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Cancer Risk Factors for Selecting Cohorts for Large-Scale Chemoprevention Trials

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Abstract Many anticipate that application of findings in molecular genetics will help to achieve greater precision in defining high-risk populations that may benefit from chemopreventive interventions. We must recognize, however, that genetic susceptibility, environmental factors, and complex gene-environment interactions are all likely to be risk determinants for most cancers. Cohort studies of twins and cancer indicate that having "identical" genes is generally not a very accurate predictor of cancer incidence. Data from twin studies support the suggestion that environmental factors such as tobacco use significantly influence cancer risk. The complexities of the genetic contribution to disease risk are exemplified by the development of Duchenne muscular dystrophy in only one of monozygotic twin girls, hypothesized to be the result of X chromosome inactivation, with the distribution patterns of the X chromosome being skewed to the female X in the manifesting twin and to the male X in the normal twin. Evidence from transgenic and genetic-environmental studies in animals support the possibility of genetic-environmental interactions. Calorie restriction modifies tumor expression in *p53* knockout mice; a high-fat, low-calcium, low-vitamin D diet increases prepolyp hyperplasia formation in *Apc*-mutated mice; and calorie restriction early in life influences development of obesity in the genetically obese Zucker rat (*fafa*). Such environmental modulation of gene expression suggests that chemoprevention has the potential to reduce risk for both environmentally and genetically determined cancers.

In view of the growing research efforts in chemoprevention, the NCI has developed a Prevention Trials Decision Network (PTDN) to formalize the evaluation and approval process for large-scale chemoprevention trials. The PTDN addresses large trial prioritization and the associated issues of minority recruitment and retention; identification and validation of biomarkers as intermediate endpoints for cancer; and chemopreventive agent selection and development. A comprehensive database is being established to support the PTDN's decision-making process and will help to determine which agents investigated in preclinical and early phase clinical trials should move to large-scale testing. Cohorts for large-scale chemoprevention trials include individuals who are determined to be at high risk as a result of genetic predisposition, carcinogenic exposure, or the presence of biomarkers indicative of increased risk. Current large-scale trials in well-defined, high-risk populations include the Breast Cancer Prevention Trial (tamoxifen), the Prostate Cancer Prevention Trial (finasteride), and the *N*-(4-hydroxyphenyl) retinamide (4-HPR) breast cancer prevention study being conducted in Milan. Biomarker studies will provide valuable information for refining the design and facilitating the implementation of future large-scale trials. For example, potential biomarkers are being assessed at biopsy in women with ductal carcinoma in situ (DCIS). The women are then randomized to either placebo, tamoxifen, 4-HPR, or tamoxifen plus 4-HPR for 2–4 weeks, at which time surgery is performed and the biomarkers reassessed to determine biomarker modulation by the interventions. For prostate cancer, modulation of prostatic intraepithelial neoplasia (PIN) by 4-HPR and difluoromethylornithine is being investigated; similar studies are being planned for oltipraz, dehydroepiandrosterone, and vitamin E plus selenomethionine. The validation of biomarkers as surrogate endpoints for cancer incidence in high-risk cohorts will allow more agents to be evaluated in shorter studies that use fewer subjects to achieve the desired statistical power. J. Cell. Biochem. 25S:29–36.

Key words: biomarkers; cancer risk assessment; gene-environment interactions; large-scale trials; prevention trials decision network; twin studies

Many in the cancer research community anticipate that application of findings in molecular genetics will help to achieve greater precision in defining high-risk populations that may benefit from chemopreventive interventions. It must be recognized, however, that in addition

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to genetic susceptibility, environmental factors and complex gene-environment interactions are also likely to be risk determinants for most cancers. Ideally, characterization of risk should consider contributions from all sources. For example, estimates of environmental exposures should be coupled with information on interindividual differences in genetic susceptibility that may result from variations in metabolism of xenobiotic chemicals or DNA repair capability, as well as factors such as duration of exposure, induction intervals, and age-specific penetrance [1]. In reality, our current knowledge and measurement capability does not provide a readily integrated approach for the reliable determination of cancer risk for most individuals.

Clarifying the relative importance of contributions from "nature" versus those from "nurture," and their interactions, has been the underlying theme of many studies during the last several decades. One interesting observation in a study of obesity in pet dogs was that the incidence of obesity among dogs owned by obese people was higher than among dogs owned by people of normal physique (44% versus 25%) [2], suggesting that nurture—in this case, overfeeding and perhaps inadequate exercise—had a measurable influence over and above any "natural" genetic susceptibility of the animals to obesity. Concordance for disease in twin pairs provides some insight into the relative contributions of environmental and genetic factors to disease occurrence. Low concordance rates among monozygotic (MZ) twins suggests a greater influence of environment; a large difference in concordance between MZ and dizygotic (DZ) twins may be evidence of a greater genetic influence [3]. Evidence from numerous epidemiologic studies that have investigated cancer incidence in identical twins indicates that having "identical" genes is generally not a very accurate predictor of cancer incidence and mortality, suggesting that environmental factors significantly influence cancer risk. A recent cohort study of white male U.S. veterans from 1946 to 1990, which assessed the effect of inherited predisposition to cancer in 5,690 MZ twin pairs and 7,248 DZ twin pairs, found a 40% greater degree of concordance for death from cancer among MZ twins than among DZ twins [4]. However, approximately one-third of concordant MZ twins died from smoking-associated cancers, supporting the influence of environment on cancer risk. Further, death from cancer in an MZ twin did not indicate that his cotwin would soon die from cancer; in fact, fewer than 1 of 8 MZ twins whose cotwin died from cancer could be expected to die from cancer if they lived until age 63 years, and only 1 of 32 (3%) could be expected to die from cancer of the same site.

Findings from this study are consistent with those of earlier investigations among MZ and DZ twin pairs in Sweden [5,6], Finland [7], Denmark [8], and the United States [9]. Even though sample sizes were small in the European studies, results generally suggested that inherited predisposition does not explain a large proportion of either all cancer incidence or all cancer mortality [4]. For example, in the Danish study, no significant genetic predisposition could be demonstrated for cancers of the breast, colon, rectum, or leukemia between MZ and DZ twin pairs [8]. Although cotwins of MZ breast cancer patients exhibited a significantly higher number of breast cancer cases than expected, the same was also true for cotwins of DZ breast cancer cases, suggesting that environmental similarities may have contributed to the increased risk of breast cancer in these twin pairs. Data from one Swedish study showed no concordance with respect to cancer at all sites, but demonstrated a significantly increased concordance rate for cervical cancer among MZ twins [5]. Also, significant associations were found between cervical cancer incidence and certain behavioral characteristics such as smoking, alcohol consumption, and use of drugs, suggesting that similar environment most likely influenced cervical cancer risk. In another Swedish study, concordance rates for prostate cancer in MZ twin pairs were more than four times greater than rates for DZ twin pairs, indicating that genetic factors might be of importance in prostate cancer development [6].

Genetic linkage studies have found evidence of genetic susceptibility for Hodgkin's disease, the first common cancer for which strong evidence of an inherited component has been obtained, and for nasopharyngeal carcinoma (NPC) [10]. For NPC, the most common cancer in certain parts of China, increased risk likely is also the result of environmental factors, including the high consumption of salted fish [10,11]. Further, epidemiologic evidence links viral agents, particularly Epstein-Barr virus, to NPC development [12]. A recent study of Hodgkin's disease in young adult twin pairs

reported an increased concordance rate among MZ twin pairs (10/179) relative to DZ twin pairs (0/187) for Hodgkin's disease, but not for non-Hodgkin's lymphomas and other cancers [13]. These findings contribute to the evidence that genetic susceptibility is an important underlying factor in the development of Hodgkin's disease in young adults. It is noteworthy that patients with a history of infectious mononucleosis, a disease associated with exposure to Epstein-Barr virus, have a threefold increase in risk for Hodgkin's disease [14], and earlier studies have supported the hypothesis of an infectious etiology [15]. Thus, genetic susceptibility does not exclude the possibility that environmental factors may play a role in Hodgkin's disease pathogenesis.

The complexities of genetic contributions to disease risk are exemplified by the development of Duchenne muscular dystrophy, a disease carried on the X chromosome, in only one of MZ twin girls; this was hypothesized to be the result of specific X chromosome inactivation [16]. Analysis of an X-linked DNA polymorphism suggested that both twins were heterozygous for Duchenne muscular dystrophy. However, the distribution patterns of the X chromosome appeared to be skewed dramatically to the female X in the manifesting twin and to the male X in the normal twin. Thus, even though these identical twins shared the same genes, the genetic traits were not shared because of the skewed distribution patterns of active X chromosomes between the twins, a characteristic observed in varying degrees in other MZ twin girls.

Evidence from transgenic and ecogenetic studies in animals support the possibility of gene-environment interactions. For example, early research in the obese Zucker rat determined that growth and degree of obesity were established by early nutrition-genetic interaction. Underfeeding was the predominant influence during the first 30 days of life; by 12 weeks of age, however, genetic differences became the major determinant of body weight [17]. For cancer, the use of animal models such as *p53* knockout mice and *Apc*-mutated mice provides opportunities to investigate the potential modulating effects of specific environmental factors on cancer development. *p53* knockout mice—transgenic mice with both alleles of the *p53* tumor suppressor gene knocked out by gene targeting—rapidly develop spontaneous tumors. A study that evaluated the effects of caloric restriction (60% of *ad libitum* intake) in these mice demonstrated that tumor development in male mice was significantly delayed by caloric restriction, possibly because of cell cycle modulation [18]. Mice that carry specific mutations in the *Apc* gene, the murine homolog of the human *APC* gene, also are potentially useful models for ecogenetic studies. *Apc* mutations include a chain-termination mutation introduced into the 15th exon of the *Apc* gene (*Apc*1638 mice) and a mutation called Apc^{Min} (*Min*/+ mice; *Min*, multiple intestinal neoplasia) [19]. These *Apc*-mutated mice develop intestinal tumors in a manner similar to individuals with familial adenomatous polyposis (FAP). Recent findings from a study in *Apc*1638 mice fed a Western-style diet that contained high fat and phosphate and reduced calcium and vitamin D showed that prepolyp hyperplasia (PH) formation was significantly increased by the Western-style diet (33 PH) compared with *Apc*1638 controls (3 PH). This is the first animal model that rapidly produces colonic lesions without a chemical carcinogen, and that rapidly responds to dietary manipulation [20].

Thus, environmental modulation of gene expression is evident, even with known gene mutations, suggesting that chemoprevention has the potential to reduce risk for both environmentally and genetically determined cancers. In fact, recent research on chemoprevention of spontaneous tumorigenesis in *p53*-knockout mice demonstrated that tumor development can be delayed by dehydroepiandrosterone (DHEA) in mice with greatly increased genetic susceptibility to cancer [21]. As the scientific community's ability to characterize genetic susceptibility and its interaction with possible environmental influences continues to advance, the development of effective chemopreventive approaches specifically targeted to individuals at high risk for cancer will be critical.

THE PREVENTION TRIALS DECISION NETWORK

In view of the growing research efforts in chemoprevention, the National Cancer Institute's (NCI) Division of Cancer Prevention and Control (DCPC) has developed a Prevention Trials Decision Network (PTDN) to formalize the evaluation and approval process for large-scale chemoprevention trials and to streamline

management of the growing body of data generated by researchers in chemoprevention. The PTDN relies on the expertise of three subcommittees—the Large Trials Committee (LTC), the Endpoints and Biomarker Committee (EBC), and the Agent Development Committee (ADC)—in formulating its recommendations as to those trials that appear to be most promising with respect to advancing cancer prevention and control goals. In addition, a fourth subcommittee has been established that is responsible for addressing minority recruitment and retention issues, including the development of strategies to enhance accrual of minority populations to large-scale prevention trials.

Large-scale, randomized prevention trials are considered to be the most definitive scientific approach available to determine if promising interventions actually do reduce cancer incidence. Such trials are used both to test hypotheses that are generated based on data from epidemiologic and experimental studies and to confirm the efficacy of chemopreventive agents that have been evaluated for biological effect in early-phase clinical studies. Large-scale trials must be carefully planned, reviewed, implemented, and monitored. The LTC is responsible for making recommendations regarding which large trials should be initiated and has developed a uniform review mechanism for all proposed large trials that includes a well-defined scoring system. Scoring is based on prioritization criteria that include importance of the study hypothesis and a reasonable uncertainty about whether it is true or false (equipoise); strength of the study design; potential public health impact; and evaluation of how the trial would fit into DCPC's trial portfolio as a whole.

The EBC identifies biomarkers currently being used in clinical trials and develops strategies for validation of these biomarkers for eventual use as intermediate endpoints for cancer in large-scale trials. Also, the EBC identifies new biomarkers that should be studied in ongoing clinical trials. The availability of valid, reliable biomarkers is key to designing efficient large-scale trials. When biomarkers that reflect relatively early and site-specific carcinogenic changes are used as intermediate endpoints, fewer trial subjects are required to achieve statistical power, and interventions can be evaluated in shorter studies than when cancer incidence is the sole endpoint. A biomarker database, part of the DCPC Human Intervention Studies (HINTS) database, is being established for systematic collection of data on biomarkers used in clinical trials, including information on sensitivity, specificity, and predictive value for subsequent cancer incidence.

The ADC is responsible for the identification and prioritization of promising chemopreventive agents, and for development of a process to advance the priority agents into prevention trials. This subcommittee works closely with the EBC and the LTC to coordinate overlapping research and development efforts. The ADC has drafted clinical development plans for a number of agents including aspirin, calcium, β-carotene, fluasterone (a DHEA analog), difluoromethylornithine (DFMO), all-*trans*-*N*-(4-hydroxyphenyl) retinamide (4-HPR), genistein, glycyrrhetinic acid, carbenoxolone, ibuprofen, *N*-acetyl-*l*-cysteine (NAC), oltipraz, perillyl alcohol, piroxicam, finasteride, selenium, sulindac, tamoxifen, vitamin D_3 and analogs, and vitamin E. These plans summarize agent development status in terms of preclinical and clinical testing and suggest strategies for moving the agents into phase II and phase III clinical trials.

The comprehensive HINTS database is being established to support the PTDN's decision-making process and will help to determine which agents investigated in preclinical and early-stage clinical trials should move to large-scale testing. Information being entered into this database includes data on active trials sponsored by DCPC, including trial monitoring and minority accrual; data on promising biomarkers and those under study in trials; and data on all chemopreventive agents in NCI-sponsored clinical trials, as well as those undergoing preclinical toxicology studies. To date, the database includes information on approximately 300 phase I, II, and III clinical trials and studies supported or being considered for support by DCPC, 80 biomarkers, and 40 chemopreventive agents.

LARGE-SCALE PREVENTION TRIALS

The conduct of randomized, prospective large-scale clinical trials is a logical step in moving from basic science to the application of research results for disease prevention. These trials, however, are expensive and represent major challenges in design, implementation, and analysis; they involve thousands of subjects, multi-institutional arrangements, and take years to com-

plete. The long duration of large-scale trials allows for confirmation of the limited toxicity and efficacy data determined in early-stage clinical studies and for the possible appearance of adverse effects or agent efficacy not previously detected [22]. Cohorts for large-scale chemoprevention trials include individuals who are determined to be at high risk as a result of genetic predisposition, carcinogenic exposure, or the presence of markers indicative of increased risk. Selected examples of current large-scale chemoprevention trials in well-defined high-risk populations are described below.

Initiated in 1992, the Breast Cancer Prevention Trial (BCPT) is a 10-year study testing the ability of tamoxifen to prevent the development of breast cancer in healthy women at increased risk for developing the disease. For this trial, a woman's risk is determined by age, number of first-degree relatives with breast cancer, age at first live birth, number of benign breast biopsies, age at menarche, and presence of atypical hyperplasia. Based on previous clinical trial experience with tamoxifen, it has been estimated that tamoxifen may reduce the incidence rate of breast cancer in high-risk women by at least 30%. This study is focused on women with a risk for breast cancer at least equal to that of a 60-year-old, because the potential benefits of tamoxifen must be weighed against an increased risk for endometrial cancer and other possible adverse effects. Approximately 16,000 high-risk women over age 35 are being randomized to receive oral tamoxifen (20 mg/day) or placebo for an initial period of 5 years [23,24].

In Milan, Italy, a large-scale clinical trial that began in 1987 is testing the effectiveness of 4-HPR as a chemopreventive agent in breast cancer patients who are at risk for a new primary tumor in the contralateral breast. By June 1993, about 3,000 patients were randomized to receive either 4-HPR (200 mg/day) for 5 years or no treatment; the study protocol includes a 2-year followup for both the intervention and control groups [25]. Results for contralateral breast cancer occurrence are not yet available. However, one interesting result from this study is that no ovarian cancers were found in the women who received 4-HPR for 5 years, compared with 6 ovarian cancers in the controls, suggesting that chemoprevention with 4-HPR may also be possible for ovarian cancer [26].

The Prostate Cancer Prevention Trial (PCPT) is a large-scale, double-blind, randomized multicenter trial designed to investigate the ability of finasteride to prevent the development of prostate cancer in men ages 55 and above. The risk for clinically significant prostate cancer begins to rise significantly after age 55; the incidence rate for men aged 50–54 is 134.6/100,000 compared with 337.5/100,000 for men aged 55–59 [27]. Participants must have no evidence of prostate cancer by digital rectal exam and a serum prostate specific antigen (PSA) of 3 ng/ml or less. They may have some symptoms of benign prostatic hyperplasia (BPH), but symptoms may not be so significant that a transection is anticipated within a year of entry [28]. Because the development of early-stage prostate cancer appears to be strongly influenced by androgens, particularly dihydrotestosterone (DHT), it is hypothesized that the inhibition of DHT synthesis by administration of finasteride will lead to a significant reduction in the number of individuals who develop prostate cancer. Half of the approximately 18,000 men in this trial will receive 5 mg of finasteride orally per day for 7 years; the remaining participants will receive a placebo.

BIOMARKER STUDIES AND LARGE-SCALE TRIAL DESIGN

Clinical biomarker studies have the potential to provide valuable information for refining the design and facilitating the execution of future large-scale trials. These studies identify intermediate biomarkers with the potential to serve as surrogate trial endpoints, establish a dose-biomarker response relationship for the chemopreventive agent(s), and select a safe chemopreventive agent(s) dose for a large-scale trial. Demonstrating the correlation between intermediate biomarker modulation and decreased cancer risk in clinical biomarker studies will begin to validate the biomarker as a surrogate endpoint for future large-scale trials. Final validation of the biomarker will be included as an objective in large-scale trial design.

Findings from current biomarker studies being carried out for breast and prostate cancers may prove to be important for planning further chemoprevention trials for these cancers. Ductal carcinoma in situ (DCIS)—a preinvasive neoplastic lesion at high risk for progression to invasive cancer—and prostatic intra epithelial neoplasia (PIN)—an abnormal epithelial prolif-

eration in the prostate ducts believed to represent a preinvasive form of prostate cancer—are considered to be biomarkers for breast cancer and prostate cancer, respectively [29,30]. Other types of markers may be identified within these lesions. For example, aneuploidy, a genetic biomarker, has been identified in over 50% of DCIS [29]. Proliferation markers such as proliferating cell nuclear antigen (PCNA) and Ki-67 antigen expression may be associated with both PIN and DCIS. Some markers will be generally useful regardless of the chemopreventive approach used; others, such as upregulation of progesterone receptor (PR) expression in response to tamoxifen, are uniquely useful for specific agents [31].

For breast cancer, potential biomarkers are being assessed at biopsy in women with either biopsy-proven DCIS or carcinoma <10 mm diameter who are scheduled for surgery. After assessment at biopsy, the women are randomized to placebo, tamoxifen, 4-HPR, or tamoxifen plus 4-HPR for the 2–4 weeks between core biopsy and surgical excision. Biomarkers are reassessed when surgery is performed to determine biomarker modulation by the chemopreventive agent. The intermediate biomarkers being investigated in this study include DCIS grade, DNA ploidy, PCNA, Ki-67 antigen, S-phase fraction, and nuclear polymorphism [32]. Short-term trials, such as this one, are appropriate for biomarkers that are readily modulated by the chemopreventive treatment.

For prostate cancer, clinical biomarker studies are investigating the modulation of both PIN and associated biomarkers by 4-HPR and DFMO in patients recruited from high-risk groups. For example, in one study of 4-HPR, patients with biopsy-proven prostate cancer scheduled for radical prostatectomy are administered 4-HPR for up to 8 weeks. The intermediate biomarkers being evaluated in this study include PIN grade, ploidy, PCNA, Ki-67 antigen, and nuclear polymorphism. Another 4-HPR study is being conducted in men who have a negative biopsy for carcinoma and serum PSA ≥ 4 ng/ml; these men receive 4-HPR for 1 year. Endpoints of this study include modulation of PIN and nuclear matrix protein, as well as prevention of positive biopsy or doubling of PSA [32]. The chemopreventive effects of DFMO are being investigated in men who have a serum PSA of 3–10 ng/ml, including patients with prostatic carcinoma and PIN. Modulatory effects on histopathology as well as serum PSA, prostatic acid phosphatase (PAP), and testosterone are being monitored [33]. Similar studies are being planned for oltipraz, DHEA, and vitamin E plus selenomethionine.

FUTURE PROSPECTS

The ability to identify individuals at high risk for cancer, regardless of the source of the risk, is accompanied by the responsibility to offer interventions that have potential to reduce the risk of carcinogenesis. Chemoprevention, which has progressed to the point where it is considered to be an extremely promising approach to cancer prevention, may be a way to reduce risk for susceptible individuals and, ultimately, for the general population. Determining the clinical effects of potential chemopreventive agents in randomized trials has become a major objective of cancer prevention research [34]. Because agents undergoing evaluation in clinical chemoprevention trials generally are either nontoxic or of extremely low toxicity, such trials can combine two or more agents in factorial study designs that allow cost-effective determination of the main effects of each agent as well as any interactions in a single trial. Future trial design should emphasize such efficient approaches to optimize use of available resources, including high-risk target populations. Chemopreventive agents with proven efficacy in clinical trials will be valuable components of protocols for reducing cancer risk in susceptible individuals.

Fundamental data on the interactions of environmental factors with genetic predisposition to cancer generally are not available for cancer risk determination. Further research to model gene-environment interactions as well as rigorous statistical analysis of retrospective epidemiologic studies could provide insight into possible interactions. However, prospective trials of individuals with known genotypes and varying environmental exposures could provide particularly valuable data on the influence of environment on cancer risk in susceptible individuals [35]. In the foreseeable future, it may be possible to develop risk profiles for individuals, based on medical history, genetic factors, and environmental exposures, including lifestyle, or some combination of these that would provide a sound rationale for defining specific interventions to modulate risk for individuals at high risk for cancer [36,37]. A precise

determination of individual risk status will allow the most appropriate interventions to be identified, leading to the consequent development of efficient and effective future chemoprevention regimens [36]. To succeed, this effort will require collaboration by scientists from diverse disciplines and will need to address important issues such as validation of risk profiles and adherence to interventions. Ultimately, the assessment of individual cancer risk and the implementation of appropriate chemopreventive measures could become part of standard medical practice and would be available to all individuals as part of routine health care.

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