Non-small cell lung cancer

Studies on pathogenesis, tumour targeting and treatment outcomes

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To all lung cancer patients and their loved ones

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1 ABBREVITATIONS

8-OHdG	8-hydroxydeoxyguanosine
BDI	Baseline Dyspnea Index
CCI	Charleson Comorbity Index
COPD	Chronic Obstructive Pulmonary Disease
CT	Computed Tomography
DL_{CO}	Carbon Monoxide Diffusing Capacity
DNA	Deoxyribonucleic Acid
EGFR	Epidermal Growth Factor Receptor
EORTC	European Organisation for Research and Treatment of Cancer
FEV_1	Forced Expiratory Volume in one second
FVC	Forced Vital Capacity
FDG	[^{18}F]2-fluoro-2-deoxy-glucose
FNA	Fine Needle Aspiration
HAD	Hospital Anxiety and Depression scale
HRQoL	Health-Related Quality of Life
MMP	Matrix-Metalloproteinase
MPO	Myeloperoxidase
NADPH	Nicotinamide Adenine Dinucleotide Phosphate
NOS	Nitric Oxide Synthase
NSCLC	Non-Small Cell Lung Cancer
QLQ	Quality of Life Questionnary
PET	Positron Emission Tomography
PMN	Polymorphonuclear Neutrophils
ROS	Reactive Oxygen Species
SF-36	Short Form (36) health survey
SOD	Superoxide Dismutase
SUV	Standard Uptake Value
TNM	Tumour node metastasis
VATS	Video-Assisted Thoracic Surgery
VEGF	Vascular Endothelial Growth Factor

2 LIST OF ORIGINAL PUBLICATIONS

This thesis is based on the following original publications, which are referred to in the text by their Roman numerals:

I Ilonen IK, Räsänen JV, Sihvo EI, Knuuttila A, Sovijärvi AR, Sintonen H, Salo JA. Pneumonectomy: post-operative quality of life and lung function. *Lung Cancer* 2007;58:397-402.

II Ilonen IK, Räsänen JV, Sihvo EI, Knuuttila A, Salmenkivi K, Ahotupa MO, Kinnula VL, Salo JA. Oxidative stress in non-small cell lung cancer: Role of nicotinamide adenine dinucleotide phosphate oxidase and glutathione. *Acta Oncologica* 2009; 48:1054-61.

III Ilonen IK, Räsänen JV, Knuuttila A, Sihvo EI, Sintonen H, Sovijärvi AR, Salo JA. Quality of life following lobectomy or bilobectomy for non-small cell lung cancer, a two-year prospective follow-up study. *Lung Cancer* 2010; 70:347-51.

IV Ilonen IK, Räsänen JV, Knuuttila A, Salo JA, Sihvo EI. Anatomic thoracoscopic lung resections for non-small cell lung cancer in stage I is associated with less morbidity and shorter hospitalization than thoracotomy. *Acta Oncologica* (in press)

V Koivistoinen A, Ilonen IK, Punakivi K, Räsänen JV, Helin H, Sihvo EI, Peräkylä H, Bergman M, Salo JA. Targeting peptides specific for non-small cell lung cancer identified by ex vivo phage display. (submitted)

3 ABSTRACT

Lung cancer accounts for more cancer-related deaths than any other cancer. In Finland, five-year survival ranges from 8% to 13%. The main risk factor for lung cancer is long-term cigarette smoking, but its carcinogenesis requires several other factors.

The aim of the present study was to 1) evaluate post-operative quality of life, 2) compare clinical outcomes between minimally invasive and conventional open surgery, 3) evaluate the role of oxidative stress in the carcinogenesis of non-small lung cancer (NSCLC), and 4) to identify and characterise targeted agents for therapeutic and diagnostic use in surgery.

For study I, pneumonectomy patients replied to 15D quality of life and baseline dyspnea questionnaires. Study III involved a prospective quality of life assessment using the 15D questionnaire after lobectomy or bi-lobectomy. Study IV was a retrospective comparison of clinical outcomes between 212 patients treated with open thoracotomy and 116 patients who underwent a minimally invasive technique. Study II measured parameters of oxidative metabolism (myeloperoxidase activity, glutathione content and NADPH oxidase activity) and DNA adducts. Study V employed thephage display method and identified a core motif for homing peptides. This method served in cell-binding, cell-localisation, and biodistribution studies.

Following both pneumonectomy and lobectomy, NSCLC patients showed significantly decreased long-term quality of life. No significant correlation was noted between post-operative quality of life and pre-operative pulmonary function tests. Women suffered more from increased dyspnea after pneumonectomy which was absent after lobectomy or bi-lobectomy. Patients treated with video-assisted thoracoscopy showed significantly decreased morbidity and shorter periods of hospitalization than did open surgery patients. This

improvement was achieved even though the VATS patients were older and suffered more comorbid conditions and poorer pulmonary function. No significant differences in survival were noted between these two groups. An increase in NADPH oxidase activity was noted in tumour samples of both adenocarcinoma and squamous cell carcinoma. This increase was independent from myeloperoxidase activity. Elevated glutathione content was noted in tumour tissue, especially in adenocarcinoma. After panning the clinical tumour samples with the phage display method, an amino acid sequence of ARRPKLD, the Thx, was chosen for further analysis. This method proved selective of tumour tissue in both in vitro and in vivo cell-binding assay, and biodistribution showed tumour accumulation.

Because of the significantly reduced quality of life following pneumonectomy, other operative strategies should be implemented as an alternative (e.g. sleeve-lobectomy). To treat this disease, implementation of a minimally invasive surgical technique is safe, and the results showed decreased morbidity and a shorter period of hospitalisation than with thoracotomy. This technique may facilitate operative treatment of elderly patients with comorbid conditions who might otherwise be considered inoperable. Simultaneous exposure to oxidative stress and altered redox states indicates the important role of oxidative stress in the pathogenesis and malignant transformation of NSCLC. The studies showed with great specificity and with favourable biodistribution that Thx peptide is specific to NSCLC tumours. Thx thus shows promise in imaging, targeted therapy, and monitoring of treatment response.

4 INTRODUCTION

Lung cancer is the leading cause of cancer-related deaths worldwide in both men and women, respectively causing 1.3 million deaths annually (Parkin et al 2005). Lung cancer has the second highest cancer incidence in Finnish men, with 1556 cases in 2008, and the fourth in women, with 722 cases (Finnish Cancer Registry, 2009). The main risk factor for lung cancer is cigarette smoking (Wynder and Graham 1950). Other known significant risk factors in the pathogenesis of lung cancer include exposure to radon gas, asbestos and air pollution, as well as genetic factors (Pass et al 2010). One of the key factors initiating the pathogenesis of lung cancer is through direct exposure to reactive oxygen species (ROS) and via activation of polymorphonuclear neutrophils (Federico et al 2007). This leads to alterations in cell-signaling and mutations, and ultimately to carcinogenesis. The two main types are non-small cell lung cancer (NSCLC) and small cell lung cancer. Of the three main types of NSCLC, the most frequent type is adenocarcinoma, followed by squamous cell carcinoma and large cell carcinoma (Brambilla et al 2001).

The diagnosis of lung cancer is usually made at the end-stage of the disease, and is thus associated with poor prognosis (Tammemagi et al 2004). Modern diagnosis and staging includes computed tomography (CT) of the chest and upper abdomen. The use of fiberoptic bronchoscopy, tumour biopsy, positrone-emission tomography and mediastinoscopy in the diagnosis and staging is often customised from patient to patient.

The most significant determinant for treatment and survival is the stage of the disease. Surgical resection is the main therapy for local NSCLC in patients with stage IA to IIB (Scott et al 2007) and in selected IIIA patients (Robinson et al 2007). In unresectable stage III patients, concurrent chemoradiation is the treatment of choice (Jett et al 2007),

whereas advanced stage IV is treated with palliative treatment (Socinski et al 2007). Lung cancer is usually diagnosed at an advanced stage, hence the poor outcome despite improved diagnosis and treatment. Overall the five-year survival of lung cancer patients ranges from 8% to 13% (Hakulinen et al 2010). For pathological stages IA and IB, five-year survival ranges from 73% to 58%; for stages IIA and IIB, from 46% to 36%; IIIA, 24%; and IIIB and IV, 13% to 9% (Goldstraw et al 2007).

Since its introduction in the early 1990s (Roviaro et al 1992), video-assisted thoracic surgery has received mixed responses and its adaptation has been slow. It gained greater acceptance following reports from larger series (McKenna et al 2006) and long-term results (Swanson et al 2007; Yamamoto et al 2010). Studies have shown video-assisted thoracic surgery to be associated with lower morbidity (Paul et al 2010) and shorter hospitalisation (Flores et al 2009) without sacrificing oncologic safety.

Progression-free and overall survival are classic endpoints for evaluating treatment outcomes, but this fails to take into account the subjective measure of quality of life. In recent years, this issue has received greater attention to accommodate patient needs. Surgery for NSCLC is challenging, as patients are often elderly with comorbid conditions and decreased performance status. The issue of decreased quality of life following surgery for NSCLC is well known (Brunelli et al 2007; Kenny et al 2008), but has otherwise received little attention, possibly because of the poor prognosis of the disease. In clinical practice, one of the key factors in determining eligibility for surgery has been pre-operative pulmonary function tests (Reilly 1997). Such tests, however, serve as a poor surrogate for estimating post-operative quality of life (Brunelli and Salati 2008).

The use of targeted therapy has increased significantly in recent years (Pass et al 2010) to achieve better treatment outcomes with lower morbidity than that of normal cytotoxic agents. Linear peptides offer many advantages over monoclonal antibodies that are currently used in clinical

practice, including lower molecular weight, less immunogenity, a more stable configuration, and easier synthesis and modification (Adessi and Soto 2002). These peptides serve as homing molecules for both imaging agents and cytotoxic agents.

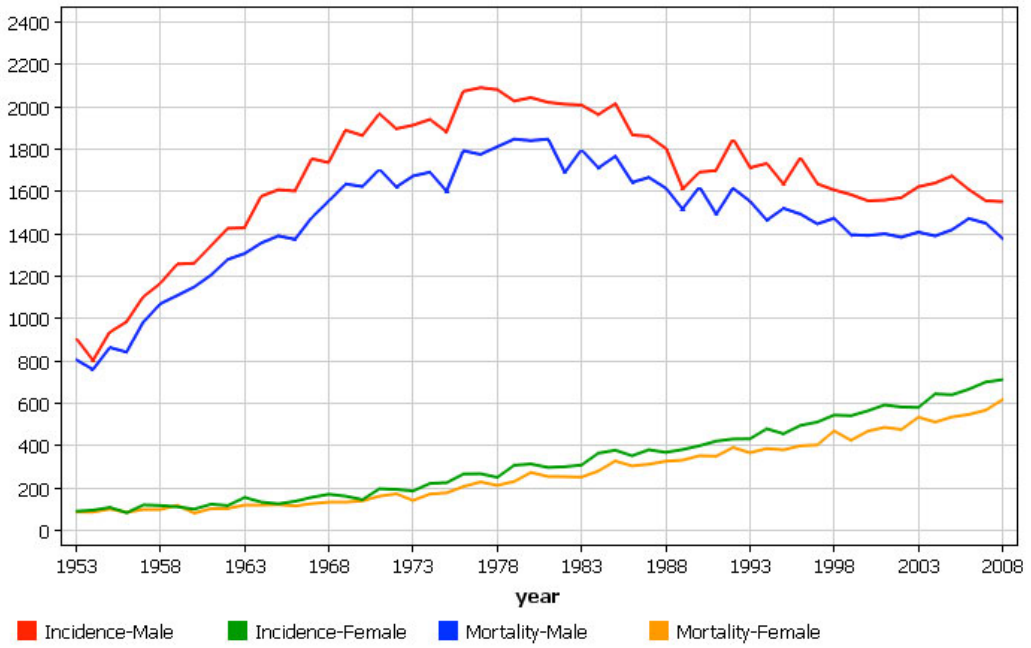
5 REVIEW OF THE LITEATURE

5.1 Epidemiology

Lung cancer has been the most common cancer in the world since 1985, with approximately 1.35 million new cases diagnosed annually. Both the incidence and mortality of lung cancer are higher in developing countries than in developed ones (Parkin et al 2005). The incidence among men in Europe has seen a significant reduction in the past five years, but among women, and in other parts of the world, it continues to increase (Ferlay et al 2007). In Finland, lung cancer has the second highest incidence after prostate cancer among men; in 2008, 1575 new cases were diagnosed. In women, lung cancer is the fourth most common cancer after breast, bowel and endometrial cancer; in 2008, 722 new cases of lung cancer were diagnosed (Finnish Cancer Registry, March 2010). The incidence of lung cancer in Europe accounts for about 21% of all cancer cases in men (Borras et al 2003). As the prevalence of male smokers has declined since the mid-1970s, the incidence of lung cancer has also decreased. In contrast, the incidence of lung cancer among women has been increasing, and estimates indicate that the trend will continue (Finnish Cancer Registry, March 2010). The incidence and mortality of lung cancer in Finland appears in Figure 1. The prognosis of lung cancer has remained poor despite progress in both diagnostic and treatment measures, as five-year survival ranges from 8% to 13% (Hakulinen et al 2010). Globally, lung cancer is the most common cause of cancer death (Parkin et al 2005), yet none of the lung cancer screening programmes available has yielded any clear benefit (Field and Duffy 2008). The preliminary results of the most recent study by the national lung cancer screening trial, however, show that

low-dose CT in screening is associated with 20% fewer lung cancer deaths than the plain chest X-ray (National Lung Screening Trial Research Team et al 2011).

Figure 1 Incidence and mortality of lung cancer in Finland between 1953 and 2008 according to gender (Engholm et al 2010).



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5.2 Risk factors

Cigarette smoking is the main cause of lung cancer (Doll and Hill 1950; Wynder and Graham 1950). Estimates suggest that NSCLC is attributable to cigarette smoking in about 90% of men and in 80% of women (D'Addario et al 2009). Over 4000 individual constituents of cigarette smoke have been identified (Hoffmann and Hoffmann 1997), of which 60 are established carcinogens (Hecht 2003), including nitrosamines, polycyclic aromatic hydrocarbons, aldehydes, oxidants, metals, and aromatic

amines (Hecht 2002). Of all smokers, only 10-15% develop lung cancer, and 10-15% of lung cancer patients are non-smokers (Mattson et al 1987). Some researchers have postulated that by eliminating tobacco smoking, 20% of all cancer deaths could be prevented (Pisani et al 1999). The change in cigarette design has resulted in a major shift in histological classification from squamous cell carcinoma to adenocarcinoma (Gray 2006). Enviromental exposure to cigarette smoke is also a risk factor, which may increase the probability in non-smokers living with smokers up to 26% (Hackshaw et al 1997), and may contribute to up to 2% to 3% of all cases of lung cancer (Besaratinia and Pfeifer 2008).

Genetic susceptibility to lung cancer is supported by the familial aggregation of a disease, which was first described in 1963 (Tokuhata and Lilienfeld 1963). Several susceptible genes have been associated with moderately increased risk for NSCLC (Schwartz et al 2007). Studies have demonstrated that men over 50 years have no clear inherited genetic predisposition (Braun et al 1994), but patients under 50 years, heritage seems to be a significant factor (Li and Hemminki 2004). The clinical implications for these findings, however, remain unclear.

Gender contributes significantly to the risk for lung cancer (Zang and Wynder 1996), as the response odds ratio for developing lung cancer in women was 1.2- to 1.7-fold higher than in men. The same study found that non-smoking women were twice as susceptible to lung cancer as men.

The risk for lung cancer is significantly associated with age, as two thirds of patients are over 65 and over 40% of patients are over 70 years of age (Ries 1994). Only about 10% of the cases are diagnosed in patients under 50 years (Jemal et al 2009).

Asbestos is historically the most widely accepted occupational cause of lung cancer (Doll 1955). The use of asbestos has been banned in Finland since 1994, and

asbestos renovations are currently subject to a license requiring appropriate protection equipment. Thus, exposure to asbestos is currently minimal. Arsenic has also been identified early as a significant risk factor for lung cancer (Blot and Fraumeni 1975). Other occupational causes for lung cancer include radon gas (Darby et al 2005), silica (Steenland et al 2001), beryllium and other chemical agents (Pass et al 2010). In addition, environmental air pollution, such as nitric oxide, fine particulate matter and combustive by-products, increase life-long risk for lung cancer (Boffetta 2004).

Lung cancer is associated with several genomic alterations. Mutation of the p53 tumour suppressor gene is the most common genetic lesion in human cancers (Steen 2000), is mutated in two thirds of lung cancers (Bennett et al 1993) and overexpressed in about half (Carbone et al 1994). Mutations in the proto-oncogene *ras* gene family, K-*ras*, occur in 30% of adenocarcinomas of the lung (Westra et al 1993) and are thus considered a precursor lesion to adenocarcinoma of the lung (Cooper et al 1997). One of the key signalling pathways for NSCLC is epidermal growth factor (EGFR), which interacts with various processes related to the carcinogenesis of NSCLC (Ciardiello and Tortora 2001). EGFR is overexpressed in 40% to 80% of NSCLC tumours (Rusch et al 1997), and EGFR mutation is more common in Asian populations (25% to 50%) than in North American and Western European populations (Sequist et al 2007). Mutated EGFRs, primarily in adenocarcinomas, show a higher activation type than the wild-type receptor (Lynch et al 2004).

5.3 Inflammation and lung cancer

Carcinogenesis is a long process that depends on multiple factors, including the initiation, promotion and progression of transformed cells to cancer. The association

between inflammation and carcinogenesis is well established (Federico et al 2007; Weitzman and Gordon 1990). The main component of oxidative stress, reactive oxygen species (ROS), consists broadly of oxygen-containing chemical species with reactive properties, such as superoxide ($O2^{\bullet -}$) and hydrogen peroxide (H_2O_2). Cells constantly generate ROS via enzyme-catalysed and non-enzyme reactions, mostly in mitochondria (Chance et al 1979). The most significant source of exogenous ROS derives from polymorphonuclear neutrophils (PMN), which are abundant in the respiratory system, with the pulmonary vasculature being their main reservoir (Doerschuk 2001). Inhaled particles activate PMN in the lungs (e.g. cigarette smoke, silica and asbestos), thereby inducing the release and formation of ROS in PMN (Borm et al 2004; Knaapen et al 2004). Chronic inflammation of the lungs, as in chronic obstructive pulmonary disease (COPD), is also independently associated with increased risk for lung cancer (Islam and Schottenfeld 1994). Besides the activation of PMN, cigarette smoke contains high levels of oxidants and compounds that initiate carcinogenesis, which contribute to the formation of ROS (Pryor et al 1983). Free radicals of cigarette smoke occur in two phases: the gas and tar phases. Of these, the gas phase contains more reactive radicals (Church and Pryor 1985). Exposure to cigarette smoke in the respiratory tract induces direct carcinogenesis (Subapriya et al 2002). As a result, cancer cells are generally under intensive oxidative stress, thereby inducing enhanced ROS generation, increased accumulation of ROS-mediated products and over-expression of antioxidant enzymes (Pelicano et al 2004). Overexposure to ROS causes multiple alterations in cellular molecules (Federico et al 2007). Also, established oncogenes such as c-myc induce the formation of ROS in malignant cells (Vafa et al 2002) and the polymorphism of pro-inflammatory genes which contribute to individual risk for NSCLC (Shih et al 2006).

Myeloperoxidase (MPO) is the most abundant protein in PMN, a dominant lysosomal enzyme which produces hypochlorous acid (HOCl) from H_2O_2 and chloride for defence against micro-organisms (Klebanoff 2005). MPO is associated with PNM activation (Weiss and LoBuglio 1982), and increased serum levels of MPO, being more sensitive than other PNM activation markers, are associated with current cigarette smoking (Andelid et al 2007).

The initial enzyme in ROS production is nicotinamide adenine dinucleotide phosphate (NADPH) oxidase, which transports electrons to reduce oxygen to a superoxide. To date, seven different isoforms have been identified in non-phagocytic mammalian cells (Bedard and Krause 2007). NADPH oxidase also serves as a mediator in numerous physiological and pathological processes, including cell signaling, inflammation and mitogenesis (Brar et al 2002). In cancer cells, ROS may act in cell signaling, promoting cell survival over apoptosis (Szatrowski and Nathan 1991). Hydrogen peroxide, produced by Nox1, can induce the activation of several pro-angiogenic factors (Arbiser et al 2002). Isoforms of NADPH oxidase expressed in lung tissue include NADPH oxidase 2, dual oxidase 1 and NADPH oxidase 4 (van der Vliet 2008). In NSCLC, studies have reported down-regulation of dual oxidase 1 and 2 expression (Luxen et al 2008).

Reduced glutathione is an intracellular free thiol group containing tripeptide antioxidant, which serves as an electron donor, thereby reducing disulfide bonds. Reduced glutathione is synthesised mainly in cytoplasm and serves in numerous antioxidant and detoxification reactions (Schafer and Buettner 2001). Being the most prevalent intracellular radical scavenger, reduced glutathione is considered one of the most important antioxidant defences against the inhaled reactive components of cigarette smoke (Biswas et al 2006). Reduced glutathione also modulates cell proliferation (Navarro et al 1997). The content of

glutathione in the epithelial lining fluid of the respiratory tract is 100-fold higher than in the blood (Cantin and Begin 1991). Increased glutathione content is associated with NSCLC tumours (Blair et al 1997), and researchers believe that altered glutathione metabolism contributes to resistance in radiation and chemotherapy (Yang et al 2006). The rate-limiting enzyme of glutathione, glutamate-cysteine ligase, is overexpressed in NSCLC (Soini et al 2001).

Some of the main evidence of the carcinogenetic properties of ROS is the induction of genotoxic effects on cellular deoxyribonucleic acid (DNA) (Burcham 1998; Wiseman and Halliwell 1996), as with OH^--specific DNA base lesion 8-hydroxydeoxyguanosine (8-OHdG) in the alveolar epithelium (Knaapen et al 1999). Exposure to cigarette smoking is associated with a 30-50% increase in urine 8-OHdG content (Loft et al 1992), indicating that cigarette smoking is major cause of oxidative DNA damage and carcinogenesis. Impaired DNA repair capacity is also associated with lung cancer (Hansen 2008).

5.4 Diagnosis

The symptoms of lung cancer are non-specific and usually present at an advanced stage of the disease. Of all patients, approximately 5-10% are asymptomatic at presentation (Chute et al 1985; Hansen 2008). One of the most important factors behind the poor prognosis of lung cancer is the delay in diagnosis (Tammemagi et al 2004). Common symptoms of lung cancer include cough (45-75%) (Kvale 2006), haemoptysis, thoracic pain (25-50%) (Patel and Peters 1993), dyspnea, loss of appetite, loss of weight, frequent pneumonias, malaise and weakness (Pass et al 2010). Rarer symptoms include Horner's syndrome for Pancoast tumours that invade the thoracic wall, superior vena cava syndrome and paraneoplastic symptoms that usually occur with small cell lung cancer. Pleural

metastasis occurs in 10% of all lung cancer patients (Patel and Peters 1993).

The diagnosis is usually based on physical examination, radiological findings and a biopsy sample. Chest radiography is the preferred initial radiographic study due to its availability, low cost, low radiation exposure, and high sensitivity (Heelan 1991). The main imaging study for staging is CT of the chest and upper abdomen, hence its great importance in treatment planning.

Fiberoptic bronchoscopy is valuable tool for establishing diagnosis and treatment planning for intrabronchial tumours (Savage et al 2001). This technique allows endoscopic acquisition of brushing, lavage, as well as direct and fine-needle aspiration (FNA) biopsies for pathological analysis. The combination of bronchial lavage and transbronchial FNA in central lesions can yield sensitivity as high as 88% (Rivera et al 2007). However, the yield is strongly associated with the size and location of the tumour, as with peripheral lesions under 2 cm, sensitivity decreases to 34% (Rivera et al 2007). The complication rate for bronchoscopy is under 1%, including cough, hypoxemia, cardiac arrhythmia, bleeding, pneumothorax, and iatrogenic infection.

A biopsy of the primary tumour is usually obtainable by using CT or ultrasound-guided biopsy. Transthoracic FNA has been the method of choice for diagnosing peripheral tumours, as sensitivity is as high as 90%, and specificity approaches 100% (Rivera et al 2007). The false-negative rate, however, ranges from 20% to 30%. Most common complications for transthoracic FNA include bleeding and pneumothorax. Only 5% of patients who receive pneumothorax from FNA require chest tube placement. Tumour markers are not routinely used in the diagnosis of lung cancer (Pass et al 2010).

5.5 Histologic classification

Lung cancer is divided into two main groups according to histological appearance: NSCLC and small cell lung cancer (Beasley et al 2005). NSCLC accounts for approximately 70-80% of lung tumours, whereas small cell lung cancer accounts for 20-30% (Travis et al 1995).

The main subtypes of NSCLC include adenocarcinoma, squamous cell lung cancer and large cell carcinoma (Brambilla et al 2001). Adenocarcinoma is the most common subtype of NSCLC, especially among women (Radzikowska et al 2002), and accounts for 30% to 50% of all cases (Travis et al 1995). However, the incidence of squamous cell carcinoma and small cell lung cancer among women is rising (Devesa et al 2005). The second most prevalent NSCLC subtype is squamous cell carcinoma, constituting 20% to 35% of all lung cancer cases (Travis et al 1995). A subtype of adenocarcinoma, bronchioloalveolar carcinoma, is most common in female non-smokers, and thus may respond differently to treatment (Raz et al 2006). As of recently, bronchioloalveolar carcinoma is no longer part of the lung cancer classification system, and has been replaced by adenocarcinoma in situ or adenocarcinoma (Travis et al 2011). No differentiation has been observed between large cell carcinoma which would allow classification into squamous cell carcinoma, adenocarcinoma or small cell lung cancer (Hansen 2008). Large cell carcinoma tumours usually occur in the lung periphery.

Exposure to cigarette smoke contributes significantly to the histology and location of the tumour (Godschalk et al 2002), as squamous cell carcinoma is more associated with smoking than adenocarcinoma (Khuder 2001), and is usually anatomically located more centrally than adenocarcinoma or large cell carcinoma, since two thirds of squamous cell carcinoma tumours present as central lesions (Tomashefski et al 1990). Moreover, the effect of

smoking cessation is more prominent in the prevention of small cell lung cancer and squamous cell carcinoma carcinogenesis than adenocarcinoma (Devesa et al 2005).

5.6 Staging

Initial staging of NSCLC is important in order to identify patients who may benefit from therapies that target curative treatment. TNM staging is generally based on the size of the primary tumour (T), regional lymph nodes (N) and metastasis (M) characteristics. The revised TNM staging used in the present studies was published in 1997 (Lababede et al 1999) and appears in Table 1. Stage grouping is the combination of TNM subsets into seven stage categories of disease that reflect on the survival expectations of the best outcome for stage I to the worst in stage IV (see Table 2). As of 2010, however, an updated seventh version of the classification has been implemented in clinical practice (Rami-Porta et al 2009). Changes include the following: the T1 and T2 categories are divided by tumour diameter (T1a, T1b, T2a, and T2b) and malignant pleural effusion from T4 to M1a. In addition, if a patient has a satellite tumour in the same lobe, it falls into the T3 category, or T4 if it is in the ipsilateral lobe, or M1a if it is in the contralateral lobe. There were no changes in N classification, but the M category has been subdivided into M1a and M1b. These changes accomplished a more accurate alignment for treatment and prognosis (Goldstraw et al 2007; Rami-Porta et al 2009).

Table 1 The TNM classification of lung cancer (Union for International Cancer Control) (Lababede et al 1999)

T0	No evidence of primary tumour
Tis	Carcinoma in situ
T1	Tumour is 3 cm or less in its greatest dimension, surrounded by lung or visceral pleura, without bronchoscopic evidence of invasion more proximal than the lobar bronchus* (i.e., not in the main bronchus)
T2	Tumour with any of the following features of size or extent: More than 3 cm in its greatest dimension Involves main bronchus, 2 cm or more distal to the carina Invades the visceral pleura Associated with atelectasis or obstructive pneumonitis that extends to the hilar region, but does not involve the entire lung.
T3	Tumour of any size that directly invades any of the following: chest wall (including superior sulcus tumours), diaphragm, or the mediastinal pleura or pericardium; or tumour in the main bronchus less than 2 cm distal to the carina, but without involvement of the carina; or associated atelectasis or obstructive pneumonitis of the entire lung.
T4	Tumour of any size that invades any of the following: mediastinum, heart, great vessels, trachea, oesophagus, vertebral body, carina; or tumour with a malignant pleural or pericardial effusion [†], or with satellite tumour nodule(s) within the ipsilateral primary tumour lobe of the lung.
Regional Lymph Nodes (N)	
NX	Regional lymph nodes cannot be assessed
N0	No regional lymph node metastasis
N1	Metastasis to ipsilateral peribronchial and/or ipsilateral hilar lymph nodes, and intrapulmonary nodes involved by direct extension of the primary tumour
N2	Metastasis to ipsilateral mediastinal and/or subcarinal lymph node(s)
N3	Metastasis to contralateral mediastinal, contralateral hilar, ipsilateral or contralateral scalene, or supraclavicular lymph node(s)
Distant Metastasis (M)	
MX	Presence of distant metastasis cannot be assessed
M0	No distant metastasis
M1	Distant metastasis present [‡] Specify site(s)

* **T1**: Uncommon superficial tumour of any size with its invasive component limited to the main bronchus is classified as T1.

† **T4**: Most pleural effusions associated with lung cancer are due to tumour. However, for some patients in whom cytopathologic examination of pleural fluid (on more than one specimen) is negative for tumour, the fluid is non-bloody and is not an exudate. In such cases where these elements and clinical judgment dictate that the effusion is unrelated to the tumour, the patients should be staged as T1, T2, or T3, excluding effusion as a staging element.

† **M1**: Separate metastatic tumour nodules in the ipsilateral non-primary tumour lobe(s) of the lung are also classified as M1.

Table 2 The International Staging System for Lung Cancer (Lababede et al 1999)

Stage Subset	TNM
Stage 0	Carcinoma in situ
Stage IA	T1 N0 M0
Stage IB	T2 N0 M0
Stage IIA	T1 N1 M0
Stage IIB	T2 N1 M0
	T3 N0 M0
Stage IIIA	T3 N1 M0
	T1 N2 M0
	T2 N2 M0
	T3 N2 M0
Stage IIIB	T4 N0 M0 T4 N1 M0
	T4 N2 M0
	T1 N3 M0 T2 N3 M0
	T3 N3 M0 T4 N3 M0
Stage IV	Any T Any N M1

Note: Staging is irrelevant for Occult Carcinoma, designated TX N0 M0.

Because of multiple treatment variations for NSCLC, pre-operative staging is paramount for treatment planning. The preliminary staging assessment is usually based on a CT of the chest, as it is routinely taken from every patient

with NSCLC. The main role of the primary tumour assessment is to differentae the T_{1-3} from the T_4 lesions. However, CT can discriminate between advanced chest wall tumours and primary tumours in only 62% of cases (Webb et al 1991), and the sensitivity for mediastinal invasion can range from 60% to 75%. Magnetic resonance imaging (MRI) is currently used almost exclusively to evaluate superior sulcus (Pancoast) tumours for possible involvement of the brachial plexus nerves, spinal cord, chest wall, and blood vessels.

As for curative surgery, the most important factor is usually mediastinal lymph node involvement in N2 lymph nodes. Benignity or malignancy in enlarged mediastinal lymph nodes of > 1 cm in short-axis diameter cannot be reliably evaluated solely on CT, as one third of 2- to 4-cm lymph nodes were benign in NSCLC patients (McLoud et al 1992). Moreover, undetected mediastinal metastases may result in unnecessary thoractomies in up to 28% of all patients undergoing surgery (Fischer et al 2009b). The sensitivity and specificity of CT for evaluating mediastinal lymph nodes is 51% and 86%, respectively (Silvestri et al 2007). In addition, patients with adenocarcinoma and clinical N2 involvement are more susceptible to brain metastasis (Tanoue 2008).

Positron emission tomography (PET) is used to evaluate [^{18}F]2-fluoro-2-deoxy-glucose (FDG) uptake in both the tumour and possible distant metastasis. As glucose intake is elevated in malignant cells, the isotope is trapped after phosphorylation at a higher concentration than in the surrounding tissue. This imaging modality is thus complimentary to CT and provides the functional aspect of the tumour and metastasis. As a result, these two modalities are usually combined in the same device to yield a more accurate evaluation of the tumour. Specific criteria for abnormal finding include a standard uptake value (SUV) of > 2.5 or uptake in a lesion that is greater than the background uptake in the mediastinum. Pooled

sensitivity and specificity for PET in a solitary pulmonary nodule were 87% and 83%, respectively (Wahidi et al 2007). The value of PET in small nodules of under 8-10 mm is significantly inferior (Herder et al 2004). Decreased intake of FDG in the primary tumour is associated with carcinoid tumours and well-differentiated adenocarcinoma tumours, as well as with false-negative outcomes (Higashi et al 1998; Lowe et al 1998). For mediastinal metastasis, PET has sensitivity of 74% and specificity of 85% (Silvestri et al 2007). Despite these limitations, implementation of PET can prevent non-therapeutic thoracotomies (Fischer et al 2009a; Reed et al 2003).

In lung cancer patients with enlarged and/or PET-positive mediastinal lymph nodes, a cervical mediastinoscopy is used pre-operatively to evaluate the nodal histological status (Detterbeck et al 2007). Tissue confirmation of the mediastinum is unnecessary in patients with small cell lung cancer, stage IV NSCLC or malignant pleural effusion, as it is irrelevant for their appropriate treatment (Detterbeck et al 2007). The sensitivity of mediastinoscopy alone is 78% (Detterbeck et al 2007). Invasive staging of the mediastinum is discouraged for peripheral T1 lesions with normal mediastinum in CT, as the false-negative rate is around 10% (Detterbeck et al 2007). More recently, endobronchial and endoscopic ultrasound has been introduced to stage mediastinum mini-invasively (Wallace et al 2008), although it has not replaced mediastinoscopy as the main diagnostic approach for mediastinum (Annema et al 2010).

5.7 Treatment

The treatment strategy for NSCLC depends on the TNM stage and performance status of the patient. In addition, comorbid conditions, especially pulmonary and cardiac related, must be taken into account.

5.7.1 Surgery

Surgery is the main treatment choice when curative treatment can be achieved in stages IA to IIB (Scott et al 2007). Selected IIIA and IIIB patients with multimodal therapy are also eligible for curative surgery (Jett et al 2007; Robinson et al 2007). If a patient has incidental N2-disease at surgical resection and surgical resection is feasible, completion of the surgery and mediastinal lymphadenectomy is recommended, followed by adjuvant chemotherapy (Robinson et al 2007). In stage IV, surgery may be possible for patients with solitary brain and adrenal metastasis (Burt et al 1992; Hu et al 2006). A predicted post-operative forced expiratory volume in one second (FEV_1) of 0.81 is usually a prerequisite for surgery (Reilly 1997). Depending on the tumour invasion and patient performance status, surgical treatment options may include lobectomy, bi-lobectomy, pneumonectomy, segmentectomy and sleeve resection. Limited resections such as segmentectomy and wedge resection are associated with a three-fold increase in local recurrence and 30% higher overall mortality (Ginsberg and Rubinstein 1995). Thus, for patients with adequate pulmonary function test results, a lobectomy should be considered the minimum operation. However, in special situations, such as small peripheral well-differentiated adenocarcinoma, even a wedge resection is adequate (Nakamura et al 2004). Recently, studies have shown that sublobar resections are associated with similar disease-free survival of lobectomy in stage IA, but not in IB patients (El-Sherif et al 2006). Since 1995, the diagnostic utilities have been refined to diagnose early-stage NSCLC. Thus, interest in sublobar resections has grown, and they are now the subject of investigation (Blasberg et al 2010).

Every lung resection for NSCLC with curative intent requires a systematic mediastinal lymph node sampling because of frequent N2 node involvement in the presence of benign N1-level nodes (Daly et al 1993; Goldstraw et al

1994). However, the survival benefit of systematic mediastinal dissection is highly debated (Watanabe and Asamura 2009). Recently, a large randomised trial showed no survival benefit for systematic lymphadenectomy over lymph node sampling (Darling et al 2011).

5.7.2 Radiation therapy

Radiation therapy may be used alone in unresectable local NSCLC as a definitive therapy (Komaki et al 1985), but the local control rate is far from optimal, ranging from 15% to 17% (Arriagada et al 1991). Induction chemotherapy, followed by definitive chemotherapy offers better survival than radiation alone: five-year survival up to 22% (Lee et al 1998; Sause et al 1995). Patients with resectable NSCLC may benefit from post-operative radiation therapy in the presence of N2 disease (Lally et al 2006; Robinson et al 2007). Pre-operative neoadjuvant radiation therapy in NSCLC has shown no benefit to downstaging, improved local control, or higher survival rates, except for superior sulcus (pancoast) tumours (Rusch 2006). Radiation therapy is also used for palliative treatment, especially for brain and bone metastasis of both NSCLC and small cell lung cancer (Pass et al 2010).

5.7.3 Adjuvant and neoadjuvant chemotherapy

Although surgical resection remains the main treatment for early-stage NSCLC, the five-year survival rates for stages I and II range from 73% to 36% (Goldstraw et al 2007). Resected NSCLC recurs mainly in extrathoracic sites as a result of disseminated micrometastasis. The rationale behind post-operative adjuvant chemotherapy has been postulated for decades (Holmes and Gail 1986), but has only recently been shown to improve survival (Douillard et al 2006; Winton et al 2005). As a conclusion to these recent studies, patients with IIA, IIB or IIIA should be offered platinum-based adjuvant chemotherapy

that favours cisplatin-vinorelbine combination chemotherapy (Douillard et al 2006).

The role of pre-operative (neoadjuvant) chemotherapy has also been postulated since early 1990s in order to improve the rate of complete resection and to decrease the chance of micrometastasis (Burkes et al 1992). Not until the early 2000s have studies conducted with newer platinum-based agents seen more positive outcomes in stage IIIA (pN2) patients (Betticher et al 2003). The role of neoadjuvant therapy is unclear in stage IIIB (Barlesi et al 2005) and is not supported in stage IIIA (Robinson et al 2007). Further studies are needed to reliably determine the scope for neoadjuvant therapy for NSCLC (Burdett et al 2007).

5.7.4 Palliative treatment

Generally, patients with stage IV and stage IIIB (N3) or small cell lung cancer disease do not benefit from surgery, so treatment of these patients is palliative. The standard palliative chemotherapy regimen in NSCLC, when a patient has an adequate performance status, is a platinum-based doublet regimen (Socinski et al 2007). As older patients (over 70 years) generally have more comorbid conditions affecting their performance status, delivery of this double-agent regimen may often be challenging. In these scenarios, delivery of single-agent chemotherapy is associated with increased survival and HRQoL (Matsui et al 2005), and is recommended for elderly patients whose performance status is inadequate for a double-agent regimen (Socinski et al 2007). Second-line chemotherapy planning depends heavily on the response to first-line therapy and patient characteristics (Sculier and Moro-Sibilot 2009).

As small cell lung cancer is considered a systemic disease (Ohe 2004), chemotherapy is the mainstay treatment (Stupp et al 2004). Current first-line treatment

combines etoposide with cisplatin or carboplatin.

Palliative treatment entails management of various symptoms, including malignant pleural effusion, pain, dyspnea, and other symptoms (Pass et al 2010).

5.8 Prognosis

The survival of NSCLC patients depends heavily on numerous factors other than the stage of the disease. Patients who have smoked more than 20 pack-years have worse five-year survival than do patients who smoked less (Bryant and Cerfolio 2007). In addition, non-smokers survive significantly longer than do smokers (Bryant and Cerfolio 2007). The most important prognostic factor, however, is the extent of the disease at the time of diagnosis. For stages IA and IB, five-year survival ranges from 73% to 58%; for stages IIA and IIB, 46% to 36%; IIIA, 24%; and IIIB and IV, 13% to 9% (Goldstraw et al 2007).

5.9 Video-assisted thoracic surgery

The first reports of the use of video-assisted thoracic surgery (VATS) for radical therapy in NSCLC came in the 1990s (Roviaro et al 1992). The diagnostic use of VATS was adopted early, including in Finland (Salo 1994). Doubts have emerged about the safety and benefits reported for VATS, but reports from larger studies (McKenna et al 2006) and long-term results (Swanson et al 2007; Yamamoto et al 2010) added momentum to the greater acceptance and subsequent adoptation of this technique. The benefits of VATS over thoracotomy include fewer hospital days (Flores et al 2009), faster recovery (Aoki et al 2007), lower complication rate (Paul et al 2010), higher tolerance in fragile elderly patients (Cattaneo et al 2008), improved post-operative pulmonary function (Endoh et al

2009), higher chemotherapy tolerance (Nicastri et al 2008), and lower total treatment costs (Casali and Walker 2009). A VATS segmentectomy is comparable to a VATS lobectomy in terms of short- and long-term results (Shapiro et al 2009). Critics of VATS have argued against the possibly lower oncologic safety, but recent long-term survival analyses have demystified these claims. Because of these recent proceedings, VATS lobectomy is considered an acceptable procedure for selected stage I NSCLC patients (Yan et al 2009).

5.10 Impact of surgery on quality of life

Post-operative health-related quality of life (HRQoL) is an important treatment outcome that has been taken into account only in recent years. Because NSCLC patients have a median age of 70 and usually have comorbid conditions affecting their post-operative recovery, pre-operative patient selection based on predicted post-operative HRQoL is imperative. Traditional methods for assessing post-operative recovery include pulmonary function tests such as spirometry, radiospirometry and diffusion capacity. However, these tests have only relative value, with lung diffusion capacity being the most significant test (Brunelli and Salati 2008). Patients are also willing to accept a short-term decrease in HRQoL during post-operative recovery, but not long-term post-operative disability, although this is seldom taken into account pre-operatively (Cykert et al 2000). Most studies show a steep decline in post-operative HRQoL in NSCLC in the short-term and a rise in the following 6-12 months (Dales et al 1994; Win et al 2005; Zieren et al 1996). A query of long-term survivors of lung, colon, and prostate cancer showed that lung cancer patients showed no difference in their length of survival (Schag et al 1994). However, more recent studies show that the decrease in HRQoL fails to return to

baseline in 24 months of follow-up (Kenny et al 2008). A summary of HRQoL studies appears in Table 3. Adjuvant therapy impacts post-operative HRQoL only temporarily and modestly (Bezjak et al 2008). Moreover, post-operative adjuvant radiation therapy only modestly impacts HRQoL (Kepka et al 2010). The extent of the lung resection correlated to post-operative HRQoL, especially with pneumonectomy (Schulte et al 2009). When pneumonectomy patients were compared to sleeve lobectomy patients, the first-month results were comparable, but after three months, the sleeve lobectomy patients exhibited higher HRQoL in multiple dimensions. Studies have shown post-operative HRQoL among elderly patients with NSCLC to be acceptable (Balduyck et al 2009; Burfeind et al 2008; Salati et al 2009). HRQoL following a VATS lobectomy is similar to an open thoracotomy with less post-operative pain (Aoki et al 2007; Handy et al 2010). In addition, persistent smoking is associated with decreased HRQoL (Garces et al 2004).

Table 3 Quality of life following surgery for non-small cell lung cancer studies, without special emphasis on patient selection

Author (year)	Type	No. of patients	Instrument	Follow-up (months)
Zieren 1996	Retrospective/prospective	52/20	EORTC QLQ-C30 & Spitzer index	12 (20 pts)
Handy 2002	Prospective	103	SF-36	6
Myrdal 2003	Retrospective	112	SF-36 & HAD	-
Win 2005	Prospective	110	EORTC QLQ-C30 + LC13	6
Brunelli 2007	Prospective	156	SF-36	3
Kenny 2008	Prospective	173	EORTC QLQ-C30 + LC13	24

EORTC European Organisation for Research and Treatment of Cancer
HAD Hospital Anxiety & Depression scale
SF-36 Short Form (35) health survey
QLQ Quality of Life Questionnaire

5.11 Targeted therapy

Targeted therapy agents are specifically selective molecules that modify a cell's pathological molecular signalling or target drug delivery. The objective is to achieve good therapeutic effectiveness and minimise any toxic effects associated with traditional cytotoxic agents (Cascone et al 2007). Currently, commercially available targeted agents in Finland for NSCLC include Tyrosine kinase inhibitors (erlotinib and gefitinib), epidermal growth factor receptor (EGFR), monoclonal antibody (cetuximab), and vascular endothelial growth factor (VEGF) inhibitor (bevacizumab).

Most studies that employ targeted agents have been carried out in advanced NSCLC. The first large study to demonstrate the survival benefit of targeted agents combined bevacizumab with chemotherapy in advanced NSCLC patients, yielding a median two months' survival benefit (Sandler et al 2006). Unfortunately, this agent has several limitations as it can only be used in patients with non-squamous histology, no brain metastasis and no haemoptysis. A large study designed to investigate Bevacizumab in an adjuvant setting is presently underway. Maintenance Erlotinib after chemotherapy has been shown to increase progression-free survival in advanced NSCLC (Cappuzzo et al 2010). However, despite the initial response, EGFR tyrosine kinase inhibitors eventually develop resistance (Kobayashi et al 2005). The addition of cetuximab to standard palliative chemotherapy has been shown to prolong survival in advanced NSCLC in a phase III study (Pirker et al 2009).

More recently, promising phase II results have been presented with boretzomib, a proteasome inhibitor that, together with gemcitabine-karboplatin palliative treatment, yields longer survival than chemotherapy alone (Davies et al 2009).

5.11.1 Phage display

Since 1985, phage display has served as a tool for basic research and drug discovery, allowing tens to hundreds of millions of variable peptides to be presented on surface of filamentous phages for the selection of pepetides with high affinity (Nilsson et al 2000; Smith 1985). Phage display is used to screen protein interactions in order to identify novel proteins and peptides that show selective binding. As a result, peptide display serves as a useful tool for drug discovery and has been used in cancer research to identify selective peptides in breast cancer (Shukla and Krag 2005), hepatocarcinoma (Zhang et al 2007), prostate cancer (Newton et al 2006), and metastasis (Yang et al 2008). Peptide display has also been used in lung cancer to identify tumour blood vessels (Lee et al 2007) and cell lines (Oyama et al 2003; Zang et al 2009).

5.11.2 Homing peptides

The non-specific toxicity of anticancer drugs towards normal tissue limits their clinical usage and leads to significant morbidity. To minimise morbidity and to improve therapeutic efficacy, tumour-selective peptide ligands have been proposed as a homing ligand for anticancer agents, which could lead to more accurate drug delivery (Zhou et al 2004). They can also be used in imaging with PET-CT (Enback and Laakkonen 2007). Since their introduction into clinical practice, monoclonal antibodies have now become an integral part of cancer drug therapy (Green et al 2000). However, monoclonal antibodies have significant limitations, including their size, heterogenous expression and immunogenecity (Esteva 2004). Linear peptides have many advantages over monoclonal antibodies, such as lower molecular weight, less immunogenity, a more stable configuration, and easier

synthesis and modification (Adessi and Soto 2002). Currently no linear peptides are used in cancer therapy.

6 AIMS OF THE STUDY

I. To evaluate the long-term HRQoL of patients who underwent pneumonectomy for NSCLC and to compare the results with post-operative pulmonary function tests.

II. To observe changes in HRQoL following lobectomy and bi-lobectomy in patients who underwent surgery for NSCLC.

III. To evaluate the role of oxidative stress and radical scavenger capacity in the pathogenesis of NSCLC by measuring the parameters of oxidative metabolism and DNA adducts in the NSCLC tumour and normal lung of the NSCLC patient, and then comparing the results to a control group.

IV. To compare patient outcomes between open thoracotomy and VATS in stage I NSCLC patients, with special emphasis on post-operative complications and discharge from hospital.

V. To describe the properties and possible applications of THX-targeting peptides, discovered with the phage display method.

7 PATIENTS AND METHODS

7.1 Patients

Study I

Between January 1997 and October 2003, 98 patients were treated for NSCLC with pneumonectomy in the Department of Cardiothoracic Surgery, Helsinki University Central Hospital. In June 2004, 34 patients were alive, of whom 31 replied to a 15D quality of life questionnaire (QLQ) and Baseline Dyspnea Index (BDI) questionnaire. Post-operative pulmonary function tests were obtained from 20 patients in March 2005. The median interval from surgery was 33 months (range 8-86 months). The median age was 67 years (range 47-78 years) at the time of the questionnaire.

Study II

In this study, 22 NSCLC patients were sampled for tumour and adjacent normal lung tissue between January 2003 and May 2006. Of these patients, 11 had adenocarcinoma and 11, squamous cell carcinoma as their histology, which was verified collectively by one pathologist. Control samples derived from 10 patients 7 of whom underwent surgery for recurrent pneumothorax, 2 for hamartoma, and 1 for vascular anomaly. The median age for NSCLC patients at the time of surgery was 62.5 years (range 40-82 years), and for the control patients, 38.5 years (range 16-71 years).

Study III

Between May 2002 and September 2005, a total of 53 NSCLC patients underwent lobectomy or bi-lobectomy in

the Department of Cardiothoracic Surgery, Helsinki University Central Hospital. The median age at the time of surgery was 63 years (range 44-80 years). Follow-up questionnaires were mailed to patients at three months, one year and two years post-operatively. A total of 48 patients replied at three months post-operatively (2 died and 3 were lost to follow-up), 42 patients replied at one year (4 died and 2 were lost to follow-up), and 36 patients replied at two years (4 died and 2 were lost to follow-up). During the two-year follow-up period, survival was 81% (43/53 patients).

Study IV

A total of 622 patients underwent surgical treatment for lung cancer between January 2000 and February 2010 at the Division of General Thoracic and Oesophageal Surgery in the Department of Cardiothoracic Surgery, Helsinki University Central Hospital. Of these patients, the 328 included in the study were clinically staged as stage I NSCLC and underwent lobectomy, bi-lobectomy or segmentectomy. A total of 212 patients were treated with thoracotomy, and 116 patients with VATS. The median age of the thoracotomy patient group was 65 years (range 37-80 years), and in the VATS patient group, 69 years (range 47-83 years). The mean Charleson Comorbidity Index (CCI) in the thoracotomy patient group was 0.87 ± 1.14, and in the VATS patient group, 1.14 ± 1.09.

Study V

Samples of lung tumour tissue were collected from 34 patients, 24 of whom were diagnosed with adenocarcinoma or squamous cell cacrcinoma and were included in this study.

7.2 METHODS

Tissue-sample collection

In study II, all samples were taken during surgery from resected specimens. Non-malignant control samples of the NSCLC patients were taken 50 mm distal from the tumour. These were immediately frozen and stored at -80°C. One pathologist collectively verified the pathologic classification of these samples using standard routine histology and immunohistochemistry.

In study V, fresh tumour samples were collected from the resected specimen and processed as soon as possible.

Analysis of myeloperoxidase activity, cluthathione content, NADPH oxidase activity and 8-hydroxydeoxyguanosine content

Myeloperoxidase (MPO) activity was determined by modifying a method by Suzuki in which the enzyme catalyses the oxidation of 3,3',5,5'-tetramethylbenzidine with H_2O_2 to yield a blue chromogen with a maximum wavelength of 655 nm (Suzuki et al 1983). MPO activity is expressed as units/milligram protein (U/mg protein).

The glutathione content was estimated with Saville's method (Saville B 1958). Glutathione concentrations are expressed as nmol/mg protein.

Nicotinamide adenine dinucleotide phosphate (NADPH) oxidase (NOX) activity was measured with lucigenin-enhanced chemiluminescent of superoxide anion as described by Li et al (Li and Shah 2002); the results are expressed as mV/mg protein.

DNA was isolated using a non-enzymatic method in which pure DNA was dissolved in high-performance liquid chromatography (HPLC)-grade water with deferoxamine mesylate added to reduce artificial oxidation. DNA hydrolysation of nucleotides was carried out during incubation with nuclease P1, and was further hydrolysed to

nucleosides with alkaline phosphatase; the nucleosides were then separated using a C_{18} reverse-phase column. The amount of 8-OHdG was determined with HPLC equipped with an electrochemical detector; deoxyguanosine (dG) was determined with a UV detector; the 8-OHdG concentration is expressed as the ratio of 8-OHdG per 10^5 dG (8-oxo-dG/10^5 dG).

Health-related quality of life (HRQoL)

HRQoL was measured with the 15D questionnaire, a generic, 15-dimensional, standardised, self-administered HRQoL instrument which can serve as both a profile and a single index score measure (Sintonen 2001). The 15D questionnaire consists of the following 15 dimensions: moving, seeing, hearing, breathing, sleeping, eating, speech, eliminating, usual activities, mental function, discomfort and symptoms, depression, distress, vitality, and sexual activity; each dimension has five levels. For each dimension, the respondent must choose one of the five levels that best describes his/her state of health at the moment (the best level = 1; the worst level = 5). A set of utility or preference weights elicited from the general population in a three-stage valuation procedure is used in an additive aggregation formula to generate an overall score (i.e., the 15D score, a single index number). The maximum score is 1 (no problems on any dimension), and the minimum is score 0 (being dead). The questionnaire has been validated with other similar generic HRQoL instruments (Hawthorne et al 2001). General population reference values are derived from the Health 2000 Survey and are matched to age distribution of the results studied (Aromaa and Koskinen 2004).

Mahler's BDI is a multidimensional scale for grading dyspnea according to three categories (''functional impairment'', ''magnitude of task'', and ''magnitude of effort'') on a scale of 0-4, where 4 represents the best, and 0, the worst value (Mahler et al 1984). Ratings for each of

the three categories are totaled to form the baseline total dyspnea score (range 0-12).

Pulmonary function tests

For studies I and III, pre-operative pulmonary function test (PFT) data drawn from spirometric studies (FEV_1, FVC, and FEV_1/FVC) and pulmonary diffusion capacity measurements (DLco) were recorded from the patients' medical records. The diffusion capacity of the lungs for carbon monoxide (DLco) was measured pre-operatively with the Master Lab single-breath method (Erich Jaeger, Wurzburg, Germany). Predicted post-operative FEV_1 was calculated based on pre-operative FEV_1 dynamic flow-spirometry together with radiospirometry (Veneskoski and Sovijarvi 1986). The patients' results from the PFTs were compared to those from a gender- and height-standardised general population as percentages of the mean value (Viljanen et al 1982).

For study I, spirometry was carried out post-operatively for 20 patients with a flow-volume spirometer connected to a microcomputer system (Medikro 202; Medikro Oy; Kuopio, Finland); measurements were performed according to European Respiratory Society (ERS, 1993) guidelines. The results were recorded from the envelope curve of at least three superimposed, forced expiratory flow-volume curves, FEV_1, forced vital capacity (FVC), and flow rate in the middle of FVC(FEF_{25-75}). The bronchodilatation test was carried out by administering a single-dose inhalation of salbutamol through an inhalation chamber (Volumatic, GlaxoSmithKline, Brentford, UK).

Ex vivo peptide identification and synthesis

Fresh tissue was prepared, washed, and exposed to the fd-tet M13-fUSE5 phage library. These samples, which contained attached phage, were exposed to K91kan *E.coli*

bacteria. Barbas et al (Barbas et al 2001) describe the procedure, which used cell lines of NSCLC (NCI-H23, NCI-H520, NCI-460 and A549) as controls. Peptide synthesis was carried out manually or with peptide synthesisers (either Applied Biosystems 433A or Advanced Chem Tech 396DC) using standard FMOC chemistry. Alexa488 conjugate was used for microscopy analysis.

In-vitro cell-binding assay

The tested cell lines included NCI-H23, NCI-H520, A549, PAMSC, and NIH-3T3; six parallel samples were analysed, and each study was carried out at least twice. A scrambled version of Thx RARKLPD served as a negative control.

Localisation assay

For in vitro analysis, confocal microscopy was used with the Thx-Alexa488 conjugate. Cultured A549 cells were exposed for 5 min to 50 nmol of Thx conjugate and then analysed after PBS washing. In vitro localisation assay was performed using the method described by Laakkonen et al (Laakkonen et al 2004). In brief, A549 cells were subcutaneously injected into murine. After four weeks, the biodistribution and internalisation of the Alexa-conjugated Thx peptide was tested in parallel with the Alexa conjugate of the TAT peptide as a positive control and saline as a negative control.

Ex vivo biodistribution

Subcutaneous injections (1×10^7 of A549 and NCI-H520 cells) went into both flanks of each mouse. An intravenous injection of DTPA-Europium-Thx conjugate (275 nmol) in saline was given to the mice and left to circulate for 15 min. The biodistribution profile of Thx-conjugated DTPA Europium was analysed from the tissue lysates using inductively coupled plasma mass spectrometry (ICP-MS).

Statistical methods

Values are expressed as median and range unless otherwise stated. All reported p-values are based on two-tailed tests without adjustment for multiple comparisons. Statistical significance of $p < 0.05$ was considered significant. Statistical calculations were carried out with SPSS software, version 18.0 for Macintosh (SPSS, Inc., Chicago, IL, USA).

Studies I, II & III

Differences between the control population and the patient group were analysed with the independent sample *t*-test, and within the patient group with the Mann-Whitney *U*-test. The Wilcoxon signed rank test served for statistical comparison with time. Linear correlation was analysed with Sperarman's correlation co-efficient. Bonferroni's correction was used in study III for multiple comparisons.

Study IV

Propensity scores were estimated using a logistic model that included the following preoperative variables: age; gender; Charlson comorbidity index score; forced expiratory volume in one second of predicted, pre-operative staging; and pack-years smoked. Patients were paired between groups according to their propensity score. Fischer's exact or Chi-squared tests were employed for comparison of categorical variables. The two-sample rank sum test and the Mann-Whitney U test served for continuous variables. Survival was compared using the log rank test.

8 RESULTS

8.1 Health-Related Quality of Life (HRQoL), following lung cancer surgery

The mean post-operative total 15D score in the pneumonectomy patients was significantly lower (0.804) than that of the age-matched general population (0.884; $p = 0.001$). Significantly inferior results ($p < 0.030$) were noted in mobility, breathing, usual activities, discomfort and symptoms, depression, distress, vitality, and sexual function. In women, the dimension of breathing was significantly lower ($p = 0.009$), especially after right-sided pneumonectomy ($p = 0.020$). No significant differences were noted when compared to right- and left-sided pneumonectomy ($p = 0.920$), patients with disease progression ($p = 0.726$), or significant post-operative complications ($p = 0.412$).

The mean pre-operative total 15D score in lobectomy and bi-lobectomy patients (0.898) did not differ from that of the age-matched general population (0.887; $p = 0.438$). However, significant differences ($p < 0.030$) were noted pre-operatively in the following dimensions: breathing, mental function, discomfort and symptoms, and distress. In follow-up questionnaires completed 24 months after surgery, the total 15D score was significantly lower (0.820; $p = 0.001$), and the following dimensions yielded significantly lower values ($p < 0.030$): mobility, breathing, usual activities, and sexual activity. 15D scores were not significantly lower in patients with post-operative complications than in patients without complications. A total of 15 patients had disease progression, but no significant difference was observed in the 15D score results of those patients who remained progression-free. Women exhibited more depression than did men three months

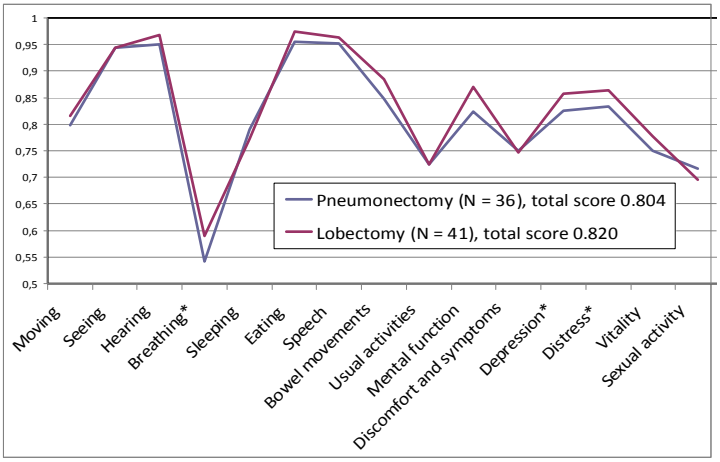
post-operatively ($p = 0.004$); no other differences were observed between genders.

Differences in post-operative HRQoL between pneumonectomy and lobectomy patients appear in Figure 2. No significant difference was noted in the total 15D score. However, pneumonectomy patients suffered more dyspnea, depression, and distress.

The median baseline dyspnea index (BDI) in the pneumonectomy patients was 7.0 (range 2-11). Right-sided pneumonectomy in women was associated with significantly lower values in magnitude of task, magnitude of effort, and BDI total score. Significant correlation was noted between BDI score and total 15D score ($p = 0.002$) in pneumonectomy patients. In the same study, significant correlation to BDI score was noted in the following dimensions of 15D: breathing ($p = 0.001$), usual activities ($p = 0.001$), mental function ($p = 0.016$), discomfort and symptoms ($p = 0.002$), distress ($p = 0.043$), and vitality ($p = 0.009$).

Neither of the two studies revealed any significant correlations with pre-operative spirometric studies in predicting post-operative total HRQoL. In study I, however, post-operatively measured or predicted FEV_1 correlated with magnitude of effort in BDI ($p = 0.001$) and the dimension of breathing in the 15D ($p = 0.018$).

Figure 2 Comparison of post-operative 15D results between pneumonectomy and lobectomy patients.

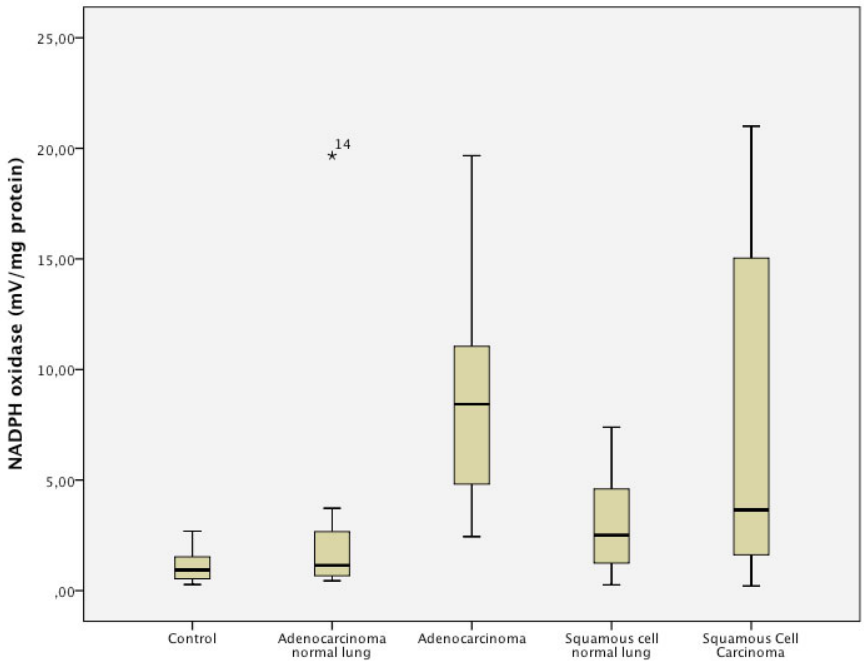


*significant difference ($p < 0.03$)

8.2 Oxidative stress in the pathogenesis of non-small cell lung cancer

The activity of NADPH oxidase (NOX) was lowest in the control group (0.94 mV/mg, range 0.28-2.69 mV/mg), increasing significantly in both the normal lung tissue of the NSCLC patients (1.83 mV/mg, range 0.26-19.67,mV/mg $p = 0.044$) and the NSCLC tumour (7.30 mV/mg, range 0.21-47.20 mV/mg, $p = 0.001$). The difference was also significant when compared to normal lung to tumour tissue ($p = 0.001$). The results of the NADPH oxidase activity divided by histology appears in Figure 3. A significant difference in NADPH oxidase activity was observed between the normal lung and tumour of the adenocarcinoma patients ($p = 0.005$). This was not apparent in the squamous cell cancer patients. No significant differences were noted between adenocarcinoma and squamous cell carcinoma.

Figure 3 NADPH oxidase activity levels in non-small cell lung cancer, divided by histology



No significant difference was noted in myeloperoxidase (MPO) activity between the control (4.60 U/mg, range 0.40-50.50 U/mg) and normal lung of the NSCLC patients (7.60 U/mg, range 0.00-23.30 U/mg) or the NSCLC tumour samples (1.60 U/mg, range 0.00-12.50 U/mg). However, a significant difference was noted between the normal lung and tumour tissue of the NSCLC patients ($p = 0.001$). This phenomenon was evident in both the adenocarcinoma ($p = 0.007$) and squamous cell carcinoma ($p = 0.007$) samples.

Glutathione content was lowest in the control (8.25 nmol/mg, range 5.40-16.10 nmol/mg) and normal lung tissue of the NSCLC patients (7.40 nmol/mg, range 0.20-36.90 nmol/mg). Glutathione content was significantly higher in the tumour samples (15.35 nmol/mg, range 0.10-

43,70, p = 0.009). In adenocarcinoma in particular, glutathione content was significantly higher in the tumour tissue (20.80 nmol/mg, range 5.80-36.90 nmol/mg) than in the normal lung samples (11.60 nmol/mg, range 3.10-36.90 nmol/mg). This difference was evident in the squamous cell carcinoma patient samples.

No difference was noted for 8-oxo-hydroxydeoxyguanosine (8-OHdG) content when compared to the control (1.59 8-oxo-dG/10^5 dg, range 0.80-4.67 8-oxo-dG/10^5 dg), the normal lung tissue of the NSCLC patients (1.69 8-oxo-dG/10^5 dg, range 0.00-72.90 8-oxo-dG/10^5 dg) or the NSCLC tumour samples (1.13 8-oxo-dG/10^5 dg, range 0.00-188.30). However, significant differences were noted when normal lung (p = 0.017) and tumour samples (p = 0.001) were compared between adenocarcinoma and squamous cell carcinoma.

Significant correlations were observed in the control patient group between glutathione content and NADPH oxidase activity (0.81, p = 0.005), and a negative one between glutathione and 8-OHdG content (-0.69, p = 0.029). In the normal lung tissue of the NSCLC patients, a negative correlation was noted between the glutathione and 8-OHdG content (-0.75, p = 0.001), which turned positive in the tumour samples (0.56, p = 0.007).

Patients with squamous cell carcinoma histology had significantly higher numbers of pack-years smoked (50 years, range 37-100 years) than did adenocarcinoma patients (30 years, range 0-50 years) (p = 0.003).

8.3 VATS lung resections for non-small cell lung cancer

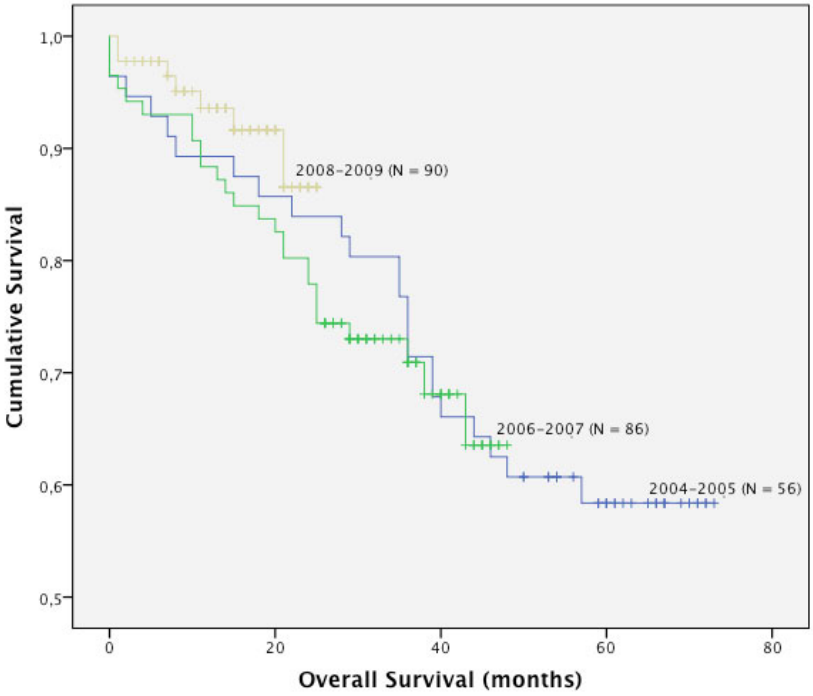
Clinical stage I NSCLC patients were assigned to the VATS group according to the surgeon's preference. Patients who underwent VATS were significantly older, with a median age of 69 years (range 47-83 years), than thoracotomy patients, with a median age of 65 years (range 37-80 years)

(p = 0.001). Patients treated with VATS also exhibited a poor pulmonary diffusion capacity of 70.0 ± 18.1% of predicted than did the thoracotomy patients (80.8 ± 21.2%) (p = 0.001). No significant differences were observed in the following pre-operative measures: gender, pack-years, tumour histology and percentage of predicted FEV_1. Of the 116 patients who underwent VATS, 16 patients (14%) were converted to conventional thoracotomy due to visualisation in six, dense adhesions in five, bleeding in four, and sudden hypotension in one patient. Six in-hospital deaths occurred in the thoracotomy group, and three in VATS group, of which two were converted to thoracotomy. Two completed VATS patients (2%) and 21 thoracotomy patients (9%) were observed overnight in the intensive care unit. No intraoperative deaths occurred. Patients who underwent VATS had significantly shorter post-operative stays (7.5 ± 6.7 days) than did the thoracotomy patients (10.7 ± 6.9 days) (p = 0.001). This was also evident in the propensity-matched thoracotomy patients (10.8 ± 7.4 days) (p = 0.001). Patients treated with VATS also had lower complication rates (15.5%) than did the thoracotomy group (26.9%) (p = 0.020). The VATS patients experienced fewer prolonged post-operative air leaks (over seven days) (4.3%) than did the thoracoromy group (8.5%). While the thoracotomy patients were in surgery, the mean sampled lymph node stations was 4.6 ± 1.8, whereas for the VATS patients, the mean sampled stations was 2.8 ± 1.7 stations (p = 0.001). No differences in overall survival or progression-free survival at 2 years were observed between the VATS and thoracotomy groups.

Because implementation of VATS lobectomy and segmentectomy for NSCLC began in 2006, this method has seen greater use. In 2008-2009, 71% of stage I NSCLC patients were treated with VATS, compared to 50% in 2006-2007. As a result, the mean overall post-operative stay in 2008-2009 was 8.4 ± 7.4 days, whereas in 2004-

2005, it was 10.6 ± 5.6 days (p = 0.001). Mean pulmonary function tests scores were lower in patients treated in 2008-2009 in both the percentage of predicted FEV_1 (72.5% ± 19.8) and DL_{CO} (71.2% ± 17.7), compared to the respective values for patients treated in 2006-2007: (80.6 ± 20.2) (p = 0.012) and (80.6 ± 22.1) (p = 0.029). However, no differences were observed in treated patient age, complication rate, or CCI, and no survival differences were noted between these two-year treatment interval groups (Figure 4).

Figure 4 Kaplan-Meier survival analysis divided by two-year treatment intervals



8.4 Identification and properties of novel peptides

In ex vivo screening of clinical NSCLC tumour samples, libraries produced a peptide with a core motif of $^{A}/_{S}$RXPXXX. After five panning rounds, the ARRPKLD

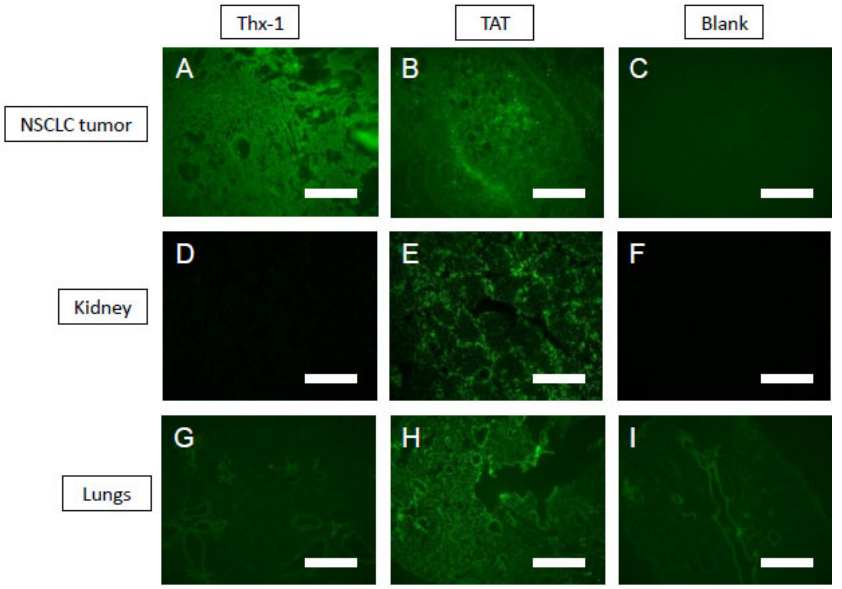
sequence was selected for in vitro and in vivo binding studies. This sequence is hereafter referred to as Thx.

In cell-binding assays, Thx bound selectively to NCI-H23, NCI-H520, A549 and NCI-H460 NSCLC cell lines, but not to control cell lines PASMC and NIH3T3. The scrambled version of the Thx peptide (RARKLPD) failed to bind to NSCLC cell lines.

In vitro analysis of cellular location by microscopy revealed that Thx entered cells within 15 minutes and was detected in a subset of perinuclearly located lysosomes.

Localisation and distribution in vivo of the Thx peptide with confocal microscopic analysis revealed a selective accumulation of Thx-Alexa488 in the tumour tissue, whereas the control peptide, TAT-Alexa488, was present in all analysed tissues and organs (Figure 5).

Figure 5 Tissue localisation pattern and distribution of Thx Alexa488 in mice in vivo at 15 minutes.



Confocal microscopic analysis of the sections shows selective accumulation of Thx-Alexa488 in the tumour tissue, whereas TAT-Alexa488 is present in all tissues and organs. The lung sections show a typical autofluorescence background. The kidney sections shown were taken from the central region of the kidney. At 15 min p.i., most or all Thx-Alexa488 appears to be removed from the central parts of the kidneys (scale bars 300 µm). NSCLC = non-small cell lung cancer

9 DISCUSSION

9.1 Quality of life following surgery

Studies I and III both show a significant decrease in overall HRQoL as measured by the 15D QLQ instrument. In addition, both studies show that post-operative HRQoL is independent of pre-operative lung function testing. Moreover, both studies revealed that the decrease in HRQoL is permanent and affects the dimensions independent of physical activity.

The limitations of study I included the fact that it was retrospective and lacked pre- and postoperative serial data. However, the goal of the study was to compare long-term survivors of NSCLC treated with pneumonectomy. Pneumonectomy is currently used very rarely, as 54 (20.2%) of 243 patients underwent this procedure for NSCLC at the Helsinki University Central Hospital for NSCLC between 2000 and 2004, and only on 12 (3.2%) of 379 patients did so between 2005 and 2009. The majority of patients in study I were progression-free (77.4%), so the results of study I reliably reflect the long-term HRQoL. The limitations of study II included the fact that cancer patients do not complete surveys when the disease has progressed or they become too ill (Fairclough 2010). This could lead to the overestimation of HRQoL due to the better health of the respondents. Moreover, seven patients (13%) were lost to follow-up, which also affected the results.

To our knowledge, study I was the first to use two independent instruments (the 15D and baseline dyspnea index) to evaluate the long-term HRQoL of NSCLC patients treated with pneumonectomy. A retrospective study of 112 patients with the short form 36 (SF-36)

questionnaire showed that 22 patients treated with pneumonectomy exhibited less physical activity and more emotional problems than did lobectomy patients (Myrdal et al 2003). A one-year follow-up study by *Balduyck et al* (Balduyck et al 2007) showed that pneumonectomy patients exhibited poorer physical function and more thoracic pain, as measured by the EORTC QLQ C30, than did patients treated with lobectomy. A study by the same author compared the post-operative HRQoL of pneumonectomy (n = 20) and sleeve lobectomy (n = 10) patients, as measured by EORTC QLQ generic C30 and lung cancer-specific (LC13) questionnaire modules (Balduyck et al 2008), and showed that pneumonectomy was associated with significantly more dyspnea, more general pain, more thoracic pain, and shoulder dysfunction. Schulte et al showed that, in contrast to lobectomy patients, pneumonectomy patients (n = 28) had inferior post-operative HRQoL which did not return to baseline after 12 months (Schulte et al 2009). Our findings are in line with the findings (presented here) that pneumonectomy patients experience more difficulties than do lobectomy patients.

One of the key elements of our study was that we conducted post-operative pulmonary function testing, which revealed poor correlation with pneumonectomy patients' current overall HRQoL. However, post-operative or predicted FEV_1 showed a correlation with the dimension of breathing in the 15D and magnitude of effort in the BDI, which was predictable. One must conclude that pre- and post-operative spirometry does not serve as a substitute for predicting post-operative HRQoL or for evaluating current HRQoL. Some researchers have proposed that lung diffusion capacity should be evaluated routinely in all NSCLC patients undergoing surgery, as it may predict post-operative complications in patients with normal FEV_1 (Brunelli and Salati 2008).

The main finding of study II was the sustained decrease

in overall HRQoL over the two-year follow-up period, a result previously established by *Kenny et al* (Kenny et al 2008) and *Schulte et al* (Schulte et al 2009). As expected, NSCLC patients pre-operatively scored inferior values in dimensions of breathing and distress. However, contrary to the results of Kenny et al (Kenny et al 2008), our results showed no significant difference in HRQoL between patients with disease progression and those who remained progression-free.

9.2 Oxidative stress in the carcinogenesis of non-small cell lung cancer

As study II demonstrated, NSCLC is associated with significantly altered cell homeostasis due to oxidative stress. The main finding of this study was the modestly elevated NADPH oxidase activity in the normal lung tissue of NSCLC patients and the great elevation in malignant tumour tissue, especially in adenocarcinoma. This phenomenon was evident in previous studies of prostate and colon cancer (Kumar et al 2008; Laurent et al 2008). Because the higher NADPH oxidase activity independently of the MPO activity, the elevated activity could not be due to increased PMN activity. However, the higher MPO activity in the normal lung tissue of NSCLC patients reflects the significant burden of oxidative stress, which is not present in the established tumours. The reason for this is unknown, but this may reflect the altered oxidative homeostasis of lung cancer patients (Masri et al 2005).

In this study, we also noticed a high concentration of glutathione in the tumour tissues, especially in the adenocarcinoma patients. The same finding was established by Blair et al (Blair et al 1997). This may in turn indicate tumour invasion and resistance to cytotoxic agents. Glutathione also prevents the formation of 8-OHdG (Spear and Aust 1995), which is in line with our results for the negative correlation of glutathione and 8-

OHdG in non-malignant samples from both the control patients and the normal lung of the NSCLC patients. This correlation became positive in tumour samples, thus revealing uncontrolled cell homeostasis in the NSCLC tumour. The difference in 8-OHdg content in the lung depends heavily on current smoking (Suzuki et al 2003). Consequently 8-OHdG content was higher in patients with squamous cell carcinoma who smoked significantly more.

The limitations of study II include the following: no co-efficients were implemented for current smoking, so the results do not reflect this factor. Also, the clinical stage varied between adenocarcinoma and squamous cell carcinoma patients, due to the natural pathology of the disease. The control patients were neither age- nor gender-matched to patients with NSCLC, which would have proved an impossible task to achieve in a timely manner.

9.3 Clinical outcomes following implement-tation of VATS

The main finding of study IV was that the clinical outcomes with VATS were either similar to or better than those of open surgery via thoracotomy. Fewer complications and a shorter post-operative stay favoured VATS. Moreover, the implementation of VATS was considered safe, resulting in more efficient use of hospital resources and a safer method for high-risk patients.

Patients who underwent VATS were more fragile and were at greater risk for post-operative morbidity and mortality than were patients who underwent thoracotomy, as measured by higher age, more comorbid conditions and poorer pulmonary function test results. The most important contribution of VATS to rehabilitation is the reduction of post-operative pain (Demmy and Curtis 1999; Whitson et al 2008) and improved post-operative pulmonary function (Endoh et al 2009). This result has

been also documented in previous studies (Roviaro et al 2004; Yamamoto et al 2010). The quality of life following VATS has also been superior to that of patients treated with thoracotomy (Aoki et al 2007).

VATS patients also suffered fewer overall complications. The most significant difference was that VATS patients experienced fewer post-operative air leaks and less bleeding, possibly due to the increased use of staplers in lung parenchyma. Another result was fewer cardiac arrhythmias, namely atrial fibrillation in patients treated with VATS. In larger trials, this difference has not been significant (Park et al 2007). Our study is too underpowered to analyse differences between different types of complications. Other studies have addressed the evaluation of post-operative morbidity following VATS and have shown similar results to those in our study (Paul et al 2010; Villamizar et al 2009). We found no differences between VATS and thoracotomy patients in overall and 24-month survival, which is in line with the results of previous reports (Yamamoto et al 2010; Yang et al 2009).

Our conversion rate from VATS to open thoracotomy, at 13.8%, was similar to that of earlier reports (Flores et al 2009; Handy et al 2010). In our series, the most common reason for conversion was the inability to visualise tumour reliably. Because two of the converted patients died after a long intensive care period, the operative morbidity of open surgery should be weighed in high-risk patients undergoing VATS. We dissected fewer lymph node stations with VATS, as opposed to thoracotomy. With experience, lymph node dissection with VATS could be equivalent to that in thoracotomy (Sagawa et al 2002). However, the patients treated with VATS were older with more comorbid conditions, and were thus ineligible for adjuvant chemotherapy. As a result elective sampling was carried out in these patients in order to minimise operative morbidity. Moreover, no survival benefit was observed in mediastinal dissection over sampling (Darling et al 2011).

The use of VATS in stage I NSCLC during the implementation period rose to 63.3%, which reveals a similar progress to that reported by Seder et al (Seder et al 2009). In previous reports, the use of VATS in stage I could rise to 90% of all stage I NSCLC patients who underwent surgery (Walker et al 2003). VATS is currently considered the standard operative treatment for non-complicated stage I NSCLC patients (Demmy and Nwogu 2008).

Limitations of this study include its retrospective analysis. However, this study presents real-life results of VATS implementation. Moreover, inherited bias is a possibility, as VATS patients underwent surgery exclusively by one of two surgeons. To compensate for the retrospective nature of this study, we used propensity-score matching, which is a class of multivariate statistical method that identifies groups of patients with similar chances of receiving one treatment or another from within a given study population (Blackstone 2002). However, this is not equivalent to a prospective, randomised study. To weigh pre-operative comorbid conditions, we implemented CCI. Previous studies have shown CCI to be a better predictor of post-operative survival in NSCLC patients than individual comorbid conditions in patients (Birim et al 2003).

9.4 Homing peptides for non-small cell lung cancer

Study V identified and characterised of the heptameric linear homing peptide for NSCLC, the Thx (ARRPKLD). The strength of the study was that Thx was identified from clinical tumour samples, as opposed to cultured cell lines in vitro (Hui et al 2008; Zang et al 2009), or phage libraries infused intra venously in vivo (Arap et al 2002; Krag et al 2006) or ex vivo (Maruta et al 2007).

The binding selectivity of Thx was measured both in

vitro and in vivo. These two tests revealed that Thx binds selectively to NSCLC tumours. In addition, the performance of Thx-DTPA-Eu chelator-conjugate suggests that Thx could be developed into a clinical agent for imaging. In internalisation assays, Alexa-Thx showed selective uptake and appeared to be located in the vacuolar structures of the cell cytoplasm. This suggests that after binding, Thx is internalised in lysosomes for degration. Similar internalisation has been previously described for other similar-sized peptide (Langel 2006).

Limitations of the study include the fact that the binding site for Thx remained unidentified. Knowledge of this would enable improvements in its affinity and stability through chemical modifications. Possibilities for the possible binding site are many. Similarities were found in SwissProt amino acid analyses between Thx and similar proteins linked to NSCLC. This suggests that these proteins may have co-binding cabability, being the binding site for Thx. Previously identified MTA1 protein, which correlates with the invasiveness and metastatic capability of NSCLC, containts the ARRPXLX sequence (Futamura et al 1999). In addition, the C-terminal domain of breast cancer suppressor protein (BRCA1) contains a similar sequence, RRPKL (Holt et al 1996), which studies have shown to be expressed in NSCLC and to bind to c-myc (Wang et al 1998).

10 SUMMARY

Non-small cell lung cancer causes more cancer-related deaths than any other cancer, with five-year survival ranging from 8% to 13%. In this thesis, we studied the pathogenesis, tumour targeting and clinical outcomes of this disease.

Quality of life following surgery for NSCLC decreases significantly in dimensions both related and unrelated to physical activity. In the two-year follow-up period, the quality of life does not return to the pre-operative state after lobectomy or bi-lobectomy. The long-term decrease in quality of life was noted with pneumonectomy, and its implementation should be avoided if possible. Pre-operative pulmonary function tests do not serve as a substitute for predicting post-operative quality of life.

Simultaneous increased exposure to oxidative stress, the formation of DNA adducts, and an uncontrolled redox state in the tumour reveals the important role that they play in the carcinogenesis of non-small cell lung cancer. The role of increased NADPH oxidase activity, together with the decrease in glutathione content, seems to be of great importance in carcinogenesis.

The introduction, with VATS, of minimally invasive lobectomy and segmentectomy yielded reduced morbidity, faster recovery, and shorter hospitalisation in stage I NSCLC patients with more comorbid conditions and poorer pulmonary function than in thoracotomy patients. This result was achieved without sacrificing long-term progression-free or overall survival. By greater implementation of VATS, radical surgery may be offered to otherwise inoperable patients.

We identified and characterised a new homing peptide from the clinical patient samples with the phage display method. The study yielded a peptide, Thx, for tumour

targeting of NSCLC. This peptide offers many advantageous properties for both diagnostic and therapeutic usage.

11 CONCLUSIONS

I. Long-term quality of life for NSCLC after pneumonectomy is significantly worse than in the age-standardised normal population. Pre- or post-operative pulmonary function tests have no value for predicting post-operative overall quality of life. Pneumonectomy should be avoided and other means should be implemented, if possible.

II. Quality of life for NSCLC patients following lobectomy or segmentectomy does not return to its pre-operative state in the two-year follow-up period. Pre-operative counselling should be offered to achieve post-operative recovery and adaptation.

III. Increased NADPH oxidase activity, independent of myeloperoxidase activity and current smoking, suggests its important role in the carcinogenesis of NSCLC.

IV. Minimally invasive surgery for NSCLC, via VATS, offers many advantages over thoracotomy, including decreased morbidity, faster recovery and shorter hospitalisation, without compromising oncologic radicality.

V. Linear peptide, ARRPKLD, identified from clinical NSCLC tumour samples with the phage display method, shows significant promise for implemention in both diagnostic and therapeutic use in NSCLC.

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14 APPENDIX

TERVEYTEEN LIITTYVÄN ELÄMÄNLAADUN KYSELYLOMAKE (15D©)

Ohje: Lukekaa ensin läpi huolellisesti kunkin kysymyksen kaikki vastausvaihtoehdot. Merkitkää sitten rasti (x) sen vaihtoehdon kohdalle, joka parhaiten kuvaa nykyistä terveydentilaanne. Menetelkää näin kaikkien kysymysten 1-15 kohdalla. Kustakin kysymyksestä rastitetaan siis yksi vaihtoehto

KYSYMYS 1. Liikuntakyky

1 ( ) Pystyn kävelemään normaalisti (vaikeuksitta) sisällä, ulkona ja portaissa.
2 ( ) Pystyn kävelemään vaikeuksitta sisällä, mutta ulkona ja/tai portaissa on pieniä vaikeuksia
3 ( ) Pystyn kävelemään ilman apua sisällä (apuvälinein tai ilman), mutta ulkona ja/tai portaissa melkoisin vaikeuksin tai toisen avustamana.
4 ( ) Pystyn kävelemään sisälläkin vain toisen avustamana.
5 ( ) Olen täysin liikuntakyvytön ja vuoteenoma.

KYSYMYS 2. Näkö

1 ( ) Näen normaalisti eli näen lukea lehteä ja TV:n tekstejä vaikeuksitta (silmälaseilla tai ilman). ‘
2 ( ) Näen lukea lehteä ja/tai TV:n tekstejä pienin vaikeuksin (silmälaseilla tai ilman).
3 ( ) Näen lukea lehteä ja/tai TV:n tekstejä huomattavin vaikeuksin (silmälaseilla tai ilman).
4 ( ) En näe lukea lehteä enkä TV:n tekstejä ilman silmälaseja tai niiden kanssa, mutta näen kulkea ilman opasta.
5 ( ) En näe kulkea oppaatta eli olen lähes tai täysin sokea.

KYSYMYS 3. Kuulo

1 ( ) Kuulen normaalisti eli kuulen hyvin normaalia puheääntä (kuulokojeella tai ilman).
2 ( ) Kuulen normaalia puheääntä pienin vaikeuksin.
3 ( ) Minun on melko vaikea kuulla normaalia puheääntä, keskustelussa on käytettävä normaalia kovempaa puheääntä.

4 ( ) Kuulen kovaakin puheääntä heikosti; olen melkein kuuro.
5 ( ) Olen täysin kuuro.

KYSYMYS 4. Hengitys

1 ( ) Pystyn hengittämään normaalisti eli minulla ei ole hengenahdistusta eikä muita hengi- tysvaikeuksia.
2 ( ) Minulla on hengenahdistusta raskaassa työssä tai urheillessa, reippaassa kävelyssä tasamaalla tai lievässä ylämäessä.
3 ( ) Minulla on hengenahdistusta, kun kävelen tasamaalla samaa vauhtia kuin muut ikäiseni.
4 ( ) Minulla on hengenahdistusta pienenkin rasituksen jälkeen, esim. peseytyessä tai pukeu- tuessa.
5 ( ) Minulla on hengenahdistusta lähes koko ajan, myös levossa.

KYSYMYS 5. Nukkuminen

1 ( ) Nukun normaalisti eli minulla ei ole mitään ongelmia unen suhteen.
2 ( ) Minulla on lieviä uniongelmia, esim. nukahtamisvaikeuksia tai satunnaista yöheräilyä.
3 ( ) Minulla on melkoisia uniongelmia, esim. nukun levottomasti tai uni ei tunnu riittävältä.
4 ( ) Minulla on suuria uniongelmia, esim. joudun käyttämään usein tai säännöllisesti unilääkettä, herään säännöllisesti yöllä ja/tai aamuisin liian varhain.
5 ( ) Kärsin vaikeasta unettomuudesta, esim. unilääkkeiden runsaasta käytöstä huolimatta nukkuminen on lähes mahdotonta, valvon suurimman osan yöstä.

KYSYMYS 6. Syöminen

1 ( ) Pystyn syömään normaalisti eli itse ilman mitään vaikeuksia.
2 ( ) Pystyn syömään itse pienin vaikeuksin (esim. hitaasti, kömpelösti, vavisten tai erityisapuneuvoin).
3 ( ) Tarvitsen hieman toisen apua syömisessä.
4 ( ) En pysty syömään itse lainkaan, vaan minua pitää syöttää.
5 ( ) En pysty syömään itse lainkaan, vaan minulle pitää antaa ravintoa letkun avulla tai suonensisäisesti.

KYSYMYS 7. Puhuminen

1 ( ) Pystyn puhumaan normaalisti eli selvästi, kuuluvasti ja sujuvasti.
2 ( ) Puhuminen tuottaa minulle pieniä vaikeuksia, esim. sanoja on etsittävä tai ääni ei ole riittävän kuuluva tai se vaihtaa

korkeutta.

3 ( ) Pystyn puhumaan ymmärrettävästi, mutta katkonaisesti, ääni vavisten, sammaltaen tai änkyttäen.

4 ( ) Muilla on vaikeuksia ymmärtää puhettani.

5 ( ) Pystyn ilmaisemaan itseäni vain elein.

KYSYMYS 8. Eritystoiminta

1 ( ) Virtsarakkoni ja suolistoni toimivat normaalisti ja ongelmitta.

2 ( ) Virtsarakkoni ja/tai suolistoni toiminnassa on lieviä ongelmia, esim. minulla on virtsaa- misvaikeuksia tai kova tai löysä vatsa

3 ( ) Virtsarakkoni ja/tai suolistoni toiminnassa on melkoisia ongelmia, esim. minulla on satunnaisia virtsanpidätysvaikeuksia tai vaikea ummetus tai ripuli.

4 ( ) Virtsarakkoni ja/tai suolistoni toiminnassa on suuria ongelmia, esim. minulla on sään- nöllisesti "vahinkoja" tai peräruiskeiden tai katetroinnin tarvetta.

5 ( ) En hallitse lainkaan virtsaamista ja/tai ulostamista.

KYSYMYS 9. Tavanomaiset toiminnot

1 ( ) Pystyn suoriutumaan normaalisti tavanomaisista toiminnoista (esim. ansiotyö, opiskelu, kotityö, vapaa-ajan toiminnot).

2 ( ) Pystyn suoriutumaan tavanomaisista toiminnoista hieman alentuneella teholla tai pienin vaikeuksin.

3 ( ) Pystyn suoriutumaan tavanomaisista toiminnoista huomattavasti alentuneella teholla tai huomattavin vaikeuksin tai vain osaksi.

4 ( ) Pystyn suoriutumaan tavanomaisista toiminnoista vain pieneltä osin.

5 ( ) En pysty suoriutumaan lainkaan tavanomaisista toiminnoista.

KYSYMYS 10. Henkinen toiminta

1 ( ) Pystyn ajattelemaan selkeästi ja johdonmukaisesti ja muistini toimii täysin moitteettomasti.

2 ( ) Minulla on lieviä vaikeuksia ajatella selkeästi ja johdonmukaisesti, tai muistini ei toimi täysin moitteettomasti

3 ( ) Minulla on melkoisia vaikeuksia ajatella selkeästi ja johdonmukaisesti, tai minulla on jonkin verran muistinmenetystä

4 ( ) Minulla on suuria vaikeuksia ajatella selkeästi ja johdonmukaisesti, tai minulla on huomattavaa muistinmenetystä
5 ( ) Olen koko ajan sekaisin ja vailla ajan tai paikan tajua

KYSYMYS 11. Vaivat ja oireet
1 ( ) Minulla ei ole mitään vaivoja tai oireita, esim. kipua, särkyä, pahoinvointia, kutinaa jne.
2 ( ) Minulla on lieviä vaivoja tai oireita, esim. lievää kipua, särkyä, pahoinvointia, kutinaa jne.
3 ( ) Minulla on melkoisia vaivoja tai oireita, esim. melkoista kipua, särkyä, pahoinvointia, kutinaa jne.
4 ( ) Minulla on voimakkaita vaivoja tai oireita, esim. voimakasta kipua, särkyä, pahoin- vointia, kutinaa jne.
5 ( ) Minulla on sietämättömiä vaivoja ja oireita, esim. sietämätöntä kipua, särkyä, pahoinvointia, kutinaa jne.

KYSYMYS 12. Masentuneisuus
1 ( ) En tunne itseäni lainkaan surulliseksi, alakuloiseksi tai masentuneeksi.
2 ( ) Tunnen itseni hieman surulliseksi, alakuloiseksi tai masentuneeksi.
3 ( ) Tunnen itseni melko surulliseksi, alakuloiseksi tai masentuneeksi.
4 ( ) Tunnen itseni erittäin surulliseksi, alakuloiseksi tai masentuneeksi.
5 ( ) Tunnen itseni äärimmäisen surulliseksi, alakuloiseksi tai masentuneeksi.

KYSYMYS 13. Ahdistuneisuus
1 ( ) En tunne itseäni lainkaan ahdistuneeksi, jännittyneeksi tai hermostuneeksi.
2 ( ) Tunnen itseni hieman ahdistuneeksi, jännittyneeksi tai hermostuneeksi.
3 ( ) Tunnen itseni melko ahdistuneeksi, jännittyneeksi tai hermostuneeksi.
4 ( ) Tunnen itseni erittäin ahdistuneeksi, jännittyneeksi tai hermostuneeksi.
5 ( ) Tunnen itseni äärimmäisen ahdistuneeksi, jännittyneeksi tai hermostuneeksi.

KYSYMYS 14. Energisyys
1 ( ) Tunnen itseni terveeksi ja elinvoimaiseksi.

2 ( ) Tunnen itseni hieman uupuneeksi, väsyneeksi tai voimattomaksi.
3 ( ) Tunnen itseni melko uupuneeksi, väsyneeksi tai voimattomaksi.
4 ( ) Tunnen itseni erittäin uupuneeksi, väsyneeksi tai voimattomaksi, lähes "loppuun palaneeksi".
5 ( ) Tunnen itseni äärimmäisen uupuneeksi, väsyneeksi tai voimattomaksi, täysin "loppuun palaneeksi".

KYSYMYS 15. Sukupuolielämä
1 ( ) Terveydentilani ei vaikeuta mitenkään sukupuolielämääni.
2 ( ) Terveydentilani vaikeuttaa hieman sukupuolielämääni.
3 ( ) Terveydentilani vaikeuttaa huomattavasti sukupuolielämääni.
4 ( ) Terveydentilani tekee sukupuolielämäni lähes mahdottomaksi.
5 ( ) Terveydentilani tekee sukupuolielämäni mahdottomaksi.

15 ORIGINAL PUBLICATIONS