Chemoprevention of Lung Cancer

Stéphane Vignot1 · Jean-Philippe Spano2 · Sylvie Lantuejoul3 · Fabrice André4 · Thierry Le Chevalier1 · Jean-Charles Soria1 (✉)

1 Department of Medicine, Institut Gustave Roussy, 39 rue Camille Desmoulins, 94805, Villejuif, France soria@igr.fr
2 Oncology Department, SOMPS, Hopital La Pitié-Salpêtrière AP-HP, Paris, France
3 Cellular Pathology, Lung Cancer Research Group INSERM 9924, CHU Michallon, Grenoble, France

1 Introduction ........................................ 146
2 Lung Carcinogenesis .................................. 147
  2.1 Basic Concepts .................................... 147
  2.2 Molecular Basis of Lung Carcinogenesis .......... 148
    2.2.1 Genetic Susceptibility ....................... 148
    2.2.2 Chromosomal Alterations .................... 148
    2.2.3 Oncogenes .................................... 149
    2.2.4 Tumour Suppressor Genes .................... 151
    2.2.5 Cyclooxygenase Activity and Carcinogenesis .... 153
3 Chemoprevention ................................... 153
  3.1 Definition ...................................... 153
  3.2 Interventional Strategies: Primary, Secondary and Tertiary Chemoprevention 154
  3.3 Lung Chemopreventive Agents .................... 154
    3.3.1 Primary Chemoprevention .................... 154
    3.3.2 Secondary Chemoprevention ................... 156
    3.3.3 Tertiary Chemoprevention .................... 157
4 Future of Chemoprevention: Developing New Agents .......... 157
5 Conclusion ...................................... 159

References ........................................ 160

Abstract Lung cancer remains a major cause of mortality worldwide, despite advances in surgery, radiotherapy and chemotherapy. Most patients present with advanced disease, and early detection approaches are still experimental. Chemoprevention strategies are therefore essential. Chemoprevention can be defined as the use of specific natural or synthetic chemical agents to reverse, suppress or prevent progression to invasive cancer. The present review will provide an update on lung cancer clinical chemoprevention trials as well as the molecular basis of lung carcinogenesis. A better knowledge of lung carcinogenesis is obviously fundamental to improve chemoprevention strategies. Identification of molecular defects involved in premalignant lesions and/or invasive cancer could lead to clinical studies with new molecular-targeted agents (mainly tyrosine kinase inhibitors, farnesyl-transferase inhibitors and/or antiangiogenic molecules) and the development of surrogate biomarkers. Such biomarkers would be essential to detect high-risk patients,
select adequate chemoprevention strategies and monitor drug efficacy. New chemoprevention trials are planned with collaborative efforts of researchers involved in fundamental or clinical studies.

1 Introduction

Despite tobacco control campaigns, tobacco-related cancers remain a great concern. In particular, lung cancer is a major cause of mortality worldwide, especially in developing countries, where tobacco consumption is still rising. An estimated global annual incidence of over 1.2 million cases and an overall mortality of over 1.1 million cases are presumed [1]. Estimates of cancer incidence and mortality in Europe in 1995 were 377,000 new cases of lung cancer and 330,000 deaths from this disease [2]. In the United States, an estimated 171,900 new cases of lung cancer were expected annually in 2003, meaning an estimated 157,200 deaths per year from lung cancer [3]. The most effective treatment for non-small cell lung cancer (NSCLC) remains surgical resection, but at the time of diagnosis about 70% of NSCLC patients present with advanced diseases and/or visceral metastases. For these, no curative surgery is possible and treatment is based on chemotherapy with classical cytotoxics and/or new molecular-targeted therapies. Improving the survival rate of patients with lung cancer will not be achieved only by improving these strategies. Indeed, major efforts are currently being deployed to facilitate earlier detection of lung cancer in high-risk patients and to develop chemopreventive approaches. Chemoprevention is defined as the use of natural or synthetic agents to reverse, prevent or delay carcinogenic progression to invasive cancer. This strategy requires the understanding of molecular events leading to lung cancer in order to identify genetic factors involved in lung cancer progression. Modern chemopreventive medicine is thus tightly related to a better comprehension of the carcinogenic process. The understanding of the molecular and biological basis of lung cancer has significantly expanded over the last 20 years. The present review will provide an overview of the current genetic changes associated with lung carcinogenesis. This review will also summarize the outcome of the major lung clinical chemoprevention trials.
2 Lung Carcinogenesis

2.1 Basic Concepts

Two fundamental concepts should be considered because they underlie all chemoprevention strategies: multistep carcinogenesis and field cancerization.

*Multistep Carcinogenesis.* According to the multistep carcinogenesis concept (Fig. 1), cancer develops in a stepwise fashion, with an accumulation of molecular alterations progressing from preinvasive lesions to invasive disease.

![Fig. 1. The multistep carcinogenic process](image-url)
The earliest events of this process are mutations, deletions or polysomy at the cellular genomic level. These genetic modifications are not initially translated into cellular morphologic alterations or tissular structural changes [5]. Additional events are necessary to induce phenotypic, then physiologic modifications at the tissular level (uncontrolled proliferation, invasion, metastasis, etc.). It has been suggested that 10–20 genetic events are necessary in the setting of lung cancer [6], the most relevant of these events will be described below.

Field Cancerization. Carcinogen exposure (e.g. cigarette smoke) to an entire epithelium (field) such as the lung will result in diffuse tissue damage. Thus, genetic changes and/or premalignant lesions in one area of the exposed field imply an increased risk of developing cancer in any other site within the same field [7]. Treatment or control of precancerous lesions is then a potential means to avoid invasive lesion development.

2.2 Molecular Basis of Lung Carcinogenesis

2.2.1 Genetic Susceptibility

Over 80% of lung cancers are attributed to tobacco and its carcinogenic products. However, epidemiological studies show that only 15% of smokers will ultimately develop lung cancer. The fact that 85% of smokers do not develop lung cancer indicates differences in susceptibility [8]. A study of genes implicated in activation or detoxification of tobacco carcinogens showed that enzymatic genetic polymorphisms may play a role in lung and head-and-neck cancer incidence. In this setting, it has been suggested that a high activity of cytochrome P450 could be a risk factor of lung cancer [6] and that specific mutations associated with cytochrome P450 genes could be implicated in lung cancer susceptibility [9]. Besides, the null genotype of detoxification enzyme glutathione S-transferase (GST) and M1 GST also seems to be a risk factor of lung and head-and-neck cancers [10–12]. Furthermore, recent case-control studies have shown that defective repair of genetic damage and increased sensitivity to mutagens have been associated with increased individual susceptibility to lung cancer [13]. In this setting, the DNA excision repair pathway might also be implicated [14].

2.2.2 Chromosomal Alterations

Fewer than 10% of lung cancers are diploid, and the large majority of patients with lung cancer present chromosomal abnormalities not only in tumour cells but also in histologically normal adjacent tissues [15]. The
amount of DNA (DNA index) has been correlated with the severity of dysplasia in precancerous bronchial lesions, and with greater tumour size, poor differentiation and node invasion in invasive lung lesions [16]. Various chromosomal imbalances were identified in lung cancers and in in vitro epithelial bronchial tumour cell lines. The most common chromosomal abnormalities in lung cancer are allelic deletions or LOH (loss of heterozygosity) at sites characterized by tumour suppressor genes which will be described below: 3p (FHIT and others), 9p (9p21 for p16INK4, p15INK4B and p19ARF), 17p (17p13 for p53 gene and others), 13q (13q14 for retinoblastoma gene and others). 3p and 9p losses have been associated with smoking and are recognized as early events of lung carcinogenesis. They remain detectable many years after smoking cessation [17]. The loss of 17p13 is less common, suggesting that p53 alterations are rather a late event. The frequency and the number of chromosomal abnormalities parallel the phenotypic progression from premalignant lesions to invasive cancer [18]. Deletions affecting 3p, 5q, 8p, 9p, 17p and 18q chromosomal regions are also among common changes in lung cancer.

2.2.3 Oncogenes

Activation of oncogenes are related to genetic modifications including mutation, amplification or chromosomal rearrangement as well as to epigenetic changes such as hypermethylation. More than 100 oncogenes have been identified to date; among them, several are implicated in lung carcinogenesis (Table 1). RAS, C-MYC, epidermal growth factor receptor (EGFR, also named HER1) and HER2/neu play an important role in lung cancer. Telomerase activity is also involved in this process.

- RAS mutations are detected more frequently in adenocarcinomas and large cell lung carcinomas or carcinoid tumours than in squamous cell carcinomas where mutation level is often lower [19]. The RAS family encodes 21-

<table>
<thead>
<tr>
<th>Oncogene</th>
<th>Usual alterations</th>
</tr>
</thead>
<tbody>
<tr>
<td>RAS</td>
<td>Mutations in adenocarcinoma, large cell lung cancer and SCLC</td>
</tr>
<tr>
<td>C-MYC</td>
<td>Genetic amplification both in SCLC and NSCLC</td>
</tr>
<tr>
<td>EGFR</td>
<td>Overexpression in NSCLC (prognostic factor) and in SCLC</td>
</tr>
<tr>
<td>HER2</td>
<td>Overexpression in NSCLC (prognostic factor)</td>
</tr>
<tr>
<td>Cyclin E, D1, B1</td>
<td>Deregulation in premalignant lesions and in NSCLC</td>
</tr>
<tr>
<td>GRP/Bombesin</td>
<td>Overexpression in SCLC; higher level of expression in women</td>
</tr>
<tr>
<td>Telomerase</td>
<td>Overexpression in NSCLC (prognostic factor) and in almost all SCLC</td>
</tr>
</tbody>
</table>

NSCLC, non-small cell lung cancer; SCLC, small cell lung cancer.
kDa proteins able to bind guanosine triphosphate (GTP) to form RAS-GTP complex which transduces proliferation signals. This activation in RAS-GTP induces transcription factors C-FOS, C-JUN, C-MYC and DNA synthesis. Activating RAS mutations are mostly identified at codon 12 of the K-RAS gene induced by tobacco carcinogens like benzo[a]pyrene and nitrosamine, more rarely at codons 13 and 61, and infrequently in N- and H-RAS genes.

- Oncogenic activation of C-MYC occurs in 20% of small cell lung carcinoma (SCLC) and in 10% of NSCLC in relation with a genetic amplification. L- and N-MYC are also frequently overexpressed in NSCLC (35%) and this profile is very usual in aggressive neuroendocrine lung cancer [20]. Interestingly, patients with lung cancer present with a high C-MYC level in histologically normal or altered lung surgical margins [21]. This suggests that C-MYC expression is an early event in lung carcinogenesis.

- EGFR (HER1) and HER-2/neu are tyrosine kinase receptors both involved in lung cancer progression and overexpressed in NSCLC. EGFR overexpression has been associated with poor survival, an advanced stage, a poor differentiation, a high proliferation index and an increased risk of metastasis [22, 23]. HER-2/neu overexpression is also a pejorative prognostic factor, especially associated with a higher degree of chemoresistance [24]. EGFR and HER2/neu overexpression is mainly due to an increase of both transcription and translation, with only a low percentage of tumours presenting a gene amplification similar to the one observed in breast carcinomas with HER2/neu.

- More recently, the role of cyclins E, D1 and B1 as potential oncogenes in lung cancer has been highlighted [25–27]. Cyclin D1 and cyclin E overexpression is responsible for deregulation of RB phosphorylation in about 50% of lung carcinoma and is an early event (it can be detected by immunohistochemistry in half of dysplastic lesions) [28].

- Expression of neuroendocrine factors, including gastrin-releasing peptide/bombesin-like peptides (GRP/bombesin), and their receptors, has been reported in lung cancers. The GRP-receptor autocrine loop appears particularly important in SCLC. GRP mRNA expression was detected more frequently in females than in males, suggesting that this gene may be a factor in the increased susceptibility of women to tobacco-induced lung cancer [29].

- Telomerase is expressed in 80%–85% of NSCLC and in almost all SCLC [30, 31]. Telomerase is the key enzyme stabilizing the telomeres, which are highly complex terminal chromosome structures, whose correct function is crucial for normal cell survival. Telomerase is preferentially expressed in tumour cells with short telomeres and is not expressed in most somatic cells which usually have longer telomeres. The expression level is a prognostic factor in early-stage NSCLC [32] and its activity has been correlated with stage and node invasion [33]. Telomerase activity is detected in precancer-
ous lesions of the lung, reflecting the early involvement of the molecule in lung tumourigenesis [34].

2.2.4 Tumour Suppressor Genes

Tumour suppressor gene inactivation may be due to mutation, loss of chromosomal material (one or two alleles) or epigenetic changes such as methylation of the promoter regions.

The main tumour suppressor genes involved in lung carcinogenesis (Table 2) are those implicated in cell cycle control, apoptosis and differentiation.

- p53. This is a tumour-suppressor gene which has been called ‘the guardian of genome’. It acts as a transcription factor implicated both in the G1 arrest control and in apoptosis. It reduces RB phosphorylation and induces a stop at the G1-S checkpoint to allow a DNA repair or to drive the cell to apoptosis mediated by BAX/BCL2. Its properties are abrogated as a result of mutations or pathway alterations [35, 36]. About 70% of lung cancers present a p53 mutation which induces its abnormal stabilization. Mutations are detected in 70%–100% of SCLC and in 45%–75% of NCLC [37, 38]. In preinvasive lesions, p53 aberrant expression was found from the level of mild dysplasia (25%) to that of CIS (75%), and with RAS mutation, p53 mutation is in one of the most powerful tools for early lung cancer diagnosis and detection [39, 40]. The most common p53 mutation is a GC to TA transversion. A strong correlation was observed between the frequency of these mutations and the global duration of tobacco exposure.

- Cell cycle control: RB protein is the main effector of G1 arrest mediated by p53 in the context of DNA damage or oncogenic stress. RB protein expression is lost in 80% of SCLC but only in 15% of NSCLC and never in preinvasive lesions [41, 42]. In contrast, RB inactivation through deregulation of its

<table>
<thead>
<tr>
<th>Tumour suppressor gene</th>
<th>Usual alterations</th>
</tr>
</thead>
<tbody>
<tr>
<td>p53</td>
<td>Mutations observed in NSCLC and SCLC</td>
</tr>
<tr>
<td>RB</td>
<td>Frequent loss of expression in SCLC (occasionally in NSCLC, almost never in preinvasive lesion)</td>
</tr>
<tr>
<td>p16</td>
<td>Inactivation by either mutations, deletions or promoter methylation</td>
</tr>
<tr>
<td>FHIT (3p14.2)</td>
<td>Frequent deletions</td>
</tr>
<tr>
<td></td>
<td>LOH observed in premalignant lesions</td>
</tr>
</tbody>
</table>

LOH, loss of heterozygosity; NSCLC, non-small cell lung cancer; SCLC, small cell lung cancer.
phosphorylation is common in NSCLC. Two mechanisms are responsible for this deregulation: the loss of the CDK inhibitor p16INK4, which negatively controls the cyclin-dependent kinase (CDK)–cyclin activity, and the overexpression of cyclin D1. Inactivation of p16INK4 in NSCLC is mainly caused by exon 1 or 2 mutation (15%), homozygous deletions (30%–40%) or promoter methylation (30%–40%) [43], and there is a strict inverse relation between RB and p16 expression. Hypermethylation of p16 can be detected in bronchial epithelium from chronic smokers with a high risk, suggesting inactivation of p16 occurs early in lung tumourigenesis [44]. p16 methylation status which can be also detected with a very high sensitivity (one allele methylated detected over 10⁴ normal unmethylated alleles) in exfoliated cells, represents a promising tool for early detection of lung cancer [45, 46].

- Apoptosis regulation: FHIT is a tumour-suppressor gene implicated in the apoptotic process [47, 48] whose locus is 3p14-2, a fragile genomic region, frequently lost in lung cancers (more than 70%) [49]. It has been shown as a preferential target of tobacco smoke, since 80% of FHIT LOH were found in cancers and preinvasive lesions of smokers [50]. Alterations of the apoptotic pathway can also be related to two other genes: BAX and BCL2 [51–53]. BAX is an apoptotic gene whose dimeric protein product formation induces apoptosis. BCL2, conversely, is a survival (antiapoptotic) gene, and the dimer BAX/BCL2 induces a neutralization of BAX and a loss of apoptosis. BAX/BCL2 deregulation (e.g. the inversion of the BAX/BCL2 ratio) has been studied on preneoplastic lesions [40]. A ratio below 1 indicates hyperexpression of BCL2 and loss of BAX as compared with normal bronchial epitheliums, and has been shown to increase with the severity of the preneoplastic lesions from low-grade to high-grade lesions.

- Differentiation regulation by retinoids and their receptors: Vitamin A and its analogs (retinoids) are differentiation and proliferation modulators of epithelial cells. They are able to invert airway cancerous progression by complex mechanisms. These mechanisms essentially consist of retinoids’ capacity to regulate gene expression through nuclear transduction signal modulation mediated by nuclear retinoid receptors. These receptors act as ligand-activated transcription factors. It has been demonstrated that retinoid acid receptor (RAR)–β expression, one of these receptors, is lost in early stages of head-and-neck carcinogenesis (premalignant lesions of the oral cavity and tumours adjacent dysplastic tissues) and in lung carcinogenesis [54]. This receptor expression could be restored by 13-cis retinoic acid (13-cRA) administration. These results have been confirmed by in vivo studies [55].

Additionally, although a high proportion of loss of heterozygosity in 5q, near the APC (adenomatous polyposis coli) gene was established—and the loss of heterozygosity at APC locus occurs in 80% of dysplastic epithelia, 67% of in situ carcinomas and 50% of invasive cancers—the tumour-suppressor gene located at 5q has not been identified definitively [56].
2.2.5 Cyclooxygenase Activity and Carcinogenesis

Cyclooxygenase (COX-1 and -2) catalyses the synthesis of prostaglandins from arachidonic acid. Most tissues express COX-1 constitutively. On the other hand, COX-2 is inducible, and increased concentrations are observed in the context of inflammation and in the setting of invasive cancers such as NSCLC. The COX2 gene is an immediate early response gene that is induced by growth factors, oncogenes, carcinogens and tumour-promoting phorbol esters, whereas the constitutive COX1 is unaffected by these factors. COX-2 is upregulated in malignant tissue and seems to be important in carcinogenesis, as suggested by various experimental systems. For example, COX-2 expression and prostaglandin production have been shown as crucial for tumour growth and development in epithelial cancer such as colon cancer. COX-2 is frequently expressed in tissue samples from NSCLC and premalignant lesions [57] [expression evaluation by immunohistochemistry, RNA in situ hybridization or reverse transcriptase polymerase chain reaction (RT-PCR)]. Expression of COX-2 is associated with worse prognosis, at least in patients with early-stage disease [58]. In contrast, adjacent histologically normal epithelium and histologically normal epithelium from smokers without cancer show low levels of expression. Treatment with non-steroidal anti-inflammatory drugs (NSAIDs), which are COX inhibitors, reduces the growth of NSCLC cells in tissue culture and in xenograft studies with effects on proliferation, apoptosis, invasion, angiogenesis and tumour lymphocyte infiltration [59, 60]. Besides, expression of COX-2 in premalignant disease suggests it could be a good target for chemoprevention studies.

3 Chemoprevention

3.1 Definition

Chemoprevention, a term coined by Sporn in 1976, can be defined as the use of specific natural or synthetic chemical agents to reverse, suppress or prevent progression to invasive cancer [61]. The foundation of chemopreventive medicine is the translation of basic biological research into clinical chemical interventions, which attempt to halt the process of carcinogenesis. Its principles build on the concepts of field cancerization and multistep carcinogenesis. These basic principles also include the theory of the potential reversibility of some precancerous lesions, and the importance of the relationship between cancer cells and their environment (the concept of carcinogenic progress modulator genes) [4, 62].
Those concepts were validated by clinical trials studying the reversal of premalignant lesions such as leukoplakia using 13-cRA. Furthermore, this treatment was effective in preventing secondary tumours from occurring in patients who had been cured of head-and-neck cancer [63, 64]. In fact, vitamin A deficiency was first reported to be associated with changes in epithelial histology in 1925 and it was afterwards related with bronchial metaplasia and an increased incidence of cancer.

3.2 Interventional Strategies: Primary, Secondary and Tertiary Chemoprevention

Chemoprevention targets the carcinogenic process at earlier and potentially more reversible stages than those observed in the setting of invasive overt cancer. Chemopreventive strategies can be defined as follows. Primary chemoprevention’s aim is to prevent the occurrence of cancer in healthy individuals at high risk: drugs are used to avoid cancerization of healthy epithelium submitted to carcinogenic agents such as tobacco. Secondary chemoprevention’s aim is to prevent cancer in patients with premalignant lesions (intraepithelial neoplasia). Reversal of bronchial metaplastic lesions may prevent progression to lung cancer. Tertiary chemoprevention’s aim is to prevent second primary tumours in patients cured from an initial cancer who have a very high risk of developing a secondary primary tumour according to the concept of field cancerization.

3.3 Lung Chemopreventive Agents

Nearly 2,000 natural and synthetic agents are presumed to have chemopreventive activity in experimental systems. Some of them have been studied in clinical trials: retinoids, N-acetyl-cysteine, \( \beta \)-carotene, calcium, \( \alpha \)-tocopherol, selenium, tamoxifen, finasteride and NSAIDs [65–71]. The rationale for prevention of lung cancer is similar to that in head-and-neck cancer. In both diseases, chronic exposure to tobacco is the major risk factor and dysplastic epithelial lesions are thought to be a premalignant stage. As summarized in Table 3, all chemopreventive trials in current smokers are negative, and only a few are positive in former smokers.

3.3.1 Primary Chemoprevention

All lung primary chemoprevention trials are negative, or even show a deleterious effect of \( \beta \)-carotene in active smokers. Interestingly, a randomized phase II study with isotretinoin in heavy smokers suggested that smoking cessation was more important than the actual prevention with retinoids [67].
The ATBC trial (α-Tocopherol, β-Carotene Cancer Prevention Study Group) tested α-tocopherol and β-carotene in 29,233 50- to 69-year-old heavy smoker Finnish men. The subjacent rationale of this ATBC trial was the existence of epidemiological data showing inverted association between plasmatic or dietetic β-carotene levels and lung cancer incidence. Patients were randomized in four groups and received, for between 5 and 8 years, β-carotene (20 mg/day), α-tocopherol (50 mg/day), both, or placebo. Unexpectedly, both groups who received β-carotene supplementation showed an 18% increase in the incidence of lung cancer and an 8% excess in global mortality compared with placebo [69].

The CARET (β-Carotene And Retinol Efficacy Trial) [70] secondarily confirmed deleterious effect of the β-carotene combined with retinyl palmitate in chemoprevention of men and women at high risk for lung cancer. The patient population smoked at least 20 pack-years or had extensive occupation-
al exposure to asbestos. This trial was stopped after 21 months because of a 17% increase in mortality and a 28% increase in lung cancer incidence in the active treatment arm.

The Physician’s Health Study group also evaluated the role of β-carotene versus placebo in prevention of lung cancer in 22,071 American physicians. Neither a benefit nor a deleterious effect on lung cancer incidence was identified in this study [71].

In China, a study evaluating β-carotene, α-tocopherol and selenium in the prevention of gastric and oesophageal cancer showed an insignificant decrease in the risk of lung cancer in a small cohort of patients [72].

In vitro and in vivo data provide some indications to understand the negative interaction between β-carotene and tobacco observed in the ATBC and CARET trials. It is possible that carcinogenesis mechanisms would be raised if elevated tissular β-carotene concentrations interact with highly oxidative tobacco smoke [73]. Other studies suggest a procarcinogenic effect of β-carotene implicating cytochrome P450 modifications in some circumstances [74, 75]. With these results it is admitted that the next primary chemoprevention trials should focus on former smokers. Indeed, the pursuit of tobacco consumption during a chemoprevention trial is not only deleterious at the level of the airway epithelium but could also lead to an inversion of the anticipated effect of the chemopreventive agent.

3.3.2 Secondary Chemoprevention

Randomized trials testing retinoids in precancerous lung lesions are negative in their vast majority. A randomized trial tested etretinate efficacy for 6 months versus placebo to decrease the number of metaplasia observed in sputum [76]. The reduction was of 32% in the etretinate arm versus 30% in the placebo arm. Isotretinoin or fenretinide use in this same setting did not provide satisfactory results [67, 77]. But it has been demonstrated that isotretinoin will decrease lung metaplasia index in the arm of patients who stopped smoking [67].

A randomized trial testing, in 755 workers exposed to asbestos, the use of β-carotene associated with retinol versus placebo did not demonstrate any improvement of cytological atypia observed in spittle, despite a 58-months follow-up. Many authors consider today that cytological atypia analysis in spittle or metaplasia analysis on bronchial biopsies is a not a very satisfactory modality to evaluate efficacy of chemopreventive agents on precancerous lesions of the bronchial tree. Indeed, spontaneous improvement of these indexes (mostly at smoking cessation) are frequent and make very difficult the evaluation of chemopreventive effect. To palliate this problem, biomarkers have been recently added to evaluate retinoid efficacy (at least at the molecular level if not clinical). Thus, two recent publications showed 13-cis retino-
ic acid efficacy on RAR-β receptor re-expression in lung metaplastic areas of smokers who received at least 6 months retinoid treatment [78, 79].

### 3.3.3 Tertiary Chemoprevention

In a randomized study, 307 patients with completely resected stage I NSCLC, received either 12 months of treatment with retinol palmitate or no treatment. At a median of 46 months of follow-up, patients who received retinol palmitate had a 35% lower incidence of second primary tumours than the control group (3.1% vs 4.8%) [68].

Nevertheless, the EUROSCAN trial did not confirm these initially encouraging results [65]. This double-blind, placebo-controlled, randomized trial tested for 2 years retinol palmitate, N-acetylcysteine or both in 1,023 patients treated for a lung cancer.

U.S.-Intergroup NCI I91-0001 was a randomized, double-blind, placebo-controlled study using low-dose 13-cRA after complete resection of stage I NSCLC (postoperative T1 or T2, N0). It included 1,304 patients who all had undergone surgery 6 weeks to 3 years prior to registering. After a median follow-up of 3.5 years, there were no statistically significant differences between the placebo and isotretinoin arms with respect to the time to second primary tumours, recurrence or mortality. Secondary multivariate and subset analyses suggested that isotretinoin was harmful in current smokers and beneficial in never smokers [80].

### 4 Future of Chemoprevention: Developing New Agents

The previous studies prove that strategies should be reassessed, and that new agents should be investigated. Recently, a new class of retinoids has been identified that seems to be more effective in growth inhibition and induction of apoptosis of lung cancer cell lines [81]. Such agents could be more efficient in lung chemoprevention than the retinoids that have been investigated so far. The way of administration should also be reconsidered: Is the oral administration the more relevant way? It is possible that other routes of administration (inhalational route in particular) may finally provide an effective way of prescribing retinoids [82].

Although retinoids are the most-frequently used pharmacological agents in chemoprevention trials, they are pretty toxic. Therefore, other molecules with a best-therapeutic index are currently in development to be used as chemopreventive agents.

Results of a randomized phase IIb study of anethole dithiolethione (ADT), an organosulphur compound originally developed as a radio-protec-
tant more than 30 years ago, have been recently published [83]. In total, 112 current and former smokers, with at least one site of bronchial dysplasia, were randomly assigned to receive placebo or ADT at 25 mg orally thrice daily for 6 months. Progression rate of pre-existing dysplastic lesions by two or more grades and/or the appearance of new lesions was statistically significantly lower at 8% in the ADT group than in the placebo group (17%). At the clinical level, the disease progression was statistically significantly lower in the ADT group (32%) than in the placebo group (59%). Adverse events were mostly gastrointestinal symptoms that resolved with dose reduction or discontinuation of the medication.

An epidemiological case-control study of chemoprevention of lung cancer among smokers found that daily intake of NSAID (aspirin or ibuprofen) for at least 2 years is associated with a 68% reduction of relative lung cancer (relative risk 0.32; $p<0.01$) [84]. Specific inhibitors of COX-2, one of two enzymes catalysing prostaglandin synthesis, and inducted by growth factors, oncogenes or carcinogens, are in study. Elevated levels of prostaglandin, whose proangiogenic effect was demonstrated, have been observed in head-and-neck cancers. COX-2 overexpression in epithelial cells inhibits apoptosis, favours genetic damage accumulation and allows the transformation of carcinogens into active metabolites. COX-2 mRNA levels are 150 times higher in head-and-neck cancers than normal oral mucous of healthy persons, and 50 times higher in normal epithelium adjacent to the tumour [85].

Development of molecular-targeted therapies is the next step in the therapy and prevention of cancer. As described before, EGFR is overexpressed in lung cancer and in premalignant lesions and seems therefore to be an accurate target for chemoprevention. Mutations in the RAS family are very frequently observed in lung carcinogenesis and targeting this pathway could also be attractive. However, the question is not only the identification of the best-targeted drug but also the adequate strategy to prove its efficiency. Chemopreventive studies are time-consuming studies that require many patients. In this setting, they would also become very expensive studies. Targeting high-risk populations and making use of potential intermediate biomarkers could significantly reduce the time and resources required for chemoprevention trials. Recent efforts have focused on the definition of these biomarkers of early carcinogenesis. The aim is to define at the biological level the epidemiological variable ‘increased risk of cancer’. Defining such risk biomarkers has multiples advantages. These biomarkers would allow the follow-up of cancer at the molecular level and not merely at the phenotypic level. They could potentially be used as intermediate endpoints to evaluate the efficacy of chemopreventive strategies. Overall this approach has multiple advantages, such as shortening of the follow-up period and reducing the size of the cohort to treat [4]. However, there is still a need to definitively validate biomarkers with hard clinical criteria (secondary cancer appearing, cancer-related mortality, etc.).
Actually, new chemoprevention trials have been designed. They will focus on studying promising new biological agents in randomized phase II setting. Patients will have tissue and serum collected at specific points in the hope of developing a risk model for lung cancer development. These types of trials would be accrued within 3 years with endpoints assessed in 5–6 years. Any promising evidence would be applied into larger phase III trials for definitive testing. Members of the Lung Cancer Biomarkers Chemoprevention Consortium (an NCI-funded programme) propose to test two drugs: ZD1839, an EGFR inhibitor, and R115777, a farnesyl transferase inhibitor. Two randomized, placebo-controlled, double-blind, multi-institutional phase II trials are planned to investigate the reversal of premalignant bronchial lesions. The primary endpoint will be improvement in bronchial histology and the secondary endpoint will be Ki67 status, a proliferation indicator. Patients must have had a previous, definitively treated tobacco-related cancer (lung, head-and-neck, bladder, oesophagus), a 30-pack-year smoking history and confirmed sputum atypia. They will then be treated for 6 to 12 months, with serial bronchoscopies.

Finally, future chemoprevention studies will probably consider association of targeted agents to maximize preventive effect. Trials using tyrosine-kinase inhibitors (especially EGFR), farnesyl-transferase inhibitors and/or antiangiogenic molecules are expected.

In addition, it is obvious that one of the best preventive approaches is avoiding tobacco consumption. Health policy campaigns are essential in this area. They should be enriched by studies on pharmacological agents able to fight nicotine dependence in persons at risk. In that regard, analysis of the genetic polymorphism implicating D2 dopamine receptor and the enzyme cytochrome P2A6 [86], involved in nicotine dependence, are very promising.

### 5 Conclusion

The incidence and mortality associated with lung cancer has not been significantly modified over the last 25 years, despite the introduction of new cytotoxic drugs and development of multidisciplinary approaches combining surgery, chemotherapy and radiotherapy. Such considerations highlight the need to develop and reinforce chemopreventive approaches. Preliminary results demonstrating a retinoid efficacy to prevent cancer or revert premalignant lesions in the oral cavity and the larynx have not been confirmed in the setting of lung carcinogenesis. New agents have been identified through a better understanding of lung carcinogenesis and are currently being evaluated for chemoprevention: COX-2, EGFR and farnesyl transferase inhibitors. Complete characterization of molecular determinants of lung or head-and-
neck carcinogenesis is essential to enable rational and targeted development of chemopreventive agents. Modern chemoprevention trials should include an evaluation of biological markers of carcinogenesis in order to establish molecular risk models. This new approach of chemoprevention, based on a better comprehension of carcinogenic mechanisms and the use of targeted agents, is quite costly but very promising.

Search strategy and selection criteria: Data for this review were identified by searches of PubMed and references from relevant articles. Articles were found using the search terms 'lung cancer', 'chemoprevention', 'carcinogenesis', 'oncogenes' and 'retinoids'. Only papers published in English were included.

References


