Chapter 8

Discussion
Summary of data

Chapter 1 summarizes the epidemiological data on lung cancer and introduces methods that may help reduce lung cancer mortality. Lung cancer mortality remains high, mainly because when patients become symptomatic the disease has often progressed beyond the stage in which curative therapy is possible. Apart from the most obvious solutions: reducing the incidence of lung cancer by measures to dissuade people from smoking and improving therapy for non-operable lung cancers, there are two other potential means by which a reduction in lung cancer incidence and mortality can be achieved, namely chemoprevention and early detection by screening.

In chapters 2-4 of this thesis we examined the effect of 6 months use of fluticasone, an inhalational corticosteroid and potential chemopreventive agent, in smokers with bronchial premalignant lesions. Chapter 2 describes a double-blind randomised placebo-controlled trial of the effect of fluticasone on surrogate end-point markers of carcinogenesis, i.e. histology of premalignant bronchial lesions, as well as (previously validated) molecular markers: hTERT expression and p53 immunohistochemistry. Our data, as well as those of a study by Lam et al., do not reveal any effect of fluticasone on either histology or molecular markers of bronchial mucosa. The study by Lam et al., however, did find some effect of inhaled corticosteroids on the number of indeterminate pulmonary nodules detected by CT-scanning. The authors suggest this could indicate a true chemopreventive effect since the nodules may represent areas of atypical adenomatous hyperplasia (AAH), a putative precursor of peripheral adenocarcinoma. However, the precise nature of the indeterminate nodules detected in the study of Lam et al. is unknown (as their name implies) since histology was not available. The data presented in chapter 3 of this thesis, concerning the CT-detected lesions in our study population, do not support an effect of corticosteroids on indeterminate pulmonary nodules as did the findings of the Lam et al. study. Although in the treatment arm of the fluticasone study more subjects had a decrease and fewer had an increase in number of nodules compared with the placebo arm, this trend did not reach statistical significance. This result should be interpreted with caution; the number of subjects with indeterminate nodules, although comparable with the Lam et al. study, was low, as the selection of study subjects was not based on the presence of CT detected abnormalities. In chapter 4 we examined the smoking cessation rate of study participants, which was comparable to that in other screening studies; around 20%. More male than female subjects quit smoking. Being diagnosed
with abnormalities did not seem to influence the subjects’ high-risk behaviour since there was no significant difference in cessation rate between subjects with and without premalignant bronchial lesions.

The final three chapters examine techniques that may enable early detection of (pre)malignancy.

**Chapter 5** evaluates a recently added function of the Onco-LIFE autofluorescence bronchoscopy system. Autofluorescence bronchoscopy is a useful aid for finding pre-invasive bronchial lesions, which can then be biopsied for pathological assessment\(^5\). A concern is its low specificity, which can lead to an increase in the number of biopsies and a concomitant increase in complications and procedure time. The Onco-LIFE instrument aids the clinician by providing a numerical value for the \(R/G\) ratio; the ratio of red light (reflected) to green light (fluorescence) of a small area in the middle of the screen. We examined what \(R/G\) ratio should be used as cut-off by correlating the \(R/G\) score of suspicious locations with the histology of the subsequently taken biopsies of this area. Using data of 3292 biopsies as training data we obtained an optimal cut-off of 0.54 for lesions with moderate to high-grade dysplasia. This cut-off was then used for a prospective validation study on 25 male patients. A combination of visual scores (using white light bronchoscopy) and \(R/G\) ratio yielded a sensitivity of 80% and a specificity of 88% for high-grade and moderate dysplasia. Quantification of the signal by the \(R/G\) ratio can reduce inter-observer variability and aid clinicians who are learning to use autofluorescence bronchoscopy.

In **chapter 6** we focus on methylation of the promoter of CADM1, a tumour suppressor gene encoding a member of the immunoglobulin superfamily that was originally described in lung cancer\(^8\). Methylation-specific PCR comprising three regions of the CADM1 promoter was informative for high-grade premalignant cervical lesions\(^9\). However, when this test was used on a panel of lung tumours of different histology as well as tumour distant normal lung tissue, many normal samples tested positive as well. Moreover, even normal samples from patients who did not suffer from lung cancer at the time of surgery frequently tested positive for CDAM1 promoter methylation. This marker is therefore not sufficiently specific for early detection of lung cancer in an unselected population.

**Chapter 7** deals with a multicenter European study (EUELC) in which 12 centres throughout Europe prospectively recruited lung cancer patients before surgery. These patients were followed for 3 years during which at 6 months intervals materials such as blood, sputum and bronchial biopsies were collected\(^10\). So far, two studies have appeared from the data collected during this EUELC collaboration: one on inactivation of the FHIT gene and
its correlation with progressive disease, i.e. recurrence, metastasis or second primary tumour in follow-up\textsuperscript{11} and a case/control study of family history of lung cancer being a risk factor for lung cancer\textsuperscript{12}.

For the study described in \textbf{chapter 7} we used baseline tumour and tumour distant normal samples from the EUELC study to investigate the prognostic value of hTERT mRNA levels in both tumour and normal tissues. Results of earlier studies have not been conclusive; some found a correlation between increased tumour hTERT level or telomerase activity and decreased (disease free) survival, whereas others revealed no correlation and one study even found an increased hTERT level to be indicative of longer disease free survival. Our study, using the largest patient group to date, did not find a correlation between tumour hTERT levels and disease free survival. Surprisingly, there was a correlation between hTERT mRNA levels in tumour distant normal tissue and progressive disease. Similar results had been obtained from another sub-study within the EUELC project that revealed a correlation between FHIT methylation in normal lung tissue and progressive disease\textsuperscript{11}.
Future prospects

New techniques

Techniques that enable a more comprehensive examination of the tumour genome, epigenome and transcriptome are constantly being developed. Recently, in a tumour sequencing project 623 genes of 188 adenocarcinomas were investigated using traditional sequencing technology and several genes previously known to be involved in other cancers were implicated in lung carcinogenesis. Newly developed massively parallel sequencing (MPS) platforms will enable whole-genome sequencing on large numbers of tumours. A small-cell lung cancer cell-line has recently been one of the first to be analysed by MPS. More comprehensive analysis of premalignant bronchial lesions is also possible by such novel methods; SAGE (serial analysis of gene expression) has been performed on biopsies from bronchial carcinoma in-situ, which allowed comparison of gene expression levels with that of invasive SCC. Integration of data obtained from whole-genome sequencing and copy number alteration analysis, as well as methylation- and expression studies of tissue from tumours and premalignant lesions is likely to result in a better understanding of tumour biology, identification of new targets for chemoprevention and treatment, and novel biomarkers for screening and staging.

Chemoprevention

To definitively disprove the notion that use of corticosteroids impedes lung carcinogenesis in humans, a large randomised controlled trial, with lung cancer mortality as the endpoint, would have to be performed. However, given that so far the results of trials using surrogate end-points (the fluticasone studies that are part of this thesis and the study by Lam et al.) have not been encouraging, it is unlikely that such a trial will be performed. At this time there is still at least one ongoing chemoprevention trial of inhalational corticosteroids using CT detected pulmonary nodules as a surrogate end-point. Any future studies could benefit from inclusion of molecular markers that are part of the pathway affected by corticosteroids. Since this pathway is currently unknown, more research should be directed at finding out the exact mechanism by which corticosteroids reduce the size and number of tumours in A/J mice.

Other substances which show promise for lung cancer chemoprevention have been reviewed by Keith.
Myo-inositol is a substance that, like corticosteroids, is chemopreventive in A/J mice. In a non-controlled phase I trial, its use was associated with a greater reduction of bronchial dysplasia than in the control group of a similar study, possibly because of inhibition of PI3K. A placebo controlled multi-center study of myo-inositol is currently recruiting participants.

A search on the clinicaltrials.gov website using the search terms ‘lung cancer’ and ‘chemoprevention’ yields a number of agents currently being investigated in clinical phase II trials. These include celecoxib and sulindac (COX inhibitors), iloprost (a prostacyclin analog), Zileuton (a leukotriene modifier), selenium, PPARγ agonists, mTOR inhibitors and phytochemicals extracted from green tea and broccoli sprouts. Most of these studies have been set up the same way as the fluticasone study in this thesis. Smokers are used as test subjects and as surrogate end-point markers these studies use histology and molecular markers of bronchial biopsies obtained by autofluorescence bronchoscopy and CT detected indeterminate nodules. Other trials use parameters from lung function tests or biomarkers from sputum, exhaled breath condensate or blood as surrogate end-point markers. Preliminary results presented at the ASCO 2010 conference of one large trial, a tertiary chemoprevention study of selenium in patients with previously resected NSCLC, are disappointing.

Hecht et al. argue that a combination of chemopreventive substances is most likely to be successful. There is circumstantial evidence for the chemopreventive effect of the combination of prostacyclins and the EGFR tyrosine kinase inhibitor gefitinib since one study found that gefitinib had a chemopreventive effect in mice that had been genetically modified to overexpress prostacyclin synthase in lung tissue, but not in wild-type mice of the same strain.

Screening

At this time low-dose chest CT scanning is the most promising screening modality and trials are currently underway to find out whether it will have an impact on mortality. To limit the degree of overdiagnosis and increase sensitivity for centrally located early squamous cell carcinoma, screening performance can be improved by using other tests in conjunction with CT scans. These should utilise materials that can be easily obtained, such as sputum, exhaled breath or blood. Candidate tests are automated sputum cytometry (to detect malignancy-associated changes) and assays that detect molecular markers specific for the carcinogenesis process.
Identifying markers that predict the risk of carcinogen-exposed bronchial epithelium to develop into full-blown cancer is an exciting though challenging effort. Recent publications describe detection of chromosomal aneusomy and 3p LOH as methods for predicting development of pre-invasive bronchial lesions. When there is molecular evidence of carcinogenesis in the large airways these could be subjected to surveillance using autofluorescence bronchoscopy. New developments in bronchoscopic technology like optical coherence tomography and microendoscopy may in the near future enable bronchoscopic diagnosis of early cancer without biopsy, which also will contribute to knowledge of the natural course of preneoplastic lesions.

The CADM1 study in this thesis shows that when testing for a property that is causally related to cancer (methylation of a tumour suppressor gene promoter), normal tissue from a surgical specimen may test positive almost as frequently as tumour tissue. Therefore, when testing potential molecular markers for their suitability as early detection markers they should be tested against normal controls to ensure that a test has sufficient specificity. Both the hTERT and the CADM1 study use normal lung tissue from the same surgical specimen as the tumour as normal control. Some authors believe this is inappropriate, as this histologically normal tissue may be affected on a molecular level by field carcinogenesis. In the CADM1 study, we tested whether such a ‘field effect’ could be the cause of a positive test of control samples by also analysing normal lung tissue from non-lung cancer controls. These also frequently tested positive for methylated CADM1. These data indicate that lung cancer research will benefit from a tissue bank containing frozen lung tissue specimens obtained from lung cancer free patients with a known smoking history.

The chapter on hTERT expression (as well another EUELC project study) suggests that normal lung tissue removed during lung cancer surgery may yield clinically important information; molecular analysis of histologically tumour-free lung tissue from the resected lobe could aid in predicting disease recurrence. The mechanism behind a prognostic effect of tests using molecular markers of carcinogenesis in tumour adjacent normal lung tissue may lie in their ability to detect field cancerization, as was previously suggested for head and neck carcinoma. Experiments using lung cancer susceptible and -resistant mice show that gene expression profiles of normal lungs can predict their genetic predisposition to lung cancer.
Staging and predicting response to chemotherapy

Gene expression$^{34}$ or -methylation arrays can predict disease recurrence in surgically treated patients. From these predictive patterns found by using microarray technology, panels of molecular markers may be selected, which could guide the decision of whether or not a patient should receive (neo-) adjuvant chemotherapy. In non-operable patients the reaction to biologicals like tyrosine kinase inhibitors can already be predicted from presence of mutations in EGFR and KRAS genes$^{35}$. Circulating tumour cells can be isolated by ‘CTC chip’ technology and may be used for non-invasive serial sampling of tumour$^{36,37}$. In this way, development of resistance to chemotherapy can be assessed.
References


