contrast with observations from prospective cohort studies that found elevated serum androgens (androstenedione, testosterone) are associated with increased risks of breast cancer among both pre- and postmenopausal women.

Taken together, these various observations might lead to speculation about whether the effects of estrogen-plus-progestin HRT regimens on mammographic densities could be unrelated to the mechanisms by which such regimens increase breast cancer risk. Both the observational and intervention studies have indicated relatively acute changes in mammographic density upon either starting or stopping HRT use. It has been speculated whether these effects might be due specifically to the progestogenic component, which might cause the intralobular tissue to loosen and to become more edematous (Campagnoli et al. 2005), as also occurs naturally during the menstrual cycle. According to this speculation, mammographic density variations might merely reflect differences in tissue water content, but would be necessarily related to the types of physiological changes (e.g., in tissue proliferation and/or apoptosis) that might enhance tumor development.

An alternative speculation would be that combined estrogen-plus-progestin HRT regimens do enhance breast tumor development, but largely through mechanisms that are independent of estrogen and/or progesterone receptors. One well-documented effect of combined (oral) HRT regimens including synthetic progestins is their capacity to reduce the hepatic synthesis and circulating levels of IGF-I (Campagnoli et al. 2005). From a physiological perspective, however, such decrease in IGF-I would be expected to reduce the risk of breast cancer, and possibly also mammographic density, which is not what the majority of epidemiological studies have shown.

Regarding serum IGF-I levels, findings are not fully consistent with respect to breast cancer risk, with some studies showing an increase in risk only among premenopausal women, others only among older women, and some studies showing no relationship at all. Likewise, cross-sectional relationships did not uniformly show a direct association between circulating IGF-I and mammographic densities, although results from some studies did suggest a possible relationship especially among premenopausal women.

It is perhaps for PRL that findings are most coherent, so far, with clear positive associations of serum PRL levels with both mammographic density and breast cancer risk, among both pre- and postmenopausal women. The number of studies showing these associations, however, is still relatively small.

In summary, although there are some intriguing parallel findings relating exogenous hormones (estrogen-plus-progestin HRT), tamoxifen, or endogenous hormones (PRL) to both increased mammographic density and increased breast cancer risk, there are also major discrepant observations regarding the role of sex hormones in the regulation of mammographic density. It is therefore impossible, at present, to propose mammographic density measures as an intermediate measure of risk, integrating the effects of exogenous and/or endogenous hormones on breast tumor development.

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Part VI

Cancer Prevention and Target Organs II: Cancer of the Digestive Tract

Chemoprevention of Oesophageal Cancer and the AspECT Trial

15

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Abstract Oesophageal cancer is on the rise and often present in an advanced state. Advances in surgical techniques, chemotherapy and radiotherapy have not changed the prognosis of oesophageal cancer over the last 20 years. With the unravelling of molecular biology of carcinogenesis in the oesophagus, there is a need for a paradigm shift from cancer treatment to prevention. Barrett's oesophagus is the commonest pre-malignant condition for development of oesophageal adenocarcinomas and is eminently suitable for the study of chemoprevention strategies. Now in its third year, the AspECT trial is the biggest, multicentre, randomised controlled clinical trial looking at the long-term chemoprevention effect of esomeprazole with or without aspirin. More than 85% of the participants tolerated the medications at the initial intended doses, and the drop-out rate has been 7%; the interim analysis is due in 2011.

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15.1 Introduction

Oesophageal cancer is notorious in presenting late and has a bad prognosis with over 85% 5-year mortality (Eloubeidi et al. 2003). Though squamous cancer of the oesophagus is the major variety worldwide, oesophageal adenocarcinomas is a resurgent problem in most Western populations with a relentless rise in incidence. Studies have shown that this rise in incidence is a true rise rather than just a reflection of improved surveillance.

Developments in surgical and neoadjuvant chemo- and radiotherapy have failed to make any real improvement in overall survival of patients with oesophageal cancer, apart from increasing the 1-year survival rate. This is in contrast to the improvements seen with other gastrointestinal cancers such as colon cancer, where survival rates are increasing in part due to better therapy.

With this background, the way forward to genuinely make a difference is to prevent progression from Barrett's oesophagus to neoplasia. The world of chemoprevention is evolving fast in a variety of cancers and oesophageal cancer is one of them.

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15.2 Barrett's Oesophagus

Barrett's oesophagus is a pre-malignant condition affecting 1% of the population in the West. Even though most patients with Barrett's will not develop oesophageal cancer, the incidence of adenocarcinoma is 0.45%–1% (Shaheen et al. 2000; Jankowski et al. 2002), conferring a 40-fold increased risk compared with the general population. The risk rises to 40%–50% within 5 years for those with high-grade dysplasia.

In the United States, adenocarcinomas develop in Barrett's oesophagus at a rate estimated to be one case per between 175 and 441 patient-years of follow-up (Cameron et al. 1985; Spechler et al. 1984). The rate of cancer development in Barrett's may also depend on the epidemiological cohort studied: i.e. the rate of cancer development is twice as high in white males as in black females (Bani-Hani et al. 2000). The extent as well as severity of dysplastic change may also be important (Buttar et al. 2001).

Though there have been suggestions from some studies that the length of Barrett's segment may modify the risk (Avidan et al. 2002), a recent meta-analysis found that there was no significant difference in cancer risk in short segment Barrett's oesophagus [odds ratio (OR) 0.55; 95% confidence interval (CI): 0.19–1.6); p=0.25] compared to conventional Barrett's segment (Thomas et al. 2007). The same meta-analysis also found that there were no significant overall differences in the cancer development rate per patient-year of follow-up among UK [7/1,000 (CI: 4–12)], United States [7/1,000 (CI: 5–9)] and European [8/1,000 (CI: 5–12)] studies. However, if two large studies were excluded (having flawed inclusion criteria in which patients with benign hiatus hernias rather than Barrett's were analysed) then the UK cancer development rate would have gone up to 10/1,000 (CI: 7–14).

15.3 Need for Chemoprevention Approach Rather Than Surveillance

An examination of National Cancer Institute's Surveillance, Epidemiology, and End Results database (USA) showed that the number of cases of oesophageal adenocarcinoma increased at a greater rate between 1975 and 2001 than any other major type of cancer in the United States, outpacing even those of melanoma, breast cancer and prostate cancer in terms of the rapidity of rise (Pohl and Welch 2005).

Although highly touted in the literature, surgical and endoscopic ablation therapies are limited by several factors, including high rates of symptom recurrence, persistently abnormal pH values, need for repeat surgery, and, in the case of endoscopic therapy, residual Barrett metaplasia that can progress to high-grade dysplasia or cancer (Ragunath et al. 2005).

Some forms of surveillance for dysplasia in Barrett's oesophagus are being offered in suitable patients almost worldwide in a hope to diagnose early neoplasia and thus offer curative treatment when prognosis is good. In the United States and the Europe there have been recent updates in guidelines for such programmes (Wang and Sampliner 2008; Boyer et al. 2007).

However, there are several pitfalls that mar the results expected from screening programmes due to the inherent weakness of these surveillance programmes.

At diagnosis, oesophageal adenocarcinoma is frequently accompanied by Barrett's metaplasia, though only approximately 5% of patients who present with oesophageal adenocarcinoma have an antecedent diagnosis of Barrett's metaplasia. The majority of patients presenting with oesophageal adenocarcinoma will therefore not benefit from refinements to endoscopic surveillance programmes for Barrett's metaplasia (Dulai et al. 2002; Corley et al. 2002).

Studies in Barrett's cohort under surveillance have shown that half of patients who developed high-grade dysplasia/adenocarcinoma had no dysplasia on their first two endoscopies (Sharma et al. 2006).

Histological diagnosis of dysplasia and its grading remains confounded by subjective variation. Although speciality pathologists may be able to make reproducible diagnoses of high-grade dysplasia (Montgomery et al. 2001), community pathologists only reproduce specialty pathologist diagnoses of high-grade dysplasia about 30% of the time (Alikhan et al. 1999).

About 40% of patients with oesophageal adenocarcinoma have no history of reflux symptoms (Inadomi et al. 2003). Consequently, screening programmes that target only patients with heartburn can have only limited impact on cancer mortality rates, and there is little evidence that these programmes have prevented deaths from oesophageal adenocarcinomas. No study has established the reliability of surveillance in detecting curable dysplasia, and a number of reports have documented the development of incurable malignancies in some patients despite adherence to endoscopic surveillance programmes (Conio et al. 2003; Peters et al. 1994).

Given the fact that there is no linear correlation to progression from mild to moderate to high-grade dysplasia and finally to adenocarcinoma, the optimum time interval between surveillance endoscopies are rather driven by cost-effectiveness analysis, which are again derived from computer modelling. Such computer modelling uses a lot of assumed data and is thus fraught with generating misleading information (Provenzale et al. 1999; Inadomi et al. 2003).

15.4 The AspECT Trial

The AspECT Trial (Aspirin Esomeprazole Chemoprevention Trial) is a pragmatic, multicentre, phase III, randomised, open label trial and is currently recruiting participants. It started enrolling in September 2005 in the UK. It is organised by the National Institute for Cancer Research and has been reviewed and funded by external grant authorities in the United Kingdom (namely Cancer Research UK, Medical Research Council, National Cancer Research Institute, and the University Hospitals of Leicester). Of a target population of 2,500, in the middle of September 2008, 2075 patients have enrolled. It is the second-fastest recruiting Randomised Controlled Trial (RCT) in UK and is already the largest Barrett's RCT in world. The follow-up will last at least 8 years, with 2 years of initial recruitment, for a total of 10 years. Patients will receive endoscopy and biopsy examinations every 2 years.

The trial has a 2×2 design and the patients are randomised into four groups. Table 15.1 shows the format of the study.

Aspirin does confer a risk of dyspepsia and gastrointestinal bleeding. Concomitant proton pump inhibitor (PPI) use reduces the risk. However, in the AspECT study provision has been made to take this into consideration. Therefore, if new dyspeptic symptoms arise, a dose reduction protocol will be used for aspirin, decreasing from 300 mg/day, then 100 mg/day, and ultimately 75 mg/day. If a gastrointestinal bleed occurs (melaena, haematemesis, decreased haemoglobin), then immediate and permanent cessation of the aspirin will occur. No washout period will be required for individuals already on aspirin or PPI to allow baseline blood tests and biopsy samples to be assessed easily.

The primary end point is all-cause mortality. We suspect that low-dose aspirin therapy will benefit people with Barrett's metaplasia because some studies suggest that this group has a higher-than-average incidence of ischaemic heart disease and cardiac death, with 42% dying of vascular-related disease compared with 32% in a sex- and age-matched population in the UK (Solaymani-Dodaran et al. 2005). A recent study from Leicester, UK, showed that patients with Barrett's died more commonly from chest infec-

Table 15.1 The 2 × 2 factorial design of the AspECT study

	Low-dose PPI	High-dose PPI
No aspirin	Esomeprazole 20 mg Symptomatic treatment *n* = 625	Esomeprazole 80 mg Strong acid suppression *n* = 625
Aspirin	Esomeprazole 20 mg +300 mg or less aspirin *n* = 625	Esomeprazole 80 mg +300 mg or less aspirin *n* = 625

PPI, proton pump inhibitor

tion and ischaemic heart disease than oesophageal adenocarcinoma (Moayyedi et al. 2008).

The other main objective of the study is to see whether aspirin or esomeprazole has any role in reducing transformation of Barrett's mucosa to high-grade dysplasia or adenocarcinoma, or influencing the mortality in such patients.

Secondary objectives are the identification of clinical and molecular risk factors for the development of Barrett's adenocarcinoma and the evaluation of cost-effectiveness of aspirin, PPI treatment, or both in the prevention of Barrett's adenocarcinoma.

The study also will investigate the molecular changes found in the Barrett's segment and adjoining oesophageal and gastric tissues to identify possible biomarkers. In addition, microarray analysis is underway to identify novel DNA single nucleotide polymorphism (SNP) signatures and target regions for expression analysis and investigation of clonality.

Preliminary analysis of data returns in early 2008 indicate that tolerability is good, with 85%–90% of subjects remaining on the randomised medications at the intended doses, with only 1% needing a reduction of the esomeprazole dose from 80 mg; 3% needed an increase in dose of PPI to 40 mg from 20 mg for symptom relief; 2% needed their dose of aspirin reduced; and another 3% had to come off aspirin. Of enrolled patients, 7% have opted out of future participation.

An indirect benefit has also been shown in terms of improvement in the quality of the surveillance programme for Barrett's oesophagus in centres participating in the AspECT trial. Not only are more biopsies being taken (ideally quadrantic biopsies every 2 cm) in patients undergoing surveillance as part of the AspECT trial, but also in those that are under surveillance but did not participate in the trial (Das et al 2008).

15.5 Trials Related to AspECT

Allied to the AspECT trial, two other large trials are also on their way to study two other aspects of Barrett's oesophagus.

The BOSS trial (Barrett's Oesophagus Surveillance versus no Surveillance) investigates the effect of regular surveillance against no surveillance in Barrett's oesophagus patients. The BOSS trial has been designed to determine whether providing regular endoscopies for patients with Barrett's oesophagus, in order to detect progression of disease towards precancerous conditions, is cost-effective. This trial will have 2,500 patients with 1,250 randomised to no surveillance and the others randomised to two yearly follow-ups with endoscopy. Everyone will get yearly phone and questionnaire follow-up to check for symptoms of oesophageal cancer.

The HiGH trial (High Grade Dysplasia Histology), the first rigorous trial to assess patient outcomes of different management options for

high-grade dysplasia in Barrett's oesophagus, aims to recruit 300 patients and randomly allocate surgically fit patients to continued surveillance endoscopy with endoscopic treatments or surgical resection. Those who are not suitable for surgery will be allocated either to surveillance or endoscopic treatments [endoscopic mucosal resection (EMR) and HALO system promoted by BarRX Medical, Sunnyvale, CA].

15.6 The Evidence Base for Using Aspirin and Esomeprazole

15.6.1 Aspirin

Aspirin has been around for a long time and has been extensively used in the treatment of a variety of conditions. A substantial proportion of the population uses the drug. It is also cheap.

The actual incidence of oesophageal adenocarcinomas is still low, and thus to really make a perceptible change in the absolute risk of developing adenocarcinomas, the number needed to treat (NNT) would be high. For example to reduce the absolute risk from 0.5% to 0.25% per year, the NNT (=1/absolute risk reduction) is 400 per year. With such a large NNT the intervention has to be cheap and safe to be acceptable for practical implementation.

Over-expression of COX-2 in vitro has been shown to have a number of cellular effects including increasing proliferation, reducing apoptosis (Tsujii and DuBois 1995), promoting angiogenesis (Jones et al. 1999), decreasing E-cadherin expression, and increasing invasive potential (Tsujii et al. 1997), and all of these factors could be involved in the transformation from a benign to malignant phenotype in Barrett's.

Inducible COX-2 enzyme expression is greater in Barrett's tissue than oesophagitis or normal controls (Abdalla et al. 2005) and the expression increases as tissue progresses along the metaplasia–dysplasia–adenocarcinoma sequence (Morris et al. 2001).

Chemoprevention possibilities of non-steroidal anti-inflammatory agents (NSAIDs) have been shown in multiple epidemiological studies to be associated with a significantly reduced risk of cancer, with an OR of 0.57 (95% CI, 0.47–0.71) (Corley et al. 2003). This decreased risk has also been substantiated with the observation that known biomarkers such as aneuploidy and tetraploidy were also reduced with NSAIDs (Vaughan et al. 2005). However, in the Chemoprevention for Barrett's Oesophagus Trial (CBET), a phase II multicentre randomised placebo-controlled trial of celecoxib in patients with Barrett's oesophagus and low- or high-grade dysplasia, the administration of 200 mg of celecoxib twice daily for 48 weeks of treatment did not appear to prevent progression of Barrett's dysplasia to cancer (Heath et al. 2007).

Thus, whilst there is good evidence for a role of aspirin and COX inhibitors in modulating molecular biology of carcinogenesis in Barrett's oesophagus (Table 15.2) and its role has support from retrospective and observational studies, there is little evidence of its chemoprevention efficacy from large prospective studies. This will hopefully be addressed by the AspECT study.

15.6.2 Esomeprazole

Acid and bile are thought to be responsible for damaging the superficial squamous mucosa, provoking an inflammatory response, and initiating the development of an acid/bile resistant, mucin-secreting lineage from the squamous epithelial stem cells (Vaezi and Richter 1996; Jankowski et al. 1999). Acid exposure in vivo and in vitro has been shown to activate the mitogen-activated protein kinase (MAPK) signalling pathway, with subsequent transcription

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Table 15.2 Cell signalling pathways modulated by proton pump inhibitors and aspirin

Major pathways		Relevant molecular markers
Cell cycle/checkpoint controls		p53, cdc2, p27, Rb
Apoptosis/caspase pathway controls		FasL, TNF, Bcl–2, c-myc
Growth factor phosphorylation[a]		EGFR, GRB/SOS, PKC, AKT
Cytokine signalling pathway		Stat 1–6, NF-κB and Smad transcription
MAP kinase pathway[a]	Raf–1 path MEKK path	ERK SAPK, JNK, p38
Chromatin regulation and methylation		-
B-catenin and WNT signalling pathway		-
COX–2 receptors		-
Cell adhesion molecules		Cadherin/integrin

[a]Denotes pathways modulated by proton pump inhibitors

factor expression (Souza et al. 2002; Table 15.2). Fitzgerald et al. used an ex vivo model to demonstrate that intermittent acid exposure favoured an undifferentiated phenotype and greater cell proliferation—a pro-carcinogenic combination—whereas continuous acid exposure had the reverse effect (Fitzgerald et al. 1996).

Studies have shown that Barrett patients treated with PPIs developed dysplasia less frequently than those treated with histamine H2-receptor antagonists, which are less effective at controlling gastric acid secretion (El-Serag et al. 2004). Furthermore, a significantly increased rate of cell proliferation and pro-proliferative cell cycle abnormalities have been detected in biopsies of Barrett epithelium from patients treated with H2-receptor antagonists compared with biopsies from patients treated with PPIs (Peters et al. 2000; Umansky et al. 2001). Long-term PPI use has become the mainstay of treatment of patients with Barrett's oesophagus.

Data from two retrospective cohort studies suggest that PPI therapy significantly reduces the likelihood of developing dysplasia (El-Serag et al. 2004; Hillman et al. 2004). This provides a rationale to treat even asymptomatic Barrett's oesophagus patients with PPI. The benefit of acid suppressive therapy as a means of preventing cancer has not been documented prospectively. Studies have suggested that normalisation of oesophageal acid exposure may decrease markers of proliferation (Ouatu-Lascar et al. 1999; Hillman et al. 2008). However, there are currently no data that directly support the use of high-dose anti-secretory therapy to delay or prevent the development of oesophageal adenocarcinoma.

Long-term use of PPIs has been shown to be safe and effective (Klinkenberg-Knol et al. 2000) and thus, PPI therapy would seem to be an efficacious, well-tolerated and safe treatment for Barrett's patients. However, the advent of more potent inhibitors of acid production in the past 20 years has done nothing to slow the rising incidence of oesophageal carcinoma. Indeed, in some studies patients receiving medical treatment for gastro-oesophageal reflux disease (GERD) had a higher risk of development of oesophageal cancer (adjusted odds ratio 2.9) than the untreated (Lagergren et al. 1999). This negative effect of acid suppression could be mediated through gastrin that plays a complex

role in regulation of epithelial proliferation and differentiation.

The long follow-up period and large size of the AspECT trial should give us more definitive answers. Additionally, the use of high and standard dose arms in the trial will shed light on the optimal goal of acid control and if higher cancer rates are really associated with intensive acid suppression.

15.7 Conclusion

Despite improvements in surgery, chemotherapy and radiotherapy, the prognosis for advanced oesophageal cancer remains dismal. Years after the initiation of a number of surveillance programmes aiming to pick up the early stages of this cancer, the majority of cases still present late. Furthermore, adenocarcinomas diagnosed in patients participating the targeted surveillance programme account for a minority of all cases of oesophageal adenocarcinoma.

Emphasis is shifting to prevention strategies. Barrett's oesophagus is a well-characterised premalignant condition that lends itself to the study of chemoprevention very well. AspECT is the largest randomised controlled trial in Barrett's oesophagus, looking into chemoprevention of one of the most notorious malignancies of the Western world. Two well-studied agents, aspirin and esomeprazole, are being used in a 2×2 study design, with planned follow-up of 8 years. There is a good evidence base from observational and retrospective studies for possible chemoprevention actions of both of these agents, but prospective studies are lacking. The study will also address genetic and epigenetic factors relating to carcinogenesis in Barrett's oesophagus, and the related HiGH and BOSS trials will also assess the natural history of dysplasia development in Barrett's segment and also the effectiveness and safety of various surgical and endoscopic interventions in the same setting.

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Part VII

Cancer Prevention and Target Organs III: Prostate Cancer

Review of Diagnostic Markers for Prostate Cancer

16

Fritz H. Schröder

Abstract This review concentrates mainly on currently available markers for prostate cancer and cannot cover the multiple marker substances which are now in experimental and clinical development. Prostate-specific antigen (PSA) is still the main diagnostic tool despite its serious limitations, which will be addressed. Studies of new diagnostic markers and also most studies of PSA are subject to attribution or assignment bias, which limits the accuracy of the resulting information. Usually a more or less arbitrarily chosen cut-off value is used as a "gold standard" to determine the indication for the decisive test, a prostatic biopsy, and the assumption is made that no cancers are present below that cut-off value. This assumption has been proved wrong by findings in the control arm of the Prostate Cancer Prevention Trial (PCPT), where more than 5,000 men were biopsied independent of their PSA status. As an example: a PSA cut-off value of 4.0 ng/ml, a commonly used biopsy indicator, missed about 75% of all biopsy-detectable cancers. On the other hand, sextant biopsies in all men led to a detection rate of 21.9%, evidence of the diagnosis of many cases in men who otherwise would never have had any clinical signs of prostate cancer (overdiagnosis). The only way out of this dilemma is a better understanding of the natural history of those cases with low PSA values that would not be considered suspicious with the use of currently available risk indicator nomograms. The European Randomised Study of Screening for Prostate Cancer (ERSPC) offers such an opportunity. Results are summarised in this chapter. Evidence is provided that men diagnosed in the low PSA ranges (<3.0 ng/ml) usually present with more favourable cancers which, when identified, are often eligible for active surveillance after application of the appropriate nomogram. In addition, the data in the setting of the ERSPC study show that biopsy in such men can safely be delayed until PSA rises to above a cut-off value of 3.0 ng/ml. The limitations of PSA discussed herein clearly point to the need to find better diagnostic and prognostic markers for prostate cancer.

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Multiple markers are available for the diagnosis of prostate cancer, and multiple new markers are under development. Prostate-specific antigen (PSA) has become the mainstay not only in the early diagnosis of prostate cancer (Catalona et al. 1991) but also as a parameter of aggressiveness or indolence of diagnosed cancers, as well as an indicator of future clinical progression with the highest accuracy in cases that have been treated by potentially curative strategies. However, PSA has major limitations in diagnosing prostate cancer that are mainly due to the fact that the enzyme is prostate spe-

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cific but not prostate cancer specific. In the age group in which prostate cancers are most commonly diagnosed (55+), benign enlargement of the prostate (benign prostatic hyperplasia, BPH) is also prevalent. BPH leads to elevations of PSA that overlap with those PSA levels which are due to the presence of localised prostate cancer. Specifically, the PSA area of 4.0–10.0 ng/ml had therefore been termed to be the "grey zone" of PSA. Molecular sub-forms of PSA, such as fPSA and human glandular kallikrein 2 (hK2), contribute to the differentiation of BPH and to the identification of aggressive prostate cancer where PSA reflects prostatic volume more than total PSA. Therefore, the ratio between the two substances can be used to improve on the predictive value of total PSA specifically in the grey zone of PSA. PSA, the molecular sub-forms of PSA and the other kallikreins have been subject to extensive recent reviews (Stenman et al. 2005; Lilja et al. 2008; Schröder et al. 2005; Steuber et al. 2007; Mullins et al. 2008).

New markers are under development. The limitations of this review will not permit us to deal with markers that are currently being tested at the tissue level and as serum and urine markers that are in preclinical development. The paper concentrates on the PSA-related issues, with the inclusion of other readily available and commonly used clinical parameters. This paper would, however, be grossly incomplete without mentioning at least three marker substances that are considered promising in terms of either replacing or implementing the value of PSA. The PCA3 test is based on the *DD3* gene that was shown to be prostate cancer specific. The gene can be studied at the RNA level in the urine after prostatic massage. It has been shown to reduce the number of biopsies needed in men who have had a previous negative biopsy based on an elevated PSA (Hessels et al. 2003; Van Gils et al. 2007). The full diagnostic value of PCA3 is still unknown but is under study. Another example is early prostate cancer antigen (EPCA-2), which has been studied in serum in a pilot study (Leman et al. 2007). This first study shows a high rate of sensitivity and specificity for prostate cancers and seems capable of differentiating between locally confined and locally advanced prostate cancer. The data need to be confirmed. As with other new markers, the effect of attribution bias arising from the use of another substance such as PSA as the gold standard for cancer detection is present in this study. It arises from the fact that men with low PSA values who have not undergone biopsy are likely to harbour unknown numbers of prostate cancers that might be detected with the new marker substance. Another potentially important marker relates to the expression of the gene fusion of TMPRSS 2–ERG that is detectable in urine. One recent study that is also subject to attribution bias that limits the conclusions drawn by the authors is available. The study shows that positive and negative predictions of PCA3 in the urine are significantly improved by the addition of TMPRSS 2–ERG fusion status (Laxman et al. 2008). Obviously, confirmation of these findings in a prospective setting is necessary. The recently discovered fusion gene has also been studied in various settings as a marker for aggressiveness.

The remainder of this manuscript will concentrate on PSA-driven detection of prostate cancer in conjunction with other readily available clinical information.

16.1 Characteristics of PSA-Driven Screening

The value of PSA as a potential screening test was first described in 1991. At that time a normal value of 4.0 ng/ml was determined on the basis of the distribution of the marker in the male population. Later it became evident that many prostate cancers are present in the lower PSA ranges (Kranse et al. 1999). Only recently,

due to information becoming available from the control arm of the Prostate Cancer Prevention Trial (PCPT), has the true relationship between PSA and biopsy-detectable prostate cancer become evident (Thompson et al. 2003). More than 8,000 men in the control arm of this study were eligible for biopsies at yearly follow-up screens if they presented with a PSA level of 4.0 ng/ml or higher or an abnormal rectal examination. In addition to that, they were offered biopsies at the end of the study period of 7 years. A large group of at least 4,692 men above the age of 55 years were all eventually biopsied. This set-up allowed for the study of sensitivity and specificity of the procedure followed with minimal attribution bias. Table 16.1 summarises some of the data presented by Thompson et al. (2006). The traditional cut-off of 4.0 ng/ml has a sensitivity of 24.8% to detect any prostate cancer. This means that below the cut-off of 4.0 ng/ml, 75.2% of all biopsy detectable cancers can be found. The same cut-off identifies correctly 40.4% of cancers with Gleason scores exceeding or at 7.0. Of these potentially aggressive tumours, 59.6% are missed. It is evident from the data shown in Table 16.1 that there is no cut-off value identifiable where sensitivity and specificity match in a satisfactory fashion. The justified conclusion is that PSA cannot be used as a dichotomous parameter; the relationship between biopsy-detectable prostate cancer and PSA values is continuous. The situation described here has also been named the "PSA dilemma".

16.1.1 PSA and Cancer Aggressiveness

There seems to be no immediate sensible way out of the PSA dilemma. If a suitable marker to identify potentially aggressive or indolent disease would be available, this would be the right indication for its use. Unfortunately, this is not (yet) the case. Recent information from the European Randomised study of Screening for Prostate Cancer (ERSPC; section Rotterdam), however, has shown that low PSA values are significantly more frequently associated with potentially insignificant or clinically indolent prostate cancers (Postma et al. 2007). In this report 550 radical prostatectomy specimens were analysed. Prostate cancer volumes were measured planimetrically, Gleason scores were determined and the specimens were evaluated according to previously published definitions of "minimal tumour". It turned out that the proportion of minimal disease in the first screening round and in the second screen 4 years later was inversely related to the PSA level. Below a PSA of 3.0 ng/ml in the first and second round, respectively 67% and 56% of cases, were

Table 16.1 Performance characteristics of PSA in detecting prostate cancer (PC). From the PCPT trial (Thompson et al. 2006)

PSA level[a] (ng/ml)	Any PC vs no PC		Gleason>7 vs Gleason<7 or no PC	
	Sensitivity %	Specificity %	Sensitivity %	Specificity %
1.1	82.0	40.6	92.8	37.8
2.1	54.4	70.8	75.6	67.3
3.1	35.8	85.1	57.6	82.3
4.1	24.8	92.3	40.4	90.0
10.1	1.0	99.5	2.4	99.5

[a]*n*=4,692, men age>55, all biopsied

classified as "minimal tumour". These data suggest that PSA together with other ancillary parameters may be useful to identify potentially insignificant cancers that may be clinically identical with "indolent" disease.

These findings were pursued further in a 12-year follow-up study of ERSPC data. It turns out that the risk of being diagnosed with prostate cancer that becomes progressive after 12 years or that is identified at a second screen as being potentially aggressive is extremely low (Schröder et al. 2008a, b). PSA together with other potential predictors of potentially indolent (insignificant) prostate cancer was therefore used to validate a predictive nomogram that was previously described by Kattan et al. (2003). The validation procedure showed that more than twice as many screen-detected prostate cancers have potentially insignificant characteristics in radical prostatectomy specimens when compared to clinically diagnosed cases. This has led to the description of a nomogram for the identification of indolent disease (Steyerberg et al. 2007).

16.2 How to Improve on PSA in the Detection of Prostate Cancer?

It is now obvious that the initial notion that PSA could be used with an arbitrarily defined cut-off value requires revision. The present knowledge that many prostate cancers in the low PSA ranges have the characteristics of being "potentially indolent" allows different applications of this important marker. An obvious next step to improve on the diagnostic value of PSA would be an attempt to improve on its relative specificity (avoiding unnecessary biopsies) by correcting for the presence of BPH usually reflected in an enlarged prostate. Also, other potential risk indicators such as age, family history, presence or absence of micturition complaints, the presence or absence of an abnormal digital rectal examination (DRE), or an abnormal lesion on transrectal ultrasonography (TRUS) were studied in a multivariate analysis. Many predictive nomograms have therefore arisen and are subject to a recent review (Schröder and Kattan 2008). This review shows that predictive nomograms are of great importance but will only produce reliable results if utilised in the population from which they have been derived. The same principle is obviously true for a risk calculator that was recently produced on the basis of data derived from the ERSPC, section Rotterdam (Kranse et al. 2008) and which is available on the Internet (www.uroweb.org). A screen shot of level 3 of the risk indicator is shown in Fig. 16.1. Level 1 of this instrument utilises parameters that are available to any man at risk (considering age, family history and micturition complaints). While these parameters do give an indication of the risk of a positive biopsy, they are overruled by the PSA value that is subject to indicator 2. On the other hand, once urological examination has been carried out, the additional availability of DRE, prostate volume and the results of transrectal ultrasonography improve the prediction of PSA alone in a significant fashion (Fig. 16.1). A large number of unnecessary biopsies can be saved utilising this risk indicator in a setting where men in the age group 55–75 request opportunistic screening.

16.2.1 PSA Use in Men Less Than 50 Years Old

PSA use as a biopsy indicator below the age of 50 is unlikely to be useful because of the low prevalence of prostate cancer in this age group. However, recent data show that just one determination of PSA in this age group may be predictive of prostate cancer and aggressive

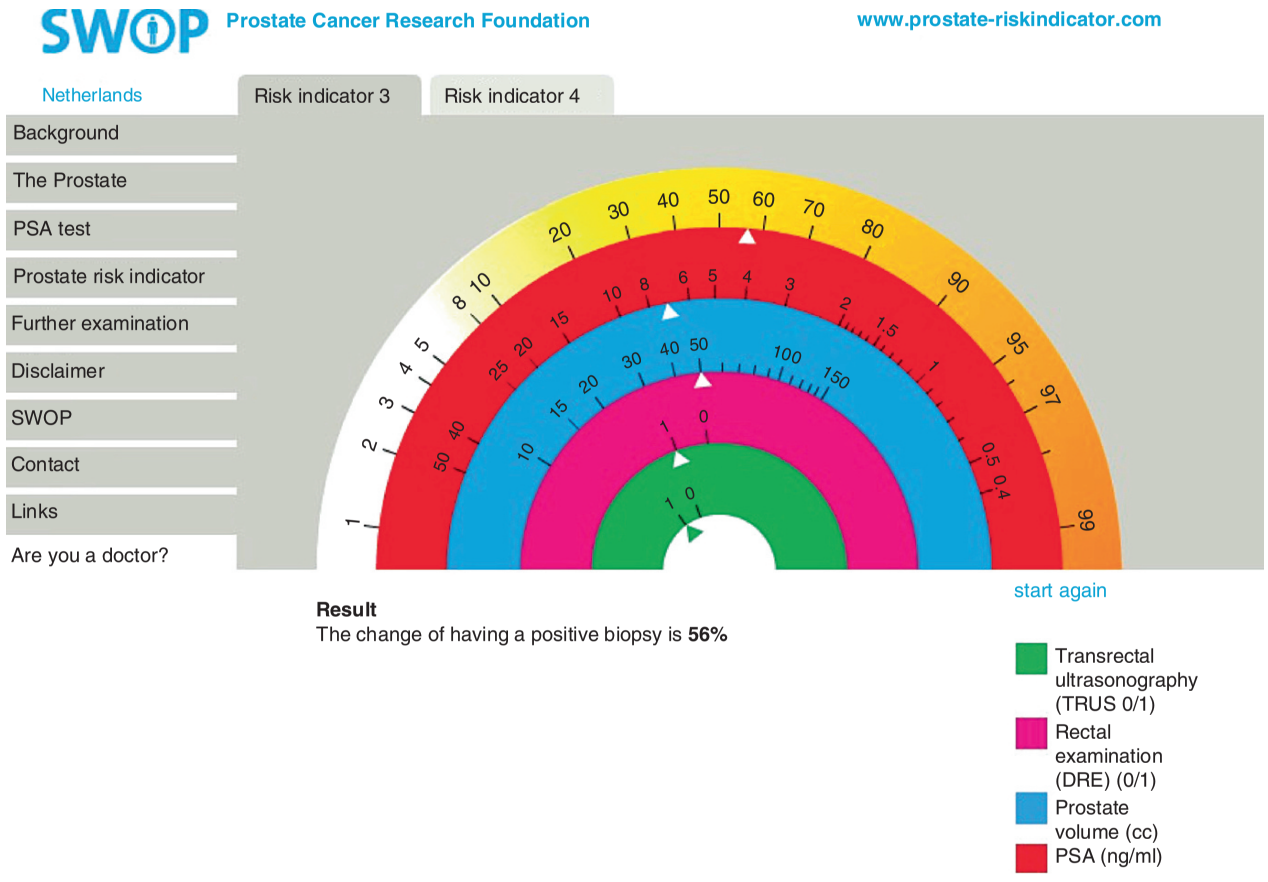

Fig. 16.1 The risk of finding prostate cancer at sextant biopsy of the prostate

prostate cancer 15–25 years later (Ulmert et al. 2008a, b). These observations are likely to be of value for the development of future strategies for the early detection of prostate cancer.

16.3 Selective Detection of Aggressive Prostate Cancer

The application of the nomogram for the prediction of indolent disease described by Steyerberg et al. (2007) to 1,629 cancers detected in two subsequent screening rounds in ERSPC Rotterdam identified 825 cancers that were suitable for nomogram use (that were "potentially indolent"). The application of the nomogram to all 1,629 cancers predicted that 485 of those (30%) could be classified as potentially indolent. This proportion amounted to 23% versus 44% of those cancers detected in the first versus the second screening round. It is likely that many of these cancers are "overdiagnosed" meaning that they would not have surfaced clinically or led to the death of their carrier without screen detection. It has been calculated that 54% of all cancers detected with the screening regimen of the ERSPC study can be classified as "overdiagnosed" (Draisma et al. 2003). Overdiagnosis is the result of the diagnosis of indolent tumours or of prostate cancers that may have aggressive patterns but are diagnosed in men who will intercurrently die of some other cause. It hasbeen shown that potentially indolent cancers are usually treated, which results in potential "overtreatment" (Cooperberg et al. 2007). At least in the European setting it is unlikely that screening for prostate cancer will be acceptable unless overtreatment is curbed. This highlights the importance of the recent development of the nomogram described above and the finding that 30% of screen-detected cancers canbe identified as potentially indolent (Roemeling et al. 2007).

In dealing with this situation, two options exist. One could attempt to identify selectively aggressive prostate cancers and leave the rest untreated. The other option is to try to identify overdiagnosed cases and treat the remainder. On the background of the available information that is summarised in Fig. 16.2 it seems more sensible to follow the second strategy. If potentially overdiagnosed cases are identified as potentially indolent using available nomograms, active treatment can be avoided. The remaining cancers will then become eligible for treatment.

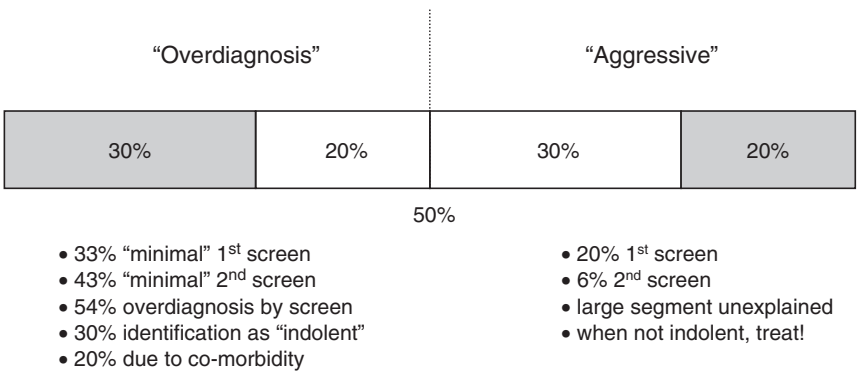

Fig. 16.2 Parameters for the identification of overdiagnosed versus aggressive disease with screening for prostate cancer

16.3.1 PSA Velocity

PSA velocity is the increase of PSA over time expressed in absolute terms per year of observation. Recent evidence is contradictory. However, as shown by Etzioni et al. (2007) it is unlikely that PSA velocity will be useful in diagnosing prostate cancer. It is, however, likely that the speed of increase of PSA over time relates to prostate cancer aggressiveness (Carter et al. 2006). This is also confirmed by data from ERSPC that are summarised in Table 16.2 (Schröder 2006). While this paper studying PSA velocity in men with initial PSA values of less than 4.0 ng/ml shows a relationship of increasing PSA velocity with cancer aggressiveness, the table clearly shows that any cut-off used will lead to missing substantial numbers of prostate cancers in a PSA range that otherwise would not be eligible for biopsy. Closest to the recommendation by Carter et al. (2006) is the PSA velocity of more than 0.50 ng/ml per year. Had this PSA velocity been used as a biopsy indication, 58 of the 167 cancers detected with a PSA cut-off of 4.0 would have been missed and only 65 (59.6%) of the 89 aggressive PCs would have been found (24, or 40.4%, would have been missed).

16.4 Needs and Expected Future Developments

The most important need is that level 1 evidence for the effectiveness of screening is shown. Two major studies, the Prostate, Lung, Colon and Ovary screening trial (PLCO trial) in the United States and the ERSPC trial in Europe, are set to produce this information during the coming years. Screening will only become generally accepted if it is clearly shown that prostate cancer mortality is reduced and that early detection measures are effective on an individual level, achieving the same goal. In the meantime, it is essential to improve screening regimens in anticipation of the need to make proper recommendations for screening procedures to governments and health-care providers. The on-going randomised studies produce large amounts of applicable evidence in this respect. The capability of identifying potentially indolent disease and of avoiding overtreatment is essential. Also, improvements in selectively identifying aggressive disease could improve the algorithm shown in Fig. 16.2 in such a way that attempts to identify indolent disease may become unnecessary. This is, however, unlikely to happen in the near future considering the present state of knowledge. Important recent progress has been made

Table 16.2 Prostate cancer detection in 588 men 4 years after a negative screen (PSA < 4.0 ng/ml, no biopsy). Comparison of PSA progression to PSA > 4.0 ng/ml and three PSA velocity (PSAV) cut-offs. All men were biopsied at the second screening. (Schröder 2006)

PSA/PSAV	No PC	PC (*n*)	Total (*n*)	PPV (2:3)	Aggressive
(ng/ml/year)	1	2	3		*n* (%)
PSA>4.0	421	167	588	28.4	89 (53.3)
PSAV>0.25	392	158	550	28.7	87 (55.1)
PSAV>0.50	278	109	387	28.2	65 (59.6)
PSAV>0.75	154	49	203	24.1	32 (65.3)
PSAV>1.00	76	34	110	30.9	22 (64.7)

PPV, positive predictive value

with respect to the identification of prostate cancer susceptibility genes (Gudmundsson et al. 2008; Eeles et al. 2008; Thomas et al. 2008). However, recent evidence suggests that these genes may not be predictive of aggressive cancer (Mucci et al. 2008). Progress in this field is rapid and could lead to breakthrough information.

16.5 Conclusions

The field of diagnostic markers for prostate cancer is moving rapidly. On the other hand, PSA—which has been established as a diagnostic tool since 1991—is still the main parameter indicating prostate biopsy as the determining diagnostic tool. This review shows that PSA cut-off values have limited use since recent data show that the relationship between PSA and the rate of positive biopsies is continuous and does not allow us to identify a cut-off value that relates sensitivity and specificity of detection in a scientifically acceptable fashion.

If a cut-off value is chosen, available nomograms and risk calculators may allow for a rational use of PSA. PSA also has been shown to relate to aggressive or, on the other hand, potentially indolent prostate cancer. Recently developed nomograms which include other predictors suggest 30% of screen-detected prostate cancers are potentially indolent. This allows the application of active surveillance in a scientifically justified fashion. The speed of rise of PSA over time (PSA velocity or PSA doubling) is more indicative of aggressive disease than of value in a diagnostic sense.

PSA determinations at an early age (age 40–50 years) may be useful in predicting future prostate cancer and the occurrence of future aggressive prostate cancer. These observations may be useful in designing screening strategies.

On-going randomised screening trials are likely to produce level 1 evidence for or against screening in the near future. The improvement of diagnostic strategies and the avoidance of overtreatment will be requirements of health-care authorities and providers if screening is to be introduced in health-care policy and financing.

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17 Selenium and Vitamin E Cancer Prevention Trial: A Nutrient Approach to Prostate Cancer Prevention

Barbara K. Dunn, Anne Ryan, and Leslie G. Ford

Abstract Prostate cancer continues to be both a major health threat, especially among African-American men, and a public health burden. However, growing evidence suggests that selenium and vitamin E may decrease the risk of this disease. The Selenium and Vitamin E Cancer Prevention Trial (SELECT), a phase III randomized, placebo-controlled study, is designed to determine whether selenium and vitamin E, alone or in combination, decrease the risk of prostate cancer in healthy men. SELECT opened to accrual on 25 July 2001 in more than 400 clinical sites across the United States, Puerto Rico, and Canada; the goal was to randomize 32,400 men. Accrual was completed in June 2004, 2 years ahead of schedule, with a total of 35,534 men randomized. Eligibility requirements include age of at least 55 years (African-American men at least 50 years), and no evidence of prostate cancer as determined by a serum PSA level of no more than 4 ng/ml and a digital rectal exam (DRE) not suspicious for prostate cancer. Participants were randomized to receive selenium (200 μg/day of *l*-selenomethionine) and/or vitamin E (400 IU/day of *all-rac*-alpha-tocopheryl acetate) supplementation for a minimum of 7 years (maximum of 12 years). The rationale for choosing these agents was based on preclinical data as well as analyses of secondary endpoints in cancer prevention clinical trials. The primary endpoint of SELECT is occurrence of prostate cancer based on community standards of diagnosis. Several other non-cancer endpoints are also being explored.

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17.1 Introduction

17.1.1 A Preventive Approach to the Problem of Prostate Cancer

Prostate cancer is the most common malignancy and the second most common cause of cancer-related death among men in the United States. An estimated 186,320 new cases and 28,660 deaths due to this disease are projected for 2008 [1]. Therapy for early stage cancer,

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either surgery or radiation, although effective, frequently results in adverse effects on quality of life [2]. Treatment of advanced or recurrent disease is at best palliative. Together, these challenges to the treatment of existing prostate cancer have stimulated an interest in developing preventive approaches to this widespread disease.

Androgens, long recognized as key contributors to prostate carcinogenesis, have been targeted in prostate therapy, leading to widespread use of anti-androgenic therapy in the treatment of advanced or recurrent disease [3, 4]. This etiologic and therapeutic association of androgens with prostate cancer coalesced with the interest in developing preventive modalities, leading to the hypothesis that an anti-androgenic agent would intervene in the carcinogenic pathway so as to decrease the risk of prostate cancer in men at risk of but *without* diagnosed disease. The anti-androgenic drug finasteride, already in use for benign prostatic hyperplasia, inhibits 5-alpha-reductase, the enzyme that converts testosterone to its more potent form, dihydrotestosterone. This mode of action suggested finasteride as an intervention in the first prevention trial, the Prostate Cancer Prevention Trial (PCPT). This phase III trial randomized 18,882 men aged 55 years or older without evidence of disease based on a normal digital rectal exam (DRE) and prostate-specific antigen (PSA) to finasteride (5 mg daily) or placebo for 7 years [5]. Finasteride reduced the 7-year prevalence of prostate cancer by 25% compared to placebo, although this favorable outcome was accompanied by an apparent 1.3% increase in the prevalence of high-grade prostate cancer. Accrual to PCPT was accomplished in a shorter than expected timeframe via the National Cancer Institute (NCI) cooperative group system at over 200 participating sites. This effective accrual infrastructure was intact at the time that the Selenium and Vitamin E Cancer Prevention Trial (SELECT) was initiated.

17.1.2 SELECT: Selection of Study Interventions

17.1.2.1 Selenium

Selenium, a nonmetallic trace element, is an essential nutrient for human health. In the United States the typical dietary intake is 80–120 μg daily and the recommended daily dietary allowance is 0.87 μg/kg, or 55 μg; 98% of an oral dose is absorbed. Selenium functions as a cofactor to antioxidant enzymes, particularly glutathione peroxidase. Other modes of action include induction of apoptosis, inhibition of cell proliferation, alteration of carcinogen metabolism, cytotoxicity due to selenium metabolites formed as a result of high selenium concentration, and an influence on testosterone metabolism [6, 7].

Laboratory evidence from a variety of experimental models has demonstrated the ability of selenium to inhibit tumorigenesis. In particular, selenium has been shown to inhibit the growth of DU-145 human prostate carcinoma cells in vitro [8] as well as to play a role in decreasing the incidence of prostate cancer in male rats pretreated with the chemical carcinogen 3,2′-dimethyl-4-aminobiphenyl [9].

Epidemiological evidence indicates an inverse correlation between selenium status and risk of some types of cancer, including prostate cancer [10–12]. Additional support for such an inverse association between selenium and cancer comes from two large randomized trials [12, 13]. The Nutrition Intervention Trial in over 29,000 individuals 40–69 years old from the general population of Linxian, China showed that supplementation with 50 μg selenium daily, 30 mg vitamin E, and 15 mg beta-carotene daily was associated with a 13% decrease in mortality from cancer at all sites and a 21% decrease in mortality from stomach cancer [12]. The second trial, also in Linxian, tested a multivitamin/mineral, including 50 μg selenium plus 15 mg beta-carotene daily, in 3,000 individuals with

esophageal dysplasia [13]. Total cancer mortality was 7% lower and esophageal cancer was 14% lower in the supplemented group. Specific attention to the impact of selenium in preventing prostate cancer appears in a clinical trial that was designed to address the effect of this nutrient on skin cancer, the Nutritional Prevention of Cancer (NPC) Study [14]. In this trial 1,312 patients with a history of skin cancer were randomized to receive 200 μg elemental selenium daily in the form of selenized yeast or placebo and were followed an average of 4.5 years with primary endpoints of assessing the occurrence of new basal or squamous cell carcinomas of the skin and other cancers. Secondary endpoints included all-cause mortality and total cancer mortality, total cancer incidence, and the incidences of lung, prostate, and colorectal cancers [15, 16]. Interestingly, although no difference was noted in the rate of skin cancer, the incidence of prostate cancer was decreased by two-thirds in men in the selenium supplemented group. Stratified analysis of a small number of cases suggested that the decrease in prostate cancer was greater in men with low baseline selenium, men younger than 65 years, and those with low serum PSA [15]. These secondary subset analyses generated the underlying hypothesis of the SELECT trial with regard to the potential benefit of selenium in decreasing risk of prostate cancer.

17.1.2.2 Vitamin E (Alpha-Tocopherol)

Vitamin E, a family of naturally occurring, essential, fat-soluble vitamin compounds, is the major lipid soluble antioxidant in cell membranes, acting as a free radical scavenger that inhibits lipid peroxidation, and has biological activity relevant to carcinogen-induced DNA damage [7, 17]. Of the eight variants of vitamin E, alpha-tocopherol is the most active form and is also one of the most abundant, being widely distributed in nature, in foods, and making up the predominant form in human tissue. In the United States the average dietary vitamin E intake in men is estimated to be 10 mg, and in women 7 mg, daily. The recommendation of the National Research Council for a daily dietary allowance is 15 mg for both men and women [17–19]. Its oral absorption is 20%–50%.

Laboratory studies have shown that vitamin E inhibits the growth of a variety of human cancer cell lines, including those of the prostate. In animal experiments, vitamin E prevents various chemically induced tumors, including some that are hormonally mediated [20, 21]. Prostate cancer is among those affected, with vitamin E slowing the growth of such tumors in vivo in rats receiving various doses of chemotherapeutic agents. Several mechanisms have been proposed to underlie the anti-carcinogenic effect of vitamin E. These include the following activities of vitamin E: acting as a free radical scavenger/antioxidant; blocking nitrosamine synthesis; exerting antiproliferative effects, inducing the detoxification enzyme nicotinamide adenine dinucleotide phosphate:quinine reductase; and inhibiting fatty acid metabolism, protein kinase C activity, and arachidonic acid and prostaglandin metabolism [7].

Data from epidemiologic studies are inconsistent with respect to a possible beneficial association of vitamin E status (alpha-tocopherol plasma or serum levels) or intake and prostate cancer [7]. In an example of a positive study, serum or plasma vitamin E concentrations measured before diagnosis appeared to be lower in prostate cancer cases than in controls [22–24]. However, other observational studies fail to support such associations [25]. Secondary analysis of data from a large randomized trial, the Alpha-Tocopherol Beta-Carotene (ATBC) Cancer Prevention Trial offered the most convincing evidence that vitamin E is associated with a decrease in the risk of prostate cancer. Conducted in Finland by the National Public Health Institute of Finland and the United States NCI, the ATBC

study was a randomized, double-blind, placebo-controlled trial of alpha-tocopherol (50 mg synthetic *dl*-alpha-tocopherol acetate) daily and beta-carotene (20 mg) daily alone or in combination in 29,133 male smokers 50–69 years old at study entry [26]. ATBC was designed to determine whether these nutritional interventions would reduce the risk of lung cancer among 29,133 male smokers aged 50–69 years [27]. Paradoxically, the incidence of lung cancer increased among men receiving beta-carotene. Yet, of the 14,564 patients assigned to the alpha-tocopherol supplementation arm of the trial, 99 incident prostate cancers were observed compared with 147 in the 14,569 assigned to the non-alpha-tocopherol arm, representing a statistically significant 32% decrease in the prostate cancer incidence [95% confidence interval (CI), 12–47; p=0.002]. This preventive effect appeared to be stronger in clinically evident cases (stages B–D disease), with participants receiving alpha-tocopherol showing a 40% (95% CI, −20 to −55) decreased occurrence of such disease. Furthermore, despite being based on fewer events, prostate cancer mortality also showed a similarly strong effect, with a statistically significant decrease of 41% (95% CI, −1 to −64) among the 14,564 men receiving vitamin E compared to the men not receiving vitamin E [26]. These findings, which were pre-specified as a secondary endpoint in the ATBC trial, were hypothesis-generating, offering strong support for testing vitamin E in a prospective clinical trial of prostate cancer prevention.

17.2 SELECT: Study Design

Based on the observations described above, the NCI together with the Southwest Oncology Group (SWOG), which is providing administrative and scientific oversight, designed and implemented SWOG Protocol S0000, the Selenium and Vitamin E Cancer Prevention Trial, SELECT. The SELECT study coordinators include Scott Lippman, MD for medical oncology, Eric Klein, MD, and Ian M. Thompson, Jr., MD for urologic oncology.

17.2.1 Study Objectives

The primary objective of the SELECT trial is to assess the effects of selenium and vitamin E alone and in combination on incidence of prostate cancer. Several pre-specified secondary endpoints will also be assessed: prostate cancer-free survival; all cause mortality; the incidence and mortality of other cancer types such as lung and colorectal; overall cancer incidence and survival; and disease potentially impacted by chronic administration of selenium and vitamin E. The collection of serious cardiovascular event data is also being carried out in order to monitor the safety of vitamin E with regard to the risk of hemorrhagic stroke [7, 28]. Other trial objectives include periodic quality of life assessment, serum micronutrient measurement, prostate cancer risk assessment, and the evaluation of biological and genetic markers associated with the risk of prostate cancer [29].

17.2.2 Selection of Study Agents

The ATBC Study led to the choice of vitamin E for SELECT, but a debate arose over the best dose and formulation to use. The selection of alpha-tocopherol (*all rac (dl)*-alpha-tocopheryl acetate) was based on the observed association of long-term supplementation with this form of vitamin E with reduction in prostate cancer incidence in the ATBC trial [25, 26]. The racemic mix of alpha-tocopherol, which includes the *d* and *l*-isomers, was to be used. A 400-mg daily dose was chosen because of potential benefits of this dose on other non-cancer diseases (Alzheimer's disease and age-related macular degeneration),

as well as its inclusion in widely used vitamin supplements, suggesting its safety [19, 30, 31]. (National Institutes of Health Office of Dietary Supplements, Vitamin E, http://ods.od.nih.gov/factsheets/vitamine.asp; revised October 2004). The selection of the selenium formulation was less straightforward. On the advice of an NCI-sponsored panel of experts, *l*-selenomethionine was chosen over selenized yeast, despite the latter being the form used in the hypothesis-generating trial of Clark et al. [14]. These recommendations were based on marked batch-to-batch variability in various forms of selenium in the selenized yeast, the lack of commercial availability of the selenized yeast used in the NPC study [14], and laboratory analysis that determined that the predominant selenium species in currently commercially available selenized yeast is *l*-selenomethionine.

17.2.3 Study Cohort

The study cohort in SELECT consists of 35,533 healthy men with a DRE not suspicious for cancer and serum total PSA of 4.0 ng/ml or less (Fig. 17.1). Elevated risk of disease is based on age eligibility of 55 years or older in Caucasian men and 50 years or older in African-American men (since 50 to 55-year-old black American men have a prostate cancer incidence rate comparable to that of 55- to 60-year-old white men). The complete list of eligibility criteria appears in Table 17.1.

17.2.4 Study Design

SELECT is a prospective randomized double-blind, placebo-controlled, 2×2 factorial study of selenium and vitamin E alone and in combination in eligible healthy men. Randomization should lead to equal participant distribution among the four study arms and to avoidance of hidden sources of bias in participant characteris-

Table 17.1 SELECT eligibility criteria

Age ≥ 55 years (African American men ≥ 50 years)
Total PSA ≤ 4.0 ng/ml
DRE not suspicious for cancer
No previous prostate cancer or high-grade PIN
Normal blood pressure
No current anticoagulation therapy
Willing to restrict off-study supplement use

PIN, prostate intraepithelial neoplasia

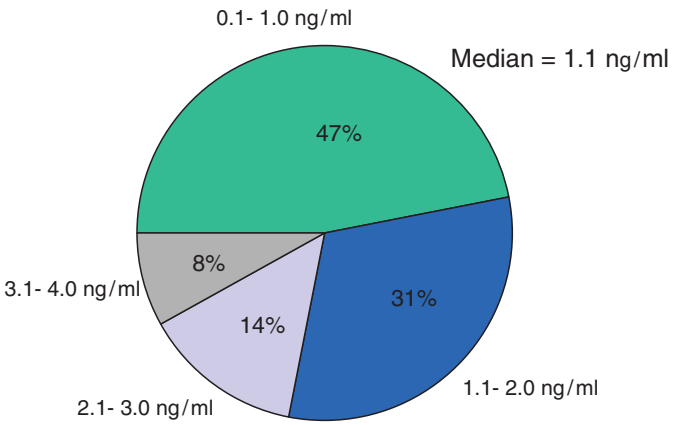

Fig. 17.1 Baseline PSA

tics. The study interventions include selenium 200 μg (*l*-selenomethionine) and vitamin E 400 mg (*racemic* (*dl*)-alpha-tocopheryl acetate). These two nutrients are being administered to participants in designated arms of the SELECT trial (Fig. 17.2). Thus, the intervention consists of a daily oral dose of one study supplement plus placebo matched to the other supplement or both study supplements or both matched placebos according to the randomization plan. Study duration is planned to be 12 years with a 5-year uniform accrual period, and a minimum of 7 and maximum of 12 years of intervention depending on the time of randomization. A pre-determined follow-up schedule is shown in Fig. 17.2.

17.2.5 Statistical Considerations

A planned sample size of 32,400 men was based on a number of underlying assumptions [32]. These included estimates of the incidence of prostate cancer among men in the placebo group based on the latest official nationwide Surveillance, Epidemiology and End Results (SEER) data from 1991–1995, which in the first 3 years of the trial would be similar to the rate from the PCPT [5, 17]. The incidence of prostate cancer in the SELECT population was anticipated to be higher than the relevant SEER age-related incidence primarily because most men in the study would probably receive annual screening with DRE and PSA, unlike men in the SEER database. In addition, the SELECT population was expected to include a substantial percentage of intensively recruited African-American men, which would also contribute to a higher rate of disease.

SELECT will be analyzed as a four-arm study (Fig. 17.2), with five pre-specified comparisons being incorporated into the primary study analysis. These include: (1) vitamin E versus placebo, (2) selenium versus placebo, (3) combined vitamin E plus selenium versus placebo, (4) combined vitamin E plus selenium versus vitamin E and (5) combined vitamin E plus selenium versus selenium. This study design will allow detection of a 25% decrease in the incidence of prostate cancer for selenium

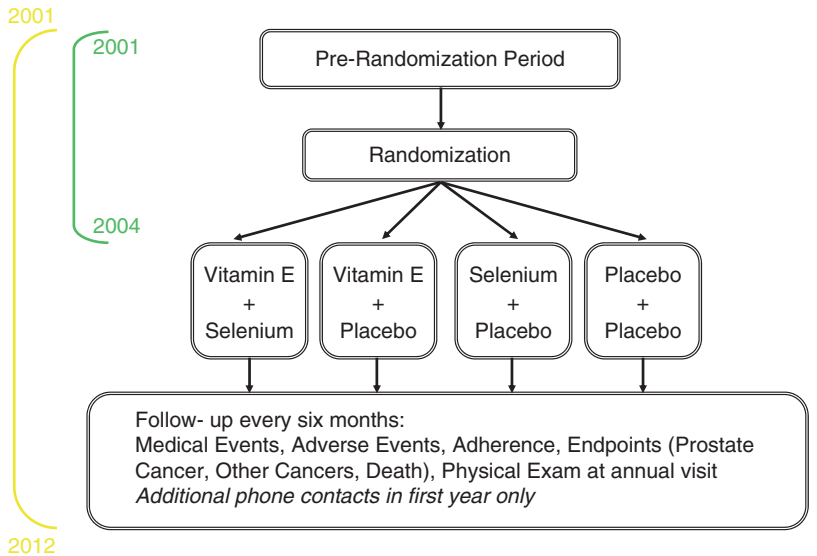

Fig. 17.2 Study schema

or vitamin E alone with an additional 25% decrease for combined selenium and vitamin E compared with either agent alone. The study also allows for the potential interaction of vitamin E and selenium. Additional statistical analysis is planned to include tests for vitamin E versus no vitamin E, selenium versus no selenium and interactions of the two agents. Secondary analyses of the NPC study [14] and ATBC study [26] showed that selenium and vitamin E were associated with reductions in prostate cancer incidence of greater than 60% and greater than 30%, respectively, during the interventions. Based on these observations, a treatment effect of 25% was estimated for either selenium or vitamin E.

17.2.6 Evaluation of Prostate Cancer Endpoint

Prostate cancer will be assessed on a recommended routine clinical diagnostic evaluation, including yearly DRE and serum PSA measurement. Prostate biopsy will be performed at the discretion of study physicians according to local community standards. However, the study protocol recommends biopsy for study participants with DRE suspicious for cancer and/or elevated serum PSA. Unlike the PCPT, no biopsy is required at the end of SELECT. A histologic diagnosis will be made by the study site and confirmed by the SELECT pathology review committee. This centrally reviewed histological diagnosis of prostate cancer is required in all cases except those based on total PSA greater than 50 ng/ml and positive bone scan.

17.2.7 SELECT in Contrast to PCPT

Although both of the phase III clinical prevention trials in prostate cancer address the question of preventing prostate cancer in men at increased risk based on age, there are a few key differences between the earlier PCPT and SELECT. These are shown in Table 17.2. Importantly, an end-of-study biopsy, which was implemented in 7,472 participants (3,652 in the finasteride and 3,820 in the placebo group) in PCPT and allowed assessment of prevalent clinically non-evident disease, is not being carried out in SELECT. The secondary endpoints of the two trials are also different. The end-of-study biopsy in PCPT provided data on prevalent cases which, when juxtaposed in relation to scheduled measurements of PSA levels, led to exploration of this parameter as a potential screening modality [5, 33]. In contrast, screening is not a focus of SELECT. Data collected in the context of SELECT will emphasize issues related to the molecular epidemiology and risk of prostate and other cancers [29].

17.2.8 Ancillary Studies

Several ancillary studies have been planned to address secondary endpoints and toxicity concerns. The Prevention of Alzheimer's Disease with Vitamin E and Selenium (PREADVISE) study is to be run through the University of Kentucky's Sanders-Brown Center on Aging and Alzheimer's Disease Research Center

Table 17.2 Differences between PCPT and SELECT

	PCPT	SELECT
Endpoint	7-year prevalence	Incidence
End-of-study biopsy	Yes	No
Disease ascertainment	Pathology proven biopsy required	Community standard recommendation
Secondary endpoints	Prostate and screening test issues	Other cancers

(ADRC) in Lexington, Kentucky. The impact of SELECT agents on eye disease will be addressed in the Prevention of Cataract and Age-Related Macular Degeneration with Vitamin E and Selenium (SEE) study to be conducted in the Division of Preventive Medicine, Harvard Medical School, Boston, MA. The Respiratory Ancillary Study (RAS), designed to examine the prevention of lung function decline with selenium and vitamin E, will be run by the Division of Nutritional Sciences at Cornell University, Ithaca, New York. Prevention of Colorectal Adenoma with Selenium and Vitamin E will be explored in the Adenomatous Colorectal Polyps (ACP) study at the Arizona Cancer Center in Tucson, AZ.

In addition to pre-planned ancillary studies, a biorepository is being assembled from specimens collected from SELECT participants [29]. Baseline and bloods at year 5 are being collected for plasma, buffy coat/white blood cell, and red blood cell isolation. Toenail samples collected at baseline will be used to measure baseline levels of selenium. Additional tissue samples will include all prostate biopsies; all of these will be clinically indicated, in contrast to the PCPT where exit biopsies were also performed.

17.3 Study Implementation

SELECT was activated on 25 July 2001 at over 400 study sites throughout the United States, Canada, and Puerto Rico. The accrual goal of 32,400 men was reached 28 months earlier than projected. As a result, the trial was closed to accrual on 24 June 2004 with a final accrual total of 35,533 participants. The projected end date is 2012. Of the participants, 73% were recruited via the cooperative groups, 29% of the total (10,371) coming from the Community Clinical Oncology Program. Another 9% were enrolled at Veterans Administration sites and 17% at miscellaneous facilities.

A major initiative in this trial was to attain a population of men that would be representative of the population that gets prostate cancer. Given the higher incidence of this disease in African-American men and the earlier age of onset in this population compared to white men, SELECT investigators set a goal of recruiting at least 20% African-Americans. To accomplish this, broad-ranging efforts were implemented through preexisting networks, including churches, fraternities, and African-American media [34]. Although 14% of the United States population is African-American, a goal of 20% was chosen so as to oversample this group. Also, the lowered age of eligibility for African-American men, which reflects the earlier age of onset in this group, also contributed to increasing the percentage of participants from this racial population. Success in accrual of minority populations is shown in Table 17.3.

Maximum age of eligibility was based on anticipated lifespan that would allow useful data to be collected from enrollment. Although the age distribution of participants is concentrated in the group of younger eligible men, men with the full range of ages above the minimum at baseline were recruited (Table 17.3). Participants with a wide spectrum of education levels were recruited (Table 17.3). Importantly, although nearly 50% of participants had a PSA below 1.0 ng/ml at baseline, a range of PSAs within the standard normal range was represented at entry (Fig. 17.1).

17.4 Future Challenges in the Conduct of SELECT

Despite rapid initial accrual and good minority representation among participants, several

Table 17.3 SELECT participant characteristics

Characteristics	% of participants (n=35,534)
Race	
White	78
African American (including Hispanic)	14
Hispanic (non-AA)	5
Asian/Pacific island	1
Other/unknown	1
Age, years	
50–54	5
55–64	58
65–74	31
75+	6
Education level	
Grade school	3
High school	20
Some college	26
College degree	19
Graduate school	31

challenges remain to the further conduct of SELECT. The long-term nature of this trial, 7–12 years with follow-up, lends special importance to measures to promote retention of trial participants. In this protracted timeframe, staff turnover, continued funding, as well as participant response to publicizing by the media of results of other, related studies that might influence the decision to remain on study must also be addressed. Finally, the SELECT population, although healthy at baseline, were recruited when in their middle to advanced years, so that further aging over the course of study is expected to be accompanied by health issues, re-location due to health needs, and retirement.

A National Participant Advisory Board consisting of 10 men enrolled in the SELECT trial holds the responsibility of representing to the investigators the views of all participants on issues involved in conducting the trial. These participants offer regional representation by being allied with various study sites. In addition to attending twice yearly training sessions, they review retention and adherence materials, recommend and advise on retention and adherence strategies, and write articles for the participant newsletter.

Successful completion of SELECT will provide insight into the potential of two widely investigated nutrients regarding their role in prostate cancer prevention.

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18 Prostate Cancer Prevention by Short-Term Anti-androgens: The Rationale Behind Design of Pilot Studies

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Abstract This paper sets out to review evidence that low-grade prostate inflammation is a precursor of prostate cancer development and the mechanisms by which it may account for the more than 50 years natural history from first infection to cancer. Though as yet there is no clear-cut specific associated infection, there is clear evidence that some sexually acquired infections damage the prostate and increase serum PSA with slow recovery back to normal. The demonstration that low-level solar exposure is protective provides a possible mechanism due to vitamin D's known benefit through action to boost macrophage-mediated immune surveillance. This observation and data demonstrating that non-steroidal anti-inflammatory drugs (NSAIDs) protect against prostate cancer provide the justification for trials of these two agents combined with short course intermittent anti-androgen therapy in populations at high risk of prostate cancer.

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18.1 Introduction

Twelve years ago one of us (T.O.) first proposed the hypothesis that prostate cancer was caused by a life-time of "sub-clinical" prostatic inflammation of unknown cause (Oliver 1995; Oliver and Gallagher 1995). These articles also speculated on the basis of the first results from use of intermittent hormone therapy that short-term (1–3 months) androgen blockade given at age 45 (to the one-third of the population known from post-mortem studies to have latent prostate cancer at that stage) might be a very effective form of chemo-prevention (Oliver and Gallagher 1995). The hypothesis was speculative and based on a considerable paucity of hard facts. It mainly rested on three borderline significant and less reproducible aetiological similarities in common between prostate and cervix cancer—i.e. risk of malignancy was increased by (1) early onset of sexual activity (Key 1995), (2) reduced by circumcision (Oliver et al. 2001), and (3) increased in Afro-Caribbeans (Chinegwundoh et al. 2006). Further support came from the observation that 1 in 5 patients with advanced metastatic

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prostate cancer treated by intermittent androgen blockade (IAB) survived for prolonged periods (5 or more years) without further need for treatment (Oliver and Gallagher 1995). Subsequently, studies on a cohort of predominantly 25- to 35-year-old South African gold miners demonstrated those individuals with increased PSA levels had both earlier initiation of sexual activity and evidence of chlamydial infection (Oliver et al. 2001). This provided additional support for the hypothesis that repeated exposure to sexually transmitted diseases (STD) might cause lasting damage to the prostate. The subsequent demonstration by others that at the age of 45 those with a PSA above the median level had a 3.75 higher risk of death from prostate cancer (Fang et al. 2001) provided further evidence of the value of PSA as a screening tool for prostate cancer detection (Lane et al. 2007). These observations also raised questions about whether this association was because the PSA level also reflects the existence of cancer at this early stage or the degree of persistent sub-clinical prostatitis (Lilja et al. 2007), and that this latter underlying pathology is associated with subsequent cancer development.

Today the concept of persistent sub-clinical infection and inflammation as a major cause of the early stages of cancer development is increasingly accepted (Danesh et al. 1997; Balkwill and Mantovani 2001; Riss et al. 2006). This paper will review this data and examine how lifelong sub-clinical or intermittent vitamin D deficiency could potentiate this damage, and finally how these observations could lead to possible therapeutic strategies for treating patients with raised PSA and no evidence of prostate cancer on biopsy, so-called "PSAitis". This is the name coined by Tannock (2002) for the modern epidemic of worried well men who live in fear of their next biopsy and for whom at present there is no known treatment.

18.2 Modern Evidence of a Contributory Role for Sexually Acquired Infection to Causation of Prostate Cancer But Lack of Specificity of Organism

During the past 15 years two groups have focussed on developing the idea that sexually acquired infection has a direct causal link to prostate cancer. First, Dennis et al. (Dennis and Resnick 2000; Dennis et al. 2002, 2004; Dennis and Dawson 2002) have, through a series of informative papers, updated Key's original meta-analysis and provided added support for all of the original conclusions except one, i.e. that early onset sexual activity increases the risk of prostate cancer (Table 18.1). However, included in the review of the 12 papers was analysis showing that 4 of the 12 studies showed there was some significant data supporting the hypothesis that young age of onset of sexual activity increased the risk of prostate cancer, in another 4 the association was non-significant, and in 3 there was a non-significant protective effect of early onset sexual activity, although there were major differences in the cut-point of age that showed this effect. One study published in Japanese could not be examined.

Two other points emerged of relevance from the analysis in Table 18.1. First, less clear cut is the association with vasectomy in more recent studies. A possible confounding variable that might explain this comes from the report of Hays et al. (2000) who showed that prostate cancer was increased in men who used the sex industry, but only those who did not regularly use condoms. The series showing less strong association of vasectomy in Dennis' review tended to be the more recent ones with data collected after the acquired immunodeficiency syndrome (AIDS) epidemic had led to major changes in condom use.

The second observation related to the association of prostate cancer with a diet high in

Table 18.1 Overview behavioural related risk factors in prostate cancer epidemiology

Risk factor	Risk ratio	Odds ratio
	(Key 1995)	(Dennis and Dawson 2002; Dennis et al. 2002, 2004)
Animal fat	1.54	1.4
Retinoid intake	0.66	NA
Age 1st sex/ No. of partners	1.31/1.21	1.09/1.2
Any STD	1.4	1.4
Prostatitis	NA	1.6
Vasectomy	1.54	1.4

animal fat. Again this was confirmed by Dennis et al. (2004) but was less significant or even negative in the most recent series. There are at least two confounding variables not taken into consideration in most animal fat studies. First, burnt fat, proven in the association of barbecued meat and colon cancer, if carcinogenic (Wu et al. 2001), could influence internal organ cancer, as is known with inhaled tobacco tar; second, fat deposits are the repository of the long legacy of organochlorine pesticides (Sharpe 1995). The worldwide decline in their use over the last 40 years could be a factor in both prostate and breast cancer showing less association with animal fat recently. In breast cancer there is better evidence that this could be a real influence, as Westin demonstrated in Israel that when this class of chemical was banned for 10 years there was a significant reduction of breast cancer associated with an increased intake of animal fat in women less than 65 years old (Westin and Richter 1990). These observations are potentially even more significant if true for prostate cancer (Fleming et al. 1999) as organochlorine pesticides have long been known to be immunosuppressive (Bekesi et al. 1983) and could contribute to persistence of sub-clinical prostatitis.

The second group to make major contributions to the STD hypothesis in the last 15 years has been the Baltimore group and specifically the work of Sutcliffe and co-workers (2005, 2006a, b, c, 2007a, b, c). Most significantly they have confirmed that a proven exudative urethritis-inducing STD—such as *Chlamydia*, *Trichomonas* or gonorrhoea—causes on average a 40% increase in PSA, which only declines 3 months later by less than 5% (Sutcliffe et al. 2006c). Unfortunately, despite large-scale studies, there has been no consistent organism associated with prostate cancer, but a continued expansion of the number of organisms that have had strong association confirmed in one study and refuted in a repeat study, often by the same group. Most recently the BK polyoma virus has been associated with the transition from proliferative inflammatory atrophy (PIA) to prostatic intra-epithelial neoplasia (PIN) (Das et al. 2008).

A possibly significant exception is work on *Trichomonas vaginalis*. This widespread organism produces minimal symptomatology in the male, though it is associated with non-specific urethritis and has its growth effected by zinc, which is concentrated in the prostate. More importantly in the study of study of Sutcliffe and colleagues, regular aspirin consumption was associated with a reduction of the Trichomonas-associated risk (Sutcliffe et al. 2006b).

18.3 Modern Ideas on the Role of Inflammation as Causative of Malignant Transformation and Its Relevance to Prostate Cancer

The concept that persistent inflammation can lead to malignant transformation has been increasingly recognised (Danesh et al. 1997). This began with anecdotal observation of osteogenic sarcoma

developing after unhealed osteomyelitis in the pre-antibiotic era, though in more modern times, the *Helicobacter* association with stomach cancer, hepatitis B and liver cancer, and tumour necrosis factor (TNF) enhancement of ovarian cancer growth are more thoroughly worked out examples of the modern concept of cancer as a wound that does not heal (Riss et al. 2006).

Today, with five reviews in major journals, the concept of chronic inflammation of the prostate as a major cause of prostate cancer has become mainstream (De Marzo et al. 2007). As discussed in the previous section, the failure to associate a single major pathogen with cause, unlike *Helicobacter* gastritis in stomach cancer, has so far resulted in few therapeutic endeavours and led to the conclusion that the association is multi-factorial, including non-specific infections, autoimmunity and dietary/environmentally acquired chemical toxins. The demonstration in a rat model that the development of proliferative inflammatory atrophy precedes PIN (Borowsky et al. 2006) has demonstrated how a non-specific inflammation can become a major driving force in the clonal development of this disease.

There have been two major recent developments that could be of considerable therapeutic importance in reducing the level of mitotic activity in the prostate. First, the report by Platz et al. (2005) who have shown that regular consumption of aspirin and/or a non-steroidal analgesic reduced the risk of developing prostate cancer and that its effect was more marked in individuals under the age of 70. However, more worrying was the observation that with prolonged use beyond 4 years the benefit was reversed and the incidence of prostate cancer increased, suggesting that the maximum benefit might come from an intermittent schedule.

The second development has been the increasing numbers of studies showing increased prostate cancer incidence associated with factors related to vitamin D deficiency. Though the picture from study of single serum samples (in part due to the major fluctuations that occur between summer and winter) and from study of vitamin D receptor polymorphisms has been confusing (Hamasaki et al. 2001; Rukin et al. 2007a), more consistent results have come from studies attempting to quantitate lifetime sun exposure (Luscombe et al. 2001; Bodiwala et al. 2003; Rukin et al. 2007a). While possibly adding an extra layer of complexity to the interpretation by involving light-induced melatonin, a more likely mechanism for this association which has been little explored at the present time relates to vitamin D's known role in immune surveillance. This is best known from the study of tuberculosis (Liu et al. 2006; Martineau et al. 2007a, b). A lifetime's episodic immune deficiency from sub-optimal vitamin D levels could go a long way to explaining the association of chronic inflammation with prostate cancer. It could also explain the higher prevalence of prostate cancer in Afro-Caribbeans living in northern climates and inner cities, as it takes 2 h of sun exposure for a dark-skinned Afro-Caribbean to achieve the vitamin D boost achieved in 15 min by a Caucasian (Clemens et al. 1982).

18.4 PSA Screening and Impact on Over-Diagnosis of Prostate Cancer

There is little doubt that PSA screening leads to earlier diagnosis of less advanced prostate cancer, though at the cost of reducing the therapeutic cost-benefit ratio. As currently practiced it has been estimated that PSA-detected cancers are detected and the patients suffer the side-effects of treatment at least 10 years before the disease would present clinically, though more than half of those diagnosed would not ever have presented clinically and 4 out of 5 PSA positives do not have cancer on the biopsy but remain at risk and need further biopsies over the next 5 years, as there is no known treatment for this PSA

elevation, although it does sometimes remit spontaneously (Venkitaraman et al. 2007).

18.5 Evidence of Harm from Prostate Biopsy

In the hands of an experienced operator with good experience of using local anaesthesia for the procedure, there should be very little in the way of acute complications these days. However, all patients have to be covered by antibiotics and deaths have been reported. Scientifically more interesting is the issue of what is happening after the biopsy during the period of 4–6 weeks when there is a surge in PSA (Bhanot et al. 2003) and also an increase in circulating tumour cells (Oliver 1995) and persistence of haemospermia (Manoharan et al. 2007). Now that the numbers of biopsies at each session because of the use of local anaesthesia have increased to 12, it is not known whether this has any significance in patients that ultimately accept surveillance. The advent of urine-based assays such as the PCA3 test (Marks et al. 2007) may one day allow such issues to be resolved.

18.6 Lack of Evidence of Major Therapeutic Gain from Current Approaches to Radical Treatment of Prostate Cancer

There have been two major problems affecting all efforts directed to early diagnosis of prostate cancer. First, PSA testing leads to over-diagnosis and therefore active monitoring to select out those in need of the morbidity of radical treatment is increasingly justified for a large proportion of those diagnosed (Cuzick et al. 2006; Scardino 2008). Furthermore, as shown in Table 18.2, which summarises the results of the major randomised trials of immediate versus deferred therapy both for surgery and for androgen blockade, there is a consistent but only low-level survival advantage which has to be balanced against the burden of side-effects for immediate treatment. The past year has seen the publication of the first results from The International Study of Intermittent Androgen Blockade for Carcinoma of the Prostate (ISICaP) group that has undertaken an individual patient data meta-analysis of IAB studies. This has provided the first solid evidence that short-course (3 month) IAB offers a potentially safe alternative to continuous anti-

Table 18.2 Overview of randomised trials of immediate versus deferred therapy in prostate cancer

	Immediate		Deferred (or watchful waiting)		
Surgery	**No. of cases**	**Dead**	**No of cases**	**Dead**	**Diff**
(Bill-Axelson et al. 1977; Byar 1973)	347	23.9%	348	30.5%	−6.6%
Median follow-up 8.2 years					
(Byar 1980) VACURG 3	61	89%	50	92%	−3.0%
Median follow-up 25 years					
Endocrine therapy					
(Byar 1973) VACURG 1	1,418	77%	483	75.8%	+1.2%
Total since VACURG 1	1,310	67%	1,305	72%	−5.6%
(Kirk 1997; Studer et al. 2004)					

VACURG, Veterans Association Cancer Urology Group

androgen therapy (Shaw et al. 2007) which has been supported by the first meta-analysis of the early results of on-going randomised trials (Conti et al. 2007). More importantly from the point of view of the chemo-preventive potential of short-course treatment, this analysis has also demonstrated that a higher proportion of earlier cases remain progression free for 3 years or more off treatment (Table 18.3).

18.7 Possible New Approaches to Chemo-prevention of Prostate Cancer

Given the observations made earlier, there is little doubt that measurement of PSA at the age of 45 would enable detection of a sub-group with a high risk of dying by the age of 65 of prostate cancer. However, given the extent of over-treatment such a screen would produce, it would only be acceptable if treatment at this stage had as low a morbidity as colposcopy for cervical cancer and as high a cure rate at a cost that could be met out of the savings from over-treatment at present. It is clear that some progress has been reported in developing lower morbidity diagnostic tests on urine (Marks et al. 2007), though so far they have only been used as an adjunct to selecting those in need of biopsy. Little progress has been made with using semen-based tests because there is a major problem in getting ejaculate to screen in the elderly population as decline in ejaculation is one of the earliest manifestations of andropause (Walz et al. 2007) and is increasingly recognised as being more prevalent in men with prostate cancer (Leitzmann et al. 2004; Oliver 2004; Sutcliffe et al. 2005). Despite this, in those where ejaculation is possible, there is an adequate number of cells to examine morphology (Gardiner et al. 1996) and perform RT-PCR tests on them (Clements et al. 1999). Furthermore, it is possible to use such screenings to quantify the degree of chronic inflammation (Andrade-Rocha 2007) and assess the impact of treatments such as Cox-2 inhibitors on the degree of inflammation (Lackner et al. 2006).

Table 18.3 Intermittent hormone therapy (HT) (Shaw et al. 2007)

Univariate analyses	No. of cases	Proportion off treatment at 2 years (%)
Initial PSA		
<10 ng/ml	91	41
10–75 ng/ml	295	25
>75 ng/ml	60	23
Duration of HT		
<4 months	148	35
4–8 months	102	24
>8 months	219	**27**
Treatment		
aA	68	11
LHRH	39	31
MAB	404	32

aA, anti-androgens; LHRH, LHRH analogues only; MAB, maximum androgen blockade

18.8 Choice of Patient Populations for Chemo-prevention Studies

18.8.1 Patients with Low-Grade Prostate Cancer Volunteering for Surveillance Protocols

Currently there is no adjunctive treatment given to patients with low-grade prostate cancer and elevated PSA who opt for surveillance. There are limited data suggesting that a low proportion of such patients undergo a fluctuation of PSA, and some men actually fall below the upper limit of normal and remain so. Given the earlier discussion on the role of low-grade inflammation, if it

could be treated there is a chance of an increased incidence of this PSA regression of early cancer, as is seen in cervical cancer with the use of condoms (Richardson and Lyon 1981; Hogewoning et al. 2003). On the basis of the previous discussion, it is clear that non-steroidal anti-inflammatory drugs (NSAIDs) and vitamin D supplements would be sensible options. However, given the results from intermittent hormone therapy studies even using anti-androgens alone, there could be a case for adding 1–3 months anti-androgen monotherapy to NSAIDs and vitamin D into a combined attack on these early cancers, as this would give the added benefit of thymus regeneration after anti-androgen therapy (Oliver et al. 1995).

18.8.2 Patients with Persistent PSA Elevation After a Negative Biopsy Developing Prostate Cancer

There is increasing recognition that there is a high prostate cancer risk of PSA-positive biopsy-negative individuals at subsequent biopsy (Yanke et al. 2005), hardly surprising given its likelihood that the raised PSA may indicate subclinical prostatic inflammation. Such patients would also be suitable for a study of the combination of vitamin D and NSAIDs. In addition, they would be a useful source for validating urine and semen-based assays for malignant transformation, though given the high frequency of ejaculatory problems at this age, it may be necessary to also use PDE5 inhibitors combined with testosterone replacement. At first sight such a suggestion might seem inappropriate, given the longstanding worry about hormone-replacement therapy for men lighting up latent prostate cancer. However, there is increasing evidence that there is a need to re-appraise the role of testosterone in prostate cancer (Prehn 1999; Slater and Oliver 2000; Morgentaler 2006; Morales 2006a, b) because of data showing lower levels in high-grade prostate cancer (Morales 2006a, b) and safety of use in men at high risk of prostate cancer (Rhoden and Morgentaler 2003). One other aspect that needs to be addressed is whether there needs to be more intensive treatment of infection and in particular anaerobic infection and *Trichomonas vaginalis* using metronidazole.

18.8.3 Patients with STDs

The report of Sutcliffe—that exudative STDs produce a 40% increase in PSA and that this declines very slowly over the next 3 months—suggests that with only a 5% decline there could be a very interesting opportunity for further investigation. The decline rate of PSA in these patients would be a useful indicator to follow the impact of vitamin D and NSAIDs and work out dosing schedules.

18.8.4 Patients with Male Factor Infertility

With Lackner and colleagues' (2006) publication showing rising sperm counts after treating abacterial leucocytospermia with a Cox-2 inhibitor, it is clear that this could open up a whole new approach to male infertility, an effect which might also be even more marked in combination with vitamin D. At first sight this might not have much relevance to preventing prostate cancer; however, as male infertility is maximal after 35 years of age and the data of Lackner et al. suggest that prostate health may be critical in this population—and were treatment at this age with a low-risk protocol of vitamin D and NSAIDs to become universal for male infertility—the measurement of PSA 10 years later, at age 45, could provide evidence for a surrogate marker of later prostate cancer risk. Even more interesting given the increasing evidence that testicular germ cell cancers are associated with testicular

atrophy from multiple causes including viruses (Oliver 1990; Oliver 2007), a protocol of combined vitamin D and NSAIDs could have relevance for the prevention of testis cancer in men with testicular epithelial neoplasia.

18.9 Conclusion

Evidence that low-grade prostate inflammation is a precursor of prostate cancer development is increasingly accepted, though as yet there is no clear-cut specific associated infection. The demonstration that low-level solar exposure is protective provides a possible mechanism due to vitamin D's known benefit through action to boost macrophage-mediated immune surveillance. This observation and data demonstrating that NSAIDs protect against prostate cancer provide the justification for trials of these two agents combined with short course intermittent anti-androgen therapy in populations at high risk of prostate cancer.

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Part VIII

Cancer Prevention: Metabolic Aspects

19 Anti-angiogenic Activity of a Novel Class of Chemopreventive Compounds: Oleanic Acid Terpenoids

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Abstract Angiogenesis is the base for solid tumour growth and dissemination, and anti-angiogenic drugs have been demonstrated to be active in clinical trials. In addition, it has become increasingly clear that inflammation is a key component in tumour insurgence. Chemoprevention focuses on the primary or secondary prevention of cancer using natural or synthetic agents that usually show mild or no collateral effects. We have noted that angiogenesis, particularly 'inflammatory angiogenesis', is a common target of many chemopreventive molecules, where they most likely suppress the angiogenic switch in pre-malignant tumours, a concept we have termed 'angioprevention'. We have shown that various molecules, such as flavonoids, antioxidants and retinoids, act in the tumour microenvironment inhibiting the recruitment and/or activation of endothelial cells and phagocytes of the innate immunity. We have recently assessed the activity of novel compounds derived from the oleanolic acid triterpenoid, called CDDO-Me and CDDO-Imm. These compounds show a potent anti-angiogenic activity at low dosages. In vivo they inhibit angiogenesis in the Matrigel sponge assay and in KS-Imm (an immortalized Kaposi's sarcoma cell line) tumour growth. In vitro they are able to prevent endothelial cell tubulogenesis when cultured on Matrigel. In human umbilical vein endothelial (HUVE) cells these compounds can inhibit the activation of the extracellular signal-regulated kinase ERK1/2 pathway after stimulation with vascular endothelial growth factor (VEGF). Moreover, from immunofluorescence experiments we observed that treatment with these triterpenoids prevents nuclear factor NF-κB translocation into the nucleus and thereby the activation of downstream pathways. The particularly potent anti-angiogenic activity seen in vivo suggest that CDDO-Me may be interacting with an important network of molecular and cellular targets, on endothelial cells, and could be employed for 'angioprevention'. These substances are being assessed in phase I trials in humans in the United States.

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19.1 Introduction

Angiogenesis is a multiphase process now recognised to be a limiting step for transition from a dormant tumour to a tumour able to grow and metastatise. Folkman (1971) was the first to understand that tumour mass needs oxygen and nutrients to grow and that by 'putting an embargo on the tumour' it would not progress. New vessels in fact ensure the necessary conditions to expand and are a way to spread through the organism.

This process is not isolated, but is well integrated in a network of pathways involved in diverse mechanisms, including responses to hypoxia and inflammation.

Tumour cells proliferate with high doubling rates and those that are in the 'core area' lack oxygen. The hypoxic condition leads the tumour to release chemical signals to prevail upon surrounding tissues to create new vessels.

Inflammation plays an important role in benign-to-malign transition (Brigati et al. 2002). Cells of immune response, such as macrophages and neutrophils, release factors and cytokines in tumour microenvironment favouring the angiogenic switch.

Thus the tumour microenvironment is the source and the target of many stimuli. Different cells cohabit and exchange signals, influencing their behaviour and recruiting cells from tissues and the bloodstream.

In the anti-tumour therapy scenario, radiotherapy and chemotherapy are currently the main strategies to defeat cancer. Unfortunately their activity is not selective for altered tissues and can lead to toxicity in normal tissues. Moreover, tumour cells present a high rate of mutation, so drug-resistance easily occurs. Endothelial cells, by contrast, are genetically more stable and tend to mutate less frequently.

Anti-angiogenic compounds present three main advantages: their biological target is more stable; they cause low systemic toxicity because they affect proliferating endothelial cells and generally do not interfere with the rare instances of normal and physiological angiogenesis; they rarely lead to drug resistance (Boehm et al. 1997).

Angiogenesis inhibitors can act directly or indirectly. In the first case they bind and block pro-angiogenic factors, whereas in the latter case they inhibit pro-angiogenic factor receptors (soluble and membrane-bound) and the activation of downstream cascades.

In this context it is important to discover new targets to deliver therapy selectively to the cells of interest and new markers to monitor the efficacy of response.

19.2 Angioprevention and Anti-angiogenic Therapy

Inhibition of neo-vessel formation represents an intriguing tool to prevent neoplastic progression of solid tumours.

Pro-angiogenic stimuli are released by tumour cells and immune cells and alter endothelial cell behaviour in the microenvironment (Albini and Sporn 2007). Thus, these different cellular populations influence each other and promote the formation of a microenvironment suitable for tumour development.

Angiogenesis is the result of many steps, each one represents a potential target for inhibition.

In general, anti-angiogenic therapy's are already formed neovessels. Tumour vessels do not have a normal organisation because endothelial cells establish leaky interactions both with other endothelial cells and with surrounding cells (e.g. pericytes). The tumour vasculature is abnormally tortuous and disorganised and blood flow is chaotic and does not always go in the same direction (McDonald and Baluk 2002). All these features make chemotherapy ineffective because drugs have difficulty penetrating into

the tumour mass. Thus one main task of anti-angiogenic therapy is vessel normalisation.

Another strategy is angioprevention, which consists of inhibition of neovessel formation. Angiopreventive compounds affect the early stages of neovascularisation, for instance inhibiting recruitment and migration of endothelial cells to the tumour site and interfering with their organisation in capillary-like structures. This is one of the possible mechanisms of action of chemopreventive drugs (Tosetti et al. 2002).

19.3 Triterpenoids: New Promising Angiogenesis Regulators

It is increasingly clear that prevention strategy is more desirable from both a public health and economic point of view. Healthy people lead a more productive life and cost less to the government. Thereby the attention of research is focussed on the discovery of preventive compounds.

Likewise, it is generally accepted that a correct lifestyle represents a good weapon to prevent different diseases, from cardiovascular to oncologic. Good dietary habits are an important aspect of lifestyle because they guarantee the right calorie needs, while supplying molecules which may co-operate for the correct functioning of the organism.

Many research groups are studying molecules already housed in food and their potential effect in oncology patients.

Various molecules, such as flavonoids, antioxidants and retinoids, act in the tumour microenvironment inhibiting angiogenesis and neo plastic progression.

At the moment our group is studying a new class of molecules. Terpenoids are synthetic molecules that mimic the chemical structure of oleanolic acid, a substance present in citrus fruit peels CDDO (2-cyano-3,12-dioxoolean-1,9-dien-28-oate) was the first terpenoid to be synthesised. In particular we are focussing our attention on two CDDO analogues, CDDO-Me (methyl 2-cyano-3,12-dioxoolean-1,9-dien-28-oate) and CDDO-Im (2-cyano-3,12-dioxoolean-1,9-dien-28-oic imidazolide; Fig. 19.1).

Scientific literature sustains the idea that these compounds are promising in the oncology scenario.

CDDO and CDDO-Im are able to reverse the TRAIL-resistant phenotype in human breast cancer cell lines and to inhibit cell growth. (Hyer et al. 2005; Lapillonne et al. 2003). CDDO decreases the inflammatory response (Suh et al. 1999). CDDO-Me has a strong inhibitory effect on lung cancer cells proliferation (Zou et al. 2004) and interferes with the nuclear factor NF-κB pathway in leukaemia cells (Shishodia et al. 2006).

These compounds show potent anti-angiogenic activity at low dosages (Albini and Sporn 2007). In vivo they inhibit angiogenesis in the Matrigel sponge assay and growth of Kaposi's sarcoma xenografts in nude mice (Vannini et al. 2007).

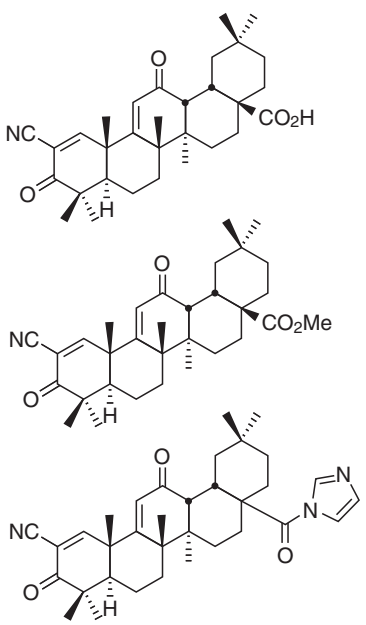

Fig. 19.1 Chemical structure of triterpenoids. *Top*: CDDO; *middle*: CDDO-Me; *bottom*: CDDO-Im

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In vitro they are able to prevent endothelial cell tubulogenesis when cultured on Matrigel. In human umbilical vein endothelial (HUVE) cells these compounds can inhibit the activation of the extracellular signal-regulated kinase ERK1/2 pathway after stimulation with VEGF. Moreover, from immunofluorescence experiments we observed that treatment with these triterpenoids prevents NF-κB translocation into the nucleus and thereby the activation of downstream pathways. The particularly potent anti-angiogenic activity seen in vivo suggests that CDDO-Me may be interacting with an important network of molecular and cellular targets on endothelial cells and could be employed for 'angioprevention'. In the United States these substances are being assessed in phase I trials in humans.

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Part IX

Aspirin and NSAIDs in Cancer Prevention: Attempts at an International Consensus

20 Pharmacologic Effects of NSAIDs and Implications for the Risks and Benefits of Long-Term Prophylactic Use of Aspirin to Prevent Cancer

Michael J. Thun and Bonny Blackard

Abstract This paper briefly reviews the pharmacologic effects of nonsteroidal antiinflammatory drugs (NSAIDs) that influence the risks and benefits of using these drugs prophylactically for cancer. It describes the metabolism of arachidonic acid through the cyclooxygenase (COX) pathway, the physiologic functions of prostanoids (prostaglandins, prostacyclin, and thromboxane A_2) produced by this pathway, and the pharmacologic consequences of blocking the enzymatic activity of the two COX isoforms. We mention other proposed mechanisms by which NSAIDs may directly or indirectly affect non-COX pathways. The diverse pharmacologic effects of NSAIDs, when combined with the relatively low probability that an individual with average risk will develop any single type of cancer over a lifetime, severely limit the tolerance for toxicity if aspirin or related drugs are to be administered prophylactically to large numbers of otherwise healthy people. Further research is needed to identify a drug, dose, treatment regimen, and patient population(s) where the benefits of prophy- lactic treatment will exceed the risks. A singular advantage of aspirin over all other NSAIDs is the potential to combine reduced risk of certain cancers with cardiovascular benefit. However, many elements that are needed to achieve this remain unresolved.

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20.1 Introduction

Aspirin and other nonsteroidal antiinflammatory drugs (NSAIDs) are a chemically diverse group of compounds that share the ability to block the metabolism of arachidonic acid through the cyclooxygenase (COX) pathway. Their pharmacologic effects are thought to derive principally from decreased formation of downstream tissue-specific signaling lipids produced by this pathway. These compounds, collectively called prostanoids, include prostaglandins, prostacyclin, and thromboxane A_2 (Fig. 20.1) [1]. They differ from systemic hormones in that they are not stored in tissues but are produced on demand, act locally in the tissue of origin (autocrine)

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and/or adjoining tissues (paracrine), and are then rapidly inactivated in the systemic circulation.

Biosynthesis of prostanoids begins with arachidonic acid (AA), which is normally bound to phospholipids in the cell membrane but is released by phospholipases (principally A_2) in response to inflammatory or other stimuli (Fig. 20.1) [1]. Free AA can be metabolized by several pathways (Fig. 20.1). Only metabolism through the COX pathway leads to the production of prostanoids, and only the inhibition of COX activity has unequivocally been proved to result from therapeutic concentrations of NSAIDs. Alternative pathways for AA metabolism include the lipoxygenase (LOX) pathway [which generates leukotrienes, lipoxins, and hydroxyeicosatetraenoic acid (HETE) compounds], metabolism by various cytochrome P450 enzymes, and nonenzymatic degradation by free radicals to isoprostanes (Fig. 20.1). Even though NSAIDs may not affect these alternative pathways directly, they do increase the availabil-

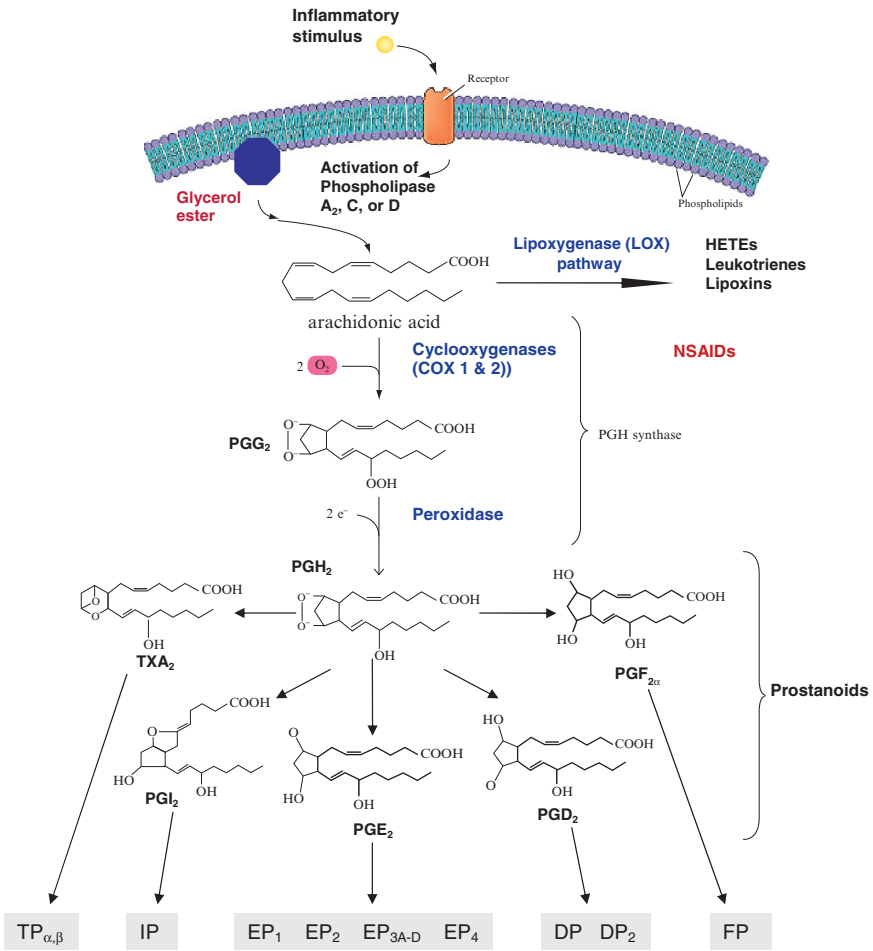

Fig. 20.1 Metabolism of arachidonic acid through the COX pathway (modified from [1]). Membrane-bound receptors shown in *gray boxes at bottom* transduce different effects in different tissues

ity of AA substrate by blocking its metabolism through the COX pathway.

The biologic effects of prostanoids differ markedly in different tissues, both because tissue-specific isomerases affect the end product, and because there are multiple membrane-bound receptors that transduce different effects [1]. For example: thromboxane A_2 (TXA_2) promotes the aggregation of platelets, vasoconstriction, and other factors involved in hemostasis; prostacyclin (PGI_2) in vascular endothelial cells causes vasodilatation and inhibits platelet aggregation; prostaglandin E_2 (PGE_2) in gastric epithelium helps to protect the gastric mucosa against acid, whereas PGE_2 in inflammatory tissues depresses the humoral immune response and can promote inflammation, wound healing, and neoplasia. Given these diverse physiologic functions, it is not surprising that blockade of the COX pathway by NSAIDs results in complex and often conflicting pharmacologic effects that can be can be therapeutic, toxic, or both depending upon the treatment regimen and patient characteristics.

The defining pharmacologic effect of NSAIDs is their ability to inhibit the first step of the COX pathway by blocking the activity of the prostaglandin G/H synthases, commonly known as cyclooxygenases [2]. There are at least two isoforms of this enzyme. COX-1 is expressed constitutively in most cells of the body where its products maintain many of the housekeeping functions described above. A second isoform, COX-2, discovered in 1992, is induced in many tissues in response to inflammation, wound healing, and neoplasia. The functions of COX-2 can be perceived as detrimental in the context of chronic inflammation, but beneficial in vascular endothelium where the isoform is expressed constitutively and is the major source of PGI_2.

The diverse chemical structures of NSAIDs are shown in Figs. 20.2 and 20.3. The so-called traditional NSAIDs (tNSAIDs), depicted in Fig. 20.2, nonselectively block both COX-1 and COX-2, especially at high doses. Aspirin is the oldest and best known of the tNSAIDs. At low doses (<100 mg), aspirin selectively inhibits COX-1 in platelets, whereas at higher, antiinflammatory doses, aspirin and other tNSAIDs inhibit both COX-1 and COX-2. Aspirin is the only NSAID that binds covalently (irreversibly) to COX-1 and permanently inhibits platelet aggregation for the life of the platelet. The widely used analgesic acetaminophen is not classified as an NSAID because it has not been demonstrated to inhibit COX-1 or COX-2.

The structures of a number of newer drugs, collectively called coxibs, are shown in Fig. 20.3. These compounds were developed to be selective inhibitors of COX-2, with the goal of sparing COX-1 and thereby minimizing gastrointestinal toxicity. Rofecoxib proved to be the most potent and selective inhibitor of COX-2 among the currently available Coxibs. It was withdrawn from the market because of unanticipated cardiovascular toxicity.

The pharmacologic consequences of blocking the COX pathway are complex and depend on the dose and treatment regimen as well as on the specific NSAID. For example, low-dose aspirin (<100 mg daily) has no antiinflammatory effects but irreversibly inhibits COX-1 production of thromboxane A_2 in platelets, thereby decreasing the risk of thrombotic cardiovascular events but increasing the risk of bleeding. Antiinflammatory doses of nearly all NSAIDs reduce the pain, swelling, redness and fever that are hallmarks of inflammation, but also cause gastrointestinal irritation, bleeding, and, particularly at older ages, renal dysfunction.

The anticancer effects of NSAIDs appear to be mediated in experimental models by the restoration of apoptosis, induction of cell cycle arrest, inhibition of proliferation, and inhibition of angiogenesis. There is no scientific consensus about the underlying mechanism(s), however, and it remains unclear whether these effects are caused predominantly by modulation of prostanoids, by factors outside the COX pathway, or by some combination of the two. Supra-physiologic concentrations of aspirin have been reported to

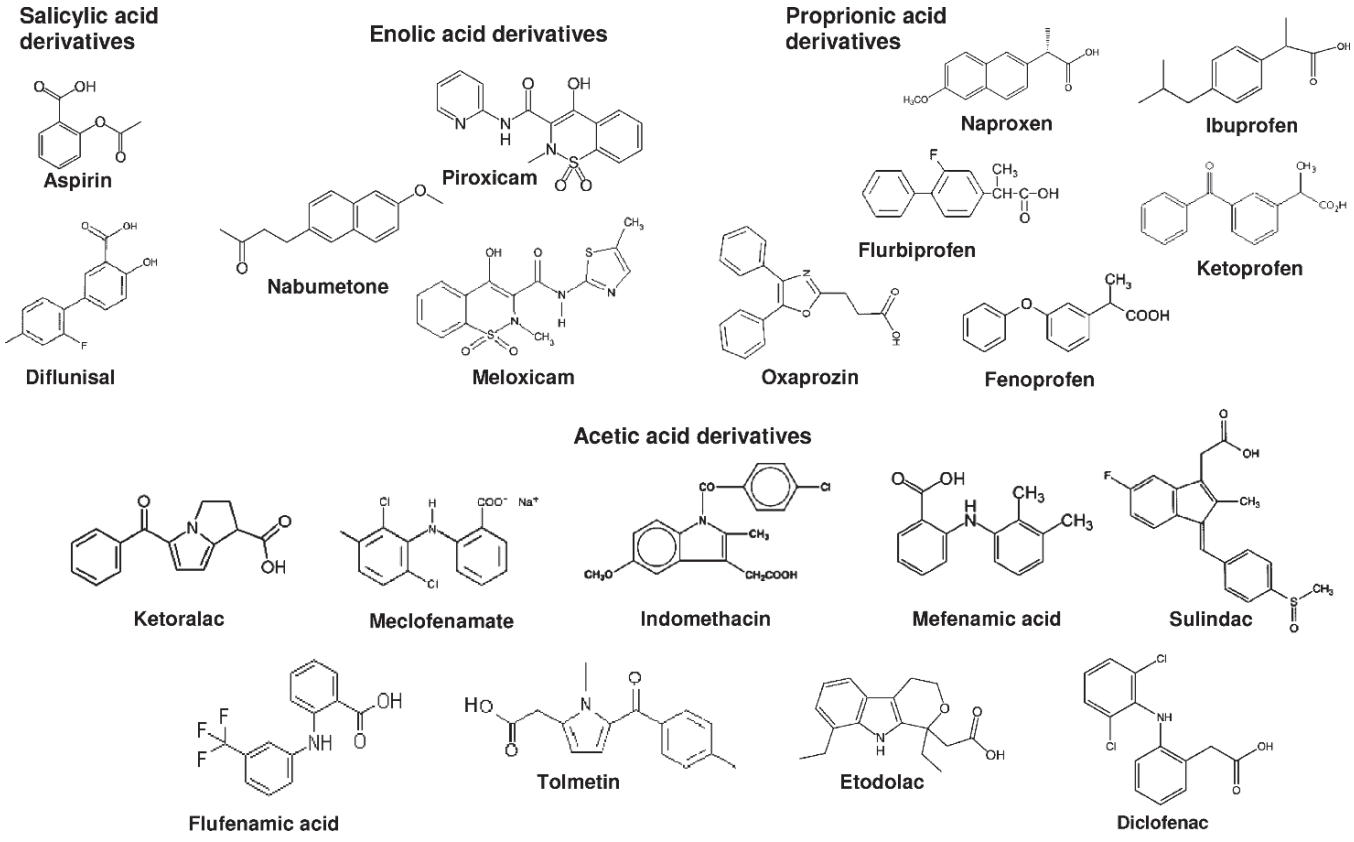

Fig. 20.2 Chemical structures of traditional NSAIDs (tNSAIDs)

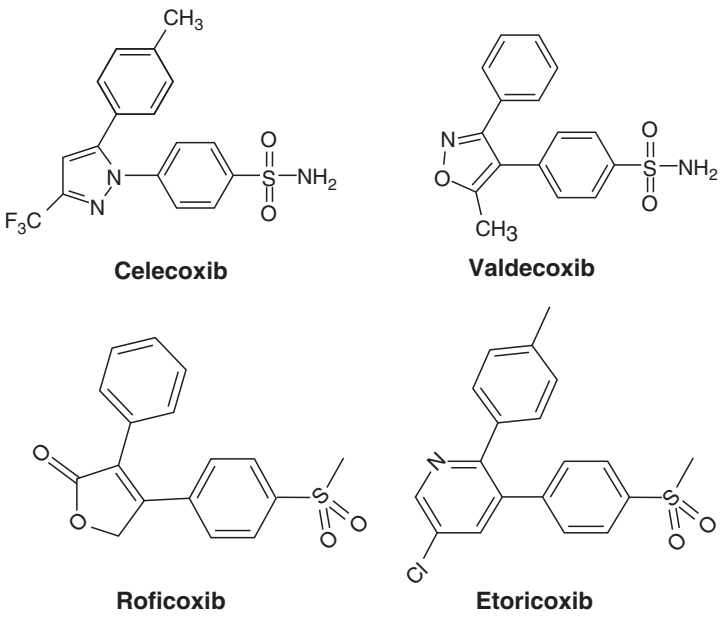

Fig. 20.3 Chemical structures of selective COX-2 inhibitors

induce apoptosis through COX-independent mechanisms that involve 15-LOX-1 [3] and genes that are both pro- and antiapoptotic (*PAR-4* and *Bcl-XL* respectively) [4, 5]. Chan and others have proposed that the tumor-suppressive effects of NSAIDs are attributable not to the reduction in prostaglandins but to increased levels of arachidonic acid stimulating apoptosis through production of ceramide [6]. Others propose that NSAIDs may induce apoptosis by the activation of caspases [7], p38 MAP kinase [8], or the release of mitochondrial cytochrome c [9].

20.2 Risk–Benefit Considerations

Drug toxicity poses a serious challenge to the development of "safe" and effective interventions to prevent cancer. "Safety" in this context signifies that the net benefits exceed the net risks, not that the intervention is without risk. Constraints from toxicity are greatest when interventions to prevent a specific type of cancer are applied to the general population, and large numbers of otherwise healthy people must be treated for many years to prevent this endpoint from occurring in a small fraction of those treated. For example, only 6% of the United States population, on average, will be diagnosed with colorectal cancer over a lifetime. The benefits of aspirin to prevent colon cancer are offset by the increase in side effects due to bleeding. However, several strategies can be pursued to shift the balance of risks and benefits from prophylactic treatment with aspirin. These include finding the least toxic treatment regimen, documenting other common cancers besides colon cancer that can be prevented, and selecting patient populations for whom the benefits of treatment will outweigh the risks.

Aspirin has a singular advantage over other NSAIDs in that it is the only COX inhibitor that

has been shown to be highly effective at reducing the risk of thrombotic cardiovascular events, even at very low doses. The development of low-dose aspirin as the optimal antiplatelet therapy takes advantage of several unique characteristics of aspirin and platelets. Aspirin is the only NSAID that binds irreversibly to COX-1 in platelets; platelets migrate through the portal circulation where the concentration of salicylic acid is higher than in the systemic circulation; and platelets lack a nucleus and cannot regenerate active enzyme. In contrast to aspirin, rofecoxib and other potent selective COX-2 inhibitors increase cardiovascular risk, effectively precluding their use for cancer prevention.

It is admittedly more difficult to determine the lowest effective dose of aspirin for cancer prevention without a better understanding of the specific target tissue(s) and mechanisms that mediate these effects. However, it would be possible to conduct longer-term clinical trials to determine whether aspirin at various doses (80 mg or 325 mg) inhibit the development of common cancers (particularly colorectal, breast, and prostate). Future trials should test daily administration of aspirin, rather than every other day, since daily treatment is optimal to inhibit platelet aggregation and matches the current clinical recommendation for the prevention of heart disease.

It may also be possible to broaden the potential benefits of long-term prophylactic treatment with aspirin if intervention can be shown to decrease the risk of breast and prostate cancer as well as colorectal cancer. Whereas the benefits of preventing colorectal cancer with aspirin treatment are counterbalanced by the side effects of gastrointestinal bleeding and hemorrhagic stroke, this would not be the case if other common cancers were also inhibited. Even if a higher dose of aspirin were needed to accomplish this, the net effect might be favorable, since the absolute risk of bleeding increases only slightly across the range from a baby aspirin (80 mg) daily to an adult aspirin (325 mg) daily.

20.3 Conclusions

Prophylactic treatment with aspirin continues to have promise for the prevention of colorectal and perhaps other cancers. Major gaps in the evidence, however, currently preclude any clinical recommendation. Additional research is needed to identify the specific dose, treatment regimen, and patient population(s) where the benefits of prophylactic treatment will exceed the risks.

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Aspirin and NSAIDs for the Prevention of Colorectal Cancer

21

John A. Baron

Abstract With few exceptions, epidemiological studies have found that individuals who take nonsteroidal antiinflammatory drugs (NSAIDs) have a reduced risk of colorectal adenomas and carcinoma. Similarly, randomized studies in patients with familial adenomatous polyposis have uniformly found that NSAIDs can lead to polyp regression and prevention of new polyps, and trials in patients with sporadic adenomas document that aspirin reduces the risk of adenoma recurrence. Together these data provide convincing evidence for the chemopreventive efficacy of NSAIDs in the large bowel.

21.1 Introduction

Although aspirin has been widely used for over a century, some of its effects—and those of other nonsteroidal antiinflammatory drugs (NSAIDs)—are only now being clarified. Of course these drugs are well known for their analgesic, antipyretic, and antiinflammatory effects, but over the past 30 years their antineoplastic potential has become an intense focus of research.

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The possibility that NSAIDs may have anticarcinogenic effects derived from observations that many cancers overproduce prostaglandins and the knowledge that aspirin and other NSAIDs inhibit the production of these compounds (Lupulescu 1996). Subsequent data from human epidemiological studies and experimental animal investigations suggest that NSAIDs may interfere with carcinogenesis in the large bowel and other organs.

However, for several reasons, the observational investigation of the association between NSAID use and cancer risk may be complicated. For one thing, there is a reasonable chance of substantial confounding. The disorders that prompt individuals to use NSAIDs could plausibly be related to cancer risk or lead to enhanced diagnosis of cancer, and individuals who use cardioprotective aspirin may be particularly health conscious, and so have a lower risk of some cancers. Another problem is that widely available, over-the-counter (OTC) drugs such as aspirin and ibuprofen may be taken in irregular patterns, making it hard to accurately measure intake and distinguish use from that of other similar drugs such as acetaminophen. This measurement error can distort observed associations in observational studies. Moreover, reporting of use may vary according to the education or other characteristics of subjects—or even worse, in

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case-control studies, by case status. Another difficulty is that early symptoms of undiagnosed cancer may prompt affected individuals to use NSAIDs for symptomatic relief. This would create an apparent association between NSAID use and cancer risk. Conversely, the upper gastrointestinal symptoms and bleeding associated with aspirin use may lead to earlier diagnosis of cancer, particularly gastrointestinal cancer.

It is difficult for any single observational study to avoid all of these potential biases or even to assess the extent to which the resulting distortions affect a study. Many of the potential problems can be avoided through careful research strategies, and a series of observational studies of various designs in different populations can elucidate the effect of NSAIDs on cancer risk. Nonetheless, because of the potential limitations of observational research, clinical trials provide the strongest evidence regarding the chemopreventive potential of NSAIDs.

21.2 Colorectal Neoplasia: Observational Studies

Epidemiological studies have repeatedly indicated an inverse association of use of NSAIDs, particularly aspirin, with risk of colorectal cancer (Baron 2003; Rostom et al. 2007; Dube et al. 2007). The first epidemiological study to report such an effect was a population-based case-control study from Australia (Kune et al. 1988). The risk of colorectal cancer was lower in subjects who used aspirin: the association was seen in men and women, and for both colon and rectal cancer. There was also a nonsignificant reduction in risk associated with use of nonaspirin NSAIDs. A later report by Rosenberg et al. provided confirmation and further detail. This study reported approximately a 50% lower risk of large bowel cancer in subjects who regularly used NSAIDs (principally aspirin) (Rosenberg et al. 1991). Both colon and rectal cancer showed the effect, and there was a similarly reduced risk for both men and women. The reduced risk was not seen among former users (cessation more than 1 year before interview).

The vast majority of subsequent observational studies have reported broadly similar findings regarding the association of NSAID use with risk of colorectal cancer, showing an inverse association that is not materially affected by gender, age, race, family history of colorectal cancer, or anatomic site in the large bowel (Baron 2003; Dube et al. 2007; Flossmann and Rothwell 2007; Rostom et al. 2007). The inverse association of colorectal cancer risk has been reported for aspirin specifically, for nonaspirin NSAIDs, and for all NSAIDs considered as a single group (Dube et al. 2007; Flossmann and Rothwell 2007; Rostom et al. 2007). The fact that use of acetaminophen is not associated with a decreased risk of colorectal cancer (Baron 2003) suggests that the association is specific to aspirin (or other NSAIDs) and not to some sort of response bias. Most studies have reported that the inverse association of NSAID use with risk of colorectal cancer dissipates after stopping regular use of the drugs for a few years (Baron 2003; Chan et al. 2008; Sansbury et al. 2005). A protective effect of aspirin use on the risk of large bowel adenomas has also been repeatedly documented (Baron 2003; Flossmann and Rothwell 2007; Tangrea et al. 2003).

One study conducted in a California retirement community failed to demonstrate a protective effect of NSAIDs on colorectal cancer risk (Paganini-Hill et al. 1991). This investigation studied the elderly (the median age of subjects was 73 years) and so its findings raise the possibility that the effects of aspirin on large bowel neoplasia are less pronounced or absent in older individuals. This pattern has been suggested by findings in other studies (Baron and Sandler

2000), although the corresponding interactions in these analyses were not statistically significant, and this issue has not been fully investigated.

For aspirin, and NSAIDs generally, a pattern of decreasing risk with increasing dose has usually been seen in the observational studies that have considered the matter (Baron 2003; Chan et al. 2005; Chan et al. 2008; Collet et al. 1999; Juarranz et al. 2002; Hoffmeister et al. 2007). A few studies, however, have not seen such a pattern (Allison et al. 2006; Baron 2003; Larsson et al. 2006). Whatever the dose–response, patients who take aspirin for cardiovascular protection seem to have a reduced a risk of colorectal cancer (Baron 2003; Chan et al. 2005; Chan et al. 2008; Larsson et al. 2006).

There appears to be a relationship between duration of NSAID use and risk of colorectal cancer. Most studies that have addressed the issue have reported decreasing risks with increasing duration of use (Chan et al. 2008; Flossmann and Rothwell 2007; Hoffmeister et al. 2007), although a few studies have not seen such trends (Baron 2003). The most careful data, reported from large cohort studies, suggests that around 10 years of use is required for a meaningful reduction in the risk of colorectal cancer to become apparent (Chan et al. 2005; Chan et al. 2008).

21.3 Clinical Trial Data: Sporadic Colorectal Neoplasia

Clinical trial data confirm many of these observational findings. A combined analysis of the long-term follow-up of two English trials is particularly compelling (Flossmann and Rothwell 2007). The two trials were the British Doctors Aspirin Trial (5,139 male subjects randomized to 500 mg aspirin or no treatment in a 2:1 ratio for 5 years) and the UK-TIA Aspirin Trial (2,449 subjects randomized to 300 mg, 1,200 mg or placebo for 1–7 years). There was a small, nonsignificant reduction of colorectal cancer risk during the first 10 years after randomization [hazard ratio 0.92; 95% confidence interval (CI), 0.56–1.49]. Subsequently, risk was more substantially reduced: the relative risk for 10–19 years after randomization was 0.60 (95% CI, 0.42–0.87).

The Physicians' Health Study (Gann et al. 1993; Sturmer et al. 1998) assessed the effect of 325 mg of aspirin every other day on cardiovascular endpoints in over 22,000 men, and reported on colorectal neoplasia in secondary analyses. There was an increased risk of diagnosed colorectal cancer within 3 years of randomization—an effect that would be expected if aspirin use led to the diagnosis of cases present (but unrecognized) at study entry. However, the relative risk fell over time after randomization (p for trend = 0.11) to a relative risk nonsignificantly below 1.0 for the period 10 or more years after randomization (Sturmer et al. 1998). Overall, there was no reduction in risk of colorectal cancer. Another large trial of aspirin use was the Women's Health Study (Cook et al. 2005), which randomized almost 40,000 women to 100 mg aspirin every other day or placebo, with treatment extending for around 10 years. As in the Physicians' Health Study, there was no reduction in risk of colorectal cancer over a follow-up of about 10 years; the relative risk for aspirin was 0.97 (95% CI, 0.77–1.24). These negative findings are consistent with the epidemiological findings and the combined analysis of the English trials: the chemopreventive effect of aspirin on colorectal cancer risk emerges only about 10 years after the initiation of aspirin treatment.

Adenomas occur earlier in colorectal carcinogenesis, and shorter time periods for an aspirin

effect would be expected for these lesions. Indeed, two trials of adenoma patients have shown that aspirin reduces the risk of subsequent adenoma occurrence after only 1–3 years of aspirin treatment and follow-up (Baron et al. 2003; Benamouzig et al. 2003). An adenoma trial among patients with a history of colorectal cancer showed similar findings (Sandler et al. 2003). Several large trials have documented that a similarly short period of use of the Cox-2 selective inhibitors celecoxib and rofecoxib are effective in reducing the risk of adenomas in patients with a recent history of these lesions (Baron et al. 2006; Bertagnolli et al. 2006; Arber et al. 2006).

In the Adenoma Prevention with Celecoxib trial, there were suggestions that higher-dose celecoxib reduced the risk of adenoma more than a lower dose. However, in the Aspirin/Folate Polyp Prevention Study, 3 years of aspirin 81 mg was effective in reducing risk, but 325 mg was not (Baron et al. 2003). A similar perverse dose–response trend has been reported from the smaller French trial (R. Benamouzig, personal communication).

Several intervention studies have assessed whether NSAIDs cause regression of sporadic adenomas. The data are suggestive, but interpretation is hampered by weak study designs and incomplete reporting (Baron and Sandler 2000).

21.4 Special Populations

Familial adenomatous polyposis (FAP) is a rare genetic syndrome in which affected patients develop thousands of colorectal adenomas by young adulthood and have a near universal risk of colorectal cancer if the large bowel is left intact. In the 1980s, small, uncontrolled, unblinded studies suggested striking efficacy of NSAIDs against neoplasia in FAP. Sulindac (300 mg to 400 mg/day) led to almost complete resolution of polyps. Polyps recurred with cessation of treatment. Indomethacin, on the other hand, did not lead to regression of adenomas, suggesting that there was no general effect of NSAIDs (Baron and Sandler 2000).

Formal clinical trials have confirmed this efficacy. Sulindac has been tested in several placebo-controlled trials, leading to a 40%–50% reduction in the number of polyps (Baron 2003). In one trial that used particularly careful measurement techniques in 22 patients, 150 mg of Sulindac b.i.d. for 9 months of treatment resulted in a 44% decrease in the number of polyps and a 35% decrease in the size of the polyps found. In the placebo group, there were increases in both parameters (Giardiello et al. 1993). Celecoxib and rofecoxib have also shown efficacy in reducing the number of polyps in randomized trials, though the reductions reported in these trials seem less marked than for sulindac (Higuchi et al. 2003; Steinbach et al. 2000).

These data are exciting indications that NSAIDs may actually lead to the regression of established neoplasia. Like the observational data discussed above, these studies also indicate that the neoplastic effect of NSAIDs is rapidly reversible after cessation of NSAID use. This pattern parallels findings for sporadic neoplasia discussed above. Also, reports of FAP patients who had regression of adenomas on NSAIDs but developed carcinoma nonetheless emphasize the fact that cancer protection from NSAIDs is incomplete (Lynch et al. 1995).

Patients with ulcerative colitis have a greatly increased risk of colorectal cancer, and one of the drugs used to treat this condition, sulfasalazine, incorporates a salicylate moiety. This drug seems to exert a chemoprotective effect (Croog et al. 2003).

Summary

On balance the literature on aspirin and NSAID prevention of colorectal neoplasia is extremely compelling. With only rare exceptions, studies with different designs, populations, locations, agents and investigators have reached the same conclusions—aspirin and other NSAIDs appear to decrease the risk for colorectal neoplasia by approximately half. Studies have shown that drugs of this class decrease the risk not only of colorectal cancer but also of adenomas. Experimental studies using sulindac in patients with FAP show regression of adenomas; less consistently, polyp regression has been observed in patients with sporadic adenomas. Ten or more years of regular use seem to be required before the incidence of invasive cancers is decreased. The literature in humans is supported by data from experimental studies in animals.

There is compelling evidence that aspirin and other NSAIDs interfere with carcinogenesis in the large bowel. With only rare exceptions, observational studies with different designs, populations, locations, agents and investigators have found that aspirin and other NSAIDs appear to decrease the risk for colorectal neoplasia. The reality of a protective effect is buttressed by clinical trial data showing effects on both colorectal cancer and sporadic adenomas. Ten or more years of regular aspirin use seem to be required before the incidence of invasive cancers is decreased. Furthermore, the NSAIDs sulindac, celecoxib, and rofecoxib have actually led to the regression of existing colorectal polyps in patients with FAP. Animal data support the findings from human studies.

As a body of research, the findings discussed here from epidemiological studies and clinical trials have clarified the effect of NSAIDs on carcinogenesis in the large bowel. However, it is probably premature to now begin to use these drugs widely for cancer prevention. To reach that point, a weighing of the risks and benefits of the drugs needs to be made, together with a judgment regarding the benefits of alternative means of prevention. For colorectal cancer, for example, aspirin may provide only limited benefit over regular colonoscopy (DuPont et al. 2007; Ladabaum et al. 2001; Suleiman et al. 2002). Nonetheless, with the increased understanding of the clinical effects of NSAIDs on cancer, the development of effective chemoprevention with these drugs appears to be a real possibility.

Several cost-effectiveness analyses of aspirin as a chemopreventive agent for colorectal cancer have been conducted. They are all in broad agreement that colonoscopy/polypectomy is more cost-effective than aspirin use, and that aspirin alone is not a cost-effective intervention alone. Of course these studies are highly dependent on the assumptions that go into them, including those related to the efficacy of aspirin, the risk of serious aspirin toxicity, and the efficacy of colonoscopic surveillance.

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Aspirin and Cancer Risk: A Summary Review to 2007

22

Cristina Bosetti, Silvano Gallus, and Carlo La Vecchia

Abstract Aspirin has been associated with a reduced risk of colorectal cancer and—based on limited evidence—to cancers of the oesophagus, stomach, breast, ovary and lung. The role of aspirin on other cancers, such as pancreatic, prostate and bladder cancer and non-Hodgkin's lymphomas and myeloma is less clear, and an increase of risk has been suggested for kidney cancer. For most cancer sites, however, significant heterogeneity between studies, and particularly between study design, was found, with a reduction in risk generally stronger in case-control studies than in cohort ones.

22.1 Introduction

Epidemiological data on a favourable role of aspirin on the risk of colorectal and other common cancers have accumulated since the late 1980s (Berkel et al. 1996; Greenberg and Baron 1996; IARC 1997). A possible target of the cancer chemopreventive effect of aspirin and other non-steroidal anti-inflammatory drugs (NSAIDs) is the inhibition of cyclooxygenase (COX). COX (in particular the isoform COX-2) has been reported to be abnormally expressed in many cancer cell lines, and has been implicated in the process of carcinogenesis, tumour growth, apoptosis and angiogenesis (Fiorucci and Antonelli 2001; Taketo 1998a, b; Thun et al. 2002).

Epidemiological studies on the association between aspirin and cancer risk published up to 2005 have been previously reviewed (Bosetti et al. 2002, 2006a). An overall quantitative estimate of the risk from all case-control and cohort studies published upto 2005 on aspirin use and cancer risk has been also provided, based on a meta-analysis published by Bosetti et al. (2006a). Major evidence from these meta-analyses is summarized in the present work, and studies published thereafter have been added.

22.2 Materials and Methods

The studies considered were all original cohort and case-control investigations on

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cancer including information on aspirin and other NSAID use published up to December 2007. The main characteristics and results of each study are described in Tables 22.1, 22.2, 22.3, 22.4, 22.5, 22.6 and 22.7. These included the first author and year of publication, the total numbers of cases, the relative risk (RR) and corresponding 95% confidence interval (CI) for regular aspirin use (or ever use, if the former was not given), and a brief comment.

22.3 Results

22.3.1 Colon and Rectal Cancer

Over 25 case-control and cohort studies have been published on aspirin and colorectal cancer (Table 22.1), the most frequent cancer among non-smokers in Western countries (Allison et al.

Table 22.1 Main findings of epidemiological studies on aspirin and colorectal cancer

Study	No. of cases	RR	95% CI	Comment
Case-control studies				
Kune et al. 1988	715	0.53	0.40–0.71	Current vs non-current use
Rosenberg et al. 1991	1,326	0.50	0.40–0.80	Regular use for >3 months
Suh et al. 1993	830	0.3–0.4		Use >2/day. Significant trend with dose
Muscat et al. 1994	511	0.64[a] 0.32[b]	0.42–0.97 0.18–0.57	Protection unrelated to the indication. Significant trend with duration in men
Peleg et al. 1994	216	0.25	0.09–0.73	Use in the four previous years >131 g. Significant trend with dose
Reeves et al. 1996	184	0.79	0.45–1.36	RR=0.4, significant, for other NSAIDs
La Vecchia et al. 1997	1,357	0.70	0.50–1.00	Significant trend with dose
Rosenberg et al. 1998	1,201	0.70	0.50–0.90	Continued use. Significant protection for other NSAIDs
Friedman et al. 1998	1,993	0.70	0.60–0.80	RR=0.60 for recent use. Similar protection, for other NSAIDs
Neugut et al. 1998	256	0.35	0.17–0.73	
Juarranz et al. 2002	196	0.98	0.90–1.00	RR for a continuous increment. RR=0.30, significant, for other NSAIDs
Sansbury et al. 2005	643	0.47	0.27–0.80	RR=0.49, significant, for any NSAIDs
Sansbury et al. 2005	294 black 349 white	0.41 0.48	0.22–0.77 0.28–0.83	Regular use of NSAIDs
Vinogradova et al. 2007	5,681	0.94	0.83–1.02	UK General Practice Research database. Any NSAID use
Cohort studies				
Paganini-Hill et al. 1989	181	1.50	1.10–2.20	Daily use
Thun et al. 1991, 1993	1,388	0.60	0.34–1.01	ACS/CPS II. >16 times/month for >1 year. Significant trend with frequency but not duration

(continued)

Table 22.1 (continued)

Study	No. of cases	RR	95% CI	Comment
Schreinemachers and Everson 1994	169	0.85	0.63–1.15	NHANES I. Incidence
Giovannucci et al. 1994	251	0.68	0.52–0.92	Male Health Professional. Trend with the length of follow-up
Giovannucci et al. 1995	331	0.56	0.36–0.90	Nurses' Health Study. Reduced risk for >20 years of use
García Rodríguez and Huerta Alvarez 2001	2,002	0.90	0.80–1.10	Nested case-control study. R=0.6, significant, for 300 mg/day for >6 months. RR=0.7, significant, for other NSAIDs
Ratnasinghe et al. 2004	193	1.07	0.71–1.60	NHANES I and II. Mortality
Chan et al. 2005	962	0.77	0.67–0.88	Nurses' Health Study. Regular use. Significant dose- and duration-response relations. RR=0.47 for >14 aspirins/week and for >20 years of use
Larsson et al. 2006	705	0.65	0.45–0.94	Swedish prospective cohort including 74,250 men and women. Use >20 years. No association for shorter use
Mahipal et al. 2006	636	0.76	0.58–1.00	Iowa Women's Health Study. Regular use
Allison et al. 2006	631	0.96	0.8–1.2	Women's Health Initiative
Jacobs et al. 2007	1,801	0.68	0.52–0.90	ACS CPS II Nutrition Cancer. Current daily users

ACS, American Cancer Society; CI, confidence interval; CPS, Cancer Prevention Study; NHANES, National Health and Nutrition Examination Survey; NSAIDs, non-steroidal anti-inflammatory drugs; RR, relative risk

[a] Men

[b] Women

[c] Update of Giovannucci et al. 1995

2006; Asano and McLeod 2004; Chan et al. 2005; Friedman et al. 1998; García Rodríguez and Huerta-Alvarez 2001; Giovannucci et al. 1995; Giovannucci et al. 1994; Herendeen and Lindley 2003; Jacobs et al. 2007; Juarranz et al. 2002; Kune et al. 1988; La Vecchia et al. 1997; Larsson et al. 2006; Levi et al. 2004; Mahipal et al. 2006; Muscat et al. 1994; Neugut et al. 1998; Paganini-Hill et al. 1989; Peleg et al. 1994; Ratnasinghe et al. 2004; Reeves et al. 1996; Rosenberg et al. 1998; Rosenberg et al. 1991; Sansbury et al. 2005; Schreinemachers and Everson 1994; Suh et al. 1993; Thun et al. 1991, 1993; Vinogradova et al. 2007).

The results of epidemiological studies conducted on different populations, using different methods and types of controls, and based on more than 13,000 cases, indicate that (regular) aspirin use is associated with a reduction in the risk of colorectal cancer. The pooled RR estimate was 0.59 (95% CI, 0.54–0.64) from 11 case-control studies, 0.85 (95% CI, 0.78–0.92) from 7 cohort ones, and 0.71 (95% CI, 0.67–0.75) from all studies combined (Table 22.8). The estimates from case-control and cohort studies were, however, heterogeneous, and a significant heterogeneity was observed also within case-control and cohort studies.

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Table 22.2 Main findings of case-control studies of aspirin and digestive tract cancers other than colorectal

Study	No. of cases	RR	95% CI	Comment
Oesophagus				
Case-control studies				
Farrow et al. 1998	293[a] 221[b]	0.37 0.49	0.24–0.58 0.28–0.87	>1 tablet/week for >6 months. No relation with frequency and duration
Sharp et al. 2001	159[b]	0.08	0.01–0.56	English centres
		0.77	0.21–2.94	Scottish centres Daily use for >1 month
Jayaprakash et al. 2006	163	0.54	0.36–0.80	Regular users from Buffalo, NY
Cohort studies				
Thun et al. 1993	157	0.59	0.34–1.03	ACS/CPS II. >16 times/month for >1 year
Funkhouser and Sharp 1995	15	0.10	0.01–0.76	NHANES I. Incidence. Occasional use
Ratnasinghe et al. 2004	37	0.48	0.18–1.29	NHANES I and II. Mortality
Lindblad et al. 2005	909	0.93	0.76–1.15	Nested case-control study. Current use. RR=0.76 for >3 years. Similar RR for non-aspirin NSAIDs
Stomach				
Case-control studies				
Farrow et al. 1998	261[c] 368[d]	0.80 0.46	0.54–1.19 0.31–0.98	>1 tablet/week for >6 months. No relation with frequency and duration. Stronger reduced risk for non-aspirin NSAIDs
Zaridze et al. 1999	448	0.60	0.41–0.90	>2 days/week for >6 months.
Akre et al. 2001	480	0.70	0.60–1.00	>1 tablet/month. RR=1.1 for non-aspirin NSAIDs
Cohort studies				
Thun et al. 1993	266	0.53	0.34–0.81	ACS/CPS II. >16 times/month for >1 year
Schreinemachers and Everson 1994	39	0.93	0.49–1.74	NHANES I. Incidence
Ratnasinghe et al. 2004	48	0.82	0.38–1.81	NHANES I and II. Mortality
Lindblad et al. 2005	1,023	1.15	0.98–1.36	Nested case-control study. Current use. RR=0.83 for non-aspirin NSAIDs
Pancreas				
Case-control studies				
Menezes et al. 2002a	194	1.00	0.72–1.39	>1 tablet/week for >6 months. No relation with dose and duration
Cohort studies				
Schreinemachers and Everson 1994	30	0.67	0.33–1.36	NHANES I. Incidence

(continued)

Table 22.2 (continued)

Study	No. of cases	RR	95% CI	Comment
Anderson et al. 2002	80	0.57	0.36–0.90	IWHS. RR=0.40, significant, for >6 times/week. RR=1.19 for non-aspirin NSAIDs
Schernhammer et al. 2004	161	1.20	0.87–1.65	Nurses' Health Study. >2 tablets/week. RR=1.58 for >20 years of regular use
Jacobs et al. 2004	4,577	0.97	0.86–1.09	ACS/CPS-II. Daily use
Ratnasinghe et al. 2004	78	0.87	0.42–1.77	NHANES I and II. Mortality
Biliary tract				
Case-control study				
Liu et al. 2005				
Gallbladder	368	0.37	0.17–0.88	From Shanghai, China
Bile duct	191	0.48	0.19–1.19	
Ampulla of Vater	68	0.22	0.03–1.45	

ACS, American Cancer Society; CI, confidence interval; CPS, Cancer Prevention Study; IWHS, Iowa Women's Health Study; NHANES, National Health and Nutrition Examination Survey; NSAIDs, Non-Steroidal anti-inflammatory drugs; RR, relative risk

[a] Oesophageal adenocarcinomas

[b] Squamous cell oesophageal carcinomas

[c] Gastric cardia adenocarcinomas

[d] Non-cardia gastric adenocarcinomas

Table 22.3 Main findings of case-control studies of aspirin and lung cancer

Study	No. of cases	RR	95% CI	Comment
Case-control studies				
Moysich et al. 2002	868	0.57	0.41–0.78	>1 per week for >1 year
Muscat et al. 2003	1,038	0.84	0.62–1.14	Regular use. Risk reduction only in smokers
Kelly et al. 2007	1,884	1.1	0.9–1.4	Hospital-based study. No relation with value was duration
Hernández-Díaz and García Rodríguez 2007	4,419	0.76 1.15	0.61–0.94 0.79–1.34	All NSAIDs Aspirin. Nested in The Health Improvement Network (THIN), UK
Olsen et al. 2008	573	0.86	0.65–1.14	Significant trends in risk with dose
Cohort studies				
Paganini-Hill et al. 1989	111	1.35[a] 0.29[b]	0.73–2.32 0.07–1.14	Daily use

(continued)

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Table 22.3 (continued)

Study	No. of cases	RR	95% CI	Comment
Thun et al. 1993[c]	NR	1.11[a] 1.07[b]	0.98–1.25 0.88–1.30	ACS/CPS II. >16 times/month for >1 year
Schreinemachers and Everson 1994[c]	163	0.68	0.49–0.94	NHANES I study. Incidence
Akhmedkhanov et al. 2002	81	0.66	0.34–1.28	Nested case-control study. >3 times per week for >6 months. RR=0.68 for NSAIDs
Holick et al. 2003	328	0.89	0.47–1.67	Health Professionals Study. Use >2/week
Ratnasinghe et al. 2004	410	0.81	0.62–1.07	NHANES I and II. Mortality
Hayes et al. 2006	403	1.23	0.92–1.65	IWHS. Regular use
Jacobs et al. 2007	1,730	0.98	0.76–1.25	ACS CPS II Nutrition Cohort. Current daily users
Feskanich et al. 2007	1,446	1.00	0.86–1.00	Nurses Health Study. Multivariate RR

ACS, American Cancer Society; CI, confidence interval; CPS, Cancer Prevention Study; IWHS, Iowa Women's Health Study; NHANES, National Health and Nutrition Examination Survey; NR, not reported; NSAIDs, non-steroidal anti-inflammatory drugs; RR, relative risk

[a] Men

[b] Women

[c] Respiratory cancers

Table 22.4 Main findings of epidemiologic studies of aspirin and breast and ovarian cancer

Study	No. of cases	RR	95% CI	Comment
Breast				
Case-control studies				
Harris et al. 1996	511	0.69	0.46–0.99	
Neugut et al. 1998	252	0.80	0.35–1.80	
Coogan et al. 1999	6,558	0.70	0.50–0.80	RR=0.9 and 0.8 for other NSAIDs, in comparison with cancer and non-cancer patients
Cotterchio et al. 2001	3,133	0.73	0.61–0.87	RR=0.76, significant, for all NSAIDs combined. RR=0.68 for >8 years of NSAIDs use
Terry et al. 2004	1,442	0.80	0.66–0.97	Stronger protection for more frequent users (RR=0.72) and in hormone receptor-positive tumours (RR=0.74)
Swede et al. 2005	1,478	0.85	0.74–0.97	Regular use. RR=0.74 for daily use. No trend with duration
Zhang et al. 2005[a]	7,006	0.86	0.64–1.16	Regular use. No trend with duration. RR=0.74 for any NSAIDs and 0.85 for ibuprofen

(continued)

Table 22.4 (continued)

Study	No. of cases	RR	95% CI	Comment
Cohort studies				
Friedman and Ury 1980	2	0.20	0.05–0.80	Computer-stored drug-dispensing data
Paganini-Hill et al. 1989	214	0.96	0.75–1.21	Daily use
Thun et al. 1993	ND	0.88	0.62–1.24	ACS/CPS II. >16 aspirin/month for >1 year
Schreinemachers and Everson 1994	147	0.70	0.50–0.96	NHANES I. Incidence
Egan et al. 1996	2,414	1.01	0.80–1.27	Nurses' Health Study. No trend with frequency or duration of use
Harris et al. 1999	393	0.60	0.47–0.77	Similar protection for other NSAIDs
Johnson et al. 2002	938	0.82	0.71–0.95	IWHS, post-menopausal women. RR=0.71 for more frequent use. RR=0.98 for other NSAIDs
Harris et al. 2003	1,392	0.81	0.66–0.99	WHI. >5 years of use. Similar RR for all NSAIDs combined; stronger protection for ibuprofen
García-Rodríguez and González-Pérez 2004b	3,708	0.77	0.62–0.95	Nested case-control study. RR=1.0 for non-aspirin NSAIDs and 0.76 for paracetamol. Greater reduced risk for low doses. No trend with duration
Ratnasinghe et al. 2004	131	0.82	0.49–1.36	NHANES I and II. Mortality
Jacobs et al. 2005b	3,008	1.01	0.84–1.20	ACS/CPS-II Nutrition Cohort. >60 pills/month. RR=1.06 for ibuprofen and 1.16 for other NSAIDs. No association with long-term regular use
Marshall et al. 2005	2,391	0.98	0.86–1.13	Daily use. No trend for frequency and duration among regular users RR=0.89 in hormone receptor-positive cancers
Jacobs et al. 2005b	3,066	0.83	0.63–1.10	ACS CPS II Nutrition cohort. Current users only
Gill et al. 2007	1,830	1.05	0.88–12.5	The multiethnic cohort. Current long-term aspirin users
Ovary				
Case-control studies				
Tzonou et al. 1993	189	0.51	0.26–1.02	Unspecified analgesics (mainly aspirin). Significant trend for frequency
Cramer et al. 1998	563	0.75	0.52–1.10	
Tavani et al. 2000	749	0.93	0.53–1.62	No trend with frequency and duration
Rosenberg et al. 2000	780	0.8	0.5–1.2	RR=0.5 for >5 years of use. RR=0.5 for other NSAIDs
Moysich et al. 2001	547	1.00	0.73–1.39	RR=0.87 for >7 aspirins/week
Akhmedkhanov et al. 2001	68	0.60	0.26–1.38	No trend with duration

(continued)

Table 22.4 (continued)

Study	No. of cases	RR	95% CI	Comment
Schildkraut et al. 2006	586	0.72	0.56–0.92	Population -based study from North Carolina
Cohort studies				
Fairfield et al. 2002	333	1.00	0.80–1.25	Nurses' Health Study. No trend with dose and duration. RR=0.60, significant, for NSAIDS and 0.81 for paracetamol >5 days/month
Lacey et al. 2004	116	0.86	0.52–1.4	RR=0.56 for more frequent use. No association for paracetamol and other NSAIDs
Endometrium				
Case-control study				
Moysich et al. 2005	427	0.91	0.66–1.21	From Buffalo, NY. Inverse relation for obese women

ACS, American Cancer Society; CI, confidence interval; CPS, Cancer Prevention Study; IWHS, Iowa Women's Health Study; ND, not defined; NHANES, National Health and Nutrition Examination Survey; NSAIDs, non-steroidal anti-inflammatory drugs; WHI, Women's Health Initiative; RR, relative risk

[a] Update of Coogan et al. 1999

Aspirin and NSAIDs have been shown to suppress the development of adenomatous polyps in patients with familial adenomatous polyposis (Asano and McLeod 2004; Herendeen and Lindley 2003), and a few randomized trials (Baron et al. 2003; Benamouzig et al. 2003; Greenberg et al. 1993; Sandler et al. 2003) showed that aspirin reduces the risk of colorectal adenomas in populations with a history of colorectal cancer or adenomas. Similar protections against the risk of adenomas were found in various case-control and cohort studies (Thun et al. 2002). Results from intervention studies on colorectal cancer are, however, inconclusive. Thus, the Physicians' Health Study randomized trial found no effect of aspirin (325 mg) on invasive colorectal cancer incidence, although it was based on short treatment and limited follow-up (Gann et al. 1993; Sturmer et al. 1998). Similarly, the Women's Health Study (WHS) randomized trial (Cook et al. 2005) did not report any protection for long-term aspirin use on colorectal cancer, at a dose of 100 mg/day.

22.3.2 Other Digestive Tract Cancers

There are suggestions that the potential favourable role of aspirin and other NSAIDs on colorectal cancer might extend to other gastrointestinal cancers such as oesophagus, stomach, pancreas and biliary tract (Table 22.2; Akre et al. 2001; Anderson et al. 2002; Corley et al. 2003; Farrow et al. 1998; Funkhouser and Sharp 1995; Jacobs et al. 2004; Jayaprakash et al. 2006; Lindblad et al. 2005; Liu et al. 2005; Menezes et al. 2002a; Ratnasinghe et al. 2004; Schernhammer et al. 2004; Schreinemachers and Everson 1994; Sharp et al. 2001; Thun et al. 1993; Wang et al. 2003; Zaridze et al. 1999).

Overall, the RR of oesophageal cancer was 0.41 (95% CI, 0.29–0.57) from 2 case-control studies, 0.83 (95% CI, 0.70–0.98) from 4 cohort studies, and 0.72 (95% CI, 0.62–0.84) overall (Table 22.8). Corresponding figures for gastric cancer were 0.67 (95% CI, 0.56–0.80), 0.93 (95% CI, 0.82–1.05) and 0.84 (95% CI, 0.76–0.93), from 3 case-control, 4 cohort studies, and

Table 22.5 Main findings of case-control studies of aspirin and prostate cancer

Study	No. of cass	RR	95% CI	Comment
Case-control studies				
Norrish et al. 1998	317	0.85	0.61–1.19	>1 per week. RR=0.88 for NSAIDs and 0.87 for non-aspirin NSAIDs. Stronger reductions for metastatic cancers
Neugut et al. 1998	319	1.60	0.82–3.11	
Menezes et al. 2002b	1,096	1.08	0.87–1.35	
Irani et al. 2002	639	0.95[a]	0.75–1.20	
Bosetti et al. 2006b	1,261	1.10	0.81–1.50	Use for >1 times week for >6 months. No trend with duration
Menezes et al. 2006	1,029	1.05	0.89–1.25	Data from Buffalo, NY. No association with dose nor duration
Cohort studies				
Paganini-Hill et al. 1989	149	0.95	0.60–1.51	Daily use
Thun et al. 1993[b]	NR	0.82	0.56–1.19	ACS/CPS II. >16 aspirin/month for >1 year
Schreinemachers and Everson 1994	123	0.95	0.66–1.35	NHANES I. Incidence
Leitzmann et al. 2002	2,479	1.04	0.86–1.26	Male Health Professional. >2 times/week reported in 4 consecutive questionnaires. RR=0.73 for metastatic cancers
Habel et al. 2002	2,574	0.76	0.60–0.98	>6 tablets almost ever day
Perron et al. 2003	2,221	0.82	0.71–0.95	Nested case-control study. Daily dose of >80 mg for 8 years. RR=0.72 for >4 years and >325 mg daily. No association with other NSAIDs
García-Rodríguez and González-Pérez 2004a	2,183	0.70	0.61–0.79	Nested case-control study. Current use. No trend with duration. RR=1.14, significant, for non-aspirin NSAIDs and 0.95 for paracetamol
Ratnasinghe et al. 2004	121	1.11	0.60–2.05	NHANES I and II. Mortality
Platz et al. 2005	141	0.76	0.54–1.16	Ever use. Lower reductions for current use. No trend with duration. Similar results for non-aspirin NSAIDs and paracetamol
Jacobs et al. 2005a	4,853	0.95	0.82–1.10	ACS/CPS II Nutrition Cohort. >60 pills/month. Significant reduced risks with long-term regular use. RR=0.92 for ibuprofen and 0.98 for other NSAIDs

ACS, American Cancer Society; CI, confidence interval; CPS, Cancer Prevention Study; NHANES, National Health and Nutrition Examination Survey; NR, not reported; NSAIDs, non-steroidal anti-inflammatory drugs; RR, relative risk

[a] Crude estimate

[b] Genital cancers (including prostate and testis)

Table 22.6 Main findings of case-control studies of bladder and kidney cancer

Study	No. of cases	RR	95% CI	Comment
Bladder				
Case-control studies				
Castelao et al. 2000	1,514	0.85	0.66–1.09	>2 per week for >1 month. Trend with cumulative lifetime exposure. RR=0.81, significant, for any analgesic and 1.03 for paracetamol
Fortuny et al. 2007	376	0.6	0.4–0.9	Any NSAID. Population-based from Spain
Cohort studies				
Paganini-Hill et al. 1989	93	1.10	0.65–1.85	Daily use of aspirin for an undefined time
Schreinemachers and Everson 1994	35	1.06	0.54–2.09	NHANES I. Incidence
Ratnasinghe et al. 2004	40	3.36	0.60–2.05	NHANES I and II. Mortality
Jacobs et al. 2004	827	0.83	0.58–1.19	ACS CPS II Nutrition Cohort. Current daily users
Genkinger et al. 2007	607	0.99	0.83–1.88	Health Professionals Follow-up study, incidence
Kidney				
Case-control studies				
McLaughlin et al. 1985[c]	495	0.5[a]	0.2–1.0	More than 14 times per month for >36 months
		1.8[b]	0.7–4.1	
McCredie et al. 1988[a]	360	1.2	0.7–1.9	>0.1 kg lifetime use
Mellemgaard et al. 1994[a]	368	1.4[a] 1.3[b]	0.8–2.7 0.7–2.6	Ever use. RR=3.1 and 4.0, respectively for men and women using >10,000 g. Similar risk for ever use of paracetamol
McCredie et al. 1995[c]	1,732	0.4	0.2–0.8	<1 kg lifetime use
		1.1	0.9–1.3	>0.1 kg lifetime use
Gago-Dominguez et al. 1999[c]	1,204	1.5	1.2–1.8	>2 per week for >1 month. Trend with dose. Similar increased risk for other analgesics
Cohort studies				
Paganini-Hill et al. 1989[c]	NR	6.3	2.0–20.0	Daily use
Schreinemachers and Everson 1994	32	0.60	0.29–1.24	NHANES I. Incidence
Ratnasinghe et al. 2004	37	2.27	0.93–5.54	NHANES I and II. Mortality
Jacobs et al. 2007	344	1.13	0.69–1.87	ACS-CPS II Nutrition cohort. Current daily users

ACS, American Caner Society; CI, confidence interval; CPS, Cancer Prevention Study; NHANES, National Health and Nutrition Examination; NR, not reported; NSAIDs, non-steroidal anti-inflammatory drugs; RR, relative risk

[a] Men

[b] Women

[c] Renal cell carcinoma only

Table 22.7 Main findings of case-control studies of aspirin and lymphatic and haematopoietic neoplasms

Study	No. of cases	RR	95% CI	Comment
Case-control studies				
Bernstein and Ross 1992	619	1.63	1.19–2.24	NHL. Continuous use of aspirin or other pain relieves for >1 month. RR=1.96 for >13 months
Kato et al. 2002	376	2.38	0.75–7.56	NHL. >10 years of use. RR=1.39 for paracetamol, 5.64 for ibuprofen and 4.84 for other NSAIDs
Zhang et al. 2004	601	0.7	0.4–1.2	NHL. No trend with duration RR=1.4 for long duration. RR=1.0 for non-aspirin NSAIDs
Chang et al. 2004	565	0.60	0.42–0.85	HL. >2 tablets/week for >5 years. RR=0.97 for non-aspirin NSAIDs and 1.72 for paracetamol
Baker et al. 2005	625	0.82[a] 0.93[b]	0.65–1.04 0.71–1.23	NHL. Regular use. RR=0.57 and 1.71, for paracetamol use in men and women, respectively
Zhang et al. 2006	529	0.9	0.6–1.3	NHL. Hospital-based. RR 0.4 (95% CI, 0.1–2.0) for use 10 years
Flick et al. 2006	1,000	1.00	0.67–1.47	NHL. Population-based from San Francisco Bay area. Long term use
Moysich et al. 2007	117	0.99	0.65–1.49	MM. Regular aspirin users
Cohort studies				
Thun et al. 1993	ND	0.89[a] 0.93[b]	0.66–1.20 0.65–1.33	Lymphatic and haematopoietic cancers. ACS/CPS II. >16 aspirin/month for >1 year
Schreinemachers and Everson 1994	48 49	0.89 0.67	0.51–1.55 0.34–1.31	Lymphomas Leukaemias NHANES I. Incidence
Cerhan et al. 2003	131	1.21	0.81–1.87	NHL. IWHS. RR=1.68 for exclusive use of aspirin. RR=1.38 for non-aspirin NSAIDs
Kasum et al. 2003	81	0.45	0.27–0.75	Leukaemias. IWHS. >2 times/week. RR=1.31 for non-aspirin NSAIDs
Ratnasinghe et al. 2004	94 63	0.97 1.08	0.52–1.81 0.58–2.01	Lymphomas Leukaemias NHANES I and II. Mortality
Jacobs et al. 2007	683 449	0.89 0.67	0.58–1.37 0.43–1.27	NHL Leukaemia. ACS CPS II Nutrition cohort. Current daily users

ACS, American Cancer Society, CI, confidence interval; CPS, Caner Prevention Study; HL, Hodgkin's lymphoma; IWHS, Iowa Women's Health Study; MM, multiple myeloma; ND, not defined; NHL, non-Hodgkin's lymphoma; NSAIDs, non-steroidal anti-inflammatory drugs; RR, relative risk

[a] Men

[b] Women

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Table 22.8 Pooled relative risk (RR) and 95% confidence interval (CI) for aspirin users by cancer site (from Bosetti et al. 2006b)

Cancer, study design	No. of studies	No. of cases	RR[a]	95% CI	Heterogeneity test; *p*-value: Between studies	Heterogeneity test; *p*-value: Between study design
Colon and rectum						
Case-control studies[b]	11	9,232	0.59	0.54–0.64	0.008	<0.0001
Cohort studies	7	5,146	0.85	0.78–0.92	0.006	
Oesophagus						
Case-control studies	2	643	0.41	0.29–0.57	0.26	0.0001
Cohort studies	4	1,118	0.83	0.70–0.98	0.18	
Stomach						
Case-control studies	3	1,557	0.67	0.56–0.80	0.42	0.0012
Cohort studies	4	1,376	0.93	0.82–1.05	0.002	
Pancreas						
Case-control studies	1	194	1.00	0.72–1.39	-	0.81
Cohort studies	5	4,926	0.96	0.92–1.01	0.04	
Lung						
Case-control studies	2	1,906	0.70	0.56–0.88	0.086	0.006
Cohort studies[c]	6	1,003	0.96	0.91–1.02	0.012	
Breast						
Case-control studies	6	13,822	0.80	0.73–0.87	0.66	0.0012
Cohort studies[c]	12	14,738	0.94	0.90–0.98	<0.0001	
Ovary						
Case-control studies	6	2,896	0.82	0.69–0.99	0.51	0.07
Cohort studies	2	449	0.98	0.80–1.20	0.59	
Prostate						
Case-control studies	5	3,632	1.02	0.90–1.16	0.44	0.48
Cohort studies[c]	10	14,844	0.97	0.94–1.01	<0.0001	
Bladder						
Case-control studies	1	1,514	0.85	0.66–1.09	-	0.05
Cohort studies	3	168	1.23	0.83–1.81	0.21	
Kidney						
Case-control studies	5	3,796	1.21	1.07–1.36	0.004	0.003
Cohort studies	3	69	1.45	0.87–2.41	0.002	
Non-Hodgkin's lymphomas						
Case-control studies	4	2,221	0.98	0.85–1.14	0.01	0.81
Cohort studies[c,d]	2	142	1.08	0.78–1.51	0.39	

[a] Weighted average of the each study estimate, with a weight proportional to the study precision, i.e. to the inverse of the variance of the estimate

[b] Does not include Juarranz et al. (2002)

[c] Does not include Thun et al. (1993)

[d] Includes also Schreinemachers and Everson (1994) and Ratnasinghe et al. (2004)

overall respectively. For both oesophageal and gastric cancer there is evidence of a dose-dependent inverse relation with aspirin use, although the results from case-control and cohort studies were heterogeneous. The stronger inverse relation reported in case-control than in cohort studies may be partly attributed to potential greater biases of case-control studies. Since in fact aspirin and other NSAIDs may cause gastrointestinal bleeding, patients with early symptoms of undiagnosed oesophageal and gastric cancer may be less prone to use these drugs. However, gastrointestinal bleeding caused by aspirin and other NSAIDs may have increased the frequency of medical examination, and thus the detection of early cancers otherwise undiagnosed, with the consequence that the inverse association between aspirin and digestive tract cancers could have been obscured in both case-control and cohort studies.

The studies on pancreatic cancer gave a pooled RR of 0.96 (95% CI, 0.92–1.01), 1.00 (95% CI, 0.72–1.39) from 1 case-control study, and 0.96 (95% CI, 0.92–1.01) from 5 cohort studies (Table 22.8). The data are therefore too limited to draw any definitive conclusion on the association between aspirin and pancreatic cancer risk, although they allow us to exclude a strong association.

A study from China (Liu et al. 2005) also showed a favourable effect of aspirin in the risk of cancers of the gallbladder, extra-hepatic bile ducts and ampulla of Vater.

22.3.3 Lung Cancer

The relation between aspirin and lung cancer risk has been investigated in at least 6 case-control and 9 cohort studies, whose results are given in Table 22.3 (Akhmedkhanov et al. 2002; Feskanich et al. 2007; Harris et al. 2003; Hayes et al. 2006; Hernández-Díaz and García Rodríguez 2007; Holick et al. 2003; Jacobs et al. 2007; Kelly et al. 2007; Moysich et al. 2002; Muscat et al. 2003; Olsen et al. 2008; Paganini-Hill et al. 1989; Ratnasinghe et al. 2004; Schreinemachers and Everson 1994; Thun et al. 1993).

The overall RR of lung cancer was 0.70 (95% CI, 0.56–0.88) from 2 case-control, 0.96 (95% CI, 0.91–1.02) from 6 cohort studies, and 0.94 (95% CI, 0.89–1.00) overall (Table 22.8). The pooled estimates from the two study designs were, however, heterogeneous, as well as the results of cohort studies. Thus, although there is a suggestion from case-control studies of a beneficial role of aspirin on lung cancer risk, the evidence remains inconsistent.

22.3.4 Breast and Ovarian Cancers

Studies on aspirin on breast cancer are shown in Table 22.4 (Coogan et al. 1999; Cotterchio et al. 2001; Egan et al. 1996; Friedman and Ury 1980; García Rodríguez and González-Pérez 2004b; Gill et al. 2007; Harris et al. 1999; Harris et al. 1996; Jacobs et al. 2005b; Johnson et al. 2002; Marshall et al. 2005; Neugut et al. 1998; Paganini-Hill et al. 1989; Ratnasinghe et al. 2004; Schreinemachers and Everson 1994; Swede et al. 2005; Terry et al. 2004; Thun et al. 1993; Zhang et al. 2005).

Epidemiological evidence on aspirin and breast cancer gave a pooled RR of 0.80 (95% CI, 0.73–0.87) from 6 case-control studies, 0.94 (95% CI, 0.90–0.98) from 12 cohort studies, and 0.90 (95% CI, 0.87–0.94) overall (Table 22.8), although in the presence of a significant heterogeneity between study design, and within cohort studies. Further quantification is needed, especially to clarify the long-term effects.

With reference to ovarian cancer, ovulation (and related inflammation) has been suggested to have a role in ovarian carcinogenesis (Balkwill and Mantovani 2001; Ness and Cottreau 1999). Epidemiological studies on aspirin use are still scanty, but there is an indication that aspirin may have a favourable effect on ovarian cancer (Table 22.4; Akhmedkhanov et al. 2001; Cramer et al. 1998; Fairfield et al. 2002; Lacey et al.

2004; Moysich et al. 2001; Rosenberg et al. 2000; Schildkraut et al. 2006; Tavani et al. 2000; Tzonou et al. 1993).

The pooled RR of ovarian cancer for aspirin use was 0.82 (95% CI, 0.69–0.99) from 6 case-control studies, 0.98 (95% CI, 0.80–1.20) from 2 cohort ones, and 0.89 (95% CI, 0.78–1.02) overall (Table 22.8). The evidence is therefore too inconsistent for any conclusion.

22.3.5 Prostate Cancer

The role of aspirin and other NSAIDs on prostate cancer risk is considered in Table 22.5 (Bosetti et al. 2006b; García-Rodríguez and González-Pérez 2004a; Habel et al. 2002; Irani et al. 2002; Jacobs et al. 2005a; Leitzmann et al. 2002; Menezes et al. 2002b, 2006; Neugut et al. 1998; Norrish et al. 1998; Paganini-Hill et al. 1989; Perron et al. 2003; Platz et al. 2005; Ratnasinghe et al. 2004; Schreinemachers and Everson 1994; Thun et al. 1993).

The pooled RR for prostate cancer was 1.02 (95% CI, 0.90–1.16) from 5 case-control studies, 0.97 (95% CI, 0.94-1.01) from 10 cohort studies, and 0.98 (95% CI, 0.95–1.01) overall (Table 22.8). Men taking aspirin on a regular basis may be more likely to have had frequent medical contacts and consequently prostate-specific antigen measurements, and thus to have received a diagnosis of prostate cancer. Such a bias may well influence—to a variable extent—the results of cohort studies, too. The relation between aspirin use and prostate cancer, if any, remains inconclusive, but the data allow us to exclude strong associations.

22.3.6 Bladder and Kidney Cancers

The main results on aspirin and bladder and kidney cancers are reported in Table 22.6 (Castelao et al. 2000; Fortuny et al. 2007; Gago-Dominguez et al. 1999; Genkinger et al. 2007; Jacobs et al. 2004; Jacobs et al. 2007; McCredie et al. 1988; McCredie et al. 1995; McLaughlin et al. 1985; Mellemgaard et al. 1994; Paganini-Hill et al. 1989; Ratnasinghe et al. 2004; Schreinemachers and Everson 1994). The epidemiologic evidence on aspirin and bladder cancer is inconsistent, although an excess risk can now be excluded (RR=0.85; 95% CI, 0.66–1.09, from 1 case-control study; 1.23, 95% CI, 0.83–1.81, from 3 cohort studies; and 0.95, 95% CI, 0.77–1.17, overall; Table 22.8).

Overall, the pooled RR for kidney cancer was 1.21 (95% CI, 1.07–1.36) from 5 case-control, 1.45 (95% CI, 0.87–2.40) from 3 cohort studies, and 1.22 (95% CI, 1.08–1.37) overall (Table 22.8). Thus, although based on a limited number of studies, the epidemiological evidence suggests an increased risk of kidney cancer for regular use of aspirin and other NSAIDs use. This increased risk may, however, be due to residual confounding by phenacetin use, which is likely to have been used in combination to aspirin and other NSAIDs.

22.3.7 Lymphatic and Haematopoietic Cancers

A few studies have investigated the relation between aspirin and the risk of non-Hodgkin's lymphoma (NHL) (Table 22.7; Baker et al. 2005; Bernstein and Ross 1992; Cerhan et al. 2003; Flick et al. 2006; Jacobs et al. 2007; Kato et al. 2002; Ratnasinghe et al. 2004; Schreinemachers and Everson 1994; Zhang et al. 2006; Zhang et al. 2004).

The pooled RR for NHL was 0.98 (95% CI, 0.85–1.14) from 4 case-control studies, 1.08 (95% CI, 0.78–1.51) from 2 cohort studies, and 1.00 (95% CI, 0.88–1.14) from all studies combined (Table 22.8). The slight increased risk for NHL observed in some studies may be related to the immunomodulatory effects of aspirin and other NSAIDs, although a residual confounding by an underlying chronic inflammation among

patients with long-term anti-inflammatory drug use cannot be excluded (Signorello et al. 2003).

A few studies examined the relation between aspirin use and the risk of other lymphatic and haematopoietic neoplasms (Table 22.7; Chang et al. 2004; Kasum et al. 2003; Ratnasinghe et al. 2004; Schreinemachers and Everson 1994; Thun et al. 1993). A population-based case-control study from Connecticut (Chang et al. 2004) on 565 patients with Hodgkin's lymphomas reported a significantly lower risk for regular aspirin use (RR=0.60). No association was found for non-aspirin NSAIDs (RR=0.97), while a significant increased risk was reported for paracetamol (RR=1.72). The Iowa WHS cohort (Kasum et al. 2003), including more than 28,000 post-menopausal women and 81 incident leukaemia cases, reported a reduced RR for leukaemias in regular users of aspirin (RR=0.45, 95% CI, 0.27–0.75), but not of other NSAIDs (RR=1.31). The incidence of leukaemias was not significantly reduced in the National Health and Nutrition Examination Survey (NHANES) I cohort (RR=0.67) (Schreinemachers and Everson 1994), and no association with leukaemia mortality was found in the NHANES I and II cohorts (RR=0.97) (Ratnasinghe et al. 2004). In a Danish cohort study (Friis et al. 2003), low-dose aspirin users had a RR of 1.3 (95% CI, 1.0–1.6), based on 69 cases of leukaemia.

22.4 Discussion

Table 22.8 gives the pooled risk estimates from epidemiological studies on aspirin and several cancer sites. Besides a reduction in risk for cancer of the colorectum, there is evidence—although more limited, and mainly from case-control studies—that aspirin has a favourable effect on other digestive tract cancers, including those of the oesophagus and stomach, on hormone-related neoplasms, such as the breast and ovary, and on lung cancer. The role of aspirin on other cancers, such as pancreatic, prostate and bladder cancers and NHL is less clear, and an increased risk has been suggested for kidney cancer, possibly related to the associations with other or unspecified NSAIDs.

For most cancer sites, however, a significant heterogeneity between estimates from various studies was found. Moreover generally stronger reduction in risk in case-control studies than in cohort ones were reported. Estimates from cohort studies are considered more reliable and valid, since these studies are generally less prone to information or selection bias. However, cohort studies may have less detailed information on drug use, and their often short follow-up does not allow us to measure the long-term effects of aspirin, whereas a detailed lifelong history of aspirin and other NSAIDs use can generally be obtained from case-control studies.

Further, notwithstanding the large amount of epidemiological evidence, substantial uncertainties remain about the proper aspirin dose and duration of treatment. For colorectal cancer it seems that doses over 300 mg/day (García Rodríguez and Huerta-Alvarez 2001; Peleg et al. 1994; Suh et al. 1993) and many years of use (Chan et al. 2005; Giovannucci et al. 1995; La Vecchia et al. 1997) are needed to reduce the risk. Only a few studies, however, included information on dose and duration of use in relation to other cancers.

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