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REVIEW ARTICLE

Nutritional aspects regarding lung cancer chemoprevention

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Summary

Lung cancer is still one of the major causes of cancer-related deaths and its mortality figures argue powerfully for new approaches to control this leading cancer threat. Chemoprevention can be defined as the use of specific agents to reverse, or prevent premalignancy from progressing to invasive cancer. The use of foods and dietary supplements present a safe chemopreventive strategy.

Data for this review were identified by searches of PubMed and references from relevant articles. Articles were identified by use of the search terms "lung cancer", "chemoprevention", "carcinogenesis", and "retinoids". Only papers published in English were included.

Trials in lung cancer chemoprevention have so far produced either neutral or harmful primary endpoint results, whether in the primary, secondary, or tertiary settings. Lung cancer was not prevented by β-carotene, α-tocopherol, retinol, retinyl palmitate, N-acetylcysteine, or isotretinoin in smokers. Ongoing trials may help define new avenues for chemoprevention.

The concept of chemoprevention in lung cancer is still in its infancy, but in the future it may have a significant impact on the incidence and mortality of lung cancer. In addition to epidemiologic studies, basic science research to detect mechanisms and evaluate the chemopreventive potential of food components is necessary. The overwhelming evidence of a major role of nutrition in carcinogenesis, the many leads that nutritional intervention may reduce cancer incidence, and the growth and increasing sophistication of clinical trials networks point to a very promising future for nutritional intervention trials leading to substantial public benefit.

Key words: carcinogenesis, chemoprevention, diet, lung cancer, nutrition, retinoids

1. Introduction

Lung cancer is a major cause of mortality and morbidity worldwide. Aggressive local control by surgical resection and/or radiation therapy is currently the mainstay of lung cancer therapy for early-stage disease. Systemic chemotherapy has been used in order to prolong symptom-free survival in patients deemed unresectable or with metastatic disease. These interventions have produced slight declines in mortality rates in recent years. Although it appears unlikely that additional marked improvements with these practices alone will occur in the near future [1,2], new molecular targeted agents are currently being studied in all treatment settings including that of chemoprevention.

Individuals at high risk for specific cancers such as non-small cell lung cancer (NSCLC) have, apart from smoking cessation, no options to reduce their steadily increasing risk. The poor lung cancer survival statistics indicate that there is a strong need for additional measures. Progress in chemoprevention is reliant on the collaborative efforts of researchers in basic science and clinical settings, who study the biology of lung cancer with the goal of uncovering new mechanisms of carcinogenesis.

Because of their safety and the belief that they are not "medicine," food-derived products have already been used as chemopreventive agents, in widespread,

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long-standing trials in populations at normal or high risk of developing lung cancer.

This review focuses on several issues related to lung cancer chemoprevention, emphasizing the emerging role of nutrition and diet. Also, the most known chemopreventive agents as well as the results of clinical chemoprevention trials are reviewed.

2. Chemoprevention

Chemoprevention as first described by Sporn in 1976 [3], is defined as the use of specific natural or synthetic chemical agents to reverse, suppress, prevent, or delay a carcinogenic progression to invasive cancer. The two fundamental concepts underlying chemoprevention, common to most epithelial cancers, are field cancerisation and multistep carcinogenesis.

2. a. Field cancerisation and multistep carcinogenesis

According to the multistep carcinogenesis concept, cancer develops in a series of steps, with accumulation of molecular changes progressing through preinvasive histological changes to invasive disease [4]. The earliest events of this process are mutations, deletions, or polysomy at the genomic level. These genetic modifications are not initially translated into cellular morphological changes or tissue structural changes [5]. Additional events are necessary to induce phenotypical, then physiological, modifications in the tissue, such as uncontrolled proliferation, invasion, and metastasis. Studies of the airways of lung cancer patients show that extensive hyperplasia and dysplasia occur throughout the bronchial epithelium, accompanied by aneuploidy. These multiple lesions are not usually genetically distinct from the patient's tumor and presumably arise independently. It has been suggested that multiple (10-20 or more) genetic events are necessary for lung carcinogenesis [6].

On the other hand, field cancerisation is a concept used to explain how diffuse tissue damage, which is been caused by carcinogenic exposure in an area of epithelium or region, such as the lung, leads to multiple lesions appearing in the whole region. Practically it means that an individual with a small dysplastic lesion in his airway has a high risk of developing cancer anywhere in his airway epithelium. Histological changes associated with chronic smoking and cancer include loss of cilia, cellular atypia, reserve cell hyperplasia, squamous metaplasia and dysplasia, and carcinoma *in situ*. Genetic changes and premalignant and malignant lesions in one part of the exposed area imply increased risks of developing cancer in other sites within the area [7]. The essence of chemoprevention is intervention within this multistep carcinogenic process, i.e. treatment or control of precancerous lesions- bronchial dysplasia- may be a way to avoid the development of invasive lung cancer.

By using pharmacologic or natural compounds, chemoprevention aims to block, reverse, or inhibit this process by blocking DNA damage, retarding or reversing malignant phenotype, or inducing apoptosis in the damaged area [4].

2. b. Primary, secondary, and tertiary chemoprevention strategies

Generally chemoprevention can be organized into 3 settings: primary, secondary, and tertiary prevention. Primary prevention is defined as an intervention intended to delay or prevent the development of initial cancer in healthy individuals who are at high risk (e.g. current or former smokers). Examples of this strategy are smoking prevention and cessation treatments.

Secondary prevention aims to prevent development of cancer in individuals with precancerous lesions (e.g. intraepithelial neoplasia, leukoplakia, dysplasia), and tertiary prevention targets patients who have had previous cancers and involves decreasing the morbidity of established disease. Prevention of second primary cancer or reccurence in patients cured of an initial cancer is a good example of tertiary prevention.

3. Diet

Since the publication of 2 reports in the early 1980s [8,9], there has been intense interest in the role of nutrition in the etiology and prevention of cancer. In the past 25 years, there have been hundreds of observational studies of diet and cancer, and the vast majority shows that individuals who consume more fruit and vegetables have lower cancer risk [10,11]. The relationship between diet and lung cancer has been extensively explored in epidemiological studies and there are many leads to support an association between a high intake of fruits and vegetables and a reduced risk of lung cancer [11]. However, eating more fruit and vegetables, like reducing dietary fat, is challenging. Much effort has been made to identify the specific components of these foods that may be responsible for the lower lung cancer risk.

Numerous studies have shown that the incidence of cancer can be inversely related to the intake of many food groups [11-14]. The serum concentra-

tion of many micronutrients is also inversely related to the incidence of lung cancer [15-19]. Based on these epidemiological studies, it has been suggested that micronutrients and macronutrients present in our diet may act as cancer inhibiting substances.

As a society and as physicians, we are far more inclined to prescribe than to proscribe. So, while the evidence supports modification of food patterns in order to lower cancer risk, we remain reductionists, forever seeking out the easiest solution, preferably a magic molecule in a pill. Arguably, because of their safety, food-derived products are highly interesting for development as chemopreventive agents that may find widespread, long-term use in populations at normal risk. Numerous diet-derived agents are included among the more than 40 promising agents and agent combinations that are being evaluated clinically as chemopreventive agents for major cancer targets including lung. Many of these micronutrients have antioxidant capacity including vitamin E, selenium, isothiocyanates, allyl sulphur compounds, green and black tea polyphenols, soy isoflavones, lycopene, perillyl alcohol, vitamin D and calcium [20].

Attention has also been focused on cooking practices. Increased lung cancer risk has been noted as a consequence of high intake of heterocyclic amines, which are produced when meats are cooked at high temperatures [21].

Recent epidemiologic investigations on diet and lung cancer risk point to the importance of including smoking behaviour in the analysis. Ideally, it is important to take also diet-gene interactions into account [22,23]. Studies suggest that low levels of vitamin E can increase the GSTM1 associated risk [24]. Interactions with dietary enzyme factors such as folate and subsequent folate metabolism have also been suggested [25]. Although it is possible that a single polymorphism or dietary interactions may significantly alter the relative risk, it is likely that many interactions, each having a subtle effect, can result in synergistic interactions that greatly affect the overall risk. Determining the risk profile of an individual, based on his inherited polymorphisms and his potential dietary interaction will be a complex undertaking. Testing these hypotheses will require studies with a very large sample size to achieve the statistical power.

4. Chemopreventive agents

Chemopreventive agents' requirements include experimental or epidemiologic data showing chemopreventive efficacy, a mechanistic rationale for the chemopreventive activity observed and safety on chronic administration. Such agents that have been tested in patients for lung cancer prevention include all-trans-retinoic acid (ATRA), 13-cis-retinoic acid (13-cisRA) or 9-cis-retinoic acid (9-cisRA), fenretinide (*N*-[4-hydroxyphenyl] retinamide), beta-carotene, alpha-tocopherol, and selenium.

4. a. Retinoids

Vitamin A and its analogues (retinoids) refer to natural or synthetic compounds that share a similar chemical structure consisting of a cyclic end group, a polyene side chain, and polar end group. They have complex biological effects, including modulation of differentiation, proliferation, apoptosis, and immune status in both normal and neoplastic epithelial cells [26,27]. This complexity is a function of not only the diversity of the retinoid ligands, but also the variety of nuclear receptors that mediate their activity [26,27]. They invert cancerous progression in the airway by complex mechanisms, which depend on the retinoids' capacity to regulate gene expression through nuclear transduction signal modulation mediated by nuclear retinoid receptors.

Two classes of nuclear retinoid receptors are known- retinoic acid receptor (RAR) and retinoid X receptor (RXR). Each receptor contains alpha, beta, and gamma subtypes, and several of these subclasses have multiple isoforms produced through differential promoter usage and alternative splicing of receptor transcripts [27-29]. These receptors act as ligand-activated transcription factors; this means that they are ligand activated and following the binding to retinoids, the retinoid target genes become transcriptionally activated or repressed. The target genes regulate cell growth, differentiation and death (apoptosis) [30].

RXRs are active only as heterodimers or homodimers. RXRs can form homodimers or heterodimers by binding with RARs or a host of other receptors, all of which are members of the steroid hormone superfamily of receptors. RARs form only heterodimers and only with RXRs. These different receptor dimerizations confer effector specificity to different cells. Each receptor is thought to bind to specific response elements named retinoic acid response elements (RAREs) that govern the expression of genes and modify posttranscriptional mechanisms [26,27].

It has been shown that expression of the retinoic acid receptor (RARβ) is inhibited in the early stages of head and neck carcinogenesis (premalignant lesions of the oral cavity) and in lung carcinogenesis. Expression of this receptor is restored by administration of 13-cisRA. These results have been confirmed by studies *in vivo* [31].

More than 1,000 retinoids have been synthesized. Current efforts are concentrating on the development of receptor-selective and function-selective retinoids through molecular targeting strategies and structure activity relationship studies based on binding and transactivation assays. Primary targets and related examples of receptor-selective retinoid agents currently include RARα/AM80, RARβ/CD2317, RARγ/CD437, RXR/LG268, and the pan-agonist RARs/LGD1550 [32].

So far, these strategies have proved successful in helping generate retinoid agents that offer not only good efficacy but also good tolerability. For example, N-(4-hydroxyphenyl) retinamide (fenretinide or 4-HPR), despite its inability to bind directly to nuclear receptors, showed some preventive activity in experimental animals and was also active in lung cancer cell lines by inhibiting growth and inducing apoptosis [33]. In a group of smokers, 4-HPR was able to modulate telomerase expression [34]. Furthermore, 9-cisRA has a high affinity for both RAR and RXR receptors and was able to modulate the suppression of RARβ in ex-smokers [35]. In the same study 13-cisRA, which cannot be bound directly to nuclear receptors, was unable to induce a substantial change in RARβ expression. ATRA binds only to the RARs and may therefore not be the best candidate for lung cancer prevention studies. On the other hand the discovery that ATRA can induce differentiation and clinical remission in patients with acute promyelocytic leukaemia has shown the potential of a biologically based approach [36]. In this context targretin or bexarotene, which was associated with a particular favourable outcome of patients with advanced NSCLC, has to be mentioned [37]. So far there is only limited evidence that other retinoids such as acitretin and etrenitate have a preventive potential [38].

Despite these critical points, the use of retinoids has not been effective and has possible harmful effects in the chemoprevention of NSCLC, especially in current smokers. In order to find an explanation for these results, studies of the interaction between the products of cigarette smoking and high blood concentrations of retinoids have been performed. Results from Arora et al. indicate that the oxidative metabolites from cigarette smoke have a direct effect on the nuclear receptors and the retinoid-signaling pathway [39]. The oxidative metabolites induce cytochrome P450 enzymes, lowering the serum levels of retinoid acid and down-regulating RXR and RARβ. Nicotine by itself inhibits RARβ expression via methylation and induction of orphan receptor TR3 (a subfamily of transcription factors belonging to the nuclear receptor superfamily). RARβ is a potent inhibitor of the proliferation-signaling protein AP-1 and a promoter of apoptosis, so down-regulation of the different nuclear receptors, as well as defects in the RA/RARβ-regulated genes, results in retinoic acid resistance and enhanced mitogenic activities and cell proliferation.

The results of EUROSCAN, a large tertiary chemoprevention study showed no reduction in the development of second primary tumour or tumor recurrence [40]. A similar result has been obtained in the US NCI intergroup trial with 13-cis RA, which did not improve the rate of development of second primary tumour or mortality [41]. Subgroup analyses suggested that 13-cis RA might have been harmful in smoking patients and beneficial for those patients belonging to the category of never smokers.

As mentioned earlier, the explanations for the lack of preventive effects of retinoids in these studies are provided by the observation that RARβ is frequently suppressed in preneoplastic bronchial lesions and the distinct patterns of binding of different retinoids to the nuclear receptors.

Current systemic therapy with retinoid compounds is limited by substantial toxicities that result from activation of multiple signalling pathways. These toxicities involve numerous systems, including the skin and mucosa (dryness of skin or mucous membranes, desquamation, peeling, pruritus, dermatitis, and cutaneous photosensitivity), liver (reversible elevations of hepatic enzymes), skeleton (arthralgias, ligament calcification, skeletal hyperostosis, increased bone fragility and risk of fracture), central nervous system (headache), nausea or dyspepsia and reversible abnormalities in serum lipids (hypertriglyceridemia) [42,42]. Occasionally, many of these side effects can be ameliorated by the concomitant use of alpha-tocopherol without any loss of retinoid activity [44]. Whereas overall toxicity may be less with fenretinide, this retinoid has the additional adverse effect of impaired visual adaptation to darkness ("nightblindness"), an effect that appears to be related to the lowering of retinol levels [45]. This reversible ocular toxicity of fenretinide occurs in approximately 25% of patients, is asymptomatic in 50% of affected patients, and can be averted or minimized by administering it in shorter intervals [45,46].

Finally, there are indications that new routes of administration, such as the inhalational route, may provide an effective way of prescribing retinoids [38,47]. In this way high topical concentrations may be achieved and systemic toxicities avoided.

4. b. Carotenoids

Based on the hypothesis that the reduced risk of lung cancer associated with a high intake of fruit and vegetables is due to β-carotene and other antioxidants, dietary carotenoids were the first micronutrients that

epidemiological studies have verified to be inversely related to lung cancer risk [13,48].

Carotenoids are a family of conjugated polyene molecules, with proved antioxidant properties and important contribution in epithelial growth and differentiation; certain carotenoids also serve as precursors to retinol. They constitute a class of over 600 compounds found predominantly in fruits and vegetables. β-carotene has been the most extensively studied. This molecule has been reported to have a number of actions including important antioxidant activity and enhancement of immune function. Original epidemiologic data showed a correlation between low dietary β-carotene and increased risk of lung cancer.

However, clinical trials involving supplementation of pharmacologic doses revealed detrimental effects. Two are the most known intervention trials focused on lung cancer -the Alpha Tocopherol Beta Carotene Trial (ATBC) in Finland [49] and the Carotene and Retinol Efficacy Trial (CARET) in the USA [50]. In the CARET trial the intervention stopped 21 months earlier, because of evidence of no benefit and possible harm (mean follow up 4 years) [51]. There were 28% more lung cancers and 17% more deaths in the active intervention group. Because CARET administered a combination of β-carotene and retinyl palmitate, it was not possible to distinguish whether the adverse effects were due to β-carotene, retinyl palmitate, or the combination. These results were remarkably similar to the ATBC trial [52]. The β-carotene supplementation was associated with an increase in lung cancer risk. The adverse effect of β-carotene appeared to be stronger in those who were heavy smokers of at least 20 cigarettes per day, than in those who smoked 19 cigarettes or less per day.

Possible explanations for this effect include an inhibition of absorption of other nutrients by large doses of β-carotene and the autocatalytic pro-oxidant activity of β-carotene under high oxygen tension such as that occurring in the lungs of smokers [53-55]. Studies in ferrets showed that, as in humans, they absorb β-carotene into the bloodstream and transport it to the lungs as well as to other tissues [55,56]. The large amounts of β-carotene in lung tissue in combination with cigarette smoke are broken down into oxidative metabolites [57,58]. One possible explanation of the harm seen in the chemoprevention trials can be a procarcinogenic effect of the toxic oxidative carotene metabolites. On the other hand, another study indicate that β-carotene is sensitive to cigarette smoke oxidation but does not lead to prooxidant effects in human bronchial epithelial cells, but it has a direct effect on the nuclear receptors and the retinoid signaling pathway [39].

It is true that the findings of CARET and ATBC were a surprise since they conflicted with the epidemiological data. However, both trials administered high doses of β-carotene (20-30 times the average daily intake). These results emphasize the importance of carefully controlled intervention trials in determining the role of dietary supplements or any intervention agent. Because of the discouraging results of the large intervention trials and also of the rapidly expanding understanding of lung cancer, there has been a shift in focus to small clinical trials evaluating the effect of potential intervention agents on biomarkers of carcinogenesis. Regardless of the mechanism, β –carotene is now being avoided in heavy smokers and is no longer being used for lung cancer chemoprevention.

Despite the negative effects of β-carotene, carotenoids continue to be of interest as potential prevention agents. The biochemistry and biophysics of lycopene, a simple hydrocarbon precursor of β-carotene, found in tomatoes and their products, has been examined [59]. Lycopene is a potent antioxidant (25% better than β-carotene) and is the second most common dietary carotenoid. The most common source of lycopene in the diet is cooked or processed tomatoes that contain about 30 mg/kg and epidemiological studies of the dietary intake or serum concentration of lycopene found an inverse relationship with cancer risk [59,60]. *In vivo* animal trials assessing its chemopreventive effects in a multiorgan carcinogenesis model found that the pulmonary adenoma and carcinoma formation were reduced with lycopene [61,62]. Before large intervention trials can be justified, however, small-sized human trials will need to be performed to determine if the agent has biologic activity and toxicity.

4. c. Alpha- tocopherol (Vitamin E)

Alpha-tocopherol, the predominant form of vitamin E, is also an antioxidant, which scavenges reactive oxygen species and free radicals, and protects against oxidative damage. It has also been shown to have potent inhibitory activity on cell proliferation in various cancer cell lines including lung cancer, and high-molecular-weight DNA analysis revealed fragmentation consistent with apoptosis [63].

Epidemiological and dietary studies suggest a potential preventive role for vitamin E [48]. In the only published trial-the ATBC study [49] - vitamin E supplementation had no effect on lung cancer incidence. In the same study, there was an association between blood levels of α-tocopherol and incidence of lung cancer [64]. A 19% reduction of lung cancer incidence was observed in the highest *versus* the lowest quintile of serum α-tocopherol. It was also found

that vitamin E is more protective in younger men with fewer years of smoking, suggesting that high levels of serum α-tocopherol, if present during the early critical stages of carcinogenesis, may inhibit lung cancer development. It is worth noting that higher mortality was observed, due to hemorrhagic stroke among the participants who received α-tocopherol; this is possibly related to the known effects of vitamin E on platelet function.

However, its efficacy in the chemoprevention of lung cancer has not been fully demonstrated, and further evaluation in prevention trials is needed before firm recommendations can be made. In limited studies, vitamin E had possible protective effects on other cancers.

4. d. Selenium

Selenium is a component of the oxidative enzyme glutathione peroxidase. The proposed mechanisms of action of this micronutrient include antioxidant defense, anticarcinogenic, antiproliferative, and proapoptosic actions. Selenium as L-selenomethionine, has been shown to inhibit cell growth, induce apoptosis *in vitro*, and delay carcinogenesis at higher dose levels in animal models.

Interest in the chemopreventive effects of the trace element selenium has spanned the last 3 decades. Of more than 100 studies that have investigated the effect of selenium on tumor burden in carcinogen-exposed animals, two-thirds have observed a reduction in tumor incidence and/or preneoplastic endpoints [65,66]. Many prospective studies of serum selenium concentrations and lung cancer risk have been published. In one study it was found a significant inverse association between serum selenium and subsequent lung cancer occurrence in men within the cohort studied in the Finnish Mobile Health Examination Survey [67,68]. However, this study showed no association between estimated selenium intake and lung cancer risk [69]. A strong inverse association between toenail selenium level and lung cancer incidence in men and women was observed in a longitudinal observational study from the Netherlands [70]. Other published studies suggested inverse trends in lung cancer risk with increasing selenium status but were nonsignificant because of small numbers of cases [71-74].

The major clinical trial that stimulated further interest in the role of selenium in chemoprevention is the one by Clark et al., who designed a trial to determine selenium effects on the incidence of skin basal or squamous cell carcinomas; the nutritional supplementation with selenium showed no consequences on skin cancer incidence. However, secondary analyses revealed that it was associated with significantly fewer lung cancers. These observations led to an ongoing intergroup trial examining the effects of selenium on the reduction of lung cancer-associated second primary lung tumors (SPTs) [75].

A recent update of the Nutrition Prevention of Cancer (NPC) trial indicated that selenium supplementation did not significantly decrease lung cancer incidence in all of the population, but a decrease among individuals with baseline plasma selenium in the lowest tertile was observed [76]. The NPC sample had high percentages of former and current smokers. It is of particular interest that selenium supplementation appears to have chemopreventive effects in persons with relatively heavy tobacco use histories (median of 49 pack-years) and low baseline selenium concentrations. Thus, both current and former smokers may benefit from selenium supplementation, especially if they have low plasma selenium concentrations.

The exact mechanism by which selenium exerts a chemopreventive effect is not known. In the lungs of rodents, several forms of selenium have inhibited carcinogen-induced covalent DNA adduct formation, retarded oxidative damage to DNA, lipids and proteins, inhibited tumor cell growth, altered DNA, RNA, and protein synthesis, increased apoptosis, changed cell cycle and p53 and COX-2 expression, modified transcriptional factors activator protein P and nuclear factor κB, decreased aberrant crypt foci, and decreased Mtase activity [77]. DNA hypermethylation and decreased apoptosis are two possible mechanisms that have been implicated in lung carcinogenesis. At present, there is evidence that selenium modulates these biomarkers [78,79].

Several forms of selenium have been used to determine the mechanisms that explain the chemopreventive activity of selenium In the NPC trial, selenized baker's yeast was selected as a vehicle for selenium because it contains high concentrations of organic, bioavailable forms of selenium. Selenized baker's yeast contains a mixture of ~60% selenome-thionine; the remaining selenium compounds (40%) are a mixture of other organic selenoproteins [80]. Other as-yet-unidentified selenium-based agents are likely to be complex organic selenium compounds with chemopreventive properties that are not known, continuing the active debate on the most efficacious form of selenium to use in chemoprevention trials.

Overall, on the basis of the current reanalysis, selenium supplementation appears efficacious in decreasing lung cancer incidence in subjects whose baseline plasma selenium is ~106 ng/ml or below. Future research is needed to help confirm the role of

selenium in lung cancer prevention, using multiple forms of selenium alone and in combination with other chemopreventive agents.

4. e. Others

Several agents have been considered for the chemoprevention of lung cancer. Isothiocyanates, which occur as thioglycoside conjugates in a wide variety of cruciferous vegetables, have also been shown to have an inverse relationship with the incidence of lung cancer [81]. Isothiocyanates can influence P-450 enzyme levels and enhance detoxification. *In vivo* animal model systems have shown that isothiocyanates have activity in decreasing the incidence of lung cancer. A recent series of newly diagnosed lung cancer cases had significantly lower isothiocyanate intake when compared with controls [82]. In addition, the combination of glutathione-S-transferase (GST) null genotype and smoking was associated with increased lung cancer risk, suggesting that smokers with low intake of isothiocyanates and a null GST genotype carry an extra risk.

Allyl sulphur compounds, present in onions and garlic, were able to induce apoptosis in cell cultures and Japanese studies have coined green tea polyphenols as potential preventive agents for NSCLC [83,84].

Other potential chemopreventive agents that have been studied include the monoterpenes limonene and perillyl alcohol, the isoflavone genistein (which is found in high concentrations in soybeans and soy products) and the lipoxygenase and cyclo-oxygenase inhibitors (which are found in grapes and other fruits).

5. Lung chemoprevention studies

5.a. Primary chemoprevention-First generation trials

Three are the major trials concerning the primary chemoprevention of lung cancer. The first one - the Alpha-Tocopherol, Beta-Carotene (ATBC) study took place in Finland, in the 1980s. In this study it was tested whether α-tocopherol or β-carotene supplementation would reduce the incidence of lung and other cancers; 29,133 male smokers 50 to 69 years of age from southeastern Finland were randomly assigned to one of four daily supplementation regimens in a 2x2 factorial design: α-tocopherol (50 mg) alone, β-carotene (20 mg) alone, both α-tocopherol plus β-carotene, or placebo. Intervention continued for 5 to 8 years [49].

A total of 894 new lung cancer cases were identified in the final report of the ATBC study [52]. Lung cancer incidence was unaffected by α-tocopherol (a nonsignificant 1% increase); however, β-carotene supplementation significantly increased incidence rates by 16% (482 new cases in β-carotene group vs. 412 in no β-carotene group). Lung cancer mortality patterns followed incidence for both supplements. Total mortality was also unaffected by α-tocopherol (a nonsignificant 2% increase), although deaths from hemorrhagic strokes were significantly elevated by 50% [49]. Supplementation with β-carotene resulted in a significant 8% increase in total mortality, primarily due to more deaths from lung cancer and ischemic heart disease. Detailed analysis of the β-carotene-induced lung cancer elevation suggested that this effect was most pronounced in men who smoked heaviest and drank the most [52].

The second chemoprevention trial that started in 1985 is the Beta-Carotene and Retinol Efficacy trial (CARET). This study was designed to examine whether the daily β-carotene (30 mg) plus retinol (25,000 U) supplementation could prevent new lung cancers in persons at high risk [85]. Subjects were recruited and randomly assigned at 6 study centers from 2 risk groups: men over 45 years of age with occupational asbestos exposure, and men or women 50 to 69 years of age who were heavy smokers (i.e. at least a 20 pack-year history of cigarette smoking, either current smokers or recent quitters). The overall trial population then consisted of 18,314 individuals, including 34% females. Intervention was terminated after an average follow-up time of 4 years.

A total of 388 new lung cancers were diagnosed and 974 deaths occurred during the intervention phase of the CARET study. Compared to placebo, the supplemented group had significantly increased rates of both lung cancer (28% increased) and total mortality (17% increased). Detailed analyses by subgroups suggested that the increased risk attributed to β-carotene supplementation was most pronounced in current smokers and in participants with the highest alcohol intake [51].

The third chemoprevention study, the Physicians' Health Study (PHS) that took place in the USA in the 1980s, was designed to test the potential effects of aspirin and β-carotene on both cardiovascular disease and cancer. The PHS recruited 22,071 male physicians, who were 40 to 84 years of age and randomly assigned them using a 2×2 factorial design to 1 of 4 groups: aspirin alone (325 mg on alternate days); β-carotene alone (50 mg on alternate days); both aspirin plus β-carotene; and placebos. The randomized aspirin component was terminated early due to a significant 44% reduction in risk of first myocardial infarct in the aspirin group [86], while the randomized β-carotene component continued until 1995.

The 2,566 new cancers (excluding nonmelanoma skin cancers) identified during this trial were essentially evenly distributed between the β-carotene and placebo groups (a nonsignificant 2% lower rate was seen in the β-carotene group), as were all important cardiovascular events (no difference by β-carotene group status) and total mortality (a nonsignificant 2% increase in the β-carotene group) [87]. Although lung cancer was relatively uncommon in this population, in which only 11% of participants were current smokers, the 170 new lung cancers diagnosed were distributed evenly between the β-carotene and no β-carotene groups. Event rates did not differ by supplementation status when subgroups based on smoking status were examined (i.e., nonsmokers, former smokers, current smokers).

5.b. Secondary chemoprevention

Premalignant markers detectable by sputum cytology studies or found in bronchial metaplasia have been investigated as early predictors of lung cancer. Reversal of these premalignant lesions through treatment modalities may prevent progression to lung cancer. Several agents have been investigated for the treatment of sputum atypia [88-90], or bronchial squamous metaplasia [91,92]. A total of 5 randomized trials have been conducted.

One study reported improvement of bronchial epithelium metaplasia in smokers given folate and vitamin B12 [93]. This result is questionable because of the small sample size, substantial spontaneous and interobserver variability in atypia assessments, and complex or non-standard statistical methods. A reanalysis of these data using standard analytical methods found no significant difference between the two groups [94].

The 4 other phase IIb trials were conducted in smokers with metaplasia or sputum atypia for secondary prevention and all have been negative [95-98]. These trials evaluated α-tocopherol, β-carotene, retinal, retinyl palmitate or isotretinoin in smokers. Only smoking cessation correlated with a significant reduction in squamous metaplasia and cell proliferation and isotretinoin plus smoking cessation further reduced metaplasia, but so far neither metaplasia nor sputum atypia are established intermediate endpoints for chemoprevention trials [97]. Collectively, these results demonstrated that retinoids added no significant benefit to the effects of smoking cessation in the reversal of squamous metaplasia or dysplasia.

However, because of problems of the consistency of endpoints, positive results must be viewed with caution. Larger trials of biological endpoints are needed to confirm treatment efficacy. It is possible that lessons learnt from studies in the upper aerodigestive tract are applicable to lung cancer. Low-dose 13-cisRA was shown to decrease premalignant disease in the oral cavity when given as a maintenance regimen [99]. These studies have led to translational lung cancer trials based on the biological activity of 13-cisRA. Trials targeting intermediate biological markers, including molecular indicators of genetic damage, may well be the most promising for the control of lung cancer.

Kurie and colleagues reported the results of a large trial in former smokers who received 9-cisRA or 13-cisRA with α-tocopherol. The endpoint of the trial was upregulation of RARp, the loss of which in bronchial epithelium is considered a biomarker of preneoplasia. Of 177 evaluable patients, those treated with 9-cisRA were found to have restoration of RARp expression and reduction of metaplasia ($p=0.01$) [100]. On the basis of these results, further investigations with 9-cisRA in former smokers are needed.

5. c. Tertiary chemoprevention

Patients with cancer of the aerodigestive tract who have been successfully treated are at a significantly higher risk for developing additional tumors within the same area [101-104]. The concept of multistep precancerous progression explains the development of multiple independent tumor sites within the aerodigestive tract [104].

Second primary tumours (SPTs) must be distinguished from recurrent lesions and are defined as: 1) a new cancer of a different histologic subtype; 2) a cancer, regardless of site, that occurs after an interval of more than 3 years; or 3) a cancer presenting as a solitary mass that is of squamous cell histologic type, develops within 3 years, and occurs in the absence of local or regional disease accompanied by evidence of dysplasia or carcinoma *in situ* within the bronchial epithelium [105].

In patients with resected NSCLC, SPTs occur at the rate of 2-4% per year. Retinoid treatment reduces the incidence of SPTs in patients with lung cancer who have undergone resection, showing similar effects to those observed in patients with head and neck cancer.

In a randomized study, the adjuvant effect of high-dose retinyl palmitate (300,000 IU per day) was evaluated in 307 patients with early-stage lung cancer, randomly assigned after surgical resection to active drug or no further treatment [106]. After a median follow-up of 46 months, the SPT rate was 39% in the retinyl palmitate arm and 48% in the no-treatment control arm. A statistically significant difference in favor of treatment was observed regarding time to SPT develop-

ment within the aerodigestive tract. This initial trial led to other investigations in lung cancer prevention.

The EUROSCAN trial, however, did not confirm these initially encouraging results [40]. This randomized study by the European Organisation for Research and Treatment of Cancer's head and neck and lung cancer groups, studied the effects of vitamin A (retinyl palmitate) and N-acetylcysteine in patients with early-stage head and neck or lung cancer. In the trial, 2,592 patients with cancer of the larynx, oral cavity or NSCLC received retinyl palmitate (300 000 IU daily in year 1; 150 000 IU daily in year 2), N-acetylcysteine (600 mg daily for 2 years), both drugs, or placebo. There were no differences in endpoints between the 3 active treatment groups and the placebo group in terms of lung cancer incidence, occurrence of SPTs, or survival. There was a significant difference in time to development of SPTs within the carcinogen-exposed area in favor of the retinoid-treated group. Most of the patient population (93%) were regular smokers, at least half having had tobacco exposure greater than 43 pack-years.

The last of these trials carried out through the Oncology Intergroup involving all NCI Cooperative Oncology groups studied the efficacy of isotretinoin (13-cisRA) in the prevention of SPTs after complete resection of stage I NSCLC (US Intergroup NCI I91-0001). In this randomized, double-blinded, placebo-controlled trial, more than 1,000 patients received 3 years of intervention and an additional 4 years of follow-up [41]. Time to SPT was the primary endpoint. Additional study objectives were to look at the qualitative and quantitative toxicity of daily low-dose of the retinoid and compare the overall survival rates of the two groups. After a median follow-up of 3.5 years, no statistically significant differences were observed with respect to time to SPTs, recurrences, or mortality. Secondary multivariate analyses suggested that isotretinoin did not improve overall survival from SPTs or recurrences or mortality in patients with stage I NSCLC and possibly, in subset analyses, that isotretinoin was harmful in current smokers and beneficial in never smokers. Possible reasons for this finding include potential adverse interactions of retinoic acid with tobacco smoke. For example, tobacco carcinogens can suppress RAR expression and can induce retinoic acid metabolism and DNA methylation. Retinoic acid and smoking can increase gastrin-releasing peptide (GRP) expression and smoking can increase GRP receptor expression. Finally, the tobacco carcinogen benzo[a]pyrene and retinoic acid can induce NF-kB activation. These smoking-related genetic and epigenetic changes are more dominant in the lungs of active smokers than in the lungs of former smokers, which may help explain the difference in recurrence between these two subgroups [41].

5.d. Second generation ongoing trials

A number of other phase III trials using nutritional agents have been initiated and the results of these efforts will be available over the next years. These second generation trials include: 1) the PHS II trial (which tests β-carotene, α-tocopherol, ascorbic acid, and/or daily multivitamins in the prevention of cancer, cardiovascular, and eye diseases) [107]; 2) the Supplementation en Vitamins et Mineraux Antioxidants SU.VI.MAX trial (which studies the combination of ascorbic acid plus α-tocopherol, β-carotene, selenium, zinc in the prevention of all-site cancers and ischemic heart diseases) [108]; and 3) the a-tocopherol arm of the WHS, which continued after β-carotene was stopped [109]. There is an ongoing randomized phase III trial to determine the effectiveness of selenium in preventing the development of secondary primary lung tumors in patients with previously resected stage I NSCLC, comparing the incidence of specific cancers, mortality from cancer and overall survival of participants treated with selenium vs. those treated with placebo (ECOG-E5597) [110].

Phase III cancer prevention trials using bioactive food constituents as the study agent have had the following as their primary rationale: a convergence of epidemiologic research results; an intriguing secondary endpoint in a phase III trial done for another purpose; or laboratory evidence including largely empirical results showing cancer prevention in animal models. It is likely that in the future, the rationale will necessarily include phase II clinical trial results showing biologic activity suggestive of a benefit in humans and mechanistic evidence based upon modern basic science approaches to biomedical research [111].

6. Considerations - ascertainments

1. Because of their expected safety and because (unlike agents such as synthetic pharmaceuticals) they are not perceived as "medicine," food-derived products may find widespread, long-term use in the populations at normal risk; thus they are highly interesting for development as chemopreventive agents. Of course, characterization of efficacy and safety, biomarkers of efficacy and risk, and suitable cohorts for clinical intervention are critical in order to proceed in chemoprevention with diet-derived agents.

2. Many food-derived agents are extracts containing multiple compounds or classes of compounds

(e.g., tea, soy isoflavones or other soy fractions, curcuminoids). The NCI has advocated a science-based approach to their evaluation and development. Usually, a single or a few putative active compounds contained in the food-derived agent are isolated or synthesized and codeveloped with the food extract. Once it has been determined that the cancer-related targets and effects of the putative active components and the extract are similar (e.g., dose-response curves are parallel), the more expensive and possibly more toxic purified agent may be dropped from development in favor of the more nearly natural product. Alternatively, the purified product may be more potent and, even if more toxic, suitable for use in higher risk populations, such as patients with premalignant disease or previously treated cancers.

3. Also important concept in the development of food-derived chemopreventive agents is careful characterization of the active substance(s) and the technology to ensure reproducible preparations. For example, definition of growth conditions (e.g., hours of sunlight or soil nutrients) may be important, as may be the precise extraction conditions and spectrophotometric characteristics of the preparation to ensure the similarity of different preparations of the agent.

4. The first generation nutritional trials have shown us the potential pitfall in reliance or over-interpretation of results from observational studies. Best example is that of β-carotene and lung cancer chemoprevention. When nearly all the published prospective observational studies showed strong associations between low dietary β-carotene intake and/ or low serum β-carotene levels and increased lung cancer risk, both the ATBC and CARET trials showed that β-carotene supplementation actually increased lung cancers. Observational epidemiology cannot be relied on alone for making health recommendations regarding vitamins and minerals, and, in order to direct public health policy, results from randomized clinical trials are needed.

5. In order to come to safe conclusions, it is recommended that the future trials intervene longer than the typical 5 to 6 years of most of the first generation studies. The most common factors that determine the duration of an intervention trial are: the purpose of the study, the overall study size, the kinetics of test agents, including both biochemical half-lives and biologic half-lives with particular emphasis on kinetics within the specific target tissue of interest and logistics (cost, compliance).

6. The study agent should be used in efficacious doses, since at- or near-physiologic doses are the appropriate choice in the setting of a public health fortification plan, while higher doses might be considered if individual supplementation is contemplated. The data to date support that modest doses are the safest and this is the most efficacious approach. The potential of single chemopreventives is limited by potency and toxicity at efficacious doses. Simultaneous or sequential administration of multiple agents can increase efficacy and reduce toxicity. For example, differences in the chemopreventive mechanisms among the agents can provide additive or synergistic efficacy; thus, adequate efficacy may be observed at lower and presumably less toxic doses of the individual agents.

7. Critical issue remains the selection of study endpoints. Intermediate endpoints, variously and loosely defined as biomarkers or surrogate endpoint biomarkers (SEBs) are used as intermediate indicators of cancer incidence reduction in chemoprevention studies. SEBs are required to be integrally involved in the process of carcinogenesis, such that the changes of expression correlate highly with disease course. Markers must be differentially expressed in normal and premalignant or high-risk tissue. They must also occur in sufficient amounts to permit their biological and statistical assessment, assayed dependably and quantitatively, and measured without difficulty. Lastly, their expression should be able to be modulated by efficacious chemopreventive interventions but not vary spontaneously or have an appreciable spontaneous remission rate.

The best model for validating such intermediate endpoints is to embed them within large randomized controlled trials, which, by design, have cancer as the primary endpoint. Since relatively few prevention trials with cancer endpoints have been conducted, there have been limited opportunities to validate such intermediate endpoints.

Cytologic and histopathologic markers that are used as SEBs include nuclear features, nucleolar features, and tissue architecture. These markers are now being quantified using stoichiometric stains viewed by computer-assisted imaging systems. Quantitative cytology and histopathology allow for an objective, reproducible measure of what is observed by the pathologist. A class of biomarkers of increasing importance assesses proliferation and growth regulation and includes RARβ and other retinoid receptors, proliferating cell nuclear antigen (PCNA), Ki-67, TGFβ and EGFR. Other markers such as the genomic instability markers may be very important, by reflecting the sum of the changes in all other categories. DNA abnormalities (eg, DNA hypomethylation) and chromosome aberrations (micronuclei from chromosomal damage, polysomy, and deletions at 3p, 5q, 9p, 11q, 13q, and 17p) have been proposed as promising markers for lung cancer trials. Because of the complexity and multifaceted nature of carcinogenesis, it is unlikely that any one of

these markers alone will be able to encapsulate all the information necessary to be a viable endpoint. Actually, a panel of markers will be required to gather sufficient information to assess the effects of preventive agents.

There is enthusiasm and urgency for using intraepithelial neoplasias (IENs) as prevention endpoints. IENs are defined as noninvasive lesions with genetic abnormalities, loss of cellular control functions, and at least some phenotypic characteristics of invasive cancer; they should also be highly predictive of invasive cancer [112]. But using IENs in cancer risk reduction studies is challenging because the multifocal and multiclonal nature of carcinogenesis makes epithelial sampling for the detection of IENs problematic, and relatively small percentages of IENs actually progress to cancer.

8. The unexpected increases in lung cancer development and total mortality among participants who received β-carotene and/or retinol in the ATBC and CARET trials established a new example about potential side effects from what were previously considered benign interventions. It is important to monitor other major causes of morbidity and mortality except the effects on cancer alone.

9. Finally, there is a need to examine whether oral administration is the most appropriate route for these agents. We should not rule out other routes of administration (e.g. inhalational), which may prove more effective.

7. Conclusions

All the prospective, randomized, controlled trials in lung cancer chemoprevention have so far produced either neutral or harmful primary endpoint results. Lung cancer was not prevented by β-carotene, α-tocopherol, retinol, retinyl palmitate, *N*-acetylcysteine, or isotretinoin in smokers. Secondary results from phase III trials involving selenium and vitamin E and the results from the US Intergroup NCI I91-0001 trial supporting treatment with isotretinoin in never and former smokers, present a promising direction for future clinical studies.

The continuing magnitude and severity of the lung cancer problem make it imperative to enhance smoking cessation campaigns and to make progress in early detection and chemoprevention. With the expanded understanding of the molecular and biological mechanisms of lung cancer development, new specific targets for prevention are being identified. The identification of appropriate high-risk patient groups, who will develop lung cancer, is crucial and will enable smaller studies to be designed. Also important is to integrate the growing biological knowledge in a rational timeframe that can be done by the identification and validation of intermediate endpoints, which will be sufficiently predictive of lung cancer development.

The use of foods and dietary supplements present a safe chemopreventive strategy. In addition to epidemiologic studies, basic science research to detect mechanisms and evaluate the chemopreventive potential of food components is necessary. Talalay's research on phase II enzyme induction by molecular components of broccoli sprouts is the prototype of what is required to demonstrate chemopreventive potential of foods [113]. The overwhelming evidence of a major role of nutrition in carcinogenesis, the many leads that nutritional intervention may reduce cancer incidence, and the growth and increasing sophistication of our clinical trials networks points to a very promising future for nutritional intervention trials leading to substantial public benefit.

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