

# Biomarkers in Cancer Chemoprevention

## Workshop report\*

### Introduction

Cancer chemoprevention is the inhibition or reversal of carcinogenesis (before invasion) by intervention with pharmacologically active agents. While minimizing exposure to carcinogens and changes in lifestyle will eventually reduce cancer incidence, chemoprevention offers an alternative approach with the potential for more immediate results, especially in subjects known to be at high risk of cancer.

Numerous oncogenes, tumour-suppressor genes and phenotypic preneoplastic cellular changes are being discovered, allowing improved definition of events in the process of carcinogenesis. Late events are critical in the actual occurrence of cancer, and interventions during these stages of carcinogenesis are thus theoretically very attractive. With better understanding of possible ways to perturb carcinogenesis, preventive intervention becomes increasingly practical for many cancer sites.

### Objectives of the workshop

The objectives of the workshop on Biomarkers in Cancer Chemoprevention were:

- (i) To summarize the current state of knowledge on biomarkers indicative of individual susceptibility or of carcinogenic exposure and on intermediate biomarkers predictive of invasive cancer occurrence that are relevant to studies on cancer prevention;
- (ii) To prepare a consensus report on the role of biomarkers in studies of cancer prevention, covering:
  - validation of already existing biomarkers for the purposes of cancer prevention,
  - interpretation of the results of studies of cancer prevention using biomarkers,
  - future activities for development of new biomarkers;
- (iii) To improve the future use of biomarkers in development of new cancer chemopreventive agents.

The workshop built upon a previous IARC meeting on the application of biomarkers in cancer epidemiology (Toniolo *et al.*, 1997), but was more specifically oriented to the application of biomarkers to cancer chemoprevention.

### Definition of biomarkers

The term 'biomarkers' refers to indicators of exposures and/or events in biological systems or samples. Biomarkers potentially relevant to cancer chemoprevention include exposure, intermediate-effect, drug effect, tumour and susceptibility markers. Of these, the workshop restricted its attention to exposure, intermediate-effect and susceptibility markers. Many of the characteristics and issues pertaining to each type of biomarker are different and the three types of biomarker are considered separately. However, there is no strict boundary between them, and several markers can be considered as belonging to two or even to all three categories (examples of this would be DNA damage and its repair and mutations in tumour-suppressor genes).

In chemoprevention, an exposure biomarker is any substance or structure that reflects endogenous or exogenous exposure to carcinogenic risk factors, which can be measured in the human body or its products and which may be

\*This paper is the consensus report of the workshop *Use of Biomarkers in Chemoprevention of Cancer*, which was held at the Deutsches Krebsforschungszentrum, Heidelberg, Germany, in February 2000.

predictive of the incidence or outcome of disease. A biological marker of intermediate effect is an indicator of the development in an individual of a carcinogenic change short of invasive cancer. A biological marker of cancer susceptibility is an indicator of a heritable ability of an organism to respond to the challenge of carcinogenic agent(s) or event(s).

### **Biomarkers in chemoprevention**

Much of the initial work on biomarkers has been performed in cellular or whole animal models, but there has been no systematic assessment of lessons learnt from such studies to guide the search for biomarkers that might be useful in humans.

Intermediate-effect biomarkers of potential value in chemoprevention are already available for most of the accessible human cancers (e.g., mouth, colon, lung, breast, prostate) but not for cancers at inaccessible sites such as the pancreas. There is need to capitalize on advances in molecular and cellular biology, imaging and microsurgery to reach the relevant organs and access cells and cellular products that may harbour biomarkers.

In practice, biomarkers already play an important role in the evaluation of chemopreventive agents in phase II trials. However, the intermediate-effect biomarkers used in phase II trials have not been validated in relation to subsequent cancer occurrence, and therefore are not being used in phase III trials.

Even large trials in humans are dependent on identifying individuals at high risk for the relevant disease. High-risk populations include those known to be heavily exposed to an important etiological agent (such as heavy smokers at high risk for tobacco-induced cancers); individuals with recognized genetic predisposition, from either high- or low-penetrance genes; and those with already detectable preneoplastic lesions. Biomarkers of both exposure and susceptibility are being developed that could reduce the need for very large samples of subjects, even in phase III chemoprevention trials.

### **Validity of biomarkers**

Utilization of validated biomarkers would markedly facilitate the development and testing of

potential chemopreventive agents. Validation of a biomarker involves the understanding of the molecular mechanisms related to chemopreventive action, external exposures, the outcome (cancer or preneoplastic alterations) and the biomarker itself. Several questions should be addressed in the validation of biomarkers:

1. Does the chemopreventive intervention affect the biomarker?
2. Is the biomarker consistently found in populations and/or the disease?
3. Is there molecular understanding as a basis for the use of the biomarker?
4. Is the biomarker associated with a chemopreventive agent or risk factors for cancer?
5. Is the biomarker associated with the outcome (cancer)?
6. Is the chemopreventive intervention effect on the main outcome (cancer) mediated by the biomarker?
7. Are the chemopreventive or risk factor effects mediated by the biomarker?

Many issues related to the validation of biomarkers were discussed in Toniolo *et al.* (1997) and the reader is referred to the relevant chapters (Schulte & Perera, 1997; Pearce & Boffetta, 1997; Vineis, 1997; White, 1997; Schatzkin *et al.*, 1997; Boone & Kelloff, 1997; McMichael & Hall, 1997).

The use of non-valid biomarkers in cancer chemoprevention can lead to invalid inferences and generalizations and ultimately to erroneous assessment and use of cancer-chemopreventive agents. Anything less than perfect validity of the biomarker will lead to imperfection in our assessment of chemoprevention. It would be helpful to specify appropriate targets for the levels of sensitivity, specificity, variability and reproducibility of biomarkers that may be applicable in chemoprevention research.

## Exposure biomarkers

Exposure biomarkers may include endogenous or exogenous agents and their metabolites or adducts in tissues or body products, whether in physiological or pathological amounts. Structural changes in the cell or organism which reflect exposure are also included. Exposure is thus defined in a very broad sense and encompasses any influence that might predict the incidence or outcome of disease, including dietary factors, hormonal status, redox status, agent–gene interactions, and others. This is in line with, although still more narrow than, previous definitions, such as that of Armstrong *et al.* (1992) where exposure is referred to as ‘any of a subject’s attributes or any agent with which he or she may come in contact that may be relevant to his or her health’.

Whether a biomarker is considered an exposure marker may depend on its intended use. For example, HBV antibody seropositivity may be regarded as an exposure biomarker for viral exposure, or as an acquired predisposition marker used to select individuals who are at increased risk of hepatocellular cancer. Another example would be the presence of an HPV-related gene in a cervical biopsy, which may be regarded as an exposure biomarker, since it discriminates between HPV-exposed and non-exposed persons. It may, however, also be used as an intermediate-effect biomarker, since it is usually accompanied by a cellular effect (cervical dysplasia) along the pathway to cervical cancer.

Biomarkers of exposure are sometimes defined more narrowly as biomarkers of external carcinogen exposures. These have been subdivided into biomarkers of exposure, of target tissue dose, and of biologically effective dose. Although exposure biomarkers are meant here to encompass all of these, biomarkers which fall outside this definition are also embraced, for example, because they are related to protective exposures.

In chemoprevention, the most important exposure biomarkers concern exposures directly related to the intervention agent and to the target exposure to be modified by the intervention. It must be assumed that exposures to other risk factors or preventive factors are evenly balanced between the arms of the study, which should be the case provided randomization has been effective, but it may be considered prudent to

monitor such equivalence by the use of exposure biomarkers.

### *The contribution of exposure biomarkers to chemopreventive interventions*

Exposure biomarkers may contribute to intervention studies in several ways:

- In the selection of target populations: to identify subgroups of the population who have been exposed to an agent. Any exposure marker which could help in the selection of the relevant target population, making sure they have been exposed (e.g., patients excreting aflatoxin adducts in their urine, cotinine status, viral infection status, etc.), would qualify.
- In monitoring of the target population for confounders: to check that randomization has distributed evenly all important risk factors which are supposed not to change differentially in the different arms of the study during the intervention (e.g., use of nonsteroidal anti-inflammatory drugs (NSAIDs) in a colon cancer intervention trial, alcohol intake in a trial on lowering aflatoxin carcinogenicity in liver cancer, smoking status in a trial on intervention with antioxidants against lung cancer). Measurements may be made repeatedly during the intervention study.
- In the measurement of outcome:
  - (a) to measure quantitatively the efficacy of the intervention, determined as the effect on the targeted ‘exposure’ risk factor (if applicable), e.g. decreased adduct levels, decreased oxidative stress factors;
  - (b) to monitor chemopreventive agent compliance, uptake and/or distribution;
  - (c) to assess inter-individual and between-group differences in efficacy of the chemopreventive intervention.
- In the interpretation of results: e.g., to evaluate the influence of sub-group variables (as defined by biomarkers for other exposures) which may have influenced the outcome.

*Etiological considerations and choice of additional exposure markers*

Initially it is important to evaluate an exposure biomarker in relation to the following questions:

- Is the marker causally related to the disease or the intervention?
- What is the rationale for using the biomarker in question, more specifically:
  - Does the marker reflect the targeted 'exposure' (in a broad sense)? and
  - Is the exposure related to the natural history of the disease process?

For instance, if oxidative stress is targeted by intervention with an antioxidant, it is necessary to consider the evidence for the existence of such oxidative damage and for its causal relationship to the relevant disease. An exposure biomarker should in this case reflect the oxidative stress in the target organ, either as a direct measurement (e.g., in biopsies, if possible) or in a surrogate sample known to reflect the variation at the target. Other exposures influencing the targeted exposure should also be monitored, e.g., the target organ, plasma or serum status of dietary or endogenous antioxidants and biomarkers of more general reactive oxygen radical exposures of DNA, lipids or proteins.

*Steps in the development of new exposure biomarkers*

The development of a new exposure biomarker should take the following course:

1. Observations on risk and protective factors in human or experimental systems.
2. Mechanistic studies in experimental systems to establish the relationship of the biomarker to the causal chain of molecular and cellular events.
3. Development of experimental model systems such as transgenic mice.
4. Development of the biomarker methodology for exposure determination.

5. Testing of the exposure biomarker methodology in animal and human pilot studies.
6. Exploration of markers in large-scale intervention studies.

At any stage of this process, there can be feedback on etiology, prevention, diagnosis, prognosis, and thus an iterative return to step 1.

During steps 4 and 5, a range of characteristics have to be evaluated which constitute the best approximation of the validity of a biomarker. They may be expressed on a continuous scale. These should encompass the approaches to validation considered by Toniolo *et al.* (1997).

*Examples of exposure biomarkers*

Exposure biomarkers may be used for any of the following purposes:

1. Assessment of exposure to external carcinogens (e.g., body levels of external agent (carcinogen), DNA adducts, protein adducts)
2. Assessment of exposure to harmful endogenous agents (e.g., indices of oxidative damage to DNA or resulting from lipid peroxidation, hormonal levels and levels of hormone-regulated products)
3. As compliance markers, including markers for the intervention agent, for 'adherence' to the risk group, etc.
4. To assess endogenous or exogenous factors which are believed to interact with biological processes leading to cancer (e.g., preventive agents, markers of diet or lifestyle)

During the workshop, a number of examples of exposure biomarkers were discussed related to the six cancer sites considered.

Relevant exposure biomarkers for the skin should reflect exposure to sunlight and biomarkers of defence against radical-induced damage. Development of erythema on the skin is a good marker for excess sun exposure and is very consistent with the adducts formed in DNA. The number of naevi in less sun-exposed locations of the body

is believed to reflect mainly childhood exposure to sunlight and if so, might be used as a childhood exposure marker.

For colorectal cancer, NAT2 fast acetylation, GSTM1 null and high CYP1A2 status are potential modifiers of exposure to heterocyclic aromatic amines from cooked meat.

For breast cancer, endogenous levels of hormones or hormone-regulating proteins seem to be relevant exposure markers. Further development of these biomarkers should have high priority. Exposure to certain carcinogens belonging to the polycyclic aromatic hydrocarbon group or to the heterocyclic aromatic amine group gives rise to breast cancer in rat models and might be investigated in humans by using available or developing exposure biomarkers.

For prostate cancer, exposure biomarkers for endogenous androgens, androgen-regulated proteins and antiandrogens are currently available or under development. Selenium speciation biomarkers and plasma tocopherols might also be important exposure biomarkers for studies on prostate cancer.

For liver cancer, good biomarkers for cumulative exposures to alcohol are not available at present, whereas markers of exposure to aflatoxin B<sub>1</sub> and hepatitis B and C viruses may be adequately assessed in blood samples. Exposure biomarkers for direct or indirect DNA damage (e.g., 8-hydroxydeoxyguanosine (8-OH-dG) and etheno adducts) resulting from radical-mediated damage including lipid peroxidation might also prove useful in intervention studies on liver cancer.

For aerodigestive tract cancers, several biomarkers for exposure to polycyclic aromatic hydrocarbons and tobacco-specific nitrosamines have been developed. A simple biomarker for tobacco exposure, plasma cotinine, has the advantage of not being influenced by chemopreventive treatments. The apparent contradiction between elevated plasma  $\beta$ -carotene level as a negative predictor of lung cancer risk in smokers and the direct risk increase caused by  $\beta$ -carotene supplements calls for the use of other biomarkers of fruit and vegetable intake. Markers for other carotenoids are already available. Exposure biomarkers for various groups of dietary polyphenols have also been developed and biomarkers for other potentially modulating factors like isothiocyanates, indoles or terpenoids are available or under development.

#### *Further research needed*

In the area of exposure biomarkers, there are several areas where more research is needed, either in biomarker validation or in the development of new biomarkers.

##### *(a) Exposure biomarker validation*

There is a need for better and more systematic validation before exposure biomarkers are applied, requiring more interlaboratory validation efforts. The development of more than one marker for each end-point, using different analytical techniques, can disclose some of the analytical problems, which are difficult to identify with common validation procedures. Some aspects of validation are difficult to assess in pilot human studies, for example interactions with host factors or with dietary habits, and the inclusion of validation programmes into large human intervention studies is therefore important. An important aspect of the biological validation of an exposure biomarker is evaluation of underlying hypotheses on the relationship between the exposure and the effect. An example is whether oxidative stress or any DNA adduct formation is truly detrimental in all cases; if such processes are also used in endogenous signalling pathways, for instance in the induction of defence and repair, measurement of such end-points may lead to misclassification. With increasing sensitivity of methods, dose ranges may be studied which are well below those used in animal studies of dose-response relationships for genotoxic compounds. High priority should be given to the conduct of short-term human intervention studies for this type of biological validation of the hypotheses often implicitly underlying the use of exposure biomarkers.

##### *(b) Development of new exposure biomarkers*

In order to identify dietary or endogenous components which might influence newly identified molecular targets for chemoprevention, there is a need to develop *in vitro/in vivo* screening assays (for example, for screening of COX-2 inhibitors, compounds possessing hormonal activity, etc.). Such screening methods could be developed for exposure factors influencing a range of known or anticipated steps in carcinogenesis. The use of sophisticated animal model systems such as

transgenic mice or even higher eukaryotic systems should be encouraged.

High priority should also be given to the development of biomarkers of exposure to substances which help the body to defend itself. In particular, better markers of dietary exposure and their interaction with host factors are needed.

The development of better (validated) oxidative stress markers at the DNA level, encompassing both direct and indirect damage, should have high priority due to the implication of redox factors in carcinogenesis at several stages. Specifically, the evaluation of surrogate markers in blood and their correlation with target organ exposures should be investigated in animal models and in human pilot studies. This would cover part of the strong need for more robust methods for DNA adduct measurements in human samples. However, also for other types of adducts, including those with small or bulky alkylating agents, more robust methods are required which can be applied in clinical laboratories. In particular, the sensitivity of the available assays needs to be improved. Furthermore, there is a strong need for biomarker methodologies which could accurately assess exposures to factors that work through mechanisms other than DNA adduct formation. Methods for measuring potential protective dietary factors, oxidative stress factors, viral infections and hormonal levels, which may all be characterized to some extent as epigenetic factors in carcinogenesis, should be extended and refined. Another area of interest is protein or RNA modification, which could be more widely used for biomarkers.

### Intermediate-effect biomarkers

An intermediate-effect biomarker is a detectable lesion or biological parameter with some of the histological or biological features of preneoplasia or neoplasia but without evidence of invasion, which either is on the direct pathway from the initiation of the neoplastic process to the occurrence of invasive cancer, has a high probability of resulting in the development of cancer, or is a detectable biochemical abnormality which is highly correlated with the presence of such a lesion. Thus intermediate-effect markers include (a) detectable precancerous changes in an organ (confirmed by histology), (b) an alteration of a gene that is considered to play a causative role, and

(c) other indicators of carcinogenesis, such as the expression of a marker that represents the cause of a cancer (e.g., HPV DNA positivity). Causation is not a requirement for inclusion in this group, but the expectation is that a relevant biomarker can eventually be linked, through a biological mechanism, to the cancer.

A hierarchy of intermediate-effect biomarkers can be perceived. Those that are known to be on the causal pathway to cancer are at the top, and can be truly called intermediate-effect markers. Then there are those markers for which present knowledge indicates only a probability of association with cancer, but it is uncertain as to whether they are on the causal pathway; these can only be called intermediate markers. However, whether one is talking about 'tissue lesions' (e.g., liver foci, skin papillomas, dysplastic lesions) or molecular markers (e.g., alterations in *ras* or *p53* genes), it is essential to know the statistical relationship between the lesions and cancer incidence.

A subset of intermediate-effect markers, which can be modulated, have been called surrogate end-point biomarkers (Kelloff *et al.*, 2000). An expectation is that if reduction in, but not abolition of, surrogate end-point biomarkers is shown, it can be demonstrated that those that remain do not have markers of progression, i.e. the bad actors have been removed.

Intermediate-effect markers contribute to chemopreventive interventions:

- in the selection of target populations: identification of subgroups of the population in whom precancerous lesions are detectable (e.g., patients with adenomatous polyps)
- in the measurement of outcome: surveillance of the efficacy of the intervention (e.g., women who test HPV DNA-positive in whom the subsequent incidence of high-grade cervical lesions is changed)
- in the interpretation of results: assessment of inter-individual and inter-population differences in efficacy of chemopreventive agents
- in the design of relevant animal models: creating informative animal models at high risk for

development of precancerous lesions (e.g., transgenic systems).

- in the characterization of molecular pathways involved.

*General considerations regarding use of intermediate-effect biomarkers in chemoprevention*

It has not been convincingly shown that the use of chemopreventive agents in men and women with any type of preneoplastic lesion can substantially reduce the subsequent development of a truly invasive cancer. In general, not enough is known about the natural history of precancerous lesions to identify those that will progress to invasive cancer if allowed to do so. It would be valuable to establish biomarkers of potential for such progression that could be used in chemoprevention research. Conventional histopathology alone has not proved reliable for such purposes.

*Examples of intermediate markers*

Skin cancer *in situ* is generally considered irreversible, and qualifies as an intermediate-effect marker. Markers of sun damage to the epidermis (sunburn cells or *p53* mutations) or melanocytes are indicators of early effects which are reversible. Actinic keratoses are later events, but may spontaneously regress after cessation of exposure to ultraviolet radiation. Naevi are risk factors that may be precursors of melanoma. They are clonal. Large atypical naevi are likely to be precursors, but it has not yet been determined whether acquired naevi represent actual premalignant lesions.

Adenomas are the most commonly used intermediate-effect biomarker for colorectal cancer. In chemoprevention trials, adenomatous polyps are used as biomarkers of risk, but can also be treated as surrogate end-points. There is a need to consider if chemoprevention will remove those adenomas that are most likely to progress to cancer. Oxidative DNA base modifications may be usable as markers, but are really biomarkers of exposure, not of outcome. The value of aberrant crypt foci or crypt fission as risk markers or as surrogate end-points is uncertain, as is the role of *ras* mutations in stools and of mucosal proliferation.

The degree of mammary density as a proportion of breast, measured on mammograms, is associated

with increased risk of breast cancer. Ductal carcinoma *in situ* (DCIS) may be a marker of risk but may not be a precursor in the classic sense. However, DCIS is being used as a marker of drug effect in phase IIa studies, when an agent is given for three weeks, with modulation of cellular progression as end-point.

The natural history of prostate intraepithelial neoplasia (PIN) is not clear. High-grade PIN is associated with the subsequent risk of prostate cancer, but it is difficult to be certain that invasive cancer was not present at the time of diagnosis of PIN, while some of the architectural variants of PIN may be confused with intraductal spread of a concomitant adenocarcinoma. Chemoprevention studies have been conducted by enrolling men before planned prostate surgery and assessing the effect of intervention on high-grade PIN in the six-week interval before surgery. People with PIN have also been enrolled in studies and followed for cancer.

For liver (hepatocellular) cancer, it is possible that tests for *p53* mutations may indicate long-term changes, but accumulation of the *p53* protein is not an early event in hepatic carcinogenesis in areas with low exposure to aflatoxin. Preneoplastic phenotypically altered lesions (falling short of either benign or malignant neoplasia) are very small and cannot be detected by conventional biochemical approaches in tissue homogenates. However, their assessment may be useful in short-term chemoprevention trials in individuals scheduled for liver transplantation. Cell proliferation is not a good marker for the earliest emerging types of preneoplastic lesion. Glycogenotic-basophilic cell lineages are found in humans with hepatocellular cancer.

Atypical cells in sputum are only weakly predictive of lung cancer. Chemopreventive agents have been found to modulate (upregulate) nuclear retinoic acid receptor  $\beta$  (RAR $\beta$ ). Many of the non-calcified nodules identified by helical computerized tomographic (CT) scanning show atypical adenomatous hyperplasia. These may be related to bronchioalveolar carcinoma. Immunostaining of A2/B1 heterogeneous nuclear ribonucleoprotein (hnRNP) is not correlated with histological change. Metabolically active preneoplastic cells seem to most actively express this protein.

*Other relevant considerations*

- Prevention of invasion is the fundamental goal in cancer chemoprevention.
- There is an increasing role for computer-assisted imaging for morphometric analysis to assess intermediate biomarkers in chemoprevention research.
- The term intraepithelial neoplasia (IEN) is being used to describe lesions such as colorectal adenomas, breast ductal carcinoma *in situ*, prostatic intraepithelial neoplasia, cervical intraepithelial neoplasia and actinic keratoses. Lesions such as Barrett's oesophagus, bronchial dysplasia, bladder dysplasia and oral leukoplakia are not neoplastic and should not be described as IEN, but may qualify as intermediate-effect biomarkers. Early biological events that could be potential targets of chemoprevention include alterations in protein kinases, transcription factors, enzymes involved in carbohydrate and lipid metabolism, factors that control angiogenesis, and altered components of the immune system.
- Animal models, particularly those using transgenic mice, are useful in the development of intermediate-effect biomarkers, but caution is required in extrapolating the results to the human situation.
- It is necessary to determine how good DNA modifications or adducts are as biomarkers, and how best to assess oxidative stress: by DNA adducts, protein oxidation, or other methods. Methods for measuring oxidative DNA base alterations are probably not yet sensitive enough. The use of classical DNA repair assays such as unscheduled DNA synthesis is not sufficient as the repair system is very refined and there is a large degree of heterogeneity in DNA repair within the genome. Active genes are repaired much faster and with different enzymology than inactive genomic regions. Techniques are required to measure this kind of repair in populations. Methods to assess DNA repair in individual cells are now emerging, and should be applied in population studies.

**Susceptibility markers in chemoprevention**

A biological marker of cancer susceptibility is an indicator of a heritable ability of an organism to respond to the challenge of carcinogenic agent(s) or event(s). In particular with respect to cancer chemoprevention, susceptibility markers are also indicators of an ability to respond to the cancer-preventive action. The marker can indicate an increased susceptibility to chemoprevention as well as a resistance to it. Thus the response can take the form of an enhanced sensitivity or of an adverse effect.

The very limited spectrum of susceptibility biomarkers available in the past, such as the phenotypically obvious features sex and skin colour, is expanding dramatically with the advances in the field of genetics. The traditional distinction between high- and low-penetrance gene defects obscures a continuum of susceptibility at the biological level. At the operational level, however, it is possible to identify a small number of genes in which pathological mutations are sufficiently predictive of cancer risk to influence clinical management. Any allelic variant of the 'major' genes, alteration in other interactive genes or in environmentally sensitive polymorphisms which would not be sufficiently penetrant to determine clinical practice will be categorized as low-penetrance biomarkers. As knowledge expands and the capacity to test multiple genes simultaneously becomes commonplace, high-penetrance 'genotypes' comprising several low-penetrance genetic variations may be recognized.

Susceptibility markers require specific types of validation, for example phenotype-genotype correspondence (the lack of such correspondence for CYP2E1 casts doubt on the usefulness of this marker for chemopreventive trials) and expression in relevant tissues.

Validated biomarkers of genetic or acquired susceptibility can be conceptualized as 'effect modifiers' in epidemiological studies. From a biological perspective, effect modification conceptually answers the question as to why two similarly treated (exposed) individuals or groups of individuals respond differently.

### *Use of susceptibility biomarkers in chemoprevention trials and interventions*

Markers of susceptibility can contribute at different levels of chemopreventive trials and interventions.

#### 1. Selection of high-risk target populations

The strategy of chemoprevention may be targeted to high-risk individuals. Patients diagnosed with cancer are at increased risk of a second primary cancer and are highly motivated to take part in trials. Individuals in families with genetic syndromes have increased risk of developing certain cancers. For colorectal cancer, the two major syndromes are hereditary non-polyposis colorectal cancer (HNPCC) and familial adenomatous polyposis (FAP). HNPCC accounts for 5% and FAP for 1% of all persons with the disease. The lifetime risk of developing colorectal cancer is almost 100% for FAP and around 70% for HNPCC. Randomized chemoprevention trials (with NSAIDs and nutritional supplements) in FAP and HNPCC families are in progress.

Results of chemoprevention trials in genetically high-risk individuals may apply to the general population if the high-risk cohort represents common disease pathways and the relevant biological pathways through which the chemopreventive agent operates are the same in the susceptible and the non-susceptible. By the same token, if pathways differ, an agent ineffective in the genetically susceptible may, nonetheless, have efficacy in the general population.

This subject-selection strategy has advantages but also disadvantages.

#### *Advantages*

- Individuals at risk may be easily traced through clinical or genetic registers.
- Perceived benefits to the individual and the families should increase compliance.
- Expensive interventions such as colonoscopy are available as part of routine health care.
- High penetrance reduces the number of participants and duration of treatment needed to achieve statistical power.

#### *Disadvantages*

- The high-risk cohort may not represent common disease pathways.
- Large-scale genotyping may overload the capacity to provide adequate pre-test genetic counselling.
- Compliance may be reduced if there is a perception of threat to health insurance if an individual's genetic status is revealed.
- Fear of cancer may encourage drop-out or non-compliance before a randomized trial is complete, especially if the agent under consideration is readily available to the general population.

#### 2. Target of chemoprevention

Susceptibility markers can be the target of chemopreventive interventions at the phenotypic level. This is the case when the chemopreventive agent is chiefly aimed to modify the expression (phenotype) of a susceptibility factor. Many phase I or phase II metabolic pathways are inducible by a number of potential chemopreventive agents such as components of fruits and vegetables. For example, cruciferous vegetables can be administered as inducers of CYP1A2 and other inducible enzymes.

#### 3. Modification of effect of chemoprevention

It is plausible that genetic susceptibility factors may modify the effect of chemopreventive agents. Such effect modification would be responsible for inter-individual and inter-population differences in the efficacy of chemopreventive interventions. In humans, however, no results are currently available showing such effect modification. Nevertheless, evidence that some possible chemopreventive agents (e.g., carotenoids) operate through induction of metabolizing enzymes in experimental systems supports the notion of a role for metabolic polymorphisms in the modulation of the response to chemoprevention.

#### *Animal models in the design of new susceptibility biomarkers*

The recent development of mouse strains with overexpressed or inactivated cancer-related genes has provided researchers with new models for testing chemoprevention strategies that could

counteract specific genetic susceptibilities to cancer. The multiple intestinal neoplasia (Min) mouse, which carries a fully penetrant dominant mutation of the *Apc* gene, was first reported in 1990 (Moser *et al.*, 1990). Mice that are heterozygous for the *Apc* mutation develop scores of grossly detectable adenomas throughout the small intestine, and less so in the colon. Studies with Min and Cox-2 knock-out mice have provided strong evidence that Cox-2 plays a major role in intestinal carcinogenesis, and that NSAIDs which target the Cox-2 protein have great potential as chemopreventive agents.

Mutation of the *p53* gene is the most commonly observed genetic lesion in human cancer; more than 50% of all human tumours examined have identifiable *p53* mutations or deletions. Donehower *et al.* (1992) reported that homozygous *p53*-knock-out mice were viable but highly susceptible to spontaneous tumorigenesis (particularly lymphomas) at an early age. Hursting *et al.* (1994) reported that calorie restriction significantly delayed the onset of spontaneous tumorigenesis in *p53*<sup>+/-</sup> mice. Heterozygous *p53*-knock-out mice may be analogous to humans susceptible to heritable forms of cancer due to decreased *p53* expression, such as individuals with Li-Fraumeni syndrome. These mice exhibit increased sensitivity to mutagenic carcinogens, and thus may be susceptible to low-dose chronic carcinogen regimens that more closely mimic human exposures. Thus these mice may have great potential for developing models for studying modulatable biomarkers relevant to human cancer chemoprevention.

#### *Design issues when susceptibility markers are integrated into chemoprevention trials*

##### 1. Selection of study populations

Differences in the distribution of allelic variants of putative susceptibility genes across populations can be used to define the study population by ethnic or geographical origin, with the aim of select-

ing the study population with the highest allele frequency. When there is a strong age-dependence in the penetrance of the susceptibility marker, the age range of the study population can be chosen accordingly to target the chemoprevention to the subjects with highest risk.

##### 2. Sample size and statistical analysis

Calculation of study sample size needs to take into account the gene penetrance and the prevalence of the susceptibility allele(s) when the susceptibility allele(s) is(are) used to identify the target study population. In the case of risk modification by other susceptibility allele(s) or risk factors, the prevalence of combined genetic and other risk factors needs to be considered. When a susceptibility allele is treated as a modifier of the effect of chemoprevention in the intervention groups, the prevalence of the susceptibility allele needs to be considered in addition to the risk of disease in the study population. In general, the sample size will be determined by the subgroup with the lowest expected proportion of subjects.

Susceptibility alleles considered to modify the disease risk of the study group or the effect of chemoprevention need to be accounted for in the analysis of the efficacy of the chemoprevention.

#### *Ethical issues in relation to susceptibility markers*

A large literature has developed dealing with the health implications of predictive testing for late-onset disease using high-penetrance biomarkers. Chemoprevention trials targeted at carriers of low-penetrance biomarkers raise new challenges. On the one hand, disclosure of genetic status in relation to metabolic polymorphisms may cause unnecessary anxiety and create difficulty for clinicians asked to explain the results. On the other hand, confidential genotyping to stratify populations before enrolment would involve non-disclosure of genetic information, a practice liable to generate objections from research ethical committees.

## Recommendations

1. Studies should be conducted in homogeneous, well defined, high-risk groups (i.e., subjects with dysplastic lesions).
2. Costly five-year studies are not the ideal start for programmes, especially if they involve heterogeneous populations (mixture of high- and low-risk responders, due to genetics, diet, etc.).
3. There is an urgent need to develop chemopreventive options for susceptible high-risk individuals.
4. Research is needed to validate intermediate markers as indicators of effect with a high probability that the marker is on the causal pathway to cancer.
5. Research should be encouraged into the cellular and molecular biology and pathogenesis of preneoplasia in order to identify and validate intermediate biomarkers relevant to chemoprevention.
6. Techniques, assays and scientific results need to be compared and validated among different laboratories to develop reliable methods.
7. Chemopreventive agents should be sought that will yield (a) clinical benefit from the arrest or reversal of surrogate lesions and (b) enhanced quality of life. Continued monitoring for adverse effects that might not be observed in short-term surrogate end-point studies is important.
8. There is a need to explore the ethical dimension of genetically targeted chemoprevention. In particular, there is a dilemma in relation to disclosure of genetic information about low-penetrance biomarkers. Should information about biomarkers of little or no relevance to the individual be disclosed and if not, what methods should be employed to protect confidentiality? If a biomarker or combination of biomarkers becomes predictive of a disease, how will the information be made available to the participants?
9. Reproducible high-risk genotypes comprising several lower-penetrance genetic variants should be identified so as to expand the potential to target high-risk individuals for chemoprevention trials.
10. Concentration on populations with identifiable genetic polymorphisms would help in assessing the public health impact of chemoprevention.
11. It is important to assess whether agents that are potentially beneficial in some individuals may be harmful in others, and whether chemoprevention can be restricted to only those who will benefit from the agent.
12. Studies should have large enough sample size to permit assessment of gene-environment and gene-gene interactions. Such large studies require adequate long-term funding. However, with appropriate combination of exposure and susceptibility markers, it may be possible to concentrate on subjects especially at risk and reduce the sample sizes needed.
13. Meta-analyses and pooled analyses should be used to combine small studies. However, caution should be applied in interpreting their results.

## References

- Armstrong, B.K., White, E. & Saracci, R. (1992) *Principles of Exposure Measurement in Epidemiology*, Oxford, Oxford University Press, p. 4
- Boone, C.W. & Kelloff, G.J. (1997). Biomarker end-points in cancer chemoprevention trials. In: Toniolo, P., Boffetta, P., Shuker, D.E.G., Rothman, N., Hulka, B. & Pearce, N., eds, *Application of Biomarkers in Cancer Epidemiology* (IARC Scientific Publications No. 142), Lyon, IARC, pp. 273–280
- Coggon, D. & Friesen, M.D. (1997). Markers of internal dose: chemical agents. In: Toniolo, P., Boffetta, P., Shuker, D.E.G., Rothman, N., Hulka, B. & Pearce, N., eds, *Application of Biomarkers in Cancer Epidemiology* (IARC Scientific Publications No. 142), Lyon, IARC, pp. 95–101
- Donehower, L.A., Harvey, M., Slagle, B.L., McArthur, M.J., Montgomery, C.A., Jr, Butel, J.S. & Bradley, A. (1992) Mice deficient for p53 are developmentally normal but susceptible to spontaneous tumours. *Nature*, 356, 215–221
- Hursting, S. D., Perkins, S. N. & Phang, J.M. (1994) Calorie restriction delays spontaneous tumorigenesis in p53-knockout transgenic mice. *Proc. Natl Acad. Sci. USA*, 91, 7036–7040
- Kelloff, G.J., Sigman, C.C., Johnson, K.M., Boone, C.W., Greenwald, P., Crowell, J.A., Hawk, E.T. & Doody, L.A. (2000) Perspectives on surrogate end points in the development of drugs that reduce the risk of cancer. *Cancer Epidemiol. Biomarkers Prev.*, 9, 127–137
- McMichael, A.J. & Hall, A.J. (1997) The use of biological markers as predictive early-outcome measures in epidemiological research. In: Toniolo, P., Boffetta, P., Shuker, D.E.G., Rothman, N., Hulka, B. & Pearce, N., eds, *Application of Biomarkers in Cancer Epidemiology* (IARC Scientific Publications No. 142), Lyon, IARC, pp. 281–289
- Moser, A.R., Pitot, H.C. & Dove, W.F. (1990) A dominant mutation that predisposes to multiple intestinal neoplasia in the mouse. *Science*, 247, 322–324
- Pearce, N. & Boffetta, P. (1997) General issues of study design and analysis in the use of biomarkers in cancer epidemiology. In: Toniolo, P., Boffetta, P., Shuker, D.E.G., Rothman, N., Hulka, B. & Pearce, N., eds, *Application of Biomarkers in Cancer Epidemiology* (IARC Scientific Publications No. 142), Lyon, IARC, pp. 47–57
- Schatzkin, A., Freedman, L.S., Dorgan, J., McShane, L., Schiffman, M.H., & Dawsey, S.M. (1997). Using and interpreting surrogate end-points in cancer research. In: Toniolo, P., Boffetta, P., Shuker, D.E.G., Rothman, N., Hulka, B. & Pearce, N., eds, *Application of Biomarkers in Cancer Epidemiology* (IARC Scientific Publications No. 142), Lyon, IARC, pp. 265–271
- Schulte, P.A. & Perera, F.P. (1997). Transitional studies. In: Toniolo, P., Boffetta, P., Shuker, D.E.G., Rothman, N., Hulka, B. & Pearce, N., eds, *Application of Biomarkers in Cancer Epidemiology* (IARC Scientific Publications No. 142), Lyon, IARC, pp. 19–29
- Toniolo, P., Boffetta, P., Shuker, D.E.G., Rothman, N., Hulka, B. & Pearce, N., eds (1997) *Application of Biomarkers in Cancer Epidemiology* (IARC Scientific Publications No. 142), Lyon, IARC
- Vineis, P. (1997) Sources of variation in biomarkers. In: Toniolo, P., Boffetta, P., Shuker, D.E.G., Rothman, N., Hulka, B. & Pearce, N., eds, *Application of Biomarkers in Cancer Epidemiology* (IARC Scientific Publications No. 142), Lyon, IARC, pp. 59–73
- White, E. (1997) Effects of biomarker measurement error on epidemiological studies. In: Toniolo, P., Boffetta, P., Shuker, D.E.G., Rothman, N., Hulka, B. & Pearce, N., eds, *Application of Biomarkers in Cancer Epidemiology* (IARC Scientific Publications No. 142), Lyon, IARC, pp. 73–93