

## Review articles • Artykuły przeglądowe

## Lung cancer chemoprevention – focus on surrogate markers

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*Lung cancer remains the leading cause of cancer-related mortality worldwide. Recent therapeutic improvements are unlikely to change the dramatic prognosis of this disease. This review focuses on completed lung cancer chemoprevention studies and presents future perspectives in relation to recent achievements of molecular biology. Special emphasis is given to surrogate endpoints as these are increasingly being incorporated into currently performed chemoprevention clinical trials.*

**Prewencja farmakologiczna raka płuca z uwzględnieniem zastępczych wskaźników oceny skuteczności**

*Rak płuca jest najczęstszą przyczyną zgonu wśród nowotworów złośliwych na świecie. Pomimo znacznych postępów w metodach leczenia, rokowanie w tym nowotworze jest nadal niepomyślne. Niniejsza praca podsumowuje wyniki dotychczas przeprowadzonych badań klinicznych w zakresie prewencji farmakologicznej raka płuca i przedstawia perspektywy rozwoju tych badań w świetle postępów biologii molekularnej. Szczególną uwagę zwrócono na omówienie zastępczych wskaźników oceny skuteczności, coraz częściej stosowanych w badaniach prewencji farmakologicznej.*

**Key words:** lung cancer, chemoprevention, surrogate endpoints

**Słowa kluczowe:** rak płuca, prewencja farmakologiczna, zastępcze wskaźniki oceny skuteczności

**Background**

Lung cancer remains the leading cause of cancer-related deaths worldwide [1, 2]. Recent therapeutic improvements in this disease have not substantially changed the prognosis, with only 10–15% patients surviving 5 years from the diagnosis. There is an urgent need for new strategies that will impact on the global incidence of lung cancer. Chemoprevention is an attractive concept of basic and clinical research. Chemoprevention strategies are expected to change the grim lung cancer statistics. The aim of this article is to review the existing clinical data and to present the future perspectives on lung cancer chemoprevention. Special emphasis is given to the controversial issue of surrogate endpoint validation and its application in the design of chemoprevention studies.

Tobacco remains the leading cause of lung cancer worldwide, as about 90% of cases in men and 70–80% of cases in women are related to active smoking. Epidemiological studies suggest a different susceptibility of individuals to the genotoxic activity of tobacco exposure. This is largely due to the polymorphism of genes coding liver activation (cytochrome P450) and

detoxication enzymes (glutathione transferases) [3]. Familial occurrence of lung cancer is relatively rare; however there is some evidence of dominant, high penetrance oncogenes involved in the development of lung cancer.

Currently, most new lung cancer patients are ex-smokers, and this situation will probably continue for the next decades [4]. It is therefore important to emphasize the need for the development of further strategies aimed at prevention, inhibition or reversal of the carcinogenesis process. These strategies should optimally involve a selected population of high-risk individuals and use appropriate surrogate markers for efficacy evaluation [5].

**Completed chemoprevention trials in lung cancer**

Retrospective epidemiological studies performed over the last two decades indicate that a diet abundant in fruit and vegetables may decrease the incidence of lung cancer [6]. The possible compounds considered to be linked to decreased risk of lung cancer include retinoids – vitamin A (retinol) derivatives, and carotenoids – conjugated polyene molecules with antioxidant properties. Retinoids regulate gene expression and signal transduction through two classes of nuclear retinoid receptors – RAR and RXR. In both classes, at least three subtypes of receptors exist, namely  $\alpha$ ,  $\beta$ , and  $\gamma$ . These findings, together with a number of laboratory studies that have elucidated the possible mechanisms of the action of vitamin A

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derivatives, led to the development of chemoprevention trials conducted in the last two decades. These trials were designed to evaluate chemoprevention strategies on different levels: primary (healthy high-risk individuals, e.g. smokers), secondary (pre-malignant lesions) and tertiary (second primary tumors [SPTs] in previously treated patients).

Three randomized, large phase III clinical studies have been conducted in a primary prevention setting: the Alpha-Tocopherol, Beta-Carotene (ATBC) study [7], the Beta-Carotene and Retinol Efficacy Trial (CARET, [8]) and the Physicians' Health Study [9]. The ATBC trial involved more than 29,000 previous and current smokers, with a smoking history of five or more cigarettes per day. Study subjects were randomized to beta-carotene, vitamin E or both. In the CARET study, more than 18,000 patients with a history of asbestos exposure (over 15 years) or smoking history of at least 20 cigarette pack-years were randomized to beta-carotene and vitamin A, or placebo. The Physicians' Health Study differed significantly from the previous two trials – smokers constituted only 11% of the study population. In this trial, about 22,000 US male physicians with no history of cancer, myocardial infarction or stroke were randomly assigned to beta-carotene or placebo.

In the ATBC study, an increase of 18% in the incidence of lung cancer was found in the treatment arm, and there was a paradoxical harmful effect of beta-carotene/vitamin E. In the CARET study, lung cancer occurred more frequently in the study group, yielding a relative risk of 1.28. Interestingly, a subgroup analysis of current versus former smokers led to strikingly different relative risks of lung cancer incidence of 1.42 and 0.80, respectively, compared to controls. In the Physicians' Health Study, no effect of beta-carotene on lung cancer incidence was found (relative risk of 0.93). The former

two studies showed that the use of high dose beta-carotene in current smokers leads to an increased risk of lung cancer incidence and mortality.

Another important prospective primary chemoprevention study included former crocidolite (blue asbestos) workers from Western Australia. The subjects who expressed their interest in the program were randomly allocated to 30 mg of beta-carotene or 25,000 IU of retinol daily. The first report from this study compared overall and disease-specific mortalities in both intervention groups combined vs the outcome in subjects who did not participate in the trial [10]. Interestingly, the adjusted relative risk of death from all causes was 0.64 in the intervention groups (95% CI: 0.47-0.88), indicating overall protective effect. The adjusted relative risks of developing lung cancer and mesothelioma were non-significant (0.67; 95% CI: 0.33-1.37 and 0.77; 95% CI: 0.38-1.55, respectively), and both risks differences decreased with the duration of retinoid exposure. The obvious selection bias and the relatively small number of subjects in this observational part of the study hamper the interpretation of the results. In the main publication reporting trial results, no lung cancer risk differences were found between former asbestos mine workers randomized to beta-carotene or retinol [11]. However, the risk of developing malignant mesothelioma was significantly lower in the retinol group compared to that using beta-carotene (0.24; 95% CI: 0.07-0.86).

The secondary prevention trials were aimed at the reduction of sputum atypia (two studies, [12, 13]) and metaplasia (two studies, [14, 15]). These studies included smokers and evaluated the impact of alpha-tocopherol, beta-carotene, retinol, retinyl palmitate and isotretinoin. No significant reduction of the evaluated markers was observed (Table I), and smoking cessation was the only

**Table I. Completed chemoprevention trials in lung cancer**

[ref]	Prevention setting	Study group	Number of patients	Agents	Endpoints	Result (RR; p-value)
[7]	I	Smokers	29.133	BC/Vit. E	Lung cancer incidence	Harmful (1.18; 0.01)
[8]	I	Smokers, asbestos	18.314	BC+RA	Lung cancer incidence	Harmful (1.28; 0.02)
[9]	I	US physicians	22.071	BC	Lung cancer incidence	NS
[10]	I	Asbestos	2.199	BC/Retinol	Lung cancer incidence Mesothelioma incidence	NS NS
[12]	II	Sputum atypia	150	Etretinate	Sputum atypia	NS
[13]	II	Sputum atypia	755	BC+RA	Sputum atypia	NS
[14]	II	Metaplasia	152	Isotretinoin	Metaplasia index	NS
[15]	II	Metaplasia	139	Fenretinide	Metaplasia index	NS
[19]	III	NSCLC	1486	Isotretinoin	Rate of second primary tumors	NS
[20]	III	NSCLC+HNC	2.592	N-acetylcysteine/ Retinyl palmitate	Rate of second primary tumors	NS
[18]	III	NSCLC	307	Retinyl palmitate	Rate of second primary tumors	Positive (?; 0.045)

Abbreviations:

I – primary prevention, II – secondary prevention, III – tertiary prevention;

RR – relative risk, NS – not significant, BC – beta carotene, Vit. E – vitamin E, RA – retinoid acid;

NSCLC – non-small cell lung cancer, HNC – head and neck cancer

factor related to decreased metaplasia index and cell proliferation [15].

Three phase III studies of tertiary chemoprevention have been conducted in lung cancer, with high expectations after positive results of short-term retinoid assessment in head and neck cancer [16, 17].

The smallest of the three studies, reported by Pastorino et al. [18], showed a borderline ( $p=0.045$ ) increase of time to SPT or recurrence in a group of 307 stage I NSCLC patients randomly assigned to 12 month administration of retinol palmitate or placebo. This effect was not translated into survival benefit.

In the phase III Lung Intergroup Trial, low-dose isotretinoin was compared to placebo in a cohort of 1166 NSCLC pathologic stage I patients, randomized six weeks to three years after surgery [19]. No reduction in the rate of SPTs was observed, and neither was there a reduction in recurrence or morbidity rate in the study group.

In the Euroscan, EORTC open-label study, retinyl palmitate and/or N-acetylcysteine was assessed with a 2 x 2 factorial design in 2592 lung cancer and early stage head and neck patients who underwent curative therapy [20]. The study was designed in both tumor types, due to similar etiological and biological factors. This study failed to show a clinical benefit of either agent on the incidence of SPTs, event-free or overall survival. Importantly, in the two above-mentioned studies, smoking cessation was associated with better survival of the trial participants.

Reduction in SPTs after definite surgery for stage I NSCLC is sought in a currently ongoing US intergroup clinical study with planned accrual of 1960 patients, evaluating the role of selenium supplementation. The rationale for this study came from a number of epidemiological studies suggesting an association between low selenium concentration and the risk of cancer [21, 22]. The recently updated Nutritional Prevention of Cancer Trial indicated that selenium supplementation in non-melanoma skin cancer patients results in a non-significant reduction of lung cancer incidence [23]. In the subgroup analysis, this finding was significant in patients with lowest pre-study selenium plasma levels, however the number of events in the study did not allow for any firm conclusions. The precise mechanism of the chemopreventive action of selenium remains unknown, although several molecular targets for this effect have been postulated [24]. Completed lung cancer chemoprevention studies are summarized in Table I.

### **Biology of lung cancer premalignant lesions and rationale for selection of surrogate markers**

The chemoprevention studies in NSCLC involved more than 70,000 individuals and lasted more than a decade. The overwhelming logistical and economic burden of these trials and their neutral or even harmful results obviate the need for redefinition of the endpoints in the design of future studies. It is speculated that a surrogate endpoint may be a laboratory examination or a physical sign which is a prerequisite of a meaningful endpoint,

such as lung cancer incidence or mortality [25, 26]. Surrogate endpoints may greatly improve the efficiency and reduce the costs of clinical trials, mainly through decreasing required sample size and shortening study duration. However, the appropriate validation of surrogate markers and their predictive value for the true clinical endpoints of the intervention remain to be confirmed.

Recently, much attention was given to identifying and validating molecular events preceding the development of lung cancer as potential surrogate markers in chemoprevention studies. It is not the intention of this review to cover the molecular biology of premalignant lesions (recent excellent reviews include: Winterhalder, 2004 [5] Franklin, 2000 [27], Wistuba, 1999 [28], Niklinski, 2002 [29]) but rather to point out these abnormalities, which are of potential relevance in the design and conduct of chemoprevention trials in lung cancer.

### **Preneoplastic bronchial lesions detected on the level of tissue and cell morphology**

Several preinvasive changes, divided into squamous and non-squamous, have been described and correlated to the development of lung cancer [27]. Squamous premalignant lesions include metaplasia, dysplasia and carcinoma *in situ*; the latter two are included in the current WHO classification of lung neoplasms [30]. On the cellular level, these changes may be reflected by atypia, often detected by sputum cytology. As some studies indicate that metaplasia is often reversible while dysplasia is not [28], the latter seems to be a more appropriate indicator for lung cancer risk assessment and monitoring of an intervention. To quantify metaplasia in a more consistent manner, a metaplasia index was developed [31]. This index was also used in the two previously mentioned lung cancer secondary prevention trials [14, 15]. The proportion of metaplastic and dysplastic changes in bronchial epithelium seems to depend on the studied population and risk definition. Results of a study performed at the M. D. Anderson Cancer Center demonstrated that among high-risk participants (history of over 20 pack-years who quit smoking for one year), metaplasia was most prevalent [32]. In contrast to this finding, more than half of the patients in the Colorado study on high-risk patients (smoking history of over 30 pack-years, chronic obstructive pulmonary disease and sputum atypia) had moderate or high-grade dysplasia or carcinoma *in situ* [33]. Sputum atypia as a marker for lung cancer risk assessment is currently a subject of investigation in this study. Preliminary results demonstrated a relative risk of 2.4 for developing lung cancer in patients with moderate or severe atypia in sputum compared to their counterparts without sputum atypia. By adding information on the DNA hypermethylation status of eight genes in the sputum, the relative risk of lung cancer development increased to 7.9 [34].

Pathologic criteria of premalignant lesions may not adequately reflect the risk for all lung cancer types.

Currently, no morphological abnormality preceding the development of small-cell lung cancer (SCLC) has been identified. Diffuse idiopathic neuroendocrine cell hyperplasia has been linked to the development of carcinoid tumors, although there are no strong data to support this relation. Atypical adenomatous hyperplasia (AAH) has been described as peripheral epithelial cell proliferation with minimal cytologic atypia and stromal response [27]. This lesion is regarded as a preinvasive counterpart of adenocarcinoma, but there have been no prospective studies to clearly establish this link and quantify the risk. A practical difficulty is the frequent occurrence of these lesions in the small bronchioles, not accessible by bronchoscopy.

#### Cytogenetic and molecular biomarkers

A number of molecular abnormalities on DNA, RNA or protein level were described in premalignant bronchial lesions as possible candidates for lung cancer risk assessment. These findings were also correlated to morphologic premalignant changes detected by fluorescent bronchoscopy and microdissection techniques [35, 36]. Candidate risk markers include Ki67, p53, retinoid acid receptors, *c-myc*, *erbB-1*, *erbB-2*, cyclin D1, cyclin E, *bcl-2/bax*, and gene hypermethylation markers [37]. Multiple marker assessment and multivariate analysis in the same population of individuals may yield additional information on the risk of lung cancer development [38]. Such an analysis is now also technically feasible with microchip gene array or tissue array technology.

Despite the above developments, for several reasons these biomarkers cannot yet be considered as useful candidates for surrogate markers in chemoprevention studies. First, adequately low interobserver and interlaboratory variability must be established for any test to be reproducible. Second, these markers have to be validated in properly designed nested case-control studies in untreated high risk cohorts, and require adequate follow-up. An example of such a study currently under way is the Colorado High Risk Cohort Study, initiated in 1993. In this study, high-risk individuals with sputum atypia are evaluated annually with sputum cytology and tumor suppressor gene methylation analysis of the sputum, and many individuals are followed up by fluorescence bronchoscopy.

Molecular markers may also be used to quantify the risk of lung cancer in relation to genetic susceptibility, based on the polymorphism of carcinogen activation and detoxification, as well as DNA repair enzymes [39, 40]. In a recent case-control study including 136 individuals, low activity of DNA oxidative damage repair enzyme OGG (8-oxoguanine DNA *N*-glycosylase) was linked with significantly higher odds for lung cancer development [41]. Interestingly, the estimated risk associated with low OGG activity was very high and independent of the risk caused by smoking. This study, as well as others focusing on cytochrome P450 and glutathione S-transferase

isoenzyme polymorphisms, may better define the high-risk population and facilitate the proper evaluation of chemoprevention measures.

#### Novel agents in chemoprevention trials

The above-mentioned molecular events improve our understanding of lung cancer development, but also serve as molecular targets for an early intervention. There are a number of new agents considered as possible candidates for chemoprevention and of those several are already tested within clinical trials.

Eicosanoid pathway plays an important role in the development of invasive malignancy from epithelial premalignant lesions in many cancer types, including NSCLC. Cyclooxygenase-2 (COX-2) is an inducible enzyme responsible for the formation of prostaglandins and thromboxanes from arachidonic acid. The high activity of this enzyme in tumors is also linked with their aggressiveness through upregulation of angiogenesis, upregulation of matrix metalloproteinases (invasiveness) and inhibition of apoptosis [42]. In a case-control epidemiological study, the two-year use of non-selective anti-inflammatory drugs or aspirin was associated with an almost 70% reduction of lung cancer risk [43]. Selective COX-2 inhibitors (coxibs) are thus considered potential chemoprevention agents and are currently tested in a series of clinical trials. However, the recent withdrawal of rofecoxib from the market due to high rate of cardiovascular events clearly indicates the need for careful toxicity evaluation of other selective COX-2 inhibitors. A potential candidate for chemoprevention is lipooxygenase (LOX), an enzyme involved in the conversion of arachidonic acid to leukotrienes [5]. LOX inhibitors are already used in the treatment of asthma and other inflammatory diseases. Another potentially chemopreventive substance, already validated in a phase I/II chemoprevention study, is prostacyclin PGI<sub>2</sub> analogue (iloprost), as its high levels were found to reduce tumor formation in mice exposed to lung cancer carcinogens [44].

The members of epidermal growth factor receptor (EGFR) family mediate the signal transduction for EGF, an important mediator of epithelial proliferation involved in the pathogenesis of NSCLC. Moreover, recent data indicate that EGFR tyrosine kinase mutational activation is the leading molecular abnormality in a subpopulation of NSCLC patients with specific features – non-smoking females with adenocarcinoma [45, 46]. Novel agents targeting EGF family include its tyrosine kinase inhibitors (gefitinib, erlotinib and dual inhibitors – e.g. GW572016) and monoclonal antibodies against their receptors (cetuximab and trastuzumab targeting respectively EGFR [erbB1] and erbB2). Due to the frequent heterodimerization of EGF family members, dual inhibition of erbB1 and erbB2 seems an attractive strategy of chemoprevention and treatment of lung cancer.

Mutation of *K-ras* gene occurs in 20-40% of NSCLCs and is an important molecular event in tumors with intact

EGFR pathway. Ras protein expression may be inhibited through farnesyl transferase inhibitors, agents that also block the expression of downstream signal mediators rho and raf. Thus, also farnesyl transferase inhibitors may be considered potential candidates for chemoprevention trials.

Dithioloethiones are organosulfur compounds acting mainly through the increased expression of phase II detoxication enzymes (glutathione transferases) and through free-radical scavenging. This class of agents includes oltipraz and anethole dithioloethione. The latter compound was evaluated in a phase II randomized chemoprevention study in smokers with bronchial dysplasia monitored by serial fluorescent bronchoscopy [47]. Although no histological regression of dysplasia was observed in this study, the dysplasia progression was significantly delayed in the subjects receiving anethole dithioloethione as compared with placebo.

#### Surrogate markers – surrogate evidence?

The need to include surrogate end-points is increasingly important in cancer chemoprevention studies. There is, however, considerable controversy about the usefulness of these endpoints for clinical interventions, and this controversy is supported by numerous studies showing an apparent improvement in surrogate endpoint but no benefit or even a harmful effect on clinical outcome, such as incidence or mortality [48].

An example of a study that demonstrated the paradoxically harmful clinical relevance of a potentially useful surrogate marker is the Cardiac Arrhythmia Suppression Trial (CAST [49, 50]). In this study, the reduction of ventricular ectopic contractions (a surrogate) by one of three class I antiarrhythmic agents (encainide, flecainide and moricizine) was expected to result in a decreased cardiovascular-related mortality (true endpoint). At the time the trial was conducted, the drugs had already been approved by the Food and Drug Administration (FDA) and were used in daily practice. Although the effect on a surrogate was achieved, the number of deaths was significantly higher in the groups randomly assigned to one of the three drugs, compared with placebo. Clearly, a harmful effect measured by overall survival was demonstrated for each drug, indicating the unreliability of surrogate endpoints used earlier in the drug approval process.

To be valid a surrogate endpoint must closely correlate with the true clinical endpoint. More importantly, it must also predict the effect of intervention on clinical outcome [25]. This frequently unrecognized principle may not be met for many studies, and therefore should be validated before a phase III clinical intervention trial is set. In statistical terms, these requirements may be indicated by two coefficients: the proportion of treatment effect explained by a surrogate marker (PE) and the ratio of the effects of treatment upon the final and surrogate endpoint (RE, [51]). A number of methods have been developed to calculate these parameters and their

confidence intervals in order to evaluate the validity of the surrogate endpoint [51, 52]. These parameters may also be used to compare the usefulness of different surrogates in the studied population in terms of their adherence to clinical outcomes.

The lack of the intervention effect on a surrogate endpoint may also lead to the exclusion of a potentially valuable treatment. In a study evaluating interferon-gamma in immunocompromised children with chronic granulomatous disease (CGD), this treatment was expected to result in a more effective generation of oxygen burst in mononuclear cells as a response to infection. Surprisingly, the trial showed a significant reduction of the rate of serious infections, without any impact on a surrogate endpoint [48, 53].

#### Conclusions

Chemoprevention studies in lung cancer completed within the last two decades indicate the need for the implementation of surrogate endpoints in the design of future trials in order to perform these studies effectively and with fast turnover. A large number of potentially active chemoprevention agents are available today. Given the large number of new molecular targeted therapies potentially reducing the incidence of lung cancer in high-risk populations, there is a growing demand to properly evaluate these endpoints before commencing the trial. The necessity for the proper validation of surrogate endpoints is indicated by numerous examples of misleading preliminary findings.

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