Minimally invasive diagnostics and immunotherapy of lung cancer

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Chapter 1

General introduction,
aim and outline of the thesis

LUNG CANCER: EPIDEMIOLOGY AND CLASSIFICATION

Lung cancer has a major impact on the global burden of disease. In 2012, it caused the death of approximately 1,5 million persons, which makes lung cancer the most common cause of cancer-related mortality in males (24%) and the second leading cause in females (14%) worldwide[1,2]. In the Netherlands, 10.357 lung cancer related deaths occurred (6.179 men and 4.178 women) in 2014. In 2015, 12.217 new cases of lung cancer were been reported, 6.861 males (56%) and 5.356 females (44%). When analyzing trends in incidence and mortality of lung cancer in the Netherlands in the past 25 years (**Figure 1 and Figure 2**), an evident but moderate decline is observed in males with respect to the number of newly diagnosed lung cancer cases and number of deaths caused by lung cancer. However, the opposite is the case for women, in whom a sharp increase in incidence and mortality is observed. This relative increase of lung cancer prevalence and mortality is caused by the increase in tobacco smoking by Dutch women. Smoking is the single most predominant risk factor responsible for lung cancer. Although the number of smokers is gradually decreasing in the Netherlands, almost a quarter of the Dutch population of 12 years and older still smoked in 2015, of whom 74% on a daily basis[3].

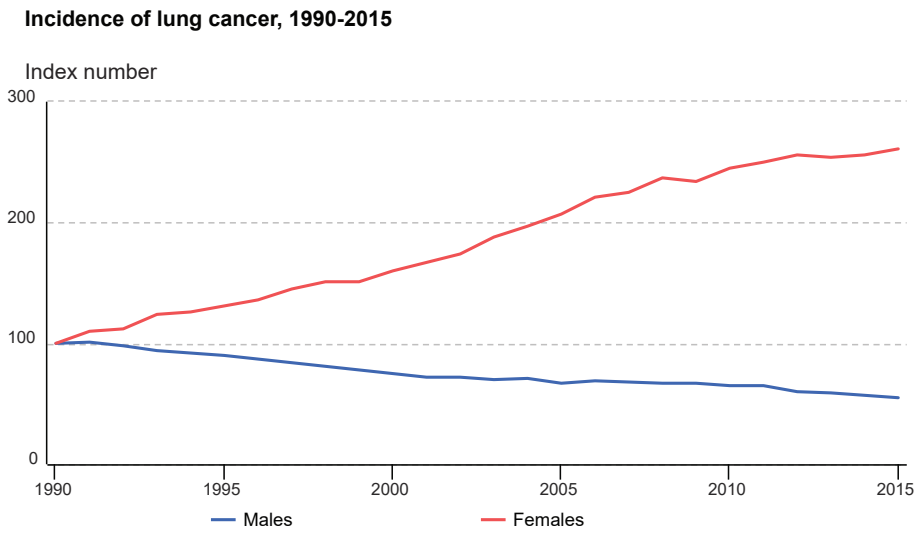

Figure 1. Incidence of lung cancer in the Netherlands from 1990 – 2015
(Source: volksgezondheidenzorg.info)

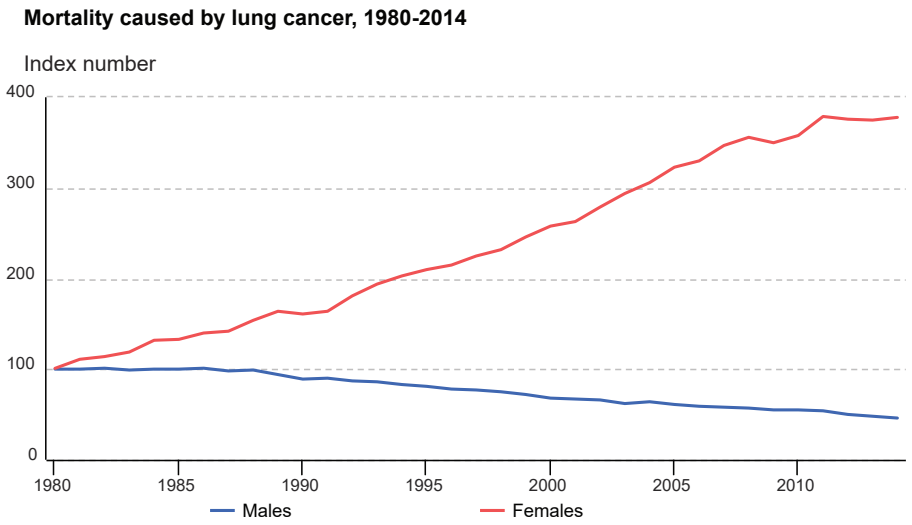

Figure 2. Mortality of lung cancer in the Netherlands from 1990 – 2015 (Source: volksgezondheidenzorg.info)

Lung cancers are classified according to histological type as small cell lung cancer (SCLC) and non-small cell lung cancer (NSCLC). The majority of cases are NSCLC, which comprises approximately 80% of all lung cancers[4]. NSCLC has three main subtypes: squamous cell carcinoma, large-cell carcinoma and adenocarcinoma, the latter accounting for the majority (40%) of NSCLC cases[5,6]. A routine but critical part of diagnosing lung cancer is to assess the stage of disease in order to select the appropriate treatment and to determine prognosis. Hence, newly diagnosed lung cancer patients undergo multiple diagnostic procedures to determine the extent of disease, such as contrast-enhanced chest computed tomography (CT), fluorodeoxyglucose-positron emission tomography (FDG-PET) scan and minimally invasive endosonography[7,8].

DIAGNOSING NSCLC: THE ROLE OF ENDOSONOGRAPHY

An essential part of preoperative staging of NSCLC is the assessment of the mediastinum for lymph node involvement. Endoscopic ultrasound (EUS) and endobronchial ultrasound (EBUS) are minimally invasive endosonography techniques that operate by placing an ultrasound probe in the esophagus (EUS) or airways (EBUS). They serve to visualize mediastinal lymph nodes and by means of fine needle aspiration (FNA) tissue sampling can be performed. Both EUS and EBUS have a high sensitivity for detecting mediastinal lymph node metastases (89% for both procedures). A combined EUS/EBUS approach was shown to reduce the number of unnecessary thoracotomies (18% vs 7%,

p=0.02), while displaying a comparable sensitivity compared to surgical staging (85% vs 79%, p=0.47), which was considered the gold standard of mediastinal tissue staging before the introduction of endosonography in pulmonary oncology[7,9]. In recent international guidelines, endosonography procedures (EUS and/or EBUS) are advocated as the initial tissue sampling procedure in NSCLC staging[8,10,11].

CURRENT TREATMENT OF NSCLC

With the use of non-invasive and (minimally) invasive staging modalities, lung cancer patients can be staged according to international guidelines on TNM Classification for Lung Cancer[12] which serve to select the appropriate therapy modality for NSCLC patients. Patients with stage I/II NSCLC are treated by surgical resection of the tumor with systematic lymph node dissection which is mandatory to verify the extent of disease and to plan adjuvant treatment[13]. Stereotactic body radiotherapy (SBRT) can be an alternative treatment for early stage NSCLC, especially in those patients who are not considered medically fit to undergo surgery[14]. In case of locally advanced disease (stage III), patients are generally treated with chemoradiotherapy, preferably in a concurrent fashion[15]. In case of advanced NSCLC (stage IV), patients are treated with palliative chemotherapy. As first-line treatment, a platinum-based compound combined with a third generation cytotoxic agent is recommended[16]. For advanced NSCLC patients with tumors that carry epidermal growth factor receptor mutations or anaplastic lymphoma kinase gene rearrangements, small-molecule kinase inhibitors have shown a clear beneficial clinical effect and a substantial improvement of prognosis[17]. When first line treatment with palliative chemotherapy or a small-molecule compound is successful (clinical response or stable disease), patients are eligible for maintenance therapy, either by switching to or continuation of a single compound of the combination chemotherapy (e.g. pemetrexed, a multifolate inhibitor) or a small-molecule inhibitor[15,18,19].

IMMUNOTHERAPY OF NSCLC

Despite the current improvements in NSCLC staging and the availability of the aforementioned therapies, survival rates for NSCLC remain exceptionally poor. In the Netherlands, an analysis from a 20 year period (1989 -2009) indicated that only a very modest improvement from 14.8% to 17.4% in five-year survival for all NSCLC patients has been achieved[20]. An important contributing factor to this dismal survival statistic is the fact that the majority of NSCLC patients have locally advanced or metastatic disease (stage III/IV) at first presentation. In general, these patients cannot be cured and are only

eligible for palliative treatment[15,16]. Hence, there is a clear medical need to explore novel therapies for NSCLC in order to improve current clinical outcomes.

One new anticancer therapy that has emerged in recent years is active immunotherapy which is directed at eliciting and reinforcing T-cell-mediated antitumor responses. Studies on the tumor microenvironment have shown that NSCLC, like many immunogenic cancers, is infiltrated by a plethora of immune cells (mainly T cells, macrophages and mast cells) with both tumor-promoting and suppressive effects[21]. A dense infiltrate of M1-macrophages[22,23], CD4+ T-cells and CD8+ T-cells[24,25] is associated with an improved survival rate, which indicates that the immune system is actively involved in keeping NSCLC at bay. This immunogenicity of NSCLC is an important prerequisite for active immunotherapy and is exploited by novel immunotherapeutic treatments aimed at interrupting the action of T cell regulatory molecules[26]. In particular, infusion of antibodies that target programmed death 1 (PD-1) receptor and its ligand PD-L1, has shown very promising clinical effects in NSCLC[27], which has led to the approval of the PD-1 blocking antibody nivolumab for treating metastasized non-small cell squamous carcinoma[28,29].

AIM AND OUTLINE OF THE THESIS

The first aim of this thesis is to investigate how the implementation of minimally invasive endoscopic ultrasound techniques (EUS and EBUS) in the staging algorithm of NSCLC can be optimized (**Part I**). The routine use of EUS-FNA in the preoperative staging of unselected patients with NSCLC is evaluated in **Chapter 2**. An assessment is made with respect to diagnostic test performance (sensitivity, predictive value and accuracy), the number of prevented surgical staging procedures and the value of additional mediastinoscopy if EUS-FNA fails to detect nodal metastases. An in-depth analysis of predictors for false negative EUS and/or EBUS findings in NSCLC patients is made in **Chapter 3**. These predictors may elucidate when it is justified to directly proceed to thoracotomy and omit additional surgical procedures when the mediastinum is staged negative after EUS and/or EBUS.

The second aim of this thesis focuses on the role and potential of immunotherapy in NSCLC patients (**Part II**). An overview of recent developments in the field of therapeutic peptide vaccination of cancer (with a focus on NSCLC) is presented in **Chapter 4**. The impact of immunity on the clinical outcome of NSCLC was studied by assessment of the numbers of intra-tumoral CD8+ T cells and the expression of classical and non-classical HLA molecules. The association of these parameters with overall survival is described in **Chapter 5**. Since it was shown in animal models that chemotherapy and radiotherapy have beneficial effects on the immune system in tumor bearing hosts, the systemic effects of these conventional NSCLC therapies were investigated in NSCLC patients with

respect to the composition and function of circulating immune cells in **Chapter 6.** This provides insight on whether these standard-of-care NSCLC therapies can also be combined with novel immunotherapeutic drugs in the human setting. An investigation into the expression and immunogenicity of XAGE-1b, a novel tumor antigen of the cancer testis antigen family that is preferentially expressed in NSCLC, is presented in **Chapter 7**. With this study on spontaneously induced XAGE-1b-specific humoral and cellular responses, vital background knowledge was obtained for starting a phase I clinical trial in which a XAGE-1b overlapping peptide vaccine is tested in advanced NSCLC patients. A study protocol and rationale of this study together with preliminary results are provided in **Chapter 8**. Finally, **Chapter 9** provides a general discussion and summary of this thesis.

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