Prognosis, Prediction and Risk Assessment in the Prevention and Treatment of Non-Small Cell Lung Cancer

MARTIN SANDELIN
Abstract


**Background:** Lung cancer causes more deaths than any other cancer. Smoking causes roughly 90% of lung cancer cases. Concurrent chemoradiation therapy is the standard of care for stage IIIb patients with performance status (PS) 0-1. A less toxic approach is warranted for less fit patients. To optimize care, the understanding of common clinical variables such as haematological responses to inflammation could be much improved. Adherence to guidelines for proper clinical work-up is vital to ensure patients’ optimal care, especially for predictive assays. Screening of high-risk patients is now being implemented internationally. Chronic pulmonary obstructive disease (COPD) patients, a group at high risk to develop lung cancer, could be of interest for screening.

**Methods:** Patient cohorts collected nationally and regionally by manual search in patient records or automated search in electronic patient records and national registries were analysed in relation to overall survival, comorbidities, medication, treatment, smoking status, biomarkers and adherence to guidelines. Standard statistics were applied to adjust for confounding factors.

**Results:** Induction chemotherapy results in longer overall survival than radiotherapy alone (15.6 and 11.6 months respectively). The overall survival for patients with combined anaemia, leucocytosis and thrombocytosis at diagnosis is half of what could be anticipated if blood samples are normal (8.0 and 16.0 months respectively). Fifty percent of patients were overlooked in the routine work-up with EGFR analysis. Less than 40% of the patients received EGFR-tyrosine kinase inhibitors in first-line therapy. The frequency of EGFR mutation was 9.9%. COPD patients with asthma and medicating with inhaled corticosteroids, specific serotonin reuptake inhibitors (SSRI) or beta-blockers have a significantly decreased risk of lung cancer.

**Conclusions:** Patients unfit to receive chemoradiation therapy should be considered for induction chemotherapy sequentially to radiotherapy. A patient that presents with pathological blood samples is likely to have poor prognosis and diagnostic work-up should be thorough to optimize outcome. Inadequate adherence to the national guidelines regarding treatment and EGFR analysis was shown. COPD patients medicating with ICS, beta-blockers or SSRI and with a concurrent asthma diagnosis have a decreased risk of lung cancer.

**Keywords:** chemoradiation therapy, induction chemotherapy, thrombocytosis, anaemia, leucocytosis, EGFR, guidelines, molecular pathology, ACOS, COPD, risk assessment, comorbidities

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urn:nbn:se:uu:diva-261554 (http://urn.kb.se/resolve?urn=nbn:se:uu:diva-261554)
To all those patients whose lives we cannot save...

To all those patients who agree to participate in research that will not benefit them personally...

To my wife Emma and my children Hella, Pelle & Manne...
List of Papers

This thesis is based on the following papers, which are referred to in the text by their Roman numerals.


IV Sandelin, M., Mindus, S., Thuresson, M., Lisspers, K., Ställberg, B., Stratelis, G., Johanson, G., Telg, G., Goike, H., Larsson, K., Janson, C. Factors Associated With Risk Alteration Of Lung Cancer Development In Chronic Obstructive Pulmonary Disease Patients. (*Manuscript*)

* Both authors contributed equally to the work.

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# Abbreviations

ACOS  Asthma COPD overlap syndrome  
ALK  Anaplastic lymphoma kinase  
ASCO  American Society for Clinical Oncology  
ATC  Anatomical Therapeutic Chemical Classification System  
CK5/6  Cytokeratin 5/6  
CK7  Cytokeratin 7  
COPD  Chronic obstructive pulmonary disease  
CT scan  Computed tomography scan  
ECOG score  Eastern Cooperative Oncology Group score  
EGFR  Epidermal growth factor receptor  
EGFR m⁻  Epidermal growth factor receptor mutation negative  
EGFR m⁺  Epidermal growth factor receptor mutation positive  
EGFR-TKI  Epidermal growth factor receptor tyrosine kinase inhibitor  
EML4  Echinoderm microtubule-associated protein-like 4  
ERRB2/HER2  Human epidermal growth factor receptor 2  
FISH  Fluorescence in situ hybridization  
G-CSF  Granulocyte-colony stimulating factor  
GOLD  Global Initiative for Chronic Obstructive Lung Disease  
Gy  Gray  
Hgb  Haemoglobin  
HGFR  Hepatocyte growth factor receptor  
IASLC  International Association for the Study of Lung Cancer  
ICS  Inhaled corticosteroids  
ICD-10-CM  Classification of diseases, 10th revision, clinical modification  
KRAS  Kirsten rat sarcoma viral oncogene homologue  
LABA  Long acting beta-2 agonist  
MET  Mesenchymal epithelial transition factor  
NAC  Acetylcysteine  
NSCLC  Non-small cell lung cancer  
NSCLC-NOS  Non-small cell carcinoma not otherwise specified  
PCR  Polymerase chain reaction  
PET/CT  Positron emission tomography/computed tomography  
P13K  Phosphatidylinositol-3 kinase  
Plt  Platelet  
PD-1  Programmed death-1  
PD-L1  Programmed death-1 ligand  
RET  Rearranged during transfection
<table>
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<td>c-ROS oncogene 1</td>
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<td>SCLC</td>
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<td>SSRI</td>
<td>Selective serotonin reuptake inhibitors</td>
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<td>TKI</td>
<td>Tyrosine kinase inhibitor</td>
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<td>TRL</td>
<td>Tumour-related leucocytosis</td>
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<td>Thyroid transcription factor-1</td>
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<td>VEGF</td>
<td>Vascular endothelial growth factor</td>
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<td>WBC</td>
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Introduction

There have been immense advances in the field of medicine over the past century. We have progressed from unsterile surgery to robotic surgery, from leeches to evidence-based medicine and from the very discovery of radiation to mastering it at proton beam facilities in everyday practice. However, along with increasing life expectancy, new medical challenges arise. The relationship between the increasing age of the population and the growing numbers of patients diagnosed with malignancies is already a major challenge for health care systems all over the world. Apart from the economic challenge, we are facing a future where a majority of the population will be diagnosed with cancer in their lifetime [1].

Lung cancer serves as a model for how evolving social and economic circumstances translate into new medical challenges. The first cigarette rolling machine, “The Bonsack Machine”, was invented by James Albert Bonsack during the Industrial Revolution in 1880. Cigarette smoking became increasingly common with urbanization and industrialization. A couple of decades later, the unfortunate increase of lung cancer became evident for those who chose to see, as the German pioneer, Isaac Adler, did in 1912 [2]. However, it took until the middle of the twentieth century for the scientific community to confirm the strong relationship between tobacco smoking and lung cancer in a way that convinced politicians and lawyers. In 1950, two important reports were published: Doll and Hill published “Smoking and Carcinoma of the Lung” in the British Medical Journal [3] and Wynder and Graham published “Tobacco Smoking as a Possible Etiologic Factor in Bronchiogenic Carcinoma: A Study of Six Hundred and Eighty-Four Proven Cases” in the Journal of the American Medical Association [4]. With these and other studies published during the same decade, the deadly link between cigarette smoking and lung cancer was established.

By the time the scientific community proved the hazards associated with smoking, the use of cigarettes was already an integrated part of society. It was a symbol for female emancipation, youth rebellion, war heroes, movie stars and a “natural” part of people’s social interaction at home, at work, in restaurants and airplanes as well as most other places. Smoking cessation campaigns had a long way to go, with massive obstructions from strong commercial and cultural antagonists.
Lung cancer was long treated with nihilism. Representing one of the cancers with the worst outcome, it was acknowledged both in the public and among the medical profession as a hopeless diagnosis [5]. Patients diagnosed with inoperable tumours were, at best, left to best supportive care or, if treated at larger medical centres, bold medical trials. Fortunately medical advances eventually allowed the introduction of palliative chemo- and radiotherapy treatments. During the last ten years of the twentieth century, palliative chemotherapy became the standard of care for advanced stage lung cancer [6].

Nihilism regarding lung cancer has sadly remained a fact in parts of society as well as in medical practice up until today [7]. Proof of that is the lack of active smoking cessation programmes for patients with advanced lung cancers. Why quit a bad, but for the patient often precious, habit when it is too late? This is about to change. Oncology in the 21st century brings us new hope [8]. Today, a patient with inoperable lung cancer has more reasons than ever to quit the bad habit, as do the rest of the cigarette smoking population, who want to experience a better tomorrow…
Background

Epidemiology
Lung cancer is the leading cause of cancer deaths worldwide. With an estimated approximately 1.38 million people dead (18.2% of all cancer deaths) and with 1.61 million new cases diagnosed (12.7% of all cancer cases), lung cancer also represents the most common cancer diagnosis worldwide [9]. Tobacco smoking is well established as the number one risk factor for developing non-small cell lung cancer (NSCLC) with approximately 85% of these cases caused by smoking [10]. The number of smokers is slowly decreasing in the Western world [11, 12], but unfortunately it is rapidly increasing in developing countries [11, 13, 14]. Hence, the incidence of NSCLC will escalate globally despite the increasing knowledge that smoking is a risk factor [11, 15].

In Sweden, lung cancer was the fifth most common cancer diagnosis (6.4% of all cancer cases) in 2007. Nevertheless, it accounted for the highest amount of cancer-related deaths due to the poor survival rates for the disease [16]. Since 1980, there has been a decrease in incidence in Swedish men, whereas incidence continues to increase in Swedish women [17].

The decrease of incidence in men and increase in women is true for most European countries. However, incidence rates vary dramatically between different countries within Europe. Incidence rates are 22 to 63/100,000 and 5 to 33/100,000 per year in men and women, respectively [18, 19].

There is an interesting gender difference in smoking habits, where smoking is present in 97.9% of the males diagnosed with cancer but “only” in 75.8% of the females [20].

Risk Factors for NSCLC
Tobacco Smoking
Tobacco smoking is the single most important risk factor for developing lung cancer, highly involved in at least some 85% of all lung cancer deaths per year (equalling approximately 1.2 million people) [9, 10, 20]. Smoking is heavily linked to all types of lung cancer but the link is especially strong to squamous cell carcinomas and small cell lung cancer (SCLC) whereas the link is somewhat less strong with adenocarcinomas of the lung. This can be
illustrated by the fact that the rate of adenocarcinoma in males who have never smoked diagnosed with lung cancer is 57.6%, whereas only 26.5% of men with > 60 pack years were diagnosed with adenocarcinoma [20]. Age of smoking debut, duration of smoking and average intensity all contribute to an increased risk for lung cancer. Duration of smoking is considered the greatest risk factor of the three [20-22]. Smoking cessation lowers the risk of developing lung cancer. The lower risk becomes apparent five to nine years after cessation and decreases further thereafter compared with smoking continuation [23]. It has been shown by Peto et al. that smoking cessation even in the middle-aged group eliminates most of the subsequent risk of developing lung cancer. Smoking cessation before the age of thirty eliminates more than 90% of the risk attributable to tobacco use compared to those who continue to smoke [24].

Other Risk Factors Associated with NSCLC

While smoking is a well-known and highly significant risk factor for lung cancer, other factors that could contribute to the disease are less well defined. The following entities are a selection of the more commonly discussed risk factors related to lung cancer.

**Radon**

Radon-222 (radon) was the first identified environmental cause of lung cancer and was described as early as the 1920s as a potential explanation as to why European miners developed lung cancer at a higher degree than the general population [25]. Radon-222 is a naturally occurring radioactive gas that accounts for up to half of the annual background radiation [26]. It has been suggested that radon accounts for up to 9% of lung cancer deaths in Europe [27]. Radon exposure is the second most important risk factor for developing lung cancer and the first among those who have never smoked [26, 28]. There is evidence of synergistic effects between radon and tobacco smoke and the absolute risk of developing lung cancer by the age of 75 is approximately 25 times greater for a smoker exposed to the same accumulated radon dose than for a non-smoker (12% vs. 0.5% at 100Bq/m$^3$) [27].

**Second-Hand Smoke**

Many studies have shown an increased risk of lung cancer related to second-hand smoke [29-31]. The greatest risk for lung cancer is associated with spousal and workplace exposure to second-hand smoke and this is in line with a dose-response relationship [32]. It is estimated that 3,000 – 5,000 lung cancer deaths annually are due to second-hand smoke in the USA alone [31].

**Indoor Air Pollution**

Studies performed in Asia have shown a relationship between lung cancer and coal used as fuel for cooking in poorly ventilated spaces [33]. Using coal
for indoor cooking throughout life resulted in an increased lung cancer risk (OR: 7.5; 95% CI 2.2-25.9) among non-smokers in an Indian study [34]. It is estimated that up to half of the global population uses solid fuels for cooking, often in poorly ventilated spaces [35].

**Asbestos**

Asbestos is a silicate mineral with structural properties suitable for multiple industrial and technical purposes. It is heat and chemical resistant and the fibres are highly durable [36]. Asbestos was widely used in construction and technical materials during the 20th century despite the first reports of its carcinogenic potential which were published in 1955 by Doll et al [37]. In Sweden asbestos was finally prohibited for use in 1982, but in the European Union a total prohibition was not filed until 2005. In 1994 in an Uppsala cohort, Hillerdal et al. showed that the risk for developing lung tumours is not always related to previous signs of asbestosis, thereby challenging the formerly accepted paradigm that lung cancer occurred as a continuum in patients with asbestosis [38, 39]. The risk for lung cancer and asbestosis is, however, dose-dependent as well as additive or even synergistic when smoking and asbestos exposure occur simultaneously [39-42]. It is estimated that occupational asbestos exposure accounts for 100,000-140,000 lung cancer deaths per year worldwide and is linked to 5% - 7% of all lung cancer cases [43, 44].

**Arsenic**

Exposure to arsenic has been shown to be a risk factor for NSCLC. In copper smelter workers inhaling inorganic arsenic, a rate ratio of 2.6 in non-smoking and a multiplyable rate ratio of 14.6 in smoking workers was seen [45]. It seems likely that inhaled arsenic would be a greater risk factor for NSCLC. However, Smith et al. compared the risks between inhaled vs. ingested inorganic arsenic and found that the risk rates were similar for NSCLC development [46].

**Chronic obstructive pulmonary disease**

Similar to lung cancer, chronic obstructive pulmonary disease (COPD) is also closely linked to the chronic inflammation and oxidative stress that are induced by the thousands of toxic agents and $10^{15}$ free radicals that are inhaled with every puff of a cigarette [47, 48]. COPD patients are at a greater risk of developing lung cancer than healthy non-smoking individuals [49, 50]. The PATHOS study, which investigated the same cohort examined in Paper IV of this thesis, reported an eightfold increased risk of lung cancer death for COPD patients than for a non-COPD cohort matched for age and gender [51]. When matched for smoking history, patients who develop moderate or severe COPD also have an up to 2.6 times greater risk of developing
lunge cancer compared to smokers with mild or no COPD [52]. This indicates that COPD by itself is a risk factor for lung cancer.

Emphysema of the lungs is a common finding in COPD patients. However, emphysema and COPD are not only smokers’ diseases but can also develop in susceptible non-smokers [53]. It has been shown that emphysema of the lungs is an independent risk factor for lung cancer. The inflammatory process involved in COPD is also found in non-smoking patients with emphysema [54, 55].

Preventive Measures in NSCLC

Lung Cancer Screening

In order to start a screening programme for a disease, the criteria first presented by Wilson and Junger [56] should be fulfilled. Because it is a condition with a detectable pre-clinical phase, a potentially (if detected early) curative treatment and effective methods for screening, which can also be potentially cost-effective, lung cancer seems to meet the criteria needed for a potentially successful screening programme [57].

The fact that a majority of NSCLC cases are diagnosed at a late stage without possibility of cure has led to evaluation of lung cancer screening programmes. Large trials using chest radiographic screening, with or without the addition of sputum cytology [58-61], have individually shown only modest or no beneficial effect on lung cancer mortality. However, in a more recent meta-analysis of chest radiographic screening, the intervention groups actually had an 11% increased risk of lung cancer mortality. It is unclear whether this result is due to the risks connected with frequent radiographic exams or because of the underdetection of lung cancer as a cause of death in the control groups and other possible trial design related causes [62].

The use of computed tomography scans (CT scans) has emerged as the most promising method for screening as this technology rapidly progresses. To minimize the risks with repeated exposure to ionizing radiation due to the screening programme, the use of low dose CT scans is routine in currently studied and implemented CT scan based protocols.

The largest trial showing a positive outcome is the National Lung Screening Trial (NLST) including 53,454 screened patients. The trial randomized the included patients to three annual low dose CT scans or plain chest radiographs respectively and showed a reduction in lung cancer mortality of 20% in the low dose CT scan arm at the five-year follow-up [63]. Since then, the successful outcome of the NLST study has led to guidelines in the USA recommending screening for certain groups of patients [57]. The current recommendation, based primarily on the NLST study, is to include patients between 55 and 74 who have smoked more than 30 pack years and are cur-
rent smokers or ex-smokers who have ceased within the last 15 years, in lung cancer screening programmes [64].

Although screening for lung cancer, in well-defined groups of at-risk patients, has been adopted in the USA, questions are still raised as to whether the results are also applicable in a European setting [65]. It is primarily the high number of false positive cases that raises concerns. In a meta-analysis of low dose CT scan screening of 1,000 screened individuals, nine stage I NSCLC tumours and 235 false positive nodules were detected [66]. Before the NLST study results are confirmed by ongoing European studies, it is considered reasonable to wait with screening programmes [65].

Smoking Cessation

In terms of morbidity, mortality and costs, among the most effective measures implemented in modern health care are professional smoking cessation programmes [67]. An unfortunate circumstance is that even though the results are scientifically proven, smoking cessation programmes often struggle with lack of resources, leaving many smokers to unsupervised cessation [68, 69].

Success rates with different smoking cessation methods point to the double nature of nicotine addiction. The biological addiction is strong but often the psychological factors are just as strong. The combination of nicotine replacement, pharmaceutical measures to limit the craving for and reward from nicotine, together with professional supervision, gives the best results [67]. Long-term cessation is achieved by applying these combined measures, which are reported to be successful in up to 30% of the participants at six months of follow-up [70].

Histology

Lung cancer is traditionally divided into small cell lung cancer (SCLC) and non-small cell lung cancer (NSCLC). NSCLC represents 80% - 85% of lung cancer cases [71]. NCSLC is a highly heterogeneous group consisting of the three main histologies: squamous cell carcinoma, adenocarcinoma and non-small cell lung cancer not otherwise specified (NSCLC-NOS). After a large meta-analysis was published by the Non-Small Cell Lung Cancer Collaborative Group in 1995, chemotherapy was finally shown to prolong survival compared to best supportive care [72, 73]. Until late in the first decade of the 21st century, histology in the NSCLC group did not affect the choice of treatment [74]. Standard treatment consisting of a platinum drug in combination with a third generation cytotoxic drug was used to treat all kinds of NSCLC. In 2008, Scagliotti et al. published the results of a Phase III study, in which a pre-planned subgroup analysis showed a marked difference in
survival between squamous cell carcinomas and non-squamous cell carcinomas (e.g. adenocarcinoma and NSCLC-NOS). After that study, the focus on histology began to increase [75]. Today, tumour histology is vital when choosing an individualized treatment [76, 77].

The importance of histology is also significant when using molecular pathology to individualize targeted medicine. Mutations in the EGFR gene are almost exclusively found in non-squamous NSCLC, and thus analysis can be directed towards non-squamous histologies [78, 79]. New targeted drugs are under development for both squamous cell carcinomas and non-squamous cell carcinomas. This will challenge the present algorithm for molecular pathology analysis.

The latest consensus document defining NSCLC histology was published in 2011 and makes a clear difference between the evaluation of resection specimens and specimens from diagnostic biopsies. In diagnostic NSCLC biopsies, the main histological categories since the 2011 revision are adenocarcinoma, squamous cell carcinoma and NSCLC-NOS [80]. It should be noted that adenocarcinomas are further sub-divided in surgically resected tissues, but these subgroups are, today, of minor clinical importance [74].

Adenocarcinoma

Adenocarcinoma histology is the largest NSCLC subgroup. It tends to have a peripheral distribution in the lung, sometimes retracting the pleura. Adenocarcinomas are defined as malignant epithelial neoplasms characterized by gland formation. The most important immunohistochemical antibodies that identify adenocarcinoma of primary lung origin are thyroid transcription factor-1 (TTF-1) (see Figure I) and cytokeratin 7 (CK7) [81]. If TTF-1 is present in the specimen the tumour should be termed NSCLC favouring adenocarcinoma even if squamous markers are present [82].

**Figure I.** NSCLC tumour with histological pattern of an adenocarcinoma (left). Immunohistochemical picture of the same tumour positive for TTF1 (right). Both pictures in x10 magnification. (Photo: Dr. P. Micke.)
Squamous Cell Carcinoma

Squamous cell carcinomas are the second most common subgroup in NSCLC, although they used to be the most common in the 20th century. Squamous cell carcinomas are strongly associated with smoking and are typically found in the proximal part of the lung. Keratinization, intracellular bridges and pearl formations characterize a well-differentiated squamous cell carcinoma’s histology [83]. The most important antibodies used to characterize squamous cell carcinomas by immunohistochemistry are p63, p40 and CK5/6 (see Figure II) [83-86].

![Figure II.](image)

Figure II. NSCLC tumour with histological pattern of a squamous cell carcinoma (left). Immunohistochemical picture of the same tumour positive for CK5/6 (right). Both pictures in x40 magnification. (Photo: Dr. P. Micke.)

Non-Small Cell Lung Cancer Not Otherwise Specified

The pathologist determines the histology of a tumour by light microscopy and, if that is not conclusive, immunohistochemistry is used in addition. However, even with the use of immunohistochemistry the histologies of some tumours are not clear-cut [82]. In order to preserve as much tissue as possible for subsequent molecular testing, the histological work-up should be as limited as possible [87-89]. It is recommended that a tumour with unidentifiable morphology but with positive staining for either TTF-1 (adenocarcinoma marker) or p63/p40/CK5/6 (squamous cell carcinoma markers) should be termed NSCLC-NOS favouring adenocarcinoma or squamous cell carcinoma, respectively. If no specific marker for adenocarcinoma or squamous cell carcinoma of primary pulmonary origin is positive, a panel of additional immunohistochemical markers is suggested to exclude origins other than lung cancer [89]. By applying the present recommendations for pathology work up it is proposed that approximately 5% of the analysed specimens will result in NSCLC-NOS [82, 90].
Prognostic and Predictive Factors in NSCLC

TNM Staging

The tumour-node-metastasis (TNM) staging system was first applied to NSCLC in 1974 by Mountain et al. [91] Since then, the TNM staging system has been revised several times with the latest revision in 2009 with the 7th edition. The current TNM staging system (7th edition) is based on a database including more than 100,000 cases from international cohorts [92, 93].

T in TNM

Tumour size is a prognostic factor in itself for patients undergoing curative surgery [94, 95] as well as in advanced stage disease [96]. The T status of the tumour is evaluated with regard to its size, location, separate tumour nodule(s) and ingrowth in adjacent structures [97].

N in TNM

Accurate assessment of the nodal involvement of NSCLC is crucial in patients with non-metastatic disease. Of surgically treated patients, with any T but M0, the five-year survival rates were 42% in N0 patients, 29% in N1 patients, 16% in N2 patients and 7% in N3 patients. In the same material, the non-surgically treated patients, any T but M0, had a five-year survival rate ranging from 9% in N0 patients to 5% in N3 patients [98]. Because of the strong correlation to outcome after surgery, regardless of T status, N status is regarded as the strongest prognostic factor for long-term survival in non-advanced (i.e. stage ≤ IIIa) NSCLC cases [99, 100]. The increasing use of positron emission tomography/computed tomography (PET/CT) has led to improvements in N staging [100]. Today, the American Society for Clinical Oncology (ASCO) guidelines for NSCLC diagnostic evaluation state that PET/CT complements the CT scan and is recommended for use if no evidence for M1a-b disease is present on the CT scan [101].

M in TNM

Determining the M status of the disease also determines whether the patient will be treated with curative or palliative intent [102]. In many cases the situation is relatively clear-cut (e.g. M1b disease with distant multiple metastatic lesions). However, since M1 disease was divided into M1a and M1b disease in the 7th revision of the International Association for the Study of Lung Cancer (IASLC) classification, the M status has become more complicated [102, 103]. In the work preceding the 7th edition of the IASCL TNM staging system it became clear that the prognosis for patients with malignant pleural effusion (previously classified as T4) had a dismal prognosis resem-
bling that of other situations with intra thoracic disseminating disease (con-
tralateral tumour nodules and pericardial effusions) albeit significantly better
than tumours with distant metastatic dissemination. To reflect this difference
in prognostic value, the M1 group was split into M1a (intra thoracic spread)
and M1b (distant metastatic disease) [102]. Because of the tremendous im-
 pact for the line of action, it is essential that M1(a-b) disease is adequately
assessed with either substantial clinical evidence or diagnostic work-up in-
cluding biopsies to verify suspicions [104, 105].

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**Table 1.** TNM stage groupings

TNM Stage

By assessment of T, N and M status of the tumour, a TNM stage is assigned
to the individual patient’s disease (see Figure III). The TNM staging system
outlines prognosis in subgroups of patients thereby enabling the clinician to
make evidence-based treatment decisions [96, 97, 103]. The strength of the
TNM system is the combined impact of three individually strong prognostic
factors with an ongoing validation and thereby represents the strongest pre-
dictor for outcome in NSCLC [106, 107]. Advanced stage prognosis and
treatment will be discussed under separate sections.
Performance Status

Performance status is an evaluation of the patient’s overall physical fitness at that moment. The most frequently used performance status score in NSCLC is the Eastern Cooperative Oncology Group score (ECOG score) that runs from 0 (full health) to 5 (death) [108]. It has been shown that the ECOG score has higher prognostic potential in NSCLC than the Karnofsky score and is therefore the current gold standard in performance status assessment in NSCLC [109]. The prognostic value of the patient’s performance status is shown in multiple studies [110-114]. A dilemma is the subjectivity in the evaluation of performance status. The congruency between the patient and the physician in evaluation of the patient’s performance status has been shown to be moderate, the physician generally tend to be more optimistic [115, 116]. ECOG scores are sometimes also referred to as WHO scores or Zubrod scores.

Figure III. Schematic staging overview
Weight Loss

The importance of involuntary weight loss as an individual prognostic factor was demonstrated in a multitude of malignancies by Dewys et al. [117] Although involuntary weight loss of more than 5% is strongly correlated to outcome, more recent data show that in particular sarcopenia, loss of muscle mass, assessed by CT scan, is an even more sensitive prognostic marker [118]. The current consensus regarding weight loss assessment states that involuntary weight loss of >5% over the last 6 months in any cancer patient, or, in patients with a BMI <20, a weight loss of >2%, or signs of sarcopenia accompanied with weight loss >2% in any patient, is defined as cachexia [119]. In a study by Ross et al. 58% of patients with NSCLC reported weight loss at time of diagnosis. Although weight loss was related to dismal overall survival an important finding was that patients that stabilized weight during treatment had significantly improved survival compared to patients that continued to lose weight [120].

Age

The median age of patients receiving a NSCLC diagnosis is reported to be between 68 and 69 years old [121, 122]. About 50% of the patients diagnosed with NSCLC are 65 and older and of these 30% - 40% are 70 and older [123]. While medical studies often tend to recruit younger patients (e.g. <70 years), data is limited regarding the safety and efficacy of treatments in the elderly [124, 125]. Age is reported as a prognostic factor in many studies performed on NSCLC patients [126-128]. Until recently the use of chemotherapy in the elderly population was under discussion [122]. The importance of age as an individual prognostic factor has since been reevaluated, and an increasing amount of data supports the assumption that chronological age is less important than performance status and comorbidities (i.e. biological age) [129-133].

EGFR Status

With the identification of the EGFR mutation as a driver mutation in NSCLC, the first highly predictive molecular factor was identified for treatment outcome [134]. However, the EGFR mutation is not only predictive but is also shown to be a positive prognostic factor in NSCLC. Implications of mutations in the EGFR gene in NSCLC will be discussed further under specific sections of the thesis.

Anaplastic Lymphoma Kinase (ALK) Rearrangement Status

Similar to the EGFR status, the anaplastic lymphoma kinase (ALK) status of the tumour has a strong predictive impact for treatment outcome with ALK
inhibitors [135]. There are also reports that ALK rearrangements are predictive for increased sensitivity towards pemetrexed treatment [136]. In some reports the presence of ALK positive tumour cells is connected with a higher incidence of metastatic disease [137, 138]. The prognostic importance of ALK rearrangement is still unclear. Implications of alterations in the ALK gene in NSCLC will be discussed further under specific sections of the thesis.

**White Blood Cell Count**

It is not uncommon that NSCLC patients present with elevated levels of white blood cell count (WBC) [139]. Leucocytosis can be induced by bone marrow metastasis, infection or corticosteroid treatment [140] but some patients present with increased WBCs with no apparent reason [140, 141]. Tumour-related leucocytosis (TRL) is considered a paraneoplastic syndrome and is sometimes encountered in NSCLC patients without other apparent reasons for leucocytosis. The mechanism for TRL development is mainly unregulated upregulation of haematopoietic cytokines (e.g. granulocyte-colony stimulating factor (G-CSF) [140, 142]. An elevated WBC count is reported as a negative prognostic factor in NSCLC of all stages [143, 144].

**Haemoglobin levels**

Multiple studies have shown that anaemia is a negative prognostic factor [145, 146]. It has been shown that solid tumour patients presenting with anaemia at the time of diagnosis have a higher degree of systemic inflammation than patients with normal haemoglobin (Hgb) levels [147]. Tumours are known to secrete pro-inflammatory agents and one likely explanation is that these cytokines suppress the bone marrow’s erythrocyte production by decreased sensitivity to erythropoietin and alterations in iron metabolism [147, 148].

**Thrombocytosis**

Thrombocytosis has previously been shown to correlate with negative prognosis in all stages of NSCLC as well as other tumours [149-152]. Studies have suggested that thrombocytosis can contribute to cell invasion and formation of metastases by affecting the blood vessel endothelium [153, 154]. Thrombocytosis is a common reaction to inflammatory stimuli. Studies have shown that an elevated platelet count correlates with inflammatory levels, negative outcome and more advanced disease in different solid tumours [155, 156].
Surgical treatment of lung cancer is the oldest form of treatment as well as the modality with the greatest success rate. For a long time surgery was the only recommended option for early stage disease. However, this recommendation is now debatable when it comes to small tumours in less fit patients [157, 158]. Surgery in lung cancer patients is often delicate due to comorbidities related to smoking such as COPD with impaired pulmonary function and arteriosclerosis [159, 160]. While the recommendation is still to perform lobectomy to achieve radical excision, physicians must calculate postoperative lung function so that it will not cause respiratory failure in the patient [161].

Surgery is only recommended up to stage IIIa with the exception of so-called oligometastatic resectable stage IV disease (typically solitary metastasis to CNS or adrenal gland and resectable primary tumour) [116]. Adjuvant chemotherapy should be administered to eligible patients postoperatively from stage IIa disease. It can also be considered in stage Ib but the benefits are controversial [161, 162].

Radiotherapy

Radiotherapy has a role in all stages of NSCLC. Curative ablative therapy as a single modality is used in early stage patients who have comorbidities that make them less suitable for surgery [158]. Chemoradiation therapy with curative intent is given to patients with locally advanced NSCLC and palliative radiotherapy is often given to patients with advanced stage disease to alleviate symptoms.

Radiotherapy is planned according to the intervention intent. Palliative treatment is preferably given in fewer fractions of higher intensity (albeit to a lower total dose) than radiotherapy with curative intent [163]. One exception is for stereotactic body radiation therapy in which few fractions are given to a high total dose in the target area. Evidence is accumulating to suggest that stereotactic body radiation therapy is as effective a modality as surgery in small (< 3cm) tumours [157]. Furthermore stereotactic body radiation therapy is recognized as a less invasive and likely as effective treatment for small localized tumours especially in patients at higher risk for surgical or post-surgical complications [158, 164].

Chemotherapy

The use of chemotherapy as a treatment in NSCLC started along with the treatment of other solid tumours in the middle of the twentieth century [165].
However, success was limited and it was not until the last decade of the 20th century that palliative chemotherapy was regarded as the standard of care [6]. Today, patients with a performance status ≤ 2 should be considered for platinum-based doublet chemotherapy. The choice of a third generation cytotoxic agent together with a platinum drug (i.e. carboplatinum or cisplatinum) is considered the standard of care [166]. While many factors should be considered in the palliative setting to maximize quality of life during the treatment period, the choice of third generation cytotoxic drugs is often individualized in clinical practice. The efficacy of the third generation cytotoxic drugs is considered relatively similar, although the toxic profile differs significantly [167]. There is evidence of differences in efficacy between distinctive platinum-based combinations, although it is difficult to draw solid conclusions depending on in-trial bias (e.g. use of both carbo- and cisplatinum) or contradictory results from other studies [167-171].

Lately the use of maintenance therapy has proven effective. In patients with non-squamous NSCLC who have stable disease or better after four induction cycles with a platinum-based doublet chemotherapy, monotherapy is continued and administered to progression [166]. Pemetrexed, an antifolate agent, has proven to be the drug of choice in this setting. The pemetrexed maintenance therapy can either be given as “continuation maintenance therapy” (i.e. after a platinum-pemetrexed combination) or as “switch maintenance therapy” (i.e. after a non pemetrexed containing induction therapy) [172, 173].

When chemotherapy is used in an adjuvant setting the cytotoxic potential should be maximized. The curative intent aiming for eradication of micro metastasis not removed during surgery gives less consideration to quality of life during treatment. Since the ANITA trial, the standard of care is a combination of cisplatinum and vinorelbine administered to eligible patients for up to four cycles [174]. The LACE meta-analysis further strengthens the role for cisplatinum-based adjuvant treatment [175].

Chemoradiation Therapy
The combination of chemotherapy and radiotherapy for patients with locally advanced non-resectable NSCLC has been studied in numerous randomized control trials over the years. Sequential, concurrent and adjuvant regimens with different fractioning have been evaluated (Williams, Lung Cancer, 2015). In their large meta-analysis, Aupérin et al. showed a significant benefit in overall survival when a combined therapy was given, with an advantage for concomitant platinum-based chemoradiation therapy [176]. Today, chemoradiation therapy is established as the standard of care in inoperable stage IIIa and most stage IIIb patients. The regimens used in most departments are based on the recommendations from ASTRO and ASCO, which advise a concurrent administration of a platinum-based doublet chem-
otherapy and radiotherapy 2 Gy in ≥30 fractions (usually 60-72 Gy in total) [177, 178]. Importantly, toxicity is high when combining the modalities and patients should be selected carefully.

Targeted Therapy

A novel approach to the treatment of advanced stage lung cancer was introduced during the first decade of the 21st century: targeted therapy. Instead of the traditional chemotherapy, in which the mechanism of action is exerted through a non-specific cytotoxic property of the drug targeting all dividing cells, targeted therapy is directed at specific up-regulated signal pathways of the tumour. Typically aiming at tyrosine kinase receptors at the tumour cell surface, the toxicity profiles are different from those encountered with chemotherapy and the response rates in patients with specific genetic alterations are generally high [179].

The introduction of targeted therapies in NSCLC treatment has changed the diagnostic procedure workflow at diagnosis. The need for tumour tissue has become increasingly important along with the introduction of routine molecular pathology assays. The first analysis to be introduced into routine care was the EGFR mutation analysis, typically performed with commercially available polymerase chain reaction (PCR) kits. ALK translocations were initially detected through fluorescence in situ hybridization (FISH) but today most centres screen for ALK translocations by immunohistochemistry and then confirm positive cases (approximately 2% - 4%) by FISH analysis [180, 181].

The increasing number of known potential targets for targeted therapy has proven the current sequential analysis technique obsolete. Instead of a single assay for each gene of interest, next generation sequencing is now rapidly being implemented in molecular pathology departments. By using next generation sequencing, most genetic alterations can be analysed in one single assay, with less tumour tissue than would otherwise be needed [182].

Targeted therapies have proven effective in NSCLC but the vast majority of patients still inevitably develop resistance to the targeted drugs and relapse. New generations of drugs targeting mechanisms of resistance (such as the commonly acquired T790M resistance mutation in EGFR m+ patients treated with EGFR-TKI) are under development [179]. The future strategy will likely be to combine targeted drugs (possibly also in combination with other classes of drugs such as immunotherapy drugs) to prevent the tumour from acquiring mutations causing resistance.
Other Potential Genetic Targets for Treatment

**BRAF**
Together with its isoforms ARAF and CRAF, BRAF belongs to the RAF family of protein kinases [183]. Exerting effect downstream as a MEK activator, the most common BRAF mutation, BRAF V600E (valine to glutamate substitution in codon 600), leads to excessive cell proliferation and represents approximately 50% of the BRAF mutations known to be found in up to 3% of NSCLC tumours [184, 185]. Recently reported results from small trials targeting BRAF mutations with BRAF inhibitors (e.g. vemurafenib, dabrafenib or sorafenib) in later therapeutic lines show promising effects with a response rate of 53%, disease control rate of 85% and progression-free survival of 5.0 months [186].

**ERBB2**
Human epidermal growth factor receptor 2 (HER2/ERBB2) belongs to the EGFR subfamily but unlike other members ERBB2 has no (yet known) ligand. Instead, it is activated by homo- and heterodimerization with other members of the ERBB family. In an Asian study, NSCLC cases were retrospectively analysed for ERBB2 mutations. All mutations (46 mutated ERBB2 tumours in 1,275 NSCLC cases) were found in tumours of adenocarcinoma histology (n = 1,055) resulting in a mutation frequency of 4.3% in adenocarcinoma and 3.6% in NSCLC respectively [187]. It should be noted that a study on a mainly Caucasian cohort reports a lower frequency of ERBB2 mutations (1.7% frequency in an exclusive adenocarcinoma cohort) [188].

Therapy utilizing HER2/ERBB2 targeted drugs has not yet been tested in larger trials on NSCLC. However, there are reports of promising disease control rates for different classes of drugs targeting HER2/ERBB2 but only in small cohorts [188, 189].

**KRAS**
Genomic alterations in the Kirsten rat sarcoma viral oncogene homologue (KRAS) are the most frequently detected genomic alterations in NSCLC and are found in up to 30% of NSCLC tumour samples [183, 190]. To date, no targeted therapy is available for patients whose tumour harbours a KRAS alteration. Studies are however on-going where downstream effectors in the signalling pathway are targeted. In a recent Phase I study, a MEK1/2 inhibitor (selumetinib) showed promising results on KRAS mutated patients [191]. Phase III trials are now open for inclusion and their results are eagerly awaited.
**MET**

Mesenchymal epithelial transition factor (MET) or hepatocyte growth factor receptor (HGFR) is a trans-membrane receptor tyrosine kinase active in multiple signalling pathways [192]. The MET gene, coding for the protein c-MET, exhibits mutations and amplifications that are known to be one of several mechanisms of acquired resistance to ALK and EGFR TKIs [193, 194]. However, de novo mutations and amplifications of the MET gene are also detected in up to 20% of tumours analysed [195]. There are no standards for analysis of MET and c-MET today: immunohistochemistry, FISH and next generation sequencing are used and too little is still known of the correlation between the different methods and the implications for treatment [190]. MET inhibitors are, however, in clinical trials and it has been shown in small studies that crizotinib (originally developed as a MET inhibitor) has antitumoural properties in MET amplified tumours [196, 197].

**PI3KCA**

Phosphatidylinositol 3 kinases (PI3K) activate downstream mediators in the AKT/mTOR pathway that in turn regulate cell survival, growth and motility [183]. Activation in the PI3KCA gene by amplification or mutation can lead to excessive cell proliferation and mutations are found in approximately 5% of NSCLC tumours [198, 199]. Inhibition of PI3KCA is under development but few reports are available and initial results have not looked as promising as in other targets [200].

**RET**

The rearranged during transfection (RET) gene has been found to be altered in 1% - 2% of NSCLC tumours [201, 202]. Patients are treated in phase I and II studies with different RET inhibiting drugs showing promising results with partial response or stabilization of disease for extended durations [203, 204]. Cabozantinib, a RET targeting drug approved for treatment of medullary thyroid cancer, showed promising early results in a recent study and could represent an “off-label” alternative in later treatment lines for patients with RET alterations [204].

**ROS1**

c-ROS oncogene 1 (ROS1) gene rearrangements are present in 1% - 2% of NSCLC tumour samples [205]. ROS1 rearrangements result in a fusion protein that exerts tyrosine kinase activity promoting cell growth and proliferation [206]. ROS1 rearranged tumours are primarily found in a defined patient subgroup resembling those where ALK translocations and EGFR mutations are detected, namely younger age, never smoked or light smokers and adenocarcinoma histology [205]. Although larger trials have not been performed, ROS1 rearranged tumours are reported to be sensitive to the ALK-
TKI crizotinib in smaller cohorts with response rates resembling those of ALK translocated patients [207]. Today ROS1 is routinely analysed in some centres and treatment with crizotinib is widely recommended if a rearrangement is detected despite lack of approval from the United States Food and Drug Administration and European Medicines Agency [208].

**Immunotherapy**

The immune system is in itself a powerful gatekeeper in our defence against early malignancies. However, because one hallmark of cancer is adaptation through basic evolutionary principles, manifest tumours often have down-regulated expression of surface antigens as well as upregulated immune inhibitory cell signalling [209, 210]. Although the immune system has been recognized as a potential partner in the war on cancer since the late 19th century, when William Coley reported regression of tumours after injections of bacterial toxins, no real progress has been made in utilising its potential in oncology. Various attempts have been made to overcome this “stealth” mechanism developed by the tumour. Studies based on vaccines, to enhance immune response to tumour cells, have not rendered any immediate success in the treatment of NSCLC [211, 212].

With the identification of immune checkpoints, immunotherapy has been widely recognized as the next big step forward in the search for a cure for cancer [213]. The first immune checkpoint to be described and understood was CTLA-4, which is expressed on activated T-cell surfaces and transduces inhibitory signals to the activated T-cell [214, 215]. The first report of a successful treatment of tumours, in mice, with an anti-CTLA-4 antibody was published in 1996 by Leach et al. [216]. It took until 2010 for the first phase III trial to be evaluated and a 20% long-term survival in patients with metastatic melanoma was reported [217].

Shortly after the finding and development of CTLA-4 directed therapy, the programmed death-1 (PD-1) receptor and its ligand PD-L1 checkpoint were described. In 2003 Brown et al. showed that by blocking the PD-1 ligand with antibodies, an enhanced immune response was achieved [218]. The method of blocking checkpoint signalling by targeting the receptor PD-1 or the ligand PD-L1 has been utilized since then in drug development [219, 220]. Drugs targeting the PD-1/PD-L1 checkpoint pathway have proven less toxic than CTLA-4 directed therapy and have so far received approval in the treatment of malignant melanoma and NSCLC [220].
Survival

Overall Survival

Overall survival for patients diagnosed with NSCLC is poor. The USA reports a 15% five-year survival [221] and Europe is trailing with five-year survival of approximately 11% [18]. Although overall survival is poor there are major differences in survival with regard to tumour stage at diagnosis.

In the earliest stage (Ia), five-year survival is high with reports ranging up to 90% in selected patients [95]. The five-year survival rapidly decreases with increasing tumour stage, falling to 33% in stage IIb tumours [222] and only 24% in stage IIIa disease [223].

The majority of the NSCLC cases diagnosed are, however, inoperable, including stage IIIb (locally advanced) and stage IV (metastasized) disease. These stages represent approximately 65% of cases. The median survival is short with only 20% - 30% of the patients alive one year after diagnosis [224-226] and five-year survival around 10% [103].

Survival Stage III NSCLC

Stage III NSCLC is a heterogeneous group and presents a great challenge to the lung cancer community [227]. For better prognostic information and to aid treatment decisions, stage III disease is divided into IIIa and IIIb. Both groups are potentially curable [228], but five-year survival is already low in stage IIIa and drops significantly in IIIb. In a retrospective study, Wang et al. showed the importance of an aggressive approach to reach curative treatment in stage III disease. Five-year survival was studied in a cohort of 846 patients with stage III and IV disease. Of these patients, 199, 195 and 452 were in stages IIIa, IIIb and IV, respectively. Five-year survival was reached by 28 (IIIa), 9 (IIIb) and 19 (IV) patients corresponding to 14%, 4.6% and 4.2%, respectively. Of the stage III patients reaching five-year survival, 33 (89.2%) underwent aggressive multimodal treatment, and of those 23 (62.6%) underwent pulmonary resection and systematic mediastinal lymph node dissection. One patient (1.7%) with stage III disease survived five years with radiotherapy alone. However, the same patient eventually progressed and died from the original tumour at a later stage [228], which indicates the value of multimodal treatment.

Stage IIIa

Stage IIIa disease is primarily characterized by the presence of malignant lymph nodes in, but at most, N2 position [98]. Although treatment for stage IIIa NSCLC should be performed with curative intent, five-year survival is low, ranging from 8% - 14.1% in reports from different centres [228-230] and up to 24% in a collected large database using the 7th edition TNM system [103]. However, for those patients eligible for full multimodal treatment
(i.e. induction or adjuvant chemotherapy, surgery and adjuvant radiotherapy), five-year survival is significantly better and reports of five-year survival rates of up to 40% - 50% are present when stage IIIa patients with N2 disease received all three modalities [174, 231]. The problem with the aggressive approach is that only 50% of the patients that were eligible for initial surgery (e.g. acceptable performance status and lung function) endured the entire four cycle adjuvant chemotherapy treatment consisting of cisplatinum and vinorelbine in the ANITA trial [174]. Consequently, the group of patients that eventually undergoes a third modality will be highly selected and relatively few in number. The role of postoperative radiotherapy (PORT) also remains a controversial issue [232].

Stage IIIb

Stage IIIb is primarily characterized by the presence of malignant lymph nodes in the N3 position, but extensive tumour growth (T4) in combination with metastatic lymph nodes in the N2 position is also included in stage IIIb [98, 103]. Stage IIIb disease is generally addressed as a locally advanced (infiltrative) stage and primary surgical treatment is considered futile [233, 234]. Standard treatment for stage III disease is concomitant cisplatinum-based chemoradiation therapy [227, 234]. At present, trimodal treatment (i.e. chemoradiation therapy and surgery) is not recommended in any stage IIIb subgroup [227]. However, randomized control trials have shown longer progression-free survival in patients randomized to trimodal treatment arms, although overall survival was equal to that of patients randomized to chemoradiation therapy. This is most likely due to mortality in connection with surgery [227, 235]. The expected five-year survival in stage IIIb ranges from 1% - 5% in older reports from different centres [228-230] and up to 9% in a larger database using the 7th edition TNM system [103].

Survival Stage IV NSCLC

Stage IV is a heterogeneous group characterized by a spread of the primary tumour outside the lung (to non-lymph node structures within the thorax (M1a) or any structure outside the thorax (M1b)). A patient is classified as stage IV if M > 0 independent of T and N status [102, 103]. Generally, stage IV disease is defined as treatable albeit non-curable in patients with performance status ≤ 2 [236, 237]. While the vast majority of stage IV patients will only be candidates for palliative treatment, a small subgroup of patients with limited metastatic (oligometastatic) disease to the brain or adrenal gland could benefit from multimodal treatment with curative intent [238].

The majority of NSCLC patients present with stage IIIb and stage IV disease at diagnosis without possibility of cure. Multiple studies have shown that the use of cytotoxic agents as palliative treatment in addition to best supportive care prolongs survival as well as improves quality of life [239]. Nowadays, a platinum-based chemotherapy combination where the platinum
is combined with a third generation cytotoxic agent is the standard of care for patients with a performance status $\leq 2$ and no other major contraindications or targetable genetic alterations [237, 239-241].

The five-year overall survival in stage IV disease is low, ranging from 1% - 5% in reports from various centres [228-230] and up to 13% in a larger database using the 7th edition TNM system [103].

Survival Stage IIIb/IV with Targetable Genetic Alteration

A relatively new and interesting subgroup of advanced NSCLC patients is those with a targetable alteration in the tumour genome (e.g. at present mainly EGFR mutations or ALK rearrangements). The high response rate seen in patients treated with targeted drugs has not been seen in the NSCLC field before. The difference in toxicity also allows patients with poor performance status an attempt at a potent therapy, patients who would previously have been left solely to best supportive care due to their ineligibility to receive chemotherapy. In EGFR mutation positive (EGFR m+) patients the expected survival is drastically changed compared with EGFR mutation negative (EGFR m-) patients and median progression-free survival has been reported to be more than one year in different studies [242-244]. Positive results were demonstrated when ALK inhibitors were studied in a second-line setting with a median progression-free survival of 7.7 months [245].
Aims

General Aims of the Thesis
The aim of the thesis is to study possible ways to improve the outcome for advanced stage NSCLC patients. By studying existing cohorts in the departments of oncology, pulmonology and pathology, we have focused on finding preventive factors, treatments and biological markers that can improve survival and prognostic accuracy.

Specific Aims of the Thesis

- To investigate the impact of induction chemotherapy on treatment outcomes in curatively intended radiotherapy for NSCLC (*Paper I*)
- To investigate survival outcomes with regard to choice of induction chemotherapy (*Paper I*)
- To investigate the value of haematological blood variables (e.g. haemoglobin (Hgb), platelets (Plt) and white blood cells (WBCs)) with regard to prognosis in patients treated with curatively intended radiotherapy (*Paper II*)
- To study the potentially additive negative effect on survival of pathological blood variables (Hgb, Plt, WBCs) in patients treated with curatively intended radiotherapy (*Paper II*)
- To study adherence to guidelines regarding EGFR testing in a regional cohort (*Paper III*)
- To study the characteristics of EGFR tested patients in a regional cohort (*Paper III*)
- To investigate in what way a positive EGFR mutation test changed the course of treatment for advanced stage NSCLC patients in the region (*Paper III*)
- To study how comorbidities alter the risk for lung cancer development in COPD patients (*Paper IV*)
- To study how medication alters the risk of lung cancer development in COPD patients (*Paper IV*)
Methods

Papers I & II
Radiation charts at each individual oncology department in Sweden were examined and all detectable NSCLC patients subjected to curatively intended radiotherapy were included in this study cohort. The patients included received ≥ 50 Gy during the time period between 1990 and 2000. The study was reviewed and approved by the research ethics committee.

The patients that were included in the study all had a date of histopathological diagnosis as well as date of death or last follow-up. Data were collected for age, gender, smoking history, histopathology, stage, treatment, blood samples at diagnosis (e.g. Hgb, Plt and WBCs) and cause of death. A total of 1,146 patients were included in the overall cohort eligible for further analysis.

Paper III
The study was performed as a retrospective registry study with an individual medical chart follow-up on patients with an EGFR mutation. The registry of NSCLC patients that were analysed for EGFR mutation between 2010 and 2012 was collected at the Department of Molecular Pathology, Uppsala University Hospital.

Corresponding information about all patients diagnosed with NSCLC at the same institutions was collected from the Swedish National Lung Cancer Registry. Data regarding patient characteristics (i.e. gender, smoking habits, tumour stage, histology and age) were also collected from the Swedish National Lung Cancer Registry.

To study how a positive EGFR mutation analysis changed the course of action with regard to individualized treatment, we performed a follow-up of the medical charts. Data about time from referral of biopsy for EGFR mutation analysis to date of delivered result were collected, together with given treatment in 1st, 2nd and 3rd line, tumour stage and time from diagnosis to death or last follow-up.

Paper IV
A large COPD cohort was defined by collecting data from patients diagnosed with COPD in primary care centres from the beginning of 1999 to the
end of 2009. Patient-specific data from primary care medical records and mandatory Swedish national registers were linked, thus forming a retrospective database from which 19,921 patients were included in the present study. The primary care centres were chosen to reflect Swedish demographics, but no stratification was performed based on centre-specific conditions. Information regarding socioeconomics, date of death, prescribed drugs, and in- and out-patient health care utilization was collected and merged using established software.

The COPD diagnosis was based on the physician-acquired diagnosis classified according to the Classification of Diseases, 10th revision, Clinical Modification (ICD-10-CM) as J44. Prescribed drugs were analysed in regard to pack size and daily dose in relation to Anatomic Therapeutic Chemical classification system (ATC) groups. Drug use was calculated both as any use (dose independent) and as yearly use from two years prior to COPD diagnosis until end of prescription, lung cancer diagnosis or death. Data on comorbidities (including lung cancer diagnosis) were collected from medical records or national registers as diagnoses classified according to ICD-10-CM. Patients were followed until 31st December 2009, emigration or death.

Statistics Papers I-IV

The patients’ characteristics at diagnosis (in Papers I-III the lung cancer diagnosis and in Paper IV the COPD diagnosis) are presented using standard descriptive statistics. Kaplan-Meier product-limit estimates were used for overall survival analysis. Survival curves for analysed parameters were compared using the log-rank test. The time from date of diagnosis to death or last follow-up until the end of 2008 (Papers I and II), 2012 (Paper III) and 2009 (Paper IV) was used to define follow-up time. Age was defined as age at diagnosis (in Papers I-III the lung cancer diagnosis and in Paper IV the COPD diagnosis). Overall survival was also analysed using Cox proportional hazards regression models. Univariate and multivariate analyses were performed. In Papers I and II the multivariate models were adjusted by gender, age at diagnosis, Hgb, WBCs, Plt, stage, surgery and first-line chemotherapy.

In Paper IV baseline variables collected were: age at COPD diagnosis, gender, asthma, smoking, Charlson comorbidity index at COPD diagnosis, education level, marital status, income, sick leave days prior to index, and pneumonias. Time-dependent covariates collected were: medication (inhaled corticosteroids (ICS), acetylcysteine (NAC), oral steroids, statins, tiotropium, long acting beta-2 agonist (LABA), fixed combination ICS- LABA, bisphosphonates, angiotensin converting enzyme inhibitors, beta-blockers, angiotensin receptor blockers, selective serotonin reuptake inhibitors (SSRI), and diuretics), and comorbidities (pneumonia, COPD exacerbation, hyper-
tension, heart failure, diabetes, myocardial infarction, osteoporosis, stroke, depression and ischaemic heart disease). The multivariate analysis in Paper IV included only risk factors with a p-value below 0.2 in univariate analysis. In all papers, results were presented as hazard ratios with 95% confidence intervals (95% CI). In addition, p-values were given where p <0.05 was regarded as statistically significant.
Results

Paper I
A total of 1,146 patients with non-small cell carcinoma were eligible for analysis. Of these, 566 patients were included in this study. The median overall survival of all patients was 12.0 months, while the five-year overall survival rate was 4.9%. Patients treated with induction chemotherapy (n=79) had a significantly better overall survival compared with patients treated with radiotherapy alone (p=0.097) in a univariate Cox regression analysis (Fig. IV). However, this survival advantage was dependent on which chemotherapy regimen was used. A platinum/taxane combination produced the greatest survival benefit: HR=0.49 (95% CI 0.31 to 0.75). This was statistically significant in both univariate and multivariate Cox analyses (p=0.0013 and p=0.0020, respectively).

Figure IV: Overall survival for patients treated with different chemotherapy regimens as compared with radiotherapy alone
Paper II

For patients with Hgb <110g/L and Hgb ≥110g/L, median survival was 11.2 and 14.5 months, respectively (p=0.0032). For WBC >9.0x10^9/L and <9.0x10^9/L the median survival was 11.6 and 15.4 months, respectively (p<0.0001). For Plt >350x10^9/L and <350x10^9/L the median survival was 11.2 and 14.9 months, respectively (p<0.0001). The median survival in patients with pathological results in all three markers was half of that in patients with normal levels of all three markers (8.0 and 16.0 months, respectively (p<0.0001) (Fig. V).

![Figure V](Image)

**Figure V**: Median survival for patients with single pathological biomarkers or combinations thereof

Paper III

The EGFR mutation tested cohort consisted of 83% adenocarcinomas, 5% squamous cell carcinomas, 11% NSCLC-NOS and 1% where histology was not reported. The EGFR mutation frequency was 10% (n=66) with an expected frequency distribution within exons 18-21. Mutations were enriched in women, those who had never smoked and adenocarcinoma. The analysed tissue was collected using bronchoscopic px (29%), core needle biopsy
(46%), cytology (1%), and surgical specimens (22%), with a mean tumour cell fraction of 30% (range 5% – 85%). With regard to the subgroup identified for TKI treatment, i.e. patients with stage IIIb/IV non-squamous histology, 36% were referred for EGFR mutation testing, with an increasing trend between 2010 and 2012 (27.9% – 40.1%). Of the patients with EGFR m+ and advanced disease, 38% received EGFR-TKI in first-line, 46% in later lines and 16% had not received EGFR-TKI at the time of follow-up.

Paper IV

Our study included 19,921 patients. Of these, 646 or 3.2% developed lung cancer during the follow-up period. No significant differences in gender, income, sick leave days or country of origin were detected in the patients that developed lung cancer. Patients diagnosed with lung cancer had a lower level of education and more were living with a partner than patients without lung cancer.

COPD patients diagnosed with lung cancer had fewer concurrent diagnoses of asthma, hypertension, heart failure and depression than COPD patients not stricken by lung cancer. Lung cancer incidence had no significant correlation with COPD exacerbations, pneumonias, diabetes, myocardial infarction, stroke or ischaemic heart disease.

COPD patients diagnosed with lung cancer had a significantly lower consumption of LABA, fixed combination ICS+LABA, and statins at the time of diagnosis. No difference was seen in regard to FEV1 at diagnosis.

High age and lower education were the non-medical factors linked to lung cancer in univariate analysis. A reduced risk of lung cancer was seen in patients with the comorbidities of asthma or depression. When analysed in a dose-dependent manner, tiotropium and ASA had a significant relationship with increased risk of lung cancer whereas the opposite was true for medication with SSRI.

In the multivariate analyses, a simultaneous asthma diagnosis significantly decreased the risk of being diagnosed with lung cancer. ICS, beta-blockers and SSRI had a dose-dependent relationship with a decreased risk of lung cancer (see Table II).
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<tbody>
<tr>
<td>Age per 10 years</td>
<td>1.20</td>
<td>(1.11-1.29)</td>
<td>&lt;0.001</td>
</tr>
<tr>
<td>Asthma</td>
<td>0.54</td>
<td>(0.41-0.70)</td>
<td>&lt;0.001</td>
</tr>
<tr>
<td>ICS per 1,000 days of use</td>
<td>0.75</td>
<td>(0.65-0.86)</td>
<td>&lt;0.001</td>
</tr>
<tr>
<td>Beta-blockers per 1,000 days of use</td>
<td>0.82</td>
<td>(0.69-0.98)</td>
<td>0.030</td>
</tr>
<tr>
<td>SSRI per 1,000 days of use</td>
<td>0.62</td>
<td>(0.46-0.83)</td>
<td>0.001</td>
</tr>
<tr>
<td>Tiotropium any use</td>
<td>1.52</td>
<td>(1.27-1.81)</td>
<td>&lt;0.001</td>
</tr>
<tr>
<td>Acetylcysteine any use</td>
<td>1.24</td>
<td>(1.04-1.49)</td>
<td>0.017</td>
</tr>
<tr>
<td>Oral steroids any use</td>
<td>1.91</td>
<td>(1.61-2.27)</td>
<td>&lt;0.001</td>
</tr>
<tr>
<td>ASA any use</td>
<td>1.33</td>
<td>(1.10-1.60)</td>
<td>0.003</td>
</tr>
<tr>
<td>Statins any use</td>
<td>0.78</td>
<td>(0.68-0.97)</td>
<td>0.029</td>
</tr>
</tbody>
</table>

**Table II:** Stepwise multivariate Cox regression of factors with a p-value below 0.2 in univariate analysis
Discussion

In Paper I, we found that non-operated NSCLC patients who did not receive concomitant chemotherapy, but were treated with induction chemotherapy in addition to radiotherapy, have a better overall survival than the corresponding patient category treated with radiotherapy alone. We also found that this difference is most pronounced for patients treated with a platinum/taxane combination, which proved to be statistically significant in a univariate as well as in a multivariate Cox analysis. Although the value of induction chemotherapy is well established [6] some qualities make the present study unique. Its population-based character combined with a long follow-up period makes data robust. Furthermore, to the best of our knowledge, it is one of the largest studies in which individual data have been collected retrospectively in patients receiving curatively intended radiation treatment due to non-small cell lung cancer.

The current treatment regimen proposed to patients with sufficient performance status, presenting with inoperable disease in stage IIIa/IIIb, is concurrent chemoradiation therapy. Five-year survival is improved from 10% to 15% by administering the treatments concurrently instead of sequentially [246]. However, the gain in survival comes with increased toxicity and it has been suggested that only approximately 40% of patients in stage IIIa/IIIb are suitable for a concurrent approach [247]. For the remaining patients, our data is in line with previous reports that support sequential chemoradiation therapy where a platinum/taxane combination is preferable to single therapy [246].

In Paper II we show that haematological blood variables are strongly linked with outcome in NSCLC patients receiving curatively intended radiotherapy. Although reports have been published previously with results supporting our findings [248], the size of our cohort is larger and includes a wider variety of patients. Our findings are of high clinical relevance especially in cases where more validated prognostic factors (e.g. TNM and performance status) give inconclusive or contradictory information. Unfortunately, we did not have details about patients’ performance status in the study but it is reasonable to assume that worsened performance status and multiple pathological haematological blood samples are closely linked. Further studies would be of interest to find markers that objectify performance status, which is currently known for its high impact on prognosis but also for a built-in uncertainty due to assessment limitations.
In Paper III, we defined a novel cohort of NSCLC patients that were referred to the Department of Molecular Pathology in Uppsala for EGFR mutation testing from 2010 to 2012. While the test method in 2010 was still relatively new, the guidelines concerning which patients to test and when to perform the test, had still not been widely adopted. However, both an international consensus as well as Swedish recommendations for the EGFR testing algorithm were available at the time.

At the start of the study the investigators’ perception was that, at least during 2012, a high percentage of the eligible patients would have been tested due to reflex testing (e.g. all referred samples are tested) algorithms. The result was surprising: not even 50% of the patients that fulfilled guideline criteria for EGFR mutation testing were referred to the Department of Molecular Pathology. Furthermore, many of the patients that were EGFR m+ received chemotherapy in spite of the recommended TKI treatment in first-line.

The frequency of EGFR m+ in our cohort was 9.9%. This is in line with previous reports from Europe and the USA where the frequency has ranged from 9% -16% [244, 249, 250]. In our cohort, a clear selection bias exists, with almost 85% of the tested tumours being adenocarcinomas. It is likely that the mutation frequency of 16% in the Rosell study is, at least in part, due to an even higher degree of selection bias (evident by almost 30% of those who had never smoked and 60% of the patients being under 69 years of age) [244]. If all non-squamous cell carcinoma NSCLC were tested, it is likely that the frequency of EGFR m+ patients would be lower because of the lower frequency of mutations in NSCLC-NOS patients.

Paper IV emphasises the value of risk prediction and modulation. We studied factors correlating with increased and decreased lung cancer risk in a population-based COPD cohort. COPD patients have an up to eight times greater risk of lung cancer development compared to a non-COPD cohort matched for age and sex [51]. Also, COPD development in itself is linked to an increased risk of lung cancer development, and moderate or severe COPD patients have a 2.6 times greater risk of developing lung cancer compared to mild or non-COPD patients matched for smoking habits [52]. While smoking is still a major influence on COPD and lung cancer, the need for better understanding of risk factors is vital. Not only could the number of lung cancer cases be reduced by improved guidelines, but algorithms for screening programmes could also be more efficient by using more advanced stratification of risks to improve accuracy in the screening protocol.

COPD patients with a simultaneous asthma diagnosis had a significantly decreased risk of developing lung cancer in our study. This correlation was present even after adjusting for ICS treatment, which in turn also correlated significantly with decreased risk of lung cancer. Our results indicate that COPD patients that exhibit variability in airway obstruction may be a specific subset of COPD patients. This finding is further supported by recent re-
ports of what has been termed asthma COPD overlap syndrome (ACOS) [251]. In a Japanese study by Harada et al., ACOS patients had no higher risk of developing lung cancer than a group of asthma patients with significantly lower exposure to cigarettes [252]. It could thus be hypothesized that the ACOS subgroup does not need the same radiological surveillance as is recommended for COPD patients.

Medication with SSRI, beta-blockers and ICS were all linked with decreased risk of lung cancer development in a dose-dependent manner. Our findings could be an argument for early addition of ICS treatment to reduce the risk of lung cancer in COPD patients with a GOLD stage lower than three. Prospective studies in this matter would be of interest to further investigate the protective potential of ICS in larger cohorts.

Lung cancer mortality must be fought in multiple ways. We need better understanding of the optimal care of the patients diagnosed at an advanced stage to improve survival. We must learn how to diagnose lung cancer in the early stage and better utilize the predictive and prognostic markers present during the course of the disease. Most of all we need to prevent the disease. We have one major advantage: we know the cause of the vast majority of lung cancer cases.

The conclusion should be simple to draw. Smoking must be banned globally! With today’s often acute needs for food, medication, water, sanitation, social reforms and education in developing countries, we can no longer afford the cost of what we have long since known is a cause of tremendous human suffering and expenditure of financial recourses. “The Tobacco End-game”, an initiative established 2013 by Warner et al, is addressing the important task of a global agenda on eliminate tobacco smoking [253]. By advocating a plan that targets several different factors the goal is not only tobacco control but eventually a world free from tobacco smoking.

However, even if smoking were banned, we would have to tackle the consequences for long time. It is therefore reasonable to confront lung cancer from multiple directions. We need to find the specific drivers of the tumour and develop targeted agents to administer. The field of molecular pathology has grown from simply a research interest into a field just as important as traditional pathology. Development is fast and as I write this in September 2015, the Department of Molecular Pathology at Uppsala University Hospital is starting routine analyses using next generation sequencing on NSCLC cases. Using the Illumina MiSeq platform, eight individual samples are analysed in parallel overnight, rendering results of approximately 20 potential driver genes of interest (see the eight-sample DNA cartridge in Figure VI). And we are still just at the very beginning of the molecular era.

We need to understand how to better utilize host immune reactions in response to malignant disease. It is likely that future cancer treatment is based at least in part on immune oncology. Today we are “releasing the brakes” and “pushing down the accelerator” on the immune response by PD-1 and
CTLA-4 inhibition. However, the response is still uncertain and can lead to severe immunological side effects. By improving the specificity for tumour cells, immune therapy can be the perfect choice for adjuvant therapy. Not only would it be a new form of targeted therapy, it would possibly also have the capacity to prime the immune system to create immunity to the resected tumour cells, thus limiting the need for extensive adjuvant drug therapy. Hence immunotherapy will likely be a standard of care both in advanced disease and adjuvant settings [254, 255].

Not all patients will benefit from the latest and most aggressive treatment and the need to individualize clinical work-up and treatment will increase even more, not only to limit toxicity and potentially severe side effects for the patients but also to utilize resources in an optimal way [256]. Today it becomes increasingly obvious that the greatest challenge of cancer care in the 21st century is how to afford it. From this perspective, investment in preventive measures and early detection becomes even more cost-effective. To afford the latest treatments for late stage NSCLC patients we need to decrease the number of late stage patients. This equation probably works to the

**Figure VI.** Illumina MiSeq 8-sample cartridge used for next generation sequencing of tumour material from 8 patients simultaneously.
advantage of both patients and society, but we need to act soon to avoid a detrimental “Catch 22” situation.

A suggested approach for multi-targeted reduction of lung cancer is presented in Figure VIIa. We must focus on all areas in order to win the battle. Too much focus in one field is not cost-efficient and resources will not be used effectively [257]. In terms of predictive measures, new therapy is often targeted towards specific driver mutations or immune checkpoints. A predictive test is often required in order to gain the full effect of the medication. Typically, however, both the diagnostics and the treatment are expensive and one will not be offered or implemented without the presence of the other. In cases with rare mutations, diagnostic screening and subsequent treatment might even prove cost-ineffective [258].

![Multiple ways to reduce lung cancer mortality](figure VIIa)

By applying primary preventive measures (e.g. ban tobacco smoking), many future lung cancer cases can be prevented. By adding secondary preventive measures, fewer patients will be diagnosed with late stage NSCLC [63]. Although the screening algorithm used in the NLST trial proved effective, it could still be improved. By applying more individualized risk assessment it is possible that sensitivity as well as specificity could be improved.
By combining prognostic factors for patients referred for diagnostic work-up and subsequent treatment, an individualized approach to the aggressiveness of treatment and when to introduce a palliative discussion can be more accurately applied. There is evidence of improved quality of life, less aggressive end-of-life care and even improved survival for patients receiving early palliative consultation [259]. Lung cancer caregivers should use a holistic approach to identify those patients who would benefit from aggressive work-up and treatment and those for whom this approach would be unfavourable.

Wisely used knowledge about prevention, prognosis, prediction and treatment can greatly improve the outcome for patients diagnosed with lung cancer and limit the financial burden on health care systems. In this thesis, conclusions are drawn that could be applied in all fields of the proposed system to decrease lung cancer mortality. Figure IIIb summarizes a selection of these conclusions.

**Figure VIIb.** Selected conclusions of the thesis applied to specific areas of importance to decrease lung cancer mortality.
It should be noted that these conclusions address only limited parts of the corresponding fields of interest. However, they complement current knowledge and contribute to a better understanding of the multiple ways we should fight lung cancer mortality.
Conclusions

• Non-operated NSCLC patients who did not receive concomitant chemotherapy, but who were treated with induction chemotherapy in addition to radiotherapy, have better overall survival than the corresponding patient category treated with radiotherapy alone. (*Paper I*)

• The survival difference is most pronounced for patients treated with a platinum/taxane combination as induction chemotherapy. (*Paper I*)

• The addition of induction chemotherapy, as compared with radiotherapy alone, gave the most survival benefit in ex-smokers and patients with an advanced clinical stage. (*Paper I*)

• Anaemia, elevated leucocyte count and thrombocytosis are separate negative prognostic markers for survival in all stages of NSCLC. (*Paper II*)

• When more than one of Hgb, Plt and WBC is pathological, the negative prognostic value is noticeably increased. (*Paper II*)

• In the NSCLC cohort studied, 9.9% of the tested population had EGFR mutations. (*Paper III*)

• In the EGFR m⁺ population, only 38% received EGFR-TKI as first-line treatment and 16% had not received TKI treatment at time of follow-up. (*Paper III*)

• Despite guidelines recommending up-front EGFR mutation testing of all non-squamous cell carcinoma NSCLC cases, only 38.4% of the patients in the Swedish National Lung Cancer Registry had an EGFR mutation analysis performed between 2010 and 2012. (*Paper III*)

• A concurrent asthma diagnosis correlated to a significantly decreased risk for lung cancer development in COPD patients. (*Paper IV*)
• Medication with ICS, SSRI and beta-blockers correlated significantly to a decreased risk of lung cancer development in COPD patients. * (Paper IV)
Clinical Implications and Future Directions

The studies included in this thesis answer different but important questions often encountered in clinical practice. The patient with locally advanced disease who presents with a performance status of >1 or who has other factors present that inhibit the use of concurrent chemoradiation therapy, is likely to benefit from a sequential treatment with radiotherapy after induction therapy with platinum-based chemotherapy (\textit{Paper I}). A patient who has no initial radiological suspicion of advanced disease but presents with anaemia, leucocytosis and thrombocytosis should probably be assessed more thoroughly before any decision is made about aggressive surgery (\textit{Paper II}). Can we trust the logistics of the diagnostic work-ups that we believe are undertaken? Our studies show that a substantial number of the patients that we thought were routinely tested for EGFR status never had a test done. With a multitude of new diagnostic tests on the way to becoming tomorrow’s clinical routine, we need to assure the quality of our logistics and not just take them for granted (\textit{Paper III}). At present lung cancer screening is not routine in Swedish health care. Current guidelines state that radiological examinations should be done readily on high-risk patients. We show that COPD patients with a concurrent asthma diagnosis have a lower risk of developing lung cancer than other COPD patients. It is possible that a future screening algorithm should take the ACOS subtype of COPD into account (\textit{Paper IV}).

During the years that have passed since I started to study the aims of this thesis, much has happened within the field of lung cancer. Targets for potential therapy are increasing every year, and the techniques used to diagnose patients are developing equally rapidly. An upcoming study will present current molecular pathology routines in the Uppsala health care region. Have we learned from the information presented in this thesis and improved our logistics?

Together with colleagues at the departments of thoracic surgery, oncology and pathology in Uppsala and Umeå, we have succeeded in including lung cancer in the U-CAN collaboration and thereby have laid the ground for a large clinical cohort with extensive epidemiological data and bio-banked tissue. This database will be a source for upcoming work on predictive markers for relapse and response to treatment. As a population-based database, the lung cancer cohort in U-CAN can also be of great value in linking
deeper genetic sequencing of tumour tissue to epidemiological features that can be used for prevention or treatment.

Together with devoted colleagues in the Swedish Lung Cancer Study Group, we are pushing for greater coordination of the Swedish lung cancer community. In the molecular era, when only a few percent of patients have a specific genetic alteration, a national effort is needed to find these patients for inclusion in clinical trials that are open for national inclusion. A plan for this work is about to be launched and, once it is operational, Swedish lung cancer care will be able to compete internationally again.

In collaboration with my distinguished colleagues at the Department of Molecular Pathology (Dr. J. Botling and Dr. P. Micke), we have had the opportunity to contribute to a pivotal study, led by Dr. Roman Thomas, in which a comprehensive genomic profile of small cell lung cancer could be presented and published by George et al. [260] To further pursue the findings in continued collaboration with colleagues in Uppsala and Cologne, Germany, would be of great interest.
Acknowledgements

There are so many patients and their families that I have met and still carry with me in my memories. They have let me into their lives at a time of little hope and great desolation. Their stories, their courage and their confidence have always been the greatest reward and the strongest reason to continue the search for a future cure of this dreadful malady, the “Emperor of all Maladies”. To all the patients and their families I have met in my practice, I want to express my deepest and most sincere gratitude and respect. This thesis, as well as my upcoming scientific work, is a symbol of my pronounced appreciation of what you have given me.

I wish to express my sincere gratitude and appreciation to everyone who has helped me to complete this thesis. So many people have made invaluable contributions as co-authors, supervisors, colleagues, friends and family in a variety of forms and I feel tremendous appreciation for you all! However some of you deserve special acknowledgement, including (but not restricted to):

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Lastly, I want to express my love and gratefulness to my family: my wife Emma, your support means everything. Hella, Pelle and Manne, you have given life a new meaning. My parents, Gerd and Anthe, as well as my parents-in-law, Helena and Kjel, thank you for invaluable support. Tom and Emma, Lotta and Henrik, Kaj, and Jonna and Patrik thank you all for babysitting and good company. I owe you all greatly.
Lungcancer är den tumörsjukdom som leder till flest dödsfall globalt av alla cancerformer. Av samtliga lungcancerfall står icke småcellig lungcancer (NSCLC) för 80-85% av fallen. Den i särklass viktigaste riskfaktorn för att utveckla lungcancer är rökning och ca 90% av lungcancerpatienterna röker eller har rökt. Då en stor andel av patienterna diagnosticeras med avancerad, inoperabel, sjukdom är prognosen ofta dålig och en majoritet av patienterna är döda inom ett år från diagnos.

Patienter med lokalt avancerad sjukdom som inte är operabla erhåller idag kombinerad kemoterapi och radioterapi, s.k. konkomitant radiokemoterapi, som standardbehandling. Dock är konkomitant radiokemoterapi ofta mycket krävande för patienten och endast de med gott funktionsstatus kan komma ifråga för denna typ av behandling. I Delarbete I har vi utgått från en Svensk databas där patienter som erhållit kurativt syftande strålbehandling (>50Gy) mot lungcancer har samlats in. De patienter som erhållit konkomitant radiokemoterapi har ej inkluderats i analysen. En signifikant förlängd överlevnad ses hos de patienter som behandlats med kemoterapi i nära anslutning till att de har fått radioterapi (induktionsbehandling) jämfört med de som bara erhållit radioterapi (15,6 månader respektive 11,6 månader). Denna kunskap är viktig att ta hänsyn till när behandling planeras för patienter som inte är lämpliga för konkomitant radiokemoterapi.

I Delarbete II analyseras samma material som i Delarbete I med frågeställningen om hur resultaten av blodprover för hemoglobin, leukocyter och trombocyter påverkar överlevnaden för patienterna. Det är känt att blodbildningen ofta är påverkad vid cancer och anemi, leukocyteros och trombocyteros har samtliga blivit studerade tidigare i relation till överlevnad. I vår studie visar vi en tydlig koppling mellan patologiska blodprover för hemoglobin, leukocyter och trombocyter och försämrad överlevnad. När samtliga tre blodprover var patologiska vid diagnos var medelöverlevnaden endast hälften av medelöverlevnaden hos patienterna med normala blodprover (8,0 månader jämfört med 16,0 månader). Resultatet är kliniskt relevant och ger ledning i situationer där radiologisk stadieindelning är svår. Beroende på övriga omständigheter kan patologiska fynd i de studerade blodproverna stötta klinikern att förespråka mer extensiv behandling eller en mer palliativt inriktad strategi.
De sista tio årens utveckling mot målriktad behandling, ”targeted therapy”, har lett till påtagligt förbättrat överlevnad för de patienter som har specifik patologisk aktivering av onkgener. En sådan gen är EGFR-genen som har visat sig vara muterad i ca 10-15% av NSCLC. I de fall där en mutation i EGFR-genen konstateras är responsen på de specifika tyrosinkinashämmarna som finns tillgängliga mycket god och överlevnaden för patientgruppen är påtagligt förlängd. Analys för EGFR-mutation var det första molekylärapatologiska test som blev rutin för NSCLC. Idag står vi inför en snabb expansion av antalet analyserade gener och målriktade preparat mot mutationer i flera av dessa gener är redan godkända inom andra tumörformer och kan antas erhålla godkännande även för lungcancerbehandling. Då det prediktiva värde av molekylärapatologiska tester generellt är högt för respons på behandling är det viktigt att patienter erhåller analyser enligt rådande riktlinjer. I Delarbete III presenteras resultatet från en uppföljning av implementeringen av EGFR-mutationsanalys i Uppsala (samt sjukhusen i regionen; Gävle, Falun, Västerås och Eskilstuna). Genom att inkludera alla patienter som genomgått EGFR-mutationsanalys under 2010-2012 och jämföra dessa mot Svenska lungcancerregistrets registrerade fall i samma upptagningsområde kan vi presentera data för täckningsgrad, mutationsfrekvens och selektion av patienterna. Våra resultat tyder på att EGFR-mutationsanalyserna inte tillhandahållits i enlighet med de gällande riktlinjerna. Framförallt ses en positiv selektion av adenocarcinom. I det studerade materialet är mutationsfrekvensen 10,3%. Efter genomgång av de EGFR-muterade patienternas journaler visar vi att endast 38% erhöll EGFR-tyrosinkinashämmare i första linjen men att en överväldigande majoritet (84%) erhåll EGFR-tyrosinkinashämmare i någon linje av behandlingen. Resultaten pekar på vikten av att följa upp hur riktlinjer efterlevs när nya diagnostiska metoder införs i klinisk vardag.

References


A doctoral dissertation from the Faculty of Medicine, Uppsala University, is usually a summary of a number of papers. A few copies of the complete dissertation are kept at major Swedish research libraries, while the summary alone is distributed internationally through the series Digital Comprehensive Summaries of Uppsala Dissertations from the Faculty of Medicine. (Prior to January, 2005, the series was published under the title “Comprehensive Summaries of Uppsala Dissertations from the Faculty of Medicine”.)