Aus der II. Medizinischen Klinik des Klinikums Augsburg
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Retrospective study to evaluate the prognostic effect of smoking cessation in patients with advanced lung cancer under palliative anticancer therapy

Dissertation
zum Erwerb des Doktorgrades der Medizin
an der Medizinischen Fakultät der
Ludwig-Maximilians-Universität zu München

vorgelegt von
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aus
Morogoro

2005

Mit Genehmigung der Medizinischen Fakultät
der Universität München

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Tag der mündlichen Prüfung:	03.03.2005

Preface

Whereas epidemiological studies have increased our understanding and knowledge regarding the role of cigarette smoking in the etiology of lung cancer development, less is known about whether discontinuation of smoking habits after diagnosis affects the prognosis of lung cancer patients. Many prognostic factors in lung cancer have been described such as morphological, molecular, and biochemical markers, and play important roles in determination of disease course and patients' survival. Advances in staging and classification of tumours as well as identification of potential prognostic factors will help us to make correct, scientifically based treatment decisions favourable to patients. Frequent review of established and assessment of new prognostic factors are therefore required for creating best treatment strategies.

Augsburg - Germany, November 2004 S. P. Deke

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1. Introduction

Cigarette smoking is the most important etiological factor in the causes of development of lung cancer. In the early 50s scientists published articles correlating tobacco smoking and carcinoma of the lung. Recent studies have shown a strong coherence between the rising prevalence of lung cancer and increasing cigarettes consumption. Tobacco use is estimated to be responsible for almost 90% of all cases of primary pulmonary malignancies. Cigarette smoking is also strongly associated with development of other cancer types such as oral, laryngeal, bladder cancer as well as carcinoma of the oesophagus. Smoking increases significantly the risks of development of coronary artery disease, chronic obstructive pulmonary disease (COPD), and other respiratory and vascular diseases. Smoking leads therefore to increased morbidity and mortality due to cardiovascular, neoplastic, and other related diseases [119].

Lung cancer remains the most frequently diagnosed malignant neoplasm with enormous public health implications as it is one of the leading causes of cancer mortality throughout the world. The cure rate of lung cancer using the major currently existing treatment modalities (surgery, chemotherapy and radiotherapy) is still very low and has not essentially improved for the past 20 years. While localized and early stage disease can be cured by surgery, the management of local advanced and metastasized pulmonary malignancies frequently requires multimodal therapeutic approaches under palliative aspect. In large measure, lung cancer patients are treated palliative, either primarily or secondarily. Although the implementation of new treatment regimes using surgery, chemotherapy and/or radiotherapy has improved the ability to prolong survival, the prognosis for the majority of lung cancer patients remains still poor. The palliative treatment using the present systemic or local anticancer therapies gives only moderate survival

chance to inoperable patients or those who suffer from advanced disease. The improvement of prognosis and quality of life (QOL), especially in patients with advanced lung cancer, is therefore an important clinical issue.

Cigarette smoking affects physiological processes in various ways in the body. Cigarette smoke (CS) is a complex mixture containing thousands of chemical substances, some of which are toxic or carcinogenic. Moreover, cigarette smoke contains and generates reactive oxygen species (ROS) which can lead to oxidative stress in the lung and other organs. The carcinogens, oxidants, and a number of toxic substances have direct or indirect, modulatory or damaging effects on DNA, membrane lipids, cell signalling proteins, and various macromolecules. These effects are considered as the major paradigms by which many diseases such as lung cancer and COPD develop [55,107].

Smoking cessation results have been poor among many lung cancer patients as nicotine in cigarette smoke is a strong addictive substance. Despite the fact that the survival expectancy of majority of lung cancer patients is very limited, there are different opinions as to whether smoking cessation can improve the overall survival and quality of life. At the same time, the abrupt stop of tobacco consumption in nicotine-addicted patients may result in withdrawal symptoms, leading to physical and psychovegetative reactions. This may bring about negative effects on the expected quality of life (quantity *vs.* quality of life). Although the benefits of quitting from smoking have often been explained such as moderate improvement in lung function, decrease in incidences of pulmonary symptoms and infections, as well as better socio-environmental integration, the question of prognosis and quality of life after smoking cessation among this patients' group is still open. The following work will therefore review carcinogens and oxidants in CS, as

well as discuss about important mechanisms and factors linking cigarette smoking, lung cancer development and tumour progression (metastasis, invasion, recurrence and therapy resistance). It will also focus on various effects of tobacco smoke, including involvement of smoking in COPD development, as well as possible withdrawal problems in nicotine-addicted patients. It is thereby the major aim of this study to find out how the prognosis of patients with advanced lung cancer can be influenced, if these patients stop their smoking habits prior to beginning of palliative anticancer therapy. The major groups of this study include patients who stopped, and those who continued smoking after histologically confirmed diagnosis of small-cell lung cancer (SCLC) and non-small-cell lung cancer (NSCLC).

2. Carcinogens and oxidants in cigarette smoke

2.1 Carcinogens

Carcinogens are physical, biological, or chemical factors which can cause development of malignant tumours**.** Tobacco smoke, which exists in two major phases, namely the gas phase and particulate (tar) phase, has a large number of chemical carcinogens. About 95% of CS is made up of gases, mainly nitrogen, oxygen, and carbon dioxide. Using a glass-fiber filter, the gas phase can be separated from the particulate phase, which contains about 3500 chemical compounds and the most of the carcinogens found in CS. Both the mainstream cigarette smoke (MSCS) emerging from the mouthpiece and the sidestream cigarette smoke (SSCS), which comes out from the burning cigarette tip, contain carcinogens. So far, over 50 different chemical carcinogens have been identified in CS. Some of the carcinogens found in tobacco smoke are listed in the table below.

Table 1: Some selected examples of carcinogens identified in cigarette smoke

Polycyclic aromatic hydrocarbons (PAHs)	
Anthanthrene	Benzo(k)fluoranthane+
Benz(a)anthracene	Cyclopenta(cd)pyrene
Benzo(a)pyrene+	Dibenz(a,h)anthracene+
Benzo(b)fluoranthene+	Dibenzo(a,i)pyrene+
Benzo(c)phenanthrene	5-Methylchrysene+
Benzo(ghi)fluoranthene	Indeno(1,2,3-cd)pyrene+
Benzo(ghi)perylene	2,1-BNT
Benzo(j)fluoranthene+	

Tobacco-specific N-nitrosamines (TSNAs)	
NNK+	N-nitrosoanatabine
N-nitrosodiethylamine+	N-nitrosoanabasine
N-nitrosodimethylamine	N-nitrosopyrrolidine
N-nitrosonornicotine	

Aromatic amines	Aza-arenes	Inorganic substances
4-Aminobiphenyl	Benz(a)acridine	Arsenic*
2-Aminophthaline	Benz(c)acridine	Cadmium+
o-Toluidine	Dibenz(a,h)acridine+	Chromium+
	7H-Dibenzo(c,g)carbazole+	Hydrazine+
		Nickel+
		Polonium-210+

2,1-BNT = Benzo(b)naphtho(2,1-d)thiophene
NNK = 4-(Methylnitrosoamino)-1-(3-pyridyl)-1-butanone
\+ = compounds which have convincingly shown pulmonary carcinogenecity in at least one of the following laboratory animals (mouse, rat, hamster)
* = lung or skin carcinogen as indicated by some epidemiological studies

[References: 35, 37, 52 - 56**]**

However, only some of the above mentioned carcinogens will convincingly induce lung tumours in laboratory animals or humans. Benzo[a]pyrene (BaP), the most extensively studied carcinogen among the PAHs, can induce lung tumours when administered local, systemic or via inhalation. In laboratory studies, BaP induces lung tumours in rats and mice. Dibenz[a,h]anthracene, dibenzo[a,i]pyrene, and 5-methylchrysene are stronger pulmonary carcinogens than BaP, but are found in lower concentrations in CS than BaP. The tobacco-specific N-nitrosamine NNK, which is also a potent pulmonary carcinogen, possesses the ability to induce lung tumours in all three commonly used rodent modals (rats, mice and hamsters). This compound shows a remarkable organospecificity for the lung and induces mainly adenoma and adenocarcinoma of the lung; an effect that does not depend on the route by which it is administered. CS contains substantially high amounts of NKK (80-770ng/cigarette), so that the total dose experienced by a smoker in a lifetime of smoking is close to the lowest total dose shown to induce lung tumours in laboratory animals. NNK induces pulmonary malignant tumours via formation of alkylated promutagenic DNA adducts such as O6-methylguanine (O6MG). N-nitrosodiethylamine, which also belongs to the N-nitrosamines, has shown pulmonary tumourigenic effect in hamsters. Its concentrations in CS are lower than those of other carcinogens. Among the aza-arenes, dibenz[a,h]acridine and 7H-dibenzo[c,g]carbazole have shown pulmonary carcinogenic activity when tested in rats and hamsters **[**55**]**.

Some miscellaneous organic compounds found in CS are also pulmonary carcinogens. 1,3-butadiene and ethyl carbamate have shown tumourigenic potential in mice. Inorganic substances such as nickel, chromium, cadmium, arsenic, hydrazine, and polonium-210 are all present in CS in different concentrations which show carcinogenic activity in different animal species,

including rats, mice, and hamsters. Many substances in CS may also act as co-carcinogens (promoters), among them catechols, methylcatechols, semiquinones, pyrogallol, decane and undecane. Moreover, the α,β-unsaturated aldehydes (acrolein and crotonaldehyde) abundantly present in CS are strongly toxic to cells and cilia of the bronchial system. Other compounds in CS such as formaldehyde and acetaldehyde may also have carcinogenic effect on the lung [54].

2.2 Oxidants, ROS formation, and free radicals in cigarette smoke

As a result of metabolic and biochemical processes as well as external factors ROS are continuously formed in the lung and other organs of the body. For instance, mitochondria produce a substantial amount of ROS (e.g. $\cdot O_2^-$ and H_2O_2), which are normally broken down by GSH-dependent peroxidase-catalysed reactions. ROS production is also used by reactive phagocytic cells to destroy microorganisms. Being atoms or molecules, ROS can possess unpaired or paired electrons and are characterised by a particularly high affinity to undergo redox reactions with a number of macromolecules in the body including membrane lipids, cell-signalling proteins, regulatory enzymes, and DNA. Redox modifications of these macromolecules may imply an important functional or structural change to organs. In physiological conditions cells are able to retain redox equilibrium by means of different antioxidant defence systems. Shift of this equilibrium in favour of oxidants leads to inefficient cell protection against noxious effects of ROS and may result in oxidative organ damage [88].

Tobacco smoke is one of the important sources of ROS and plays a significant role in increased oxidative DNA damage and modulation of different biochemical pathways in normal and neoplastic lung cells. Nicotine abuse leads therefore to excessive oxidative burden to lung cancer

patients. Cigarette smoke is a complex mixture of more than 4,700 chemical compounds, including high concentrations of free radicals and other oxidants. Free radicals in CS are derived from both the gas and the particulate phase. Pryor and Stone [108] have reported that the gas-phase CS contains approximately 1015 radicals per puff, primarily of the alkyl and peroxyl types. In addition, nitric oxide (NO) is present in high concentrations in CS (500 - 1000 ppm). Nitric oxide reacts quickly with superoxide anion ($\cdot O_2^-$) to form peroxynitrite ($ONOO^-$), and with peroxyl radicals to give alkyl peroxynitrites (ROONO). Cigarette smoke tar contains more than 1018 free radicals per gram. The radicals in the particulate phase of CS are more stable and predominantly organic. The quinone-hydroquinone complex forms the major free radical species in this CS phase. It is hypothesized that the tar radical system exists in an equilibrium mixture composed of quinones, semiquinones and hydroquinones. It is suggested that the quinone-hydroquinone complex forms a redox cycling system that can generate ·O2⁻ from molecular oxygen and leads ultimately to formation of hydrogen peroxide (H_2O_2) and hydroxyl radical (·OH). Whereas short-lived radicals in the gas phase of CS may be quenched immediately in the ELF redox reactions in cigarette smoke condensate (CSC), which forms in the epithelial lining fluid, may produce ROS for a considerable time. Moreover, CSC can react with or complex some metal cations (e.g. Fe^{3+}, Cu^{2+}), followed by their release or deposition in the lung. Cigarette tar semiquinone is an effective metal chelator and can bind iron to produce the tar-semiquinone + Fe¦+, which generates H_2O_2. Cigarette smoking results also in iron being released from ferritin. Since iron is strongly catalytic in many redox reactions, it can participate in generation of ROS and free radicals. In presence of free iron or copper ions, the strongly reactive ·OH is formed from the less reactive H_2O_2. Following CS exposure, additional oxidants, free radicals and ROS, are generated by inflammatory

cells in the lung. Whereas a nitric oxide synthetase (NO-synthetase) is responsible for the NO synthesis in macrophages, neutrophils do possess myeloperoxidase (MPO), an enzyme that produces HOCl, an oxidation product of chloride ions (Cl^-). In response to CS, reactive phagocytes, including monocytes, neutrophils, and alveolar macrophages (AM), do produce and release $\cdot O_2^-$ and NO, which can react together to produce a highly reactive $ONOO^-$ [10,36].

2.2.1 Evidences for cigarette smoke-induced oxidative stress

There are different ways by which oxidative stress in the body can be detected following exposure to CS. These include analysis of various antioxidant defence mechanisms, analysis of molecular and biochemical products of oxidative stress, and pulmonary inflammatory and immune responses. Detection of oxidative stress following cigarette smoking can be done in lung tissues, respiratory epithelial lining fluid (RELF), breath, blood, and excretory body fluids such as urine. Different antioxidant defence mechanisms may involve antioxidant enzymes, metal-binding molecules, and some micronutrients such as vitamins. Important enzymes involved in the antioxidant defences are the glutathione system (GSH-GSSG), superoxide dismutase (SOD), and catalase. Substances such as ascorbic acid (vitamin C), α-tocopherol (vitamin E), β-carotene, albumin-SH, uric acid, bilirubin, and iron or copper binding proteins like transferrin, ferritin and coeruloplasmin, are also essential in conferring protection against oxidative stress [34].

In a study conducted by Abou-Seif [1], smoking was associated with a decrease in plasma concentrations of vitamin E and C, uric acid, and ceruloplasmin. Moreover, while the blood glutathione peroxidase (GPx) activities were decreased, the activities of catalase, erythrocyte and plasma

SOD were elevated in smokers compared to the corresponding levels of the control individuals, showing that tobacco use is associated with oxidative stress which leads to depletion of some antioxidant systems as well as activation of antioxidant enzymes SOD and catalase which act against oxidative stress. Similar results were obtained in the study of Lykkesfeldt et al. [82]. Using an enzyme-linked assay for ascorbic acid (reduced form) and its oxidised form, dehydroascorbic acid, it could be observed that smoking was associated with depletion of the ascorbic acid pool and reduced capacity to maintain ascorbic acid in its reduced form in the plasma, suggesting that smoking leads to oxidative stress.

Despite continuous extensive repair, oxidatively modified DNA is abundant in normal and neoplastic tissues. The accumulation of damaged nucleosides takes place in both nuclear and mitochondrial DNA. The molecular biomarkers of oxidative DNA damage include modifications in DNA isolated from target tissues or cells, and urinary excretion of oxidised nucleosides and bases as repair products. In a molecular biology laboratory study conducted by Howald et al. [63], mice were subjected to CS. After 30min. single and triple CS exposure, a DNA analysis of tissues from lung, heart, and liver revealed a significant increase of the presence of the oxidative product 8-hydroxy-2'-deoxyguanosin (8-OHdG) above the control levels. While the tissues of group 1 animals were analysed immediately following the CS exposure, group 2 animals were allowed to rest for 90min. prior to excision and analysis of the tissues. For the single exposure 8-OHdG lung values, group 1 showed an increase of 40% over the control. Group 2 was greater than control by 99%. This suggests that the mouse lung continues to produce 8-OHdG during the rest non-exposure period. For the triple exposure, the 8-OHdG levels in the lung of group 1 mice increased over control by 63%, and group 2 increased by 54%. The decrease in group

2 levels of 8-OHdG is probably due to repair of some of the DNA damage during the rest period.

To evaluate the effect of cigarette smoking on oxidative stress in the human lung, Asami et al. [6] compared levels of the oxidative DNA adduct 8-OHdG in lung tissues from 14 smokers, 7 ex-smokers and 9 non-smokers. The mean level of 8-OHdG in the lung tissues from smokers was 1.43-fold higher than that of non-smokers with a statistically significant difference of $p = 0.0262$. There was also a positive correlation for the levels of this oxidative DNA damage product in normal lung tissues and the number of cigarettes smoked per day ($p = 0.0132$). Similar results were also demonstrated by Prieme et al. [106], who had carried out a study to investigate the effect of smoking on oxidative DNA modification. The analysis for the content of oxidised nucleoside 8-oxo-7,8-dihydro-2'-deoxyguanosine (8-oxodG) in 24-h urine samples collected from all test persons demonstrated that smoking was associated with increased urinary excretion rate of 8-oxodG. Upon smoking cessation, the oxidative DNA damage was significantly reduced. As with other oxidative DNA lesions, tobacco smoking has consistently been shown to increase the urinary excretion rate of 8-oxodG by 30 – 50%.

Evidence of increased oxidative stress following CS exposure can also be analysed in the blood by measuring the Trolox equivalent anti-oxidant capacity (TEAC) of plasma and the levels of products of lipid peroxidation as indices of overall oxidative stress. In a study, chronic smoking was associated with reduced plasma TEAC and increased levels of lipid peroxidation products, suggesting that smoking leads to an oxidant/antioxidant imbalance due to oxidative stress [109]. One of the important oxidant-induced products of lipid peroxidation is 8-isoprostane, a

prostaglandin-F2 isomer that is formed in vivo by free radical-catalyzed peroxidation of arachidonic acid. This product can be detected in plasma, breath, or urine of individuals subjected to oxidative stress. Montuschi et al. [97] quantified oxidative stress in lungs in patients with COPD and in healthy smokers, as reflected by 8-isoprostane concentrations in breath condensate. This is a non-invasive method to collect airway secretions. The acute effect of smoking on exhaled 8-isoprostane in healthy smokers was assessed. Exhaled 8-isoprostane was measured by a specific enzyme immunoassay in 10 healthy non-smokers and 12 smokers, 25 COPD ex-smokers, and 15 COPD current smokers. 8-Isoprostane concentrations were similar in COPD ex-smokers (40 + 3.1 pg/ml) and current smokers (45 + 3.6 pg/ml) and were increased about 1.8-fold compared with healthy smokers (24 + 2.6 pg/ml, $p < 0.001$), who had 2.2-fold higher 8-isoprostane than healthy non-smokers (10.8 + 0.8 pg/ml, $p < 0.05$). Smoking caused an acute increase in exhaled 8-isoprostane by about 50%. The study showed that free radical production is increased in patients with COPD and that smoking causes an acute increase in oxidative stress. Other biomarkers for estimation of the extent of CS-induced oxidative stress are the level of oxidised methionine in exfoliated bronchial epithelial lining cells, exhalation of hydrogen peroxide and carbon monoxide, xanthine/xanthine oxidase activity in broncho-alveolar lavage fluid (BALF), as well as plasma protein carbonyls and plasma protein sulfhydryl oxidation.

3. Factors contributing to lung cancer development

Lung cancer is largely due to "chronic" exposure of respiratory epithelial cells to carcinogens such as those in CS. As mentioned earlier, tobacco use contributes the greatest part of cases of pulmonary malignancies. The risk of developing lung cancer is directly related to the extent of cigarettes consumption, given in number of pack-years, and defined as the product of

the average number of packs of cigarettes (20 cigarettes/pack) smoked per day multiplied by the number of years smoked. However, the influence of malignancy seems to include a combination of carcinogen exposure, genetic predisposition, immunological factors, viral infections, dietary factors, and exposure to alcohol. Genetic factors determining individual susceptibility to lung cancer development may include differences in expression of important proteins (enzymes) involved in metabolic pathways of tobacco carcinogens, as well as polymorphisms in genes responsible for repair of damaged or adducted DNA.

3.1 Polymorphisms of important proteins (enzymes) involved in metabolism of tobacco carcinogens

(a) Cytochrome P450 gene products

Several studies have investigated the correlation between genetic polymorphisms and susceptibility to malignancy. These studies regarding cytochrome P450 polymorphisms in cancer have provided mechanistic insights into cancer susceptibility with the goal of identifying individuals at a high risk. Hepatic and extrahepatic polymorphic cytochromes P450 (CYP450) are involved in metabolism of tobacco carcinogens. A number of substances contained in CS can act as inducers, substrates or inhibitors of CYP450. Important CYP450 isoenzymes involved in the lung carcinogenesis by converting some procarcinogens into potent carcinogenic metabolites in tobacco smoke are CYP1A1, 1A2, **2A6**, 3A4, 2Cs (e.g. 9, 17, and 19), 2D6, and **2E1**,. In particular, lung cancer has been extensively studied with respect to interactions between CS carcinogens and their metabolizing enzymes **[**9,137**]**.

The CYP2E1 gene product, which is expressed in human lung, liver, kidney and brain, is involved in metabolism of low-molecular-weight compounds,

including ethanol, 1,3-butadiene, and tobacco smoke N-nitrosamines (N-nitrosodimethylamine, N-nitrosodiethylamine, and NNK). The possible association of CYP2E1 polymorphisms with lung cancer has been discussed elsewhere [123]. Consistent with the role for N-nitrosamines in lung cancer development, the polymorphic CYP2E1c1/c1 genotype has shown a 15-fold increase in lung cancer risk in an epidemiological study involved test individuals from different ethnic groups in the USA [147]. In this study, individuals with the susceptible CYP2E1c1/c1 genotype appeared to have developed cancer at an earlier age and with lower cigarette pack-year of exposure than did patients with the c1/c2 or c2/c2 genotypes. Therefore, the data suggest that individuals who lack a c2 allele might be at higher risk for developing lung cancer.

In another similar study, Yamaziki et al. [151] examined the roles of cytochrome P450 enzymes in the activation of the tobacco-smoke related nitrosamines N-nitrosodiethylamine, N-nitrosodimethylamine, N-nitrosonornicotine, NNK and its metabolic alcohol product 4-(methylnitrosamino)-1-(3-pyridyl)-1-butanol in human liver microsomes. They demonstrated that CYP3A4, CYP2D6 and CYP2Cs (9, 17 e.t.c) are not extensively involved in the activation of these nitrosamines, but that CYP2E1 and CYP2A6 were the most important enzymes in catalysing the metabolic activation of nitrosamines. The function and structure of CYP2E1 are highly conserved across species. CYP2E1 is slightly induced in the liver by CS, but its pulmonary expression is substantially more inducible (6.8-fold) than that of CYP1A1 (2-fold), All these studies suggest therefore that CYP2E1 may actively participate in pulmonary carcinogenesis induced by CS.

(b) Glutathione S-transferase gene products

Glutathione S-transferases (GSTs) are important enzymes in detoxification processes in rodents. GSTM1 gene codes for M or mu class glutathione transferases that are involved in detoxification of various pulmonary carcinogens including metabolic activation products of PAHs (e.g. PAH diol epoxides) and CS oxidants. Other human glutathione S-transferases include GSTA, GSTP, and GSTT (alpha, pi, and theta classes). Almost 40%-50% of the human population possess the GSTM1 null genotype [123]. An increasing evidence from various studies show that there is a relationship between GSTM1 null and lung cancer risk. It is hypothesized that the lung cancer risk is elevated in individuals possessing GSTM1 null genotype. The collective data of these studies suggest that there may be a close link of GSTM1 null with lung cancer. A recent study could show a higher lung cancer risk in females than males with GSTM1 null [130]. GSTP1-1 and GSTA1-1 also are important enzymes involved in catalysis of glutathione conjugation of CS carcinogens such as the bioactivated form of BaP, benzo(a)pyrene diol epoxide (BPDE), and other PAH diol epoxides [129]. However, the detoxification of PAH metabolites is a complex process which may need the presence of two or more gene products. The content of GSTP1 in the human lung exceeds that of GSTM1. Other enzymes such as uridine-5'-diphosphate-glucuronosyl transferase (UGT) and dihydrodiol dehydrogenase (DHD) are also involved in metabolic pathways of tobacco smoke carcinogens and may play an important role in determination of individual lung cancer risk.

3.2 Polymorphisms in DNA repair genes, and genetic changes in repair proteins

Although lung cancer is the paradigm of tobacco-induced malignancies, host-specific factors seem to modulate individual susceptibility to CS-induced carcinogenesis. Variations in DNA repair capacity (DRC) may influence the rate of removal of DNA damage and fixation of mutations. As a proxy for DRC, mRNA levels of different DNA repair genes can be used. These mRNA expression levels can be obtained by means of different assays (e.g. multiplex reverse transcriptase-PCR assay) in mitogen-stimulated or unstimulated human peripheral blood lymphocytes, or in other rapid proliferating tissues such as skin, ovary, testis, prostate, liver and intestine [32].

There are three mechanisms of DNA repair: direct repair (DR), base excision repair (BER) and nucleotide excision repair (NER). These topics have been reviewed elsewhere [114,121]. As indicated by some studies, individuals with reduced DRC are at higher risk of developing lung cancer. It is also reported that lung cancer patients have often been observed to have suboptimal cellular DRC and therefore more promutagenic DNA alterations than healthy individuals [117,143]. Several genes and repair proteins (enzymes) are involved in the DRC, some of which are the xeroderma pigmentosum complementary groups (e.g. XPA, B, D, F and G), x-ray repair cross-complementing groups (e.g. XRCC1 and 3), excision repair cross-complementing groups (e.g. ERCC1, 2, 3, 5, and 6), Cockayne's syndrome complementary group B (CSB) and apurinic/apyrimidinic endonuclease/redox factor-1 (APE/ref-1). The DNA repair protein O6-alkylguanine-DNA alkyltransferase (AGT), that encodes the human O6-methylguanine-DNA methyltransferase (MGMT), and the human cytosine-DNA methyltransferase-3B (DNMT-3B) are also important in cellular

defence against promutagenic effects of carcinogens. Furthermore, the enzyme 8-oxoguanine-DNA N-glycosidase (8-OGG) is also involved in repair of oxidative DNA damage [12, 95]. However, only some of the above mentioned DNA repair genes have extensively been studied and convincingly shown to play an important role in the DRC in the lung.

(a) Xeroderma pigmentosum complementary groups

The xeroderma pigmentosum complementary group A (XPA), a DNA binding protein in the NER pathways, modulates damage recognition of DNA. It is well known that NER deficiency is associated with a decreased DRC and hence an increased risk of lung cancer. Recently, a common single-nucleotide polymorphism (A-->G) was identified in the 5' non-coding region of the XPA gene. The two common polymorphisms of the XPA gene are A23G and G709A, with possible AA, AG and GG genotypes. On testing individuals from different ethnic groups for DRC and hence lung cancer risk, Park et al. [102] observed that male subjects with two G alleles (GG genotypes) demonstrated more efficient DRC than did those with homologous A alleles (AA genotypes). However, Wu et al. [148] found out that at least one G allele (e.g. AG, GG phenotypes) is enough to reduce CS-induced lung cancer risk to a significant level.

The DNA repair protein xeroderma pigmentosum complementary group D (XPD) is involved in both, NER and BER of DNA lesions induced by tobacco and environmental carcinogens. Polymorphisms in the DNA repair gene XPD have been associated with risk of developing different cancer types such as bladder, oesophagus and lung cancer [126,150]. Many studies have focused on the functional impact of the commonly known polymorphisms in XPD exon 10 (G-->A, Asp312Asn) and exon 23 (A-->C, Lys751Gln). On assessing XPD polymorphisms at codon 312, smoking, and

lung cancer risk, one study [149] found an increased risk of squamous-cell carcinoma (SCC) in individuals who carried at least one 312Asn variant allele compared with those who had the 312Asp/Asp genotype. At codon 751, subjects with at least one variant 751Gln allele were at a borderline increased risk of SCC of the lung compared with those having 751Lys/Lys genotype. A multiplicative interaction between cigarette smoking and the Asp312Asn polymorphism on risk of SCC was also observed. Other studies could also come out with similar results [62, 80]. Therefore, XPD codon 751 polymorphism (Lys-to-Gln amino acid change) and XPD codon 312 (Asp-to-Asn amino acid change) may affect the repair of smoking-induced DNA damage and be associated with increased lung cancer risk.

Jeon et al [68] investigated the relationship between the polymorphism in the xeroderma pigmentosum complementary group G (XPG) gene at codon 1104 and the risk of lung cancer in a study population consisted of almost equal number of lung cancer patients and healthy controls. In this age and sex matched study, the Asp/Asp genotype was more frequent in the controls than in the cases, and associated with a significantly decreased lung cancer risk. Older subjects, males, and lighter smokers were significantly more protected by Asp/Asp genotype than others against lung cancer development. Histologically, the Asp/Asp genotype showed a significant decrease in risk of squamous-cell carcinoma. These results were obtained when combined His/His and His/Asp genotype was used as the reference and suggest that the XPG codon 1104 (His1104Asp) polymorphism contributes to genetic susceptibility to lung cancer development.

(b) X-ray repair cross-complementing groups

The x-ray repair cross-complementing group 1 (XRCC1) is mainly involved in BER of DNA repair pathways. Polymorphisms of DNA repair gene XRCC1 have recently been identified; and growing evidence shows that these polymorphisms may have some phenotypic significance regarding smoking-related cancer risk. With regard to the XRCC1 gene and the cancer risk in different malignant tumours (breast, gastric, and lung cancer), polymorphisms at codons 194 (Arg-->Trip) and 399 (Arg-->Gln) have been extensively studied. At codon 399 (Arg/Gln), a nucleotide substitution of guanine to adenine leading to non-conservative amino acid change has been identified. This amino acid change is believed to be associated with increased levels of aflatoxin B1-adducts and glycophorin A somatic mutations. In a molecular biology study, XRCC1 genotypes were assessed at codon 399 in patients with adenocarcinoma of the lung and cancer-free controls in two ethnic populations. The distribution of XRCC1 genotypes in the study population differed between cases and controls; and all three possible genotypes (Arg/Arg, Arg/Gln, Gln/Gln) could be found between the subjects. The study showed an increased lung cancer risk in subjects with Gln/Gln genotype. The elevated cancer risk related to ethnicity, age and smoking [40].

(c) DNA repair protein O6-methylguanine-DNA methyltransferase and related proteins

The DNA repair protein O6-methylguanine-DNA methyltransferase (MGMT), codes for the human protein O6-alkylguanine-DNA alkyltransferase (AGT), and is responsible for the repair of O6-methylguanine-DNA (O6MG-DNA) adducts which are usually induced by NNK in CS. Overexpression of MGMT has been associated with increased repair capacity for O6MG-DNA adducts, decreased mutational activation of

K-*ras* oncogene, and hence reduced lung tissue susceptibility to NNK-induced tumourigenesis. Loss of expression of MGMT is rarely due to deletion, mutation, or rearrangement of the MGMT gene, but promoter hypermethylation (e.g. methylation at CpG sites) of MGMT has been associated with inactivation of the gene and increased G to A mutations in K-*ras* in colorectal cancer, and increased G to A transitions in the *p53* gene in NSCLC, particularly in adenocarcinomatous cell lines [43,145].

The expression of the human cytosine-DNA methyltransferase-3B (DNMT-3B) is regulated by methylation of promoter region of the gene. Polymorphisms in this NER gene are also believed to be associated with increased lung cancer risk. A C to T transition at a novel promoter region of the protein has recently been identified. This polymorphic transition increases the promoter activity. In a study, promoter polymorphism of the gene leading to CT heterozygotes was associated with over 2-fold risk increase of lung cancer compared to CC homozygotes. With regard to polymorphisms or low activity of 8-oxoguanine-DNA N-glycosidase (8-OGG), the high risk of lung cancer development seems to have a cumulative effect with smoking status. 8-OGG is expressed in the lung tissue, and involved in the BER of 8-OHdG DNA adducts that are formed during CS-induced oxidative stress [104,118]. In short, the individual overall susceptibility to lung cancer development may depend on the balance between carcinogen metabolic activation and detoxification, as well as the rate at which CS carcinogen-induced DNA damage is repaired. This is an area which needs further investigations.

4. Effects of smoking in lung cancer patients

Tobacco consumption plays a remarkably significant role in genetic damage and hence lung carcinogenesis. Continuation of cigarette smoking implies a prolonged exposure of the bronchial system to carcinogens and oxidants. This provides a possibility of induction of further molecular alterations to normal, preneoplastic, and neoplastic lung cells. Early preneoplastic morphological changes may bypass further steps and progress to invasion. Progressive molecular changes in pre-existing tumours may give rise to altered phenotypes or aggressive characters of the tumours, leading to important therapeutic and prognostic consequences.

Apart from carcinogenic effect, smoking may lead to a number of nonneoplastic lung disorders which can influence the disease course and prognosis of lung cancer patients. Many diseases caused or facilitated by smoking habits may play part in the multimorbidity and reduction of survival expectancy of lung cancer patients. One of the most important nonmalignant pulmonary diseases is COPD, which is mostly accompanied with impaired lung function.

4.1 Field cancerization theory in the lung

Considering the lung of long-term smokers as an organ with a very high exposure to carcinogens, the *field cancerization theory* can be applicable in explanation of all possible genetic changes existing in different cells of the bronchial epithelial lining and the underlying probability of development of lung cancer. This theory is commonly applied to head and neck squamous-cell carcinoma (HNSCC) and hypothesizes that the entire epithelial surface of the upper aerodigestive tract has an increased risk for development of (pre)malignant lesions due to multiple genetic abnormalities as the result of exposure to various carcinogens. In the lung, this hypothesis is favoured by

multiple molecular changes and high incidence of preneoplastic lesions found in lung cancers. This suggests that inside the carcinogen-exposed field there are multifocal clones with different phenotypes. The differences in mutation spectra arising from differing selectivity of carcinogens may provide important clues in the knowledge of the cause of lung cancer (*hotspots theory*). The theory of multifocal lesions in the field cancerization supports therefore the use of genetic markers in the differential diagnosis of recurrence of first primary tumours or metastases from second primaries after successful "radical" resections of tumours [59, 67, 89].

However, there are alternative theories regarding the occurrence of multiple (pre)malignant lesions. These theories which have been proposed in the last decade are based on the premise that cell transforming events are generally rare and that the multiple lesions arise due to widespread migration of transformed cells in the whole field of cancerization. In the concept of these theories, intraluminal migration in the lung might involve movement of neoplastic cells by bronchial mucus (micrometastases), or intraepithelial migration of the progenitor cells of the initially transformed cells. In order to get a consensus about these different theories, different ways of investigation may be used, among them analysis of differences in genetic alterations between histologically normal tumour-adjacent mucosal cells (TAMC) from smokers and those from non-smokers. Migrating tumour cells are likely to be found in TAMC from both smoking and non-smoking lung cancer patients. Thus, TAMC from smoking and non-smoking lung cancer patients with the same histology should exhibit the same molecular alterations. These alterations should not be found in smoking or non-smoking healthy individuals, as in those cases, there is no source for migrating tumour cells. Moreover, any observed alterations in TAMC should be identical with the alterations in the primary carcinoma, in case of

migration of cells from the pre-existing tumour. In case of migration of progenitor cells, at least some early tumourigenic alterations would be identical between primary tumour and TAMC. In absence of migrating cells, changes in TAMC of smoking patients should be absent in non-smoking patients and regarded as smoking-induced independent events [48, 49,142].

The other way to investigate these different theories is to investigate the clonal expansion of multiple (pre)malignant lesions by analysis of early genetic alterations in the course of development of lung cancer. Separate lesions would share common genetic alterations if they would have developed from a single clone. Clonal relationship between different multiple lesions points to migration of tumour or progenitor cells. If no clonal relationship between different lesions can be observed, it is likely that the lesions developed independently from each other [65].

4.2 Smoking and risk of development of second primary lung cancer

Many studies have been carried out to investigate the risk of developing second primary lung cancers among patients with initial lung cancer. In some of these studies, cigarette smoking has been condemned as one of the important factors for increased risk of development of second lung cancers. In addition, anticancer treatment modality has shown a certain correlation with the risk. Tucker et al. [133] found significant increase (about 3.5-fold) of second lung cancers among lung cancer patients compared to the general population. Moreover, this study revealed a correlation between the risk, treatment modality, and smoking habits. The second cancer risk seemed to increase with chest irradiation (13-fold). A relative risk of 21-fold showed an interaction between radiotherapy and cigarette smoking. While treatment with various forms of polychemotherapy had comparable overall risk increases of 9.4- to 13-fold, continuation of smoking habits had a 19-fold

risk increase among subjects treated with alkylating agents. These results did not differ from those of Johnson et al. [69] and Yoshida et al. [153], who found out that an increased relative risk of second primary cancer in patients treated for small-cell lung cancer was associated with smoking and family history of cancer. Other studies which investigated the impact of smoking cessation came out with results that showed significant decrease in risk of developing second primary cancers after successful treatment of patients with initial lung cancers [71,112].

The study of Kelley et al. [74] concerning genetic analysis on second primary cancers in lung cancer patients is an important step which increases our understanding regarding the validity of field cancerization theory in the development of pulmonary malignancies. In patients surviving small-cell lung cancer, cigarette smoking led to development of smoking-associated tumours which had genetic and morphological features consistent with non-small-cell carcinomas. These results could be supported by Godschalk et al. [49] who analysed the effect of smoking and the presence of miscoding multiple DNA adducts of oxidative stress O(4)-ethylthymidine (O(4)etT) and of the CS carcinogens (PAHs) in tumour adjacent normal lung tissues of smokers and non-smokers operated lung cancer patients. They concluded that the O(4)etT and PAH-DNA adduct levels were higher in lung DNA of smokers than non-smokers. These miscoding lesions contribute to increased genomic instability and elevated lung cancer risk in smokers, and may therefore lead to development of second tumours. All these studies have therefore provided hints that smoking among lung cancer patients may lead to development of further primary carcinomas. The only question to be answered in all these study results is whether the second primary tumours emerge as a result of pre-existing genetic alterations due to long-term smoking history of the patients, or as a result of new genetic changes due to

prolonged exposure to CS after diagnosis of the first tumours. It may be possible that both factors are responsible for development of new primary tumours.

Carcinogenesis is a process believed to take place over years or decades. This may raise an argument that patients under palliative treatment of advanced lung cancer would not succumb to new primary tumours if they continue their smoking habits as they hardly have a year to survive. But on the other hand, the time interval for development of lung cancer from preneoplastic lesions is not the same for all patients with equal-term smoking history and level. Moreover, not everybody who smokes develops a carcinoma. It is believed that only 11% of tobacco smokers develop manifest lung cancer [2], suggesting that many factors may determine individual susceptibility to eruption of carcinomas among those who are exposed to carcinogens. Synergistic effects of different factors, including pre-existing individual genetic factors, molecular changes acquired by smoking habits, as well as weaken or failure of *immunological surveillance*, may have decisive role in determination of latent time between promotion and progression. Weaken or failure of immunological surveillance may be not only due to various diseases or other internal and external factors such as CS [22,100], but also physiologically due to advanced age [7]. This might explain the pattern of age distribution of lung cancer patients, since majority of them age over 55 years. Therefore, people who have once developed lung cancer may be predisposed to development of further primary carcinomas. In this case, preneoplastic lesions may progress to malignancies after a short latent time as response to prolonged exposure to carcinogens CS. In this manner, continuation of smoking habits may accelerate lung cancer progression.

4.3 Cigarette smoke-induced modulation of macromolecules and signalling pathways: its role in tumour progression - metastasis, invasion, recurrence and therapy resistance in lung cancer

4.3.1 Metastasis, invasion and recurrence

(a) Protein kinases

There is an increasing evidence that protein kinase C (PKC), a family of numerous closely related isoforms, has a deep implication in carcinogenesis as well as in metastatic and invasive processes of different malignancies. However, little is known on the specific role of each isoform of the enzyme in these processes. PKC plays an important role in signal transduction pathways. As a cell-signalling protein, PKC has both an N-terminal regulatory and a C-terminal catalytic domain. The catalytic subunit is a target for chemoprotective antioxidants which cause down-regulation of the enzyme. On the contrary, oxidants predominantly react with the regulatory subunit and activate PKC-mediated cellular signal transduction. Activation or inhibition of PKC activity indicates to play a critical role in regulation of some cellular events such as mitogenesis, cell adhesion, apoptosis, angiogenesis, and metastasis. These are key events related to tumour promotion and invasion. The PKC-mediated tumour promotion by oxidants appears due to disruption of the balance between protein phosphorylation and dephosphorylation in a manner similar to phorbol esters and okadaic acid. Phorbol esters, which bind to and activate PKC, and okadaic acid, which binds to and inhibits protein phosphatases-1 and -2A, are among the most potent tumour promoting agents known. Oxidants in CSC lead to an increase in PKC activity and may enhance its oncogenic role. Recent experiment with mice indicated an increase in nodular metastasis in lungs three weeks after injection of experimental Lewis lung carcinoma cells (LLC-cells) treated with polyphenolic agents in CSC. Hydrochinone, catechol and other components of CS enhanced adhesion of the so called

LL/2 carcinoma cells to basement membrane components and endothelial cells, and increased tumour cell invasion and haematogenous metastasis. All these events could be inhibited by a variety of PKC inhibitors such as calphostin C, hypericin, chelerythrine, and bisindolylmaleimide. Anti-oxidant agents such as catalase and SOD were capable of reducing PKC activity and therefore inhibiting CSC-mediated membrane association and metastasis by reducing ROS production rate. In vitro, CSC increases tumour cell adhesion to endothelial cells and basement membrane, and may enhance invasion. As a target for both oxidants and antioxidants, redox modification of PKC activity may play a central and determining role in tumour promotion and progression. Therefore, redox-regulated PKC activity may be relevant to carcinogenesis as well as other pathologies caused by oxidative stress [18, 50, 51].

Protein kinase A (PKA) for instance shows an influence on invasive and metastatic characters of tumours by modulating interaction of tumour cells with extracellular matrix (ECM). PKA levels are said to be more increased in highly invasive and metastatic than non-metastatic lung cancer cells. An increase in PKA activity leads to decrease in tumour cell adhesion to some ECM components (collagen I, vitronectin, and laminin) and may therefore facilitate dissemination of tumour cells [86]. Some malignant cells, such as in breast cancer, express CD44 receptor that can bind to hyaluronan, an ECM component. The receptor-ligand complex may lead to PKA mediated signal transduction, resulting in rise in intracellular calcium and cyclic AMP; and this ends up with activation of actin cytoskeletal organisation and increases metastatic potential by enhancement of movement of malignant cells along hyaluronan rich surfaces [70,132]. Via phosphorylation of cytoskeletal subunits and associated proteins such as vimentin, an intermediate ECM protein, PKA may disrupt the filamentous cytoskeletal

architecture and influence the cellular morphology. It has been shown in an experiment that Lewis lung carcinoma cells (LLC-cells) with increased PKA activity have reduced levels of polymerized actin, tubulin, and vimentin, and show increased tendency to dislodge, move and invade. Transfection of LLC-cells to express PKA **C**α subunit was associated with increased metastatic, invasive, and recurrence potential of the previously non-motile, non-metastatic cells. When PKA was blocked, highly motile metastatic LLC-cells lost their motility and invasiveness by acquiring more stability in cytoskeletal organisation. On the other hand, the PKA-dependent motility by non-metastatic LLC-cells became increased when dephosphorylation reactions were blocked with the protein phosphatase-1 and -2A inhibitor okadaic acid **[**154,155**]**. Inhibition of PKA activity in cells with PKA-dependent motility may therefore indicate a critical measure in therapeutical intervention against metastasis in future.

Apart from protein kinases C and A, oxidants exposure also increases the activity of a variety of protein kinases involved in mitogenesis such as protein tyrosine kinases, c-jun N-terminal kinases and mitogen-activated kinases (MAPKs). Oxidative stress activates redox-sensitive transcription factors such as activator protein-1 (AP-1) and nuclear factor kappaB (NF-κB), and induces the proto-oncogenes c-*jun,* c-*fos, and* c-*myc* **[**3,124**]**. Oxidants and nicotine can activate extracellular signal-regulated kinase (ERK). Experimentally, upregulation of the expression of this MAPK isotype has led to overexpression of the bcl-2 protein and inhibition of apoptosis in lung cancer cells **[**57**]**. Mediated by nicotinic receptors, nicotine also has shown to inhibit down-regulation of PKC and ERK2 by anticancer agents and hence suppress apoptosis **[**87**]**. Nicotine therefore may act as a tumour promoter and affect cancer therapy. Subsequently, studies have shown that oxidants can inactivate protein tyrosine phosphatases by

oxidizing their active site cysteine residues. This leads to an upregulation of tyrosine phosphorylated proteins and hence increased growth-promoting effects. The effect of oxidative stress on tyrosine phosphorylation seems to be due to initiation of signals by protein tyrosine kinases and loss of control by protein tyrosine phosphatases. The tyrosine protein phosphorylation will then influence cellular events such as growth, death, and differentiation [128]. Therefore, oxidation and phosphorylation represent alternative mechanisms for stimulating cellular responses relevant to the process of tumour promotion, invasion, and metastasis.

(b) Extracellular matrix changes and cytoskeletal modification

Metastasis is an important characteristic of malignant tumours and provides a great obstacle to cancer cure. The mechanisms involved in metastatic spread are not fully clarified yet. However, increasing evidence shows that metastasis requires alterations in the surrounding extracellular matrix (ECM), as well as cytoskeletal modification for adhesion, migration, and extravasation of metastatic cells. Whereas molecular events necessary for cytoskeletal change include alterations in expression of cell adhesion molecules which interfere with other neighbouring cells or ECM, changes in properties of ECM itself may also influence movement of neoplastic cells from their in situ position. Alterations in the ECM may be due to changed biosynthesis of extracellular matrix components, or imbalance between molecules involved in the break-down (proteinases) and maintenance (antiproteinases) of the ECM. The role of matrix metalloproteinases (MMPs) and tissue inhibitors of metalloproteinases (TIMPs) in metastatic phenomenon continues to increase the interest of many scientists all over the world. Under physiological state, MMPs and TIMPs exist in equilibrium. Factors associated with an increase in activity of MMPs will lead to an imbalance between MMPs and TIMPs and may result in increased

degradation of the ECM and facilitate tumour growth, invasion and metastasis [27]. Cigarette smoke extract has shown to cause both, a decrease in biosynthesis of collagen type I and III, as well as an increase in the expression of MMPs (e.g. MMP-1, MMP-2, and MMP-3). Since radical scavengers such as ascorbic acid and α-tocopherol can prevent the CS-induced expression of MMPs, oxidative stress caused by CS seems to play a critical role in these processes [98,152]. However, metastatic processes might involve various complex molecular and biochemical pathways.

The role of PKA in cytoskeletal modification and its impact in the processes involving metastasis and invasion of tumour cells has previously been explained. Cytoskeleton can also indirectly affect metastatic properties of tumour cells through its association with intercellular adhesion molecules. Several studies have indicated the role of endothelial cell adhesion molecules in the adherence and penetration of blood vessel wall by tumour cells, allowing them to disseminate and colonize their metastatic sites. For example, E-cadherins can interact with the actin cytoskeleton through linkage proteins. Malignant cells express endothelial cell adhesion molecules; and this expression is said to be modulated by cytokines. Intergrins on tumour cells bind receptors on endothelial cells such as intercellular adhesion molecule-1 (ICAM-1) and vascular cell adhesion molecule (V-CAM). Tumour cell proteoglycans recognize and bind platelet endothelial cell adhesion molecule-1 (PECAM-1) [25]. All these effects may play an important role in the processes of metastasis and hence tumour progression.

(c) Mucin glycoprotein genes

The dysregulation of mucin expression in different cancer types is well known. Aberrant expression of mucin (glycosilation, underglycosilation, and overexpression of mucin peptides) and mucin-related antigens has been reported and considered to be a poor survival factor in adenocarcinomas arising from various organs such as colon, pancreas and breast cancer. Several genes which encode distinct mucin core peptides have been identified so far (e.*g. muc*-1*, muc*-2*, muc*-3*, muc*-4*, muc*-5b *and muc*-5ac). The expression of mucin in cancer cells, as demonstrated in an in vitro study, can decrease tumour cell aggregation, promote tumour cell invasion, block lymphocyte targeting and therefore facilitate metastasis by escape from immunological surveillance. The membrane-associated *muc*-1 gene for instance produces a corresponding muc-1 protein that prevents cellular adhesion by masking certain adhesion molecules on cell surfaces. The abundance of siliac acid residues in mucin (sialomucin) increases the antiadhesive effect of tumour cells by making the glycans more bulky and thus contributing to the rigidity of mucoprotein; and gives the glycoprotein a strong negative charge that causes repulsion of cell surfaces. The muc-1 protein strongly interferes with the function of lymphocyte activated killer (LAK) cells and allogenic stimmulated cytotoxic T-lymphocytes by masking cell-surface antigens that are involved in immune recognition processes. The amount of siliac acid present on the surface of malignant cells has been correlated with the ability of tumours to metastasize. In adenocarcinoma cells of colon, this effect was reduced on treatment of the cells with specific inhibitor of siliac acid. The alteration of siliac acid expression alters the binding of cancer cells to reticuloendothelial cells (adherence to E- and P-selectin) and to ECM (less adherence to collagen and stronger adherence to fibronectin), and can therefore promote metastasis of cancer cells. The study of Yu et al. **[**157**]** has shown a strong association

between overexpression of sialomucin with overexpression of *erb*-2 oncoprotein, which is an important negative prognostic factor in many cancer types. In lung cancer, mucin is particularly expressed in non-small-cell lung cancer (NSCLC), especially in adenocarcinoma. Results from the other study of Yu et al. [156] have shown a strong correlation between smoking habits and mucin gene expression in patients with NSCLC. Tumours of smokers had higher expression of mucin glycoprotein genes, especially *muc*-5b and *muc*-5ac mRNA. Tumours with overexpression of mucin gene were associated with early post-operative recurrence, metastasis and cancer death in NSCLC.

4.3.2 Multidrug resistance (MDR)

(a) Proteinkinase C, P-glycoprotein and other resistance-related proteins

Tumour cells may be insensitive to chemotherapeutic agents due to possession of multidrug-resistant phenotypes. It is well known that resistant phenotypes can result by exposing normal or neoplastic cells to carcinogens or antineoplastic drugs [30, 31]. Several proteins (including transport-associated proteins) are involved in multidrug resistance, among them P-glycoprotein (P-gp or P-170), multiresistance protein 1, heat shock proteins (HSPs), glutathione S-transferase-pi (GSTP), thymidylate synthetase (TS), topoisomerase II (topo-II), lung resistance-related protein (LRP) and putative regulators of resistance (Fos, Jun and ErbB1). Treatment of MDR cells with PKC activators is associated with an increased phosphorylation of P-gp and decreased intracellular drug accumulation and drug sensitivity [28, 29]. On the other hand, induction of MDR can be blocked by PKC inhibitors such as calphostin C and staurosporin [96,116], showing an evidence that PKC is involved in regulating activity of P-gp. The involvement of some PKC isoenzymes (e.g. PKCα) in the MDR phenomenon is believed to be

due to phosphorylation of serine in P-gp, thereby changing the geometric equilibrium of the P-gp ATPase and hence its drug-binding functions. However, recent work has shown that safingol, a lysosphingolipid derivative which specifically inhibits PKC activity via competitive interaction with the regulatory phorbol-binding domain of PKC, can inhibit the MDR phenotype without altering P-gp drug binding [115]. PKC may be more directly involved in activation of the *mdr*-1 gene since it has AP-1 binding site in its promoter region which may interact with PKC. AP-1 binding sites are DNA sequences at which some proteins such as the c-*fos*/c-*jun* complex specifically bind and thereby affect the transcriptional expression of cellular genes. PKC may therefore have a functional importance as a stimulator of the activity of proto-oncogenes such as c-*fos* and c-*jun*. The c-*fos* and c-*jun* oncogenes belong to resistance-related proteins in cancers of the lung and other organs. Other resistance-related proteins such as GSTP and topo-II are also phosphorylated by PKC. The promoter region of the GST gene contains an AP-1 binding site, and may therefore be regulated by the oncogenes c-*fos* and c-*jun*. Expression of these proteins has shown to associate with resistance to doxorubicin in lung cancer. Since PKC seems to play a central role in the acquired and inherent resistance of human cancers, it might be helpful to devise new strategical approaches to circumvent drug resistance. Potential PKC inhibitors such as tamoxifen, cyclosporin A, trifluoperazine, and chlorpromazine are some of important MDR reversing substances [141].

The role of CS tumour promoters catechol and hydroquinone as well as oxidants in PKC activation has previously been explained. This suggests that cigarette smoking may be involved in causing resistance of tumour cells to some anticancer drugs, among them doxorubicin, and cisplatin. Moreover, a study [140] could show that lung carcinomas of smokers express LRP more frequently compared to those of non-smokers. In this

study, a correlation was found between LRP expression and resistance to doxorubicin in patients with non-small-cell lung cancer. It was also demonstrated in this study that a relation of borderline exists between the patients' smoking habits and LRP expression. Lung carcinomas of heavy smokers were more frequently LRP positive. However, Dingemans et al. [38] studied 39 normal lung tissues for LRP expression and discovered no correlation between LRP intensity levels and the number of pack-years smoked, although a trend was noted for the higher LRP intensity levels in patients who smoked for more than 10 pack-years.

(b) DNA repair protein O6-alkylylguanine-DNA alkylyltransferase

The DNA repair protein O6-alkylguanine-DNA alkyltransferase (AGT) is one of the main determinants of resistance of tumour cells to the cytostatic effects of O6-alkylguanine-generating alkylating agents. The AGT, encoded by O6-methylguanine-DNA methyltransferase (MGMT), removes the methyl group that binds on tumour cell DNA on treatment with alkylating agents such as cyclophosphamide, and prevents hereby cell apoptosis and therefore the growth-inhibiting effect of alkylating anticancer agents. The expression of the human DNA repair protein MGMT is regulated by various exogenous and endogenous factors. Methionine for example is believed to participate in MGMT regulation. In a study, up-regulation of MGMT activity in methionine-dependent phenotypes led to increased cell proliferation in lung and brain cancer [77]. Increased activity of MGMT is a natural cellular response against enhanced DNA damage caused by different cytotoxic agents such as anticancer drugs and CS. It has recently been shown experimentally that some cell lines in lung cancer react with an increase in MGMT expression upon exposure to CS. In this experiment, the CS-induced effect of MGMT activity correlated with the number of cigarettes smoked. After smoking cessation, MGMT expression could be

lowered to a significant level. Since an increase in MGMT activity is associated with an increased repair capacity for O6MG-DNA adducts, CS may lead to resistance of tumour cells to cytostatic effects of some systemic anticancer agents. Although MGMT-induced cellular resistance against anticancer drugs is mainly shown in alkylguanine-generating alkylatig agents such as cyclophosphamide, combined increase in MGMT and topoisomerase II (topo-II) activity may expand resistance to anthracycline derivatives (e.g. daunorubicin and doxorubicin), topo-II inhibitors (etoposide, teniposide), as well as nitrosoureas (carmustine, lomustine). The MGMT-induced resistance can partially be reversed by O6-benzylguanine, a potent inhibitor of MGMT activity [90, 91,103].

(c) Heat shock proteins

Heat shock proteins (HSPs) are a family of stress proteins which are expressed during severe forms of stress and inflammation. It is believed that HSPs are expressed during inflammation in order to protect cells against oxidative damage. Upregulation of HSPs is therefore a natural response of cells towards stress-stimuli caused by various factors such as anticancer therapy and CS constituents. A number of HSPs have been identified so far and are believed to play important role in lung cancer and during inflammatory processes in the lung [19]. The HSP70 family belongs to the most abundantly investigated HSPs. Upon oxidative stress and inflammation, upregulation of HSP70 occurs in both, normal and neoplastic cells. Smoking, which is associated with oxidative inflammatory processes and hypoxic conditions in the lung, induces also overexpression of HSPs. A study could demonstrate that in tumours of patients with adenocarcinomas HSP70 expression correlates with smoking habits and the extent of CS exposure [138]. In subjects who smoked more than 20 cigarettes per day, 89% of the tumours had a high expression of HSP70. This study shows that

tumour cells also are highly subjected to oxidative stress by CS. Since HSPs belong to resistance-related factors in different cancer types [136,139], overexpression of HSP70 in tumours may render lung cancer cells insensitive to anticancer therapy. However, more research is needed in this area.

(d) Oncogene-induced phenotype transitions

Tumour progression from therapy-sensitive to therapy-resistant state has been demonstrated both in vitro and in vivo, and may involve partial or complete conversion of SCLC to NSCLC [17, 84]. An important molecular event responsible for the transition between lung cancer phenotypes is the activation of *ras* oncogene. The H-*ras* oncogene-induced transitions occur predominantly in the so called biochemical variant SCLC with profound amplification and expression of c-*myc* oncogene [8, 83]. Alterations in N-*myc* amplified SCLC cell line might involve induction of morphological, biochemical, and growth properties consistent with NSCLC phenotype [45]. These findings provide important links in studying and understanding molecular events involved in progression of SCLC. Although CS constituents such as NNK, as well as CS-induced oxidative stress may participate in the processes involved in *ras* oncogene activation, less is known about its contribution to transitional processes in lung cancer. Therefore, evaluation of the role of CS in H-*ras* gene activation and transitional phenomenon of SCLC to NSCLC is a field of great interest as this probably has a considerable impact on response to treatment and patients' prognosis.

(e) Glutathione

Apart from MGMT and other resistance-related factors (P-gp, LRP, HSPs e.t.c), glutathione (GSH) appears to participate in rendering human lung cancer cells less sensitive to cytostatic effect of chemotherapeutic agents such as carmustine [41]. Although acute CS exposure leads to GSH depletion, high levels of this vital cytoprotective antioxidant have been frequently observed in broncho-alveolar lavage (BAL) and epithelial lining fluid (ELF) of chronic smokers [99].

In the figure below, the CS-induced carcinogenesis and underlying mechanisms possible for tumour progression following prolonged CS exposure are summarized.

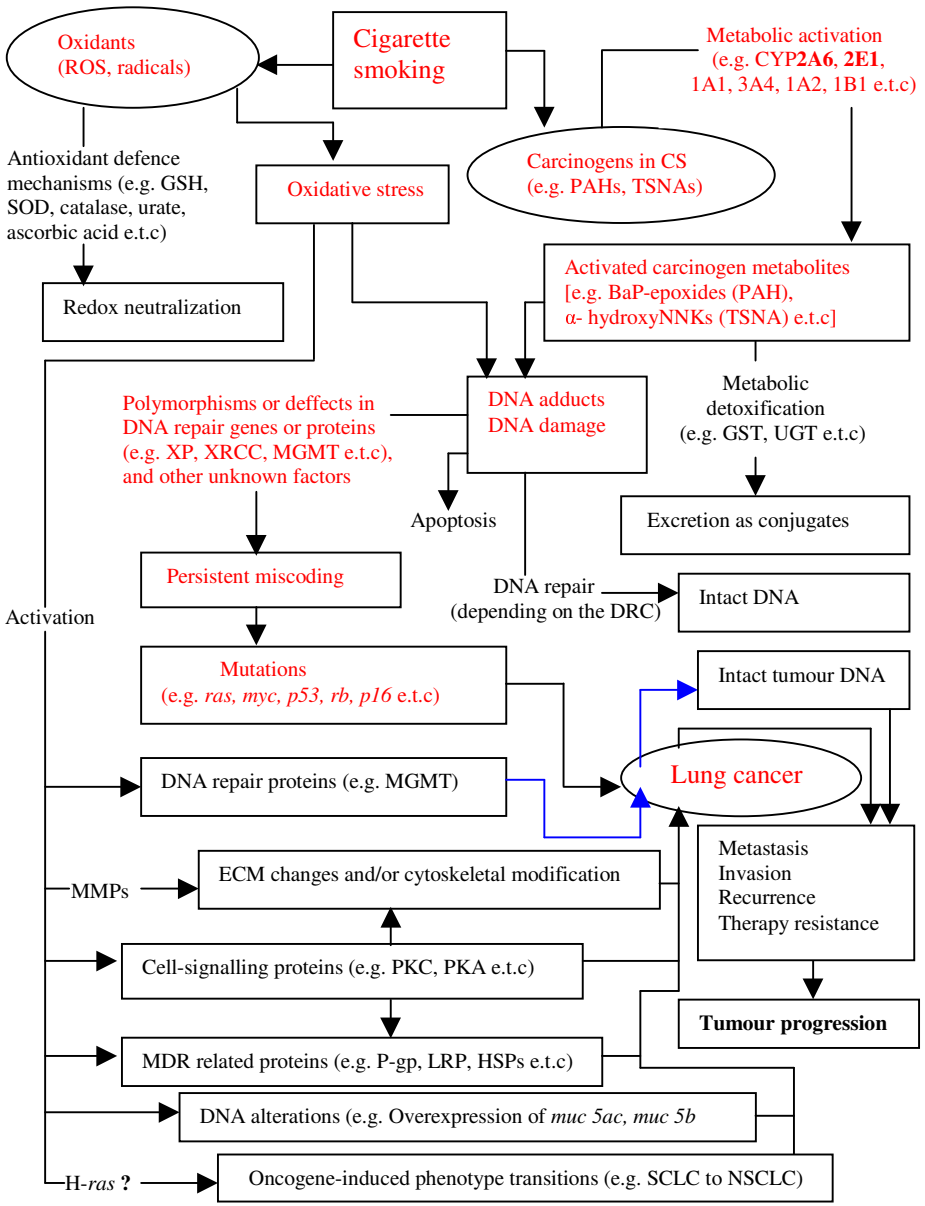


Fig. 1: Scheme showing in summary the effects of smoking (Carcinogenesis in the lung and mechanisms possible for tumour progression following prolonged exposure to CS). DRC = DNA repair capacity; SOD = Superoxide dismutase; UGT = Uridine-5'-diphosphate-glucuronosyl-transferase. Note that all remaining abbreviated words in the scheme and their meaning have repeatedly been explained in the main text of this work.

4.4 Disorders in the surfactant system due to cigarette smoke exposure

Many experimental findings on broncho-alveolar lavage (BAL) have shown that CS constituents can cause disorders in the surfactant system in the lung [46, 61]. Cigarette smoking leads to reduction of total amount of surfactant as inhaled particles in CS are incorporated with a large quantity of surfactant in phagocytes for their clearance during phagocytosis [58]. The surfactant incorporated with CS particles gets degraded in phagocytes and may be expectorated. Surfactant in the alveolar system of the lung is important and has many functions, among them defence mechanisms, and providing an adequate alveolar surface tension. The surfactant covers the alveolar epithelial membrane and provides a barrier which hinders direct contact of the membrane with particulate air pollutants and infectious agents. Tobacco smoke is also responsible for the destruction and impaired function of cilia. Cilia are important structures for the natural clearance mechanism of the air ways. Reduction of surfactant as well as destruction of cilia due to CS are therefore important factors for the increased susceptibility to bronchopulmonary infections and inflammatory processes [75], which, in turn, predispose the lung to structural changes, leading to chronic air way diseases. Although type II pneumocytes may constantly be activated to produce surfactant, the destruction rate due to frequent CS exposure may exceed its new synthesis. The ability of pulmonary surfactant to influence wall thickness and diameter of airways might be one of the mechanisms which influence airway resistance and indicates a possible role of CS-induced change in quality and/or quantity of surfactant in the pathogenesis of diseases such as COPD and bronchial asthma [60].

Important functions of surfactant are again summarized below:

1. Stabilisation of airways by preventing airway film and collapse of air walls.
2. Protection of the alveolar membrane from direct contact with air particles and microorganisms
3. Involvement in processes of bronchial clearance
4. Modulation of airway wall thickness and airway diameter by regulating airway liquid balance
5. Immunomodulatory activity due to suppression of cytokine secretion and activation of transcription factors

4.5 Cigarette smoke-induced inflammatory processes in the lung

Cigarette smoke is one of the most important contributors of oxidant pollutant-induced airways injury. Cigarette smoke induces both acute and chronic inflammatory response in the respiratory tract. While acute phlogistic reactions are usually reversible, long lasting pulmonary oxidative stress may induce irreversible lung lesions such as COPD, emphysema, and interstitial fibrosis [79]. Early inflammatory response to oxidants and pollutants in CS is driven by native cells such as alveolar macrophages (AM) and fibroblasts. AM produce ROS and a wide variety of inflammatory mediators, among them tumour necrosis factor (TNF) and interleukins (e.g. IL-1, IL-6 and IL-8). The released cytokines cause, among other things, airway constriction, and lead to increased vascular permeability. IL-8 and leukotriene B4 (LTB4) are chemoattractants which lead to cellular chemotaxis, and therefore enhance recruitment and influx of further inflammatory cells such as polymorphonuclear leucocytes (PMN). As a marker of neutrophil influx, high levels of myeloperoxidase (MPO) are observed during inflammatory processes. Inflammatory response occurs particularly through upregulation of transcription factors such as AP-1, NF-

κB and ICAM-1. The inflammatory mediators may secondarily modulate cellular synthesis of proinflammatory growth factors. While upregulation of the synthesis of the transforming growth factor-β (TGF-β) occurs through interaction with transcription factors such as AP-1, the regulatory processes of the platelet-derived growth factor (PDGF) may involve the presence of the NF-κB. On the other hand, oxidants-activated PKC, as well as inflammatory cytokines IL-1β and TNF-α, are involved in the regulation of activity of the NF-κB [11, 78, 94].

The tumour necrosis factor-α (TNF-α), which is a ubiquitous proinflammatory cytokine, is recognized as an important mediator of inflammatory events in the lung. The induction of chronic inflammatory changes in the lung by TNF-α has been associated with an increase in defence mechanisms including antioxidants [146]. TNF-α induces oxidative stress by ROS generation via leakage from the mitochondrial electron transport system and depletes GSH in human alveolar epithelial and pulmonary artery endothelial cells [105]. A proposal has been done that the TNF-α-mediated GSH depletion is due to upstream from the ceramide and sphingomyelinase pathways, which suggests that a signalling mechanism might be involved in this event. TNF-α and TGF-β levels are elevated in the broncho-alveolar lavage fluid (BALF) and sputum of COPD patients [73, 81], suggesting involvement of these pro-inflammatory factors in the pathogenesis of COPD development.

The multifunctional transforming growth factor-β1 (TGF-β1) modulates cellular proliferation and induces differentiation and synthesis of ECM components, including collagens and fibronectin in many types of lung cells [20]. Through mechanisms involving AP-1, TGF-β1 interferes with the GSH biosynthesis by modulating expression of γ-glutamylcysteine

synthetase (γ-GCS), the rate-limiting enzyme in de novo GSH synthesis. The enzyme γ-GCS consists of a catalytic heavy subunit (γ-GCS-HS) and a regulatory light subunit (γ-GCS-LS). In human, the promoter (5'-flanking) regions of both γ-GCS-HS and γ-GCS-LS genes contain a putative AP-1 binding site and an antioxidant response element (ARE) that are necessary for γ-GCS expression in response to diverse stimuli such as CS-induced oxidative stress. Differences in ELF GSH in various inflammatory lung disorders may relate to changes in molecular processes involved in regulation of GSH synthesis in lung by AP-1 and ARE. In vitro, TGF-β1 downregulates both γ-GCS-HS mRNA and GSH synthesis in human alveolar epithelial cells and pulmonary artery endothelial cells [5]. Interestingly, in an experiment with animal model, transgenic mice transfected to overexpress TGF-β1 showed decreased GSH synthesis and increased susceptibility to oxidant-induced lung injury [44]. Thus, high levels of TGF-β1 during oxidative stress may be involved in downregulation of GSH and contribute to CS-induced lung disorders such as COPD. An important result of an in vitro study showed that GSH in levels normally found in ELF suppresses fibroblast proliferation [26]. Decrease in GSH concentrations due to inflammatory processes may therefore have direct structural and functional consequences in the lung. It follows that oxidative stress plays an important role in the pathogenesis of a wide variety of lung diseases, not only through direct damage, but by involvement in molecular mechanisms which control pulmonary inflammatory and proliferative processes.

4.6 Cigarette smoking: important factor in the development of chronic obstructive pulmonary disease (COPD)

The exact mechanisms by which smokers develop COPD are not very clear yet. The major paradigms for the pathogenesis of COPD are described in the *proteinase-antiproteinase* and *oxidant-antioxidant* theories. An increasing amount of research has focused on the proposal that an oxidant imbalance occurs in smokers and in patients with chronic obstructive pulmonary disease as part of the pathogenesis of this condition. The reason for this is obvious since CS, which is the major etiological factor in the causes of development of COPD, contains a large quantity of oxidants. The traditional role for oxidants in the pathogenesis of COPD, whether inhaled in form of CS or released from activated neutrophils, is the inactivation of the natural inhibitors of neutrophil elastase, namely α1-proteinase inhibitor (α1-PI) and secretory leucoproteinase inhibitor (SLPI) [110]. Their role is the inactivation of excessive neutrophil elastase in the lung, which is liberated during inflammation and destroys elastin and other components of extracellular connective tissue matrix. Low serum levels of α1-PI have been often observed in many smokers with pulmonary emphysema. Oxidant-induced inactivation of α1-PI and SLPI produces a functional deficiency of these antiproteolytic defence forces in the airspaces, an event that is thought to be critical to the proteinase/antiproteinase imbalance that occurs as part of the pathogenesis of emphysema. However, it has been difficult to prove this theory in vivo since it is complicated by the presence of other proteinases and antiproteinases and by the fact that few studies in this field have controlled for the acute effect of smoking. One study that did assess the acute effect of smoking on the elastase inhibitory capacity of broncho-alveolar lavage (BAL) found a small but significant decrease in elastase inhibitory capacity one hour after smoking a cigarette. However other studies of chronic cigarette smokers, where the smoking history has been

controlled, have been inconclusive. The development of antioxidant therapy must take account of these fundamental molecular mechanisms in the inflammatory response to cigarette smoke in order to effectively protect the lung against both injurious and pro-inflammatory effects of oxidative stress [72, 85].

Glutathione (GSH), a ubiquitous intra- and extracellular tripeptide thiol, plays an important role in the antioxidant defence mechanisms against oxidative lung injuries due to free radicals and ROS. The synthesis of GSH requires the presence of γ-glutamylcysteine synthetase (γ-GCS) and GSH synthetase as major enzymes, as well as ATP, Mg^{2+}, and amino acids glycine, cysteine, and glutamate. Its synthesis rate depends on the controlling activity of the enzyme γ-GCS, the availability of cysteine as a substrate within the cell, and the feedback inhibitory mechanisms exerted by GSH itself on γ-GCS [93,113]. In lung, oxidants and inflammatory responses modulate gene expression of many signalling proteins in GSH metabolism, including GSH itself and γ-GCS. As previously mentioned, experimental findings suggest that the promoter (5'-flanking) regions of the γ-GCS-HS and γ-GCS-LS genes are regulated by putative c-*jun* homodimeric complex-AP-1 sequences in human alveolar epithelial cells and other cell lines [131]. It is also suggested that the transcription factor NF-κB plays a role in modulation of γ-GCS-HS [66]. Experimentally, blocking of activation at the transcriptional site of the γ-GCS-HS promoter prevents the oxidant- or cytokine-induced increase in γ-GCS-HS transcription in mouse endothelial and liver cells [134]. At the translational level, GSH synthesis is inhibited by various inflammatory agents such as cAMP and intracellular calcium that are released during inflammation. Various signalling pathways are also suggested to be involved in GSH synthesis. Investigations have determined that an activation of PKA, PKC,

and Ca^{2+}/calmodulin-dependent kinase II mediates inhibition of GSH synthesis. This inhibition of GSH synthesis was directly correlated with the phosphorylation of γ-GCS-HS on serine and threonine residues in a Mg^{2+} concentration dependent fashion [127]. Thus, the altering GSH levels during oxidative stress may be attributed to regulation of γ-GCS-HS activity due to phosphorylation-dephosphorylation. Both cytosolic and mitochondrial GSH levels may be affected by various inflammatory processes. TNF-α is known to deplete cytosolic GSH levels transiently in lung epithelial cells [111]. This depletion is thought to be due to radicals formed in mitochondria during oxidative stress. Cigarette smoke oxidants and ROS also deplete both cytosolic and mitochondrial GSH levels in lungs. It has recently been shown that mitochondrial gene transfer of glutathione reductase (GR) and overexpression of glutathione peroxidase (GPx) in various cell lines provided a protection of cells under oxidative stress [4,101].Thus, mitochondrial GSH might also play an important role in maintaining cellular antioxidant defence system and cell integrity under conditions of oxidative stress. Apart from GSH, other local and systemic antioxidant systems are affected during oxidative stress caused by smoking. In a study [42], an exposure of gas-phase CS has shown to cause a considerable depletion of various antioxidants such as ascorbate, urate, ubiquinol-10, α-tocopheral, and β-carotene. Through reduction of antioxidant capacity, the CS-induced oxidative stress can therefore lead to lung injury and epithelial permeability change. Airways obstruction will ultimately develop as a consequence of chronic recurrent inflammatory processes.

In short, the mechanisms of COPD development in smokers may therefore be explained in the chronic recurrent inflammatory responses, inactivation of anti-proteinases, and depletion of antioxidants involved during CS-induced oxidative stress. The most injurious effects of CS seem to occur

repeatedly during and immediately after acute cigarette smoking when the lung is depleted in GSH, since increased GSH levels are found in lungs of chronic smokers as a protective adaptive mechanism. However, the ability of individuals to regulate antioxidant defence mechanisms, such as the level of GSH in response to CS, may be genetically determined. Variations in these protective responses may, in part, relate to why only 15-20% of smokers develop COPD [122].Thus, alterations in alveolar and lung GSH metabolism and other antioxidant systems may play a potential role in the pathogenesis of COPD and other cigarette-related pulmonary diseases. More knowledge of the mechanisms of GSH regulation and other antioxidants in the lung could lead to better strategies in preventive and therapeutic approaches against oxidative lung inflammation and injury.

There are increasing reports suggesting that majority of cancer patients have also COPD. Since majority of these patients have long-term smoking history, the destructive pulmonary processes may involve both, the emphysematous and nonemphysematous obstruction. The most common complications of COPD are therefore pulmonary hypertension, cor pulmonale, and recurrent bronchopulmonary infections. Lung cancer patients may therefore have a limited survival not only because of the cancer itself, but also due to complications of the smoking habits. As a result, heart failure and pneumonia are important causes of death among lung cancer patients. In addition, impaired lung function due COPD and cancer, as well as reduced erythropoiesis due to bone marrow suppression during cytoreductive therapy lead together to a tremendous reduction of tissue oxygenation. This may play a critical role in the frequently observed reduction of performance status of patients with advanced lung cancer under palliative anticancer therapy. Both COPD and performance status are important prognostic factors. COPD alone can lead to a lowered survival, as

observed in a study which compared COPD patients to healthy individuals [135].

While diagnosis of COPD mostly requires examination of objective parameters such as spirometric lung function tests supported by chest radiography, its prognosis depends largely on the extent of the obstruction. The grading is based on the extent of the impaired lung function, observed on the fall in forced expiratory volume in one second (FEV1% of predicted value) and/or fall in the ratio of FEV1 to the vital capacity (FEV1/VC %). A mild COPD is characterised with FEV1 values over 70%; whereas FEV1 values ranging between 50 - 69%, and less than 50%, are regarded as moderate or severe COPD respectively. This staging has been proposed in a collective statement of the European Respiratory Society (ERS) [120].

5. Cigarette smoking, nicotine abuse and addiction

Through different ways of communication there is enough information about the carcinogenic and other disease-causing effects of cigarette smoking. Although the hazardous impact of tobacco consumption on the health is now world widely well known, many people are still smoking. This is because there are different factors which influence the use of tobacco among different age groups of people. More knowledge about pharmacological, biological, behavioural and social factors is therefore needed to evaluate different ways of solving this important public health problem.

Cigarette smoking is a genuine and complex addiction which can be compared to use of any one of the other known abused addictive substances such as heroin, cocaine, opiates, and alcohol. It is nicotine that maintains tobacco addiction. Nicotine is the key substance for the

neuropharmacological actions of tobacco smoke and has its effect on nicotinic cholinergic receptors of the central nervous system (CNS), where it mediates a number of its actions in the body. Its addictive properties appear to be mediated largely through stimulation of monoaminergic pathways as it leads to liberation of catecholamines [15]. It acts on different regions of the human brain. It can induce limbic cortical activation and an increase in neuronal activity in distributed brain regions. The same mentioned nicotine-induced effects are previously identified to participate in the mood-elevating and cognitive properties of other abused drugs such as opiates, cocaine and amphetamine [125]. These so called positive effects of nicotine are the strong reinforcements for tobacco users and play a significant role to their addiction. Nicotine is a substance with euphoriant and anxiolytic effects. It increases vigilance and intellectual performance. It is addictive and can induce pharmacological dependence and tolerance if it is repeatedly delivered to the bloodstream. It leads to physiological and psychological dependence, as well as withdrawal symptoms if its consumption is abruptly reduced or stopped. This contributes significantly to the high relapse rate upon cessation; although there are also other pharmacological and nonpharmacological factors of tobacco addiction which interfere with successful discontinuation [13]. However, the effect of nicotine in the brain is very complex and not fully understood yet. In the following table, a comparison of nicotine to other abused drugs is made.

Table 2: Comparison of addiction to nicotine and other abused drugs

	nicotine	cocaine	heroine	alcohol	caffeine
- psychoactive effects	+	+	+	+	+
- drug-reinforced behaviour	+	+	+	+	+
- compulsive use	+	+	+	+	-/+
- use despite harmful effects	+	+	+	+	-/+
- relapse after abstinence	+	+	+	+	-
- recurrent drug cravings	+	+	+	+	+
- drug tolerance	+	+	+	+	+
- physical dependence	+	+	+	+	+
- agonist useful in treating dependence	+	+	-	+	-

Adopted from reference Nr. 13

6. Effects of smoking cessation

Cigarette smoking is a global health care problem. Repetitive exposure to nicotine produces neuroadaptation leading to nicotine dependence. The resulting addiction makes smoking cessation difficult. The effects of stopping smoking or modification of smoking habits among long-term smokers are mainly observed in the lungs, air ways and CNS.

(a) Pulmonary effects of smoking cessation

After cessation or more than 25% decrease in the number of cigarettes smoked, significant improvement in spirometric performance can be registered even among heaviest smokers with a lifetime smoking history and poorest lung function [64]. In a study, subjects who stopped smoking completely had shown significant improvement in lung function one month after cessation, and continued for as long as half a year, and then remained stable [23]. Smoking cessation may also lead to structural improvement and hence amelioration of diffusing capacity of the lung [24]. In addition, other studies could register a dramatic decrease in respiratory symptoms in those who stopped smoking, a moderate decrease in those who reduced by at least 25%, and very little or no change in those who did not significantly modify their smoking habits [76,92]. This suggests that smoking cessation can prevent lung function from further damage or partly reverse the damaged lung function.

(b) Nicotine withdrawal syndrome

Upon abrupt stop or cut down of tobacco consumption among nicotine-addicted patients, psychovegetative reactions may result with manifestation of withdrawal symptoms of individually different degrees of severity, depending largely on the extent of addiction of the individual. The majority of smokers quit with minimal physical withdrawal symptoms. However, all experience some degree of psychological changes such as restlessness, increased irritation, fluctuation in mood, aggressiveness, increased anxiety, cognitive impairments, diminished stress tolerance, sleep disturbances, and craving for tobacco. The distinction between physical and psychic responses is some what artificial and difficult to delineate. However, to isolate the more physically based symptoms quitters record headache, dizziness, sweating and difficulties in concentrating. Most of the responses mentioned could be due to a drop in blood pressure that happens during the first two to three weeks of nicotine abstinence. Hunger, weight gain, constipation, probably due to the absence of gastrocolic reflex caused by CS, and creeping sensations in or beneath the skin due to spasmodic relaxation of smooth musculature in the peripheral blood vessels have also been reported **[**21,33**]**. All the above mentioned symptoms may lead to an extra burden to lung cancer patients who are generally already under immense psychological downcast. In such a situation, the compliance of patients as well as further disease course (in terms of quality of life) may be negatively influenced.

To date, the problem of nicotine withdrawal syndrome in smoking cessation programmes is partly managed by administration of nicotine as replacement therapy. Nicotine as medication for smoking cessation, and at the same time prevention of withdrawal syndrome, is currently available as chewing gum, transdermal delivery patch, inhaler, and nasal spray **[**14**]**. Recent

experimental findings in nicotine kinetics and metabolism may lead to introduction of novel pharmacological treatments in smoking cessation programmes. Further clinical analysis is needed to evaluate significance of other pharmaco-therapeutic approaches of nicotine addiction such as use of clonidine and antidepressant drugs [39, 47].

However, the exact mechanisms underlying nicotine addiction have to be more analysed. Many factors for individual differences in initiation, reinforcing effects, addiction, withdrawal and relapse of tobacco use are still not fully understood. Therefore, the importance of comparing behavioural, socio-cultural (environmental), biological, as well as pharmacological processes in the smoking problem has to be frequently assessed. Factors such as individual differences in nicotine kinetics and metabolism are some of important pharmacological processes in susceptibility to nicotine addiction and likelihood of successful smoking cessation [16]. Hence, successful management of nicotine addiction requires the use of structured, multidisciplinary, patient-oriented approach that includes nicotine replacement and withdrawal therapy, intensive monitoring, and long term follow-up; otherwise, the cessation rates will still remain very low.

7. Summary of the theoretical background

Cigarette smoking remains the proven major etiological factor for lung cancer development as indicated in many epidemiological studies carried out so far. Cigarette smoke contains a large quantity of pulmonary and extrapulmonary carcinogens. It is also an important source of reactive oxygen species, and plays a significant role in oxidative DNA damage and modulation of a number of biochemical pathways in the lung and other organs. The carcinogens and oxidative tissue damage are the paradigms of cigarette smoke-induced development of pulmonary and extrapulmonary malignant and nonmalignant diseases.

However, lung cancer development is a multifactoral process which requires carcinogen exposure, genetic and nongenetic factors. Genetic factors may lead to altered metabolism of carcinogens and mechanisms involved in repair of tobacco smoke-induced DNA damage, and therefore favour lung cancer development. Among the important enzymes in metabolism of cigarette smoke carcinogens are the cytochrome P450 (CYP450) and glutathione S-transferase (GST) gene products. In determining individual susceptibility to lung cancer development, CYP450 polymorphisms have been described in isoenzymes such as CYP2E1 and 2A6, while GST polymorphisms in isoenzymes such as GSTM1 and GSTP1. Polymorphisms in DNA repair genes or proteins relevant in lung cancer development have been described in genes such as the xeroderma pigmentosum complementary groups (XP), x-ray repair cross-complementary groups (XRCC), and in the DNA repair protein O6-methylguanine-DNA methyltransferase (MGMT).

Regarding patients with pre-existing lung cancer, continuation of cigarette smoking implies a prolonged exposure of bronchial system and other tissues or organs to carcinogens and oxidative stress. This may provide a possibility of inducing further molecular changes in normal, preneoplastic and neoplastic lung cells, as well as in structures important for tissue integrity. Early preneoplastic morphological changes may bypass further steps and progress to invasion. Progressive molecular changes in pre-existing tumours may give rise to altered phenotypes, aggressive or metastatic characters of the tumours, leading to important therapeutic and prognostic consequences. Smoking in lung cancer patients may also lead to development of nonneoplastic disorders which can influence the disease course and prognosis of lung cancer patients. Important diseases such as COPD, cardio-vascular diseases, and recurrent bronchopulmonary infections may play important role in multimorbidity and reduction of survival expectancy of lung cancer patients.

The lung of smoking lung cancer patients can be regarded as an organ with a very high exposure to carcinogenic factors (*field cancerization*), and therefore an increased risk for development of (pre)malignant lesions due to multiple genetic abnormalities caused by cigarette smoke. Smoking in lung cancer patients induces miscoding multiple DNA adducts of oxidative stress and of cigarette smoke carcinogens. These miscoding DNA adducts may undergo neoplastic transformation and progress to second lung tumours. Many studies have been carried out to investigate the risk of second cancer development in lung cancer patients. Smoking alone elevates the risk of second cancer development, but a significant risk increase has been reported when smoking was combined with treatment modalities such as radiotherapy and/or chemotherapy, or with family history of cancer.

Cigarette smoke can also induce modulation of a variety of macromolecules and signalling pathways and hence influence processes involved in tumour progression in lung cancer patients. These processes are metastasis, invasion, recurrence and resistance to therapy. Oxidative stress, whether induced by cigarette smoke or other factors such as anticancer therapy, can induce protein kinase-mediated cellular signal transduction and therefore influence regulation of some cellular events such as mitogenesis, cell adhesion, apoptosis, angiogenesis and metastasis. These are important events related to tumour promotion and invasion as shown in many studies investigated the roles of protein kinase A and C in these processes. The PKA- and PKC-mediated tumour promotion by oxidants appears due to disruption of the balance between protein phosphorylation and dephosphorylation. Via PKA and other mechanisms such as altered biosynthesis of proteinases and antiproteinases, cigarette smoke-induced oxidative stress may also influence the extracellular matrix and hence metastatic processes. Metastasis and recurrence may also be associated with other factors such as sialation of mucin in lung cancer patients. The abundance of siliac acid residues in mucin is due to overexpression of mucin glycoprotein genes. These genes are said to be highly expressed in smokers.

The problem of multidrug resistance (MDR) in the treatment of lung cancer is well known. If exposed to carcinogens or antineoplastic agents, tumour cells may possess mulltidrug-resistant phenotypes and be insensitive to chemotherapy. Some transport-associated proteins such as P-glycoprotein (P-gp or P-170), heat-shock proteins (HSPs), lung resistance protein (LRP) and others are involved in multidrug resistance. The P-gp related MDR is partly due to PKC-mediated protein phosphorylation. Both LRP and PKC are activated by cigarette smoke constituents. Cigarette smoking can induce overexpression of HSPs, the DNA repair protein O6-methylguanine-DNA

methyltransferase (MGMT), and if chronic also glutathione (GSH). In experiments these proteins have shown to cause MDR in lung cancer patients. However, the cigarette smoke-induced MDR may also involve more complicated mechanisms than these as shown in some molecular biology studies that observed H-*ras* oncogene-induced phenotype transition of SCLC to NSCLC.

Cigarette smoke is an important factor in causing oxidant pollutant-induced lung injury. Acute inflammatory reactions are usually reversible, but prolonged exposure to cigarette smoke may induce irreversible lung lesions. The inflammatory response to cigarette smoke is driven by native cells such as alveolar macrophages (AM) and fibroblasts. Inflammatory cells produce a number of proinflammatory factors such as ILs, LTs, TNF-α, NF-κB and TGF-β. Through mechanisms involving proteinases/antiproteinases, oxidants/antioxidants and GSH metabolism, chronic cigarette smoke-induced inflammatory processes are believed to be responsible for development of chronic structural lung disorders such as COPD. Cigarette smoking causes also disorders in the surfactant system and function of cilia. Cigarette smoke-induced reduction of surfactant and destruction of cilia are important factors for increased susceptibility to broncho-pulmonary infections and inflammatory processes which again predispose the lung to structural changes, leading to chronic air way diseases.

The hazardous effects of cigarette smoking are world widely known. However, many people are still smoking as nicotine in cigarette smoke is addictive. When tobacco consumption is abruptly stopped or cut down, nicotine-addicted lung cancer patients may show psycho-vegetative reactions with manifestation of withdrawal symptoms. The nicotine withdrawal syndrome may cause negative impact on the quality of life of the

patients, and is therefore the biggest obstacle for smoking cessation. Nicotine replacement and other therapeutic approaches may improve smoking cessation results. The benefits of smoking cessation have been described such as a moderate improvement in lung function, better socio-environmental integration and decrease in incidences of broncho-pulmonary symptoms and infections. However, smoking cessation among lung cancer patients may have more positive effects than these as related to different known and unknown actions of cigarette smoke in the body.

8. Patients and methods

8.1 Data acquisition and study population

The data of the study population of 302 lung cancer patients (47 women and 255 men) admitted between 1992 and 1998 in the department of pulmology of Beelitz hospital in Brandenburg (Germany) were obtained by collecting files and extracting important information for the study. The median age of the patients was 61 years, with a maximum of 84 and a minimum of 36 years. By the time of data collection, 285 (94.4%) of the patients were dead and the remaining 17 (5.6%) were not to follow up (censored).

The major criteria of inclusion for the study included histologically confirmed primary lung cancer (SCLC or NSCLC), advanced disease stages IIIa, IIIb, and IV (UICC category, corresponding to TNM-classification), primarily palliative based treatment modalities (chemotherapy, chemo-radiotherapy), and smoking habits after diagnosis (including smokers and ex-smokers). Patients who never smoked in their life time (non-smokers) are not included in this study. Patients with local advanced findings of lung cancer who were definitely treated with radiotherapy alone are also not part of this study. The criteria for the evaluation of the prognostic impact of smoking cessation were based on the overall survival time, and therapy

results. Whereas patients' smoking habits after diagnosis of lung cancer was the major factor for investigation of the prognostic impact of smoking cessation, other factors such as histology and tumour stage were also regarded as prognosis influencing parameters. However, gender and age were not matched.

In this study, there were 4 treatment regimes consisting of chemotherapy, radiotherapy and chemo-radiotherapy (table 3). 209 (69.2%) patients had got only chemotherapy, and 93 (30.8%) patients were treated with chemotherapy and radiotherapy as separate or combined modalities. Because of factors such as death, therapy withdrawal due to massive tumour progression, and lost patients' follow-up, not all patients were treated in the therapy regimes 2, 3, and 4. While 170 (56.3%) patients dropped out during the 2nd therapy regime, 268 (88.7%) patients could not be treated in the 3rd therapy regime. Only 6 (2.0%) patients were treated in the 4th therapy regime. However, all patients were treated in the 1st therapy regime (therapy regime 1); so that only the therapy results of the first therapy regime (given as outcome of therapy regime 1) have been considered in this study. The following table shows the four therapy regimes and the series in which the therapy was given.

Table 3: Therapy series in the 4 different regimes

	incidence	percent	valid percent	cumulated percent
validity				
1000	141	46,7	46,7	46,7
1100	53	17,5	17,5	64,2
1110	11	3,6	3,6	67,9
1111	4	1,3	1,3	69,2
1120	1	0,3	0,3	69,5
1200	4	1,3	1,3	70,9
1210	2	0,7	0,7	71,5
1300	9	3,0	3,0	74,5
2000	29	9,6	9,6	84,1
2100	31	10,3	10,3	94,4
2110	14	4,6	4,6	99,0
2111	2	0,7	0,7	99,7
2300	1	0,3	0,3	100,0
total	**302**	100,0	100,0	

The first column of the table represents the 4 therapy regimes and the series in which the therapy was given
0 = no therapy
1 = chemotherapy
2 = chemo-radiotherapy
3 = radiotherapy
It follows from the table above that all patients were treated in the first therapy regime, while 141 patients (1000) + 29 patients (2000) [= 170 patients] were not treated in the 2nd therapy regime

The distributions of other important parameters in the study population are summarized in the tables below. Note that percentages given in tables 4 – 8 refer to the total number of patients in the study population. Disease stages are given according to UICC category.

Table 4: Parameters of the study population based on diagnosis and smoking habits

	smokers	ex-smokers	**total**
	n	**n**	**n**
sclc	50 (16.5%)	57 (18.9%)	107 (35.4%)
nsclc	104 (34.4%)	91 (30.1%)	195 (64.6%)
total	154 (51.0%)	148 (49.0%)	**302** (100.0%)

sclc: small-cell lung cancer (= SCLC)
nsclc: non-small-cell lung cancer (= NSCLC)

Table 5: Parameters of the study population based on sex and smoking habits

	smokers	ex-smokers	**total**
	n	**n**	**n**
females	22 (7.3%)	25 (8.3%)	47 (15.6%)
males	132 (43.7%)	123 (40.7%)	255 (84.4%)
total	154 (51.0%)	148 (49.0%)	**302** (100.0%)

Table 6: Histology distribution of patients in the study population based on sex and smoking habits

	smokers	ex-smokers	**total**
females	**n = 22**	**n = 25**	**n = 47**
squamous-cell ca.	4 (1.3%)	2 (0.7%)	6 (2.0%)
adeno-ca.	8 (2.6%)	5 (1.7%)	13 (4.3%)
large-cell ca.	- (0.0%)	3 (1.0%)	3 (1.0%)
sclc	10 (3.3%)	15 (5.0%)	25 (8.8%)
males	**n = 132**	**n = 123**	**n = 255**
squamous-cell ca.	40 (13.2%)	45(14.9%)	85 (28.1%)
adeno-ca.	36 (11.9%)	28 (9.3%)	64 (21.2%)
large-cell ca.	16 (5.3%)	8 (2.6%)	24 (7.9%)
sclc	40 (13.2%)	42 (13.9%)	82 (27.1%)
total	**n = 154**	**n = 148**	**n = 302**
squamous-cell ca.	44 (14.5%)	47 (15.6%)	91 (30.1%)
adeno-ca.	44 (14.5%)	33 (11.0%)	77 (25.5%)
large-cell ca.	16 (5.5%)	11 (3.6%)	27 (8.9%)
sclc	50 (16.5%)	57 (18.9%)	107 (35.4%)

The histology distribution in the study population is once again shown in the diagram below.

Figure 2: Histogram showing histology distribution in the study population

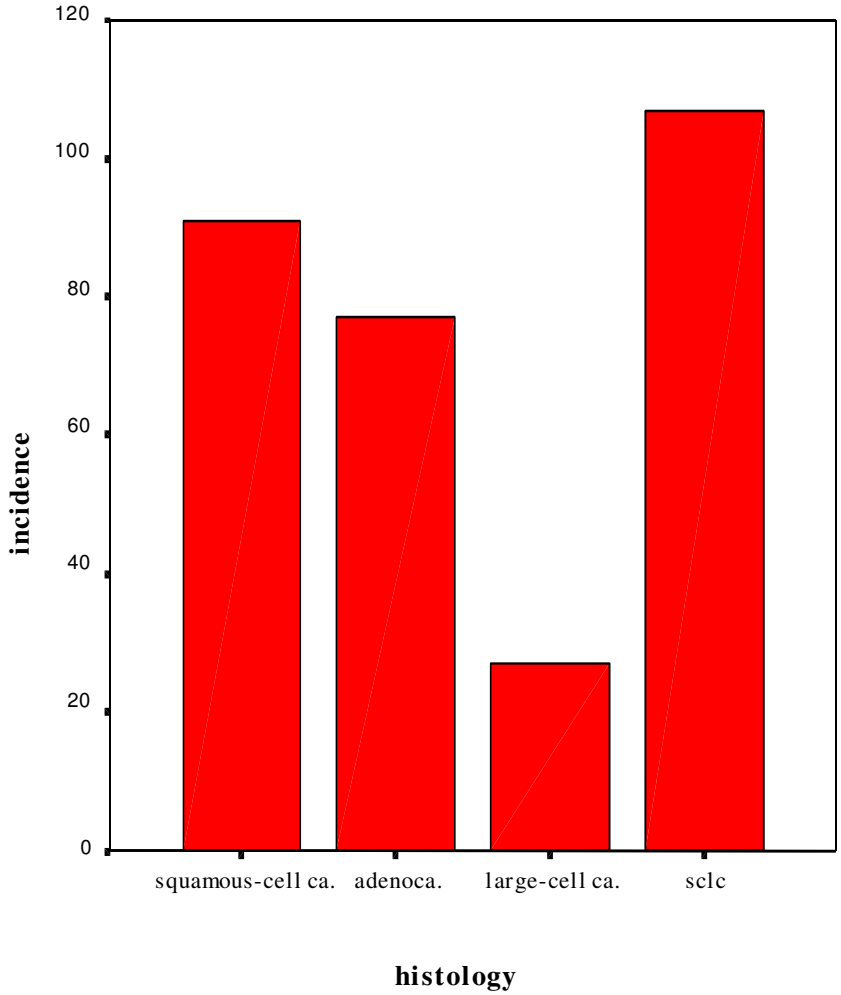


Table 7: Disease stage distribution of patients in the study population based on sex and smoking habits

	smokers	ex-smokers	**total**
females	**n = 22**	**n = 25**	**n = 47**
stage IIIa	2 (0.7%)	1 (0.3%)	3 (1.0%)
stage IIIb	1 (0.3%)	5 (1.7%)	6 (2.0%)
stage IV	19 (6.3%)	19 (6.3%)	38 (12.6%)
males	**n = 132**	**n = 123**	**n = 255**
stage IIIa	18 (5.9%)	37 (12.3%)	55 (18.2%)
stage IIIb	7 (2.3%)	11 (3.6%)	18 (5.9%)
stage IV	107 (35.4%)	75 (24.8%)	182 (60.2%)
total	**n = 154**	**n = 148**	**n = 302**
stage IIIa	20 (6.6%)	38 (12.6%)	58 (19.2%)
stage IIIb	8 (2.6%)	16 (5.3%)	24 (7.9%)
stage IV	126 (41.7%)	94 (31.1%)	220 (72.8%)

Table 8: Histology distribution of patients in the study population based on sex and disease stage

	stage IIIa	stage IIIb	stage IV	**total**
females	**n = 3**	**n = 6**	**n = 38**	**n = 47**
squamous-cell ca.	- (0.0%)	1 (0.3%)	5 (1.7%)	6 (2.0%)
adeno-ca.	1 (0.3%)	2 (0.7%)	10 (3.3%)	13 (4.3%)
large-cell ca.	- (0.0%)	1 (0.3%)	2 (0.7%)	3 (1.0%)
sclc	2 (0.7%)	2 (0.7%)	21 (6.9%)	25 (8.3%)
males	**n = 55**	**n = 18**	**n = 182**	**n = 255**
squamous-cell ca.	29 (6.9%)	5 (1.7%)	51 (16.8%)	85 (28.1%)
adeno-ca.	8 (2.6%)	4 (1.3%)	52 (17.2%)	64 (21.1%)
large-cell ca.	3 (1.0%)	1 (0.3%)	20 (6.6%)	24 (7.9%)
sclc	15(4.9%)	8 (2.6%)	59 (19.5%)	82 (27.0%)
total	**n = 58**	**n = 24**	**n = 220**	**n = 302**
squamous-cell ca.	29 (9.6%)	6 (2.0%)	56 (18.5%)	91 (30.1%)
adeno-ca.	9 (2.9%)	6 (2.0%)	62 (20.5%)	77 (25.4%)
large-cell ca.	3 (1.0%)	2 (0.7%)	22 (7.3%)	27 (9.0%)
sclc	17 (5.6%)	10 (3.3%)	80 (26.4%)	107 (35.4%)

Table 9: Histology, smoking habits and disease stage in the study population

histology	smoking habits:	stage IIIa	stage IIIb	stage IV	**total**
sclc	smokers:	6	2	42	50
	ex-smokers:	11	8	38	57
	total	**17**	**10**	**80**	**107**
squamous-cell ca.	smokers:	9	3	32	44
	ex-smokers:	20	3	24	47
	total	**29**	**6**	**56**	**91**
adenocarcinoma	smokers:	3	2	39	44
	ex-smokers:	6	4	23	33
	total	**9**	**6**	**62**	**77**
large-cell ca.	smokers:	2	1	13	16
	ex-smokers:	1	1	9	11
	total	**3**	**2**	**22**	**27**
total		**58**	**24**	**220**	**302**

Table 10: Distribution of patients in terms of smoking habits after diagnosis and the outcome of therapy regime 1

	outcome of therapy regime 1				
smoking habits	**p**	**sd**	**pr**	**cr**	**total**
ex-smokers:					
-number	48	20	69	11	148
-% of outcome of therapy regime 1	45.7	55.6	46.9	78.6	49.0
smokers:					
-number	57	16	78	3	154
-% of outcome of therapy regime 1	54.3	44.4	53.1	21.4	51.0
total					
-number	105	36	147	14	**302**
-% of outcome of therapy regime 1	100.0	100.0	100.0	100.0	100.0

Table 11: Distribution of patients in groups (cigarette groups) in terms of number of cigarettes smoked per day after diagnosis and the outcome of therapy regime 1

cigarette groups		outcome of therapy regime 1				total
		p	**sd**	**pr**	**cr**	
(ex-smokers)						
0 /d :	- number	48	20	69	11	148
	- % of outcome of therapy regime 1	45.7	55.6	46.9	78.6	49.0
(smokers)						
1 – 10 /d:	- number	23	8	51	3	85
	- % of outcome of therapy regime 1	21.9	22.2	34.7	21.4	28.1
11 – 20 /d:	- number	25	8	22		55
	- % of outcome of therapy regime 1	23.8	22.2	15.0		18.2
> 20 /d:	- number	9		5		14
	- % of outcome of therapy regime 1	8.6		3.4		4.6
total	- number	105	36	147	14	**302**
	- % of outcome of therapy regime 1	100.0	100.0	100.0	100.0	100.0

p = progression **sd** = stable disease **pr** = partial remission **cr** = complete remission

Note that the percentages given in the tables 10 and 11 in different columns of the outcome of therapy regime 1 refer to the total number of patients with the same therapy result.

Table 12: Distribution of incidences of accompanied patients' heart diseases

	incidence	**percent**	**valid percent**	**cumulated percent**
valid:				
heart insufficiency	50	16,6	68,5	68,5
arrhythmia	8	2,6	11,0	79,5
vitia	2	0,7	2,7	82,2
heart insufficiency + arrhythmia	11	3,6	15,1	97,3
heart insufficiency + vitia	1	0,3	1,4	98,6
heart insufficiency + arrhythmia + vitia	1	0,3	1,4	100,0
total	73	24,2	100,0	
missing:				
systematic	229	75,8		
total	**302**	100,0		

Table 13: Distribution of incidences of accompanied patients' non-malignant pulmonary diseases

	incidence	**percent**	**valid percent**	**cumulated percent**
valid:				
copd	89	29,5	95,7	95,7
pneumonia	2	0,7	2,2	97,8
copd + tuberculosis	1	0,3	1,1	98,9
copd + pneumonia	1	0,3	1,1	100,0
total	93	30,8	100,0	
missing:				
systematic	209	69,2		
total	**302**	100,0		

Table 14: Distribution of incidences of death causes in the study population

	incidence	**percent**	**valid percent**	**cumulated percent**
valid:				
lung cancer	285	94,4	94,4	94,4
pulmonary embolism	6	2,0	2,0	96,4
heart failure + myocardial infarction	6	2,0	2,0	98,3
unknown	2	0,7	0,7	99,0
copd	1	0,3	0,3	99,3
pneumonia	1	0,3	0,3	99,7
others	1	0,3	0,3	100,0
total	**302**	100,0	100,0	

copd: chronic obstructive pulmonary disease (COPD)

Table 12 shows that at least 50 (68.5%) of the total 73 patients who were registered to have different heart diseases had heart insufficiency caused by factors other than vitia and/or arrhythmia. The causes of heart insufficiency among these 50 patients may therefore be coronary artery disease, hypertensive heart disease, non-classified cardiomyopathy, and other diseases. Table 13 reveals COPD to be found in most of 93 patients who were registered to have different accompanied non-malignant pulmonary diseases. However, as shown in table 14, lung cancer itself remains to be the major cause of death of patients in the study population as it caused the death of 285 (94.4%) patients.

8.2 Statistical data analysis

The statistical data analysis was done using the statistical packet for social sciences (SPSS). Life table analysis for censored data according to Kaplan and Meier was used in different groups of patients for investigation of relationship of smoking status (smokers / ex-smokers) to the overall survival time. Censoring was performed for patients who were lost to follow-up. Statistically, a value of *p* less than 0.05 was considered to be significant.

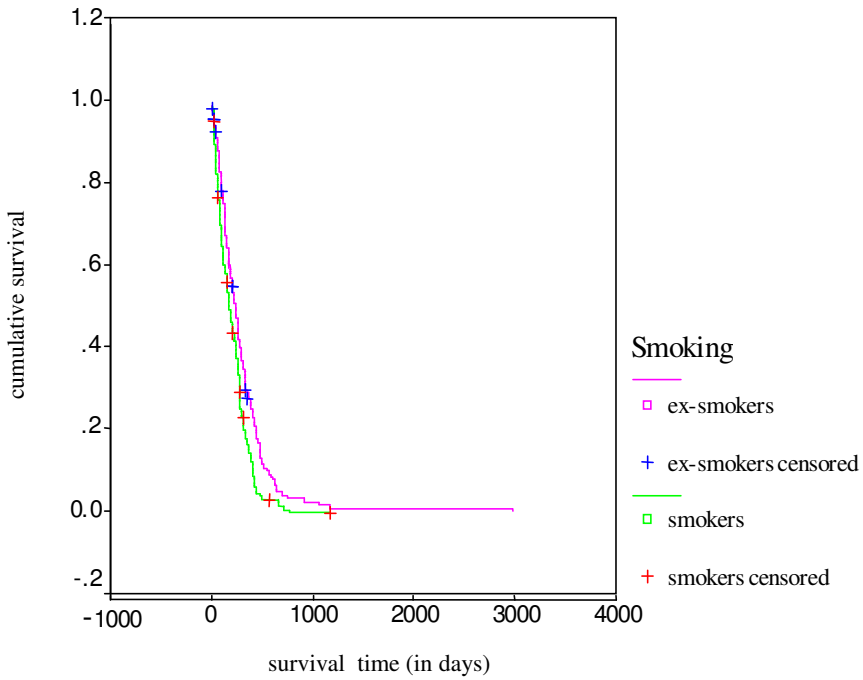
The correlation between smoking status and therapy results (outcome of therapy regime 1) was statistically evaluated by using Chi-Square test according to Pearson. A *p* value less than 0.05 was considered to be statistically significant.

9. Results

9.1 Overall survival time

Determinations of the relationship of smoking cessation to the overall survival time were done for the group with all patients in the study population (total study population), as well as for different groups formed by matching histology and/or disease stage as prognosis influencing factors. The Kaplan-Meier method of determining estimated survival functions for the factor smoking (smokers / ex-smokers) was used for the total study population and for every group of patients in the study population. However, only Kaplan-Meier graph for the total study population is illustrated in this work.

Fig. 3: Example of Kaplan-Meier graph showing estimated survival functions for the factor smoking (smokers / ex-smokers) in the total study population



Test analysis

- Smokers:

Number of Cases: 154 Censored: 8 (5.19%) Events: 146

	Survival Time (in days)	Standard Error	95% Confidence Interval
Mean:	215	15	(186; 245)
Median:	180	21	(138; 222)

- Ex-smokers:

Number of Cases: 148	Censored: 9 (6.08%)	Events: 139
Survival Time (in days)	Standard Error	95% Confidence Interval
Mean: 296	27	(243; 348)
Median: 240	21	(199; 281)

- Overall survival analysis for the factor Smoking in the total study population

	Total	Number Events	Number Censored	Percent Censored
Smokers:	154	146	8	5.19
Ex-smokers:	148	139	9	6.08
Overall:	**302**	285	17	5.63

- Test statistics for equality of survival distributions for the factor Smoking

	Statistic	df	Significance
Log Rank	7.49	1	**.0062**
Breslow	8.12	1	.0044
Tarone-Ware	8.37	1	.0038

All results (referred to *p* - values) of the statistical analysis of the effect of smoking cessation on the overall survival time in different groups in the study population are put together in the table below.

Table 15: Results of the analysis of the effect of smoking cessation on the overall survival time

	histology				
	sclc	squamous-cell ca.	adenoca.	large-cell ca.	**total**
stage (UICC)			(*p* values)		
IIIa	* 0.5436	+0.5914	+0.8153	* 0.2253	+0.8088
IIIb	♣0.0209 (s)	* 0.6939	* 0.3209	* 0.3173	♣0.0264 (s)
IV	* 0.4027	* 0.3013	* 0.1992	* 0.3070	♣0.0378 (s)
total	* 0.0788	* 0.2796	♣0.0439 (s)	* 0.1207	♣0.0062 (s)

The *p* value obtained from the group of total patients with non-small-cell lung cancer (NSCLC) was ♣0.0489 (s)

(s) = *p* value statistically significant
♣ = with a statistically significant difference, ex-smokers had longer median survival than smokers
* = with a statistically non-significant difference, ex-smokers had longer median survival than smokers
+ = with a statistically non-significant difference, smokers had longer median survival than ex-smokers

Detailed description of the results of the overall survival time

Total patients in the study population

In the total number of 302 patients in the study population there were 154 (51.0%) smokers and 148 (49.0%) ex-smokers. Without considering other prognosis influencing factors such as disease stage as well as histology, the overall survival time of smokers in the total study population differed statistically significant ($p = 0.0062$; log rank) from that of ex-smokers. The median survival of smokers was 180 +/- 21 days, and that of ex-smokers was 240 +/- 21 days.

Histology as prognosis influencing factor:

(i) Total patients with small-cell lung cancer

There were 107 patients with small-cell lung cancer in the study population, of which 50 (46.7%) patients were smokers and 57 (53.3%) patients were ex-smokers. The overall survival time between smokers and ex-smokers in patients with small cell lung cancer did not show statistically significant difference (p = 0.0788). The median survival of smokers was 258 +/- 20 days, and that of ex-smokers was 284 +/- 28 days.

(ii) Total patients with non-small-cell lung cancer

There were a total of 195 patients with non-small-cell lung cancer, of which 104 (53.3%) patients were smokers and 91 (46.7%) patients were ex-smokers. There was a statistically significant difference (p = 0.0489; log rank) in the overall survival time between smokers and ex-smokers in patients with non-small cell lung cancer. The median survival of smokers was 151 +/- 21 days, while that of ex-smokers was 181 +/- 22 days.

- Patients with squamous-cell carcinoma

There were 91 patients with squamous-cell carcinoma in the study population. 44 (48.3%) patients were smokers and 47 (51.7%) patients were ex-smokers. There was no statistically significant difference (p = 0.2796) in the overall survival time between smokers and ex-smokers in patients with squamous cell carcinoma. The median survival of smokers was 146 +/- 38 days, and that of ex-smokers was 193 +/- 38 days.

- Patients with adenocarcinoma

Patients with adenocarcinoma in the study population were 77, of which 44 (57.2%) patients were smokers and 33 (42.8%) patients were ex-smokers. The median survival of smokers with adenocarcinoma was 159 +/- 19 days, and that of ex-smokers was 245 +/- 67 days. This result showed a statistically significant difference (p = 0.0439; breslow) in the overall survival time between smokers and ex-smokers in this group.

- Patients with large-cell carcinoma

27 patients in the study population had large-cell carcinoma, of which 16 (59.3%) patients were smokers and 11 (40.7%) patients were ex-smokers. No statistically significant difference (p = 0.1207) in the overall survival time was found between smokers and ex-smoker in patients with large-cell carcinoma. The median survival of smokers was 115 +/- 14 days, and that of ex-smokers was 154 +/- 36 days.

Disease stage as prognosis influencing factor:

- Patients with disease stage IIIa

There were 58 patients with disease stage IIIa in the study population, of which 20 (34.5%) patients were smokers and 38 (67.5%) patients were ex-smokers. Smokers and ex-smokers with disease stage IIIa showed no statistically significant difference in the overall survival time (p = 0.8088). Smokers had a median survival of 311 +/- 17 days, and ex-smokers had a median survival of 293 +/- 39 days.

- Patients with disease stage IIIb

There were 24 patients with disease stage IIIb in the study population, of which 8 (33.3%) patients were smokers and 16 (76.7%) patients were ex-smokers. Patients with disease stage IIIb showed statistically significant difference (p = 0.0264; log rank) in the overall survival time between smokers and ex-smokers. While the median survival of smokers was 69 +/- 13 days, ex-smokers lived longer with the median survival of 253 +/- 59 days.

- Patients with disease stage IV

There were 220 patients with disease stage IV in the study population, of which 126 (57.3%) patients were smokers and 94 (42.7%) patients were ex-smokers. The overall survival time of smokers with disease stage VI differed statistically significant (p = 0.0378; tarone-ware) from that of ex-smokers. Smokers had a median survival of 174 +/- 19 days, and ex-smokers had a median survival of 209 +/- 23 days.

Histology and disease stage combined as prognosis influencing factors:

- Patients with small-cell lung cancer in disease stage IIIa

There were 17 patients with small-cell lung cancer in disease stage IIIa in the study population, of which 6 (35.3%) patients were smokers and 11 (74.7%) patients were ex-smokers. Patients with small-cell lung cancer in disease stage IIIa did not show statistically significant difference (p = 0.5436) in the overall survival time between smokers and ex-smokers. The median survival of smokers was 302 +/- 39 days, and that of ex-smokers was 340 +/- 63 days.

- Patients with small-cell lung cancer in disease stage IIIb

There were 10 patients with small-cell lung cancer in disease stage IIIb in the study population, of which 2 (20%) patients were smokers and 8 (80%) patients were ex-smokers. Patients with small-cell lung cancer in disease stage IIIb showed a statistically significant difference (p = 0.0209; breslow) in the overall survival time between smokers and ex-smokers. The median survival of smokers was 27 days, and that of ex-smokers was 416 +/- 213 days. However, this result should be taken with reservation since there were ex-smokers in this group of patients who could survive advanced lung cancer for more than two years. This has surely affected the result in favour of ex-smokers.

- Patients with small-cell lung cancer in disease stage IV

There were 80 patients with small-cell lung cancer in disease stage IV in the study population, of which 42 (56.5%) patients were smokers and 38 (47.5%) patients were ex-smokers. Patients with small-cell lung cancer in disease stage IV did not show statistically significant difference (p = 0.4027) in the overall survival time between smokers and ex-smokers. The median survival of smokers was 255 +/-23 days, and that of ex-smokers was 263 +/-23 days.

- Patients with squamous-cell carcinoma in disease stage IIIa

There were 29 patients with squamous-cell carcinoma in disease stage IIIa in the study population, of which 9 (31.1%) patients were smokers and 20 (68.9%) patients were ex-smokers. Patients with squamous-cell carcinoma in disease stage IIIa did not show statistically significant difference (p = 0.5914) in the overall survival time between smokers and ex-smokers. The

median survival of smokers was 311 +/- 194 days, and that of ex-smokers was 165 +/- 46 days.

- Patients with squamous-cell carcinoma in disease stage IIIb

There were 6 patients with squamous-cell carcinoma in disease stage IIIb in the study population, of which 3 (50%) patients were smokers and 3 (50%) patients were ex-smokers. Patients with squamous-cell carcinoma in disease stage IIIb did not show statistically significant difference ($p = 0.6939$) in the overall survival time between smokers and ex-smokers. The median survival of smokers was 93 +/- 22 days, and that of ex-smokers was 153 +/- 74 days.

- Patients with squamous-cell carcinoma in disease stage IV

There were 56 patients with squamous-cell carcinoma in disease stage IV in the study population, of which 32 (57.2%) patients were smokers and 24 (42.8%) patients were ex-smokers. Patients with squamous-cell carcinoma in disease stage IV did not show statistically significant difference ($p = 0.3013$) in the overall survival time between smokers and ex-smokers. The median survival of smokers was 146 +/- 39 days, and that of ex-smokers was 209 +/- 38 days.

- Patients with adenocarcinoma in disease stage IIIa

There were 9 patients with adenocarcinoma in disease stage IIIa in the study population, of which 3 (33.3%) patients were smokers and 9 (66.7%) patients were ex-smokers. Patients with adenocarcinoma in disease stage IIIa did not show statistically significant difference ($p = 0.8153$) in the overall survival time between smokers and ex-smokers. The median survival of smokers was 417 days, and that of ex-smokers was 293 +/- 37 days.

- Patients with adenocarcinoma in disease stage IIIb

There were 6 patients with adenocarcinoma in disease stage IIIb in the study population, of which 2 (33.4%) patients were smokers and 4 (66.6%) patients were ex-smokers. Patients with adenocarcinoma in disease stage IIIb did not show statistically significant difference ($p = 0.3209$) in the overall survival time between smokers and ex-smokers. The median survival of smokers was 69 days, and that of ex-smokers was 181 +/- 145 days.

- Patients with adenocarcinoma in disease stage IV

There were 62 patients with adenocarcinoma in disease stage IV in the study population, of which 39 (62.9%) patients were smokers and 23 (37.1%) patients were ex-smokers. Patients with adenocarcinoma in disease stage IV did not show statistically significant difference ($p = 0.1992$) in the overall survival time between smokers and ex-smokers. The median survival of smokers was 153 +/- 21 days, and that of ex-smokers was 140 +/- 20 days.

- Patients with large-cell carcinoma in disease stage IIIa

There were 3 patients with large-cell carcinoma in disease stage IIIa in the study population, of which 2 (66.7%) patients were smokers and 1 (33.3%) patient was ex-smoker. Patients with large-cell carcinoma in disease stage IIIa did not show statistically significant difference ($p = 0.2253$) in the overall survival time between smokers and ex-smokers. The median survival of smokers was 148 days, and that of ex-smokers was 343 days.

- Patients with large-cell carcinoma in disease stage IIIb

There were 2 patients with large-cell carcinoma in disease stage IIIb, of which 1 (50%) patient was smoker and 1 (50%) patient was ex-smoker. The 2 patients with large-cell carcinoma in disease stage IIIb did not show

statistically significant difference (p = 0.3173) in the overall survival time. The median survival of the smoker was 18 days, and that of the ex-smoker was 168 days.

- Patients with large-cell carcinoma in disease stage IV

There were 22 patients with large-cell carcinoma in disease stage IV in the study population, of which 13 (59.1%) patients were smokers and 9 (40.9%) patients were ex-smokers. Patients with large-cell carcinoma in disease stage IV did not show statistically significant difference (p = 0.3070) in the overall survival time between smokers and ex-smokers. The median survival of smokers was 115 +/- 14 days, and that of ex-smokers was 122 +/- 28 days.

9.2 Outcome of therapy regime 1 (therapy results)

The outcome of therapy regime 1, which can be regarded as the therapy sensitivity, was objectively measured by considering therapy results obtained in staging examinations of patients after therapy regime 1. Staging examinations were carried out using commonly known methods such as conventional chest radiography, sonography, and computed tomography. The therapy results are given in tables 10 and 11 as complete remission, partial remission, stable disease, or progression. The *p* value obtained from the Chi-Square test was 0.1010 (statistically not significant). This calculation was done only for the total study population without adjustment of patients for histology and/or disease stage. It follows that there is no statistically significant difference in the outcome of therapy regime 1 between smokers and ex-smokers in the total study population. However, interesting results were obtained when patients were put in cigarette groups according to the number of cigarettes smoked per day. These results are explained and interpreted in the discussion below.

10. Discussion

The objective of the present study was to determine how discontinuation of cigarette smoking influences the prognosis of 302 patients with advanced lung cancer who were under palliative anticancer therapy in the previously mentioned hospital in Germany. In the study population there were patients in different advanced disease stages (IIIa, IIIb, IV) and with different lung cancer histologies (SCLC and NSCLC). All commonly known histologies of NSCLC, namely squamous-cell carcinoma, adenocarcinoma and large-cell carcinoma, were found in different patients in the study population. Patients were treated either with chemotherapy alone, or chemotherapy and radiotherapy as separate or combined modalities. The survival times and outcome of therapy regime 1 (therapy results) were separately used as measures for the prognostic effect of smoking cessation. This retrospective study was carried out by collecting files of patients and extracting important information for the study such as smoking status, therapy modality, outcome of therapy regimes, survival time, histology, disease stage and others.

In this study there were twenty-one different groups of patients formed (table 15). One group consisted of all patients in the study population (total study population) without matching histology and/or disease stage. Five groups were only histology-matched, and three groups were adjusted for disease stage. Twelve groups were adjusted for both histology and disease stage. Histology and disease stage were regarded as potential prognosis influencing factors. In some groups of patients, smoking cessation prior to beginning of palliative anticancer therapy has shown to have positive prognostic effect on the overall survival outlook. With a statistically significant difference, ex-smokers had longer median survival than smokers in the group with all patients in the study population ($p = 0.0062$), in the group of total patients with non-small cell lung cancer ($p = 0.0489$), in the

group of total patients with adenocarcinoma (p = 0.0439), in the group of total patients with disease stage IIIb (p = 0.0264) and IV (p = 0.0378), and in the group consisting of patients with small-cell lung cancer in disease stage IIIb (p = 0.0209). All remaining fifteen groups of patients did not show any statistically significant difference in the overall survival time between smokers and ex-smokers. However, with the exception of three groups, namely the group of all patients with disease stage IIIa, the group of patients with squamous-cell carcinoma in disease stage IIIa, and the group of patients with adenocarcinoma in disease stage IIIa, ex-smokers had longer median survival than smokers in twelve of the fifteen groups of patients. In these three groups, smokers had longer median survival than ex-smokers. Since ex-smokers had longer median survival than smokers in disease stage IIIa patients with small-cell lung cancer and large-cell carcinoma, it is difficult to make a general statement on the prognostic association between smoking and disease stage IIIa; but a tendency is shown that the prognosis of patients with less advanced lung tumours may be less affected by cigarette smoking after diagnosis than that of patients with more advanced lung tumours.

The above results are therefore consistent with the fact that there are many determinants of overall survival time apart from histology and disease stage. Among these determinants are metastatic status, poor performance status, tumour size, treatment modality, and other prognostic influencing factors such as anaemia, age and gender. For obtaining more accurate tools for a rational treatment decision, Wigren et al. [144] identified in a retrospective study some important determinants of overall survival time in patients with non-small-cell lung cancer and combined them to a prognostic index. Since every patient is likely to have one or more prognostic determinants which differ from those of others, patients can be put into their corresponding

prognostic groups which may be identifiable as separate prognostic clusters. The prognostic index, which should be verified by using independent data, may be useful in daily clinical practice in the future. Nevertheless, the prognostic factors in the study of Wigren et al. were so strong that multivariate analysis did not reveal the treatment modality to have any significant influence on the survival. Therefore, this is an area of further investigations.

The outcome of therapy regime 1, which reflects the sensitivity of anticancer therapy, was measured based on the therapy results such as complete remission, partial remission, stable disease, and progression after a completed therapy regime 1. The therapy results were obtained by staging examinations of patients after the above mentioned therapy regime. There was no statistically significant difference between smokers and ex-smokers when the outcome of therapy regime 1 was tested for the total study population. However, majority of patients (78.6%) with complete remission were ex-smokers (tables 10 and 11). This percentage corresponds to 11 of the total 14 patients who reached complete remission after a completed therapy regime 1. Furthermore, the remaining 3 (21.4%) patients with complete remission belonged to the group of patients with the lowest daily cigarette consumption (1 – 10 cigarettes per day). There were no patients with complete remission from groups of patients with consumption of more than 10 cigarettes per day. When patients without complete remission after a completed therapy regime 1 were analysed, interesting results could be observed. There were 105 patients who experienced tumour progression; 48 (45.7%) patients were ex-smokers and 57 (54.3%) patients were smokers. 36 patients had stable disease, of which 20 (55.6%) patients were ex-smokers and 16 (44.4%) patients were smokers. However, only 69 (46.9%) patients from a total number of 147 patients with partial remission were ex-smokers.

More interesting results were observed in patients who smoked more than 10 cigarettes per day. In a total number of 55 patients who smoked 11 – 20 cigarettes per day, 25 (45.5%) patients had tumour progression, 8 (14.5%) patients had stable disease, and 22 (40.0%) patients experienced partial remission. There were 14 patients in the total study population with a daily consumption of more than 20 cigarettes, of which 9 (64.3%) patients had tumour progression, and 5 (35.7%) patients had partial remission. Complete remission, partial remission and stable disease are, to different extent, more favourable therapy results than tumour progression. The above results therefore indicate that ex-smokers frequently experience more favourable therapy results than smokers during anticancer therapy of advanced malignant lung tumours.

11. Conclusion

Since patients with advanced lung cancer have generally poor prognosis, identification of prognostic factors is critical for optimising treatment. It is therefore important to frequently carry out studies with the goal of identifying new potential prognostic factors that can improve survival and quality of life of this group of lung cancer patients. The purpose of this work was therefore to identify whether smoking cessation has a positive impact on the prognosis of patients with advanced lung cancer. The background of this work is based on the different effects of cigarette smoke in the body as indicated in many scientific findings which have previously been described in details in this work.

This retrospective study consisted of 302 patients with advanced lung cancer in disease stages IIIa, IIIb and IV. After histologically confirmed diagnosis of primary lung cancer, 148 (49%) patients quitted from smoking (ex-smokers), while 154 (51%) patients continued smoking (smokers). After

palliative treatment of these patients with chemotherapy and/or chemo-radiotherapy (table 3), the survival times and therapy results were statistically evaluated. Since histology and disease stage were regarded as prognosis influencing parameters, the patients' data concerning the overall survival times were analysed for the total study population and according to groups which were formed after adjustment of patients for histology and/or disease stage (table 15).

On analysing the prognostic effect of smoking cessation on the overall survival time, ex-smokers lived statistic significantly longer than their corresponding smokers in some of the groups as previously shown in the discussion. Whereas ex-smokers lived 1 month longer than smokers in the group of all patients with non-small-cell lung cancer and in the group of all patients in disease stage IV, smoking cessation could prolong the overall survival for about 2 months in the total study population, and for about 3 months in the group of patients with adenocarcinoma. A considerable prolongation of the overall survival of about 6 months could be reached by ex-smokers in disease stage IIIb.

On analysing the prognostic effect of smoking cessation on the therapy results, there was no statistically significant difference between ex-smokers and smokers in the total study population. However, when patients were put together into groups according to the number of cigarettes smoked per day (table 11), interesting results could be observed. The results have shown that only ex-smokers (78.6%) and smokers (21.4%) who smoked a maximum of 10 cigarettes per day could reach complete remission. Furthermore, ex-smokers (45.7%) less often experienced tumour progression than smokers (54.3%). In this study, the probability of reaching tumour progression depended on the number of cigarettes smoked per day and increased

significantly (64.3%) by a daily consumption of more than 20 cigarettes. This is consistent with the results of the studies of Dingemans et al. and Volm et al.[38,140].

The above results conclude therefore that cigarette smoking cessation among patients with advanced lung cancer under palliative anticancer therapy has a positive prognostic effect on the overall survival outlook. Cigarette smoke may render lung cancer cells less sensitive to anticancer therapy and therefore negatively affect the prognosis of the patients. The results also suggest that the prognosis of patients with more advanced tumours, and with NSCLC (especially adenocarcinomas), may be more negatively affected following prolonged exposure to cigarette smoke, and therefore insist histology and disease stage to be important prognostic factors.

As shown in many studies, cigarette smoke interferes with mechanisms controlling tumour invasion, metastasis, recurrence, and therapy resistance. These cigarette smoke-induced effects, which involve metabolic pathways of macromolecules such as protein kinases, resistance-related proteins and others, may partly be genetically determined and play a central role in tumour progression and hence determination of the prognostic impact of smoking cessation. However, many effects of cigarette smoke in human are unknown and still remain a challenge to the modern medicine. More research is required in this area for the better understanding of mechanisms by which cigarette smoke causes various diseases, the expected time interval in which the diseases can occur, the individual susceptibility to development of cigarette smoke-induced diseases, and the possible disease promoting and progressing effects of cigarette smoke. Genetic involvement of effects of cigarette smoke has partly been described in factors such as polymorphisms

in enzymes (e.g. CYP450) relevant in metabolism of tobacco carcinogens, and in genes responsible for repair of cigarette smoke-induced DNA damage.

I therefore advise that more and larger studies in this area should be carried out with adjustment of patients for other potential prognostic factors such as age and gender. However, if smoking cessation in the treatment of lung cancer gets recognised in the future as positive prognostic factor, any decision of asking nicotine-addicted lung cancer patients to quit from smoking should also consider the problems of nicotine withdrawal syndrome which may have negative impact on the quality of life (quantity *versus* quality of life). As already explained in this work, there are different therapeutic approaches to this problem which can improve smoking cessation results.

Conclusion (Translation in German Language) = Zusammenfassung

Die Prognose der Patienten mit fortgeschrittenem Bronchialkarzinom ist im allgemeinen schlecht. Die Identifizierung von prognostischen Faktoren ist daher entscheidend für eine Therapieoptimierung. Demzufolge ist eine häufigere Durchführung von Studien zur Identifizierung neuer potenzieller prognostischer Faktoren wichtig, um das Überleben und die Lebensqualität dieser Patientengruppe zu verbessern. Das Ziel dieser Arbeit war deshalb herauszufinden, ob eine Zigarettenabstinenz einen positiven Einfluss auf die Prognose der Patienten mit fortgeschrittenem Bronchialkarzinom hat. Der Hintergrund dieser Arbeit basiert auf der Tatsache, dass der Zigarettenrauch eine vielfältige Wirkung auf den menschlichen Körper hat. Dies ist in vielen wissenschaftlichen Studien belegt und in dieser Arbeit detailliert beschrieben worden.

Diese retrospektive Studie bestand aus 302 Patienten mit fortgeschrittenem Bronchialkarzinom in den Stadien IIIa, IIIb und IV. Nach histologischer Diagnosesicherung eines primären Bronchialkarzinoms haben 148 (49%) Patienten das Rauchen eingestellt (Ex-Raucher), während die restlichen 154 (51%) Patienten weiterhin geraucht haben (Raucher). Nach palliativer Behandlung der Patienten mit Chemotherapie und/oder Chemo-radiotherapie (Tabelle 3), wurden die Überlebenszeiten und Therapieergebnisse statistisch ausgewertet. Da Histologie und Tumorstadium als prognosebeeinflussende Parameter betrachten wurden, wurden die Patienten nach Histologie und/oder Tumorstadium in Gruppen geordnet. Die Auswertung der Gesamtüberlebenszeiten erfolgte sowohl für die Gesamtstudienpopulation als auch für die entstandenen Patientengruppen (Tabelle 15).

Wie es in der Diskussion beschrieben wurde, hat die Analyse der prognostischen Wirkung von Zigarettenabstinenz auf die Gesamtüberlebenszeit ergeben, dass die Ex-Raucher in einigen der Gruppen statistisch gesehen ein erhöhtes Gesamtüberleben haben im Vergleich zu Rauchern. Während die Ex-Raucher in der Gruppe der Patienten mit nicht-kleinzelligem Bronchialkarzinom und der Patienten in Tumorstadium IV jeweils 1 Monat länger als die Raucher lebten, erzielte eine Zigarettenabstinenz eine Verlängerung des Gesamtüberlebens um 2 Monate bei den Patienten innerhalb der gesamten Studienpopulation, und um 3 Monate bei den Patienten mit Adenokarzinom. Eine erhebliche Verlängerung des Gesamtüberlebens um 6 Monate konnte bei den Ex-Rauchern in Tumorstadium IIIb erreicht werden.

Die Auswertung der prognostischen Wirkung von Zigarettenabstinenz auf die Therapieergebnisse hat ergeben, dass es keinen statistisch signifikanten Unterschied zwischen Ex-Rauchern und Rauchern innerhalb der gesamten Studienpopulation gibt. Allerdings, nachdem Patienten anhand des täglichen Zigarettenverbrauchs in Gruppen eingeteilt wurden (Tabelle 11), konnten interessante Ergebnisse beobachtet werden. Die Ergebnisse haben gezeigt, dass nur Ex-Raucher (78,6%), und Raucher (21,4%), die maximal 10 Zigaretten pro Tag geraucht haben, eine komplette Remission erreichen konnten. Des Weiteren kam es bei den Ex-Rauchern (45,7%) weniger oft zu Tumorprogression als bei den Rauchern (54,3%). In dieser Studie war die Wahrscheinlichkeit zur Entwicklung einer Tumorprogression von der Anzahl der täglich gerauchten Zigaretten abhängig. Diese Wahrscheinlichkeit erhöhte sich signifikant (64,3%) bei täglichem Verbrauch von mehr als 20 Zigaretten. Das ist übereinstimmend mit den Ergebnissen der Studien von Dingemans et al. und Volm et al. [38,140].

Die Schlussfolgerung aus den oben genannten Ergebnissen ist, dass eine Zigarettenabstinenz bei den Patienten mit fortgeschrittenem Bronchialkarzinom, die sich einer palliativen Antikrebstherapie unterziehen, eine positive Wirkung auf die Prognose in Bezug auf das Gesamtüberleben hat. Zigarettenrauch kann die Empfindlichkeit von Lungenkrebszellen auf antineoplastische Therapie verringern, und dadurch die Prognose der Lungenkrebspatienten negativ beeinflussen. Ferner deuten die Ergebnisse darauf hin, dass die Prognose der Patienten mit mehr fortgeschrittenen Tumoren, und mit nicht-kleinzelligen Bronchialkarzinomen (vor allem Adenokarzinomen), besonders negativ durch anhaltenden Nikotinabusus beeinflusst werden kann. Somit sind Histologie und Tumorstadium als wichtige prognostische Faktoren zu betrachten.

Wie in vielen Studien gezeigt, mischt sich der Zigarettenrauch mit Mechanismen, die die Vorgänge wie Tumorinvasion, Metastasierung, Rezidiv, und Therapieresistenz kontrollieren, ein. Diese Wirkungen, die auf Einflüsse des Zigarettenrauchs auf die Stoffwechselwege von verschiedenen Makromolekülen wie Proteinkinasen, resistenz-verwandte Proteine, und andere zurückzuführen sind, können zum Teil genetisch bedingt sein, eine zentrale Rolle bei der Tumorprogression spielen, und dadurch die prognostische Bedeutung der Zigarettenabstinenz bestimmen. Allerdings sind viele Wirkungen des Zigarettenrauchs bei einem Menschen noch unbekannt und stellen eine Herausforderung für die moderne Medizin dar. Mehr Forschung wird in diesem Bereich gebraucht, um die Mechanismen des Zigarettenrauchs bei der Entstehung einer Vielfalt von Erkrankungen, den erwarteten Zeitabstand bis zur Entwicklung dieser Erkrankungen, die individuelle Anfälligkeit für zigaretteninduzierte Erkrankungen, so wie die möglichen krankheitsfördernden und –fortschreitenden Effekte des Zigarettenrauchs besser zu verstehen. Genetischer Einfluss der Wirkungen von Zigarettenrauch wurden zum Teil beschrieben in Faktoren wie Polymorphismen in Enzymen, die für den Stoffwechsel von Tabakkarzinogenen (z.B. CPY450) relevant, und in Genen, die für die Reparatur von durch Zigarettenrauch verursachten DNA-Schäden verantwortlich sind.

Deshalb weise ich darauf hin, dass mehr und größere Studien in diesem Bereich, unter Berücksichtigung auf andere potenzielle prognostische Faktoren wie das Alter und Geschlecht der Patienten, durchgeführt werden sollten. Falls Nikotinabstinenz in der Behandlung von Lungenkrebs in Zukunft als positiver prognostischer Faktor anerkannt wird, sollte jede Entscheidung einer Zigarettenabstinenz bei nikotinabhängigen Lungenkrebspatienten die Probleme eines Nikotinentzugssyndroms

berücksichtigen, da diese einen negativen Einfluss auf die Lebensqualität haben können (Lebensquantität *versus* Lebensqualität). Wie bereits in dieser Arbeit erklärt, gibt es zu dieser Problematik verschiedene therapeutische Möglichkeiten, die die Nikotinentwöhnungsergebnisse verbessern können.

12. References

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13. Acknowledgments (In German Language) = Danksagung

An erster Stelle möchte ich meinem Betreuer, Herrn Prof. Dr. med. G. Schlimok, Chefarzt der II. Medizinischen Klinik am Klinikum Augsburg, meinen tiefen Dank für seine Hilfe und Unterstützung ausdrücken. Prof. Dr. Schlimok widmete seine kostbare Zeit, um mir bei dieser Arbeit zum Erfolg zu verhelfen. Dafür rechtherzlichen Dank!

Herrn Dr. med. M. Sandherr, Oberarzt in der II. Medizinischen Klinik am Klinikum Augsburg, gilt mein besonders herzlicher Dank für seine Mitbetreuung. Seine Freundlichkeit, Geduld, Kompetenz, und vor allem konstruktive Kritik bei der Verfassung dieser Arbeit waren mir sehr hilfreich.

Herrn PD Dr. med. T. Beinert möchte ich, für seine Betreuung zu Beginn dieser Promotionsarbeit im Jahr 2000 in der II. Medizinischen Klinik mit Schwerpunkt Onkologie und Hämatologie am Universitätsklinikum (Charité) der Humboldt-Universität zu Berlin, auch danken.

Mein herzlicher Dank geht an Frau G. Siebert vom Institut für Medizinische Informatik und Biostatistik an der Humboldt-Universität zu Berlin für ihre wertvolle Unterstützung bei der statistischen Auswertung der Patientendaten und Interpretation einiger Ergebnisse.

Ferner möchte ich Herrn Dr. rer. nat. M. Fleischhacker vom Universitätsklinikum (Charité) der Humboldt-Universität zu Berlin für seine Unterstützung und hervorragendes Zusammenarbeiten in unserer wissenschaftlichen Arbeitsgruppe "Bronchialkarzinom" danken.

Vielen herzlichen Dank!

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