PERGAMON

European Journal of Cancer 36 (2000) 694–709

European Journal of Cancer

www.ejconline.com

Review

Recent advances in cancer chemoprevention, with emphasis on breast and colorectal cancer

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Received 12 July 1999; received in revised form 31 January 2000; accepted 3 February 2000

Abstract

Chemoprevention is a recently introduced and rapidly growing area of oncology that is identifying agents with a potentially preventive role in cancer. Several clinical trials have recently shown the feasibility of this approach in reducing the risk of major human cancers. In the USA, a large trial that demonstrated a reduction of approximately 50% in the risk of developing breast cancer led to Food and Drug Administration (FDA) approval of tamoxifen as a preventive agent in women at increased risk. Although the results could not be reproduced in two smaller European trials, further investigations into this agent are clearly warranted. Raloxifene, another selective oestrogen receptor modulator which has reduced the risk of breast cancer in a trial in women with osteoporosis, is being compared with tamoxifen in a large primary prevention trial in at-risk women. Retinoids are a group of compounds that have proved especially effective in reducing the occurrence of second primary tumours in subjects with skin, head and neck or liver cancer. Fenretinide, a synthetic retinoic acid derivative, has recently been shown to decrease the occurrence of a second breast malignancy in premenopausal women. Results with non-steroidal anti-inflammatory drugs (NSAIDs) have proved consistently encouraging in epidemiological studies in lowering the incidence of colorectal cancer. Clinical trials with selective cyclo-oxygenase inhibitors potentially devoid of gastrointestinal (GI) toxicity are currently underway in at-risk subjects. Calcium and selenium have also received much attention as chemopreventive agents. Originally investigated against skin cancer, selenium showed efficacy in reducing prostate, lung and colon cancer incidence. Similarly, vitamin E was effective in reducing prostate cancer incidence and mortality in a lung cancer prevention trial in heavy smokers. The challenges of conducting well-designed and unequivocal chemoprevention trials are considerable, but advances in techniques of identification of at-risk subjects and establishing surrogate endpoint biomarkers should contribute greatly to future studies. Current knowledge suggests that a pharmacological approach to preventing cancer, using natural or synthetic agents, could become an important way forward.

Keywords: Cancer chemoprevention; Biomarkers; Breast neoplasms; Colorectal neoplasms

1. Introduction

Chemoprevention is a recently introduced and rapidly growing area of oncology. The number of chemoprevention trials have increased substantially in just a few years, for there is no doubt that the prospect of being able to prevent cancer is universally attractive. The term chemoprevention was used in 1979 by Sporn and Newton who defined it as "the prevention of cancer by the use of pharmacological agents that inhibit or reverse the process of carcinogenesis" [1].

Carcinogenesis is a complex and multistage process involving interactions between genes and environmental insults that ultimately affect cell proliferation and death. Chemoprevention focuses on intervening in the processes in the cascade of carcinogenic events to prevent the final progression to neoplastic disease, unlike chemotherapy, which concentrates on containing or eradicating cells that have already undergone malignant transformation. Whilst chemotherapy is targeted at people with manifest disease, chemoprevention is directed at individuals who are apparently well, although those in high-risk groups could arguably have existing premalignant conditions. These important differences raise several considerations related to the design and execution of clinical trials of chemopreventive agents. Careful thought must be given to defining the sample population, to defining what is an acceptable intervention in a 'well' person and what are valid endpoints for measuring outcome, for if the primary aim is to avoid

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0959-8049/00/$ - see front matter
PII: S0959-8049(00)00040-X

malignancy, a successful intervention would need to be studied for many years.

The rationale behind many chemoprevention studies comes often, but not necessarily, from epidemiological and observational studies that look for an association between environmental factors and tumour occurrence. But environmental factors are hugely complex and dissecting out the primary causative factor within that association can be a long and frustrating task. It is notable that some important chemoprevention trials have contributed to our knowledge through totally negative findings.

This review discusses both the general considerations for chemoprevention trials and reviews intervention trials that have been conducted for specific cancers.

2. Target populations

Epidemiological and genetic studies have allowed us to define high-risk groups for many malignancies. Risk may be conferred by inheritance of particular genes, such as the *adenomatous polyposis coli* (*APC*) gene in colorectal cancer, or by exposure to known carcinogens, such as smoking and lung cancer. However, there are many considerations in deciding whether high-risk groups are the most appropriate cohort for chemoprevention studies. If the sample population is a high-risk group there is an advantage in that the time to occurrence of a measurable endpoint may be more predictable and shorter. But, there is also the question of how applicable results obtained from a high-risk group are to the general population. High-risk individuals could, by definition, already have premalignant changes [2]. The preventive agent may then be treating this premalignancy and stopping its progression to overt cancer, rather than preventing premalignant changes taking place. Although this distinction may be regarded as academic, since clinically the desired outcome is achieved, it makes the important mechanistic details of how chemopreventive agents are working difficult to elucidate.

Different target populations may be selected for studying different aspects of one cancer type. For example, in breast cancer chemoprevention studies, a first level may involve primary prevention trials in a wide population of healthy women who have a higher, albeit moderate risk for a combination of both genetic and reproductive factors such as those identified with the Gail model [3], or because of exposure to known promoting agents (e.g. hormone replacement therapy). Due to the limited statistical power, however, such studies are extremely costly. A second level could involve a limited population at very high risk because of highly penetrating genetic predisposition to cancer (e.g., *BRCA1* and *BRCA2* mutation carriers). Trials in this type of population may prove very efficient, but our limited understanding of the physiological function of these genes has so far prevented the rational choice of effective agents. A third level could involve secondary prevention trials in subjects with premalignant or early malignant lesions, e.g. breast atypical hyperplasia and lobular or ductal carcinoma *in situ* or micro-invasive disease, or long-term survivors after adjuvant treatment.

Individuals with colorectal adenoma may also be viewed as an ideal cohort for chemoprevention. In fact, in many large bowel chemoprevention trials adenomas can have a double duty: they are used to identify subjects at risk for large bowel neoplasia, and also serve as endpoints. Many features of adenomas make them suitable for these tasks. Patients with adenomas are fairly numerous and easy to identify, furthermore, the 'adenoma–carcinoma' [4] sequence suggests that adenomas are logical endpoints. The high risk of recurrence amongst adenoma patients means that a relatively modest number of subjects will suffice to provide an adequate statistical power. There are some limitations in the use of adenomas, however. Firstly, there is clearly heterogeneity of risk for subsequent cancer. Patients with only small adenomas may have rates of colorectal cancer that are not much greater than those of the normal population. Choosing the high-risk patients for preventive interventions makes sense from a risk–benefit point of view. However, from a population perspective, it may make more sense to answer the chemoprevention question in the numerous individuals who are each at small risk, but who collectively account for most of the cases of colorectal cancer.

Colon cancer can also provide an attractive setting for chemoprevention trials because of the frequency and variation of familial predisposition that is observed in this malignancy. Inherited colon cancer susceptibility varies from mild to severe. Conditions with extreme susceptibility include the autosomal dominantly inherited syndromes of familial adenomatous polyposis (FAP) [5] and hereditary nonpolyposis colorectal cancer (HNPCC) [6]. These are highly penetrant syndromes with extreme cancer risk. FAP arises from mutations of the *APC* gene and HNPCC from mutations of the mismatch repair genes. Specific and individual genetic diagnosis is now possible in both syndromes, thus allowing identification of genetically affected individuals for chemoprevention trials.

FAP accounts for less than 1% of colon cancer, whilst HNPCC may be present in up to 5% of cases. Familial clustering is common in the remainder of cases, which are often referred to as sporadic, but probably arise in part from inherited susceptibility. Epidemiological studies have shown that first-degree relatives have a 2- to 4-fold increased risk of acquiring colon cancer compared with the general population [7]. Ten per cent of individuals in the USA have a first-degree relative with colon cancer. This clinically identifiable higher risk group thus

constitutes a large potential cohort for chemoprevention trials. However, recruiting specific high risk populations may introduce unintentional biases if the individuals are alerted to their condition and have been educated about risk factors and lifestyle, so that they have already implemented risk-avoidance changes in lifestyle before the study has started. These biases are unavoidable, however, as ethical issues should prevail over scientific issues.

Compliance in very long-term trials is an issue and will only be achieved if the medication is easy to take, the subject can appreciate the value of the study, and the follow-up studies are simple and convenient. But, such large-scale undertakings can also yield huge amounts of data, and the effect of one or more chemopreventive agents on a variety of different cancers can be studied simultaneously. Indeed, observations on malignancies other than the primary target of the study have often brought unexpected bonuses, for example the alpha-tocopherol, beta-carotene study (ATBC) examining the influence of vitamin E on lung cancer found positive effects on the incidence of prostate cancer [8]. Similarly, the study originally evaluating the potential chemoprotective role of selenium in skin cancer found positive effects on prostate, lung and colon cancers [9], and an investigation in breast cancer patients has shown encouraging results in ovarian cancer [10]. All these observations derived from secondary endpoints are hypothesis generating and provide the rationale for *ad hoc* clinical studies.

The size and duration of a chemoprevention trial necessary to give a statistically meaningful result can be enormous. So even in a high-risk population (as selected by the Gail model) where the annual rate of breast cancer is over 6 per 1000 women, 3600 women per arm would need to be studied over a 5-year period in order to obtain a reasonable statistical power to detect a 50% risk reduction. These figures assume a 75% compliance with the trial protocol, a figure that is realistic in a long-term study involving apparently well subjects. Such an undertaking may not always be justified on the basis of currently available information and so preliminary information needs to be gathered from smaller studies of surrogate endpoints. If these are successful, they provide a stronger case for justifying a large trial.

Moreover, if an agent is to be given to huge numbers of people over extended periods then that agent must be of low toxicity, devoid of long-term effects and effective at low doses. This makes long-established drugs, and naturally occurring vitamins and minerals, attractive candidates for chemoprevention studies.

3. Surrogate endpoints in chemoprevention trials

Surrogate endpoints are biological markers or events that may be assessed or observed prior to the clinical appearance of the disease, and that bear some relationship to the development of that disease. They are intermediate in the sense that they occur sometime between a given intervention that affected the disease process and the time of the clinical diagnosis of the disease. The use of surrogate endpoint biomarkers (SEBs) in pivotal cancer chemoprevention trials may lead to a rational choice of agents which are likely to affect cancer incidence in subsequent phase III trials. For some malignancies there is a readily apparent natural surrogate marker. As mentioned, adenomatous polyps precede the development of colon cancer, thereby providing a readily identifiable clinical endpoint to judge the effectiveness of chemoprevention. However, there are complexities in considerations of the use of adenomas as endpoints of chemoprevention trials.

Adenomas that occur in prevention trials are generally small, and may not necessarily be associated with a greatly increased cancer risk. The issue for chemoprevention trials, however, is not only whether the endpoints are truly intermediate in the causal chain, but whether the intervention under study alters the adenoma recurrence risk to the same extent as it does for the colorectal cancer risk. This is a difficult matter to verify but the limited data available are encouraging. The epidemiology of colorectal adenomas (largely small adenomas) is similar in many regards to that for colorectal cancer itself. Thus, to the extent that data are available, one can tentatively conclude that external influences affect adenomas and colorectal cancer similarly. But this must always remain a general consideration — whether chemopreventive agents act in the same way on the surrogate endpoint as they do on the progression of the neoplasm.

4. Histopathological markers

As cancer is a histopathological disease by definition (although driven by germline and somatic genetic alterations), histopathological markers closest to the incidence of invasive cancer both theoretically and demonstrably hold the greatest predictive value amongst the range of intermediate efficacy markers currently available for application in cancer prevention studies. Such markers may be reductions in the number, area, or grade of incident pre-invasive neoplastic lesions (atypia of cytological specimens and dysplasia of histopathological specimens) and/or induced regression (decreases in number, area or grade) of pre-invasive neoplastic lesions. The utility and relevance of these markers are difficult to demonstrate definitively, because pre-invasive neoplastic lesions are routinely excised as a matter of the acceptable standards of care in the majority of instances, and because their risk for progression to invasive cancer is beyond the comfort level of most surgeons (e.g. ductal carcinoma *in situ*

(DCIS), colorectal adenomas, cervical dysplasia, actinic keratoses, prostatic intraepithelial neoplasia, etc.). In the case of colorectal cancer, the efficacy of these approaches are demonstrable; polypectomy certainly reduces the subsequent incidence of invasive disease, when assessed in the most rigorous methods consistent with ethical standards. The advancement of techniques such as computer-assisted image analysis that allow detection of early premalignant events and the ability to distinguish between metastases and *de novo* tumours will contribute greatly to chemoprevention studies.

In addition to histopathological SEBs, markers which characterise fundamental pathophysiological alterations from relatively early points in neoplastic progression (therefore, on subcellular, cellular and early histological organisational levels) offer important mechanistic insights, knowledge about key pharmacodynamic targets, and may represent tomorrow's validated efficacy biomarkers. Interruption in the balance of cellular population dynamics such as cell proliferation, apoptosis and sloughing, with progressive accumulation of cells, is a fundamental property of early neoplasia. Therefore, an important biomarker is 'incident' or clinically observable masses of abnormal cells arising from normal-appearing, though actually abnormal, flat mucosa. Similarly, alterations in cellular morphology, which form the basis for the pathologist's designation of 'atypia' or 'dysplasia', are key early markers of the neoplastic process within cells and tissues (occurring both in observable masses of neoplastic cells and in the fields at risk) and are, therefore, appropriate characteristics to develop as markers of preventive efficacy. The nuclear polymorphism index, which characterises tissue by several major features including nuclear area, shape and texture features and nucleolar morphometry are particularly important morphological features. The index is particularly useful when evaluated quantitatively by computer-assisted image analysis; it is then the sum of the variances of the three measures. Ultimately, key genetic markers, or combinations of these, which predispose to neoplastic progression within fields of normal appearing cells will be identified, and may be used as markers of preventive efficacy (e.g. homozygous *APC* alterations in the cellular population of the colorectum of an individual in the sporadic setting or in a genetically predisposed individual such as a FAP subject). Because these markers offer key mechanistic insights which can be applied to develop interventions, identify risk, or evaluate efficacy, investments in these markers are considered to be fundamental foundations to progress in preventing cancer.

5. Circulating insulin-like growth factor-I (IGF-I)

The IGF system plays a pivotal, permissive role in cell proliferation of both epithelial and mesenchymal tissues in at least three different ways: (1) it is highly mitogenic; (2) it protects normal and tumour cells from apoptosis; and (3) it is required in several types of cells for the establishment and maintenance of the transformed phenotype and for tumorigenesis [11]. Large, well conducted, long-term prospective studies have clearly shown that high circulating levels of IGF-I and low levels of its major binding protein (IGFBP-3) are associated with a higher risk of developing subsequent premenopausal breast cancer [12], prostate cancer [13], lung [14] and colorectal cancer [15,16]. This indicates that IGF-I in the blood is a key regulator of cell and tumour growth for the vast majority of human epithelial cancers [17]. There is growing experimental, epidemiological and clinical evidence that the IGF system is important in breast carcinogenesis. A summary of the rationale for the use of circulating IGF-I as a SEB for breast cancer is provided in Table 1. *In vitro,* IGF-I is one of the most potent mitogens for breast cancer cell lines, where it mediates the oestrogen action. Importantly, the interaction between IGF-I and oestrogen receptors (ER) is mutual, since IGF-I has been shown to function as a potent stimulatory factor of the oestrogen signalling pathway in the absence of oestrogen [18].

Several studies have recently demonstrated that the antiproliferative effect exerted by retinoids on breast cancer cell lines is mediated by the inhibition of the IGF-stimulated growth. In humans, the synthetic retinoid fenretinide or 4-HPR was shown to modulate plasma IGF-I levels [19]. As IGF-I can stimulate normal epithelial breast proliferation and promote breast cancer cell growth *in vitro* and *in vivo,* and because higher circulating IGF-I levels have been found in breast cancer women compared with healthy controls, the change in IGF-I levels may be considered as a potential surrogate endpoint of breast cancer inhibition [20]. Both anti-oestrogens and retinoids can regulate IGF-I synthesis in humans. Since they act through nuclear receptors belonging to the same steroid/thyroid/retinoid superfamily, it is likely that they interfere with IGF-I through common pathways. For instance, oestrogens have been shown to regulate *IGF-I* gene expression through the transcription factor AP-1, whilst

Table 1
Rationale for the use of circulating IGF-I as a surrogate biomarker for breast cancer

1.	Mitogenic; anti-apoptotic; tumorigenic in experimental systems.
2.	Stimulates normal mammary epithelial proliferation in primates.
3.	Mediates oestrogen effects in breast cancer cells.
4.	Activates ER pathway in the absence of E2.
5.	Has a prognostic effect in breast cancer tissue.
6.	Modulated by tamoxifen *in vitro* and *in vivo*.
7.	Reflects preventive activity.
8.	Predicts premenopausal breast cancer risk.

ER, oestrogen receptor.

retinoids can negatively regulate AP-1-responsive genes. Moreover, the hormonal regulation of IGF-I synthesis appears to be complex with oestrogens acting as dose-dependent stimulatory or inhibitory agents [21]. Specifically, physiological concentrations such as those achieved through transdermal hormone replacement therapy (HRT) tend to increase IGF-I levels, whilst pharmacological doses such as those achieved in the liver circulation after oral administration induce a decline of IGF-I levels.

6. Mammographic density

There is a consistent line of evidence that a higher mammographic density is associated with an increased risk of breast cancer (>50% relative risk (RR) = 4–5 compared with women with lucent mammograms). There is a high degree of consistency amongst epidemiological studies that the higher category of breast density (>75%) has an approximately 5-fold increased risk of breast cancer compared with lucent mammograms (no density). This conclusion is mainly based on three prospective nested case–control studies (Table 2) [22–24]. Thus, in contrast to the conflicting results provided by the qualitative classification of Wolfe, a quantitative assessment (by visual inspection) of the percentage of breast density appears to be uniformly associated with an increased risk of breast cancer. Moreover, at variance with most other risk determinants, this factor may potentially be modified by some forms of intervention [25,26] providing the endpoint for new preventive interventions.

Dense breasts at mammography are associated with a higher incidence of early preneoplastic lesions (e.g. atypical hyperplasia and DCIS) and are characterised by a predominant stromal component, supporting the contention that stromal-epithelial interactions play a significant role in breast carcinogenesis. The use of mammographic density >50% as an entry criterion for a chemoprevention trial can select a population with an incidence of breast cancer which is 4–5 times higher than the age-standardised incidence rate in that age range [27]. Whilst visual (manual) quantification of density can readily be applied as an entry criterion for defining at-risk subjects, quantitative measurement of density by instrumental or computerised methods can more reliably be applied to assess variations during intervention [22,23].

Table 2
Effect of per cent mammographic density on breast cancer risk in prospective studies

Author [ref.]	*n* cases	*n* control	% density	Adjusted odds ratio[a]	95% CI
Boyd [22]	66	31	>75	6.1	2.8–13
Byrne [23]	194	136	>75	4.4	3.1–6.1
	576	554	50–74	2.8	2.1–3.6
Kato [18]	37	99 (pre)	>66	3.6	1.7–7.9
	48	81 (post)	>66	2.1	1.1–3.8

CI, confidence interval.
[a] Odds ratio adjusted compared with controls group.

Results from a randomised trial of low dietary fat intervention have shown that density can be modulated, particularly in the perimenopausal group [26]. Tamoxifen has also been shown to be associated with a reduction of mammographic density in a recent pilot study [25]. By contrast, HRT can increase mammographic density by 17 to 73% according to different methods and studies. Thus, there is evidence for a hormonal regulation of mammographic density as well as for a modulation by active agents which can affect breast cancer risk, indicating that this factor may be another suitable surrogate endpoint for breast cancer in chemoprevention trials.

7. Chemoprevention strategies for specific cancers

7.1. Breast cancer

7.1.1. Tamoxifen studies

Tamoxifen is a non-steroidal triphenylethylene derivative which can be classified as a first generation selective oestrogen receptor modulator (SERM). Tamoxifen is widely used for palliative endocrine treatment of advanced breast cancer and as adjuvant therapy to control micrometastatic relapse and new primaries in women treated surgically for early breast cancer. It has been investigated in three large cooperative phase III trials for prevention of breast cancer in at-risk women. The results of two of these studies, the Royal Marsden Tamoxifen Chemoprevention Trial and the Italian Tamoxifen Prevention Study have recently been published in a preliminary form [28,29] and the third, the National Surgical Adjuvant Breast and Bowel Project P-1 (NSABP P-1) has been reported in full [30]. These studies are summarised in Table 3. Besides breast cancer chemoprevention, these trials are investigating other possible benefits of tamoxifen suggested by previous clinical trials of adjuvant therapy, namely decreased cardiovascular morbidity and mortality, as well as prevention of osteoporosis in postmenopausal women.

The NSABP P1 study, started in 1992, recruited 13 388 women who were at risk for breast cancer (i.e. were over 60 years old; were aged 35–59 years with an increased risk for breast cancer (using the Gail algorithm) higher than 1.66% in 5 years or had a history of lobular carcinoma *in situ*, who were randomised to tamoxifen or placebo. This trial gave such positive results that an interim analysis led to the early closure of the study.

Table 3
Summary of breast cancer, endometrial cancer, and thromboembolic disease in randomised trials of tamoxifen

	Breast Cancer Prevention Trial (BCPT) $n = 13\,388$	Royal Marsden Hospital Tamoxifen Chemoprevention Trial $n = 2494$	Italian Tamoxifen Prevention Trial $n = 5408$
Subject characteristics	High breast cancer risk (age $\geqslant 60$ years or a combination of risk factors)	Family history of breast cancer. Age 50 years or in $\geqslant 2$ relatives Median age 47 years (range 30–70)	Women who have undergone hysterectomy (48% bilateral oophorectomy) Median age 51 years (range 35–70)
Median follow-up	54.6 months	70 months	46 months
Breast cancer rate/ 1000 woman-years RR (95% confidence interval)	Placebo 6.8 Tamoxifen 3.4 RR = 0.51 (0.39–0.66)	Placebo 5.0 Tamoxifen 4.7 RR = 1.1 (0.7–1.7)	Placebo 2.3 Tamoxifen 2.1
Number and relative risk of ER+ breast cancer	Placebo 130 Tamoxifen 41 RR = 0.31 (0.22–0.45)	Not available	Placebo 10 Tamoxifen 8
Number and relative risk of endometrial cancer	Placebo 15 Tamoxifen 36 RR = 2.53 (1.35 to 4.97)	Placebo 1 Tamoxifen 4	Not applicable
Number and rates of pulmonary emboli and venous thrombosis	Pulmonary emboli (PE) Placebo 6 Treatment 18 RR = 3.0 (1.2–9.3) Venous thrombosis (DVT) Placebo 22 Treatment 35 RR = 1.60 (0.91–2.86)	DVT and PE Placebo 4 Tamoxifen 7	DVT and PE Placebo 4 Treatment 7 Superficial phlebitis Placebo 9 Tamoxifen 33

RR, relative risk; ER, oestrogen receptor; DVT, deep verious thromboembolism.

It was shown that 20 mg/day of tamoxifen reduced the risk of invasive breast cancer by 49% (two-sided $P < 0.00001$), with a cumulative incidence through 69 months of follow-up of 43.4/1000 in women in the placebo group and 22/1000 women in the treatment arm. The decreased risk occurred in women of all age groups; aged 49 years or younger (44%), 50–59 years (51%) and 60 years or older (55%). Risk was also found to be reduced in women who had a history of lobular carcinoma *in situ* (56%), or atypical hyperplasia (86%) and those with any category of predicted 5-year risk. Tamoxifen reduced the risk of non-invasive breast cancer by 50% (two-sided $P < 0.002$) and the occurrence of oestrogen-positive tumours by 69%; it had no effect on oestrogen-negative tumours. Tamoxifen did not alter the rate of ischaemic heart disease, but did produce an overall 20% reduction in the incidence of osteoporitic bone fracture of the hip, radius (Colles') and spine. Compared with the placebo group, however, women aged 50 years or older receiving tamoxifen had a 4-fold increased risk of early stage endometrial cancer, a 3-fold increased risk of pulmonary embolism and a significant excess of cataracts [30]. Notably, however, women aged 50 years or younger had no increased incidence of adverse events. The decision of stopping this trial was criticised by those who argued that the long-term effects of tamoxifen on the incidence of breast cancer and mortality will not be known. In other words, it will not be possible to distinguish a delayed incidence (therapeutic effect on pre- or early malignant cells) from a true disease eradication (prevention or reversal of initiation and promotion). This argument does not take into account: (1) the trial was not powered for mortality; (2) the informed consent included a statement on interim analyses and premature termination in case of striking differences; (3) the participants' safety is a greater priority than scientific advance.

Based on these findings, the Food and Drug Administration (FDA) has approved the use of tamoxifen to reduce the risk of breast cancer in subjects at increased risk as assessed by the Gail model. This provides the first example of a medication approved and marketed as a cancer preventive agent, a concept which is likely to be expanded in clinical practice in the near future.

However, the striking benefits of the NSABP P1 trial do not seem to be confirmed by the European trials [28,29]. The Italian study was a multi-centre, double-blind, placebo-controlled chemoprevention trial, initiated in October 1992, to evaluate the effect of a daily dose of 20 mg p.o. tamoxifen for 5 years, on the prevention of breast cancer in healthy women [29]. Eligible subjects were well women aged 35–70 years old who had

prior hysterectomy for non-malignant conditions and the primary endpoint of this study is the incidence of breast cancer. Recruitment was stopped on 31 December 1997 with 5408 women randomised. Using the definition of drop-out as the number of discontinuations other than major events (including women lost to follow-up) divided by the total number of women included in the analysis [31] the drop-out rate for the Royal Marsden study was 35.5%, for the NSABP P-1 study 28.8%, and for the Italian study 20.7%. In the Italian study most women left for voluntary reasons other than side-effects (1.5 per 100 versus 0.5 per 100 who did leave because of side-effects). Moreover, several factors external to the study contributed to the high rate of withdrawal, including bad publicity in the media after the inclusion of tamoxifen in the list of class A carcinogens by the International Agency on Cancer Research (IARC) in 1996. Because this affected the rate of accrual, the Data Monitoring Committee advised that recruitment be stopped before the planned date. However, these data, rather than detracting from the findings, should underline the fact that maintaining compliance in the long term is a formidable task even in an apparently highly motivated group and should be borne in mind when planning studies. Increasing public knowledge and appreciation of the value of chemoprevention trials can only help.

The preliminary results of the Italian study after a median of 46 months (range 0–60 months) show no difference in the incidence of breast cancer between the two arms [29]. Of the 41 cases of breast cancer that have occurred so far, 22 cases were in the placebo group and 19 cases in the tamoxifen group. Amongst women on intervention from more than 1 year, there was a trend to a beneficial effect of tamoxifen (11 in the tamoxifen arm versus 19 in the placebo arm, $P=0.16$). A borderline significant reduction of breast cancer was observed amongst women who were HRT users and received tamoxifen. Compared with the 8 cases of breast cancer occurring amongst the 390 HRT users who were on placebo, there was 1 case of breast cancer amongst the 362 HRT users who were receiving tamoxifen (RR = 0.13, 95% CI, 0.02–1.02). There was an increased risk of venous vascular events (38 women on tamoxifen versus 18 women on placebo, $P=0.0053$), mainly consisting of superficial phlebitis, and 15 versus 2 cases of severe hypertriglyceridaemia in the tamoxifen and placebo arms, respectively ($P=0.0013$).

As the combination of tamoxifen and transdermal HRT might reduce the risks and side-effects of either agent, their combined effect on several cardiovascular risk factors, including blood cholesterol levels, was tested within the trial [32]. Compared with small changes in the placebo group, tamoxifen was associated with changes in total low density lipoprotein (LDL) and high density lipoprotein (HDL) cholesterol of −9, −14 and −0.8%, respectively, which were similar in continuous HRT users and never HRT users. In contrast, the decrease induced by tamoxifen of total and LDL cholesterol was blunted by two-thirds in women who started HRT whilst on tamoxifen. Thus, the beneficial effects of tamoxifen on cardiovascular risk factors are unchanged in current HRT users, whilst they might be attenuated in women who start transdermal HRT whilst on tamoxifen. Whilst tamoxifen can reduce the risk of breast cancer associated with HRT use, HRT could reduce tamoxifen's adverse events (i.e. vasomotor and urogenital symptoms and, possibly, endometrial cancer). These findings provide the background for future investigations of the combination of tamoxifen and HRT in order to reduce the risks whilst retaining the benefit of both agents.

An interim analysis of the UK pilot prevention trial has also been published [28]. In this study, 2494 healthy women, aged between 30 and 70 years, at increased risk of breast cancer because of family history were accrued between 1986 and 1996. They were randomised in a double-blind fashion to receive tamoxifen 20 mg/day or placebo for up to 8 years. The primary endpoint was the occurrence of breast cancer. After a median follow-up of 70 months, the results demonstrate the same overall frequency of breast cancer in both arms (tamoxifen 34, placebo 36, RR 1.1 (95% CI 0.7–1.7), $P=0.8$). Interestingly, women who were already on HRT (mostly by oral route) when they entered the trial showed an increased risk of breast cancer compared with non-users, whilst the subjects who started HRT whilst on trial had a significantly reduced risk.

Comparison of the preliminary results amongst the three studies [28–30] might seem to suggest that the efficacy of tamoxifen varies depending upon the type of population and the nature of the risk, for whilst the NSABP P-1 trial recruited women with a combination of genetic and reproductive risk factors for developing breast cancer, the UK study concentrated on those with a family history of breast cancer, and the Italian study enrolled women who had undergone a hysterectomy who would presumably be at a lower risk. The different subject characteristics and the limited statistical power of the European studies makes comparison among the 3 studies inappropriate.

In general, tamoxifen is well-tolerated. Hot flushes and other vaginal symptoms are the most commonly reported side-effects; approximately 15–20% more women receiving tamoxifen develop hot flushes attributable to the drug, compared with placebo. These symptoms appear more commonly amongst younger women despite the elevated levels of oestradiol and total oestrogens reported amongst premenopausal patients receiving tamoxifen [33]. The other most frequently reported side-effects (15–20%) are vaginal discharge and dryness, urinary disturbances secondary to urogenital atrophy, nausea, gastrointestinal disturbances, rapid

pulse and weight gain. Menstrual irregularities have also been observed. Antithrombin III activity is decreased in postmenopausal patients, and this may in part account for the increased risk of venous thrombo-embolic events that has been reported in two prevention trials [29,30]. Thus, tamoxifen was reported to increase the incidence of deep vein thrombosis (DVT) and pulmonary emboli (PE) in all three trials (Table 3), but it must be remembered that these side-effects are common to all types of hormonal manipulations (including HRT, the pill and other SERMs). Ocular effects (retinal deposits, keratinopathy and cataracts) have occurred at high doses; however, some recent reports suggest a lower incidence of such disorders at the chemopreventive trial dose of 20 mg/day [34]. As regards the increased risk of endometrial cancer associated with tamoxifen administration, the NSABP P1 trial [30] has shown a 4-fold increased risk of early-stage endometrial cancer in postmenopausal women in the tamoxifen group compared with the placebo group (36 versus 15, respectively). However, all cases except one in the placebo group were at an initial stage (FIGO stage I) and no death from endometrial cancer has been reported in the tamoxifen arm.

These considerations led to a study of the biological activity of tamoxifen with a view to establishing a dosing schedule with a better risk: benefit ratio [35]. The results indicated that a dose of 10 mg on alternate days, i.e. a 75% reduction in the conventional dose of tamoxifen, does not affect the activity of the drug on a large number of biomarkers, including several surrogate markers of cardiovascular disease and circulating IGF-I. Future trials are thus warranted to assess the efficacy and the safety of tamoxifen at low doses. Finally, comparison of low-dose tamoxifen with novel SERMs with potentially improved safety profiles could provide important clues for the choice of safe and effective preventive approaches for a wide range of oestrogen-related diseases.

7.1.2. Raloxifene studies

Raloxifene is another SERM that binds with high affinity and has agonist actions on oestrogen receptors in bone and those affecting lipid production, but has antagonistic actions on receptors in breast and uterus. It has been demonstrated to maintain bone density and is used as an osteoporosis preventive agent and to lower LDL cholesterol in postmenopausal women without affecting the cholesterol [36]. It is potentially less hazardous than tamoxifen, since it has not been shown to induce endometrial cancer. However, it still does produce blood clots, and its long-term efficacy and safety profile is still unknown.

Raloxifene is currently undergoing investigation as a chemopreventive agent in breast cancer. A recent report of interim findings from the osteoporosis trials [37] showed that the incidence of newly diagnosed breast cancer was 1.7 per 1000 patient-years for subjects receiving raloxifene, compared with 3.7 per 1000 patient-years for those on placebo, giving a relative risk of 0.46 (CI = 0.28–0.75) — corresponding to a 54% reduction in incidence. Raloxifene had a marked effect on ER-positive tumours, reducing the incidence by 70% (RR 0.30; CI 0.24–0.64) with no effect on the incidence of ER-negative tumours. The final analysis of these data is awaited with interest. Raloxifene will be evaluated in comparison with tamoxifen in a large primary prevention trial in at-risk subjects in the USA. Other SERMs are also entering the field of clinical cancer prevention and a significant array of agents is likely to be tested in the next few years.

7.1.3. The synthetic retinoid (N-(4-hydroxyphenyl) retinamide or fenretinide)

Natural retinoids play a crucial role in cellular proliferation and differentiation, but their poor clinical tolerability has prevented the use of these compounds as cancer preventive agents. Toxic symptoms which may be acceptable in treating established cancer are not considered acceptable for reducing cancer risk. One of the less toxic vitamin A analogues studied for breast cancer chemoprevention is fenretinide, a synthetic amide derivative of all-*trans* retinoic acid [38]. The inhibition of chemically induced mammary carcinoma in rats by fenretinide was first described in 1979 [39]. On this basis, fenretinide was proposed for chemoprevention trials in human breast cancer. This compound has been studied extensively and proved to be less toxic than many other retinoids [38].

In contrast to retinoic acid, it selectively induces apoptosis rather than differentiation in several tumour cell systems and maintains a stable plasma concentration during prolonged administration. Whilst its mechanism of action still remains unclear, recent studies indicate that may be a selective retinoid receptor modulator which retains the inhibitory activity of retinoic acid on proliferative signals with an improved therapeutic index, an important limiting factor for other retinoids [39]. This selective binding to the nuclear receptors is likely to be the basis for its specific biological activities and its favourable pharmaceutical properties. Moreover, fenretinide appears to be a potent inhibitor of the IGF system in breast cancer cell lines and this is an important mechanism of tumour cell growth inhibition by the retinoid [40].

In recent years, fenretinide has been shown to be active *in vitro* and *in vivo* against mammary, bladder, lung, ovary, cervix, neuroblastoma, leukaemia and prostate preclinical models [41]. A characteristic feature of fenretinide is the ability to inhibit cell growth through the induction of apoptosis rather than differentiation, an effect which is strikingly different from the parent

compound all-*trans* retinoic acid and which may occur even in retinoic acid-resistant cell lines. On the basis of the selective accumulation of fenretinide in the human breast [42,43] and the good tolerability in humans [38], a phase III trial was started in 1987 aimed at reducing contralateral breast cancer. Briefly, 2972 women with a history of stage I breast cancer were randomised to fenretinide 200 mg/day or no intervention for 5 years. The primary endpoint of the study was the occurrence of contralateral breast cancer as the first malignant event. The eligible women were diagnosed with stage I invasive breast cancer or DCIS within the previous 10 years and have undergone definitive surgery without adjuvant hormone or chemotherapy.

The analysis after a median of 8 years has shown that the number of cases of contralateral breast cancer was comparable in the two arms [43]. However, there was a beneficial trend in premenopausal women (fenretinide, 27 cases; control, 42 cases; adjusted hazard ratio = 0.66, 95% CI, 0.41–1.07) and a lack of effect in postmenopausal women (fenretinide, 38 cases; control, 29 cases; adjusted hazard ratio = 1.32, 95% CI, 0.82–2.15). A test for interaction showed that the effect of fenretinide is significantly modified by menopausal status. Likewise, fenretinide significantly reduced by 35% the rate of local recurrence in premenopausal women but not in postmenopausal women (P value for the interaction between fenretidine and menopausal status is < 0.05). Interestingly, modulation of plasma IGF-I levels by fenretinide followed a similar pattern, i.e. IGF-I levels were lowered in premenopausal women only [21,22].

7.1.4. The combination of tamoxifen and fenretinide

The concept of combining multiple agents with different activities to enhance activity and minimise toxicity has been pursued for quite some time in cancer chemoprevention research [44]. For instance, synergistic efficacy has been observed *in vivo* in animal studies of tamoxifen in combination with fenretinide at lower, less toxic, doses [45]. A phase I trial of 20 mg tamoxifen per day in combination with increased doses of fenretinide has recently been performed [46]. In a recent pilot study in at-risk women, the combination of tamoxifen 20 mg per day and fenretinide 200 mg per day was also well tolerated [47]. Based on the encouraging results of previous clinical trials of tamoxifen and fenretinide in premenopausal women [30,43], a trial of a combination of low-dose tamoxifen and fenretinide in premenopausal women at increased risk for breast cancer is currently underway.

7.2. Colorectal cancer

7.2.1. Activity of NSAIDs in colon cancer prevention

Several recent studies have reported a 40–50% decrease in the relative risk of colorectal cancer in persons who are continuous users of aspirin or other non-steroidal anti-inflammatory drugs (NSAIDs) [48], suggesting that these drugs may serve as effective cancer chemopreventive agents. However, prolonged use of NSAIDs results in untoward gastrointestinal side-effects that are likely due to the inhibition of the production of gastric prostaglandins, that play a crucial role in maintaining gastric mucosal integrity.

The azoxymethane-treated rat and *Min*/*APC* mice are used as complementary animal models of colorectal carcinogenesis. Several NSAIDs — piroxicam, sulindac, aspirin, ibuprofen and others — have clear efficacy in these models resulting in 30–80% reductions in the multiplicity and incidence of adenomas and cancer [49]. Though the protective effect appears class-related, there are reproducible differences in efficacy within the class. Whether these differences are relevant to humans is unknown. At a macroscopic level, NSAIDs prevent incident neoplasia (adenomas and carcinomas), regress adenomas, and suppress the growth of carcinomas. Therefore, NSAIDs are effective when given 'early' (preceding adenoma formation), as well as 'late' (following the emergence of adenomas). On a cellular level, NSAIDs appear to increase the apoptotic index relative to proliferation in neoplastic cells, although the molecular mechanisms responsible for this effect remain poorly defined.

More than 15 observational studies, using case–control, nested case–control and prospective designs, have investigated the relationship between aspirin/NSAIDs and colorectal neoplasia. All but one suggest that NSAIDs are effective in lowering the incidence of human colorectal adenoma, reducing carcinoma incidence and/or cancer-associated mortality, regardless of age, gender, country of residence, affected colorectal segment or other underlying risk factors (e.g. diet, history of prior adenomas or socio-economic status). Potential confounding influences on aspirin's preventive efficacy have also been evaluated, such as a delay in cancer diagnoses by pain suppression and/or early detection by aspirin-induced GI bleeding. These factors are considered extremely unlikely to account for the protective association. The preventive effects of aspirin may require extended periods of exposure; some investigations reported efficacy only with aspirin use of more than 10 years duration.

Since 1983, several non-randomised case series and three small randomised trials have reported the experiences of subjects with FAP ($n = 150$) treated with sulindac or indomethacin [50]. From these reports, it is clear that NSAIDs induce the regression of prevalent adenomas in this cohort, though complete regression of all adenomas is unlikely. The effect takes several months of exposure and requires continued administration. Three case reports of cancer occurring in FAP subjects taking sulindac suggest that a more effective chemopreventive

agent, or combination of agents, may be necessary for optimal chemoprevention in this condition.

Despite this impressive body of evidence, NSAIDs are not prescribed for colorectal cancer prevention because of several key deficits in our knowledge. We do not know which specific agent(s) is most effective and, more importantly, what dose or duration of treatment should be prescribed. In addition, traditional NSAIDs are associated with several well-known risks, including gastric irritation/ulceration, renal dysfunction, anti-aggregatory effects in platelets and liver dysfunction. Balancing the risks versus benefits of NSAIDs is a challenge in the preventive setting. Insights into the action of NSAIDs on their target enzyme, cyclo-oxygenase (COX) may hold clues to solving this dilemma.

7.2.2. Role of cyclo-oxygenase-2 (COX-2) and prostanoids in colon carcinogenesis

COX catalyses the oxygenation of arachidonic acid to prostaglandin (PG) H2 as the first step in the synthesis of PGs, prostacyclins and thromboxanes in mammalian cells. Two forms of the COX enzyme have been demonstrated. COX-1 is the major enzyme form found in healthy tissues; it is constitutively expressed and plays a role in thrombogenesis and in the homeostasis of the gastrointestinal tract and kidneys. COX-2 is a distinct isoform of COX-1 encoded by a different gene. It is inducible and upregulated in pathological states, including inflammation and neoplasia, and has been associated with the elevated production of PGs observed during inflammation, pain and pyretic responses [51,52]. *COX-2* gene belongs to a class of genes referred to as immediate early or early growth response genes which are expressed rapidly and transiently after stimulation of cultured cells by growth factors, cytokines and tumour promoters; COX-2 expression is thus elevated in inflammatory cells and sites of inflammation. Most NSAIDs in current use inhibit both COX-1 and COX-2.

Previously, an increased COX-2 expression has been demonstrated in human colorectal adenocarcinomas when compared with normal adjacent colonic mucosa [53]; these findings have been confirmed by the finding of elevated levels of COX-2 protein in colorectal tumours by Western blotting and immunohistochemical staining. Markedly elevated levels of COX-2 mRNA and protein have also been observed in colonic tumours that develop in rodents after carcinogen treatment and in adenomas taken from Min mice. The observations of elevated COX-2 expression in three different models of colorectal carcinogenesis have led to the hypothesis that COX-2 expression may be related to colorectal tumorigenesis in a causal way. A recent report has demonstrated a 40% reduction in aberrant crypt formation in carcinogen-treated rats given a selective COX-2 inhibitor [54]. Another study has provided genetic evidence which directly links COX-2 expression to intestinal tumorigenesis. This recent report [55] demonstrated that *APC* mice develop hundreds of tumours per intestine. When these mice were bred with *COX-2* null mice there was an 80–90% reduction in tumour multiplicity in the homozygous *COX-2* null offspring. Additionally, when the *APC* mice were treated with a highly selected COX-2 inhibitor, there was a marked reduction in tumour multiplicity. These results suggest that COX-2 may act as a tumour promoter in the intestine and that increased levels of COX-2 expression may result directly from disruption of the *APC* gene.

A further piece of compelling evidence for a role of COX-2 and prostanoids in colorectal cancer carcinogenesis, comes from a study by Yang and colleagues [56]. This study compared the levels of five major stable metabolic products of the COX pathway in normal-appearing mucosa and in adenomas of patients with FAP to determine whether prostanoids are involved in the pathogenesis of colorectal adenomas. Of 12 patients tested, 6 had elevated levels of at least one prostanoid in the adenomas. More importantly, the relative levels of three prostanoids (PG D2, PGE2, and 6-keto-PGF1α) were elevated in adenomas compared with normal-appearing mucosa from the same patients, and the resulting ratios were correlated with the size of the adenoma. This elevation, however, was not observed until an adenoma reached an approximate size of 6–7 mm in diameter.

These observations support the hypothesis that increased COX-2 expression, with the resultant elevation of prostanoid levels in adenomas, provides a potential mechanism for tumour promotion. If this is the case, selective inhibition of COX-2 could be chemopreventive. Several selective COX-2 inhibitors have recently been developed which are under evaluation both as alternatives to conventional NSAIDs in inflammatory conditions and as chemopreventive agents, these include celecoxib, nimesulide and others [57].

7.2.3. Calcium as a chemopreventive agent in colorectal cancer

The fact that diet can influence the risk of colorectal cancer has been known for some time. There is a protective effect of fresh fruits and vegetables, whilst animal fats and red meat seem to increase risk [58]. It has been hypothesised that the carcinogenic activity of fat and meat derives from their stimulation of the production of bile acids, which are known carcinogens in animals. Calcium can bind bile acids and dietary calcium has been shown to be protective against bile-induced mucosal damage in experimental models. To assess these observations in humans, a randomised, double-blind trial of the effect of dietary supplementation with calcium carbonate was undertaken in 930 subjects with a recent history of colorectal adenomas [59]. Follow-up

endoscopies were conducted 1 and 4 years after the qualifying examination and the primary endpoint was the proportion of subjects in whom at least one adenoma was detected. The calcium carbonate group had a lower risk of recurrent adenoma. The adjusted risk ratio for any recurrence of adenoma with calcium compared with placebo was 0.85 (95% CI 0.74–0.98; $P=0.03$). At least one adenoma was diagnosed in 127 (31%) subjects in the calcium group and 159 (38%) of subjects in the placebo group. The adjusted risk ratio was 0.81 (95% CI 0.67–0.99). Thus these results show a significant, albeit modest, influence of calcium in reducing the risk of recurrent adenomas.

7.2.4. α-difluoromethylornithine (DFMO)

DFMO is an irreversible inhibitor of the enzyme ornithine decarboxylase (ODC) [60], the first and controlling enzyme in the biosynthetic pathway of mammalian polyamines. ODC shows rapid turnover, and converts ornithine to putrescine, which is in turn converted to spermidine and spermine. Polyamines are ubiquitously distributed throughout prokaryotic and eukaryotic organisms and affect a number of cellular processes. Depletion of polyamines results in a slowing of cellular growth and inhibition of carcinogenesis in essentially all models studied, and profound depletion of polyamines causes cell death [61]. Thus, inhibition of ODC is a potential site for cancer chemoprevention.

The effect of DFMO on ODC levels in rectal, rectosigmoidal and caecal colonic mucosae of 45 individuals at risk for colon cancer was studied [62,63]. Significant decreases in putrescine and spermidine, the products of the action of ODC on ornithine, were found in the rectosigmoidal colonic mucosae of subjects at 3 months ($P=0.03$ and $P=0.04$) and 12 months ($P=0.005$ and $P=0.004$). Three DFMO subjects developed ototoxicity, a known side-effect of DFMO. The authors conclude that further investigations of DFMO schedules will be a necessary step before phase III chemoprevention studies can be instigated.

Besides these promising studies, there have also been negative trials in the prevention of large bowel adenoma [64,65]. Greenberg and colleagues randomly assigned 864 patients to four groups, beta-carotene, vitamin C, vitamin E or a combination of beta-carotene plus vitamins C and E. Despite good compliance over four years there was no evidence that either beta-carotene or the vitamins reduced the incidence of adenomas.

Another smaller study giving beta-carotene alone or in addition to reduced dietary fat intake and supplementation with wheat bran in people with colorectal adenoma also failed to demonstrate an effect of beta-carotene. These data did suggest that the combined effect of low fat and wheat bran might reduce the transition from small to large adenomas, but patient numbers were too small for firm conclusions to be drawn [65].

7.3. Lung Cancer

Two major trials have been directed at the prevention of lung cancer by the chemopreventative agent beta-carotene in well nourished individuals: the ATBC cancer prevention study [8] and the beta-carotene and retinol efficacy trial (CARET) [66]. Previous epidemiological evidence had suggested that higher intake of vitamin E (alpha-tocopherol) and beta-carotene (a precursor of vitamin A) was linked to a lower incidence of cancer, especially lung cancer and cardiovascular disease. Basic research suggested plausible mechanisms by which this could occur, providing the impetus for randomised chemoprevention trials. The ATBC Study assigned 29 000 male Finnish smokers (aged 50–69 years) to receive beta-carotene (20 mg/day), vitamin E (50 mg/day), a combination of both or placebo for an average of 6 years. The CARET study enrolled 18 000 men and women at high risk of lung cancer because of a history of cigarette smoking or occupational exposure to asbestos, and gave beta-carotene (30 mg/day) in combination with retinol (25 000 IU/day) for an average of less than 4 years.

Both studies showed that beta-carotene increased the risk of developing lung cancer in groups exposed to smoking or asbestos. Although the mechanism of this effect was not clear, it has been suggested that it is related to the reported pro-oxidant effect of beta-carotene under high oxygen pressures and oxidative stress that occurs in the lungs of smokers [67]. The detrimental effect of beta-carotene appeared stronger (but not substantially different) in patients who smoked at least 20 cigarettes a day compared with a subset of moderate-intensity smokers (5–19 cigarettes per day) and was exacerbated by alcohol intake in the ATBC trial. No increase in the incidence of cancer due to beta-carotene was seen in the subset of former smokers in the CARET study. There was also no evidence that beta-carotene increases lung cancer risk in never or former smokers.

A further study has investigated the effects of beta-carotene in a mixed population. The Physicians Health Study in the USA [68] randomised 22 071 male physicians to beta-carotene (50 mg) or placebo on alternate days for an average of 12 years. Eleven per cent of the subjects were smokers and 39% were former smokers, thus 50% of the subjects had never smoked. In this broad population, supplementation with beta-carotene produced neither benefit nor harm in terms of the incidence of cancer, cardiovascular disease or death from all causes. Subset analyses of current or former smokers showed no significant differences in any of these endpoints. A reason for the differences between the findings of this trial and the two other major trials are not apparent, but this trial only included 2427 smokers, half of whom would be randomised to placebo, so the lower subject numbers may have some bearing on the findings.

This study also concomitantly carried out a secondary investigation of aspirin as a preventive agent on cardiovascular disease and cancer. The effects on heart disease were so striking that the aspirin intervention was terminated early [69]. However, it was also noted that aspirin failed to affect the incidence of colorectal cancer — a result at variance with epidemiological studies showing a protective effect of aspirin on colorectal cancer.

Another study [70] evaluated the effect of beta-carotene and retinol on sputum atypia and found no effect of treatment. The synthetic retinoid, etretinate, was equally ineffective.

However, some activity for retinol alone has been demonstrated. Retinol palmitate (30 000 IU daily for 12 months) was evaluated in comparison with no treatment in 307 patients with stage I non-small cell lung cancer (NSCLC) who had undergone curative surgery [71]. After a median follow-up of 46 months recurrence of new primary tumours occurred in 37% of the retinol palmitate group and 48% of the controls. Second primary tumours occurred in 18 of the treated group compared with 29 patients in the control group ($P = 0.045$).

These studies highlight the fact that intuitive extrapolation of epidemiological data into chemoprevention trials is not always successful. However, this may be because such observational studies are difficult to interpret, for although the association between beta-carotene and a lower incidence of cancer may be present, how can it be shown whether the results were due to a high intake of beta-carotene itself, other nutrients present in beta-carotene-rich foods, other dietary habits or other non-dietary aspects of lifestyle that accompany high intake of beta-carotene? Moreover, the negative chemoprevention studies do not entirely contradict the epidemiological data. It was noted in both the ATBC and CARET trials that higher baseline plasma beta-carotene levels were associated with lower rates of lung cancer. The consumption of foods rich in beta-carotene (giving rise to these high plasma levels) could be regarded as markers of consumption of a protective diet rich in fruits and vegetables. The question still remains of identifying these protective factors.

7.4. Bronchus and upper airways/head and neck cancer

Second tumours frequently occur in head and neck and lung cancer, thus chemoprevention can make a positive contribution, even to patients who have established disease. 13-*cis*-retinoic acid was, therefore, evaluated for its chemopreventive potential in patients who had been treated for squamous cell carcinoma of the head and neck [72]. In approximately 100 patients, 13-*cis*-retinoic acid given for 12 months produced a significant ($P = 0.005$) reduction in the number of second primary tumours (2 (4%) in the 49 patients treated with 13-*cis*-retinoic acid; 12 of 51 (24%) of patients treated with placebo). An update of this trial after a median of 54.5 months [72] showed that after completion of treatment the difference in second primary tumours between the groups diminished. However, only 3 (6%) subjects in the 13-*cis*-retinoic acid-treated group compared with 13 (25%) in the placebo-treated group, have developed second primary tumours in the upper aero-digestive tract or lungs. These encouraging results have formed the basis for two larger studies in the USA that are ongoing.

In Europe, the EUROSCAN study is a large-scale clinical trial, established in 1988, to assess the effects of retinyl palmitate and N-acetylcysteine (NAC) in patients with early stage head and neck cancer or lung cancer. Patients refractory to treatment with curative intent were randomised to retinyl palmitate, NAC, a combination of both agents or no intervention. This study is ongoing. To date 35% of patients have reported an event but there do not seem to be, as yet, positive results for either agent [74].

One negative trial in bronchial squamous cell neoplasia has been reported, where cell metaplasia was unaffected by 13-*cis*-retinoic acid [75].

7.5. Skin cancer

Two well designed chemoprevention trials have evaluated the preventative effects of selenium or retinol on skin cancer [9,76].

The study of selenium [9] gave definite negative results for the prevention of squamous cell carcinoma or basal cell carcinoma in 1312 patients with a history of non-melanoma skin cancer and living in regions of the USA where the intake of selenium was naturally low. However, this study did show as a *post-hoc* observation a statistically significant effect of selenium in preventing prostate, lung and colon cancers. The incidence of cancers were: prostate (selenium, $n = 13$; placebo, $n = 35$, RR = 0.37, 95% CI, 0.18–0.71, $P = 0.002$), lung cancer (selenium, $n = 17$; placebo, $n = 31$, RR = 0.54, 95% CI, 0.30–0.98, $P = 0.04$), colorectal cancer (selenium, $n = 8$; placebo, $n = 19$, RR = 0.42, 95% CI, 0.18–0.95, $P = 0.03$). Also the trial produced a statistically non-significant reduction in total mortality and a statistically significant reduction in total cancer mortality. Whilst derived from secondary analyses, these findings warrant further study of selenium as a chemopreventive agent.

A similar study assessed the value of oral retinol (25 000 IU) or placebo daily for 5 years in individuals who had a history of more than 10 actinic keratoses and at most two squamous cell or basal cell carcinomas [76]. A total of 2297 subjects were enrolled and followed for a median period of 3.8 years. The study showed that retinol was effective in preventing squamous cell carcinoma but not basal cell carcinoma.

Three negative studies in skin cancer have been reported [64,77,78]. Retinol and 13-*cis*-retinoic acid

were investigated in patients with at least four basal cell carcinomas and/or squamous cell carcinomas of the skin. No beneficial effects were noted with regard to the prevention of non-melanoma skin cancer with either retinol or 13-*cis*-retinoic acid [77].

In patients with previous non-melanoma skin cancer, treatment with beta-carotene did not reduce the incidence of new skin cancers over a 5-year period of treatment and observation [64].

Low-dose 13-*cis*-retinoic acid was not only ineffective after 3 years in reducing the occurrence of basal cell carcinoma at new sites in 981 patients with two or more previously treated basal cell carcinomas but was also associated with significantly more adverse events of elevated serum triglycerides, hyperostotic axial skeletal changes and mucotaneous reactions [78].

Acitretin has been shown to reduce the incidence of squamous cell carcinoma in renal transplant patients [79]. 44 patients with >10 keratotic skin lesions were randomised to placebo or acitretin (30 mg/day) for 6 months. In this period, 11% of the active treatment group and 47% of the placebos developed new squamous cell carcinomas ($P=0.01$).

7.6. All cancers

A study of nearly 30 000 subjects was carried out in Linxian, China [80]. Using a complex factorial design this study tested four main combined agent interventions of vitamins and minerals (retinol and zinc, riboflavin and niacin, vitamin C, molybdenum and beta-carotene, vitamin E and selenium) over a 5-year period in this population who have a persistently low intake of several micronutrients. No statistically significant effect on cancer incidence was achieved by any intervention. Important secondary analyses however, showed that the combination of selenium, beta-carotene and alpha-tocopherol was associated with a statistically significant lower total mortality rate (RR = 0.91; 95% CI = 0.84–0.99), a 13% reduction (borderline significant) in total cancer mortality rate (RR = 0.87; 95% CI = 0.75–1.00) and a statistically significantly lower mortality rate from stomach cancer (a major cancer in Linxian) (RR 0.79; 95% CI = 0.64–0.99). Although not definitive, these data do suggest that the combination of beta-carotene, vitamin E and selenium may affect the incidence of cancers, especially gastric cancer. It must be remembered though that this population had an initial poor intake of vitamins and minerals. It is possible that the lack of these nutrients confers a risk of developing cancer that can be overcome by increasing the dietary intake. But that is not the same as saying that an additional intake of these vitamins and minerals by already well nourished individuals will confer any active protection.

Several studies have provided evidence that retinoids have a protective function in several different types of cancer, especially in the prevention of second primary tumours. In addition to 13-*cis*-retinoic acid [72], another retinoid, polyprenoic acid, has been demonstrated to be effective in the prevention of second primary hepatomas [81]. In a study of 89 patients who were disease free after resection of a primary hepatoma or percutaneous injection of ethanol, randomly assigned to polyprenoic acid (600 mg/day) or placebo, the incidence of recurrent or new hepatomas was 27% in the polyprenoic acid group compared with 49% in the placebo group ($P=0.04$) after a median follow-up of 38 months. A recent update of the trial has shown a significant advantage in mortality rate of the retinoid arm [82].

Topically applied all-*trans*-retinoic acid has also been shown to increase the histological regression rate in women with moderate cervical intra-epithelial hyperplasia (CIN II) from 27% in the placebo group to 47% in the retinoic acid group ($P=0.041$) [83].

Collectively these studies provide evidence that retinoids may have a role in chemoprevention that warrants further investigation.

8. Future studies

It seems to be a common occurrence that chemoprevention trials that set out to investigate the specific interaction of a putative chemopreventive agent with a particular cancer provide equally useful data concerning other cancer types. Thus, the study evaluating the effect of selenium on the incidence of skin cancer [9] showed positive effects on prostate cancers. Similarly, the ATBC study of lung cancer [14,84] showed a protective role for vitamin E on the incidence and mortality of prostate cancer; 99 cases of prostate cancer occurred in the group receiving 50 mg of vitamin E daily whilst 147 cases were seen in the control arm. The role of these two agents in protecting against prostate cancer is not clear but may be linked to their anti-oxidant role, their inhibition of cell proliferation or promotion of apoptosis. These studies should provide the impetus to get to the 'final chapter in the story' of selenium, vitamin E and prostate cancer [85].

Two ongoing large-scale studies are investigating the prevention of second primary tumours of the head and neck and stage I NSCLC. Both studies are investigating the action of 13-*cis*-retinoic acid [72].

Additionally, an international trial, Euroscan, is evaluating the effect of retinyl palmitate and *n*-acetyl cysteine in more than 2500 patients with early stage cancer of the head and neck or early stage NSCLC [2]. The ongoing Prostate Cancer Prevention Trial is investigating the effect of finasteride, a testosterone analogue that competitively inhibits 5α- reductase, on the incidence of prostate cancer [86].

The challenges of conducting well designed and unequivocal trials in cancer chemoprevention are in

some ways greater than the problems of assessing a new treatment of established disease. Disease prevention rather than palliation is an ambitious goal. However, this promising area of oncology is rapidly expanding and should provide some rewarding results in terms of cancer risk reduction and mechanistic insights into the process of carcinogenesis.

Acknowledgements

This paper is largely based on a chapter prepared for the *Oxford Textbook of Oncology*, second edition, edited by R. Souhami Oxford University Press 2000, and reproduced here with the permission of the authors and publishers. The authors wish to thank Dr Serena Mora for her technical assistance and Dr Jan Hawthorn for reviewing the English style.

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