THE ROLE OF MINIMALLY INVASIVE ENDOSCOPIC TECHNIQUES IN THE DIAGNOSIS, TREATMENT AND PREVENTION OF LUNG CANCER

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DECLARATION

I hereby declare that except where specific reference is made to the work of others, the contents of this dissertation are original and have not been submitted in whole or in part for consideration for any other degree or qualification in this, or any other University.

ABSTRACT

Squamous cell carcinoma of the lung arises from pre-invasive progenitors in the central airways. The archetypal model appears to be a stepwise morphological progression until there is invasion of the basement membrane. However, their natural history is not well understood and their treatment remains controversial, with radical therapies being offered to individuals who may never develop cancer.

Autofluorescence bronchoscopy gives us the ability to follow the natural history of these lesions, with the prospect that early detection may improve survival. In this thesis, the natural history of pre-invasive disease is described in a prospective longitudinal cohort study. The data identifies a 'high-risk' cohort of patients with severe dysplasia and carcinoma *in situ*, in whom close surveillance detects multiple interval lung cancers at an early stage. The data from this indicates the need of a minimally invasive bronchoscopic treatment for these patients.

A further prospective clinical trial evaluates the role of photodynamic therapy in individuals with early invasive carcinomas of the airway who were unfit for conventional lung cancer treatment. Photodynamic laser therapy (PDT) proved to be an effective therapy for patients with small and superficial lesions. However, PDT has not been tested in randomised controlled trials, so a randomised clinical trial (the PEARL trial) was designed to evaluate whether treating high-grade pre-invasive lesions will avert progression into invasive carcinoma.

Endoscopic laser resection of primary lung carcinoid tumours was also evaluated. This thesis demonstrates that laser can be used to effectively ablate carcinoid tumours. Treatment was particular effective in small intraluminal carcinoid tumours and may be an alternative to surgical resection. Finally, the role of sedation in interventional bronchoscopy was assessed in a prospective study for patients undergoing endobronchial ultrasound and transbronchial needle aspiration. This thesis demonstrates that endoscopist led sedation is comparable to anaesthetic led sedation, but identified the need for a randomised controlled trial.

IMPACT STATEMENT

The data in this thesis will have impact at a local and international level. Chapter 2 describes how maintaining close surveillance of patients at 'high-risk' of lung cancer leads to early detection when curative treatments can be offered. It is unclear how patients with pre-invasive disease are currently managed internationally, so a publication on the clinical outcomes will disseminate the message that close surveillance in these patients is essential and should be carried out in specialist institutions. Chapter 2 further identified the clinical need to treat patients with high-grade pre-invasive disease who are at most risk. This led to the prospective study in chapter 3 to evaluate photodynamic therapy, a bronchoscopic technique using laser to activate the photosensitising drug that causes cancer cell death. The data from this chapter will be disseminated to colleagues using PDT through publication, but the outcomes also formed the foundation of a funding application to Cancer Research UK. There was a clinical need to evaluate the role of endobronchial ablation of pre-invasive disease. As a result, a collaboration with 3 other lung cancer centres in the United Kingdom and Netherlands was formed to open a randomised clinical trial that will test whether PDT ablation of carcinoma *in situ* will prevent lung cancer. Chapter 4 looked at the role of a high-power laser in ablation of carcinoid tumours of the lung. The data from this thesis has improved local learning and changed how patients with carcinoid are managed at University College London Hospital. The data from this chapter will be disseminated by way of publication and will be used to design a randomised clinical trial to prove bronchoscopic ablation of carcinoid is non-inferior to surgery. Chapter 5 is a glimpse into the future of virtual 3D animation of central airway strictures. This small pilot was already presented as a poster in British Thoracic Oncology Group and will form part of a larger piece of work in assessing complex airway strictures. Finally, this thesis reported on the different types of sedation used for bronchoscopy procedures. This data will be published, but at a local level has changed practice at UCLH in which patients are selected for general anaesthetic.

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I would like to thank Professor Sam Janes for giving me the opportunity to undertake this research and for patiently offering advice and encouragement while acting as my supervisor. He is a true inspiration and my role model who guided me to a career in interventional bronchoscopy.

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Finally, I would like to acknowledge Dr Jeremy George, to whom I am indebted for his unwavering support and for teaching me the technical skills and art of interventional bronchoscopy. He identified the need to monitor high-risk patients closely and set-up the longitudinal surveillance and photodynamic therapy studies, from which we have gained a wealth of clinical and biological data. This work has been the foundation to the chapters on early lung cancer in this thesis, which has led to an improvement in our knowledge of lung cancer pathogenesis and demonstrated the importance of interventional bronchoscopy in the management of tumours of the lung.

DEDICATION

I am very fortunate to have had incredible support from my family and dedicate this to my late grandfather and my late aunt Hasmita for their love, guidance, sacrifice and support throughout. I also dedicate this thesis to my beautiful wife Namrata for her love, support, and encouragement; but for whom this thesis would have been written several years earlier!

CONTENTS

List of Figures

List of Tables

Appendix A

Chapter 1 INTRODUCTION

1.1 Squamous cell lung carcinoma

Lung cancer is the leading cause of cancer-related death. It accounts for nearly 1.4 million deaths worldwide every year, with a 6% five-year survival. In contrast to the steady increase in survival for most cancers, lung cancer outcome has barely changed in four decades [1,2]. Although surgical resection of early stage disease offers a prospect of cure [3], the vast majority of cases are diagnosed at a late stage with no hope of curative therapy. In contrast, prospects for patients with pre-invasive or intraepithelial neoplastic lesions (stage 0), or early stage invasive cancers (Stage 1A) of the central airway are far better, with a 5-year survival of more than 70% [3–6].

Squamous cell lung cancer (SQCC) is the second most common type of non-small cell lung cancer in the US and most common in the UK. It accounts for around a third of all lung cancers and commonly arises in the central airways [7,8]. While there is promise for improving survival rates by early detection of small peripheral cancers through computed tomography (CT) screening (reducing lung cancer deaths by 16% to 20% in smokers) [9], this may not always detect small central airway cancers or indeed pre-invasive airway disease.

1.1.1 Squamous cell lung cancer pathogenesis

It is widely accepted that pre-invasive lesions are precursors of squamous cell carcinomas typically arising in the central airway bronchial epithelium [10]. These lesions may occur over wide areas of the tracheobronchial tree and are particularly prevalent in individuals who have smoked heavily or developed synchronous invasive lung cancers [11]. These observations underpin the generally held opinion

that squamous cell carcinomas (SQCC) develop through a series of morphological stages of increasing abnormality from basal cell hyperplasia, to metaplasia, dysplasia, carcinoma in situ (CIS) and then to invasive disease. The World Health Organization (WHO) summarised the pathological grading of these progenitor airway lesions in the Histological Typing of Lung and Pleural Tumours by Travis et al, summarised in Figure 1.1 [12].

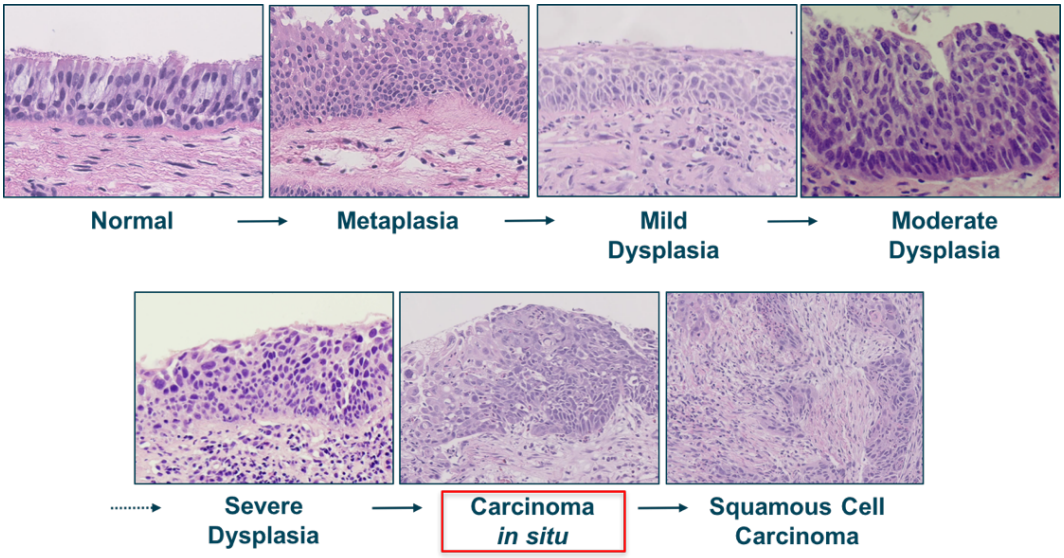


Figure 1.1 – Stepwise progression of pre-invasive lesions to squamous cell carcinoma

1.2 Characteristics of pre-invasive lung cancer

Squamous dysplasia may be mild, moderate, or severe, with severity being based on the progressive cytological aberration, loss of maturation and increasing involvement of the full thickness of the epithelium. The most important of these lesions is CIS, which sits on the extreme end of the spectrum where cytological aberration is extreme, mitoses occur at all levels, and there is no cell maturation. The usual form of CIS does not cause epithelial thickening, however a more unusual form exists where the lesion develops into an exophytic papillary growth that can cause mechanical airway obstruction, but remains free of mucosal invasion [13]. Although the WHO guidelines have been useful in distinguishing between the higher grades of dysplasia and CIS, there can be significant inter- and intra-observer variability in grading of specific pre-invasive lesions, even amongst experienced pulmonary pathologists [14,15]. This is as a result of considerable overlap between the categories and in any particular sample a range of grades may be seen. Furthermore, such lesions are not frequently encountered by pathologists, and may be incorrectly graded due to small biopsy size. Consequently, many investigators [16–18] have categorised lesions into “high-grade” and “low-grade”. This may minimise the risk of observer error in the histopathological reporting, and as described later seems to correlate to their risk of progression to invasive cancer.

Histological Grade	Pathology
Low-grade lesions	
Mild dysplasia	Mild cellular atypia limited to lower ⅓ of airway epithelium. Mild anisocytosis and pleomorphism Mitoses absent or very rare.
Moderate dysplasia	More severe cytological disarray of lower ⅔ of airway epithelium. Moderate anisocytosis and pleomorphism Mitotic figures confined to lower ⅓.
High-grade lesions	
Severe dysplasia	High degree of cellular atypia and minimal cell maturation Disarray extends entire depth of epithelium, but without reaching the surface. Mitotic figures confined to lower ⅔.
Carcinoma *in situ* (CIS)	Extreme cytological aberration and chaos Uneven chromatin, variable nuclear size and shape, multiplicity of nucleoli and dyskariosis that extend throughout airway epithelium Mitotic figures through full thickness No infiltration of the basement membrane

Table 1-1: Summary of pathological findings in pre-invasive disease of the airway

1.3 Detection of pre-invasive lesions in the airway

1.3.1 Autofluorescence bronchoscopy

The precise localisation of microinvasive carcinomas and preinvasive lesions is difficult as they are not easily visualised with conventional white light bronchoscopy (WLB) [19]. Autofluorescence bronchoscopy (AFB) improves visibility of these lesions by exploiting differences in the fluorescence and light absorption properties of normal and abnormal bronchial epithelium [20]. As pre-invasive lesions progress, they exhibit slightly weaker red fluorescence but proportionally much weaker green fluorescence (i.e. higher red:green ratio) than normal tissues when illuminated by blue light [21]. Optical systems are designed to detect a combination of these fluorescence and reflectance changes from the airway epithelium (figure 1.2). The most well-known device is the LIFE (Lung Imaging Fluorescence Endoscopy, Xillix Technologies) system, designed by Lam et al in Vancouver [22]. This system uses optical filters and was originally designed for use with fibreoptic bronchoscopes. The Pentax SAFE-3000 system (Pentax Corp., Tokyo, Japan) [23] and the Olympus autofluorescence, AFI-Lucera (Olympus, Tokyo, Japan) [24] are based on similar principles and are configured to work with video bronchoscopes.

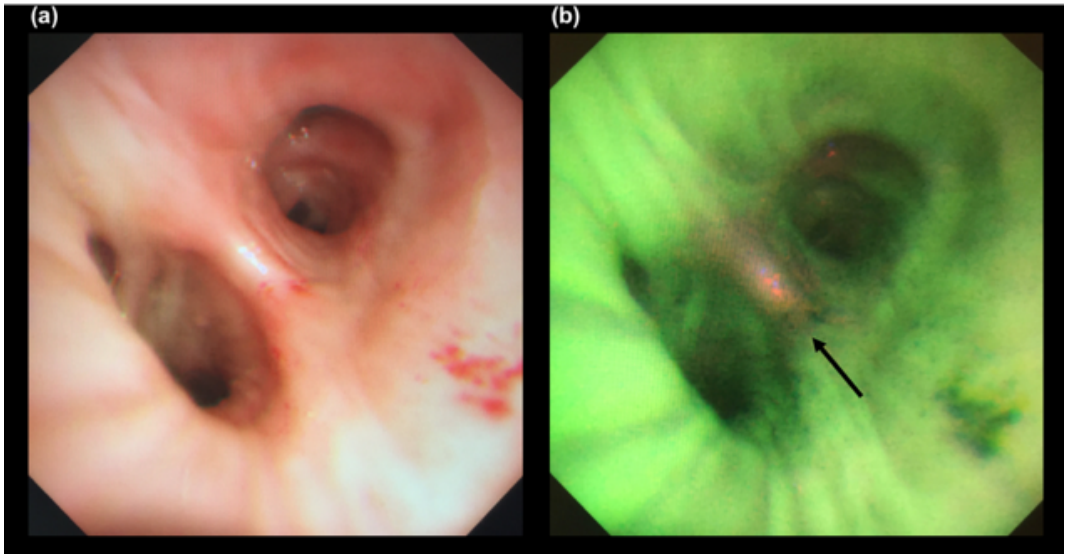


Figure 1.2 – Severe dysplasia on left upper-lower lobe carina, hardly detectable on (a) white light bronchoscopy, but easily visible on (b) autofluorescence bronchoscopy. Images from an Olympus autofluorescence bronchoscope (BF-F260), AFI-Lucera (Olympus, Tokyo, Japan).

The development of autofluorescence bronchoscopy has led to a significant increase in diagnostic sensitivity for detecting pre-invasive disease, demonstrating a 1.4–6.3 fold improvement in detection over white-light bronchoscopy (WLB) alone [22–31]. A recent meta-analysis reported a pooled sensitivity for detection of pre-invasive disease of 85% for WLB with AFB, compared to 43% using WLB alone (relative sensitivity 2.04, 95% CI 1.56-11.55) [32]. However, AFB can detect a large number of false positive lesions, such as inflammation making its specificity poor [25–27]. The same analysis by Sun et al reported a pooled specificity of 61% [32]. However, recently there has been research into improving AFB specificity using a quantitative scoring system based on red-green reflectance [33], or by combining AFB with narrow-band imaging (NBI) [34], which may in the future reduce the number of sites sampled during a procedure.

1.3.1.1 Prevalence of pre-invasive disease

AFB has been used to assess prevalence of pre-invasive disease in 'at-risk' patients. As part of the large chemoprevention studies by Lam and colleagues, combined WLB and AFB in smokers of 20 pack-years or more showed the prevalence of mild, moderate, or severe dysplasia and CIS was 40%, 14%, 6.5%, and 1.8%, respectively [35]. They further showed that women had not only a lower prevalence of high-grade lesions (14% versus 31%; odds ratio = 0.18; 95% CI 0.04–0.88), but fewer synchronous pre-invasive lesions after adjusting for smoking (p = 0.048). Ishizumi et al suggest that this prevalence has come down over time, reporting occurrence of moderate or severe dysplasia and CIS as 9%, 1.9% and 0.8%, respectively amongst a cohort of 1,581 smokers [36]. Further data come from groups evaluating patients with positive sputum cytology [19,26,31,37], and AFB in this patient cohort can identify a large number of pre-invasive and invasive lesions. Chhajed et al screened 151 patients at a high risk of lung cancer with sputum cytology and performed bronchoscopy when it showed moderate dysplasia or worse. Of 343 lesions, WLB & AFB showed mild, moderate or severe dysplasia and CIS in 14%, 15%, 2%, and 1% of the lesions detected, respectively [19]. However, since sputum cytology atypia is rarely encountered in clinical practice and its use in screening remains uncertain, few patients are likely to be diagnosed through this pathway.

1.3.2 Narrow band imaging

NBI (Olympus Optical Co., Japan) is an optical imaging technology designed to improve visualisation of microvascular structures in the mucosal and submucosal layers [38]. The bronchoscope emits light of two specific wavelengths, which are strongly absorbed by haemoglobin. This technique can detect increased vessel growth, tortuosity, and microvascular patterns in both the superficial and deeper

layers of the epithelium [39]. It is potentially useful for detection of pre-invasive disease as angiogenesis has been shown to occur early on in these lesions [40]. Only one study has directly compared NBI and AFB prospectively in pre-invasive disease [34]. The relative sensitivities of AFB and NBI, when compared to WLB were 3.7 (p=0.005) and 3.0 (p =0.03), respectively. NBI was not only as sensitive as AFB, but comparatively has increased specificity. Although based on only a small number of patients, NBI has the potential to be incorporated into clinical practice without losing sensitivity, and could improve selection of lesions requiring biopsy.

1.3.3 Optical Coherence Tomography

Optical coherence tomography (OCT) is an optical imaging technique that employs near infra-red light to visualise the epithelium down to a 3-micrometer resolution. The longer wavelength of light penetrates tissue and scattered light is measured via a probe that fits down the working channel of a bronchoscope. Three-dimensional images are constructed from these data [41–43]. In the airway it has been demonstrated that an increased thickness of the epithelial layer can differentiate normal mucosa from dysplasia, and interruption of the basement membrane can be seen when looking for micro-invasive carcinoma [41,44]. Although not currently employed in routine clinical practice, OCT may have a role as an adjunct to AFB, particularly in confirming whether there is breach of the basement membrane in cases with equivocal pathology.

1.3.4 Computerised tomography and nuclear imaging

Imaging with computerised tomography (CT) and positron emission tomography (PET) has a role in detection of parenchymal lesions, staging, and identification of metastatic disease. A high-grade pre-invasive lesion in the airway is unlikely to be detected by CT; however this imaging modality plays an important role in detecting interval lung cancers during longitudinal surveillance that would otherwise be missed by bronchoscopy [16,17]. However, Sutedja et al have used high-resolution CT (<1mm slice thickness) to evaluate both CIS and early invasive cancers as part of a diagnostic algorithm to look for peri-bronchial extension and determine whether an endobronchial treatment approach is feasible [45]. PET remains in its infancy in assessment of pre-invasive disease, however a pilot study of 20 lesions certainly looked promising [46]. We have taken this forward and assessed the diagnostic utility and predictive biomarker potential of PET in a nested cohort of 44 untreated patients with high-grade lesions (29 CIS) followed-up for 11 years [47]. Of 8 patients observed to have a PET-positive CIS lesion, 7 (87%) progressed to invasive cancer versus 6 of 21 (28.6%) patients with PET-negative CIS lesions ($p = 0.001$). We showed that PET appears to detect the 'high-risk' CIS lesions that will progress to invasive cancer, and by including this in our diagnostic algorithm, also detected separate synchronous invasive airway cancers.

1.4 The natural history of pre-invasive disease

Longitudinal studies using AFB-guided biopsy of lesions in the central airways have provided some insight into how pre-invasive disease behaves over time [16–18,48–52]. However, developing statistical models of which lesions progress or regress is not straightforward, especially in severe dysplasia and CIS [53]. There are problems

with intra- and inter-observer variability in clearly defining the severity of the dysplasia [14,15], interpreted on small bronchoscopic mucosal biopsies. Comparison of specific studies is also difficult [36,53,54]. Most studies enrol small numbers of patients using different inclusion criteria, baseline lesions, and follow-up is often relatively short. The definition of the end-point of studies also varies; investigators may define LGL progression to severe dysplasia, CIS or invasion, or combine the outcomes [55]. Finally, with the exception one study [16], CIS is often treated with concern it may progress to invasion, compromising a true assessment of the natural history of CIS. Even within studies of treated patients, different endobronchial treatments are employed. Despite these problems, we can still draw general conclusions from several of the larger longitudinal studies, Table 1-2.

Progression rates to invasive carcinoma vary depending on the initial grade of lesion [17,18,49] and it is generally accepted that high-grade lesions are more likely to progress to invasive cancer than low-grade lesions [16–18,36,49,55]. In the study by George et al, none of the low-grade lesions progressed to invasive cancer over a follow-up period of 12-85 months [16]. Breuer et al in their cohort found progression of mild or moderate dysplasia to CIS or invasion (9%) to be significantly different from severe dysplasia (32%) [49]. Similarly, other authors have reported low rates of progression of low-grade lesions to invasive cancer [18,50,56]. Interestingly, in a recent large study where strict definitions of 'site-specific' progression were used, no significant difference in development of invasive disease was observed between high- (18%) and low- (12%) grade lesions [17]. This is in part due to the high rate of progression observed in low-grade lesions and the group's usual practice of treating CIS. However, this study is one of the first to follow-up patients over a long period of time (up to 12.5years) and reflects the importance of following up low-grade lesions. In contrast, other groups have described progression of CIS to invasion as high as 43%–87% [18,48,51,52,57]. In these studies the lesions were follow-up at short time intervals, stable CIS or invasion were both

considered ‘progression’, and these lesions were subsequently treated [18,48,51,57]; making any firm conclusions impossible. Therefore, it is difficult to appreciate the natural history of high-grade lesions where endobronchial treatment hasn’t influenced its outcome. The study by George et al is unique, describing the course of 36 untreated high-grade lesions over a median of 23 months. In this cohort, 6 (17%) progressed to invasive cancers [16]. Interestingly, this is a similar incidence of progression to cancer as seen in treated high-grade lesions [17,57].

Importantly, the data from these studies show that patients with HGLs develop both synchronous and metachronous lesions, both pre-invasive and of lung cancer [16,17,51,56,58]. Van Boerdonk and colleagues performed a longitudinal study with AFB and CT in 164 patients (80 with high-grade lesions) [17]. They detected 61 cancers in 55 patients with a median time-to-event of 16.5 months. Of these, 35 were detected by AFB, where 10 interval endobronchial cancers occurred away from the initial detected site at study entry. This meant that overall 59% of cancers were metachronous and more likely to occur in individuals with high-grade lesions (HR 1.84, CI 95% 1.05-3.22). The incidence of metachronous lung cancer in those with pre-invasive lesions is similar to that seen by other authors [16,56,59]. For example, George et al detected 11 lung cancers in 9 patients with high-grade lesions, giving an estimated risk of developing lung cancer of 33% at 1 year and 54% at 2 years [16]. These studies support the theory of ‘field carcinogenesis’, and suggest pre-invasive disease, in particular high-grade lesions, are a marker of lung cancer risk. However, many of these lesions are also precursors that do progress to cancer, and thus being able to reliably predict which ones will progress to invasive disease will determine whether intervention is necessary.

Investigators	Baseline histology & lesion (no.)	Median follow-up (months)	Lesions treated	Lesion end point	Comments
Venmans et al (2000) [48]	SD (3) CIS (6)	NS	Yes	SD→INV: 100% CIS→INV: 33% CIS→CIS: 17% CIS→LGL: 50%	8 further metachronous lesions detected
Deygas et al (2000) [60]	CIS (35)	1m & 1year	Yes	CIS→LGL: 71% CIS→CIS: 9% CIS→INV: 20%	28% local recurrence (1 year) Disease free-interval 13-45months
Bota et al (2001) [18]	SD (27) CIS (31)	3–24	Yes	SD→CIS: 37% CIS→CIS: 87%	HGLs had 3m assessment prior to treatment decision
Moro-Sibilot et al (2004) [51]	SD (3) CIS (28)	24 (13–41)	Yes	SD→INV: 50% CIS→LGL: 52% CIS→CIS: 5% CIS→INV: 43%	CIS (untreated) progressed in 29%
Breuer et al (2005) [49]	SD (25)	NS	Yes	SD→LGL: 52% SD→SD: 16% SD→CIS: 32%	Progression to invasion not stated
George et al (2007) [16]	HGL (36)	21 (1–72)	No	HGL→LGL: 19% HGL→HGL: 64% HGL→INV: 17%	Time to progression 4-17months
Salaun et al (2008)[$] [52]	SD (23) CIS (31)	68 (19–117)	Yes	SD→INV: 0% CIS→LGL: 13% (untreated) CIS→LGL: 32% (treated) CIS→INV: 23%	HGLs had 3m assessment prior to treatment decision
Van Boerdonk (2015) [17]	HGL (80) CIS:14	30 (4–152)	Yes	HGL→INV: 18% (site specific progression)	Cumulative 5-year lung cancer risk 39% in HGLs

Table 1-2: Natural history of high-grade pre-invasive disease of the airway

Investigators	Baseline histology & lesion (no.)	Median follow-up	Lesions treated	Lesion end point	Comments
Bota et al (2001) [18]	SqM (36) MET (152) LGD (169)	3–24	No	SqM→CIS: 0% MET→CIS: 2% MET→INV: 1.5% LGD→CIS: 3.5%	No LGD progression to invasion
Breuer et al (2005) [49]	MET (45) LGD (64)	NS	No	MET→CIS: 9% LGD→CIS: 9%	Progression to invasion not stated
Hoshino et al (2004) [50]	MiD (32) MoD (56)	7 (5–17)	No	MiD→INV: 0% MoD→INV: 2%	
George et al (2007) [16]	LGL (17)	21 (1–72)	No	LGD→INV: 0%	
Van Boerdonk (2015) [17]	LGL (84)	30 (4–152)	Yes	LGL→INV: 12% (site specific progression)	Metaplasia included in definition of low-grade lesion

Table 1-3: Natural history of low-grade pre-invasive disease of the airway

LGL=low grade lesion, HGL=high grade lesion, INV=invasive disease, CIS=carcinoma in situ, SD=severe dysplasia, MoD=moderate dysplasia, MiD=mild dysplasia, SqM=squamous metaplasia

1.5 Predictive risk factors for progression of pre-invasive lesions

1.5.1 Clinical risk factors

Clinical risk factors and risk prediction tools have been extensively studied as decision aids in management of suspicious parenchymal lesions [61,62]. Although there are no validated models for pre-invasive disease there are some recognised associations. Active smoking, presence of synchronous lung cancer, number of baseline pre-invasive lesions, previous head and neck cancer and exposure to carcinogens including asbestos have all been shown to be risk factors for harbouring high-grade lesions in the airway [16–18,26,58,63]. Paris et al showed in a study of 241 patients that a number of these factors are independently associated with high-grade lesions, with accumulation of multiple factors conferring even higher risk [58]. However, these causative risk factors such as COPD, previous head and neck or lung cancer and smoking behaviour do not appear to consistently correlate with progression of pre-invasive disease [18,49,51,56]. This is in part related to some of the limitations of longitudinal studies described. Alaa et al examined 240 lesions under longitudinal surveillance with AFB and CT, with progression to CIS or cancer as an end-point. Metachronous severe dysplastic lesions found during follow-up ($p = 0.0001$), COPD ($p = 0.001$) or smoking history >52 pack-years ($p = 0.042$) were all associated with higher risk of developing lung cancer [64]. This appeared to indicate that having more than one lesion was a risk factor for lung cancer corroborated by Pasic et al who used an AFB scoring system showed in a cohort of 46 individuals that the number of suspicious lesions at baseline bronchoscopy correlated with developing invasive cancer. With detection of either one, two, or three suspicious lesions on AFB, lung cancer developed in 8%, 50% and 100% of cases, respectively [63]. This indicates a higher risk of lung cancer

in those individuals with multifocal airway lesions, also shown by other groups [16,17,48]. Understanding clinical risk factors that indicate progression to lung cancer is important and as with many studies the relationship of individual lesions and progression is difficult to ascertain., however, it is likely that increased understanding of molecular alterations in these lesions will play a far more important role for risk prediction.

1.5.2 Molecular risk factors

Genetic and epigenetic changes are likely to long precede the morphological transformation of pre-invasive lesions, with carcinogenesis ensuing following accumulation of successive molecular abnormalities, resulting in selection of clonal cells capable of invasion. Figure 1-3 summarises our interpretation of how pre-invasive disease exhibits the 'hallmarks of cancer'. Salaun et al followed 54 high-grade (31 CIS) lesions up to 144 months and correlated outcome with the molecular profile. The presence of 3p loss of heterozygosity (LOH) and presence of more than one site of LOH were associated with increased risk of progression to lung cancer [57]. The group further showed that presence of baseline 3p LOH was associated with a poorer survival, although treatment of endobronchial lesions may have affected lesion progression. McCaughan et al looked at alterations in chromosome 3, in 10 high- and 7 low- grade lesions within a nested cohort [65]. Progression occurred in 8 of the 10 high-grade lesions, all of whom had amplification of chromosome 3q. Similarly, Massion et al looked at genomic gains identified by 4 FISH probes (TP63, CEP3, CEP6, MYC) in 70 patients with preinvasive disease, 27 of whom developed lung cancer [66]. In a group of lesions ranging from moderate dysplasia to CIS, they showed this combination of probes offered a diagnostic sensitivity of 82% for predicting lung cancer. Van Boerdonk et al recently described

a molecular classifier based on copy number alterations of 3p26.3-p11.1 (loss), 3q26.2-29 (gain) and 6p25.3-24.3 (loss) in a group of patients with metaplasia that predicted progression to lung cancer with 97% accuracy [67]. They applied this classifier to an independent set of 36 'high-risk' patients, whereby progression to CIS or invasion was observed in 12 (3 low-grade and 9 high-grade baseline lesions) and 24 cases remained 'cancer or CIS-free' [68]. The classifier predicted progression to CIS with an accuracy of 92% (CI 77-98%). The negative predictive value of this classifier was 89%, with the gain at 3q26.2-q29 being present in virtually all lesions and hence contributing most strongly to the classification model. Although it would be useful to see a comparative cohort of progression to invasive cancer only, this study validates a copy number alteration, CNA-based classifier system as an objective determinant for progression of pre-invasive disease and likely a determinant for developing cancer.

Investigators have also utilised immunohistochemistry and other methods to detect markers that may predict progression of pre-invasive disease. These include p53 [50,69], Ki-67 labelling index [50,70], telomerase activity [50,71], apoptotic proteins [69], C-reactive protein [72] and proteomic signatures [73]. However, they have not yet been validated in prospective studies and their utility over and above histology has not been established. In contrast, genetic alterations in pre-invasive disease appears to hold far greater promise. Assessment of lesion epigenetic and genetic signatures in the future will not only provide a better understanding of carcinogenesis, but will also likely to form the basis of biomarkers that guide treatment decisions and indicate prognosis.

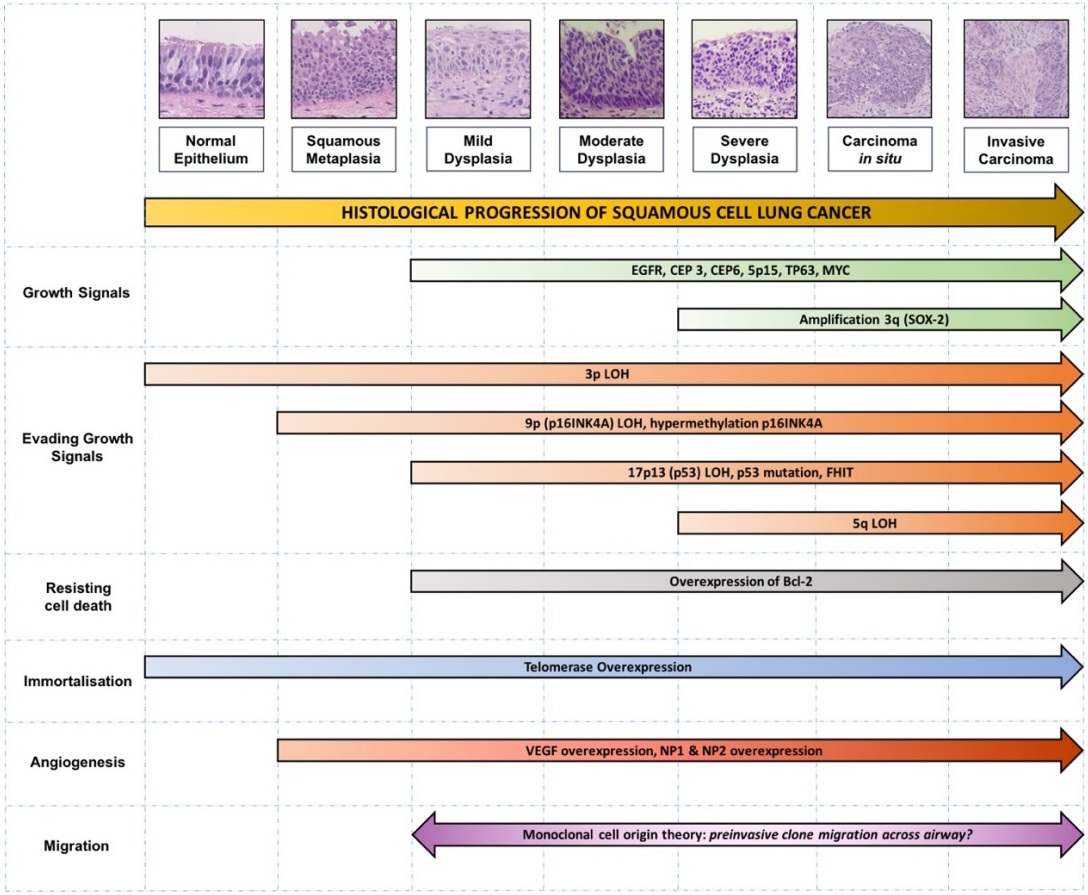


Figure 1.3– Molecular changes in the progression of pre-invasive disease

Numerous genetic and epigenetic changes have been identified as occurring at the various grades of preinvasive disease, with some occurring in histologically normal epithelium [65,68,82–85,74–81]. Figure based on the originally proposed hallmarks of cancer by Hanahan et al [86]. EGFR=epidermal growth factor receptor, CEP=chromosome enumeration probe, MYC=myelocytomatosis viral oncogene homolog gene, SOX-2=(sex determining region Y)-box 2, FHIT= fragile histadine triad, Bcl-2=B-cell lymphoma 2, VEGF=vascular endothelial growth factor, NP=neuropiln

1.6 Treatment of early central airway lung cancers

The American College of Physicians and other authors advocate surgical treatment for CIS and early lung cancers in the airway [4,6,7,87]. Despite these lesions being small, their central location means around 70% of individuals require a lobectomy, and the remaining either a bilobectomy or pneumonectomy for curative resection [4]. This approach carries appreciable morbidity and mortality, which is difficult to justify in preinvasive disease when there is no guarantee that any of these lesions will progress to invasion. George et al followed 36 high-grade untreated lesions in their cohort and showed 7 lesions regressed (19%), while a further 23 remained indolent (64%) [16]. Bota et al made a treatment decision after 3 months of follow-up for 32 CIS and 27 severe dysplasia cases [18]. Although they did not state the number that remained indolent and the follow-up period was short, 7 (22%) CIS and 17 (63%) of severe dysplasia lesions had regressed, respectively.

Surgery for patients with such early stage disease is associated with 5-year survival rates in the region of 90% [4,6]. However, patients with CIS also have a significant risk of developing multifocal preinvasive and invasive carcinoma at other sites within their lungs and consequently may not have sufficient pulmonary reserve to undergo further lung resection. This dilemma has been overcome by other investigators managing CIS with endobronchial treatments, thereby avoiding lung resection [53–55]. In one review, Banerjee et al concluded overall CIS regression occurred in 58% of individuals undergoing treatment, however 34% of CIS lesions progressed despite treatment [53].

While many investigators do report good results with a variety of endobronchial treatments, these studies are often small, with short follow-up, and frequently combine progression to invasive and high-grade preinvasive histology as a single end-point [7,53,55]. Since none of the studies have included a control arm

[48,51,55,60,88], the natural history of the lesions treated in these studies is not known, and the clinical and prognostic value of the intervention remains unclear. Nonetheless, since high-grade dysplasia and CIS are known to progress to invasive lesions in a high proportion of cases, effective treatment of airway lesions should prevent invasive cancers, leading to considerable benefit for patients and circumventing the expense and morbidity of treating advanced lung cancer. It is therefore not surprising that investigators have adopted different local ablative bronchoscopic techniques into routine practice and chemotherapeutic agents for the prevention of cancer are coming under the spotlight. A summary of the minimal invasive techniques used for local control are summarised in Table 1-4.

Investigators	Lesions (n)	Complete response (%)	Comments
Photodynamic Therapy			
Kubota et al **(1992)** [89]	29	72%	CR 89% in lesions <10mm
Furuse **(1993)** [90]	59	CIS: 100% INV: 80%	CR in lesions <1cm: 98% CR in lesions >1cm: 43%
Imamura et al **(1994)** [91]	39	64%	CR in superficial lesions: 76% CR in nodular lesions: 43%
Kato et al **(1996)** [92]	95	83%	CR in lesions <1cm: 94% CR in lesions 1-2cm: 54%
Kawaguchi et al **(2000)** [93]	59	73%	53% had no recurrence at 2-year assessment
Miyazu et al **(2002)** [94]	18	100%	EBUS to identify superficial lesions (9 lesions treated)
Furukawa et al **(2005)** [95]	114	Lesion <1cm: 93% Lesion >1cm: 58%	5-year survival (<1cm): 58% 5-year survival (>1cm): 59%
Kato et al **(2006)** [96]	264	85%	Local recurrence in 12% of cases
Endo et al **(2009)** [97]	48	94%	Tumour length <10mm Overall 5-year survival: 81%
Usuda et al **(2010)** [98]	28	100%	PDT used to treat 11 individuals with multifocal disease
Jung et al **(2011)** [99]	39	100%	PDT used in central airway lesions in individuals with multifocal disease

Brachytherapy			
Perol et al **(1997)** [100]	19	83%	Brachytherapy schedule: 7Gy x 3-5 fr. Local control in 75% (n=16) at 1-year 16% had severe bronchial necrosis or fatal haemoptysis
Taulelle et al **(1998)** [101]	22	96%	Brachytherapy schedule: 7-10Gy x 3-5 fr. Survival (median): 17months
Marsiglia et al **(2000)** [102]	34	94%	Brachytherapy schedule: 5Gy x 6 fr. Local control in 85% at 2-year median
Lorchel et al **(2003)** [103]	35	86%	Brachytherapy schedule: 5Gy x 6 fr. Bronchial stenosis observed in 36% of cases
Hennequin et al **(2007)** [104]	106	59%	Brachytherapy schedule: 7Gy x 5-6 fr. 5-year survival 48%
Electrocautery			
Van Boxem et al **(1998)** [88]	15	77%	30 watts power applied until visible necrosis
Cryotherapy			
Deygas et al **(2000)** [60]	35 (CIS only)	91%	28% local recurrence (1 year) Disease free-interval 13-45months

Table 1-4: Different endobronchial modalities used to treat early central airway pre-invasive and invasive lesions

1.6.1 Photodynamic Therapy

Photodynamic therapy (PDT) has a proven track record of successful tumour ablation [105]. It relies on activation of a photosensitiser that preferentially accumulates in transformed cells. Using a specific wavelength of light delivered endobronchially, release of reactive oxygen species causes cellular apoptosis to the lesion in question. PDT can achieve good response rates in radiographically negative airway cancers [89,90,99,106,91–98]. In an early phase II study, Furuse et al treated 59 early cancers with photofrin, a first-generation photosensitizer [90]. A complete response (CR) was seen in 85% of patients, with the remainder having either a partial or no response. A review of over 700 invasive and preinvasive lesions across 15 trials revealed a complete response rate of 30–100% and an overall 5-year survival of 61% [107]. They further showed that PDT is safe with photosensitivity being the most common complication in 5–28% of cases. The lower response rates seen in this review were largely due to the heterogeneity of cases treated and it has since come to light that PDT is most effective when there is no extra-cartilaginous disease and the tumour length is <1cm [94,95]. Furukawa et al used PDT as the definitive treatment in 114 stratified lesions (<1cm or >1cm), with long-term follow-up [95]. When persistent atypia was demonstrated at the same site the authors showed complete remission could be obtained by performing a second PDT. A complete response was seen in 93% of lesions <1cm in size compared to 58% of lesions >1cm. While the 5-year survival was not influenced by tumour size, the lower survival in both groups may be due to poor baseline performance status as patients receiving this treatment were unsuitable for surgical treatment. PDT should be considered for those in whom surgery is medically or technically not possible. PDT is becoming more common as treatment of preinvasive disease, especially in cases of multifocal disease where a tissue sparing approach is necessary. While patient selection has improved [94], its role as a

definitive treatment remains unclear and will be examined in our recently Cancer Research UK funded, randomised controlled trial (Photodynamic therapy for the prevention of lung cancer, PEARL).

1.6.2 Brachytherapy

Endobronchial brachytherapy (EBBT) involves the placement of a radioactive (commonly iridium) source via a catheter delivered through the working channel of the bronchoscope. Since the depth of treatment is usually 1cm, lesions that extend beyond the cartilage can be treated, whilst sparing normal lung tissue. It is a well-established method for the local, palliative treatment of locally advanced tumours in the central airways [108]. However, its role in the definitive treatment of preinvasive and radiographic occult cancers is yet to be proven in randomised controlled trials. Hennequin et al used brachytherapy as a monotherapy to treat 73 individuals with early cancers <10mm in length [109]. They re-evaluated at 1-2months, showing CR in 59% of cases, which was more frequently observed in shorter tumours and those undetectable on CT imaging. Median overall survival in this group was 21 months, amongst which 5% of deaths were attributable to the treatment (massive haemoptysis or airway wall necrosis). The same group later went on to show long-term local control and survival can be achieved with 5-year cause-specific survival of 49% [104]. Other groups have reported equally good outcomes, with CR rates ranging from 83-96% and 2 or 3-year survival between 45–92% [100–102,110]. In a review by Skowronek, complications occurring the in the acute setting were reported as uncommon, but include pneumothorax, bronchospasm, haemoptysis and cardiac arrhythmia or arrest [111]. Of the papers reviewed, instances of massive haemoptysis occurred in 0–18.9% of cases. Since EBBT is often used in advanced tumours, it is not always clear whether these

occurred as a result of the treatment or progression of disease. Late complications such as chronic radiation bronchitis and bronchial stenosis are observed in long-term survivors. Lorchel et al treated 35 cases of CIS or small invasive carcinomas with high-dose EBBT and demonstrated a response in 86% [103]. However, longer-term complications were observed, with bronchial stenosis occurring in 12 of 33 (36%) individuals.

1.6.3 Cryotherapy

Cryotherapy uses extreme cold to destroy abnormal tissue. In the airway this is delivered via a flexible probe that passes through the bronchoscope working channel. The probe is then cooled by delivering compressed gas to its tip, usually nitrous oxide or carbon dioxide, resulting in temperature reduction via the Joule-Thompson effect. Deygas et al used cryotherapy to treat 35 patients with CIS and early invasive cancers [60]. A complete response was seen in 32 patients (91%), observed at intervals of 1 month and 1 year. Local recurrence in this group occurred in 10 cases (28%) with a disease-free interval of 13–45months.

1.6.4 Other ablative therapies

Endoluminal electrocautery uses a high-frequency electrical current to induce a heating effect, causing coagulation and tissue necrosis. Van Boxem published the only study using solely this technique; of 13 patients, 80% achieved a complete response rate with no recurrence at a median of 21 months follow-up (16–43 months) [88]. Whilst more data is needed, electrocautery is promising with its

effect limited to superficial tissue, the ability to deliver treatment using flexible bronchoscopy and its relatively low-cost.

The neodymium-doped yttrium aluminium garnet (ND-YAG) and diode laser often takes center place in the interventional bronchoscopists tools; used commonly to ablate tumours causing central airway obstruction, see Figure 1.4. No randomised trial has been conducted using laser in early central airway cancers. Cavaliere et al reported 22 cases of early lung cancer treated within a nested cohort, and whilst excellent outcomes were reported, no long-term follow-up was conducted [112]. The depth of penetration of ND-YAG is 2-6mm and this does carry a risk of perforation into nearby structures and vasculature. Lasers such as potassium titanyl phosphate (depth ≈0.5mm-2.0mm), thulium (depth ≈0.2–0.5mm), or carbon dioxide (depth ≈0.1mm) which have shorter and more controlled depths of optical penetration, and are more likely to be useful in superficial pre-invasive lesions [113].

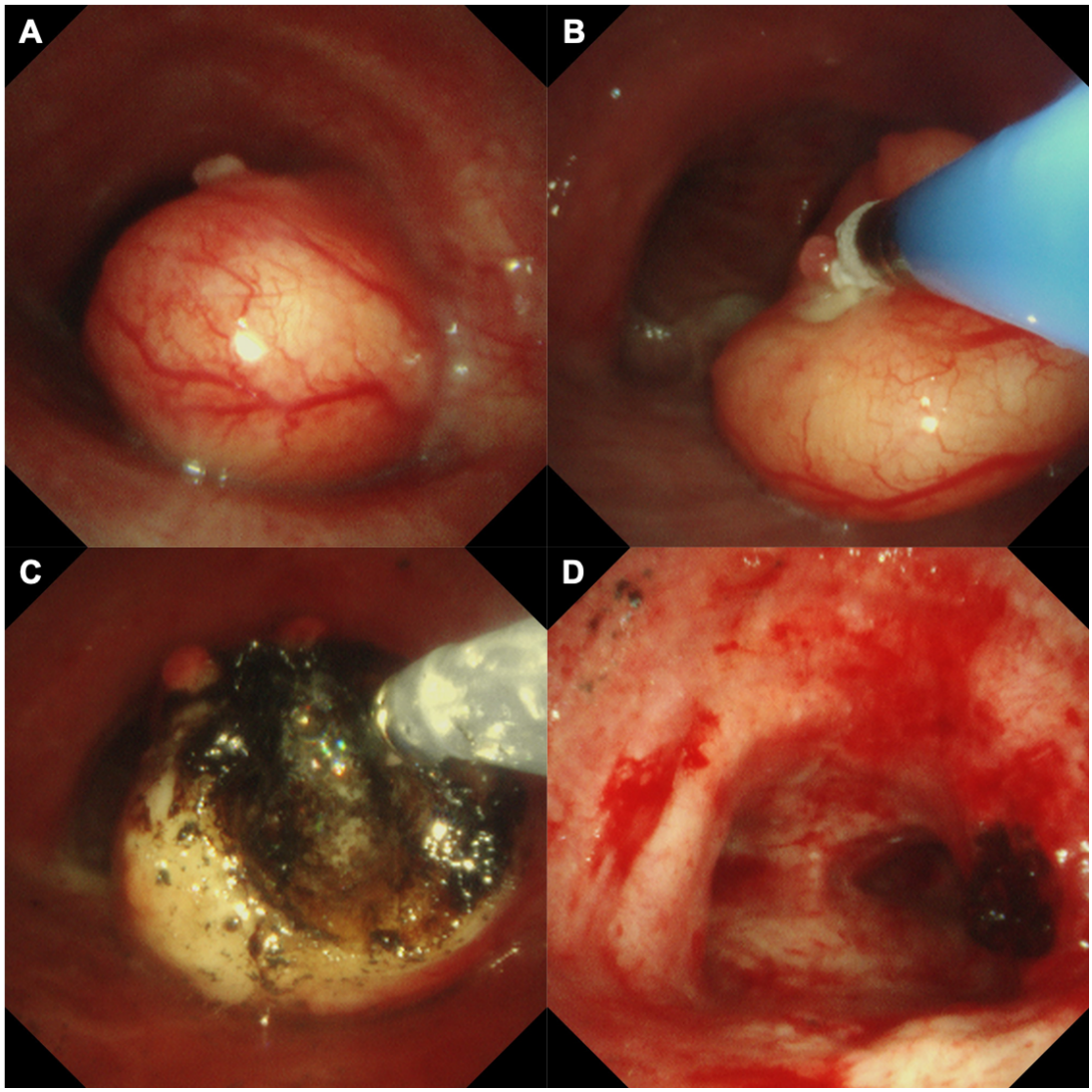


Figure 1.4 – (A) Tracheal hamartoma treated with (B) cryo-extraction and (C) diode laser ablation until (D) all residual disease vaporised

Chapter 2 LONGITUDINAL STUDY OF PATIENTS WITH PRE-INVASIVE LESIONS OF THE BRONCHUS

2.1 Introduction

It is widely believed that pre-invasive lesions are precursors to squamous cell carcinoma and that they progress through a series of morphological stages until invasion occurs. Much of the evidence for this hypothesis comes from Auerbach's post mortem studies that were conducted over 40 years ago [114]. He demonstrated that carcinoma in situ (CIS) lesions were present in up to 75% of heavy smokers and that their distribution corresponded with that seen in squamous cell carcinoma.

The potential of sputum cytology as a non-invasive test was first raised by the longitudinal studies of Saccomanno et al [115]. Sputum samples collected from uranium workers at high risk were found to contain cells with increasingly malignant features in those individuals who subsequently developed lung cancer [115]. When precursor lesions form throughout the respiratory epithelium as a consequence of carcinogenic exposure, exfoliated cells are consequently detected in the sputum. Certainly, there remains ongoing interest in high-risk populations, for example, patients with COPD are at increased risk of developing lung cancer if they have abnormal sputum, and in a cohort of 2550 patients, 17.7% of individuals who were found to have at least moderate cytological atypia had a cumulative lung cancer incidence of 10% at 3 years and 20% at 6 years, see Figure 2-1. [116]. However, sputum gives no information on specific lesions in the airway, especially

when they are multi-focal. Further problems include poor sensitivity and variation in pathologist agreement [117].

The parallel developments of sputum cytometry and fluorescence bronchoscopy have facilitated the detection of pre-invasive lesions within the airways of high-risk individuals and this has raised the clinical dilemma as to how these lesions should be managed. In view of their malignant potential, some groups have treated CIS and severe dysplasia promptly [17,18,48], but this has complicated the interpretation of their natural history. However, in Auerbach's original post mortem studies in heavy smokers, although dysplasia or CIS was found in up to 75% of heavy smokers, only 1 in 10 appeared to have a clinically significant squamous cell lung cancer [114]. Since dysplastic lesions are by definition non-invasive, one would expect them to be cured with surgical resection or radiotherapy. However, this clinical scenario is faced with 3 caveats: (i) these patients frequently have co-existing medical problems such as chronic obstructive pulmonary disease (COPD) and poor cardiopulmonary reserve making them poor candidates for surgery (ii) they are often at high-risk of developing synchronous and metachronous lesions throughout the airway, and (iii) not all pre-invasive lesions will progress to invasive cancer, with some regressing back to normal epithelium. Hence, a radical approach may not always be possible, or indeed required. However, there is universal agreement that individuals with pre-invasive disease are at risk of developing lung cancer and that early detection of pre-invasive lesions maybe critical to improving survival.

A prospective longitudinal cohort study using autofluorescence bronchoscopy and computerised tomography surveillance at University College London Hospital was conducted over a 10 year period to identify and closely monitor high-risk patients for lung cancer. In this chapter, the aim is to define the natural history of pre-invasive lesions of in this cohort, assess the clinical factors that predict

development of lung cancer, and determine whether early detection through a surveillance programme could improve outcomes. To allow useful comparison, results are drawn out of this study similar to Van Boerdonk et al, who recently presented their cohort of patients treated for high-grade pre-invasive disease [17].

The participating centre was University College London Hospital. Ethical approval was granted by the Joint UCL/UCLH Committees on Ethics of Human Research (REC 01/0148).

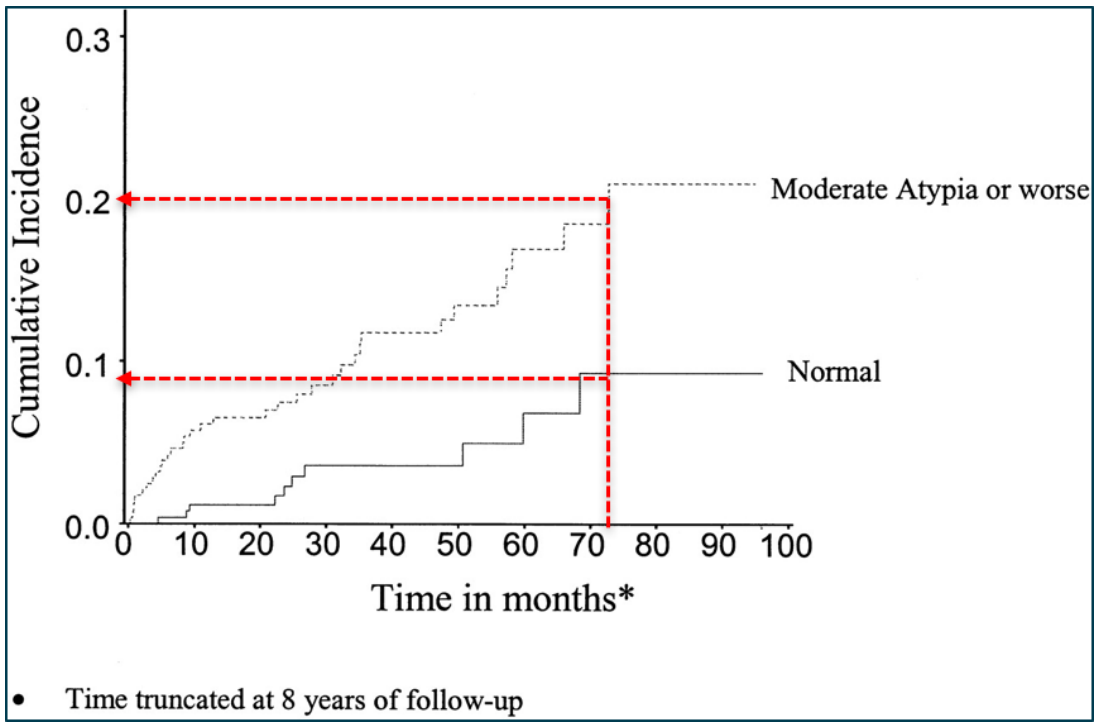


Figure 2.1– Cumulative risk of lung cancer in patients with COPD, in whom abnormal sputum is detected

Cumulative lung cancer incidence of 10% at 3 years and 20% at 6 years, Figure reproduced from Prindiville et al, Cancer Epidem Biomarker Prev, 2003. [116].

2.2 Methods

2.2.1 Patient population

Individuals were first identified by their primary and secondary care physicians with a high perceived risk of lung cancer. This may have included unexplained chest symptoms (i.e. persistent haemoptysis) and/or identification of abnormal investigations. They were then referred to our tertiary centre for an assessment where usually a clinical assessment, radiological investigation and autofluorescence bronchoscopy were performed. As described autofluorescence bronchoscopy is more sensitive at detecting pre-invasive and early invasive lesion in the tracheobronchial tree and allows us to accurately identify the extent and stage of these lesions. Once the assessment was complete, a recommendation was made for either recruitment into this study, discharge back to the referring physician, or treatment recommendations if an invasive lung cancer was detected.

Consecutive patients with a perceived high risk for lung cancer based on smoking history, abnormal sputum cytology, symptoms of unexplained haemoptysis, or incidental findings of dysplasia on flexible white light bronchoscopy underwent a CT and AFB between 1st January 2000 and 1st December 2016. Of these individuals, those that met the inclusion criteria were entered into the study.

2.2.1.1 Inclusion criteria

Presence of one or more pre-invasive lesion detected on autofluorescence bronchoscopy confirmed on histology as either: (i) mild (MID) or moderate (MOD) dysplasia defined as low-grade lesions, or (ii) severe dysplasia (SD) or carcinoma in-situ (CIS) defined as high-grade lesions.

2.2.1.2 Exclusion criteria

- Evidence of invasive carcinoma detected on autofluorescence bronchoscopy, CT, PET, and/or histopathological findings
- Patients who have previously had undergone curative treatment for invasive carcinoma of the lung were still eligible for the study
- General medical conditions precluding bronchoscopy
- Specific respiratory disease affecting ability to tolerate bronchoscopy
- Refusal or inability to give informed consent

2.2.2 Intervention

All individuals underwent annual CT Thorax with contrast and autofluorescence bronchoscopy with either the D-Light autofluorescence bronchoscope (Karl Stortz, Germany), or Olympus autofluorescence AFI-Lucera (Olympus, Tokyo, Japan). The AFB was performed under moderate sedation using either intravenous midazolam and/or fentanyl or propofol under anaesthetic supervision, using the following method (Figure 2-2):

- The bronchial tree was first inspected under white light and then under blue. All abnormal areas were initially documented and sampled when the bronchoscopic examination had been completed.
- Sites were recorded as distinct lesions when separated by mucosa with normal white light and fluorescence appearances.
- 3-5 biopsies were taken from each lesion and control site in formalin solution for standard histopathological analysis.
- Biopsies were also taken in gel-based freezing medium for molecular analysis

2.2.2.1 When pre-invasive lesions were identified

AFB was repeated at intervals according to the highest grade of pre-invasive lesion in the patient's airway:

- For SD or CIS, bronchoscopy was performed every 4-6 months
- For hyperplasia (HYP), metaplasia (MET), MID, MOD or normal epithelium, bronchoscopy was performed every 8-12 months
- The index lesion was biopsied on each occasion, as were any other lesions previously known to harbour pre-invasive pathology.
- Any newly identified lesions were also biopsied and kept under surveillance

2.2.2.2 When invasive lesions were identified

- The patient management was directed by a lung cancer multi-disciplinary meeting
- Surveillance with AFB and CT continued after treatment for the detected cancer had been completed with appropriate data censoring

2.2.2.3 When the bronchoscopy was normal

- Three biopsies were taken from the area of the index lesion(s) and a control normal site in the airway
- Patients were offered surveillance on the basis of being a high-risk cohort, but outside this current study

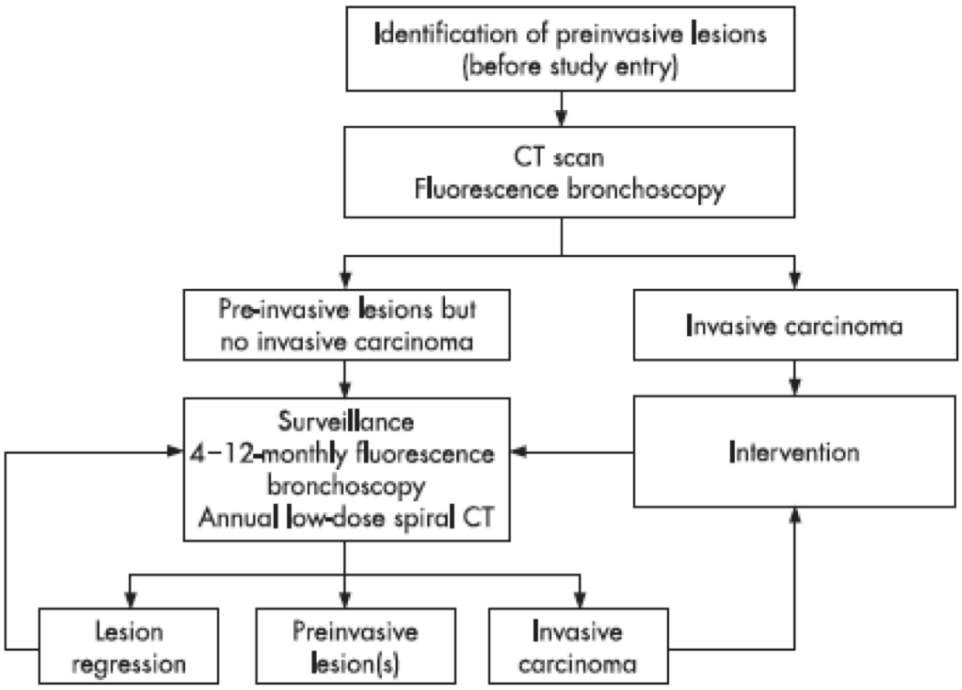


Figure 2.2– Longitudinal surveillance of patients with pre-invasive disease study schema

2.2.3 Study outcomes

2.2.3.1 Primary Outcome

The primary outcome measure was the progression to invasive lung cancer, which was defined as:

- site-specific progression, defined as a microinvasive squamous cell carcinoma originating from the index endobronchial pre-invasive lesion detected on AFB
- metachronous central airway cancer, defined as a squamous cell carcinoma occurring at another distinct site in the tracheobronchial tree detected on AFB surveillance
- metachronous parenchymal cancer, defined as an interval primary lung cancer (any pathology), which was either histologically proven or based on clinical suspicion as agreed in the lung cancer multi-disciplinary meeting.

2.2.3.2 Secondary outcomes

- Identify the clinical risk factors that affect the development of lung cancer
- Assess whether a low- or high- grade pre-invasive lesion predicts the risk of lung cancer
- Establish whether a longitudinal surveillance programme can identify lung cancers at an early stage
- Determine the cancer-free survival in patients with pre-invasive disease of the airway
- Determine the overall survival in patients with pre-invasive disease

2.2.4 Statistical analysis

For the purpose of this study, pre-invasive lesions identified were dichotomised into two groups, either (i) low-grade lesions (LGL), which include mild (MID) or moderate (MOD) dysplasia, or (ii) high-grade lesions (HGL), which include severe dysplasia (SD) and carcinoma in situ (CIS). The rationale for this is described in Chapter 1 and is consistent with the previous publication from a smaller number of patients from this cohort [16]. Patients with more than one lesion of LGL and HGL, were grouped according to the highest grade found. Individuals with insufficient follow-up (<3 months) were excluded.

Differences between groups with nominal variables were estimated using either Chi-square or Fisher's exact. Differences in numerical dependent variables were analysed using the independent two-sample t-tests or Mann Whitney U for non-parametric data. The cancer-free survival (CFS) analysed was done using the Kaplan-Meier method and calculated from the date of first AFB or histopathological confirmation when pre-invasive disease was found at a resection margin, to the date when the first cancer (bronchial or parenchymal) was detected (i.e. time to event). Censoring was performed when subjects were lost to follow-up or due to death. The overall survival (OS) was calculated for all subjects from the date of first AFB examination or histopathological confirmation on a resection margin to the date of death (time to event). Censoring was performed to the date subjects were lost to follow-up. Survival analysis was again performed using the Kaplan-Meier method. A cox proportional hazards analysis was then performed with elimination of any non-significant variables. Clinical factors that could affect cancer occurrence or death were identified and dependence between these variables was adjusted to determine the risk. For all analyses, a p values of 0.05 or less was considered statistically significant. All statistical analyses were performed using SPSS Statistics software (version 24.0; IBM).

2.3 Results

2.3.1 Study population

Of 124 individuals at risk for lung cancer, we excluded 12 patients who had microinvasive cancer at their initial AFB and CT assessment and 17 who had no lesions seen in their bronchial tree. Eighty-three subjects had one or more pre-invasive endobronchial squamous lesion(s) detected on their initial autofluorescence bronchoscopy (AFB). Of these, 6 subjects were excluded from the analysis on the basis of insufficient follow-up (<3 months), leaving a cohort of 77 subjects with pre-invasive endobronchial squamous lesions (Figure 2.3).

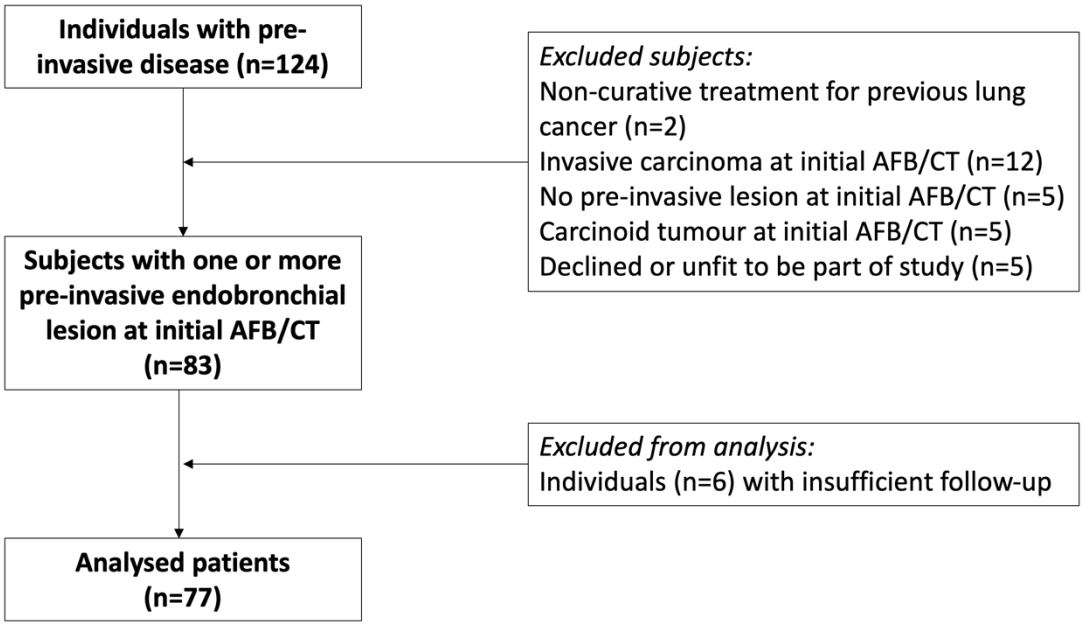


Figure 2.3–Study population flow chart

2.3.2 Patient demographics at time of referral

A total of 77 individuals (64 men and 13 women) were eligible for the study and had an average age of 65 ± 8.8 years at the time of their first AFB. Sixty-eight (88%) of patients were either current or former smokers; only 3 (4%) were never smokers with data missing for the other 6 patients. Sixty-one percent (n=47) had a diagnosis of COPD and a total of 33 individuals had previous curative treatment (surgical resection or radical radiotherapy) for non-small cell lung cancer (NSCLC) and/or head and neck squamous cell cancer. Since previously NSCLC and cancer of the head and neck are risk factors for developing lung malignancy, the patient demographics, clinical and smoking history, and surveillance data are shown in these two groups in Table 2-1. Overall, there were no significant detected differences in age, gender, smoking status, and presence of COPD between the two groups.

At the first baseline autofluorescence bronchoscopy, low grade lesions (LGLs) were found in 25 individuals and high grade lesions (HGLs) in the other 52 patients. A median of two pre-invasive lesions (range 1–5) per patient was identified on the baseline AFB. The distribution of LGL and HGL was similar in patients with and without cancer history (10 LGL and 23 HGL vs. 15 LGL and 29 HGD, respectively; p = 0.73).

Individuals were in the study for surveillance period of 53 months (range 3 – 196 months). During this time, a median number of 7 (2–28) AFBs and 5 (1–19) CTs were undertaken in each individual over the course of the study. There was no difference in how many scans or bronchoscopy a patient had whether they had previously been treated for a previous lung or head and neck squamous cell lung cancer.

Characteristic	Total	No previous NSCLC	Previous radical treatment for NSCLC	*P* value
Individuals, n	77	44	33	
Age	65±8.8	63.6±9	67.6±8	0.31
Gender				
Male	64	36	28	0.73
Female	13	8	5	
Smoking Status				
Never	3	3	0	0.29
Ex-smoker	55	30	25	
Current smoker	13	8	5	
Unknown	6	3	3	
COPD Status				
COPD	47	24	23	0.48
No COPD	9	6	3	
Unknown	21	10	11	
AFB, median no.	7 (2–28)	5.5 (2–24)	8 (2–28)	0.49
CT scans, median no.	5 (1–19)	5 (1–16)	7 (1–19)	0.13
Surveillance period (median months)	53 (3–196)	50 (3–196)	53 (5–171)	0.97

Table 2-1: Baseline characteristics of study patients

AFB=autofluorescence bronchoscopy, CT=computed tomography, NSCLC=non-small cell lung cancer

2.3.3 Lung cancers detected during bronchoscopy and computerised tomography surveillance

Over the surveillance period individuals were monitored with surveillance AFB and CT as described in the methods. Any lung cancer detected was fully treated then the patient would resume the AFB and CT surveillance programme. A total of 64 lung cancers were detected in 38 subjects within a median time to first cancer of 17 months (Table 2-2). In 87% of individuals (n=33), lung cancers were detected in the first 5 years of follow-up.

Annual CT imaging detected 19 (30%) of all the primary lung cancers found in the surveillance programme. There was no significant difference as to whether the patient had a previous lung or head and neck cancer diagnosed. Interval cancers detected on CT were only seen to occur in patients who had a baseline HGL. No CT detected cancers were found in those with LGLs.

Autofluorescence bronchoscopy detected 70% (n=45) of the cancers in longitudinal surveillance programme. Fourteen of these (22% of all cancers) were newly identified endobronchial lesions (metachronous AFB detected cancers), Table 2-2. Thirty-one (48% of all cancers) of the cancers detected with AFB, originated from the index pre-invasive lesion (site-specific progression). Of the 31 patients that developed site-specific progression of their index pre-invasive lesion, 28 had a baseline HGL (28/52 individuals; 53%) and 2 had an index LGL (2/25 individuals; 8%), see Figure 2.4. All metachronous endobronchial cancers developed only in those individuals with a baseline HGL (15/52 individuals; 28%); none were seen in those harbouring an index LGL.

A stage shift in the cancers diagnosed are shown in Figure 2.5 when compared to the Cancer Research UK dataset for 'cancer stage at diagnosis' in England [118]. Ninety-three percent of the cancers detected were at stage I (60/64 cancers). Three cancers were diagnosed at stage II and one was diagnosed at stage IIIB [119].

	Total ***N=38 individuals***	**No previous NSCLC**	**Previous radical treatment for NSCLC**
Any lung cancer detected	64	29	33
Metachronous lung cancer detected in parenchyma on CT	19 (30%)	10	9
Tracheobronchial lung cancer detected on AFB	45 (70%)	21	24
Site-specific progression	31 (48%)	17	14
Metachronous AFB detected cancers	14 (22%)	3	10

Table 2-2: Lung cancers detected stratified by previous cancer history

AFB = autofluorescence bronchoscopy; CT = computed tomography; NSCLC = non-small cell lung carcinoma. No significant differences detected on any dependent variable for groups 'no previous NSCLC' or 'previous radical treatment for NSCLC'.

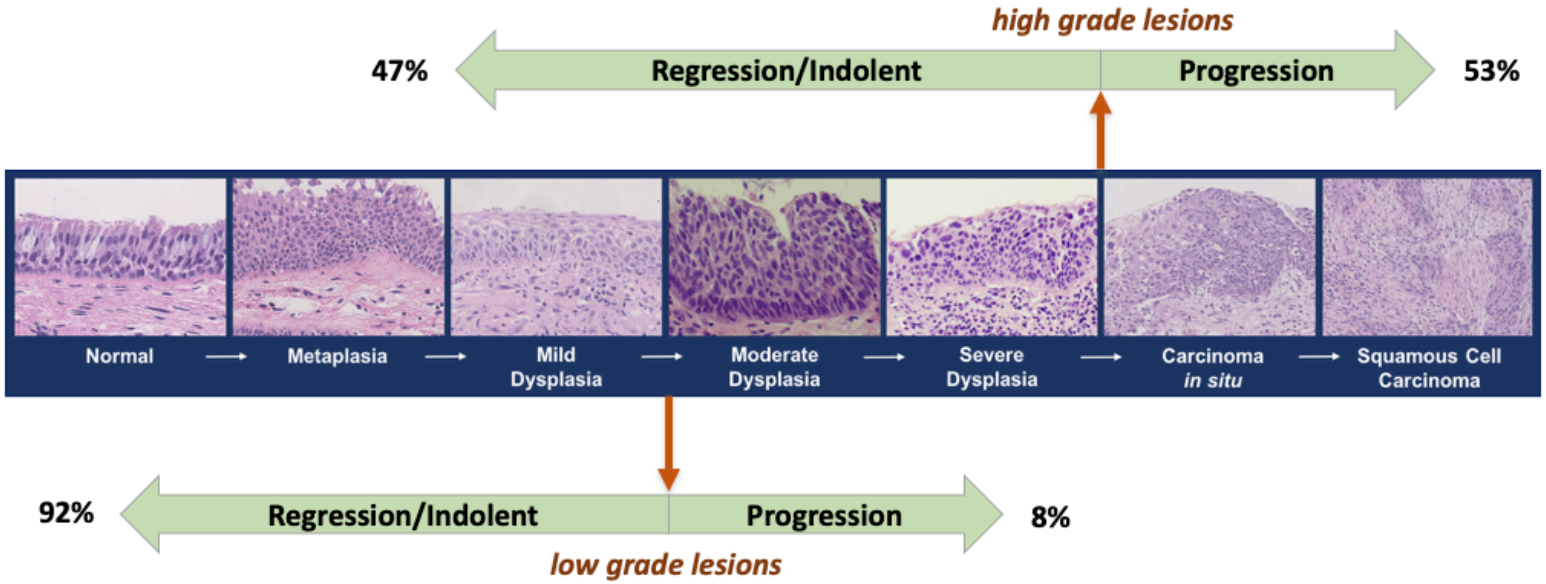


Figure 2.4 – Proportion of high- and low- grade pre-invasive lesions progressing to invasive carcinoma

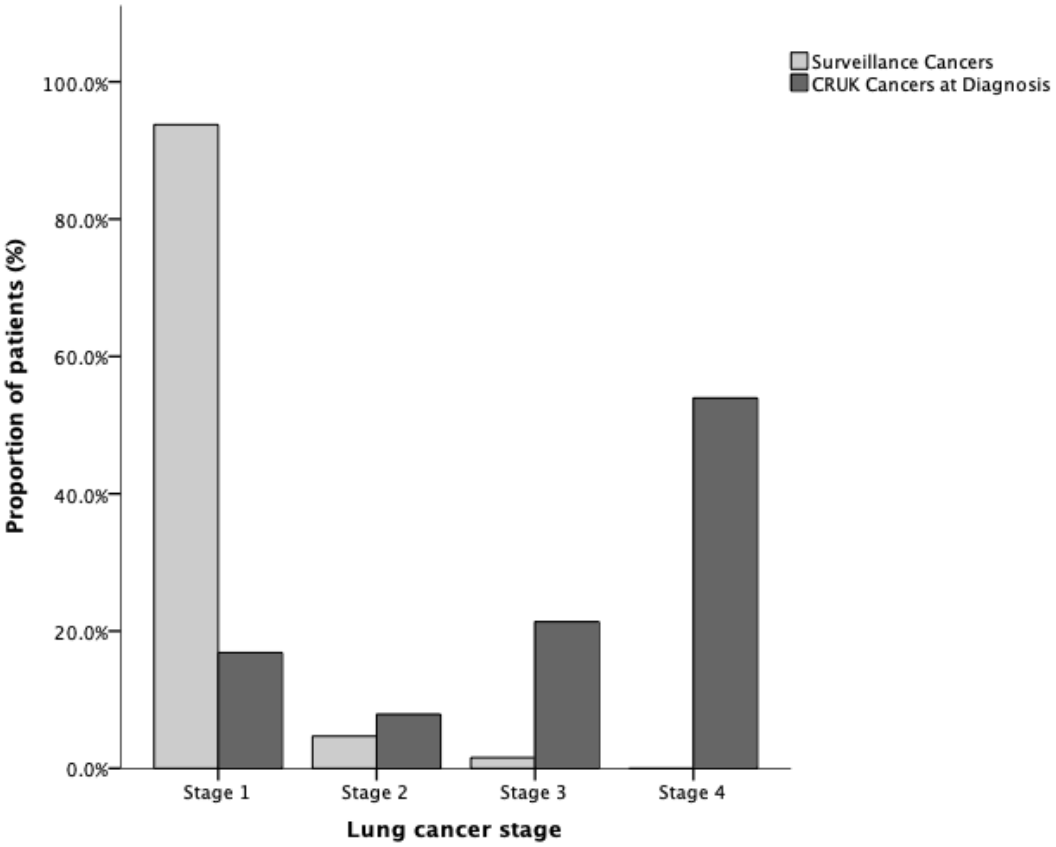


Figure 2.5 – Lung cancer stage at diagnosis for individuals in the surveillance programme compared to the routine presentation through clinic in England

Routine presentation to lung cancer through target referral pathways [118]

2.3.4 Probability of lung cancer in a 'high-risk' patients

Figure 2.6 shows the cumulative risk for all individuals in the study. The probability for developing lung cancer in this 'high-risk' un-treated cohort was 16% in the first year, 52% at 5 years, and 64% at 10 years, see Table 2-3. Patients were dichotomised into high- and low- grade depending on their baseline AFB detected lesion. For individuals who had an index HGL, the risk of any cancer developing, at any site in the lungs was 22%, 71%, and 82% at 1, 5, and 10 years, respectively. For low grade lesions these were 0%, 11%, and 22%, respectively (Table 2-3). Figure 2.7 illustrates this on a Kaplan Meier plot. The difference between HGL and LGLs is statistically significant with the log-rank for this being $p<0.01$.

A multivariate cox regression analysis was then undertaken and this showed that harbouring a baseline HGL was as an independent risk factor for developing a lung cancer (hazard ratio, 14.0; 95% confidence interval [CI], 2.8–68.5; $p = 0.001$). A history of radically treated lung or head and neck cancer, smoking status, or presence of dysplasia at resection margin after lung cancer surgery did not appear to be risk factors for cancer-free survival (Table 2-4). While a previous diagnosis of COPD did not appear to be a significant risk factor, there was a trend towards clinical significance (hazard ratio 7; 95% CI 0.9–54.5; $p=0.06$).

	Cumulative risk of developing cancer		
Time	**Overall**	**Baseline HGL**	**Baseline LGL**
1 year	16%	22%	0%
5 year	52%	71%	11%
10 year	64%	82%	22%

Table 2-3: Probability of developing lung cancer in a high-risk cohort

HGL = high grade lesion and LGL = low grade lesion

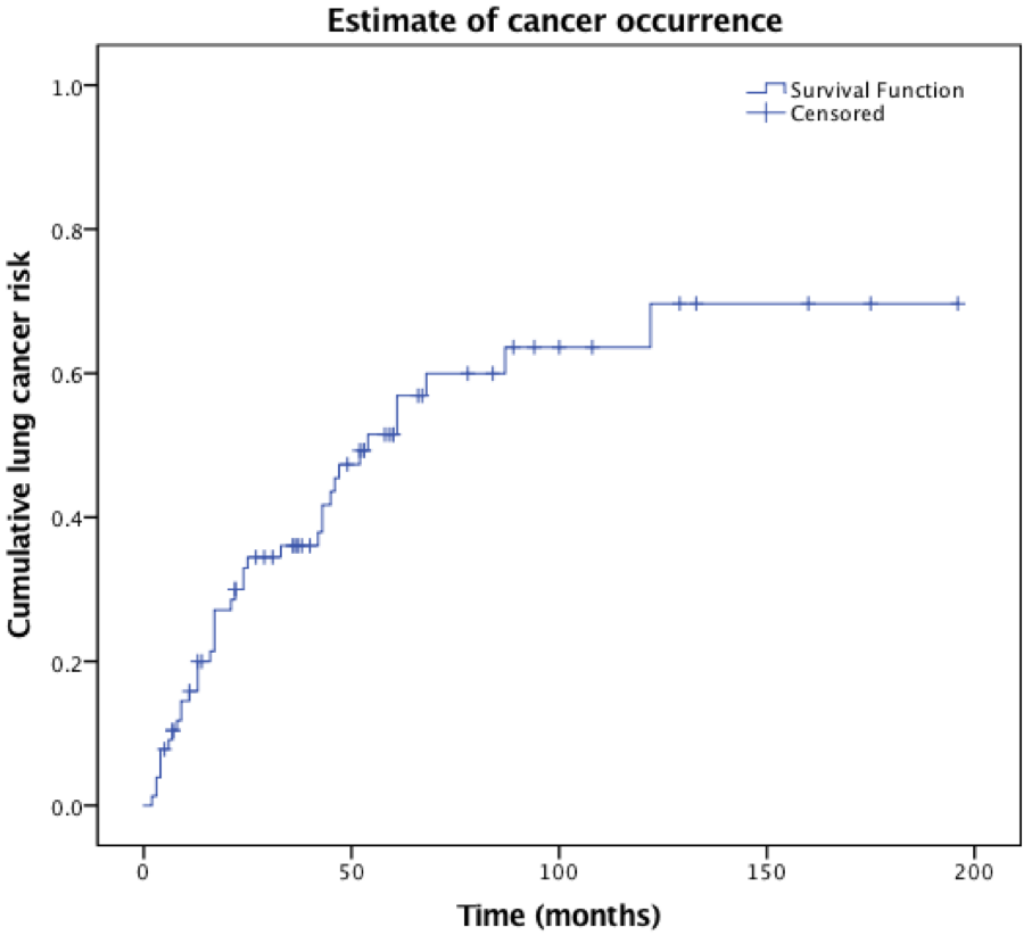


Figure 2.6– Kaplan-Meier plot to estimate of cancer occurrence in individuals with pre-invasive disease

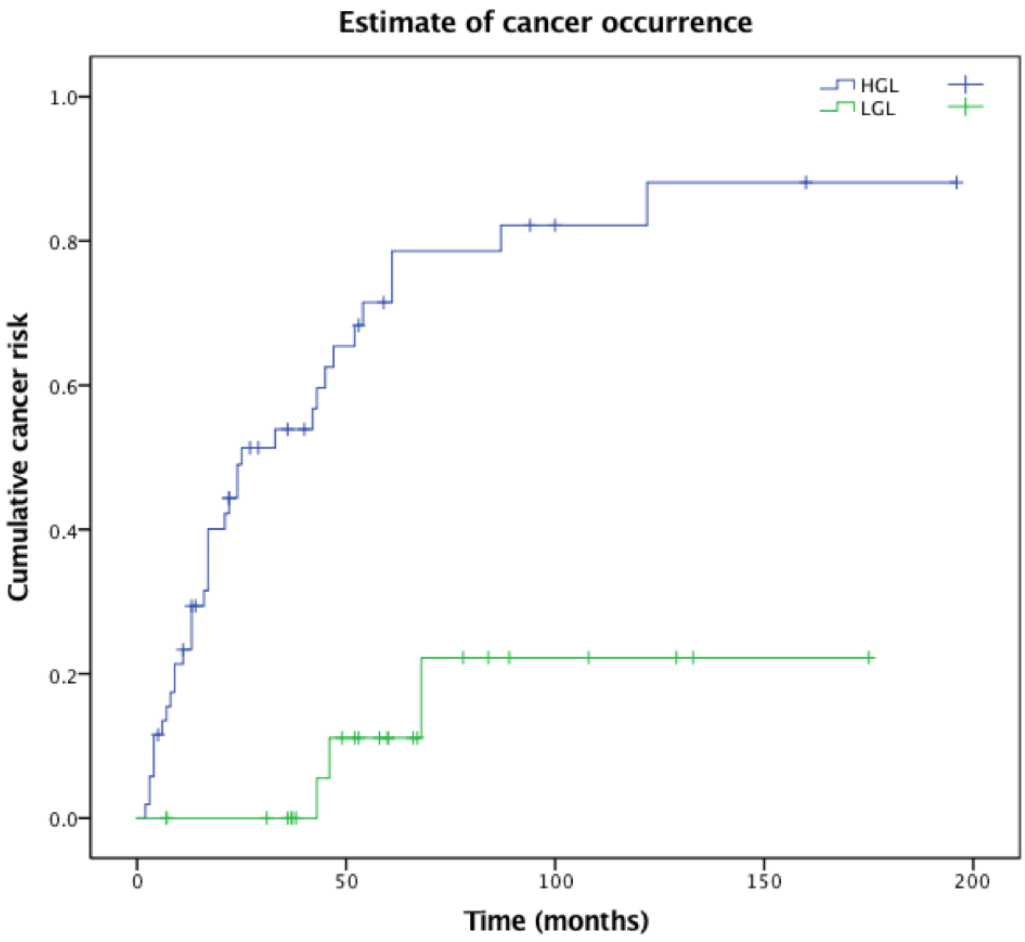


Figure 2.7– Kaplan-Meier plot to estimate cancer occurrence in individuals with baseline high- or low- grade pre-invasive airway lesions

Kaplan-Meier Log Rank <0.01

2.3.5 Causes of death in a cohort at high-risk of lung cancer

The overall 5-year and 10-year survival for these high-risk individuals is 70% and 43%, respectively. Median overall survival was 112 months (95% confidence interval [CI], 68–155 months), see Figure 2.8. Over the course of the surveillance study, 30 individuals died in total (30/77; 39% all-cause mortality). Most of these patients died as a result of lung cancer (20/77; 26% lung-cancer related mortality), and 10 died as a result of their baseline co-morbidities. Of the patients who died from lung cancer, no clear difference was observed in the groups of patients who previously had a radically treated lung or head and neck cancer (30% vs 23%; p=0.21). Using a multivariate cox regression analysis none of independent variables considered to be risks for death were found to be significant (see Table 2.4). Again a trend for increased risk of death was seen if patient had a previous diagnosis of COPD. Although, the calculated hazard ratio 5.7 (95% CI. 0.7–45) may indicate clinical significance, it was not significant (p=0.09).

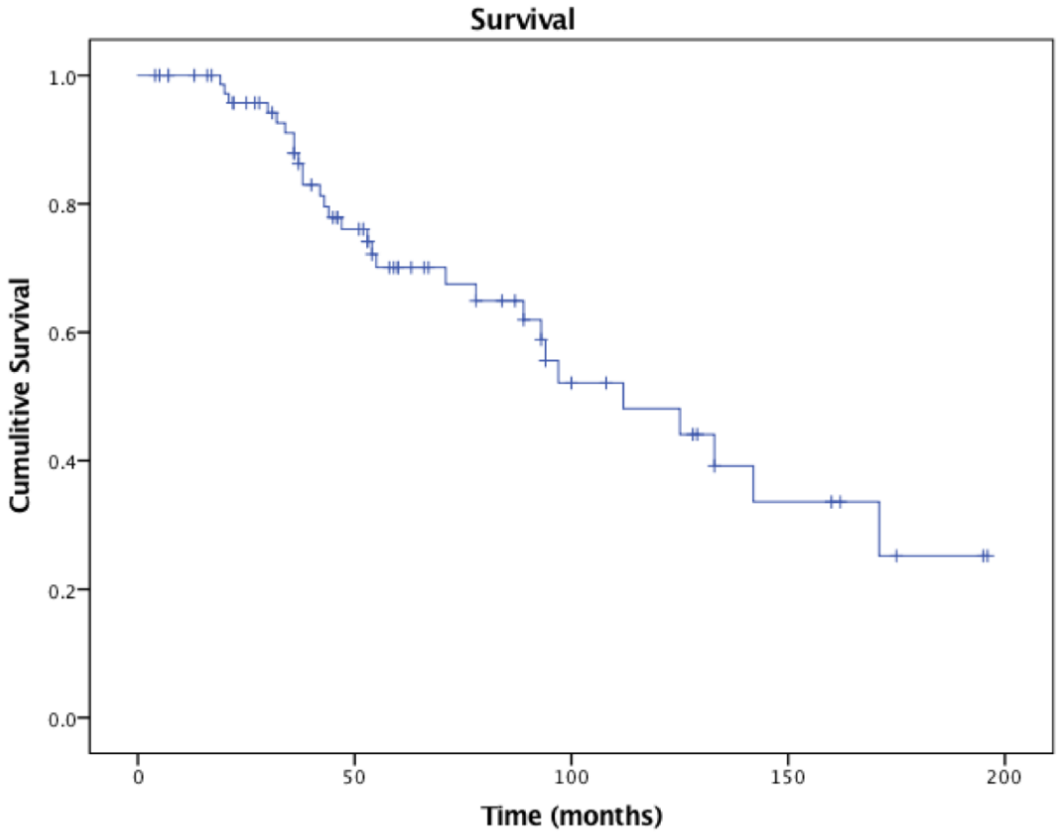


Figure 2.8– Overall survival of individuals with pre-invasive disease of the airway

Indication	Study Group	Cancer-free survival		Overall survival	
		HR (95% CI)	**p value**	**HR (95% CI)**	**p value**
Cancer history	No previous HNSCC/NSCLC vs previously radically treated HNSCC/NSCLC	0.63 (0.17–2.31)	0.48	0.27 (0.03–2.30)	0.23
Baseline histology	HGL vs LGL	14.0 (2.80–68.51)	0.001	2.16 (0.64–7.25)	0.21
Resection margin	Disease at surgical stump vs no disease at stump	1.25 (0.30–5.26)	0.76	2.64 (0.27–25.95)	0.40
COPD	No COPD vs COPD	7.00 (0.90–54.51)	0.06	5.72 (0.73–45.0)	0.09
Smoking	Ex-smoker vs Smoker	4.41 (1.10–16.31)	0.13	5.12 (0.91–21.0)	0.28

Table 2-4: Cox proportional model showing independent variables affecting cancer-Free Survival and Overall Survival

AFB=autofluorescence bronchoscopy, CT=computed tomography, NSCLC=non-small cell lung cancer, HNSCC=head and neck squamous cell carcinoma, HGL=high grade lesion, LGL=low grade lesion, CI=confidence interval, HR=hazard ratio

2.4 Discussion

In this chapter, a surveillance study that has followed a unique cohort of high-risk patients longitudinally for up to 15 years has been examined. The work demonstrates that while not all pre-invasive lesions of the airway progress, individuals harbouring this disease, especially those with high-grade lesions are at high risk for developing lung cancer. The findings from this study also show that close surveillance in a unique programme using computerised tomography and autofluorescence bronchoscopy detected cancers at a much earlier stage, when curative treatment is still possible. Finally, the work identifies a subgroup of patients with high-grade pre-invasive disease who are at most risk of developing lung cancers; not only at its original location, but anywhere in the lung.

Surveillance with annual CT and 4-12 monthly AFBs detected a total of 64 lung cancers in 38 individuals with a median time to event of 17 months. The most comparable long-term follow-up study by Van Boerdonk et al picked up 61 lung cancers in a larger cohort of 164 patients with pre-invasive disease of the central airway [17]. The higher proportion of cancers detected in this study (lung cancer detection 49% vs. 34%) is because of two major factor.: The first is the patients recruited in the Van Boerdonk study include those with baseline lesions of metaplasia (termed low-grade lesions), which are rarely seen to progress to invasive cancer (see Table 1-2 and 1-3, Chapter 1). The second is that patient with any high-grade lesions in the Van Boerdonk study have endobronchial ablative therapy to remove the lesion [17]. In this study, independent factors that could influence lung cancer occurrence such as smoking status, COPD, and a previous diagnosis of treated lung and head and neck cancer were tested. They did not appear to influence the occurrence of lung cancer, but the presence of high-grade

pre-invasive disease does seem to identify the individuals who are 'at risk' of developing cancer. Those with high-grade lesions were more likely to develop invasive cancer at the original or index (site-specific) site in the airway and were more likely to develop metachronous cancers in the central airway or lung parenchyma when compared to patients with low-grade lesions. Interestingly, 52% of all the cancers detected in surveillance, developed in a different part of the lung from the index pre-invasive lesion. Approximately one-third of all the detected lung cancers were found by computed tomography, rather than by bronchoscopy. The data indicates that high-grade pre-invasive lesions not only function as cancer precursors, but also act as a marker for lung cancer risk. These results are strikingly similar to that seen by Van Boerdonk and colleagues, who found that 59% of cancers detected in surveillance were also found at another site in the lung [17] looking at pre-invasive disease in the lung [17]. Similarly, Lee et al in their follow-up of patients with oral premalignant leukoplakia found a high incidence of metachronous pre-invasive and invasive lesions in the head and neck [120]. The findings from these studies and in this chapter support the concept of "field cancerisation", in which the entire bronchial epithelium is exposed to the carcinogenic effects of tobacco smoke [121].

Previous longitudinal studies using AFB-guided biopsy have provided some insight into how pre-invasive disease behaves over time, and it is generally accepted that high-grade lesions are more likely to progress to invasive cancer than low-grade lesions [16–18,36,49,55]. Since the progression of severe dysplasia and CIS is known to be high [18,48,51,52,57], many authors have advocated treatment with the clinical concern that invasive cancer may develop. In the present study, treatment was withheld until there was histological or radiological/clinical evidence of progression to invasive cancer in order to precisely evaluate the true malignant potential of pre-invasive lesions. Approximately half of HGLs in our study underwent site-specific progression to invasive cancer (28/52 individuals), with the

remainder of lesions remaining stable or regressing to LGLs. These findings differ from those of Venmans et al and Breuer et al who report that virtually all CIS lesions are destined to invasive carcinoma [48,49]. However, comparison of studies is complicated due to differences in study definitions, classification of pre-invasive lesions and the change in pathological definitions across the period over which these studies were performed [14,15,122,123]. In addition to inter- and intra-observer variability there maybe heterogeneity within each individual lesion.

Many pre-invasive lesions act as precursors for invasive cancer, and thus being able to reliably predict which progenitor will progress may help to determine whether intervention is necessary, and may reduce the number of AFBs and period of surveillance needed in these individuals. In line with this study, the presence of multi-focal, high-grade lesions in high-risk patients does appear to increase the overall risk of lung cancer [16,17,48], however, it is likely that increased understanding of molecular alterations and biomarkers will have the most potential in identifying high-risk lesions the future. Van Boerdonk et al applied a molecular classifier based on copy number alterations to a set of 36 ‘high-risk’ individuals, showing that the classifier predicted progression to CIS with an accuracy of 92% (CI 77-98%); with a negative predictive value of 89% [68]. While this proof-of-concept was shown in LGL progression to CIS, its clinical performance in development of invasive lung cancer would have the greatest potential. The additional research samples collected from this cohort has also informed a predictive model that confidently identifies, which lesions will progress to invasive cancer through progression-specific methylation changes on a background of widespread heterogeneity, and a strong chromosomal instability signature [124].

The treatment (surgical resection or endobronchial) of pre-invasive epithelial lesions remains controversial. The American College of Physicians and other

authors advocate surgical treatment for CIS and early lung cancers in the airway [4,6,7,87]. Surgery for patients with such early stage disease is associated with 5-year survival rates in the region of 90% [4,6]. Despite these lesions being small, their central location means around 70% of individuals require a lobectomy, and the remaining either a bi-lobectomy or pneumonectomy for curative resection [4]. This approach carries appreciable morbidity and mortality, which is difficult to justify when there is no guarantee that a pre-invasive lesion will progress to invasion. Further, as we have shown in this study, individuals are at risk of developing metachronous cancers and consequently may not have sufficient reserve to undergo further resection. This dilemma has been somewhat overcome by other investigators managing CIS with endobronchial treatments, thereby avoiding lung resection [53–55]. Van Boerdonk and colleagues in their recent study where lesions were treated with endobronchial therapy, showed a site-specific progression rate (to invasive cancer) of 18% for high-grade lesions (SD and CIS). These results are comparable to other natural history studies that included treatment of high-grade lesions with progression rates of 18-43% [17,48,49,51,53]. While many investigators do report good results with a variety of endobronchial treatments, these studies are often small, with short follow-up, and frequently combine progression to invasive and high-grade pre-invasive histology as a single end-point [7,53,55]. Since none of the studies have included a control arm [48,51,55,60,88], the natural history of the lesions treated in these studies is not known, and the clinical and prognostic value of the intervention remains unclear.

In this study, maintaining combined surveillance with CT and bronchoscopy ensured that lung cancers were identified at an early stage (stage I and II), were definitive curative treatment could be then offered to the patient. This is in stark contrast to the late disease stage presentation at diagnosis in England, where around 1 in 5 patients present early enough to have curative treatment [118]. Close surveillance in this study has allowed patients to have treatment for early cancers as has been

shown by other groups [16,17,36,53]. Individuals would then continue in the programme, which allowed further early cancers to also be diagnosed (64 cancers detected in 38 patients) in this high-risk cohort. In this study, individuals harbouring a HGL have significant risk of developing lung cancer. In our untreated cohort of HGLs, the 5-year cumulative cancer risk was 71%, as compared to 39%, as seen in Van Boerdonk et al treated high-grade disease cohort. While differences in cancer incidence could be explained by factors, such as differences in population risk, institution care delivery and follow-up duration, this difference really raises questions as to how subjects with these high-grade lesions should be managed, and it certainly would appear that treatment reduces the risk of lung cancer. It is unknown whether this effect is purely as a result of reduction in site-specific cancers and therefore raises a further interesting question of whether effective treatment of an index lesion would reduce the occurrence of cancers at remote sites in the lung. It has previously been proposed that the precancerous field is monoclonal in origin [125,126]. In 5 patients within a nested cohort of untreated high-grade lesions followed longitudinally, we have shown cells from CIS lesions are capable of migrating across histologically normal epithelium and establishing new clonal lesions [127]. By detecting a rare somatic *TP53* mutation we demonstrated multi-focal high grade lesions were derived from a common clonal ancestor; and since neighbouring mucosa was normal (p53-wild type), propose that clonal migration occurs across the airway epithelium.

This study had several limitations that need to be considered. Firstly, the relative size of the cohort is small, although probably one of the largest internationally. We report a high incidence of lung cancer in this group, because patients are mostly referred to our institution after finding abnormal sputum cytology or detection of lesions on white light bronchoscopy, hence referred to as a ‘high-risk’ cohort in this chapter. The cancer incidence would significantly differ in a screening setting where

individuals are selected based on clinical risk profiling followed by examination of the airway with AFB [128]. Secondly, it is assumed that biopsy specimens taken from pre-invasive lesions do not influence the outcome of that lesion. It has been suggested that bronchial biopsies may completely remove some lesions, but, while this possibility cannot be discounted, it is unlikely as most lesions appeared considerably larger than the area sampled by biopsy. Thirdly, there is missing data on co-morbidities that could influence some of the conclusions drawn on the importance of COPD. Finally, it is possible that treatment of invasive lesions (e.g. radiotherapy) may have affected the behaviour of adjacent pre-invasive lesions. As a result, we have carefully censored and discarded lesion data if we anticipated an effect from any treatment the individual may have received.

2.5 Conclusion

In conclusion, a high incidence of the lung cancer is found in 'high-risk' individuals with pre-invasive disease of the central airways. Close surveillance with autofluorescence bronchoscopy and computerised tomography identifies cancers at an early stage when treatment with curative intent can be offered. Individuals with changes of severe dysplasia and carcinoma in situ are at particular risk, but the treatment of these lesions remains controversial. Endobronchial treatments have the advantage of conserving lung tissue and are therefore an attractive therapy option. However, there is no data on which treatment modality is superior for pre-invasive lesions and there are major limitations of existing cohort studies, precluding any conclusions that can be drawn on efficacy of bronchoscopic intervention. Therefore, the analysis in the chapter highlighted the clinical need for establishing the efficacy of an endobronchial treatment of pre-invasive lesions in a randomised controlled trial.

Chapter 3 LONG-TERM OUTCOME OF EARLY CENTRAL AIRWAY LUNG CANCERS TREATED WITH PHOTODYNAMIC THERAPY

3.1 Introduction

Squamous cell lung cancers remain the second most common type of non-small cell lung cancer in the United Kingdom [2]. They tend to arise most commonly in the trachea and proximal bronchial tree; while this makes them roentgenographic negative [4], they can be readily detected by bronchoscopy, and specifically autofluorescence bronchoscopy remains the most sensitive method of diagnosis [25,32,129].

As discussed in the previous chapter, the early detection and accurate staging of these cancers is critical since early invasive cancers (stage 1) carry a good prognosis and are suitable for treatment with radical intent [6]. Surgery for patients with early stage microinvasive disease is associated with a 5-year survival rate in the region of 90% [3–5] and so there is no question to its value. However, we are often faced with a clinical scenario where the patients co-morbidities, their lung disease, and their performance score prevents them from having these treatments. Further, as shown in chapter 1, these individual are at risk of getting interval cancers in their lung, which make increasingly difficult to offer radical treatments such as surgery and radiotherapy. As a result, there has been significant interest in developing bronchoscopic treatment modalities for early micro-invasive cancers [55,91,112,130,131].

The common techniques employed include electrocautery, argon plasma, laser therapy, and intraluminal brachytherapy as discussed in chapter 1. However, the most common is photodynamic therapy (PDT): a dual step process that begins with

the systemic administration of a photosensitising agent with selective uptake in tumour cells [105]. After a latent period of approximately 24 to 72 hours, depending on the photosensitiser used, the drug is activated by the application of a specific wavelength of laser light directly to the tumour cells. Absorption of the photons causes transfer of energy to release oxygen species that cause cellular damage and apoptosis .

The essential requirement of curative PDT is that the disease is confined to the therapeutic range of the laser. This means that the lesion must not extend beyond the reach of the laser fibre, it must not extend beyond the bronchial wall and accurate staging should exclude nodal and extra thoracic metastases. Furukawa et al. reported it was important to accurately define the tumour extent and depth before PDT, based on their analysis of 114 treated cases of central located early lung cancers [95]. In particular they noted the importance of the depth of invasion of the bronchial wall and achieved high levels of success when the tumour was confined to the mucosa or submucosa. Further, colleagues from VU Medical, Amsterdam reported that autofluorescence bronchoscopy improved the staging of cancers that are not detected on CT, which also has an impact on the therapeutic strategy [88].

Photofrin is approved by the United States Food and Drug Administration (FDA) and has been used in the treatment of early stage lung cancers as well has oesophageal, head and neck, and advanced lung cancers [106,107,132,133]. However, the selective uptake is not limited to tumour cells and following administration patients can develop skin burns and are required to stay indoors in a darkened environment for 30 days following administration. Second generation photosensitisers, such as chlorin e6 products, have since been developed, which have a far more favourable side-effect profile [134–136].

The longitudinal surveillance programme at UCLH identified there may be a need for endobronchial therapies to tackle increased detection of the of early stage lung cancers. Consequently, patients with early stage cancers who were not suitable for conventional therapy underwent photodynamic therapy. In this chapter, a prospective phase II descriptive cohort study to evaluate the efficacy of PDT treatment for microinvasive cancers of the tracheobronchial tree is presented.

3.2 Methods

3.2.1 Hypothesis

Photodynamic therapy is an effective treatment for early stage, radiographically negative carcinomas of the bronchus who are considered unsuitable for surgery or radiotherapy with radical intent.

3.2.2 Objectives

- Evaluate the ability of photodynamic therapy to eradicate invasive carcinoma of the bronchus
- Determine any clinical or bronchoscopic factors that affect PDT efficacy
- Evaluate the rate of recurrence of disease through close bronchoscopic surveillance
- Assess the cancer-specific mortality

3.2.3 Patient selection

In our tertiary care referral centre, autofluorescence bronchoscopy (AFB) was used to localise and stage the mucosal extent of early pre-invasive lesions within the bronchial tree of patients who were referred from other hospitals because of their perceived high risk for primary lung cancer or cancer recurrence. Patients enrolled on the longitudinal surveillance bronchoscopy programme between 1st January 2000 and 1st December 2018, underwent annual CT Thorax with contrast and autofluorescence bronchoscopy with either the D-Light autofluorescence bronchoscope (Karl Stortz, Germany), or Olympus autofluorescence AFI-Lucera (Olympus, Tokyo, Japan) as described in Chapter 2.

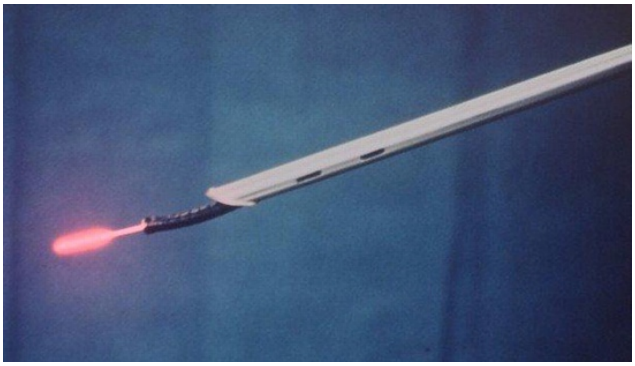
Individuals diagnosed with microinvasive squamous cell lung cancers on bronchoscopy were considered for photodynamic therapy treatment after a lung cancer multidisciplinary meeting agreed this was the (i) best treatment strategy, (ii) other treatment modalities had been exhausted, or (iii) the patient chose to have this treatment. The participating centre was University College London Hospital. Ethical approval was granted by the Joint UCL/UCLH Committees on Ethics of Human Research (REC 01/0146).

3.2.3.1 Inclusion criteria

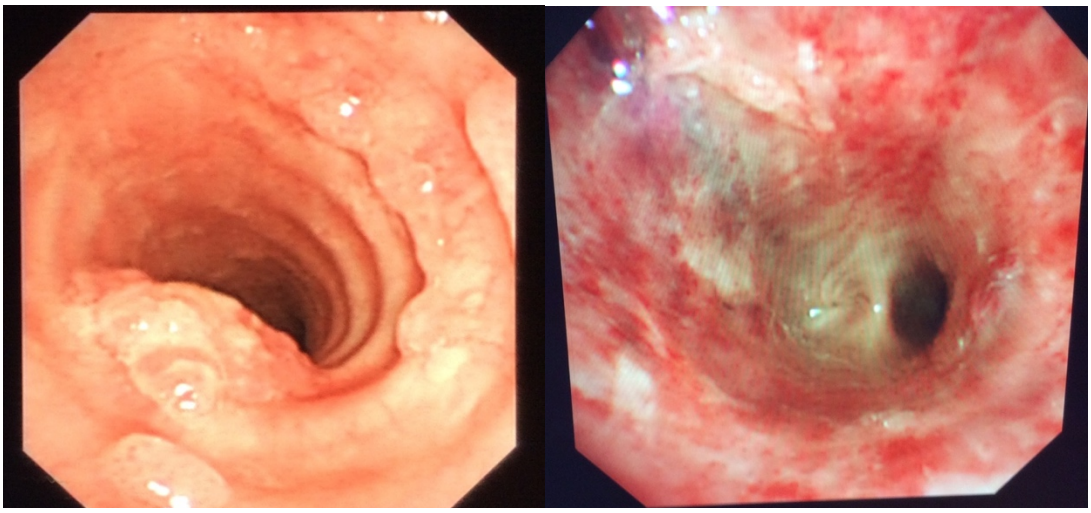
- Patients with stage 1 (T1N0M0) non-small cell carcinoma of the bronchus whose entire length is accessible to the bronchoscope.
- Medically inoperable, unable to have radiotherapy with radical intent, or those who decline conventional treatment

3.2.3.2 Exclusion criteria

- Patients who are medically fit and willing to undergo surgical resection or radical radiotherapy
- Tumour has visible extra bronchial component on CT
- CT, PET, or pathological evidence of secondary spread to peri-bronchial lymph nodes
- Specific contraindication or allergy to use of photosensitiser (Porfimer or chlorine e6)
- Failure to give informed consent



(A)



(B) (C)

Figure 3.1 – Photodynamic therapy delivery to early tracheal cancer

(A) = Photodynamic catheter in working channel of bronchoscope past through a rigid bronchoscope

(B) = Early tracheal micro-invasive carcinoma (before treatment)

(C) = Extensive response to photodynamic therapy (day 1 after treatment)

3.2.4 Intervention

The PDT protocol consisted of intravenous administration of either Photofrin (Porfimer Sodium) or Fotolon (Chlorine e6) activated by the correct wavelength using a semiconductor diode laser. At UCLH, we switched to Fotolon in 2014, as Photofrin was no longer available and the side-effect profile of Chlorine e6 was better.

3.2.4.1 Photofrin

Porfimer sodium 2mg kg^{-1} was administered intravenously in a darkened room and all precautions to protect the patient from light sensitivity were taken. Autofluorescence bronchoscopy was undertaken 40-50 hours following administration.

Application of light was performed using a cylindrical fibre-optic diffuser passed through the bronchoscope and placed across the lesion to be treated. When there was exophytic disease the tip was allowed to sit within the soft tumour. The diffuse tip length was chosen to match the length the tumour as assessed under AFB. A light dose of 200Jcm^{-1} of tumour length was delivered using a 639nm semiconductor laser and calculated from the equation below:

$$\text{Treatment time (seconds)} = \frac{\text{light dose } (200\text{Jcm}^{-1}) \text{ x treatment area}}{\text{total power output (W)}}$$

24 to 48 hours after the initial treatment, repeat bronchoscopy to remove plugs, necrotic tumour debris and debridement was undertaken. A further light dose was applied according to the treating physician's discretion if it was felt there was visible tumour still present.

3.2.4.2 Fotolon

Chlorin-e6 trisodium salt (Fotolon) at 1mg/kg body weight was administered intravenously dissolved in 200 ml of 4.5% human albumin solution.

Bronchoscopy was then undertaken following the optimal uptake of Fotolon® of 3-4 hours after administration.

The lesion was illuminated with light wavelength of 665 nm (±5 nm) delivered through 600 micron diameter quartz fibres inserted through the working channel of the bronchoscope. The bronchial lesions were treated at 150 J/cm length of diffuser at a rate of 400 mW/cm of diffuser over a period of 375s. In the case a cylindrical fibre could not be used, e.g., access to a particular lesion was difficult to cover with cylindrical illumination; a microlens fibre was utilised with a fluence of 100 mW/cm^2 given over 700s, giving a dose of 70 J/cm^2.

As above, patients underwent further bronchoscopy 24-48 hours after the initial treatment to remove any necrotic tumour debris.

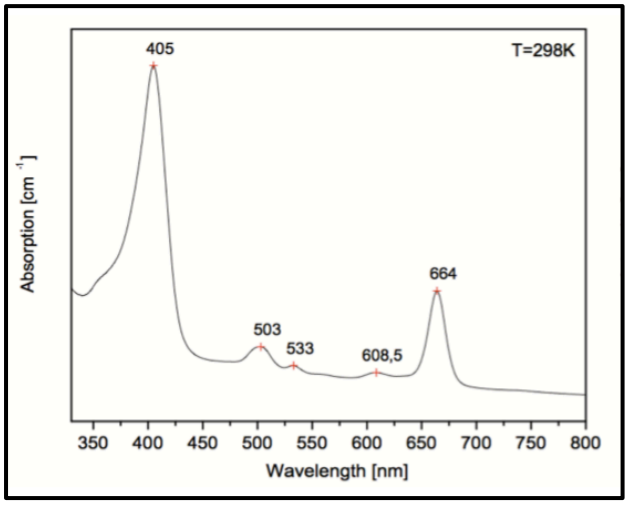


Figure 3.2 – Absorption wavelength for chlorine e6 (Fotolon)

3.2.4.3 Bronchoscopy

In all cases, PDT was delivered using flexible and or rigid bronchoscopy under deep sedation delivered by an anaesthetic professional according to the BTS guidelines [137]. The optical light delivery fibre was introduced through the working channel of the flexible bronchoscope. The patient then underwent a surveillance bronchoscopy at 8 (±4) weeks to assess the response to PDT.

When normal epithelium or a low-grade lesion was identified, i.e. when histological response was detected after PDT, a repeat AFB will be performed near as possible to 4-6 months after treatment and if the response was maintained, surveillance continued as described in Chapter 2. When micro-invasive cancer or high-grade lesion was identified the patient was managed as directed by the lung cancer multi-disciplinary meeting (MDM).

3.2.5 Outcomes

3.2.5.1 Primary Outcomes

The primary outcome measure was the response to photodynamic therapy at 8 weeks following treatment as defined by:

Complete response: refers to no pathological invasive cancer or regression to a low-grade dysplastic lesion on biopsy

Partial response: refers to no evidence of endobronchial disease present, but microscopic invasive disease or high-grade dysplasia at the treated site

No response: refers to the cancer remaining unchanged pathologically

Progression: refers to progression of the cancer detected as an increased soft tissue component seen on bronchoscopy.

3.2.5.2 Secondary outcomes

- Factors that may influence response to photodynamic therapy
- Observed complications from photodynamic therapy
- Local recurrence after treatment with PDT
- Overall survival in patients treated with PDT

3.2.6 Statistical analysis

Any categorical variables were expressed as proportions and scale variables as means. Differences between groups were estimated using chi-square, Fisher's exact, independent two-sample t-tests, and Mann Whitney U tests. For analysis, lesions response was addressed with binomial function (success = complete response to PDT and no success = partial response or no response to PDT). A univariate analysis was then conducted to look at independent factors that may affect the success of treatment. The effect size for these were presented as odds ratios and $p < 0.05$ was considered significant. Overall survival (OS) was calculated for all subjects from the date of PDT treatment or histopathological confirmation of invasive cancer, to the date of death (time to event) or to the date subjects were lost to follow-up. Survival analysis was performed using the Kaplan-Meier method. Cox proportional hazards analysis with backward-stepwise elimination of non-significant parameters was performed to identify potential risk determinants and correct for dependence between determinants. For all tests, a p value of less than 0.05 were was considered statistically significant. All statistical analyses were performed using SPSS Statistics software (version 24.0).

3.3 Results

3.3.1 Study population

Forty five individuals were referred to University College London Hospital for consideration of endobronchial therapy for early central airway lung cancer (ECALC) or carcinoma in situ (CIS). Twenty-six subjects were felt to meet the inclusion criteria for this study; with 9 individuals excluded as they were thought to be fit to have radical radiotherapy or surgical resection, 6 were thought to have extensive extra-bronchial extension of tumour, and 4 had evidence of nodal disease. Of these, 2 subjects were excluded from the analysis on the basis of one having adenoid cystic carcinoma, and the other having a human papilloma viral driven lesion of carcinoma in situ. This left a cohort of 24 subjects with early central lung cancer for treatment with photodynamic therapy (Figure 3.3).

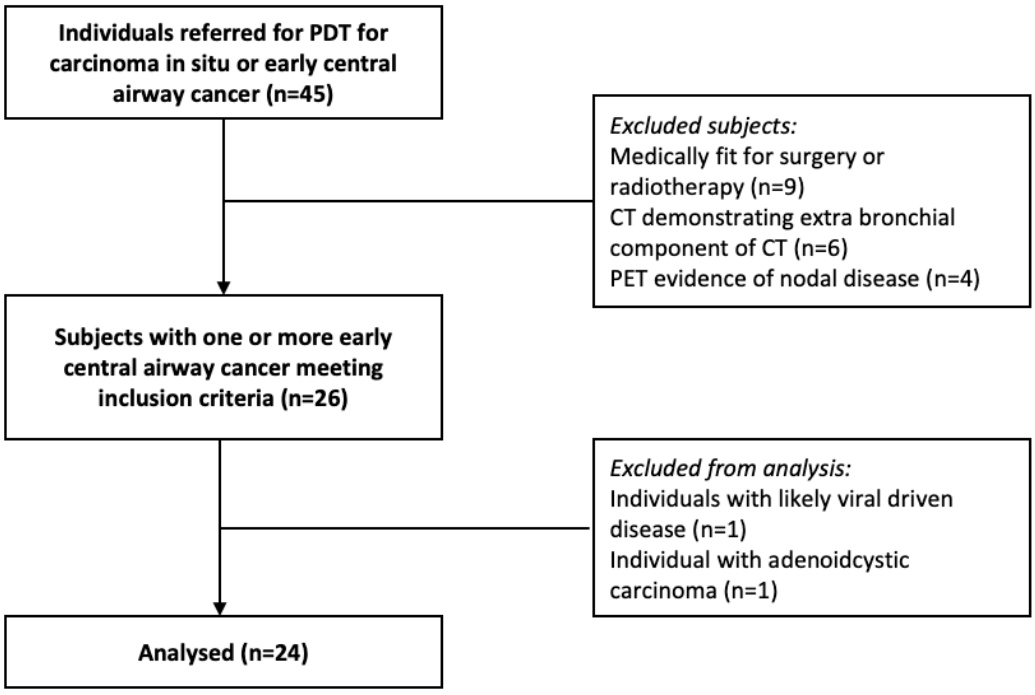


Figure 3.3 – Study population flow chart

3.3.2 Baseline characteristics

A total of 24 individuals (21 men and 2 women) were eligible for the study and were kept under clinical and bronchoscopic surveillance for a mean period of 45.9±7.9 months. The demographic and clinical variables are shown in Table 3.1. The mean subject age at study entry was 70 (55-80) years old. A total of 19 individuals had no prior diagnosis of lung cancer and their lesions were detected either through the surveillance programme as discussed in the previous chapter or because they were referred after a diagnosis was made at the patient's local hospital. Five patients had previous surgery or radical radiotherapy for previous lung cancer, with no lesions been detected either at their stump (n=2) or a metachronous site.

Since many patients were selected as they were felt to be medically unsuitable for conventional therapy, we can see there is an even distribution of subjects with a performance status of 2 or under, or above 2. There were also no significant detected differences in age, gender, smoking status, and the presence of COPD between the 2 groups

Seventeen of the individuals enrolled onto the study were discussed in the lung multidisciplinary meeting and were felt to be medically unfit to undergo a surgical resection or to have radical radiotherapy. 5 patients had previously undergone radical radiotherapy or surgery and a tissue-sparing approach was required and 2 individuals had chosen to have photodynamic therapy/conventional therapy. There was a significant difference with the choice of photosensitiser with 19 individuals treated with Photofrin (Porfimer) and 5 received Fotolon (Chlorine e6), which is taken into account as a limitation in the study analysis.

	Total	**No previous diagnosis of non small cell lung cancer**	**Previous surgery or radiotherapy for non small cell lung cancer**	***p* value**
Individuals (n)	24	19	5	
Age at baseline (years)	70 (55-80)	70 (55-78)	72 (68-80)	0.29
Gender				
Male	21	18	4	0.38
Female	2	1	1	
Smoking status				
Current smoker	6	6	0	0.28
Former smoker	18	13	5	
COPD status				
COPD	17	13	4	0.54
No COPD	7	6	1	
Performance Status				
≤ 2	16	12	4	0.63
> 2	8	7	1	

Table 3-1: Baseline characteristics of study patients

	Total	**No previous diagnosis of non small cell lung cancer**	**Previous surgery or radiotherapy for non small cell lung cancer**	***p* value**
Why PDT chosen for treatment				
Medical	17	17	0	**<0.01***
Previous lung cancer treatment	5	0	5	
Patient choice	2	2	0	
Photosensitiser				
Porfimer	19	17	3	**0.04***
Chlorine e6	5	2	3	
Surveillance period (months)	45.9±7.9	50.3±9.6	29.0±6.6	0.28

Table 3-1: Baseline characteristics of study patients (continued)

3.3.3 Lesion characteristics and response to photodynamic therapy

A total of 28 lesions were treated with photodynamic therapy in 24 subjects. Most patients received Photofrin (82% of lesions) and the predominant baseline histology was of squamous cell carcinoma. There was a similar proportion of squamous cell carcinoma baseline pathology between distributed between the two photosensitisers (p=0.74). The baseline lesion length was dichotomised into > 15 mm or <15 mm and there was no significant difference in lesion length distributed across the two photosensitisers (p=0.40).

Among the treated lesions, three quarters had a complete response to treatment with either Photofrin or Fotolon. Five lesions had a partial response, whereby regression of size of lesion or a pathological regression to high-grade preinvasive disease was seen. Two lesions did not respond to photodynamic therapy in patients treated with Porfimer (Figure 3.4).

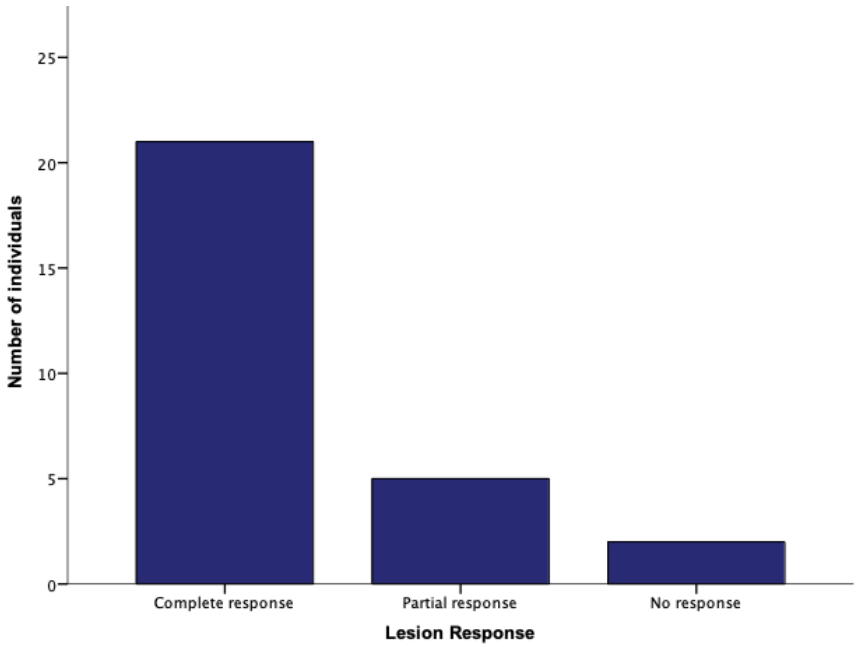


Figure 3.4 – Lesion response to photodynamic therapy

A significantly larger proportion of lesions showed a complete response to Porfimer when compared to Chlorine e6. 80% of individuals who received Photofrin had a complete response compared to 40% who received Fotolon (p=0.02), see Figure 3.5.

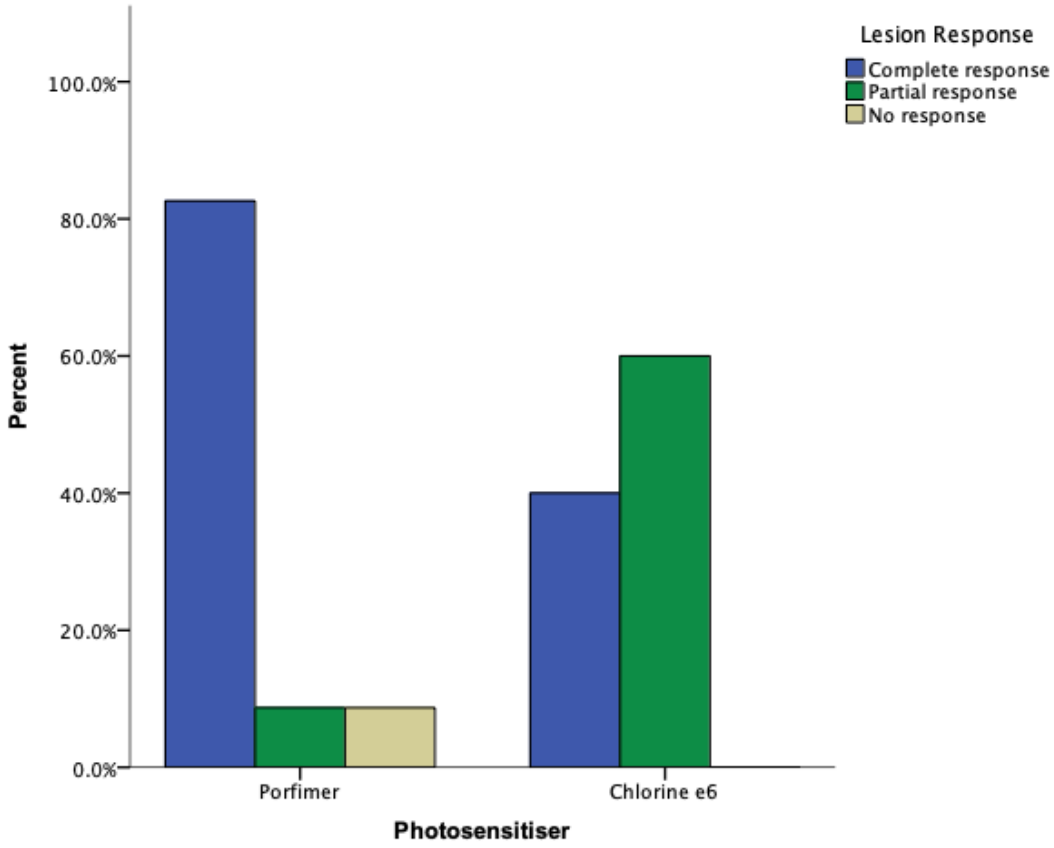


Figure 3.5 – Lesion response to either Porfimer or Chlorine e6

3.3.4 Predictors of response

Lesions were stratified to a 'successful' photodynamic therapy treatment (complete response) or 'unsuccessful' (partial or non-response) and univariate analysis was undertaken to see if there are any predictors for successful treatment, Table 3-2.

The use of Porfimer as a photosensitising agent was more successful at ablating early central airway lung cancer with an odds ratio of 7.1 (95% confidence interval [CI] 0.88-57; p=0.04) when compared to Fotolon, Figure 3.6. Analysis of the treated lesion length (as identified during bronchoscopy in mm) when categorized; demonstrated a lesion <15mm to be a predictor of successful treatment with an odds ratio 1.5 (95% confidence interval [CI] 1.2-2.1; p=0.02), Figure 3.7.

Local recurrence of cancer was detected by bronchoscopy in 23% of lesions that had a completely successful response to treatment. This group also developed metachronous cancers either within the central airway or lung parenchyma in 47% of cases, as was seen in the 'high-risk' cohort in chapter 2. A difference in photosensitiser was also detected, with a significantly increased rate of local recurrence seen with Fotolon (80%) compared to Porfimer (22%), p=0.04.

	Total	**Successful treatment (CR)**	**Not successful treatment (PR/NR)**	***p* value**
Lesion (n)	28	21	7	
Baseline histology				
Squamous cell carcinoma	26	19	7	0.40
Carcinoma in situ	2	2	0	
Treated length (mm)				
<15mm	6	8	0	**0.02**
>15mm	22	13	7	
Photosensitiser				
Porfimer	23	19	4	**0.04**
Chlorine e6	5	2	3	
Local recurrence (site specific)				
Yes	9	5	4	**0.04**
No	16	16	0	
Other site disease				
Yes	14	10	4	0.65
No	13	11	2	

Table 3-2: Independent variables affecting response to treatment for early lung cancers with photodynamic therapy

CR=complete response, PR=partial response, NR=no response

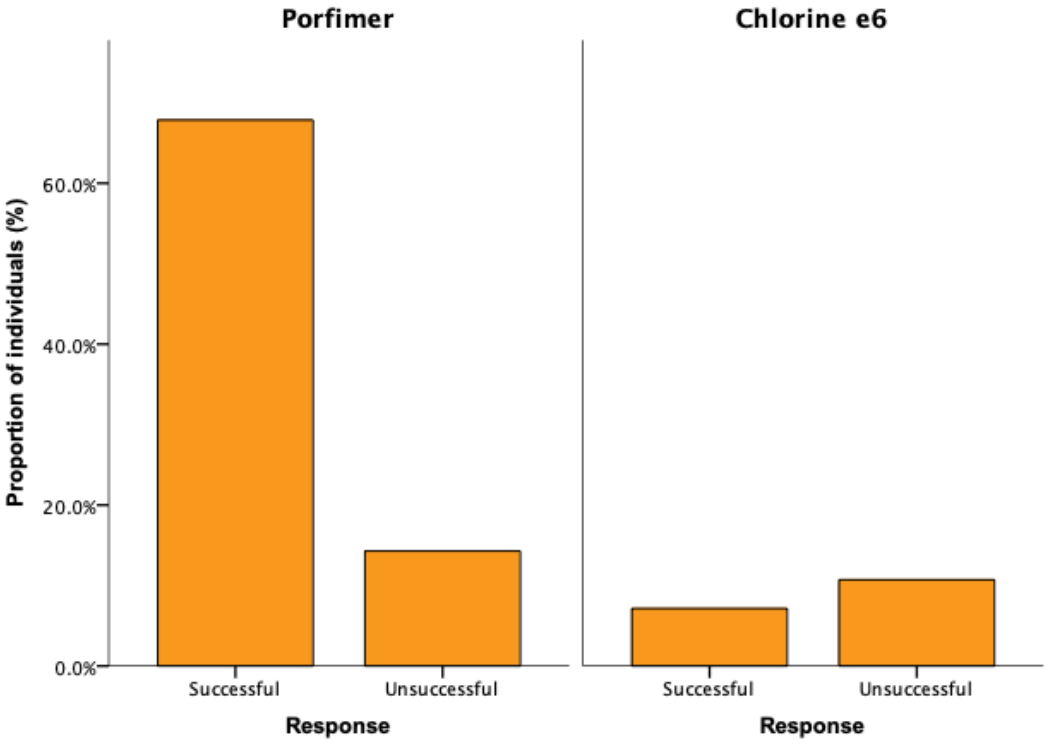


Figure 3.6 – Success of lung cancer ablation stratified by photosensitiser

Porfimer more successful than Fotolon at treated early central airway cancers (p=0.04)

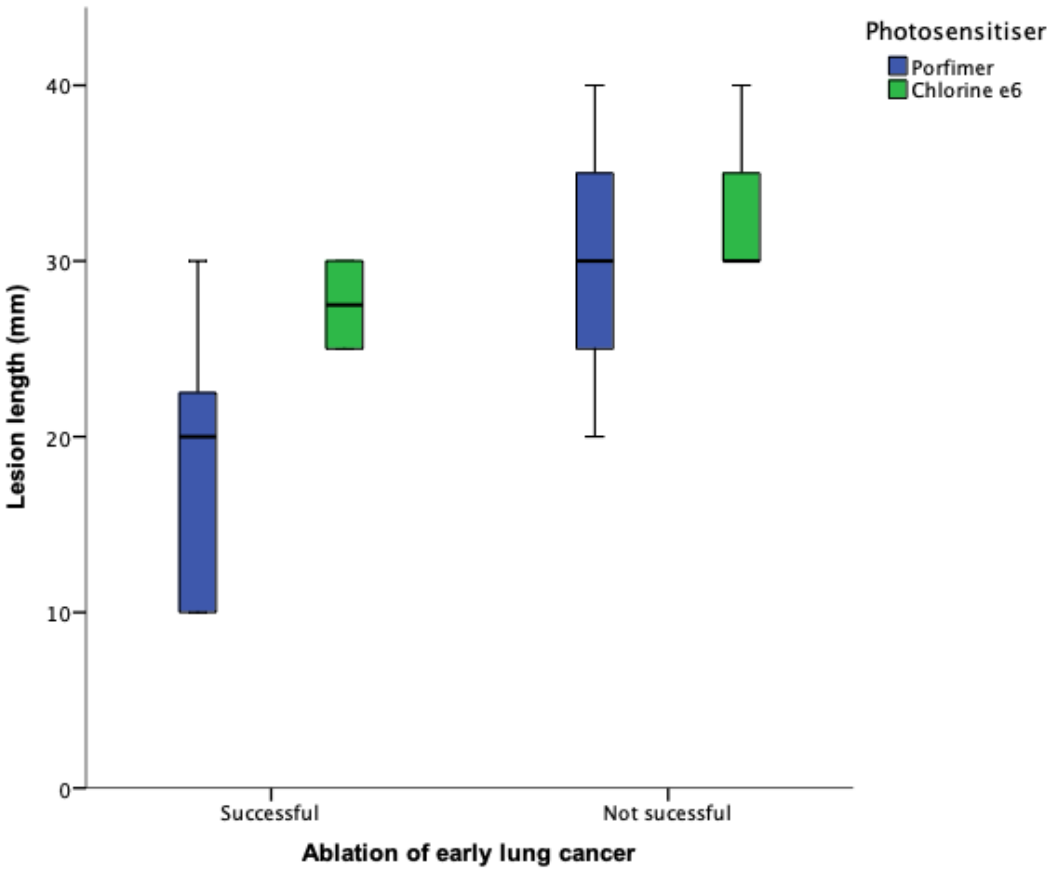


Figure 3.7 – Successful lung cancer ablation predicted by length of treated lesion

Baseline lesion length of <15 mm is more likely to lead to successful ablation (p=0.02)

3.3.5 Survival

Following photodynamic therapy of this group of patients with early central airway lung cancers, the median overall survival is estimated to be 32 months (95% confidence interval [CI] 20-44 months), Figure 3.8. Five (21%) patients are either still under surveillance, discharged after >5 years of follow-up or have left the study. Four patients (16.7%) died as a result of the initial cancer treated with PDT and a further 6 died of a metachronous cancer (25%), resulting in 40% of patients in this cohort dying as a result of lung cancer. Nine (38%) patients died from a non-cancer related death (Figure 3.9)

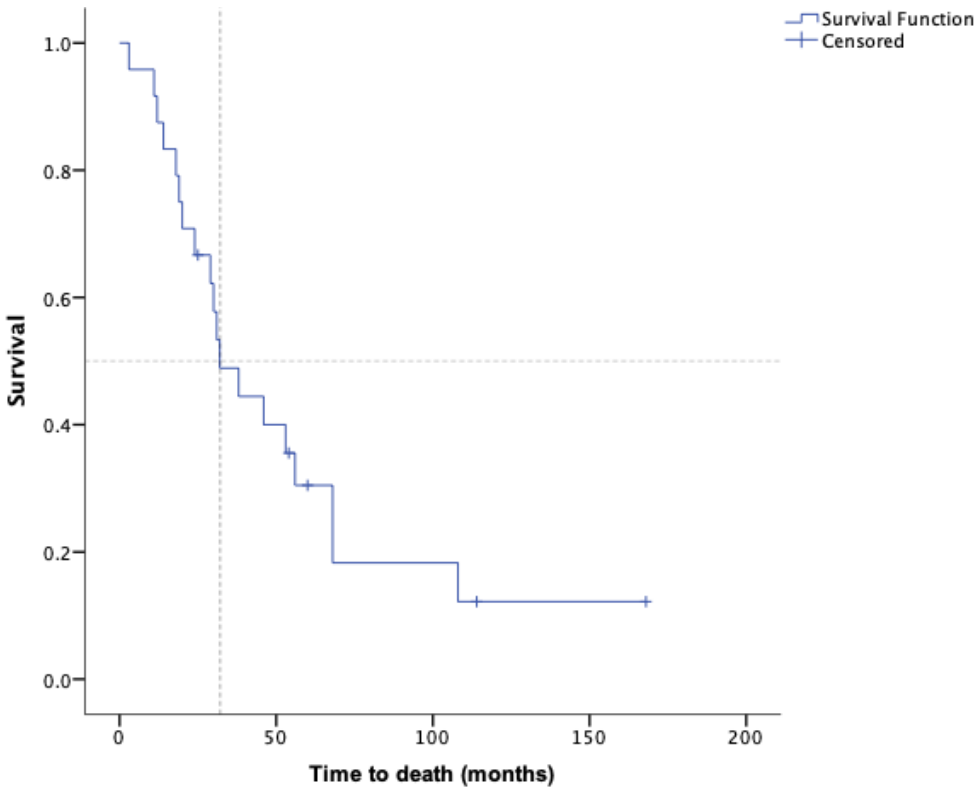


Figure 3.8 – Kaplan-Meier curve showing overall survival of patients treated with photodynamic therapy for early central airway lung cancer

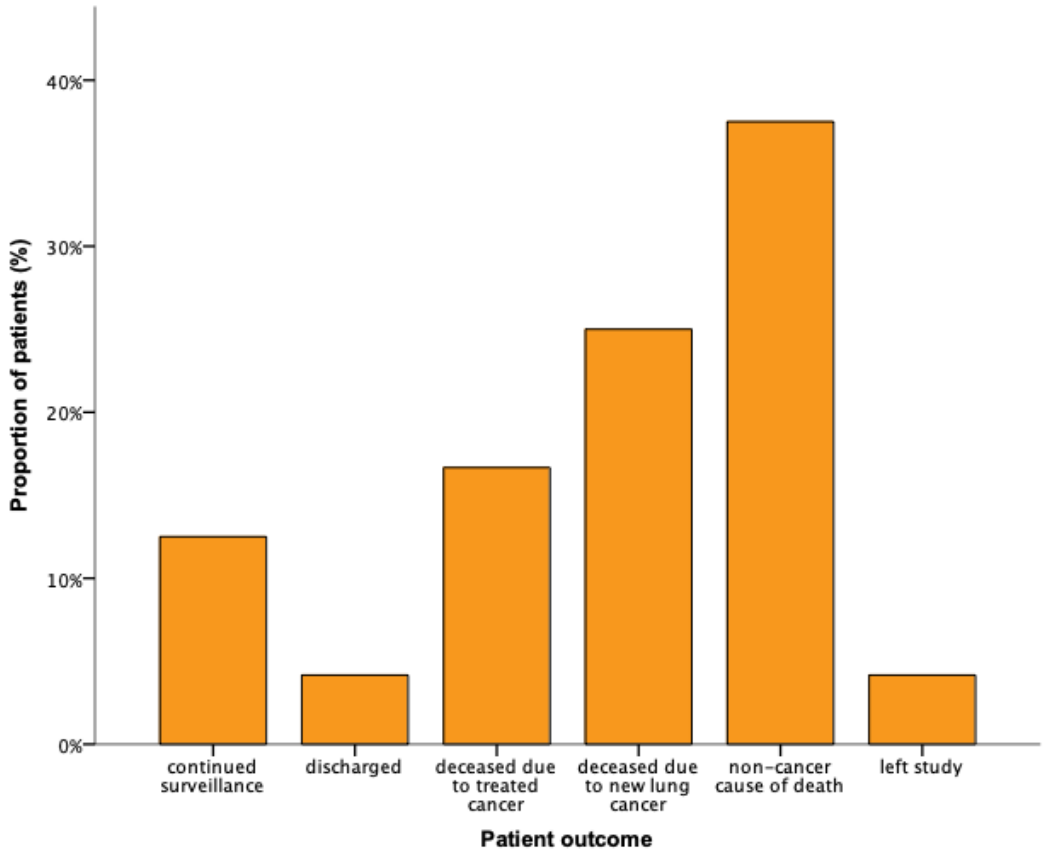


Figure 3.9 – Reason for patient leaving study

A predictor for death was individuals with a poor performance status (ECOG >2) p=0.01, Figure 3.10. A multivariate cox regression analysis with backward step-wise elimination showed that the performance status was an independent risk factor for death (hazard ratio 8.3, 95% confidence interval [CI] 1.4-48; p=0.01) as was the development of a recurrence cancer (hazard ratio 6.3, 95% confidence interval [CI] 1.1-36; p=0.04). No other associations were found between death and a history of previous lung cancer, smoking status, or a diagnosis COPD. While a difference in lesion response to photosensitiser was seen, the use of Photofrin vs. Fotolon did not appear to be a significant risk factor for death (HR 2.2, 95% confidence interval [CI]; p=0.31).

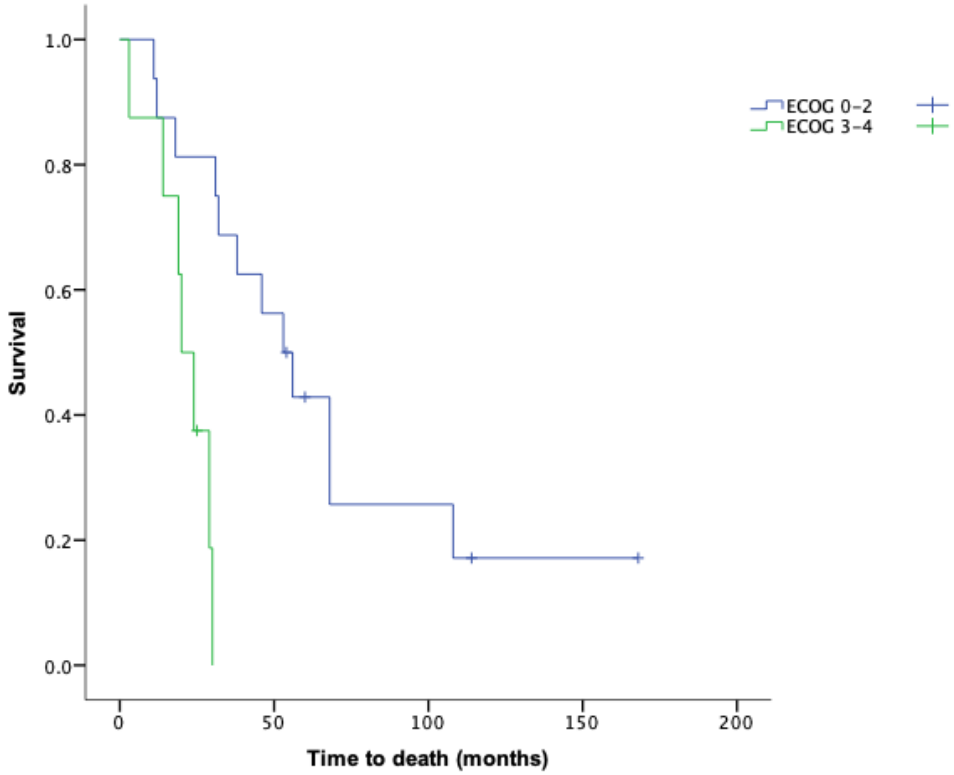


Figure 3.10 – Survival in treated patients when categorised by performance status

Significance determined by Log rank = 0.01

Complications

Overall, photodynamic therapy was quite well tolerated. Only 2 patients included in the analysis developed any complications. One patient had developed a bronchial stricture and the second developed a broncho-vascular fistula, which caused massive haemoptysis and death. While the treatment may have contributed to death, progressive invasive disease into the pulmonary vasculature was also seen on a CT shortly before the fistula developed.

3.4 Discussion

This study examines the treatment of early central airway lung cancers (ECALC) with photodynamic therapy (PDT). The work was not designed to question the value of surgical resection; rather it was designed to show that PDT could be used as an endobronchial modality in patients who are ineligible for conventional treatment or when a tissue-sparing approach is needed.

The term ECALC was defined as a malignant lesion confined to the tracheobronchial tree, with no nodal or metastatic disease, and confinement to the airway mucosa. This terminology has been adopted by many other authors and over time we have seen refinement of the patients selected for this therapy [23,98,131,135,138]. As an example, Kato et al introduced a cut-off of 1 cm in their inclusion criteria where a significantly better response is seen to PDT [96]. Similarly, Sutedja et al pre-selected patients with disease confined to the airway, where extra-bronchial spread was less likely to respond to PDT [139].

In this study, while patients were only selected when the tumour was confined to the airway, the length of lesion was not part of the exclusion criteria. Where the tumour was accessible for illumination by flexible bronchoscopy and other inclusion criteria were met, the individual was offered PDT after review in a lung cancer multi-disciplinary meeting and appropriate staging as per the British Thoracic Society and NICE guidelines for lung cancer [8]. It is also worth noting that this study has run over a period where there has been (i) improved detection (AFB) and staging (PET, EBUS) techniques that will have contributed to a better definition and patient selection, and (ii) introduction of a second generation photosensitiser, which has a better side-effect profile.

In this study we showed that a complete response was seen in 75% of 28 lesions treated with PDT. A significantly better response was seen with the early generation sensitiser, Photofrin, when compared to Fotolon (80% and 40%,

respectively). This is comparable to an early phase II study where Furuse et al treated 59 early cancers with Photofrin, a first-generation photosensitiser [90]. A complete response (CR) was seen in 85% of patients, with the remainder having either a partial or no response. In this current study the use of Porfimer was more successful at ablating early lung cancer with an odds ratio of 7.1 (95% confidence interval 0.88-57; p=0.04) when compared to Fotolon.

Furukawa et al used PDT as the definitive treatment in 114 lesions stratified by length (<1cm or >1cm), with long-term follow-up [95]. A complete response was seen in 93% of lesions <1cm in size compared to 58% of lesions >1cm. In this analysis, we looked for variables that could predict of response to PDT and similarly measured the baseline lesion length at autofluorescence bronchoscopy and stratified into <10mm or >10mm. We show that lesions under 15 mm were a predictor for complete response to PDT, adjusting for other factors. In Japan, many authors recommend PDT in ECALC lesions <1 cm only, where they have shown that the long-term complete response is such that PDT could be even be considered as a primary treatment option [90,92].

Following a complete response to treatment, on-going surveillance detected 23% local recurrence. Autofluorescence bronchoscopy was used to guide illumination in this study to prevent under-treatment as it would help visualise the extent to which the tumour extends along the airway wall. However, more difficult to assess was the degree of submucosal invasion and if there is peri-bronchial extension. High-resolution CT was used to determine if peri-bronchial extension or cartilage involvement was seen. However, this is not completely reliable and the ideal method for assessing depth of invasion would be endobronchial ultrasound, which was not used in this study. Miyazu et all showed that around 50% of lesions that look to be superficial actually have tumour extension into the cartilage and

complete cancer eradication in these cases is often not achieved [94]. This is an incredibly important factor. Although the laser wavelengths used may penetrate up to 10mm, it is likely that only truly superficial lesions can be reliably cured. While we cannot make any firm conclusions about the choice of photosensitiser due to the small numbers; local recurrence was more common with Fotolon (80%) compared to Porfimer (22%), p=0.04.

In this study, 47% individuals that had a successful treatment, still developed metachronous cancers, either within the central airway or lung parenchyma. Usuda et al treated 28 early lung cancers with PDT and similarly showed that ⅓ of their cohort developed metachronous lesions [98]. As described in the previous chapter, the data indicate that these early central lesions probably act as a marker for lung cancer risk as a result of “field cancerisation” and highlights the importance of considering a tissue-sparing strategy In these individuals.

Subjects were followed up clinically with CT and bronchoscopy for a mean surveillance period of 45.9 ±7.9 months. In this study, the reported 5-year survival rate was 42%. In comparison, Albano et al reported the survival on 191 patients with early stage lung cancers treated with either surgery or stereotactic radiotherapy if they were considered unfit for surgery. The reported 5-year survival for patients having surgery was 80%, but only 37% of those treated with stereotactic radiotherapy were alive at 5 years [140]. It is likely that the low survival reported in this study is probably due to the poor baseline performance status in this cohort, as well as other competing co-morbidities, which prevented them having conventional radical treatment in the first instance. Similarly, Furukawa et al in their long-term follow-up showed the 5-year survival to be 58% and this was not influenced by tumour size, despite seeing excellent results in lesions of <1cm [95]. In a systematic review by Moghissi et al of more than 700 early central airway

cancers, across 15 trials revealed a complete response rate of 30–100% and an overall 5-year survival of 61% [107].

Moghissi et al similarly concluded in their review that PDT is safe with photosensitivity being the most common complication in 5–28% of cases [107]. In this study, minimal complications were encountered with PDT and the treatment was well tolerated. A second generation photosensitiser, Fotolon, was brought in to use towards the end of the study. It takes less time to be taken by neoplastic cells (laser treatment can be given 3-4 hours after injection) and it has a shorter half-life, which means that patients spend only 5-7 days in a darkened environment compared to 30 days when using Photofrin.

This study has several limitations. First, the patients received different photosensitisers, which probably have difference efficacy profiles. Secondly, the study spanned the development of more sophisticated staging techniques that may influence the outcome and risk of recurrence. Third, not using endobronchial ultrasound means the study will have included patients who may have had cartilage or early extra-bronchial involvement. Finally, there is missing data on co-morbidities including the severity of COPD, which will influence survival.

3.5 Conclusion

Endobronchial therapies like photodynamic therapy have the advantage of conserving lung tissue. In this chapter, the analysis of this prospective study has shown that photodynamic therapy is effective at ablating early lung cancers in the central airway and it would appear that selection of small lesions, which are superficial, would be the best suited for this type of therapy. This leads to the natural question of whether endobronchial therapy when possible should be used at earlier stage when all these criteria are met, i.e. in carcinoma in situ, before the basement membrane has been breached. The data from the high-risk cohort in chapter 2 and this chapter led to a number of clinical questions: (i) could photodynamic therapy be used to effectively ablate high-grade pre-invasive disease? (ii) could such a treatment prevent progression into invasive cancer? (iii) would treatment of an index early central airway cancer prevent metachronous cancers from developing (iv) and would delivering such a treatment be cost effective?

In order to answer these questions funding was secured from Cancer Research UK to conduct an international, multi-centre, randomised clinical trial to address endobronchial photodynamic therapy of high-grade pre-invasive lesions could prevent lung cancer – see Appendix A.

Chapter 4 LONG-TERM OUTCOME OF PATIENTS WITH BRONCHIAL CARCINOID TUMOURS TREATED WITH LASER THERAPY

4.1 Introduction

The previous chapters have focused on squamous cell carcinoma as the underlying pathology, which is incredibly important due to its high prevalence and cause of lung cancer related death. However, a large spectrum of benign and malignant diseases of the tracheobronchial tree exist. They can cause significant morbidity and mortality and frequently present with symptoms of central airway obstruction [141,142]. In this chapter, the management of another type of primary lung tumour, bronchial carcinoid tumour is examined. Specifically, the use of a different type of endobronchial medical laser that delivers enough energy to vaporise tumour is evaluated in this study.

Carcinoid tumours of the lung arise from neuroendocrine cells or a cluster of cells (neuroepithelial bodies) found in the airway epithelium [143] and are recognised two subsets; typical carcinoid and atypical carcinoid [144]. They are part of a larger group of neuroendocrine tumours of the lung that also include high-grade small cell lung cancers, large-cell neuroendocrine tumours, but are distinguished by their morphological appearance and low mitotic index [123]. Typical carcinoids make up 2% of all primary lung tumours [145] and have an estimated incidence of up to 2 cases per 100,000, whereas atypical carcinoids are incredibly rare and make up <1% of all lung neuroendocrine tumours [146].

Carcinoid are thought to develop spontaneously, but can also be seen in some individuals with diffuse idiopathic neuroendocrine cell hyperplasia (DIPNECH); a preinvasive lesion characterised by hyperplasia that forms neuroepithelial bodies

and tumorlets (tumours of <5mm in size) [123,147–150]. Carcinoid tumours have characteristic pathological features as set put by the World Health Organisation of lung tumours [12,151], see Table 4-1. The Ki-67 antigen is also used in the pathological description and highlights the proportion of proliferating tumour cells, a common denominator to all neuroendocrine tumours. The Ki-67 labelling index reliably differentiates low- (Ki-67 <20%) and high- (Ki-67 >40%) grade neuroendocrine tumours [152].

Neuroendocrine tumour morphology: organoid, nesting, and palisading cells with florets of tumour	
Low grade neuroendocrine tumour	
Typical Carcinoid	Mitoses <2 per 10 HPFs or $2mm^2$ + no necrosis
Atypical Carcinoid	Mitoses 2-10 per 10 HPFs or $2mm^2$ ± focal necrosis
High grade neuroendocrine tumour	
Small cell carcinoma	Mitoses >10 per $2mm^2$ and other typical features
Large cell neuroendocrine	Mitoses >10 per $2mm^2$, but features of non-small cell lung cancer

Table 4-1: Pathological criteria for diagnosis of neuroendocrine tumours of the lung

Table adapted from Travis et al [150]

Although carcinoid tumours are grouped together with other neuroendocrine tumours, they have a distinct clinical differences compared to small cell and large-cell neuroendocrine lung cancer [143]. Patients are usually younger and have either no or minimal smoking history [145] and since carcinoids occur predominantly in the central airways patients often get symptoms of bronchial obstruction such as cough, haemoptysis dyspnoea, wheeze, obstructive pneumonia, and stridor [143,146,153,154]. Patients often describe symptoms for anything up to 2 years before a diagnosis is made [155], demonstrating the indolent nature of these tumours. However, although carcinoid tumours are considered indolent, they are capable of regional lymph node and distant metastasis [156], so localised disease needed to be carefully staged and managed.

The diagnostic evaluation recommended by the European Neuroendocrine Tumour Society (ENETS) recommend a CT chest, Gallium-68-1,4,7,10-tetraazacyclododecane-1,4,7,10-tetraacetic acid (DOTA) somatostatin analogue (^{68}Gallium-DOTATATE), bronchoscopy ± linear EBUS, and biochemical evaluation as appropriate [146]. Bronchoscopy is indicated for evaluation in all tumours arising in the central airways and a biopsy should be performed where possible (ENETS). However, the use of autofluorescence and endobronchial ultrasound to determine margins before surgical resection or to detect recurrence is not yet proven with limited evidence available [146,157,158].

Carcinoid tumours are not sensitive to chemo- or radiation- therapy, so once localised disease is confirmed, surgery remains the gold standard treatment [146,154,159]. For individuals with peripheral tumours this would an anatomical resection (e.g. lobectomy), and for those with central tumours either pneumonectomy maybe needed or a lung-sparing surgery such as a bronchial sleeve resection or sleeve lobectomy [146]. Local endobronchial therapy such as

laser resection is mentioned in the ENETS guideline, but the recommendations are to employ bronchoscopic therapy in patients who are considered high-risk for surgery [146,160]. However, carcinoid tumours without any extraluminal component can have an excellent long-term outcome when treated with endobronchial therapy [112,161–165], and their indolent nature means bronchoscopic therapy can be undertaken without fear of progression to metastatic disease. Endobronchial therapy can also have its use in the palliative treatment of obstructed central airways causing symptoms. Several series of bronchoscopic treatments of carcinoid tumour have appeared in the literature and have most recently been summarised in a systematic literature review [166].

In this chapter, a retrospective analysis of a prospective maintained database is performed on patients referred for laser therapy of carcinoid tumour of the central airways. The safety of semiconductor diode laser therapy and its effectiveness in treating carcinoid tumours is examined. Finally, the long-term outcome and survival for these individuals is described.

4.2 Methods

4.2.1 Objectives

- Evaluate the efficacy of semiconductor laser therapy to ablate and eradicate carcinoid tumour of the airway
- Examine the factors that affect tumour response to laser therapy
- Assess the rate of recurrence of disease by maintaining close bronchoscopic surveillance
- Evaluate the long-term outcome of individuals with carcinoid treated with laser therapy

4.2.2 Patient selection

From January 2012 to January 2018, we reviewed the cases of typical and atypical carcinoid tumour referred for endobronchial treatment to our tertiary interventional bronchoscopy centre at University College London Hospital.

Prior to considering endobronchial therapy, all subjects underwent a clinical assessment, CT chest with contrast ± ^{68}Ga-DOTATATE PET-CT, and diagnostic autofluorescence bronchoscopy and biopsy with either the D-Light autofluorescence bronchoscope (Karl Stortz, Germany), or Olympus autofluorescence AFI-Lucera (Olympus, Tokyo, Japan) before a review on their case was carried out in a lung cancer multidisciplinary meeting.

The definitive management plan to undertake endobronchial laser therapy was based on the following criteria:

Localised disease

- Any individual with intraluminal typical or atypical carcinoid tumour where extensive surgical resection would be needed or patient chose to have laser therapy.
- When the intension is curative intent, CT and PET-CT ± linear EBUS would be used to stage the patient to exclude lymph node or extra-thoracic involvement.
- Any individual thought to have (i) significant extraluminal disease, (ii) lobar/segmental disease where lung-sparing resection can be undertaken, or (iii) lymph node metastases are suspected, would be considered for surgical resection rather than endobronchial treatment.

Disseminated disease

- Individuals with disseminated disease in whom the primary goal was disobliteration of central airway disease causing symptoms would be considered for laser therapy.

4.2.3 Intervention

The definitive endobronchial therapy used was ablation with a semiconductor diode laser by an expert interventional bronchoscopist of at least 5-years' experience. The intervention employed was done on a case by case basis and physician preference, but the principles are:

- Use of general anaesthesia, neuromuscular blockade, and rigid bronchoscopy with a flexible bronchoscope used to deliver the laser treatment
- Mechanical debulking (Karl Stortz, Germany) or cryo-extraction (Cryoprobe 2.4mm, ERBE, Germany) may be used to reduce the volume of tumour before laser ablation
- Semiconductor diode laser (Biolitec, Germany) therapy used with the beam parallel to the bronchus, aiming at the tumour edge in short pulses to vaporise the disease.

Individuals then underwent a repeat bronchoscopy 2 months after the endobronchial therapy was delivered. At the discretion of the lung multi-disciplinary meeting, individuals may have undergone repeated laser ablation or may have been referred for surgical resection for residual disease.

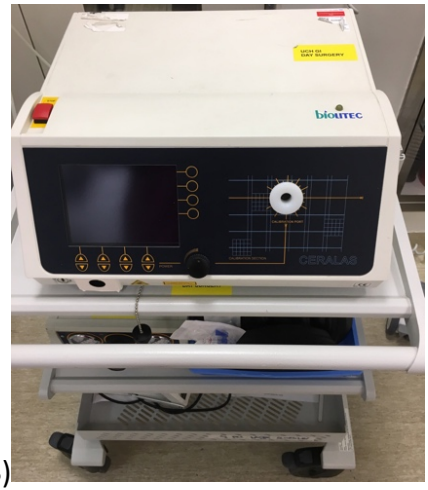
(A)  (B) 

Figure 4.1 – (A) Cryotherapy unit (ERBE) and (B) Semiconductor laser (Biolitec)

4.2.4 Outcomes

The primary outcome measure was the response to laser therapy at 12 weeks following treatment as defined by:

- **Complete response:** complete microscopic and macroscopic clearance of the carcinoid
- **Partial response:** refers to the presence of residual carcinoid tumour present after laser therapy
- **Recurrence:** site-specific disease recurrence of carcinoid detected during surveillance after a complete response to laser was achieved

The secondary outcome measured were:

- Clinical, radiological, and bronchoscopic factors that may predict successful treatment with laser
- Recurrence rate after laser therapy

- Overall survival

4.2.5 Statistical analysis

Differences between groups with nominal variables were estimated using either Chi-square or Fisher's exact. Differences in numerical dependent variables were analysed using the independent two-sample t-tests or Mann Whitney U for non-parametric data. For analysis, carcinoid response was addressed with binomial function (complete response to laser or evidence of residual disease). Analysis was then conducted to look at independent factors that may affect the success of treatment. The effect size for these were presented as odds ratios and $p < 0.05$ was considered significant. Overall survival (OS) was calculated for all subjects from the date of laser treatment to the date of death (time to event) or to the last date subjects were followed-up. Survival analysis was performed using the Kaplan-Meier method. For all tests, p values <0.05 were considered statistically significant. All statistical analyses were performed using SPSS Statistics software (version 24.0; IBM).

4.3 Results

4.3.1 Study population

Forty-four individuals were referred to University College London Hospital for consideration of definitive endobronchial laser therapy for carcinoid tumour. Following clinical assessment and the work-up described; 15 individuals had large tumours (>30mm), 12 had a clear visible extra-bronchial component, and 2 had suspicion of nodal disease, all of which were considered to be more suitable for surgical resection.

An additional 3 patients were included as part of the analysis who had disseminated disease and were being referred for laser resection of endobronchial metastases causing central airway obstruction (Figure 4.2).

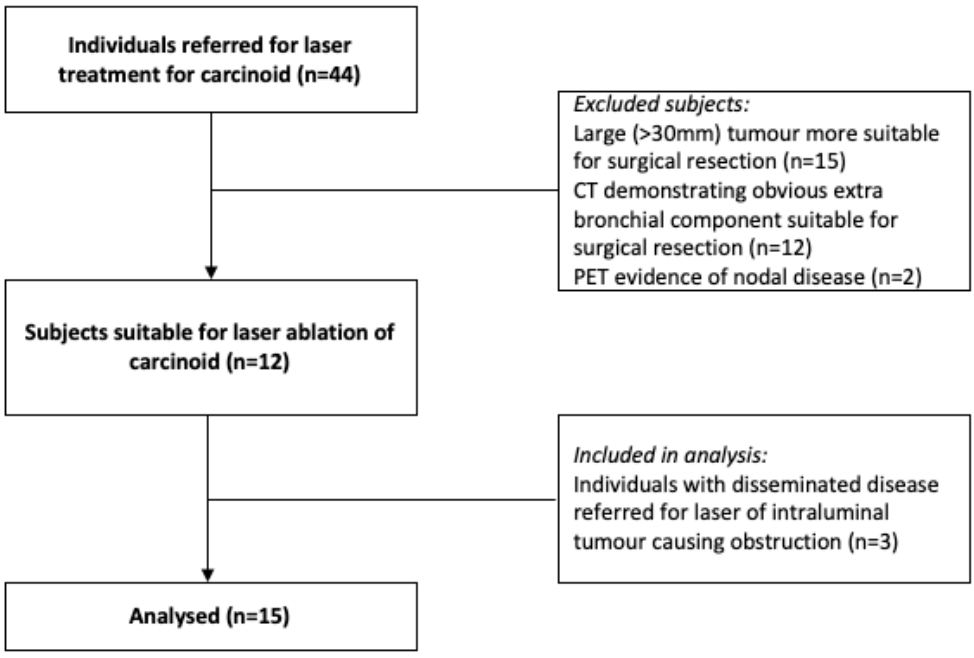


Figure 4.2 – Carcinoid study population

4.3.2 Baseline characteristics

Twelve individuals underwent definitive endobronchial laser therapy for their carcinoid tumour as an alternative to surgical resection. A further 3 patients had laser therapy to disobliterate occluded airways in the context of disseminated disease (Table 4-2). The mean age at study entry was 60 year (±4 years) and two thirds of the individuals were never smokers. The most common presenting symptom was cough and stridor (27%), the latter of which was seen in the patients with disseminated disease referred for central airway obstruction. The subjects were classified using the American Society of Anesthesiology (ASA) co-morbidity score before their procedure. Nine patients (60%) had an ASA score of 1-2 and the remaining patients had a score of 3. No patients had an ASA score of 4 or 5.

	Total	**No disseminated disease**	**Disseminated disease**
Individual (n)	15	12	3
Age (years mean)	60±4	61 (30-84)	59 (59-60)
Gender			
Male	9	7	2
Female	6	5	1
Smoking status			
Non smoker	10	8	2
Former or Current Smoker	5	4	1
Presentation			
Cough	4	4	0
Dyspnoea	2	2	0
Haemoptysis	3	3	0
Pneumonia	2	2	0
Stridor	4	1	3
Comorbidity (ASA)			
ASA <3	9	8	1
ASA ≥3	6	4	2
Surveillance period (m)	39±7.5	41.5 (11-96)	30 (6-48)

Table 4-2: Baseline characteristics of patients with carcinoid tumour

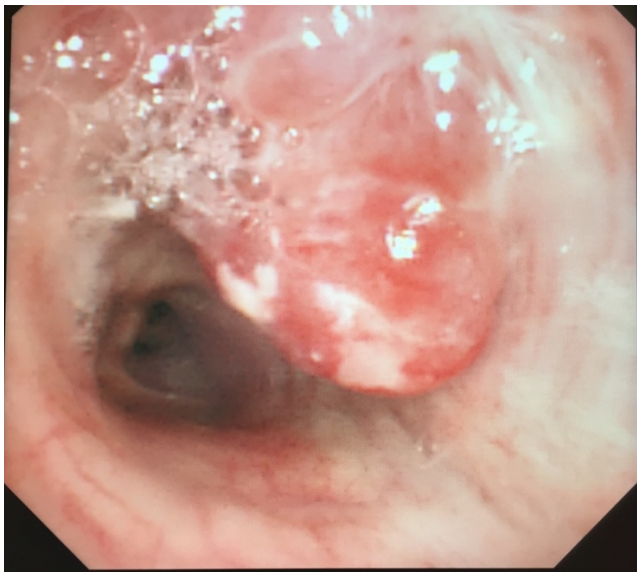
ASA = American Society of Anaesthesiology

4.3.3 Response to diode laser therapy

Seventy-three percent (n=11) of individuals assessed at their first check bronchoscopy had a complete response to laser, one of which was a metastatic deposit in the airway from a carcinoid originating in the gastrointestinal tract. Figure 4.3 shows the endobronchial appearances of one the patients with localized disease treated, where the charred base of tumour can be seen at the 1 'o' clock position after laser. Of the 12 patients having definitive treatment, one had residual tumour at the initial assessment and had further laser therapy. During surveillance, 5 individuals developed recurrence at median surveillance interval of 12 (8-36) months. Three received a total of 5 further laser therapies and had no further detectable microscopic disease. Two patients went on to have a surgical resection.

The mean tumour diameter or length (as assessed on CT or bronchoscopy) treated was 15 mm ± 7.4 mm. The tumour diameter when assigned as <20 or >20 mm predicted whether a complete response to laser was achieved (OR 3.0, 95% CI 1.0—9.4; p=0.03), Figure 4.4. While numbers are small, the presence of extra-bronchial disease (either suspicion on CT or at time of bronchoscopy) also appears to predict whether a complete response will be achieved. In this cohort, all patients who had purely intraluminal disease completely responded (i.e. 100% response) to laser therapy and those with extraluminal disease had a 20% complete response (Figure 4.5).

(A)



(B)

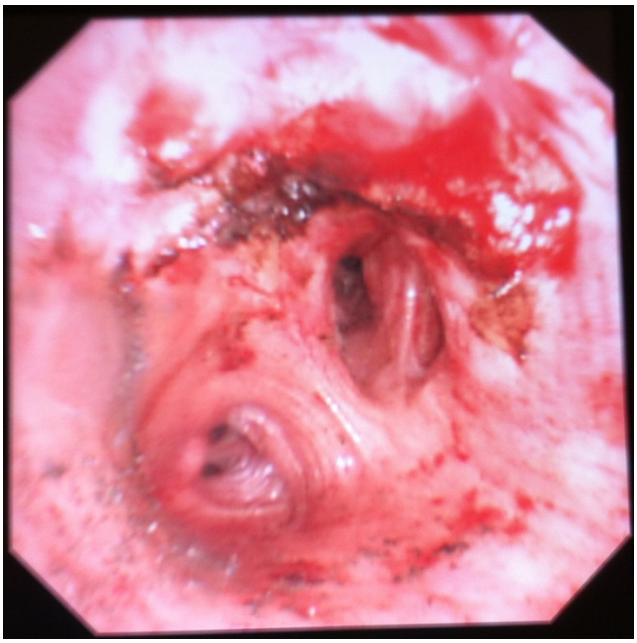


Figure 4.3 – Typical carcinoid of left main bronchus (A) before and (B) after laser treatment

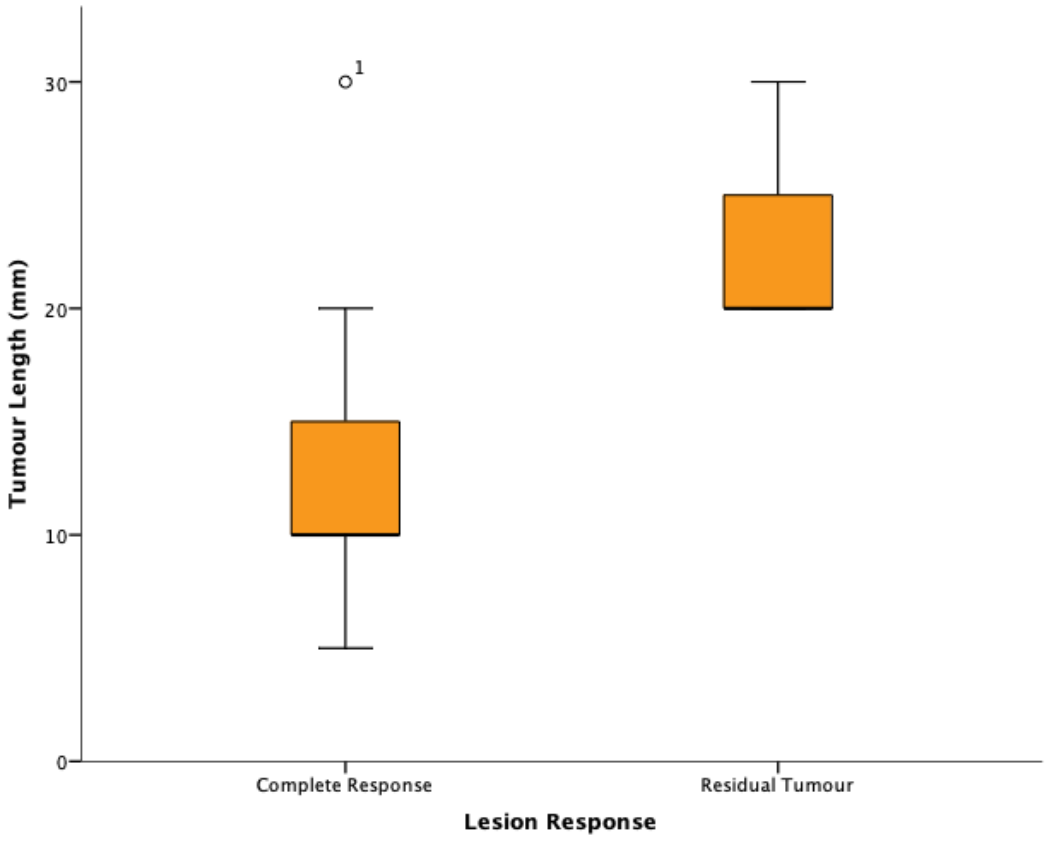


Figure 4.4 – Effect of tumour length on response to laser therapy

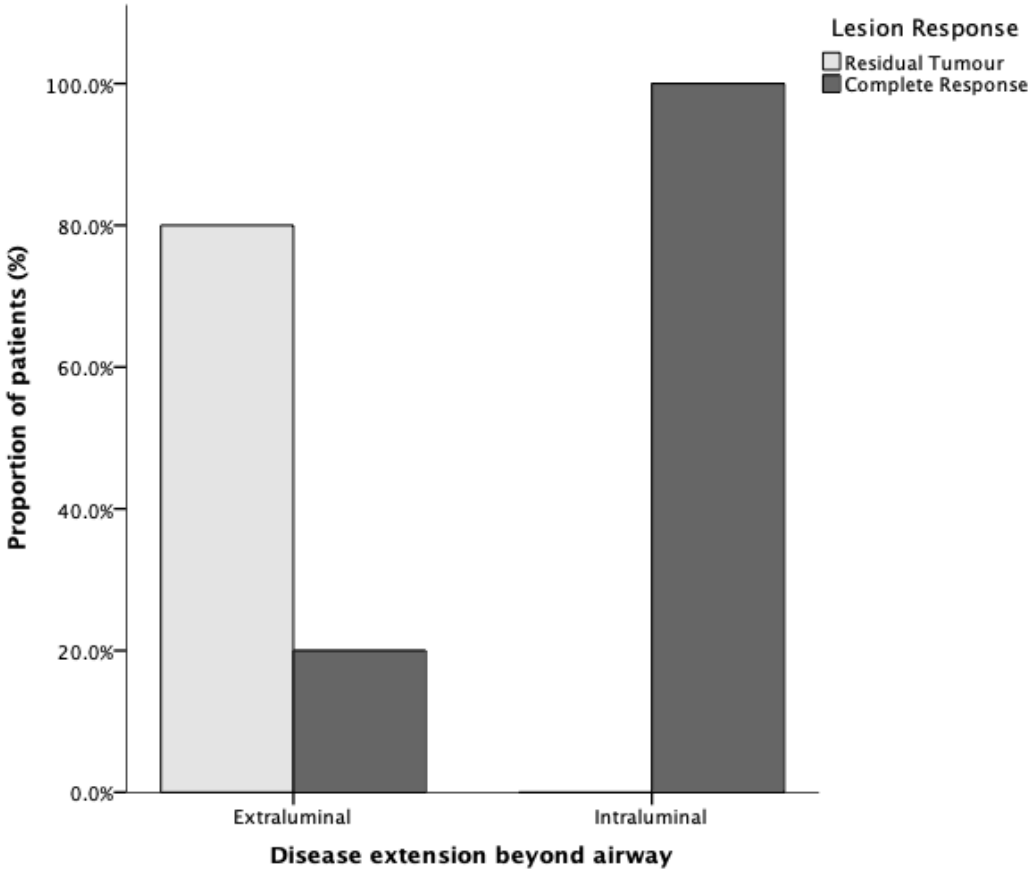


Figure 4.5 – Carcinoid response to laser therapy when suspicion of extra-bronchial disease on bronchoscopy or CT

4.3.4 Survival

All 10 individuals that had successful definitive laser treatment are alive or have been discharged. The 2 patients who went on to have surgery are also alive and under surveillance. The 3 patients with disseminated disease all died and this reflected on the survival curve in Figure 4.6.

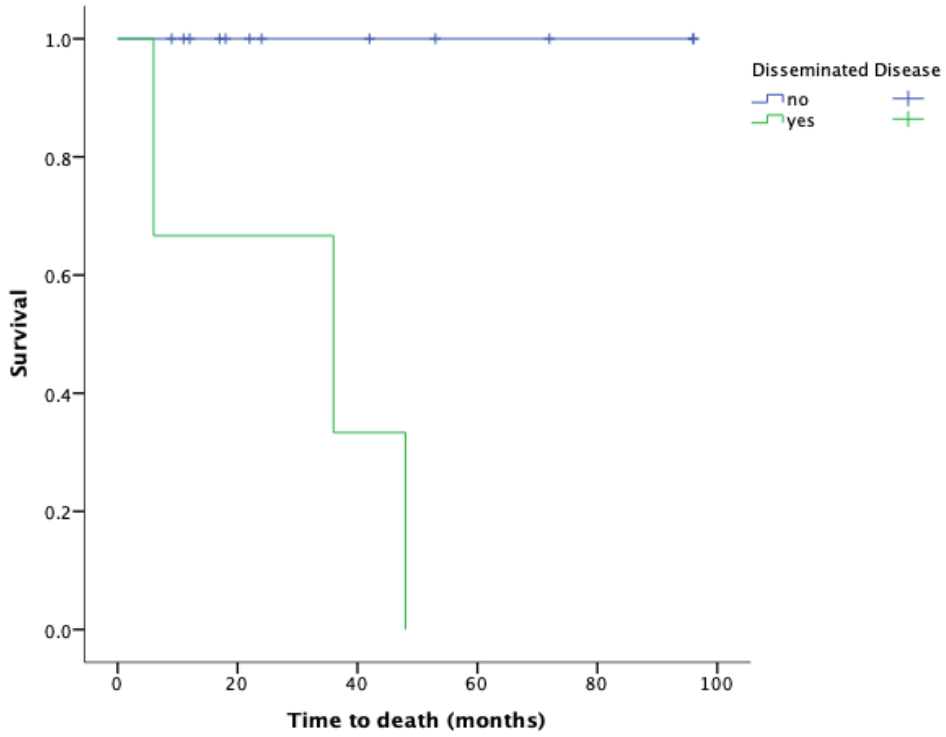


Figure 4.6 – Survival after laser therapy for carcinoid tumour in the airway

4.3.5 Complications

Twelve (80%) individuals had moderate bleeding that was controlled with topical adrenaline, intravenous tranexamic acid, and/or laser coagulation. Two patients had transient desaturation while undergoing jet insufflation. No escalation of care was needed for any of the patients. Eight patients had an elective admission after their procedure and the remainder were performed as day-cases.

4.4 Discussion

Carcinoid typically occurs in the central airways where they cause symptoms of obstruction such as stridor, retained secretions, and distal lung collapse and infection [167,168], similar to what was seen in this current study. Since they are considered malignant, curative surgical resection remains the gold standard of care [168]. However, with their central location anatomical resections (lobectomy, bi-lobectomy, and pneumonectomy) are often required, with only 3 studies reporting routine parenchyma sparing resections [166]. Therefore, it is not surprising that the use of endobronchial ablative therapy is a common discussion point in the lung multidisciplinary meeting when faced with a small, proximal typical carcinoid that is easily accessible to the bronchoscope and in range of the laser beam.

Reuling et al recently reported on a cohort of 125 individuals undergoing endobronchial therapy for carcinoid tumour in the airway, and reported excellent success with complete ablation of tumour in 72% of cases [161]. This is similar to the 73% of cases where we saw a complete response to laser ablation. However, it is important to understand if there are any factors that can affect a successful laser treatment. Early anecdotes from well-established interventional bronchoscopists describe their recipe of success for ablating airway carcinoids as a tumour growth as selecting those that are strictly endoluminal and have a small base that is accessible to laser beam [169]. Reuling et al recently looked at the factors that could predict success of endobronchial therapy for carcinoid [161]. They reported that a tumour diameter of <15 mm and the presence of intraluminal disease on CT were predictors of success following treatment. They went onto make recommendations for bronchial carcinoid, where patients with tumours of <20mm and pure intraluminal disease should be offered endobronchial therapy over

surgical resection. We corroborate these findings and show in this study that tumours that are less than 20mm are more likely to have a complete response to laser therapy (OR 3.0, 95% CI 1.0—9.4; p=0.03). Although two individuals in our study with large tumours (>20mm) responded to laser therapy; following debulking it was apparent that the tumours had arisen from small stalks. The presence of extraluminal disease evaluated at bronchoscopy was a negative predictor for complete eradication of tumour and in most cases (80%) in our study resulted in the presence of residual tumour. Similarly, Reuling et al describe similar findings in their cohort with the presence of extraluminal disease reducing the chance of successful ablation from 72% to 28% [161]. One would not necessarily expect disease that sits outside the airway wall to respond to laser therapy. The fact that some individuals with apparent extraluminal disease respond to laser therapy may be explained by an 'over call' of the extra-bronchial component. There may be collapsed lung adjacent to the tumour and/or infected secretions might give the appearance of tumour outside the airway way; i.e. there is no real extraluminal tumour. However, when there is genuine extraluminal disease, the inflammatory response that follows laser therapy may result in further cell death outside the airway wall and the coagulation effects of a semiconductor laser may also have an effect, causing interruption of the blood supplying the tumour.

Semiconductor diode laser therapy was used in this cohort of patients as the treatment of choice. In our centre, we have developed considerable experience in using laser therapy for ablation. The diode laser emits pulsed light wavelengths of between 800-900 nm, which can penetrate up to 10mm and can be used to (i) resect the tumour by causing photocoagulation of the blood vessels supplying it, or (ii) cause vaporisation by aiming the laser parallel to the tumour and using pulsed energy to cause charring and necrosis. There is minimal scatter of the near-infrared lasers, which means the focused laser energy is confined to a limited area aimed at in the airway. Since charred tissue absorbs energy and limits tumour devitalisation,

we approach larger tumours with cryo-extraction to debulk the tissue volume and ablate the residual tumour with laser. This also preserves the tissue for histological diagnosis. There are no studies that compare the use of different endobronchial therapies and in most cases, the treating clinician will use a combination of debulking techniques depending on their expertise and preference [161–164]. Bertoletti et al used cryotherapy for the treatment of 18 individuals with strictly small, intraluminal carcinoids in 2 institutions [162]. Cryotherapy directs its effects to tissues with high water content and the authors demonstrated a very good long-term response with only one failure. As a result they concluded that cryotherapy can be used as a first-line therapy and certainly strong consideration should be given to cryotherapy, which can preserve low-water content structures like cartilage and has lower risk of causing airway perforation. However, some of these patients did have preliminary coagulation with laser and so further assessment for its efficacy is needed.

Carcinoid tumours respond well to endobronchial therapy, but loco-regional recurrence can occur in up to 11% of cases [166]. However, since carcinoids have a low mitotic index, even when there is local recurrence or residual tumour, salvage surgical resection can be used to achieve a good outcome [163]. In our study, 5 individuals of the 12 with localised disease developed recurrence at median surveillance interval of 12 (8-36) months. Three received further laser therapy (in one case 3 laser treatments) and subsequently had no further detectable microscopic disease. Two patients with large tumours went on to have a surgical resection and both had extraluminal disease. Our principles have been to maintain 5 years of radiological and autofluorescence bronchoscopy follow-up after laser treatment, but it is unclear as to how long individuals should kept under surveillance after having endobronchial treatment. Many studies have shown a low recurrence rate for patients under long-term follow-up [161,163,170–172], but

there is still a need to maintain close surveillance in these patients, with some authors recommending a 10-year follow-up with CT and bronchoscopy given the slow rate of tumour growth [166].

A systematic review in endobronchial therapy for carcinoid, reported 5- and 10-year disease-free survival ranging from 73-95% in typical carcinoid [166]. So it is not surprising that this has led to endobronchial therapy for carcinoid of the tracheobronchial tree becoming a more accepted treatment approach [171–173]. In this study, the subgroup of patients with localised disease all had typical carcinoid and were all alive until either they left the study or were lost to follow-up. Although, not all the patients completed a 5-year follow-up, this highlights the expected outcomes in patients with early central typical carcinoid. This is also similar to the outcome reported by Travis et al in a survival analysis of 200 patients with neuroendocrine tumours [151]. They reported an 87% 5- and 10- year survival in individuals with typical carcinoid where localised disease was treated with surgical resection.

There are some notable limitations to this study. First, there are a small number of patients in this study, where many case notes collected from the 90's and early 00's were not of sufficient quality to include in this study. While this makes drawing strong conclusions difficult, our results are in line with the published literature [166]. Second, some individuals did not have ^{68}Ga-DOTATATE PET-CT, which is valuable in diagnosis nodal and metastatic disease. Finally, the numbers of the study are small and although this makes it difficult to draw firm conclusions, its highlights the that not all patients with typical carcinoid need to have extensive surgical resection.

Laser therapy of carcinoid tumours is challenging from the decision to treat to handling complications from endobronchial therapy, especially with the risk of bleeding. In this study, we show this is safe, but most patients had moderate

bleeding, which is expected from carcinoid tumours. We would advocate these procedures are done with an experienced interventional bronchoscopy team with access to thoracic surgery services available if needed. Our approach for treating patients with laser therapy involves always using rigid bronchoscopy to deal with bleeding complications and the large lumen allows multiple instruments including a flexible bronchoscope to be passed alongside each other.

Not every carcinoid tumour is suitable for laser therapy and individual cases should be reviewed in a lung multi-disciplinary meeting to decide the most appropriate treatment. We would advocate surgical resection in typical carcinoid tumours of the lung when there extraluminal disease, or the tumour is large (>20mm) diameter, or when there is suspected nodal metastases. In the case of large carcinoid tumours, we would consider a bronchoscopy to (i) assess if suitable for laser therapy, and (ii) to review the disease extent on autofluorescence to see if parenchymal sparing surgical resection is possible. However, not every patient needs surgery and a randomised controlled trial of typical carcinoids in the central airways would definitively prove whether endobronchial laser therapy is comparable to surgical resection.

4.5 Conclusion

In this chapter, the endobronchial treatment of carcinoid tumours in the central airways are examined. This audit of a longitudinal dataset informs us that small, intraluminal carcinoid tumours are exquisitely sensitive to bronchoscopic laser therapy and treatment results in complete ablation of the disease. Endobronchial laser treatment is safe and should be considered as a lung-sparing strategy and alternative to surgical resection.

Chapter 5 VIRTUAL ANIMATION AND 3-DIMENTIONAL AIRWAY RECONSTRUCTION USING LUNGPOINT® TO GUIDE TRACHEOBRONCHIAL STENTING

5.1 Introduction

Squamous cell lung cancer has the potential to cause obstruction of the central airways leading to cause significant morbidity and mortality [141,174]. The clinical presentation is commonly with cough, noisy breathing, dyspnea and obstructive pneumonitis. Although simple investigation with peak flow measurements, spirometry, computed tomography (CT) scanning, and bronchoscopy should enable a rapid diagnosis, it is not uncommon for patients with both benign and malignant disease to be misdiagnosed as having poorly controlled asthma [175]. The correct diagnosis is often only made at a relatively late stage when there is a risk of asphyxia. As a consequence, these cases tend to require emergency rescue with bronchoscopic endobronchial therapies including stenting.

The decision to intervene in malignant central airway obstruction usually requires careful consideration of risk vs. benefit. The National Institute of Clinical Excellence (NICE) and British Thoracic Society advocate stenting in patients with impending endobronchial obstruction [137,176], though there is no consensus as to when stents should be deployed. Intervention usually becomes necessary when airway patency is reduced by 50%. The aim of intervention is often palliation of dyspnoea or a bridge towards a more definitive treatment and treating an asymptomatic patient or one who has very limited survival may be fraught with complications.

However, intervention in even very unwell individuals can facilitate extubation and improve survival [177].

It is often difficult to determine the extent of intraluminal disease, the airway topography, and the airway calibre using the CT imaging alone. White light flexible video bronchoscopy provides a clear assessment of the degree and type of airway obstruction and allows distinction of tumour from blood and necrotic debris. Bronchoscopy and also enables exploration of the airway distal to the obstruction, to see if intervention is viable. However, a bronchoscopy in central airway obstruction is not without risk and can provoke bleeding, respiratory failure, and complete airway obstruction [175,178]. In malignant airway obstruction the therapeutic modality used will depend on whether there is intraluminal or extraluminal disease in the airway (Figure 5.1). When there is tumour in the airway lumen, debulking techniques such as laser resection, cryo-extraction, and mechanical debulking are commonly used [141]. In case of extraluminal disease, a stent that provides a radial force that overcomes the obstruction would be needed.

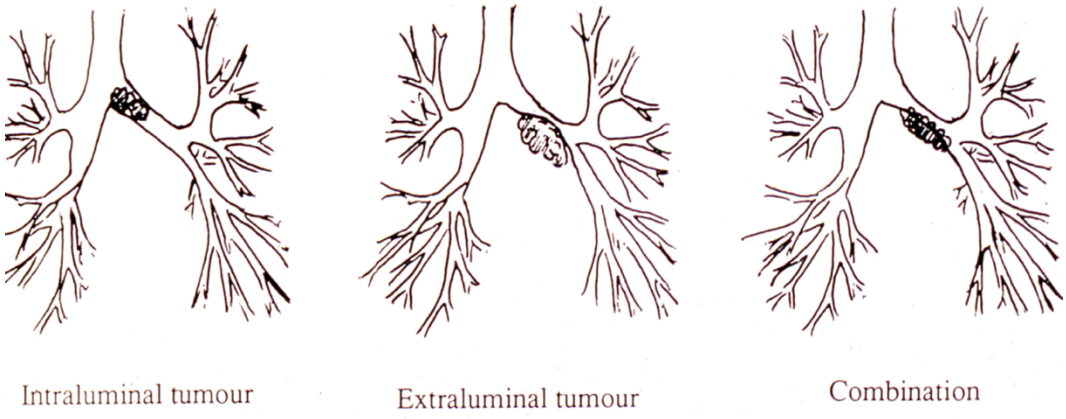


Figure 5.1 – Types of central airway obstruction

Image courtesy of Dr Jeremy George

The main indication for stenting in malignant disease with few exceptions is for palliation where long-term complications are unlikely to be encountered. Although stenting for benign disease is usually avoided as a long-term solution, it is often employed as a bridge for more definitive treatment. The techniques of stent deployment will vary between different centers, but certain common principles are employed. Patients are assessed bronchoscopically and planned for intervention in an endoscopy suite or surgical theatre. The correct stent size needs to be first chosen. A stent that is 1 to 2 mm wider than the normal native airway is generally selected. In the trachea, stents between 16 and 18 mm are usually selected, and stents between 12 and 15 mm for the main bronchi. The appropriate length is then chosen and should provide a 5-mm extension on both distal and proximal ends of the stricture. These measurements are normally confirmed at the time of a diagnostic bronchoscopy. However, due to the complications of a diagnostic bronchoscopy in airway obstruction, a non-invasive method for evaluation is needed.

In this chapter, the role of three-dimensional (3D) airway reconstruction and virtual animation bronchoscopy in airway stenting is evaluated. A navigational software, LungPoint® (Broncus Technologies) for locating and sampling peripheral lung lesions has been investigated at University College London Hospital for its capability of generating 3D virtual images and was subsequently developed for a novel use in planning airway stenting.

5.2 Methods

An audit of a prospectively collected database of malignant airway obstruction cases over a period of 12 months from January 2015 to January 2016 was performed. Cases where LungPoint® navigation was used to plan stent insertion were analysed. Through airway segmentation and endoluminal rendering of the airway, a virtual animation of the bronchoscopic view was modelled. This 3D reconstruction of the airway was used to determine the, (i) suitability for stent insertion, (ii) stent diameter, and (iii) stent length, rather than performing a diagnostic bronchoscopy. The virtual animation assessment was performed independently by two intervention bronchoscopy physicians to look for agreement.

5.3 Results

5.3.1 Case summary

Airway reconstruction with LungPoint® to plan stent insertion was utilised in 3 cases (Table 5-1). The causes of airway obstruction were due to malignancy in two cases, and fibrotic airway remodelling after tracheal transplant in the third. All three cases presented with stridor, reduced exercise tolerance and recurrent infections.

ID	Age	Cause of obstruction	Obstructed segment	Assessment using 3D virtual animation			Stent
				Stent length	Stent diameter	Endoluminal tumour identified	
1	64	Malignant	LMB	40mm	12mm	No	Alveolus® self-expanding metal stent
2	52	Malignant	Trachea	80mm	18mm	Yes	Alveolus® self-expanding metal stent
3	29	Non-malignant	Trachea	60mm	16mm	No	Polyflex® hybrid silicone

Table 5-1: Summary of cases LungPoint® used to plan airway stent insertion

5.3.2 Virtual airway animation model

Virtual airway model was successfully created in all of the cases using the patients CT scan. An assessment by 2 interventional bronchoscopists independently agreed that stenting would be appropriate in all 3 cases and agreed what stent dimensions would be appropriate.

Using the 3D airway re-constructed images and the planning software accurate stent sizing and selection was performed in each case. Figure 5.1 illustrates how the animation in Figure 5.1 shows the plan used for stenting in one individual. Figure 5.2 shows the endobronchial images before and after stent insertion.

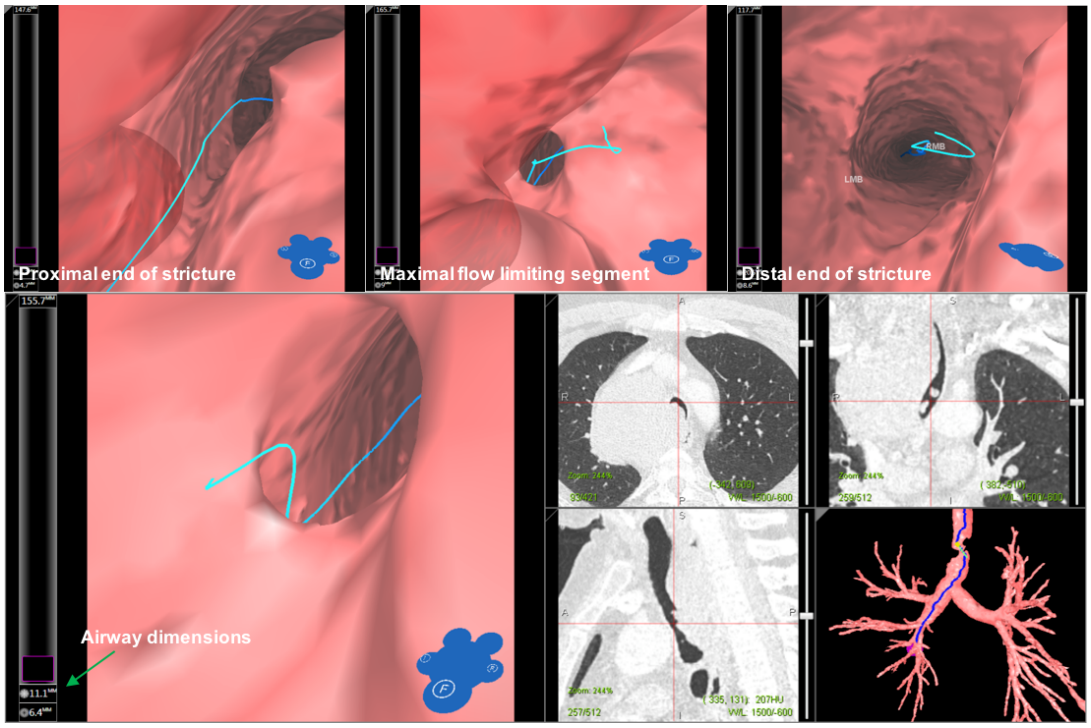
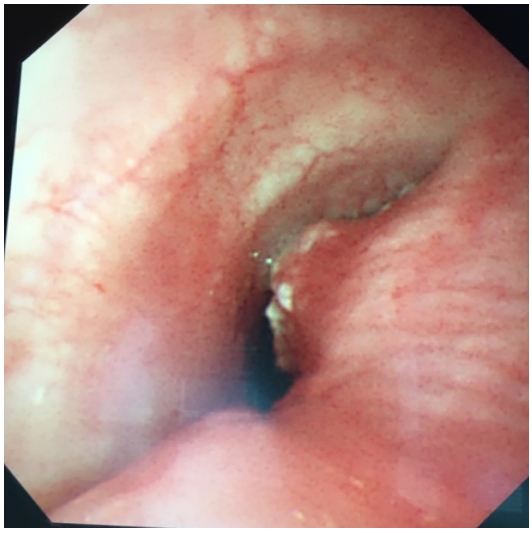


Figure 5.2 – LungPoint® software used to create airway 3 dimensional reconstruction

(a)



(b)

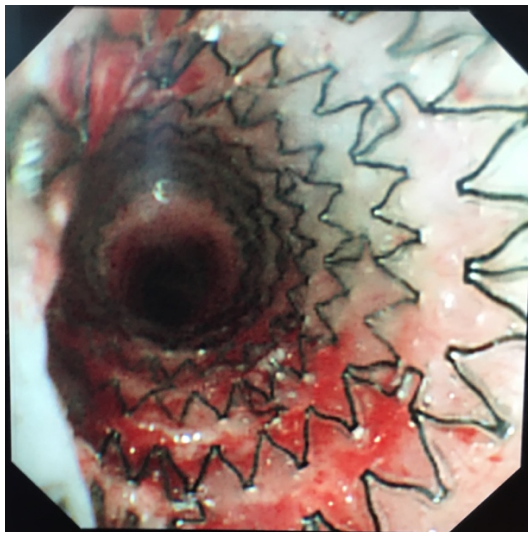


Figure 5.3 – (a) malignant tracheal obstruction treated with (b) fully covered self-expanding AERO (Merit Medical, USA) metal stent

5.4 Discussion

This is the first description of this novel use of LungPoint® navigational software and was a proof of concept pilot in three patients to see if a virtual mock-up of the airway could avoid a diagnostic bronchoscopy assessment.

Squamous cell lung cancer occurs mainly in the central airways and tends to be locally invasive. This means it can cause central airway obstruction, which often requires intervention in symptomatic patients. When there is extraluminal disease, an airway stent is the intervention modality of choice, but the decision to deploy a stent requires a number of factors to be taken into account. The most fundamental considerations relate to the patient and essentially require a judgement to be made as to whether the risks of the procedure are justified by the likely benefits [175].

Successful stenting may transform fitness as long as there is patent airway supplying vascularised lung beyond the point of obstruction, whereas patients with extensive lung damage and compromised pulmonary vessels, as often occurs in locally advanced lung cancer are unlikely to benefit. In order to make this assessment, it is important to predict the physiological and symptomatic benefits of stenting based upon the viability of the distal lung tissue and location of the obstruction. This often requires a bronchoscopy assessment before planning stent insertion. A technique to avoid the need for a diagnostic flexible bronchoscopy would save the patient from a procedure that may not be needed and may cause harm [175].

LungPoint® is a software designed for the virtual navigation to peripheral lung lesions. It segments the airways using a high-resolution volumetric CT scan and then creates a 3D virtual image. This was utilised to look at the tracheobronchial tree in cases of malignant airway obstruction in 3 cases as summarised below.

5.4.1 Case summary

Case 1 was a 64-year old woman with a squamous cell carcinoma of her left main bronchus. She had a known diagnosis of locally invasive disease, which progressed despite chemotherapy and radiotherapy. She was referred after presenting with dyspnoea and wheeze. In her 3D model a distal airway was visible and after taking measurements, a 40 mm x 12 mm stent was ordered and a stenting procedure was planned. At the time of her procedure, the stent size was confirmed using bronchoscopic measures before placing it across the compressed airway with a good result.

Case 2 was a 52-year old man who had a poorly differentiated squamous cell carcinoma of the trachea. A large mediastinal mass caused compression of the trachea and he presented with stridor to his local emergency department. Figure 5.2 shows his virtual animation. There is minimal intraluminal disease and a long length stricture of the trachea. An 18 mm x 80 mm stent was planned using the software and sited successfully before he had definitive treatment of his lung cancer.

Case 3 was a 19 year old patient with cerebral palsy and congenital heart disease had a vascular ring causing tracheal obstruction. He posed a high anaesthetic risk and a diagnostic bronchoscopy would have come at high risk. LungPoint® was used to plan an airway stent, which was ordered in and sited across his stricture successfully. He then had definitive surgical treatment to repair the vascular ring.

All 3 cases were carefully chosen and the 3D reconstruction helped physician planning of the stenting procedure, suggesting that 3D virtual animation can be used as a non-invasive planning tool to determine optimal stent size and position, avoiding a planning diagnostic bronchoscopy in inappropriate cases. This is very preliminary and further investigation in a descriptive cohort study is needed with metrics of diagnostic bronchoscopy avoidance, procedure length, and operator satisfaction.

5.5 Conclusion

The previous chapters in this thesis have focused on bronchoscopic intervention in early central airway cancer. However, when squamous cell cancer is not detected early it often presents with central airway obstruction. So, in this chapter, the aim was to use a new technology to aid the physician in planning challenging airway stenting cases. This was a proof of concept and further prospective data are needed to evaluate whether this technique could avoid diagnostic bronchoscopy, improve procedures times and improve physician confidence and satisfaction. Finally, this software opens the door to possible 3D planning and printing of stents for complex airway strictures.

Chapter 6 A COMPARISON OF SEDATION TECHNIQUES IN ENDOBRONCHIAL ULTRASOUND AND TRANSBRONCHIAL NEEDLE ASPIRATION

6.1 Introduction

Interventional bronchoscopy is a rapidly advancing field, none so less so than in the technique of endobronchial ultrasound (EBUS), which has revolutionised the practice of diagnosing and staging patients with lung cancer [8]. It is recommended as an alternative to surgical staging and it provides enough tissue to allow immunohistochemistry and molecular testing for targetable mutations [179]. Operators are expected to achieve both a diagnostic sensitivity of at least 88% and ensure they take sufficient diagnostic material to allow phenotyping and genotyping of tumours [180]. This means that physicians are placing ever increasing importance on the type of sedation used for their bronchoscopy. In this chapter, the role of anaesthetic vs. physician led sedation is compared to look at its role in interventional bronchoscopy.

Sedation in bronchoscopy aims to allay patient anxiety while reducing cough and dyspnoea to keep the patient comfortable, creating the optimal conditions for the operator [181–183]. The technique of moderate sedation (MS), usually involves the bronchoscopist delivering a combination of benzodiazapines and/or opioids to aim for a level of conscious sedation, whereby the patient is asleep, but able to maintain their airway and obey verbal commands [184]. Deep sedation (DS), general anaesthesia, and total intravenous anaesthesia (TIVA) are sometimes used

synonymously; they describe a deeper level of sedation, often using Propofol (2,6-di-isopropylphenol), a sedative that has a rapid onset and fast recovery time. Since Propofol has no reversal agent and can cause hypotension and respiratory depression, therefore most advisory bodies recommend it is delivered by an anaesthetic professional.

A number of studies have looked at the influence of sedation on diagnostic yield of EBUS, its complication rates and patient tolerance [185–191]. While Propofol appears to provide a better quality of sedation and patient tolerance when compared to Midazolam alone [185,187,192], a systematic review concluded more comparable metrics of diagnostic yield, patient satisfaction and safety when comparing MS and DS [190].

In this chapter, an audit at a single interventional bronchoscopy centre is undertaken to determine if there is a difference in diagnostic yield, specimen adequacy, operator satisfaction, and patient comfort associated with different levels of procedural sedation.

6.2 Methods

6.2.1 Hypothesis

Deep sedation delivered by a specialist anaesthesiologist results in an improved operator and patient satisfaction.

6.2.2 Patient selection

Consecutive individuals undergoing EBUS at University College London Hospital between August 2013 and December 2013 were asked to fill out a satisfaction survey after their procedure for the British Thoracic Society patient satisfaction audit. All individuals over the age of 18 with either benign or malignant mediastinal disease were included and no specific exclusion criteria were applied.

6.2.3 Study design

An audit of this prospectively collected data was undertaken. At UCLH there are specific lists for physician-led or anaesthetic led sedation. All patients had an assessment with the chest or anaesthetic physician prior to their EBUS. Data on physical status (American Society of Anaesthesiologist, ASA), current medication, and co-morbidities was recorded. Data was collected on the bronchoscopy, diagnostic yield, medications administered during the procedure, the operator satisfaction and subsequently a patient satisfaction survey was undertaken.

6.2.4 Bronchoscopy

For moderate sedation, a combination of Midazolam and Fentanyl were administered by the bronchoscopist or nurse specialist and titrated to achieve conscious sedation. Patients undergoing deep sedation would have an initial dose of Midazolam given as an intravenous bolus, followed by a target-controlled infusion of Propofol (10mg/ml solution) and Remifentanyl (2-4 micrograms/ml solution) using either a 'Schnider' or 'Marsh' model, depending on the anaesthesiologists preference [193]. Continuous electrocardiographic, pulse oximetry, respiratory rate, and intermittent blood pressure monitoring was measured for all procedures.

The EBUS was undertaken by one of 2 operating physicians and a interventional bronchoscopy clinical fellow, using the Olympus BF-UC 180F (Tokyo, Japan) or Pentax EB-1970UK (Pentax Medical, Tokyo, Japan). The EBUS was inserted orally and local anaesthetic (lignocaine 1 or 2%) was applied to the vocal cords and tracheobronchial tree to relieve coughing. The subject then underwent EBUS with transbronchial needle aspiration (TBNA) for the indications. Any further diagnostic flexible video bronchoscopy procedures needed were also carried out.

Complications were recorded and defined as hypoxia (oxygen desaturation <90% needing insertion of oral or nasal airway), hypotension (systolic <90mmHg), bleeding (if not controlled with topical adrenaline), need for intubation and ventilation, transfer to the intensive unit, and death.

At the end of the procedure, the bronchoscopist would chart their satisfaction of carrying out the EBUS. Similarly, 90 minutes after the bronchoscopy, patients were asked to document their experience in: discomfort from cough, pain, dyspnoea, odynophagia, chest pain; as well as seeing how much of the procedure they recall and if they would recommend the anaesthetic they had.

6.2.5 Analysis

The primary analysis focused on the operator satisfaction and patient comfort. Further analysis assessing the diagnostic yield (proportion of patients for whom EBUS-TBNA resulted in a specific diagnosis), and procedure duration were also undertaken. Any categorical variables were expressed as proportions and scale variables as means. Differences between groups were estimated using chi-square, Fisher's exact, and independent two-sample t-tests or Mann Whitney U for non-parametric data. All other continuously non-normally distributed parameters were evaluated using the nonparametric Mann–Whitney U-test or Kruskal–Wallis test, as appropriate. As reviewed later in discussion, the two sedation groups do have a major bias that would mean the statistical analysis used to show an effect would have to be taken into consideration. All statistical analyses were performed using SPSS Statistics software (version 24.0; IBM, Armonk, NY).

6.3 Results

6.3.1 Study population

Over the course of 4 months, data on 45 patients was collected, 31 in the moderate sedation group and 14 in the deep sedation group. The mean age at study entry was 61 years old and there were twice the number of male patients compared to female. Overall, the distribution of patient age, gender, and body mass index across the deep and moderate sedation groups were equal. There was also no significant difference for the indications for EBUS. Figure 6.1 shows the patient ASA grade at study entry in the two groups. Individuals were dichotomised into ASA grade 2 and below or ASA grade 3 or 4, and there was a higher proportion of patients with high co-morbidity scores in the deep sedation group (Table 6-1).

Patients having deep sedation had a combination of midazolam (mean 1.25mg) and infusion of propofol (mean total dose 356mg), and remifentanil (mean total dose 65.6mcg) and those in the moderate sedation group received midazolam (mean 3.7mg) and fentanyl (mean 53mcg). On average, patients in the moderate sedation group had more lignocaine than those who had deep sedation (253 ± 136 mg vs. 172±82 mg; p=0.02).

	Total	**MS Group**	**DS/GA Group**	***p* value**
Individuals (n)	45	31	14	
Age	61.2±15.4	59.7±12.6	64.4±15.3	0.35
Gender				
Male	30	19	11	0.32
Female	15	12	3	
Body mass index (mean, kgm_{-2})	25.2±4.3	25.6±4.6	24.3±3.4	0.35
ASA Grade				
1-2	26	21	5	**0.06**
3-4	19	10	9	
EBUS indication				
Diagnostic	36	25	11	0.87
Diagnosis & Staging	9	6	3	
Re-staging	0	0	0	

Table 6-1: Baseline characteristics for patients having endobronchial ultrasound under moderate and deep sedation

ASA score = American Society of Anaesthesiologists score; BMI = body mass index; GA = general anaesthesia; DS = deep sedation; MS = moderate sedation.

	Total	**MS Group**	**DS/GA Group**	***p* value**
Individuals (n)	45	31	14	
Local anaesthetic dose (mg)		253±136	172.1±82	**0.02**
Sedation doses				
Midazolam (mg)		3.7±1.2	1.25±0.75	<0.01
Fentanyl (mcg)		53.2±15	0	
Propofol (mg)		0	356±180	
Remifentanyl (mcg)		0	65.6±42	

Table 6-1 – Baseline characteristics for patients having endobronchial ultrasound under moderate and deep sedation (continued)

ASA score = American Society of Anaesthesiologists score; BMI = body mass index; GA = general anaesthesia; DS = deep sedation; MS = moderate sedation.

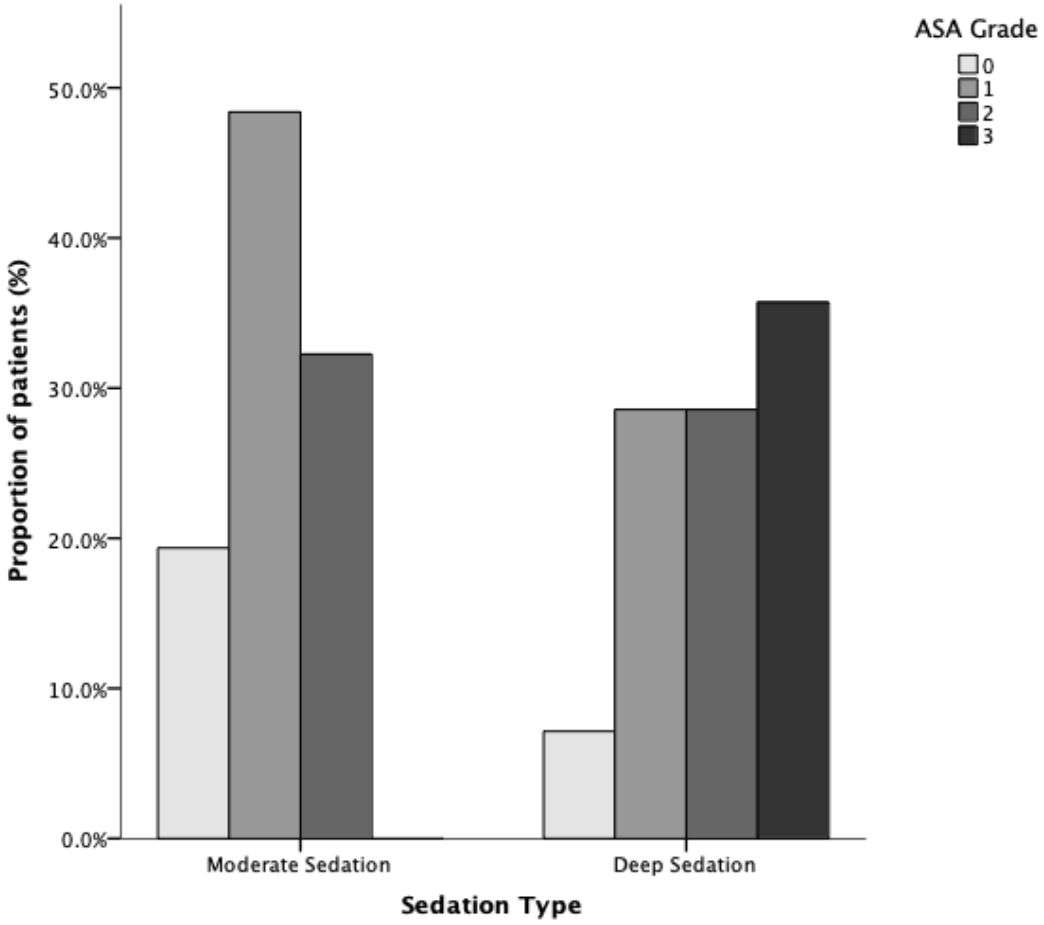


Figure 6.1 – ASA grade on study entry in moderate and deep sedation groups

ASA score = American Society of Anaesthesiologists score

6.3.2 Procedure-related performance

An overview of the total number of lymph nodes or masses sampled under EBUS-TBNA are shown in Table 6-2. A total of 35 lesions were sampled in the moderate sedation group and 19 in the deep sedation group. There was a similar distribution of nodes sampled in the hilar and mediastinal position with a median of 1 (1-4) lymph node station sampled. In the deep sedation group there was a tendency to sample more lymph nodes, but this was not significant, p=0.42. The average size of lymph node sampled in patients having moderate sedation was 16.6 ± 8mm and 14 ± 10mm in the deep sedation group (p=0.36); and the number of passes taken through each node were no different in the two groups (Figure 6.2). Sufficient tissue was taken for all procedures undertaken and the operating time in the moderate sedation group was significantly shorter than deep sedation (25 ± 12 minutes vs. 36 ± 17min; p=0.04). The diagnostic yield in the deep sedation group was higher than moderate sedation, but this was not significant (71.4% vs 61.3%; p=0.71). It was also twice as likely for the pathologist to have insufficient tissue to process in the moderate sedation group, but again this was not significant (12.9% vs. 7.1%; p=0.60).

6.3.3 Complications

There were no reported complications in the moderate sedation group. In the patients having deep sedation, 36% (n=5) had problems with desaturation and one patient (7%) had hypotension at time of anaesthetic. The relative risk of having a complication with deep sedation is 3.1 (95% confidence interval 1.7-5.5; p=0.03), but no patients in either group required an escalation of their care.

	Total	**MS group (n=31)**	**DS group (n=14)**	**p value**
Lymph nodes & masses (n)	54	35	19	
Stations				
Hilar	5	4	1	0.63
Mediastinal	34	21	12	
Hilar & mediastinal	6	5	1	
Number of lymph node sites (n)	1 (1-4)	1 (0-2)	1 (1-4)	0.42
Lymph node size (mm)	15.8±8.8	16.6±8	14±10.2	0.36
Number of passes node^{-1} (median)	5 (1-8)	5 (1-8)	5 (3-7)	0.61
Tissue collected				
Core biopsy	45	31	14	1.0
Slides	45	31	14	
Microbiology	43	29	14	
Procedure duration (min)	28.5±12.0	25.2±7.0	35.7±17.1	**0.04**

Table 6-2: Characteristics of lymph node assesses and sampled during EBUS

DS = deep sedation; MS = moderate sedation

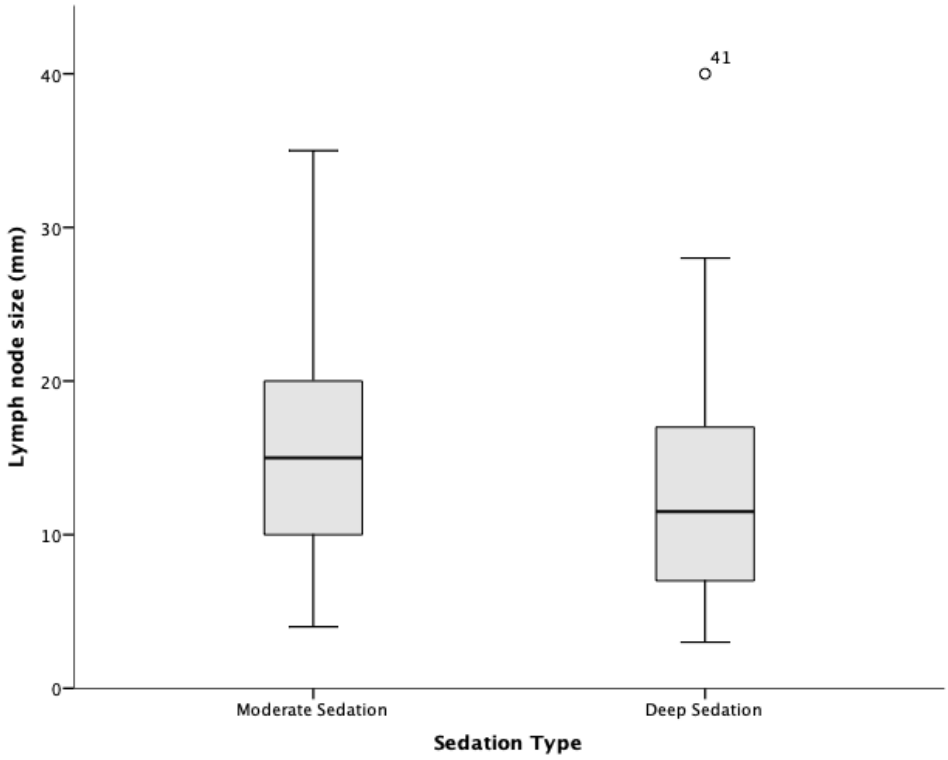


Figure 6.2 – Mean lymph node size in moderate and deep sedation groups

(p=0.36)

6.3.4 Patient & Operator Satisfaction

Patient comfort was measured with a Likert-type scale questionnaire on degree of discomfort from cough, sore throat, chest pain, and dyspnoea. These were reported to a similar degree in both the moderate and deep sedation groups (Table 6-3). Although not significant, patients were more likely to have discomfort from throat spray and insertion of the bronchoscope (p=0.09 and 0.14), respectively. Patients in the moderate sedation and deep sedation group were mostly willing to return and have another procedure, although 25% of all individuals did say they definitely wouldn't return (Table 6-4). In the deep sedation group there was a tendency towards most patients definitely returning for another procedure, although this was non-significant (64% vs. 42%; p=0.14), Figure 6.3. Further, patients in the deep sedation group were more likely to have either minimal or no recall of the procedure (64% vs. 42%; p=0.28).

Operator satisfaction was recorded on a 1–10 scale visual analogue scale (1= least satisfied and 10= most satisfied). There is no difference in the average satisfaction score in the moderate or deep sedation groups (8.48 ± 1.9 vs. 8.64 ± 1.1; p=0.69) (Figure 6.4). There is a distribution towards more satisfaction when cases are performed under deep sedation, but this was not found to be significant (p=0.20) (Figure 6.5).

	MS group (n=31)	**DS group (n=14)**	***p* value**
How much discomfort from throat spray did you have?			
None	45% (14)	71% (10)	0.09
Small amount	48% (15)	14% (2)	
Large amount	7% (2)	14% (2)	
How much discomfort from insertion of bronchoscope did you have?			
None	55% (17)	79% (11)	0.14
Small amount	36% (11)	7% (1)	
Large amount	9% (3)	14% (2)	
How much discomfort from cough did you have?			
None	13% (4)	36% (5)	0.10
Small amount	68% (21)	36% (5)	
Large amount	19% (6)	28% (4)	
How much discomfort from chest pain did you have?			
None	81% (25)	93% (13)	0.25
Small amount	16% (5)	0% (0)	
Large amount	3% (1)	7% (1)	

How much discomfort from dyspnoea did you have?			
None	64% (20)	79% (11)	0.57
Small amount	29% (9)	14% (2)	
Large amount	7% (2)	7% (1)	
How much sore throat did you have?			
None	52% (16)	57% (8)	0.77
Small amount	45% (14)	43% (6)	
Large amount	3% (1)	0% (0)	

Table 6-3: Patient comfort after having an endobronchial ultrasound under moderate and deep sedation

	MS group (n=31)	**DS group (n=14)**	***p* value**
How willing to return and have a repeat procedure?			0.14
Definitely not	7% (2)	21% (3)	
Probably not	23% (7)	0% (0)	
Not sure	13% (4)	7% (1)	
Probably would	16% (5)	7% (1)	
Definitely would	42% (13)	64% (9)	
How much of the procedure do you recall?			0.28
None	26% (8)	50% (7)	
Minimal level	16% (5)	14% (2)	
Some level	19% (6)	7% (1)	
Moderate level	16% (5)	0% (0)	
High level	23% (7)	29% (4)	
Would you have the same anaesthetic again?			0.53
Definitely not	3% (1)	7% (1)	
Probably not	13% (4)	0% (0)	
Not sure	3% (1)	0% (0)	
Probably would	19% (6)	14% (2)	
Definitely would	61% (19)	79% (11)	

Did you feel safe during you procedure?			0.74
Do not agree	3% (1)	0% (0)	
Slight agree	3% (1)	7% (1)	
Not sure	3% (1)	0% (0)	
Moderately agree	16% (5)	7% (1)	
Definitely agree	74% (23)	86% (12)	

Table 6-4: Patient satisfaction after having an endobronchial ultrasound under either moderate or deep sedation

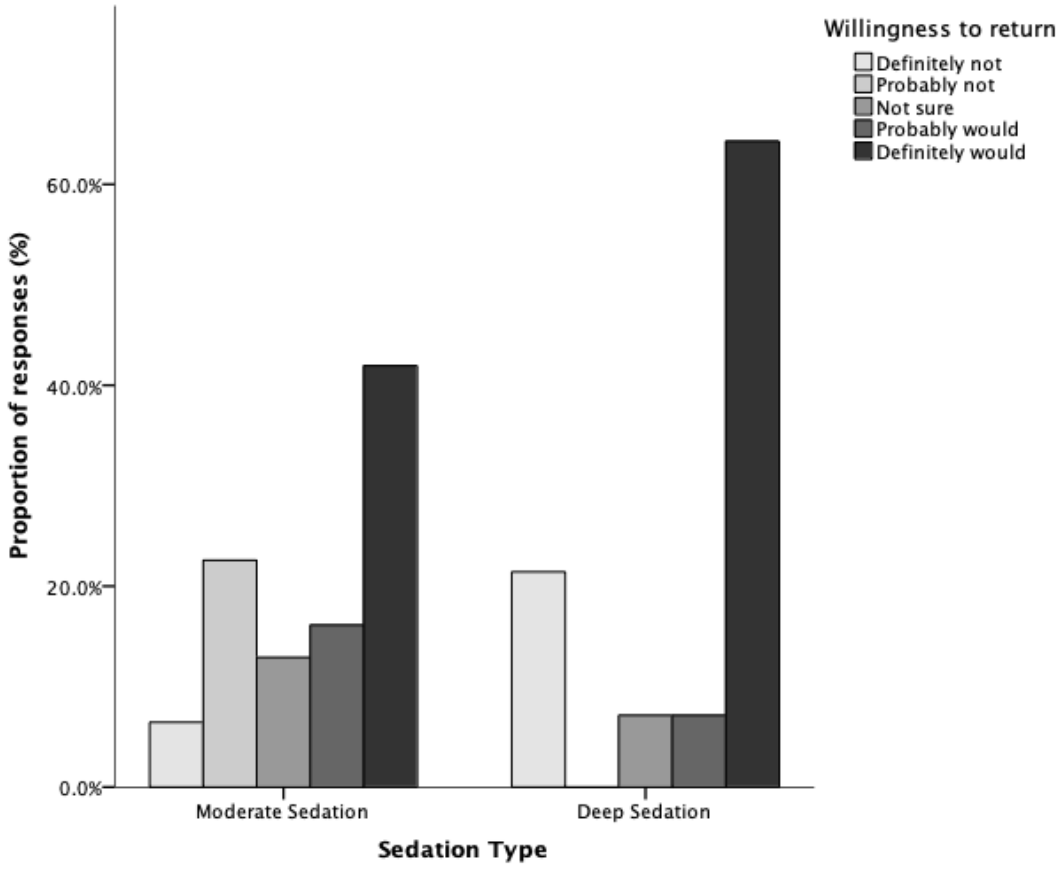


Figure 6.3 – Patient willingness to return after having an endobronchial ultrasound under moderate or deep sedation

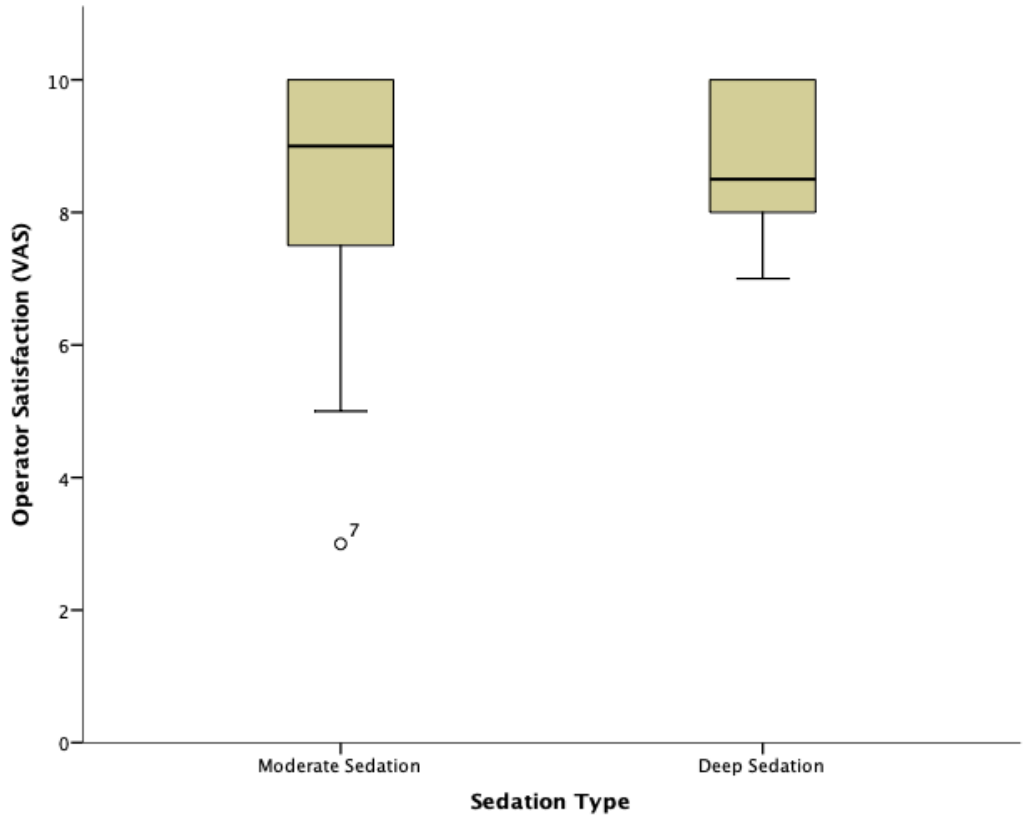


Figure 6.4 – Operator satisfaction visual analogue score (VAS) after performing endobronchial ultrasound under either moderate or deep sedation

Mean operator satisfaction in moderate (8.48 ± 1.9) vs. deep sedation groups (8.64 ± 1.1) (p=0.69)

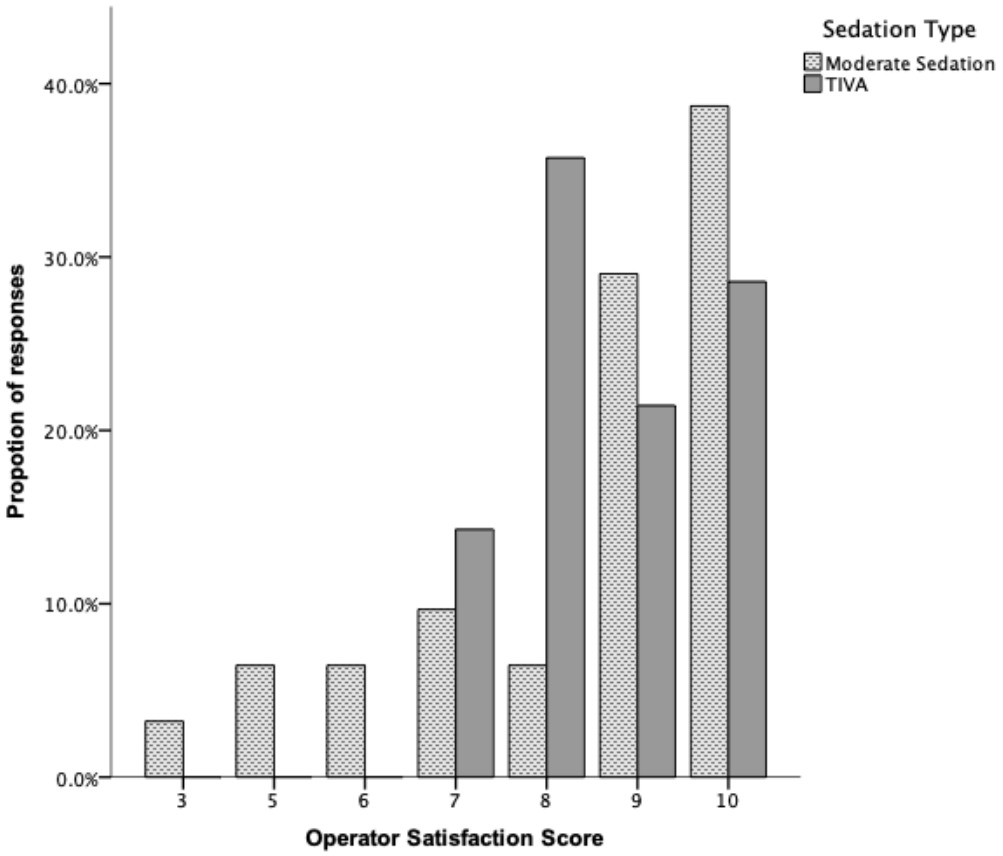


Figure 6.5 – Operator satisfaction after performing endobronchial ultrasound under moderate or deep sedation

TIVA = total intravenous anaesthesia (synonym deep sedation)

6.4 Discussion

In this study, we report patient, procedure, and operator outcomes from individuals undergoing EBUS under either moderate or deep sedation. We show that there are no differences in diagnostic yield, patient satisfaction or comfort, and no differences in operator satisfaction, when compared to moderate sedation. Deep sedation can be undertaken safely in patients with significant co-morbidities and less local anaesthetic needs to be delivered.

Endobronchial ultrasound (EBUS) is an advanced diagnostic bronchoscopy technique that has been adopted by interventional bronchoscopists and plays a central role in the diagnosis and staging of lung cancer [194]. Like many other interventional bronchoscopy procedures it requires a much higher level of technical skill, which means adequate sedation is paramount. Sedation in bronchoscopy using benzodiazepines or Propofol have individually been shown to improve patient comfort and create better conditions for the operator to perform the procedure [181–183]. However, for more complex procedures there has been much debate on whether deep sedation delivered by an Anaesthesiologist or specialist equivalent is superior to Physician led moderate sedation [185–187,195–197].

The drugs commonly used for moderate sedation are midazolam and an opioid, often fentanyl, which are delivered as intravenous boluses by the physician undertaking the procedure, which is also our practice. In deep sedation, Propofol is invariably used either alone or in combination with an opioid [186,187,189,195–197]. Coughing during a procedure makes it technically more challenging and is more common when using moderate sedation [186]. Local anaesthetic application with lignocaine helps reduce this, and in this study we found we used much less in patients having deep sedation; 172.1 mg compared to 253 mg in moderate sedation

group (p=0.02). This is likely because remifentanil, a very short acting (half-life <10min) μ-opioid agonist [198], used in combination with Propofol in our deep sedation cases has an anti-tussive effect resulting in less local anaesthetic spray. In this study, no procedures had to be abandoned in either group due to inadequate sedation. One individual in the study was referred from another hospital after moderate sedation failed to adequately sedate the patient. In a trial by Casal et al, 5 individuals in the moderate sedation group needed conversion to a general anaesthetic (non-significant). This highlights that moderate sedation can sometimes fail and in many institutions in the UK, patients will require a repeat of the procedure under anaesthetic [186].

There are currently two prospective randomised controlled trial comparing deep sedation to moderate sedation, which have shown no significant difference in the sensitivity and diagnostic yield of individuals undergoing EBUS-TBNA [186,191]. Similarly, Oztas et al in their recent retrospective study showed a similar diagnostic yield in patients undergoing moderate or deep sedation (85.7% vs. 77.6%), respectively [195]. In this study, we report similar findings, with no significant difference in the diagnostic yield. In contrast, Yarmus et al looked at the diagnostic yield in EBUS in their retrospective study comparing 2 major interventional pulmonology centres in the US; one that used moderate sedation and the other deep sedation with a laryngeal mask or endotracheal tube [187]. They reported a higher diagnostic yield when EBUS was performed under deep sedation, which was potentially as a result of being able to do more passes through each nodal station; something also noted in the Quality Improvement Registry, Evaluation and Education (AQuIRE) dataset [199]. However, the biggest confounding factor in this study was the comparison across 2 different sites, which would have influences from different study populations, operators, and pathologists. In our study, we found no significant differences in the size of the lymph nodes or the number of

passes made through each node. There was a tendency to sample more nodal stations in the deep sedation group, but this difference was not significant.

In this study, the total time taken for the procedure was shorter in the moderate sedation group; 25.2 minutes verses 35.7 minutes in the deep sedation group. This differs to that reported by Yarmus et al, who found a procedure time of 46.9 and 36.4 minutes in patients having moderate or deep sedation for their EBUS, respectively [187]. The short procedure time in our moderate sedation group is probably explained by, (i) individuals allocated to the moderate sedation list are usually for diagnostic EBUS rather than staging procedures, and (ii) that we use 2 needles for sampling, which reduces the procedure time. We also have many Consultants and registrars training in EBUS on our deep sedation lists, which naturally increases the time of the procedure.

We report an increased complication rate when patients are having deep sedation. These were all sedation-related complications and none required any escalation of the patients clinical care. Five patients (36%) had desaturation and another (7%) had hypotension during the procedure. A recent systematic review looked at safety around the two sedation methods reported 3 studies as showing no difference in escalation of care between the two groups [190]. However, an observational cohort study showed patients having deep sedation were at higher risk of escalation of care compared to those having moderate sedation (OR 4.7, 95% CI 1.0-21.6; $p<0.05$) [200]. There are two explanations for increased risk of complications in this study. Firstly, the patient population have a high co-morbidity score and the presence of co-existing lung disease may predispose to desaturation. Second, the observation period was at the time when we had increased our bronchoscopy capacity under general anaesthetic, which meant there were more anaesthetists less experienced in delivering deep sedation in bronchoscopy. Since this study we

have also started using high-flow oxygen at 30-50 $Lmin^{-1}$ (Optiflow®), which has prevented virtually all hypoxic episodes during bronchoscopy and has even been used in apnoeic ventilation.

Steinfort et al were the first to report on a patient satisfaction metric after performing EBUS under conscious intravenous sedation [201]. They reported >90% of individuals were satisfied with their procedure. In this study, we reported no difference in the patient comfort whether they had moderate or deep sedation. Casal et al reported no significant differences in patient satisfaction when assessing similar metrics such as level of discomfort from sore throat, cough, chest pain and dyspnoea. We report that patients undergoing deep sedation were twice as likely (50% vs 26%) to have no recollection of their procedure (non-significant). Overall, satisfaction of the procedure under DS was good with 79% of individuals stating they would have the same anaesthetic again compared to the 61% who had MS.

A neglected area is the physicians satisfaction of the procedure – “if I had a little more time maybe I could’ve sampled that node better” is an all too common phrase, and managing a complex procedure and sedation simultaneously can be challenging. This study is the first to look at operator satisfaction recorded on a 1–10 scale visual analogue scale and shows there is no difference in the median score for satisfaction (1= least satisfied and 10= most satisfied) in the MS or DS groups. There was a distribution towards more satisfaction with deep sedation, but this was not found to be significant. This is probably owing to the numbers, but also a single very experienced operator performed many of the procedures under MS. Fernandes et al assessed difficulties the bronchoscopist found in some of the technical skills such as EBUS scope intubation and node sampling [191]. They randomised patients to sedation vs. sedation and neuromuscular blockage and found that bronchoscopists found the procedure technically easier in those patients who had sedation and paralysis [191].

There were several limitations in this study. The most relevant is the bias introduced in the study population through our triage system. When bronchoscopy referrals come into our centre, they are reviewed by a nurse specialist, clinical fellow, and interventional bronchoscopist. Patients who are younger, have more comorbidities, and on radiology are anticipated to have a more complex procedure (e.g. smaller lymph nodes, altered anatomy, central airway obstruction) are automatically assigned to a deep sedation list. In this study, a significant proportion of patients have a higher co-morbidity score (ASA grade) in the deep sedation group. This will influence the length of the procedure, the anaesthetist decision to avoid a very deep level of sedation, and the level of comfort the patient experiences. Similar to Casal et al, this study also has a limitation whereby most of the procedures were performed or supervised by one very experienced operator. It is not clear whether our data can be extrapolated to other institutions, and if less experienced operators could achieve the same results under moderate sedation. The other limitation is there is no blinding, which is not really possible and could result in differences in not only how the operator performs the procedure, but how the patient is sedated. The study also has relatively small numbers of patients assessed over a short period of time. Trainees were able to participate in the bronchoscopy procedures, which may have prolonged procedures and affected outcomes.

This study helps answer some important questions with regard to utilisation of deep or moderate sedation for EBUS, and bronchoscopy as a whole: whether the diagnostic yield is better under general anaesthetic; whether patients are more comfortable after anaesthetist led sedation, and if the operator will feel they have had the optimal conditions to perform the procedure.

In straight-forward diagnostic flexible video bronchoscopy and EBUS performed under moderate sedation by an experienced operator in a relatively fit patient will mostly result in a good outcome. Therefore, at our institution a pragmatic approach is to use DS for younger individuals, individuals on regular sedative and opiate medications, patients with more co-morbidities will probably benefit from having a procedure under general anaesthetic. A less-experienced operator or an bronchoscopist who is training others will benefit from having an anaesthetist deliver sedation. Complex and lengthy bronchoscopy that requires accurate staging of lung cancer will be facilitated by a general anaesthetic [202]. Finally, in therapeutic bronchoscopy such as in airway stenting or ablative therapy, we would strongly advocate anaesthetist led sedation, where there may be a need for muscle paralysis and rigid bronchoscopy, and the potential for emergent intubation and ventilation.

6.5 Conclusion

Bronchoscopy is a spectrum of diagnostic flexible video bronchoscopy performed under moderate sedation to therapeutic procedures performed under general anaesthetic. EBUS-TBNA, an advanced diagnostic bronchoscopy technique can be performed with either sedation technique. The purpose of this chapter was to demonstrate safety and outcome measures in an anaesthetist led sedation.

In this study, we show that the sedation technique does not affect the patient or operator experience, and doesn't appear to have an impact on the diagnostic yield. However, we do show that general anaesthesia can be undertaken safely in patients with multiple co-morbidities and demonstrate where general anaesthesia has a role to play in bronchoscopy.

As we see a rise in novel bronchoscopy techniques we will continue to question the optimal sedation technique, which will require further investigation and robust randomised controlled trials.

Chapter 7 SUMMARY

This thesis has examined the role of bronchoscopy in the diagnosis, surveillance, treatment, and prevention of early lung cancer. Chapter 1 demonstrates that interventional bronchoscopy is a growing field with an increasing need for minimally invasive treatments for patients who are unable to undergo conventional treatment or need a tissue-sparing treatment for early central airway cancers. It reviews the different endobronchial modalities, their indications and how they are used to treat lesions in the bronchial tree. Two different medical lasers are described, a low power laser used to activate a photosensitiser in photodynamic therapy and another high power laser used for vaporising tumour.

Autofluorescence bronchoscopy has been crucial in the longitudinal surveillance of patients with pre-invasive disease of the airway. Chapter 2 shows that pre-invasive lesions are clinically important, with approximately 50% of severe dysplasia and carcinoma in situ lesions developing into endobronchial squamous cell lung carcinomas. This work also showed that high-grade lesions serve as markers of 'lung cancer risk' with 50% of all interval cancers being detected at another site in the bronchial epithelium or lung parenchyma. The role of close surveillance with autofluorescence bronchoscopy and computerised tomography in this group was supported by the remarkable stage shift at diagnosis, with >80% of cancers being detected at stage I. This is in clear contrast to the majority of patients in England who present with advanced (stage IV) lung cancer. The strategy of close surveillance and acting when there is evidence of microinvasion led to prompt treatment of early lung cancer and a 5-year survival of 70% in these individuals, which is comparable to the 73% 5-year survival seen in patients with stage IA lung cancer [203]. Therefore, the data from this chapter may guide other groups on the surveillance strategy that is necessary in this high-risk cohort.

Patients with high-grade pre-invasive endobronchial lesions were more likely to develop lung cancer at the index site or at remote sites in the lungs. In chapter 2,

the clinical parameters at baseline could not accurately predict the occurrence of cancer. However, comprehensive molecular analysis of genomic, epigenomic, and transcriptomic data from each lesion biopsied from every patient in the study led to the development of a predictive model that can identify which lesion will progress to invasive lung cancer [124].

One challenge that was evident from the longitudinal surveillance study was the optimal treatment strategy for these patients, who are at high risk of developing interval cancers. This called for a tissue-sparing strategy, so in chapter 3, the efficacy of photodynamic therapy was investigated as an endobronchial treatment for patients with early invasive central airway lung cancers. This prospective study showed that PDT was an effective therapy with minimal complications – 75% of individuals had a complete response to photodynamic therapy and an analysis in to the factors that affect a successful response to PDT showed that small (<15 mm) and superficial lesions were exquisitely sensitive to the treatment. The median overall survival for this cohort was 32 months (95% confidence interval 20–44months), which was not unexpected since the study population were patients who were unfit for conventional lung cancer treatment. The reported 5-year survival in this study was no different to patients selected for stereotactic radiotherapy (42% vs. 37%) [140]. In the cohort, 38% of patients died from non-cancer cause of death (overall lung cancer mortality was 42%). This led to the question of whether early treatment, i.e. before lesions become invasive could prevent cancer occurrence.

It is widely accepted that squamous cell lung cancers arise from pre-invasive lesions. As shown in chapter 2, not every pre-invasive lesion is destined to this course and in many individuals, lesions may not progress, or may indeed regress to normal epithelium. However, their treatment remains controversial and the American College of Chest Physicians would still advocate surgical resection of

individuals with high-grade pre-invasive disease. There is no randomised evidence to support this practice and so Appendix A presents the PEARL trial – a multi-centre randomised phase III clinical trial that will test whether photodynamic therapy can be used as a treatment to reduce the incidence of cancer in patients with high-grade pre-invasive endobronchial lesions. Data on smoking status, and co-morbidities will be collected, and individuals randomised to receive either, (i) 6–12 monthly surveillance with AFB and CT thorax, or (ii) PDT followed by surveillance with AFB and CT thorax. The primary outcome will measure the proportion of HGLs that develop into invasive lung cancers over 3 years. Secondary outcomes will look at (i) the incidence of multi-focal lung cancer, (ii) overall and lung cancer- specific survival, (iii) health-related quality of life, and (iv) cost-effectiveness. Additional biopsies will also be collected to create a unique pre-invasive tissue bank of treated patients. The PEARL trial is funded by CRUK and is the first phase III randomised trial of an endobronchial therapy for the prevention of lung cancer and is scheduled to open in 4 sites across England and the Netherlands.

The second part of the thesis examined the role of other bronchoscopy techniques in treatment of carcinoid tumours, palliation of lung cancer, and the role of sedation in interventional bronchoscopy. Chapter 4 describes the use of a high-power semiconductor diode medical laser in the treatment of carcinoid tumour of the lung. Typical carcinoid by definition is malignant, but falls on far end of the spectrum of neuroendocrine tumours characterised by a low mitosis and Ki67 index making them very much benign tumours of the lung. They also frequently occur in the central airway, which makes them accessible to endobronchial therapy. In chapter 4, an analysis of a prospective cohort is undertaken in patients referred to University College London Hospital for laser therapy of carcinoid tumours. The primary goal was to examine the safety and effectiveness of high-power medical lasers in the airway. 73% of individuals and had complete response to laser therapy and any recurrence was easily managed with further laser therapy or surgical

resection. Further, this study showed that recurrence was treated without fear of metastatic disease developing or affecting survival. Analysis of factors that could influence the success of complete ablation revealed small (<20 mm) and purely intraluminal tumours were most sensitive to thermal ablation by diode laser. Although no escalation of care was needed, a high rate of bleeding complications were seen with endobronchial therapy, which is expected of carcinoid tumours that are regarded as being incredibly vascular. A clinical trial to test non-inferiority of endobronchial laser therapy vs. surgical resection is needed. However, this chapter described that laser endobronchial therapy was safe when undertaken in a specialist interventional bronchoscopy unit and that it should be considered as a lung-sparing alternative to 'gold-standard' surgical resection.

Squamous cell lung cancer has the potential to cause malignant airway obstruction and the role of bronchoscopy as a palliative treatment was described in chapter 5. Diagnostic bronchoscopy forms one of the most crucial investigations in such patients before deciding if intervention is possible and what type of treatment modality to use. In this proof of concept study, three patients had a virtual 3D animation of their airway created and 2 independent bronchoscopists agreed on the type of procedure and stent sizing before this was corroborated with flexible video bronchoscopy. This study will be taken forward as a prospective descriptive cohort study looking at the role of 3D animation in planning procedures in central airway obstruction.

The final study conducted as part of this thesis was in the most rapidly growing interventional bronchoscopy procedure, endobronchial ultrasound and transbronchial needle aspiration (EBUS-TBNA). Physicians are placing ever increasing importance on sedation as the complexity and length of procedures increase. At University College London Hospital the growing number of procedures meant that an evaluation of sedation techniques was essential. Chapter 6

compared moderate bronchoscopist led sedation vs. deep anaesthetic led sedation in a prospective study of patients undergoing EBUS-TBNA. No significant differences were seen in the moderated vs. deep sedation group when looking at diagnostic yield (61% vs. 71%; p=0.71), patient comfort and satisfaction, or operator satisfaction. The main limitation of this study was that an interventional bronchoscopist or senior interventional clinical fellow would determine which patients are done under moderate or deep sedation based on the anticipated complexity of the EBUS-TBNA and patient co-morbidity. Despite this bias, the study did steer practice to using deep sedation on individuals that are younger, have more co-morbidities, and when trainees are attending the lists. The study also reported an increased relative risk of 3.1 (95% confidence interval 1.7-5.5; p=0.03) of complications with deep sedation, mainly desaturation. Although no patients needed an escalation of care, this led to a change in practice at UCLH, where the use of high-flow nasal oxygen (Optiflow®) has now become standard practice.

This thesis has examined a 'high-risk' cohort improving our understanding of preinvasive disease and the occurrence of squamous cell lung cancer through advanced diagnostic bronchoscopic techniques. This has been complemented by extensive biological work, which has isolated predictive biomarkers that can identify patients that will develop lung cancer. The role of minimally invasive therapies in treating cancers of the tracheobronchial tree were then described and how they will be examined in a clinical trial of high-risk patients in order to prevent the development of lung cancer.

Chapter 8 SUPPLEMENTARY WORK

University College London Hospital is a tertiary centre for interventional bronchoscopy referrals from many hospital in South England. While this thesis has focused on the bronchoscopic management of early central airway lesions, other academic work has been done alongside this in both lung cancer and bronchoscopy management of central airway obstruction. This thesis has also contributed to a considerable amount of cell biology work that has led to a better understanding of lung cancer pathogenesis. The supplementary work done and publications are described below and included in the Appendix.

8.1 Guideline

London Cancer Lung Cancer Guidelines 2014. Thakrar RM, Janes SM, Singer J, Wells P. London Cancer Network: http://www.londoncancer.org/media/81177/london-cancer-lung-clinical-guidelines-final-june-2014.pdf.

8.2 Book chapter

Tracheobronchial Stenting. Chapter 9 In: Sandhu G, Reza Nouraei SA eds. Laryngeal and Tracheobronchial Stenosis. Thakrar RM, Janes SM, George PJ. Plural Publishing.

8.3 Publications

[1] Deciphering the genomic, epigenomic, and transcriptomic landscapes of pre-invasive lung cancer lesions, Nature Medicine 2019 [124].

[2] Optimized isolation and expansion of human airway epithelial basal cells from endobronchial biopsy samples, Tissue Engineering & Regeneration 2018 [204].

[3] Phylogenetic ctDNA analysis depicts early-stage lung cancer evolution, Nature 2017 [205].

[4] Preinvasive disease of the airway, Cancer Treatment Reviews 2017 [206].

[5] Tracking the evolution of non-small cell lung cancer, New England Journal Medicine 2017 [207].

[6] Genetically modified mesenchymal stromal cell is cancer gene therapy, Cytotherapy 2016 [208].

[7] Combined cell-gene therapy for lung cancer: rationale, challenges and prospects, Expert Opinion Biology Therapy 2016 [209].

[8] Rapid Expansion of Human Epithelial Stem Cells Suitable for Airway Tissue Engineering, American Journal Respiratory and Critical Care Medicine 2016 [210].

[9] Positive 18-Fluorodeoxyglucose-Positron Emission Tomography/Computed Tomography Predicts Preinvasive Endobronchial Lesion Progression to Invasive Cancer, American Journal Respiratory and Critical Care Medicine 2016 [47].

8.4 Abstracts

[1] Photodynamic therapy for the prevention of lung cancer (The PEARL trial), Lung Cancer 2017 [211].

[2] Virtual animation and 3-dimensional airway reconstruction using LungPoint® to guide tracheobronchial stenting, Lung Cancer 2017 [212].

[3] Transbronchial cryobiopsies in the diagnosis of interstitial lung disease, Thorax 2015 [213].

[4] Role of EBUS-TBNA in the diagnosis of primary and relapsing haematological malignancy, Thorax 2014 [214].

[5] Incidental detection of early stage non-small cell lung cancer – time to implement screening, Thorax 2014 [215].

[6] Carcinoma in-situ at the bronchial resection margin – a case for routine surveillance with autofluorescence bronchoscopy, Thorax 2014 [216].

[7] Infrared spectroscopy for the detection of extended field carcinogenesis: a new paradigm for lung cancer screening, Thorax 2014 [217].

[8] MIF as the key regulator for mesenchymal stem cell homing to tumours by 3D and in vivo lung metastasis models, Thorax 2014 [218].

8.5 Clinical trials portfolio

Co-investigator on PEARL trial (Chief investigator: Sam Janes) – multi-center, international randomized controlled trial of photodynamic therapy for the prevention of lung cancer. PEARL is a CRUK funded trial and the first to look at prevention of lung cancer using PDT ablation as described in Appendix A.

Co-investigator on Longitudinal Surveillance of Pre-invasive Disease (Chief Investigator: Sam Janes) – multi-center longitudinal descriptive phase II trial looking at the natural history of pre-invasive disease in the airway described in chapter 2.

Co-investigator on UK Lung Volume Reduction (Chief Investigator: Nicholas Hopkins) – multi-center observational study examining outcomes in endoscopic lung volume reduction techniques.

Principle Investigator on Registry of therapeutic bronchoscopy in airway obstruction. Longitudinal descriptive cohort study of symptomatic and quality of life outcomes in individuals undergoing interventional bronchoscopy. Protocol in preparation.

Chapter 9 REFERENCES

[1] Jemal A, Bray F, Center MM, Ferlay J, Ward E, Forman D. Global cancer statistics. CA Cancer J Clin 2011;61:69–90. doi:10.3322/caac.20107.

[2] Siegal R, Miller K, Jemal A. Cancer statistics, 2015. CA Cancer J Clin 2015;65:5–29. doi:10.3322/caac.21254.

[3] Wright G, Manser RL, Byrnes G, Hart D, Campbell DA. Surgery for non-small cell lung cancer: systematic review and meta-analysis of randomised controlled trials. Thorax 2006;61:597–603. doi:10.1136/thx.2005.051995.

[4] Cortese DA, Pairolero PC, Bergstralh EJ, Woolner LB, Uhlenhopp MA, Piehler JM, et al. Roentgenographically occult lung cancer. A ten-year experience. J Thorac Cardiovasc Surg 1983;86:373–80.

[5] Kennedy TC, McWilliams A, Edell E, Sutedja T, Downie G, Yung R, et al. Bronchial intraepithelial neoplasia/early central airways lung cancer: ACCP evidence-based clinical practice guidelines (2nd edition). Chest 2007;132:221S-233S. doi:10.1378/chest.07-1377.

[6] Nakamura H, Kawasaki N, Hagiwara M, Ogata A, Saito M, Konaka C, et al. Early hilar lung cancer - risk for multiple lung cancers and clinical outcome. Lung Cancer 2001;33:51–7.

[7] Wisnivesky JP, Yung RC-W, Mathur PN, Zulueta JJ. Diagnosis and Treatment of Bronchial Intraepithelial Neoplasia and Early Lung Cancer of the Central Airways. CHEST J 2013;143:e263S. doi:10.1378/chest.12-2358.

[8] National Institute for Health and Clinical Excellence. The diagnosis and treatment of lung cancer (update). Clin Guidel 2011;CG121.

[9] NLST TNLSTRT. Reduced Lung-Cancer Mortality with Low-Dose Computed

Tomographic Screening. N Engl J Med 2011;365:395–409. doi:10.1056/NEJMoa1102873.

[10] Travis W, Colby T V, Corrin B, Shimosato Y, Brambilla E. Histological Typing of Lung and Pleural Tumors. WHO International Histological Classification of Tumour. 3rd ed. Berlin, Germany: Springer; 1999.

[11] Auerbach O, Stout A, Hammond E, Garfinkel L. Changes in bronchial epithelium in relation to cigarette smoking and in relation to lung cancer. N Engl J Med 1961;265:253–67. doi:10.1056/NEJM196108102650601.

[12] Travis W, Brambilla E, Burke A, Marx A, Nicholson, AGLyon: 2015. WHO Classification of Tumours of the Lung, Pleura, Thymus and Heart. Lyon: International Agency for Research on Cancer; 2015.

[13] Kerr KM. Pulmonary preinvasive neoplasia. J Clin Pathol 2001;54:257–71. doi:10.1136/jcp.54.4.257.

[14] Nicholson a. G, Perry LJ, Cury PM, Jackson P, McCormick CM, Corrin B, et al. Reproducibility of the WHO/IASLC grading system for pre-invasive squamous lesions of the bronchus: A study of inter-observer and intra-observer variation. Histopathology 2001;38:202–8. doi:10.1046/j.1365-2559.2001.01078.x.

[15] Venmans BJ, Linden HC van der, Elbers HR, Boxem TJ van, Smit EF, Postmus PE, et al. Observer Variability in Histopathologic Reporting of Bronchial Biopsy Specimens: Influence on the Results of Autofluorescence Bronchoscopy in Detection of Preinvasive Bronchial Neoplasia. J Bronchol Interv Pulmonol 2000;7.

[16] George PJ, Banerjee AK, Read CA, O'Sullivan C, Falzon M, Pezzella F, et al. Surveillance for the detection of early lung cancer in patients with bronchial dysplasia. Thorax 2007;62:43–50. doi:10.1136/thx.2005.052191.

[17] Van Boerdonk RAA, Smesseim I, Heideman DAM, Coupe VMH, Tio D, Grunberg K, et al. Close surveillance with long-term follow-up of subjects with preinvasive endobronchial lesions. Am J Respir Crit Care Med 2015;192:1483–9. doi:10.1164/rccm.201504-0822OC.

[18] Bota S, Auliac JB, Paris C, Métayer J, Sesboüé R, Nouvet G, et al. Follow-up of bronchial precancerous lesions and carcinoma in situ using fluorescence endoscopy. Am J Respir Crit Care Med 2001;164:1688–93. doi:10.1164/ajrccm.164.9.2012147.

[19] Chhajed PN, Shibuya K, Hoshino H, Chiyo M, Yasufuku K, Hiroshima K, et al. A comparison of video and autofluorescence bronchoscopy in patients at high risk of lung cancer. Eur Respir J 2005;25:951–5. doi:10.1183/09031936.05.00012504.

[20] Lam S, MacAulay C, Hung J, LeRiche J, Profio AE, Palcic B. Detection of dysplasia and carcinoma in situ with a lung imaging fluorescence endoscope device. J Thorac Cardiovasc Surg 1993;105:1035–40.

[21] Hung J, Lam S, LeRiche JC, Palcic B. Autofluorescence of normal and malignant bronchial tissue. Lasers Surg Med 1991;11:99–105.

[22] Lam S, Macaulay C, Leriche JC, Ikeda N, Palcic B. Early localization of bronchogenic carcinoma. Diagn Ther Endosc 1994;1:75–8. doi:10.1155/DTE.1.75.

[23] Ikeda N, Honda H, Hayashi A, Usuda J, Kato Y, Tsuboi M, et al. Early detection of bronchial lesions using newly developed videoendoscopy-based autofluorescence bronchoscopy. Lung Cancer 2006;52:21–7. doi:10.1016/j.lungcan.2005.11.009.

[24] Chiyo M, Shibuya K, Hoshino H, Yasufuku K, Sekine Y, Iizasa T, et al. Effective detection of bronchial preinvasive lesions by a new autofluorescence imaging

bronchovideoscope system. Lung Cancer 2005;48:307–13. doi:10.1016/j.lungcan.2004.11.023.

[25] Ernst A, Simoff M, Mathur P, Yung R, Beamis J. D-light autofluorescence in the detection of premalignant airway changes: a multicenter trial. J Bronchol 2005;12:133–8.

[26] Häussinger K, Becker H, Stanzel F, Kreuzer A, Schmidt B, Strausz J, et al. Autofluorescence bronchoscopy with white light bronchoscopy compared with white light bronchoscopy alone for the detection of precancerous lesions: a European randomised controlled multicentre trial. Thorax 2005;60:496–503. doi:10.1136/thx.2005.041475.

[27] Lam S, Kennedy T, Unger M, Miller YE, Gelmont D, Rusch V, et al. Localization of bronchial intraepithelial neoplastic lesions by fluorescence bronchoscopy. Chest 1998;113:696–702.

[28] Lam S, MacAulay C, leRiche JC, Palcic B. Detection and localization of early lung cancer by fluorescence bronchoscopy. Cancer 2000;89:2468–73.

[29] Horvath T, Horvathova M, Salajka F, Habanec B, Foretova L, Kana J, et al. Detection of Bronchial Neoplasia in Uranium Miners by Autofluorescence Endoscopy (SAFE-1000). Diagn Ther Endosc 1999;5:91–8. doi:10.1155/DTE.5.91.

[30] Sato M, Sakurada A, Sagawa M, Minowa M, Takahashi H, Oyaizu T, et al. Diagnostic results before and after introduction of autofluorescence bronchoscopy in patients suspected of having lung cancer detected by sputum cytology in lung cancer mass screening. Lung Cancer 2001;32:247–53.

[31] Hirsch FR, Prindiville SA, Miller YE, Franklin WA, Dempsey EC, Murphy JR, et al. Fluorescence versus white-light bronchoscopy for detection of

preneoplastic lesions: a randomized study. J Natl Cancer Inst 2001;93:1385–91.

[32] Sun J, Garfield DH, Lam B, Yan J, Gu A, Shen J, et al. The value of autofluorescence bronchoscopy combined with white light bronchoscopy compared with white light alone in the diagnosis of intraepithelial neoplasia and invasive lung cancer: a meta-analysis. J Thorac Oncol 2011;6:1336–44. doi:10.1097/JTO.0b013e318220c984.

[33] Lee P, van den Berg RM, Lam S, Gazdar AF, Grunberg K, McWilliams A, et al. Color fluorescence ratio for detection of bronchial dysplasia and carcinoma in situ. Clin Cancer Res 2009;15:4700–5. doi:10.1158/1078-0432.CCR-08-1644.

[34] Herth FJF, Eberhardt R, Anantham D, Gompelmann D, Zakaria MW, Ernst A. Narrow-band imaging bronchoscopy increases the specificity of bronchoscopic early lung cancer detection. J Thorac Oncol 2009;4:1060–5. doi:10.1097/JTO.0b013e3181b24100.

[35] Lam S, leRiche JC, Zheng Y, Coldman A, MacAulay C, Hawk E, et al. Sex-related differences in bronchial epithelial changes associated with tobacco smoking. J Natl Cancer Inst 1999;91:619–91.

[36] Ishizumi T, McWilliams A, MacAulay C, Gazdar A, Lam S. Natural history of bronchial preinvasive lesions. Cancer Metastasis Rev 2010;29:5–14. doi:10.1007/s10555-010-9214-7.

[37] Lam B, Lam SY, Wong MP, Ooi CGC, Fong DYT, Lam DCL, et al. Sputum cytology examination followed by autofluorescence bronchoscopy: a practical way of identifying early stage lung cancer in central airway. Lung Cancer 2009;64:289–94. doi:10.1016/j.lungcan.2008.09.016.

[38] Gono K, Obi T, Yamaguchi M, Ohyama N, Machida H, Sano Y, et al.

Appearance of enhanced tissue features in narrow-band endoscopic imaging. J Biomed Opt 9:568–77. doi:10.1117/1.1695563.

[39] Shibuya K, Nakajima T, Fujiwara T, Chiyo M, Hoshino H, Moriya Y, et al. Narrow band imaging with high-resolution bronchovideoscopy: a new approach for visualizing angiogenesis in squamous cell carcinoma of the lung. Lung Cancer 2010;69:194–202. doi:10.1016/j.lungcan.2010.04.023.

[40] HANAHAN D, FOLKMAN J. Patterns and Emerging Mechanisms of the Angiogenic Switch during Tumorigenesis. Cell 1996;86:353–64. doi:10.1016/S0092-8674(00)80108-7.

[41] Lam S, Standish B, Baldwin C, McWilliams A, leRiche J, Gazdar A, et al. In vivo Optical Coherence Tomography Imaging of Preinvasive Bronchial Lesions. Clin Cancer Res 2008;14:2006–11. doi:10.1158/1078-0432.CCR-07-4418.

[42] Fujimoto JG, Brezinski ME, Tearney GJ, Boppart SA, Bouma B, Hee MR, et al. Optical biopsy and imaging using optical coherence tomography. Nat Med 1995;1:970–2.

[43] Tearney GJ, Brezinski ME, Bouma BE, Boppart SA, Pitris C, Southern JF, et al. In vivo endoscopic optical biopsy with optical coherence tomography. Science 1997;276:2037–9.

[44] Tsuboi M, Hayashi A, Ikeda N, Honda H, Kato Y, Ichinose S, et al. Optical coherence tomography in the diagnosis of bronchial lesions. Lung Cancer 2005;49:387–94. doi:10.1016/j.lungcan.2005.04.007.

[45] Sutedja TG, Codrington H, Risse EK, Breuer RH, van Mourik JC, Golding RP, et al. Autofluorescence bronchoscopy improves staging of radiographically occult lung cancer and has an impact on therapeutic strategy. Chest 2001;120:1327–32.

[46] Pasic A, Brokx HA, Comans EF, Herder GJ, Risse EK, Hoekstra OS, et al.

Detection and staging of preinvasive lesions and occult lung cancer in the central airways with 18F-fluorodeoxyglucose positron emission tomography: a pilot study. Clin Cancer Res 2005;11:6186–9. doi:10.1158/1078-0432.CCR-04-2480.

[47] Fraioli F, Kayani I, Smith L-J, Bomanji JB, Capitanio A, Falzon M, et al. Positive (18)Fluorodeoxyglucose-Positron Emission Tomography/Computed Tomography Predicts Preinvasive Endobronchial Lesion Progression to Invasive Cancer. Am J Respir Crit Care Med 2016;193:576–9. doi:10.1164/rccm.201508-1617LE.

[48] Venmans BJ, van Boxem TJ, Smit EF, Postmus PE, Sutedja TG. Outcome of bronchial carcinoma in situ. Chest 2000;117:1572–6.

[49] Breuer RH, Pasic A, Smit EF, van Vliet E, Vonk Noordegraaf A, Risse EJ, et al. The natural course of preneoplastic lesions in bronchial epithelium. Clin Cancer Res 2005;11:537–43.

[50] Hoshino H, Shibuya K, Chiyo M, Iyoda A, Yoshida S, Sekine Y, et al. Biological features of bronchial squamous dysplasia followed up by autofluorescence bronchoscopy. Lung Cancer 2004;46:187–96. doi:10.1016/j.lungcan.2004.04.028.

[51] Moro-Sibilot D, Fievet F, Jeanmart M, Lantuejoul S, Arbib F, Laverrière MH, et al. Clinical prognostic indicators of high-grade pre-invasive bronchial lesions. Eur Respir J 2004;24:24–9. doi:10.1183/09031936.04.00065303.

[52] Salaün M, Sesboüé R, Moreno-Swirc S, Metayer J, Bota S, Bourguignon J, et al. Molecular predictive factors for progression of high-grade preinvasive bronchial lesions. Am J Respir Crit Care Med 2008;177:880–6. doi:10.1164/rccm.200704-598OC.

[53] Banerjee AK. Preinvasive lesions of the bronchus. J Thorac Oncol 2009;4:545–

51. doi:10.1097/JTO.0b013e31819667bd.

[54] Rivera MP. Preinvasive lesions of the bronchus. Clin Chest Med 2011;32:693–702. doi:10.1016/j.ccm.2011.08.008.

[55] Daniels JM a, Sutedja TG. Detection and minimally invasive treatment of early squamous lung cancer. Ther Adv Med Oncol 2013;5:235–48. doi:10.1177/1758834013482345.

[56] Pasic A, van Vliet E, Breuer RH, Risse EJ, Snijders PJ, Postmus PE, et al. Smoking behavior does not influence the natural course of pre-invasive lesions in bronchial mucosa. Lung Cancer 2004;45:153–4. doi:10.1016/j.lungcan.2004.04.029.

[57] Salaun M, Bota S, Thiberville L. Long-Term Follow-Up of Severe Dysplasia and Carcinoma In Situ of the Bronchus. J Thorac Oncol 2009;4:1185–8.

[58] Paris C, Benichou J, Bota S, Sagnier S, Metayer J, Eloy S, et al. Occupational and nonoccupational factors associated with high grade bronchial pre-invasive lesions. Eur Respir J 2003;21:332–41.

[59] Loewen G, Natarajan N, Tan D, Nava E, Klippenstein D, Mahoney M, et al. Autofluorescence bronchoscopy for lung cancer surveillance based on risk assessment. Thorax 2007;62:335–40. doi:10.1136/thx.2006.068999.

[60] Deygas N, Froudarakis M, Ozenne G, Vergnon JM. Cryotherapy in early superficial bronchogenic carcinoma. Chest 2001;120:26–31. doi:10.1378/chest.120.1.26.

[61] Callister MEJ, Baldwin DR, Akram AR, Barnard S, Cane P, Draffan J, et al. British Thoracic Society guidelines for the investigation and management of pulmonary nodules. Thorax 2015;70 Suppl 2:ii1–54. doi:10.1136/thoraxjnl-2015-207168.

[62] Gray EP, Teare MD, Stevens J, Archer R. Risk Prediction Models for Lung Cancer: A Systematic Review. Clin Lung Cancer 2015. doi:10.1016/j.cllc.2015.11.007.

[63] Pasic A, Vonk-Noordegraaf A, Risse EKJ, Postmus PE, Sutedja TG. Multiple suspicious lesions detected by autofluorescence bronchoscopy predict malignant development in the bronchial mucosa in high risk patients. Lung Cancer 2003;41:295–301.

[64] Alaa M, Shibuya K, Fujiwara T, Wada H, Hoshino H, Yoshida S, et al. Risk of lung cancer in patients with preinvasive bronchial lesions followed by autofluorescence bronchoscopy and chest computed tomography. Lung Cancer 2011;72:303–8. doi:10.1016/j.lungcan.2010.09.014.

[65] McCaughan F, Pole JCM, Bankier AT, Konfortov BA, Carroll B, Falzon M, et al. Progressive 3q Amplification Consistently Targets *SOX2* in Preinvasive Squamous Lung Cancer. Am J Respir Crit Care Med 2010;182:83–91. doi:10.1164/rccm.201001-0005OC.

[66] Massion PP, Zou Y, Uner H, Kiatsimkul P, Wolf HJ, Baron AE, et al. Recurrent genomic gains in preinvasive lesions as a biomarker of risk for lung cancer. PLoS One 2009;4:e5611. doi:10.1371/journal.pone.0005611.

[67] van Boerdonk RAA, Daniels JMA, Snijders PJF, Grünberg K, Thunnissen E, van de Wiel MA, et al. DNA copy number aberrations in endobronchial lesions: a validated predictor for cancer. Thorax 2014;69:451–7. doi:10.1136/thoraxjnl-2013-203821.

[68] van Boerdonk R a a, Daniels JM a, Snijders PJF, Grünberg K, Thunnissen E, van de Wiel M a, et al. DNA copy number aberrations in endobronchial lesions: a validated predictor for cancer. Thorax 2014;69:451–7. doi:10.1136/thoraxjnl-2013-203821.

[69] Jeanmart M, Lantuejoul S, Moro D, Sturm N, Brambilla C, Brambilla E. Value of Immunohistochemical Markers in Preinvasive Bronchial Lesions in Risk Assessment of Lung Cancer. Clin Cancer Res 2003;9:2195–203.

[70] Miller YE, Blatchford P, Hyun DS, Keith RL, Kennedy TC, Wolf H, et al. Bronchial epithelial Ki-67 index is related to histology, smoking, and gender, but not lung cancer or chronic obstructive pulmonary disease. Cancer Epidemiol Biomarkers Prev 2007;16:2425–31. doi:10.1158/1055-9965.EPI-07-0220.

[71] Lantuejoul S, Soria JC, Morat L, Lorimier P, Moro-Sibilot D, Sabatier L, et al. Telomere shortening and telomerase reverse transcriptase expression in preinvasive bronchial lesions. Clin Cancer Res 2005;11:2074–82. doi:10.1158/1078-0432.CCR-04-1376.

[72] Sin DD, Man SFP, McWilliams A, Lam S. Progression of airway dysplasia and C-reactive protein in smokers at high risk of lung cancer. Am J Respir Crit Care Med 2006;173:535–9. doi:10.1164/rccm.200508-1305OC.

[73] Rahman SMJ, Gonzalez AL, Li M. Lung Cancer Diagnosis from Proteomic Analysis of Preinvasive Lesions Lung Cancer Diagnosis from Proteomic Analysis of 2011:3009–17. doi:10.1158/0008-5472.CAN-10-2510.

[74] Jonsson S, Varella-Garcia M, Miller YE, Wolf HJ, Byers T, Braudrick S, et al. Chromosomal Aneusomy in Bronchial High-Grade Lesions Is Associated with Invasive Lung Cancer. Am J Respir Crit Care Med 2008;177:342–7. doi:10.1164/rccm.200708-1142OC.

[75] Mao L, Lee JS, Kurie JM, Fan YH, Lippman SM, Lee JJ, et al. Clonal genetic alterations in the lungs of current and former smokers. J Natl Cancer Inst 1997;89:857–62.

[76] Wistuba II, Lam S, Behrens C, Virmani AK, Fong KM, LeRiche J, et al.

Molecular damage in the bronchial epithelium of current and former smokers. J Natl Cancer Inst 1997;89:1366–73.

[77] Wistuba II, Behrens C, Virmani AK, Mele G, Milchgrub S, Girard L, et al. High resolution chromosome 3p allelotyping of human lung cancer and preneoplastic/preinvasive bronchial epithelium reveals multiple, discontinuous sites of 3p allele loss and three regions of frequent breakpoints. Cancer Res 2000;60:1949–60.

[78] Wistuba II, Behrens C, Milchgrub S, Bryant D, Hung J, Minna JD, et al. Sequential molecular abnormalities are involved in the multistage development of squamous cell lung carcinoma. Oncogene 1999;18:643–50. doi:10.1038/sj.onc.1202349.

[79] Foster N a, Banerjee AK, Xian J, Roberts I, Pezzella F, Coleman N, et al. Somatic genetic changes accompanying lung tumor development. Genes Chromosomes Cancer 2005;44:65–75. doi:10.1002/gcc.20223.

[80] Sundaresan V, Ganly P, Hasleton P, Rudd R, Sinha G, Bleehen NM, et al. p53 and chromosome 3 abnormalities, characteristic of malignant lung tumours, are detectable in preinvasive lesions of the bronchus. Oncogene 1992;7:1989–97.

[81] Hung J, Kishimoto Y, Sugio K, Virmani A, McIntire DD, Minna JD, et al. Allele-specific chromosome 3p deletions occur at an early stage in the pathogenesis of lung carcinoma. JAMA 1995;273:558–63.

[82] Thiberville L, Bourguignon J, Metayer J, Bost F, Diarra-Mehrpour M, Bignon J, et al. Frequency and prognostic evaluation of 3p21-22 allelic losses in non-small-cell lung cancer. Int J Cancer 1995;64:371–7.

[83] Lamy A, Sesboüé R, Bourguignon J, Dautréaux B, Métayer J, Frébourg T, et al. Aberrant methylation of the CDKN2a/p16INK4a gene promoter region in

preinvasive bronchial lesions: a prospective study in high-risk patients without invasive cancer. Int J Cancer 2002;100:189–93. doi:10.1002/ijc.10474.

[84] Hsu H-S, Chen T-P, Wen C-K, Hung C-H, Chen C-Y, Chen J-T, et al. Multiple genetic and epigenetic biomarkers for lung cancer detection in cytologically negative sputum and a nested case-control study for risk assessment. J Pathol 2007;213:412–9. doi:10.1002/path.2246.

[85] Gazdar AF, Brambilla E. Preneoplasia of lung cancer. Cancer Biomark 2010;9:385–96. doi:10.1016/j.micinf.2011.07.011.Innate.

[86] Hanahan D, Weinberg RA. Hallmarks of cancer: the next generation. Cell 2011;144:646–74. doi:10.1016/j.cell.2011.02.013.

[87] Fujimura S, Sakurada A, Sagawa M, Saito Y, Takahashi H, Tanita T, et al. A therapeutic approach to roentgenographically occult squamous cell carcinoma of the lung. Cancer 2000;89:2445–8.

[88] van Boxem TJ, Venmans BJ, Schramel FM, van Mourik JC, Golding RP, Postmus PE, et al. Radiographically occult lung cancer treated with fibreoptic bronchoscopic electrocautery: a pilot study of a simple and inexpensive technique. Eur Respir J 1998;11:169–72.

[89] Kubota K, Furuse K, Kawahara M, Kodama N, Yamamoto M, Ogawara M, et al. [Photodynamic therapy of roentgenographically occult lung cancer]. Kyobu Geka 1992;45:80–3.

[90] Furuse K, Fukuoka M, Kato H, Horai T, Kubota K, Kodama N, et al. A prospective phase II study on photodynamic therapy with photofrin II for centrally located early-stage lung cancer. The Japan Lung Cancer Photodynamic Therapy Study Group. J Clin Oncol 1993;11:1852–7.

[91] Imamura S, Kusunoki Y, Takifuji N, Kudo S, Matsui K, Masuda N, et al.

Photodynamic therapy and/or external beam radiation therapy for roentgenologically occult lung cancer. Cancer 1994;73:1608–14.

[92] Kato H, Okunaka T, Shimatani H. Photodynamic therapy for early stage bronchogenic carcinoma. J Clin Laser Med Surg 1996;14:235–8.

[93] Kawaguchi T, Yamamoto S, Naka N, Okishio K, Atagi S, Ogawara M, et al. Immunohistochemical analysis of Bcl-2 protein in early squamous cell carcinoma of the bronchus treated with photodynamic therapy. Br J Cancer 2000;82:418–23. doi:10.1054/bjoc.1999.0936.

[94] Miyazu Y, Miyazawa T, Kurimoto N, Iwamoto Y, Kanoh K, Kohno N. Endobronchial ultrasonography in the assessment of centrally located early-stage lung cancer before photodynamic therapy. Am J Respir Crit Care Med 2002;165:832–7. doi:10.1164/ajrccm.165.6.2108092.

[95] Furukawa K, Kato H, Konaka C, Okunaka T, Usuda J, Ebihara Y. Locally recurrent central-type early stage lung cancer < 1.0 cm in diameter after complete remission by photodynamic therapy. Chest 2005;128:3269–75. doi:10.1378/chest.128.5.3269.

[96] Kato H, Usuda J, Okunaka T, Furukawa K, Honda H, Sakaniwa N, et al. Basic and clinical research on photodynamic therapy at Tokyo Medical University Hospital. Lasers Surg Med 2006;38:371–5. doi:10.1002/lsm.20346.

[97] Endo C, Miyamoto A, Sakurada A, Aikawa H, Sagawa M, Sato M, et al. Results of long-term follow-up of photodynamic therapy for roentgenographically occult bronchogenic squamous cell carcinoma. Chest 2009;136:369–75. doi:10.1378/chest.08-2237.

[98] Usuda J, Ichinose S, Ishizumi T, Hayashi H, Ohtani K, Maehara S, et al. Management of multiple primary lung cancer in patients with centrally located early cancer lesions. J Thorac Oncol 2010;5:62–8.

doi:10.1097/JTO.0b013e3181c42287.

[99] Jung EJ, Lee JH, Jeon K, Koh W-J, Suh GY, Chung MP, et al. Treatment outcomes for patients with synchronous multiple primary non-small cell lung cancer. Lung Cancer 2011;73:237–42. doi:10.1016/j.lungcan.2010.11.008.

[100] Pérol M, Caliandro R, Pommier P, Malet C, Montbarbon X, Carrie C, et al. Curative irradiation of limited endobronchial carcinomas with high-dose rate brachytherapy. Results of a pilot study. Chest 1997;111:1417–23.

[101] Taulelle M, Chauvet B, Vincent P, Félix-Faure C, Buciarelli B, Garcia R, et al. High dose rate endobronchial brachytherapy: results and complications in 189 patients. Eur Respir J 1998;11:162–8.

[102] Marsiglia H, Baldeyrou P, Lartigau E, Briot E, Haie-Meder C, Le Chevalier T, et al. High-dose-rate brachytherapy as sole modality for early-stage endobronchial carcinoma. Int J Radiat Oncol Biol Phys 2000;47:665–72.

[103] Lorchel F, Spaeth D, Scheid P, Aletti P, Thariat J, Peiffert D. [High dose rate brachytherapy: a potentially curative treatment for small invasive T1N0 endobronchial carcinoma and carcinoma in situ]. Rev Mal Respir 2003;20:515–20.

[104] Hennequin C, Bleichner O, Trédaniel J, Quero L, Sergent G, Zalcman G, et al. Long-term results of endobronchial brachytherapy: A curative treatment? Int J Radiat Oncol Biol Phys 2007;67:425–30. doi:10.1016/j.ijrobp.2006.08.068.

[105] Simone CB, Friedberg JS, Glatstein E, Stevenson JP, Sterman DH, Hahn SM, et al. Photodynamic therapy for the treatment of non-small cell lung cancer. J Thorac Dis 2012;4:63–75. doi:10.3978/j.issn.2072-1439.2011.11.05.

[106] Corti L, Toniolo L, Boso C, Colaut F, Fiore D, Muzzio PC, et al. Long-term survival of patients treated with photodynamic therapy for carcinoma in situ and early non-small-cell lung carcinoma. Lasers Surg Med 2007;39:394–402.

doi:10.1002/lsm.20513.

[107] Moghissi K, Dixon K. Update on the current indications, practice and results of photodynamic therapy (PDT) in early central lung cancer (ECLC). Photodiagnosis Photodyn Ther 2008;5:10–8. doi:10.1016/j.pdpdt.2007.11.001.

[108] Stewart A, Parashar B, Patel M, O'Farrell D, Biagioli M, Devlin P, et al. American Brachytherapy Society consensus guidelines for thoracic brachytherapy for lung cancer. Brachytherapy 15:1–11. doi:10.1016/j.brachy.2015.09.006.

[109] Hennequin C, Tredaniel J, Chevret S, Durdux C, Dray M, Manoux D, et al. Predictive factors for late toxicity after endobronchial brachytherapy: a multivariate analysis. Int J Radiat Oncol Biol Phys 1998;42:21–7.

[110] Kawamura H, Ebara T, Katoh H, Tamaki T, Ishikawa H, Sakurai H, et al. Long-term results of curative intraluminal high dose rate brachytherapy for endobronchial carcinoma. Radiat Oncol 2012;7:112. doi:10.1186/1748-717X-7-112.

[111] Skowronek J. Brachytherapy in the treatment of lung cancer - a valuable solution. J Contemp Brachytherapy 2015;7:297–311. doi:10.5114/jcb.2015.54038.

[112] Cavaliere S, Foccoli P, Toninelli C, Feijo S. Nd:YAG Laser Therapy in Lung Cancer: An 11-year Experience with 2,253 Applications in 1,585 Patients. J Bronchol 1994;1:105–11.

[113] Bezzi M. Re-opening the Airway: Fast Methods - Laser-Assisted Mechanical Resection, Electrocautery, and Argon Plasma Coagulation. In: Diaz-Jimenez JP, Rodriguez AN, editors. Interv. Pulm. Med., New York: Springer; 2013, p. 99–123.

[114] Auerbach O, Forman JB, Gere JB, Kassouny DY, Muehsam GE, Petrick TG, et al. Changes in the bronchial epithelium in relation to smoking and cancer of the lung; a report of progress. N Engl J Med 1957;256:97–104. doi:10.1056/NEJM195701172560301.

[115] Saccomanno G, Archer VE, Auerbach O, Saunders RP, Brennan LM. Development of carcinoma of the lung as reflected in exfoliated cells. Cancer 1974;33:256–70.

[116] Prindiville SA, Byers T, Hirsch FR, Franklin WA, Miller YE, Vu KO, et al. Sputum cytological atypia as a predictor of incident lung cancer in a cohort of heavy smokers with airflow obstruction. Cancer Epidemiol Biomarkers Prev 2003;12:987–93.

[117] Holiday DB, McLarty JW, Farley ML, Mabry LC, Cozens D, Roby T, et al. Sputum cytology within and across laboratories. A reliability study. Acta Cytol 39:195–206.

[118] UK CR. Caner Research UK Lung Cancer Statistics. https://www.cancerresearchuk.org/health-professional/cancer-statistics/statistics-by-cancer-type/lung-cancer/incidence 2014.

[119] Goldstraw P, Crowley J, Chansky K, Giroux DJ, Groome PA, Rami-Porta R, et al. The IASLC Lung Cancer Staging Project: proposals for the revision of the TNM stage groupings in the forthcoming (seventh) edition of the TNM Classification of malignant tumours. J Thorac Oncol 2007;2:706–14. doi:10.1097/JTO.0b013e31812f3c1a.

[120] Lee JJ, Hong WK, Hittelman WN, Mao L, Lotan R, Shin DM, et al. Predicting cancer development in oral leukoplakia: ten years of translational research. Clin Cancer Res 2000;6:1702–10.

[121] Slaughter DP, Southwick HW, Smejkal W. Field cancerization in oral stratified

squamous epithelium; clinical implications of multicentric origin. Cancer 1953;6:963–8.

[122] Travis W, Colby T, Shimosato T, Corri B, Brambilla E, Countries in association with SL and pathologists from 14 other. WHO/IASLC Histological Classification of Lung and Pleural Tumors. Histol. Typing Lung Pleural Tumours. 3rd ed., Berlin: Springer; 1999, p. 21–4.

[123] Travis WD, Brambilla E, Müller-Hermelink H, Harris C. Pathology and Genetics of Tumours of the Lung, Pleura, Thymus and Heart. IARC Press; 2004.

[124] Teixeira VH, Pipinikas CP, Pennycuick A, Lee-Six H, Chandrasekharan D, Beane J, et al. Deciphering the genomic, epigenomic, and transcriptomic landscapes of pre-invasive lung cancer lesions. Nat Med 2019;25:517–25. doi:10.1038/s41591-018-0323-0.

[125] Braakhuis BJM, Tabor MP, Kummer JA, Leemans CR, Brakenhoff RH. A genetic explanation of Slaughter's concept of field cancerization: evidence and clinical implications. Cancer Res 2003;63:1727–30.

[126] Franklin WA, Gazdar AF, Haney J, Wistuba II, La Rosa FG, Kennedy T, et al. Widely dispersed p53 mutation in respiratory epithelium. A novel mechanism for field carcinogenesis. J Clin Invest 1997;100:2133–7. doi:10.1172/JCI119748.

[127] Pipinikas CP, Kiropoulos TS, Teixeira VH, Brown JM, Varanou A, Falzon M, et al. Cell migration leads to spatially distinct but clonally related airway cancer precursors. Thorax 2014;69:548–57. doi:10.1136/thoraxjnl-2013-204198.

[128] Tremblay A, Taghizadeh N, McWilliams AM, MacEachern P, Stather DR, Soghrati K, et al. Low Prevalence of High-Grade Lesions Detected With Autofluorescence Bronchoscopy in the Setting of Lung Cancer Screening in the Pan-Canadian Lung Cancer Screening Study. Chest 2016;150:1015–22.

doi:10.1016/j.chest.2016.04.019.

[129] Edell E, Lam S, Kennedy T, Loewen G, Keith RL. Detection and Localization of Intraepithelial Neoplasia and Invasive Carcinoma Using Fluorescence-Reflectance Bronchoscopy: An International, Multicenter Clinical Trial. J Thorac Oncol 2009;4:49–54. doi:10.1097/JTO.0b013e3181914506.Detection.

[130] Sutedja G, Postmus PE. Bronchoscopic treatment of lung tumors. Lung Cancer 1994;11:1–17. doi:10.1016/0169-5002(94)90278-X.

[131] Moghissi K, Dixon K, Thorpe JAC, Stringer M, Oxtoby C. Photodynamic therapy (PDT) in early central lung cancer: a treatment option for patients ineligible for surgical resection. Thorax 2007;62:391–5. doi:10.1136/thx.2006.061143.

[132] Schweitzer VG, Somers ML. PHOTOFRIN-mediated photodynamic therapy for treatment of early stage (Tis-T2N0M0) SqCCa of oral cavity and oropharynx. Lasers Surg Med 2010;42:1–8. doi:10.1002/lsm.20881.

[133] Manyak MJ, Ogan K. Photodynamic Therapy for Refractory Superficial Bladder Cancer: Long-Term Clinical Outcomes of Single Treatment Using Intravesical Diffusion Medium. J Endourol 2003;17:633–9. doi:10.1089/089277903322518644.

[134] Istomin YP, Lapzevich TP, Chalau VN, Shliakhtsin S V, Trukhachova T V. Photodynamic therapy of cervical intraepithelial neoplasia grades II and III with Photolon. Photodiagnosis Photodyn Ther 2010;7:144–51. doi:10.1016/j.pdpdt.2010.06.005.

[135] Kato H, Furukawa K, Sato M, Okunaka T, Kusunoki Y, Kawahara M, et al. Phase II clinical study of photodynamic therapy using mono-L-aspartyl chlorin e6 and diode laser for early superficial squamous cell carcinoma of the lung. Lung Cancer 2003;42:103–11. doi:10.1016/S0169-5002(03)00242-3.

[136] Usuda J, Tsutsui H, Honda H, Ichinose S, Ishizumi T, Hirata T, et al. Photodynamic therapy for lung cancers based on novel photodynamic diagnosis using talaporfin sodium (NPe6) and autofluorescence bronchoscopy. Lung Cancer 2007;58:317–23. doi:10.1016/j.lungcan.2007.06.026.

[137] Du Rand I a, Blaikley J, Booton R, Chaudhuri N, Gupta V, Khalid S, et al. British Thoracic Society guideline for diagnostic flexible bronchoscopy in adults: accredited by NICE. vol. 68 Suppl 1. 2013. doi:10.1136/thoraxjnl-2013-203618.

[138] McWilliams a, Lam B, Sutedja T. Early proximal lung cancer diagnosis and treatment. Eur Respir J 2009;33:656–65. doi:10.1183/09031936.00124608.

[139] Sutedja G, Golding RP, Postmus PE. High resolution computed tomography in patients referred for intraluminal bronchoscopic therapy with curative intent. Eur Respir J 1996;9:1020–3.

[140] Albano D, Bilfinger T, Nemesure B. 1-, 3-, and 5-year survival among early-stage lung cancer patients treated with lobectomy vs SBRT. Lung Cancer Targets Ther 2018;9:65–71. doi:10.2147/LCTT.S166320.

[141] Ost DE, Ernst A, Grosu HB, Lei X, Diaz-Mendoza J, Slade M, et al. Therapeutic bronchoscopy for malignant central airway obstruction: success rates and impact on dyspnea and quality of life. Chest 2015;147:1282–98. doi:10.1378/chest.14-1526.

[142] Ernst A, Feller-Kopman D, Becker HD, Mehta AC. Central airway obstruction. Am J Respir Crit Care Med 2004;169:1278–97. doi:10.1164/rccm.200210-1181SO.

[143] Hendifar AE, Marchevsky AM, Tuli R. Neuroendocrine Tumors of the Lung: Current Challenges and Advances in the Diagnosis and Management of Well-

Differentiated Disease. J Thorac Oncol 2017;12:425–36. doi:10.1016/j.jtho.2016.11.2222.

[144] Travis WD, Brambilla E, Nicholson AG, Yatabe Y, Austin JHM, Beasley MB, et al. The 2015 World Health Organization Classification of Lung Tumors: Impact of Genetic, Clinical and Radiologic Advances Since the 2004 Classification. J Thorac Oncol 2015;10:1243–60. doi:10.1097/JTO.0000000000000630.

[145] Rekhtman N. Neuroendocrine tumors of the lung: an update. Arch Pathol Lab Med 2010;134:1628–38. doi:10.1043/2009-0583-RAR.1.

[146] Caplin ME, Baudin E, Ferolla P, Filosso P, Garcia-Yuste M, Lim E, et al. Pulmonary neuroendocrine (carcinoid) tumors: European Neuroendocrine Tumor Society expert consensus and recommendations for best practice for typical and atypical pulmonary carcinoids. Ann Oncol 2015;26:1604–20. doi:10.1093/annonc/mdv041.

[147] Swarts DRA, Ramaekers FCS, Speel E-JM. Molecular and cellular biology of neuroendocrine lung tumors: evidence for separate biological entities. Biochim Biophys Acta 2012;1826:255–71. doi:10.1016/j.bbcan.2012.05.001.

[148] Marchevsky AM, Walts AE. Diffuse idiopathic pulmonary neuroendocrine cell hyperplasia (DIPNECH). Semin Diagn Pathol 2015;32:438–44. doi:10.1053/j.semdp.2015.08.002.

[149] Wirtschafter E, Walts AE, Liu ST, Marchevsky AM. Diffuse Idiopathic Pulmonary Neuroendocrine Cell Hyperplasia of the Lung (DIPNECH): Current Best Evidence. Lung 2015;193:659–67. doi:10.1007/s00408-015-9755-1.

[150] Travis WD. Pathology and diagnosis of neuroendocrine tumors: lung neuroendocrine. Thorac Surg Clin 2014;24:257–66. doi:10.1016/j.thorsurg.2014.04.001.

[151] Travis WD, Rush W, Flieder DB, Falk R, Fleming M V., Gal AA, et al. Survival

analysis of 200 pulmonary neuroendocrine tumors with clarification of criteria for atypical carcinoid and its separation from typical carcinoid. Am J Surg Pathol 1998;22:934–44. doi:10.1097/00000478-199808000-00003.

[152] Pelosi G, Papotti M, Rindi G, Scarpa A. Unraveling tumor grading and genomic landscape in lung neuroendocrine tumors. Endocr Pathol 2014;25:151–64. doi:10.1007/s12022-014-9320-0.

[153] Gustafsson BI, Kidd M, Chan A, Malfertheiner M V, Modlin IM. Bronchopulmonary neuroendocrine tumors. Cancer 2008;113:5–21. doi:10.1002/cncr.23542.

[154] Öberg K, Hellman P, Ferolla P, Papotti M, ESMO Guidelines Working Group. Neuroendocrine bronchial and thymic tumors: ESMO Clinical Practice Guidelines for diagnosis, treatment and follow-up. Ann Oncol Off J Eur Soc Med Oncol 2012;23 Suppl 7:vii120-3. doi:10.1093/annonc/mds267.

[155] Granberg D, Sissons M, Kolarova T, Goldstein G, Leyden J. Lung neuroendocrine tumor (NET) patient (pt)-reported experience: Results from the first global NET pt survey□A collaboration between the international neuroendocrine cancer alliance (INCA) and Novartis pharmaceuticals. J Clin Oncol 2015;33:e17739–e17739. doi:10.1200/jco.2015.33.15_suppl.e17739.

[156] Chong CR, Wirth LJ, Nishino M, Chen AB, Sholl LM, Kulke MH, et al. Chemotherapy for locally advanced and metastatic pulmonary carcinoid tumors. Lung Cancer 2014;86:241–6. doi:10.1016/j.lungcan.2014.08.012.

[157] Steinfort D, Finlay M, Irving L. Diagnosis of peripheral pulmonary carcinoid tumor using endobronchial ultrasound. Ann Thorac Med 2008;3:146–8. doi:10.4103/1817-1737.43082.

[158] Sarraf KM, Belcher E, Price S, Lim E. Clinical application of direct bronchial ultrasound to visualize and determine endobronchial tumor margins for

surgical resection. Ann Thorac Surg 2008;86:1339–41. doi:10.1016/j.athoracsur.2008.04.115.

[159] Tumors. NCCNN clinical practice guidelines in oncology: neuroendocrine. https://www.nccn.org/professionals/physician_gls/PDF/neuroendocrine.pdf. Accessed April 2019. Version 22016 n.d.

[160] Detterbeck FC. Management of carcinoid tumors. Ann Thorac Surg 2010;89:998–1005. doi:10.1016/j.athoracsur.2009.07.097.

[161] Reuling EMBP, Dickhoff C, Plaisier PW, Coupé VMH, Mazairac AHA, Lely RJ, et al. Endobronchial Treatment for Bronchial Carcinoid: Patient Selection and Predictors of Outcome. Respiration n.d.;95:220–7. doi:10.1159/000484984.

[162] Bertoletti L, Elleuch R, Kaczmarek D, Jean-François R, Vergnon JM. Bronchoscopic cryotherapy treatment of isolated endoluminal typical carcinoid tumor. Chest 2006;130:1405–11. doi:10.1378/chest.130.5.1405.

[163] Brokx HAP, Paul MA, Postmus PE, Sutedja TG. Long-term follow-up after first-line bronchoscopic therapy in patients with bronchial carcinoids. Thorax 2015;70:468–72. doi:10.1136/thoraxjnl-2014-206753.

[164] Van Boxem TJ, Venmans BJ, Van Mourik JC, Postmus PE, Sutedja TG. Bronchoscopic treatment of intraluminal typical carcinoid: A pilot study. J Thorac Cardiovasc Surg 1998;116:402–6. doi:10.1016/S0022-5223(98)70005-4.

[165] Fruchter O, Fuks L, Amital A, Fox BD, Abdel Rahman N, Kramer MR. Long-term follow-up of flexible bronchoscopic treatment for bronchial carcinoids with curative intent. Diagn Ther Endosc 2009. doi:10.1155/2009/782961.

[166] Reuling EMBP, Dickhoff C, Plaisier PW, Bonjer HJ, Daniels JMA. Endobronchial and surgical treatment of pulmonary carcinoid tumors: A systematic literature review. Lung Cancer 2019;134:85–95.

doi:10.1016/j.lungcan.2019.04.016.

[167] Bagheri R, Mashhadi M taghi R, Haghi SZ, Sadrizadh A, Rezaeetalab F. Tracheobronchopulmonary carcinoid tumors: analysis of 40 patients. Ann Thorac Cardiovasc Surg 2011;17:7–12. doi:10.5761/atcs.oa.08.01309.

[168] Rea F, Rizzardi G, Zuin A, Marulli G, Nicotra S, Bulf R, et al. Outcome and surgical strategy in bronchial carcinoid tumors: single institution experience with 252 patients. Eur J Cardiothorac Surg 2007;31:186–91. doi:10.1016/j.ejcts.2006.10.040.

[169] Cavaliere S, Foccoli P, Toninelli C. Curative bronchoscopic laser therapy for surgically resectable tracheobronchial tumors: Personal experience. J Bronchol 2002;9:90–5. doi:10.1097/00128594-200204000-00004.

[170] Aydin E, Yazici U, Gulgosteren M, Agackiran Y, Kaya S, Gulhan E, et al. Long-term outcomes and prognostic factors of patients with surgically treated pulmonary carcinoid: our institutional experience with 104 patients. Eur J Cardiothorac Surg 2011;39:549–54. doi:10.1016/j.ejcts.2010.08.010.

[171] Fruchter O, Fuks L, Amital A, Fox BD, Rahman NA, Kramer MR. Long-term follow-up of flexible bronchoscopic treatment for bronchial carcinoids with curative intent. Diagn Ther Endosc 2009.

[172] Dalar L, Ozdemir C, Abul Y, Sokucu SN, Karasulu L, Urer HN, et al. Endobronchial Treatment of Carcinoid Tumors of the Lung. Thorac Cardiovasc Surg 2016;64:166–71. doi:10.1055/s-0035-1549274.

[173] Reuling EMBP, Dickhoff C, Daniels JMA. Treatment of Bronchial Carcinoid Tumors: Is Surgery Really Necessary? J Thorac Oncol 2017;12:e57–8. doi:10.1016/j.jtho.2017.01.011.

[174] Ong P, Grosu HB, Debiane L, Casal RF, Eapen GA, Jimenez CA, et al. Long-term quality-adjusted survival following therapeutic bronchoscopy for malignant

central airway obstruction. Thorax 2019;74:141–56. doi:10.1136/thoraxjnl-2018-211521.

[175] Mudambi L, Miller R, Eapen GA. Malignant central airway obstruction. J Thorac Dis 2017;9:S1087–110. doi:10.21037/jtd.2017.07.27.

[176] National Institute of Clinical Excellence. The diagnosis and treatment of lung cancer (update): full guideline 2011.

[177] Mahmood K, Wahidi MM, Thomas S, Argento AC, Ninan N a., Smathers EC, et al. Therapeutic Bronchoscopy Improves Spirometry, Quality of Life, and Survival in Central Airway Obstruction. Respiration 2015. doi:10.1159/000381103.

[178] Erdös G, Tzanova I. Anesthetic Considerations - Mediastinal Mass. Eur J Anaesthesiol 2009;26:627–32. doi:10.1097/EJA.0b013e328324b7f8.

[179] Navani N, Brown JM, Nankivell M, Woolhouse I, Harrison RN, Jeebun V, et al. Suitability of endobronchial ultrasound-guided transbronchial needle aspiration specimens for subtyping and genotyping of non-small cell lung cancer: a multicenter study of 774 patients. Am J Respir Crit Care Med 2012;185:1316–22. doi:10.1164/rccm.201202-0294OC.

[180] Standard BQ. Flexible Bronchoscopy | British Thoracic Society | Better lung health for all n.d. https://www.brit-thoracic.org.uk/quality-improvement/quality-standards/flexible-bronchoscopy/ (accessed September 21, 2019).

[181] Gonzalez R, De-La-Rosa-Ramirez I, Maldonado-Hernandez A, Dominguez-Cherit G. Should patients undergoing a bronchoscopy be sedated? 2003;47. doi:10.1034/j.1399-6576.2003.00061.x.

[182] Matot I, Kramer MR. Sedation in outpatient bronchoscopy. Respir Med 2000;94:1145–53. doi:10.1053/rmed.2000.0926.

[183] Putinati S, Ballerin L, Corbctta L, Trcvisani L, Potcna A. Patient satisfaction with conscious sedation for bronchoscopy. Chest 1999;115:1437–40. doi:10.1378/chest.115.5.1437.

[184] Anesthesiologists AS of. Continuum of Depth of Sedation: Definition of General Anesthesia and Levels of Sedation/Analgesia n.d. https://www.asahq.org/standards-and-guidelines/continuum-of-depth-of-sedation-definition-of-general-anesthesia-and-levels-of-sedationanalgesia (accessed September 21, 2019).

[185] Clark G, Licker M, Younossian AB, Soccal PM, Frey JG, Rochat T, et al. Titrated sedation with propofol or midazolam for flexible bronchoscopy: A randomised trial. Eur Respir J 2009;34:1277–83. doi:10.1183/09031936.00142108.

[186] Casal RF, Lazarus DR, Kuhl K, Nogueras-González G, Perusich S, Green LK, et al. Randomized trial of endobronchial ultrasound-guided transbronchial needle aspiration under general anesthesia versus moderate sedation. Am J Respir Crit Care Med 2015;191:796–803. doi:10.1164/rccm.201409-1615OC.

[187] Yarmus LB, Akulian JA, Gilbert C, Mathai SC, Sathiyamoorthy S, Sahetya S, et al. Comparison of moderate versus deep sedation for endobronchial ultrasound transbronchial needle aspiration. Ann Am Thorac Soc 2013;10:121–6. doi:10.1513/AnnalsATS.201209-074OC.

[188] Stolz D, Chhajed PN, Leuppi JD, Brutsche M, Pflimlin E, Tamm M. Cough suppression during flexible bronchoscopy using combined sedation with midazolam and hydrocodone: a randomised, double blind, placebo controlled trial. Thorax 2004;59:773–6. doi:10.1136/thx.2003.019836.

[189] Stolz D, Kurer G, Meyer A, Chhajed PN, Pflimlin E, Strobel W, et al. Propofol versus combined sedation in flexible bronchoscopy: A randomised non-inferiority trial. Eur Respir J 2009;34:1024–30.

doi:10.1183/09031936.00180808.

[190] Aswanetmanee P, Limsuwat C, Kabach M, Alraiyes AH, Kheir F. The role of sedation in endobronchial ultrasound-guided transbronchial needle aspiration: Systematic review. Endosc Ultrasound 2016;5:300–6. doi:10.4103/2303-9027.191608.

[191] Fernandes M, Santos V, Martins N, Sucena M, Passos M, Marques M, et al. Endobronchial Ultrasound under Moderate Sedation versus General Anesthesia. J Clin Med 2018;7:421. doi:10.3390/jcm7110421.

[192] Clarkson K, Power CK, O'Connell F, Pathmakanthan S, Burke CM. A comparative evaluation of propofol and midazolam as sedative agents in fiberoptic bronchoscopy. Chest 1993;104:1029–31. doi:10.1378/chest.104.4.1029.

[193] Viterbo JF, Lourenço AP, Leite-Moreira AF, Pinho P, Barros F. Prospective randomised comparison of Marsh and Schnider pharmacokinetic models for propofol during induction of anaesthesia in elective cardiac surgery. Eur J Anaesthesiol 2012;29:477–83. doi:10.1097/EJA.0b013e3283542421.

[194] Navani N, Nankivell M, Lawrence DR, Lock S, Makker H, Baldwin DR, et al. Lung cancer diagnosis and staging with endobronchial ultrasound-guided transbronchial needle aspiration compared with conventional approaches: An open-label, pragmatic, randomised controlled trial. Lancet Respir Med 2015;3:282–9. doi:10.1016/S2213-2600(15)00029-6.

[195] Öztaş S, Aka Aktürk Ü, Alpay LA, Meydan B, Ogün H, Taylan M, et al. A comparison of propofol-midazolam and midazolam alone for sedation in endobronchial ultrasound-guided transbronchial needle aspiration: a retrospective cohort study. Clin Respir J 2017;11:935–41. doi:10.1111/crj.12442.

[196] Schlatter L, Pflimlin E, Fehrke B, Meyer A, Tamm M, Stolz D. Propofol versus propofol plus hydrocodone for flexible bronchoscopy: A randomised study. Eur Respir J 2011;38:529–37. doi:10.1183/09031936.00121610.

[197] Grendelmeier P, Tamm M, Pflimlin E, Stolz D. Propofol sedation for flexible bronchoscopy: A randomised, noninferiority trial. Eur Respir J 2014;43:591–601. doi:10.1183/09031936.00200412.

[198] Bürkle H, Dunbar S, Van Aken H. Remifentanil: a novel, short-acting, mu-opioid. Anesth Analg 1996;83:646–51. doi:10.1097/00000539-199609000-00038.

[199] Ost DE, Ernst A, Lei X, Feller-Kopman D, Eapen GA, Kovitz KL, et al. Diagnostic yield of endobronchial ultrasound-guided transbronchial needle aspiration: results of the AQuIRE Bronchoscopy Registry. Chest 2011;140:1557–66. doi:10.1378/chest.10-2914.

[200] Eapen GA, Shah AM, Lei X, Jimenez CA, Morice RC, Yarmus L, et al. Complications, consequences, and practice patterns of endobronchial ultrasound-guided transbronchial needle aspiration: Results of the AQuIRE registry. Chest 2013;143:1044–53. doi:10.1378/chest.12-0350.

[201] Steinfort DP, Irving LB. Patient satisfaction during endobronchial ultrasound-guided transbronchial needle aspiration performed under conscious sedation. Respir Care 2010;55:702–6.

[202] Wahidi MM, Sterman DH. Bringing comfort to endobronchial ultrasound bronchoscopy. Am J Respir Crit Care Med 2015;191:727–8. doi:10.1164/rccm.201502-0291ED.

[203] Nicholson AG, Chansky K, Crowley J, Beyruti R, Kubota K, Turrisi A, et al. The international association for the study of lung cancer lung cancer staging project: Proposals for the revision of the clinical and pathologic staging of

small cell lung cancer in the forthcoming eighth edition of the tnm classification for lung cancer. J Thorac Oncol 2016;11:300–11. doi:10.1016/j.jtho.2015.10.008.

[204] Gowers KHC, Hynds RE, Thakrar RM, Carroll B, Birchall MA, Janes SM. Optimized isolation and expansion of human airway epithelial basal cells from endobronchial biopsy samples. J Tissue Eng Regen Med 2018;12. doi:10.1002/term.2466.

[205] Abbosh C, Birkbak NJ, Wilson GA, Jamal-Hanjani M, Constantin T, Salari R, et al. Phylogenetic ctDNA analysis depicts early-stage lung cancer evolution. Nature 2017;545. doi:10.1038/nature22364.

[206] Thakrar RM, Pennycuick A, Borg E, Janes SM. Preinvasive disease of the airway. Cancer Treat Rev 2017;58. doi:10.1016/j.ctrv.2017.05.009.

[207] Jamal-Hanjani M, Wilson GA, McGranahan N, Birkbak NJ, Watkins TBK, Veeriah S, et al. Tracking the evolution of non-small-cell lung cancer. N Engl J Med 2017;376. doi:10.1056/NEJMoa1616288.

[208] Sage EK, Thakrar RM, Janes SM. Genetically modified mesenchymal stromal cells in cancer therapy. Cytotherapy 2016;18. doi:10.1016/j.jcyt.2016.09.003.

[209] Thakrar RM, Sage EK, Janes SM. Combined cell-gene therapy for lung cancer: rationale, challenges and prospects. Expert Opin Biol Ther 2016;16. doi:10.1080/14712598.2016.1188074.

[210] Butler CR, Hynds RE, Gowers KHC, Lee DDH, Brown JM, Crowley C, et al. Rapid expansion of human epithelial stem cells suitable for airway tissue engineering. Am J Respir Crit Care Med 2016;194. doi:10.1164/rccm.201507-1414OC.

[211] Thakrar RM, Navani N, Hackshaw A, Ngai Y, Child J, Booton R, et al. Photodynamic therapy for the prevention of lung cancer (The PEARL trial).

Lung Cancer 2017;103:S79–80. doi:10.1016/s0169-5002(17)30220-9.

[212] Thakrar RM, Dickson J, Ahmed A, George J, Sandhu G, Janes SM, et al. Virtual animation and 3-dimensional airway reconstruction using Lungpoint® to guide tracheobronchial stenting. Lung Cancer 2017;103:S67–8. doi:10.1016/s0169-5002(17)30199-x.

[213] Mikolasch T, Borg E, Thakrar R, Holmes V, Booth H, Porter J, et al. Transbronchial cryobiopsies in the diagnosis of Interstitial Lung Diseases- first UK experience. Thorax 2015;70:A27.3-A28. doi:10.1136/thoraxjnl-2015-207770.48.

[214] Thakrar R, Hardavella G, Brown J, Succony L, Falzon M, Borg E, et al. Role Of Ebus-tbna In The Diagnosis Of Primary And Relapsing Haematological Malignancy. Thorax 2014;69:A174–A174. doi:10.1136/thoraxjnl-2014-206260.349.

[215] Thakrar R, Brown J, Brazil S, Nankivell M, Lawrence D, George P, et al. Incidental detection of early stage non-small cell lung cancer - time to implement screening? Thorax 2014. doi:10.1136/thoraxjnl-2014-206260.213.

[216] Thakrar R, Brown J, Apperley H, Falzon M, Lawrence D, George P, et al. Carcinoma In-situ At The Bronchial Resection Margin - A Case For Routine Surveillance With Autofluorescence Bronchoscopy. Thorax 2014;69:A109–10. doi:10.1136/thoraxjnl-2014-206260.218.

[217] Brown J, Foreman L, Oliver K, Thakrar R, Marechal A, Rich P, et al. Infrared Spectroscopy For The Detection Of Extended Field Carcinogenesis: A New Paradigm For Lung Cancer Screening? Thorax 2014;69:A40–1. doi:10.1136/thoraxjnl-2014-206260.79.

[218] Lourenco S, Teixeira V, Kalber T, Thakrar R, Floto A, Janes S. MIF as the key regulator for mesenchymal stem cells homing to tumours by 3D and in vivo

lung metastasis models. Thorax 2014;69:A58–A58. doi:10.1136/thoraxjnl-2014-206260.114.

[219] Phoa KN, van Vilsteren FGI, Weusten BL a. M, Bisschops R, Schoon EJ, Ragunath K, et al. Radiofrequency Ablation vs Endoscopic Surveillance for Patients With Barrett Esophagus and Low-Grade Dysplasia. Jama 2014;311:1209. doi:10.1001/jama.2014.2511.

[220] Hopper C, Kübler A, Lewis H, Tan IB, Putnam G. mTHPC-mediated photodynamic therapy for early oral squamous cell carcinoma. Int J Cancer 2004;111:138–46. doi:10.1002/ijc.20209.

[221] Karakullukcu B, van Oudenaarde K, Copper MP, Klop WMC, van Veen R, Wildeman M, et al. Photodynamic therapy of early stage oral cavity and oropharynx neoplasms: an outcome analysis of 170 patients. Eur Arch Otorhinolaryngol 2011;268:281–8. doi:10.1007/s00405-010-1361-5.

[222] Jerjes W, Upile T, Hamdoon Z, Mosse CA, Akram S, Hopper C. Photodynamic therapy outcome for oral dysplasia. Lasers Surg Med 2011;43:192–9. doi:10.1002/lsm.21036.

[223] Corti L, Skarlatos J, Boso C, Cardin F, Kosma L, Koukourakis MI, et al. Outcome of patients receiving photodynamic therapy for early esophageal cancer. Int J Radiat Oncol Biol Phys 2000;47:419–24.

[224] Wei W, Chin L, Wan P, Heng S, Bhuvaneswari R, Kam W, et al. The potential application of chlorin e6–polyvinylpyrrolidone formulation in photodynamic therapy. Photochem Photobiol Sci 2006. doi:10.1039/b605772a.

[225] Duncan R. The dawning era of polymer therapeutics. Nat Rev Drug Discov 2003;2:347–60. doi:10.1038/nrd1088.

[226] Chin WWL, Lau WKO, Heng PWS, Bhuvaneswari R, Olivo M. Fluorescence imaging and phototoxicity effects of new formulation of chlorin e6-

polyvinylpyrrolidone. J Photochem Photobiol B 2006;84:103–10. doi:10.1016/j.jphotobiol.2006.02.002.

[227] Copley L, van der Watt P, Wirtz KW, Parker MI, Leaner VD. Photolon, a chlorin e6 derivative, triggers ROS production and light-dependent cell death via necrosis. Int J Biochem Cell Biol 2008;40:227–35. doi:10.1016/j.biocel.2007.07.014.

[228] Akopov A, Rusanov A, Papayan G, Molodtcova V, Urtenova M, Chistiakov I, et al. Intraoperative photodinamic therapy as a part of combined radical treatment for stage III NSCLC. Eur Respir J 2011;38:391.

[229] Lee LS, Thong PSP, Olivo M, Chin WWL, Ramaswamy B, Kho KW, et al. Chlorin e6-polyvinylpyrrolidone mediated photodynamic therapy--A potential bladder sparing option for high risk non-muscle invasive bladder cancer. Photodiagnosis Photodyn Ther 2010;7:213–20. doi:10.1016/j.pdpdt.2010.08.005.

[230] Molodtsova V, Kazakov N, Rusanov A, Chistiakov I, Urtenova M, Kulakova Y, et al. Endobronchial photodynamic therapy with chlorine E6 in III-IV stage central lung cancer. Eur Respir J 2011;38.

[231] Akopov A, Rusanov A, Gerasin A, Kazakov N, Urtenova M, Chistyakov I. Preoperative endobronchial photodynamic therapy improves resectability in initially irresectable (inoperable) locally advanced non small cell lung cancer. Photodiagnosis Photodyn Ther 2014;11:259–64. doi:10.1016/j.pdpdt.2014.03.011.

[232] Sheleg S V, Zhavrid EA, Khodina T V, Kochubeev GA, Istomin YP, Chalov VN, et al. Photodynamic therapy with chlorin e(6) for skin metastases of melanoma. Photodermatol Photoimmunol Photomed 2004;20:21–6.

[233] Fayter D, Corbett M, Heirs M, Fox D, Eastwood A. A systematic review of

photodynamic therapy in the treatment of pre-cancerous skin conditions, Barrett's oesophagus and cancers of the biliary tract, brain, head and neck, lung, oesophagus and skin. Health Technol Assess 2010;14:1–288. doi:10.3310/hta14370.

[234] van Vilsteren FGI, Alvarez Herrero L, Pouw RE, Schrijnders D, Sondermeijer CMT, Bisschops R, et al. Predictive factors for initial treatment response after circumferential radiofrequency ablation for Barrett's esophagus with early neoplasia: a prospective multicenter study. Endoscopy 2013;45:516–25. doi:10.1055/s-0032-1326423.

[235] A'Hern RP. Sample size tables for exact single-stage phase II designs. Stat Med 2001;20:859–66. doi:10.1002/sim.721.

[236] NICE NI for H and CE. Guide to the methods of technology Guide to the methods of technology appraisal 2013. NICE 2013.

[237] Briggs AH, Wonderling DE, Mooney CZ. Pulling cost-effectiveness analysis up by its bootstraps: a non-parametric approach to confidence interval estimation. Health Econ n.d.;6:327–40.

Appendix A THE PEARL TRIAL: PHOTODYNAMIC THERAPY FOR THE PREVENTION OF LUNG CANCER

A.1 Introduction

It is widely accepted that squamous cell lung cancers arise from pre-invasive lesions typically found in the central airways. Smoking exposure results in morphological progression from normal epithelium to dysplasia, carcinoma-in-situ (CIS), and ultimately to invasive carcinoma. However, as shown in chapter 2, not every pre-invasive lesion is destined to this course and in many individuals, lesions may not progress, or indeed may regress to normal epithelium.

Surveillance of pre-invasive lesions with autofluorescence bronchoscopy (AFB) allows us to document their natural history with the hope that early detection can lead to improved survival. Prospects for individuals with pre-invasive or intraepithelial neoplastic lesions (stage 0), or early stage invasive cancers (Stage 1A) of the central airway are good, with a 5-year survival of more than 70% [3–6]. However, their treatment remains controversial and, although radical therapies are advocated for individuals with high-grade pre-invasive disease (HGLs), there is no randomised evidence to support this practice.

The American College of Physicians and other authors advocate surgical treatment for CIS and early lung cancers in the airway [4,6,7,87]. Despite these lesions being small, their central location means that many individuals require extensive surgical resection[4]. However, with patients having a significant risk of developing multifocal pre-invasive and invasive carcinoma at other sites within their lungs, many will not have sufficient reserve to undergo multiple lung resections. It is also known that not all HGLs progress to invasive cancer, and in one review, Banerjee et

al concluded that overall CIS regression occurred in 58% of individuals undergoing treatment, however, 34% of CIS lesions progressed despite treatment [53]. While many investigators do report good results with a variety of endobronchial treatments, these studies are often small, with short follow-up, and frequently combine progression to invasive and high-grade pre-invasive histology as a single end-point [7,53,55]. Since none of the studies have included a control arm [48,51,55,60,88], the natural history of the lesions treated in these studies is not known, and the clinical and prognostic value of the intervention remains unclear. Consequently, the American College of Chest Physician's guidance conclude with remarks on the lack of randomised studies evaluating the efficacy of endobronchial therapies for pre-invasive disease [7].

A.2 Photodynamic therapy

A.2.1 Photodynamic therapy in treatment of early central lung cancer

Photodynamic therapy (PDT) has a proven track record of successful tumour ablation [105]. It relies on activation of a photosensitiser that preferentially accumulates in transformed cells. Using a specific wavelength of light delivered endobronchially, release of reactive oxygen species causes cellular apoptosis to the lesion in question. PDT can achieve good response rates in radiographically negative airway cancers [89,90,99,106,91–98]. In an early phase II study, Furuse et al treated 59 early cancers with photofrin, a first-generation photosensitiser [90]. A complete response (CR) was seen in 85% of patients, with the remainder having either a partial or no response. Usuda et al observed the outcome of 28 early lung cancers (including CIS) treated with PDT, where a complete response was observed

in 100% of cases at 2-month follow-up. They also showed, ⅓ of their cohort developed metachronous lesions, demonstrating the importance of utilising PDT as a tissue-sparing strategy [98].

A systematic review of over 700 invasive and pre-invasive lesions across 15 trials revealed a complete response rate of 30–100% and an overall 5-year survival of 61% [107]. They further showed that PDT is safe with photosensitivity being the most common complication in 5–28% of cases. The lower response rates seen in this review were largely due to the heterogeneity of cases treated and it has since come to light that PDT is most effective when there is no extra-cartilaginous disease and the tumour length is <1 cm [94,95].

Furukawa et al used PDT as the definitive treatment in 114 stratified lesions (<1 cm or >1 cm), with long-term follow-up [95]. When persistent atypia was demonstrated at the same site the authors showed complete remission could be obtained by performing a second PDT. A complete response was seen in 93% of lesions <1 cm in size compared to 58% of lesions >1 cm. While the 5-year survival was not influenced by tumour size, the lower survival in both groups may be due to poor baseline performance status as patients receiving this treatment were unsuitable for surgical treatment.

A.2.2 Photodynamic therapy in the treatment of pre-invasive disease

Photodynamic therapy should be considered for those in whom surgery is medically or technically not possible. However, it is more commonly being used as treatment of pre-invasive disease, especially in cases of multifocal disease where a tissue sparing approach is necessary [94]. While authors report a good response with PDT,

its role as a definitive treatment remains unclear and will be examined for the first time in this trial.

The large body of evidence for treating pre-invasive lesions comes from other organ systems. The major clinical trials in pre-invasive disease of the aerodigestive tract are summarised in Table 4-1. The documented treatment modalities in the oesophageal and head and neck studies are all using PDT. However, in the lung different endobronchial modalities are often used and there are few trials using PDT solely for pre-invasive disease. In the oropharynx, Hopper et al treated 95 lesions of severe dysplasia and CIS with PDT with a complete response maintained at 3-year assessment[19]. In a similar study HGLs treated with PDT had a response rate of 79.5% [CI 68%-88%] in a trial of 73 lesions, where patients maintained a disease-free interval for 66 months[31]. Finally, in the gut, in a landmark randomised controlled trial of surveillance versus treatment in pre-invasive disease of the oesophagus, has led to a complete change in clinical practice of Barrett's oesophagus[219]. In the lung a variable response to treatment is seen, with 57-81% of treated high-grade lesions responding to treatment. Although the results from these studies look quite promising, there is considerable heterogeneity of the studies in treatment protocols, length of follow-up, and study end-points, making firm conclusions on efficacy difficult.

Investigators	Baseline histology & lesion (no.)	Median follow-up (months)	Lesion end point/response	Comments
LUNG				
Deygas et al (2000) [60]	CIS (35)	1m & 1year	CIS→LGL: 71% CIS→CIS: 9% CIS→INV: 20%	Cryotherapy as treatment 28% local recurrence (1 year) Disease free-interval 13-45months
Kato et al (2003) [135]	CIS (39)	1-2m	CR 85% [CI 69-94]	PDT as treatment Short follow-up
Moro-Sibilot et al (2004) [51]	SD (3) CIS (28)	24 (13–41)	CIS→LGL: 52% CIS→INV: 43%	Electrocautery as treatment CIS (untreated) progressed in 29%
Salaun et al (2008)[$] [52]	SD (23) CIS (31)	68 (19–117)	CIS→LGL: 13% (untreated) CIS→LGL: 32% (treated) CIS→INV: 23%	Electrocautery as treatment HGLs had 3mo assessment prior to treatment decision
Van Boerdonk (2015) [17]	HGL (80) CIS:14	30 (4–152)	HGL→INV: 18% (site specific progression)	Electrocautery as treatment Cumulative 5-year lung cancer risk 39% in HGLs
OROPHARYNX				
Hopper et al (2004) [220]	CIS (37)	3m and 12m	CR: 85% [CI 79-92%]	PDT as treatment 18% recurrence at 12m
Karakallukcu et al (2011) [221]	CIS (73)	NS	CR: 79.5% [68-88%]	PDT as treatment 66m disease free interval
Jerjes et al (2011) [222]	SD & CIS (95)	3 year	CR: 71%	PDT as treatment 15% recurrence at 3year follow-up
GASTROINTESTINAL				
Corti et al (2000) [223]	Barretts (18)	NS	CR: 45%	PDT as treatment
Phoa et al (2014) [219]	Barretts (68)	3 year	CR: 93% (at 2months)	PDT as treatment 26.% progression to cancer in control vs 1.5% treated with PDT

Appendix A 1: Endoluminal therapy studies of pre-invasive disease of the aerodigestive tract

A.3 Photodynamic therapy using Fotolon® photosensitiser

A.3.1 Fotolon® - chemistry, pharmokinetic and pharmodynamics

Chlorin-e6-trisodium salts are a group of second-generation photosensitisers. The chlorin-e6 molecule has been adapted to improve its clinical efficacy. The conjugation of a trisodium salt of chlorin-e6 to polyvinylpyrrolidone (PVP) is Fotolon®. This has several advantages as a photosensitiser with increased stability, and solubility in water, thereby increasing its bioavailability and enhancing its photosensitising effect [224,225]. Further, this attachment to a polymeric carrier enables a prolonged circulation of the drug in the plasma leading to enhanced tumour targeting through 'enhanced permeability and retention' (EPR) effect [225]. This explains the higher selectivity of Fotolon® accumulation in malignant tissues [224]. Its activation at 665 nm has been shown to induce tumour necrosis in vivo; and this long wavelength of activation means that Fotolon® can be used for deep tissue excitation, and thus the destruction of deep-seated tumours[226]. Once activated with light, Fotolon® results in a significant production of reactive oxygen species (ROS), which is ultimately responsible for cell killing by necrosis [227].

Chlorin-e6 trisodium salt possesses photosensitising properties with rapid accumulation in tumour tissue observed at 1-3 hours following its intravenous administration and is rapidly eliminated thereafter. This allows for same day phototherapy making it more palatable for patients. The photosensitiser is best activated at a wavelength of 660-670 nm and its favourable tumour to normal tissue ratio of 8:1 is achieved at 3 hours after its administration. As a result, necrosis has been detected up to a depth of 12 mm in animal models[224,227]. The formulation of Fotolon® has been proved to be distinctly advantageous as a

diagnostic and therapeutic agent for fluorescence diagnosis and PDT of [228]NSCLC [229].

A.3.2 Clinical use of Fotolon® in the lung

Fotolon® has been used in 52 patients with stage IIIb and IV lung cancer (46 NSCLC, 6 SCLC) with major airway obstruction and has been assessed as a safe and effective way of treatment causing complete or partial (>50%) remission in 83% of patients [230]. Fotolon® has also been used intra-operatively as a part of combined radical treatment for stage III NSCLC with no complications and good clinical outcome [231]. It is currently undergoing phase IIb clinical trials in Germany in the treatment of early central airways cancer (EudraCT 2013-001876-28).

In a phase II PDT study, Kato et al treated 45 central early-stage lung cancers using chlorin conjugate, where a complete response was obtained in 85% of the lesions. Further, rates of skin photosensitivity were considerably lower than that seen with Photofrin[135]. Fotolon® has a rapid accumulation in tumour tissue observed at 1-3 hours following its intravenous administration and is rapidly eliminated thereafter. The post-treatment photosensitivity phase is only 4-7 days as compared to 6-8 weeks with Photofrin® resulting in shorter hospital admission, thus improving patient acceptability and reducing costs.

A.3.3 Clinical use of Fotolon® in other organs

Istomin et al administered Fotolon® and performed PDT in 112 women of a childbearing age with cervical intraepithelial neoplasia grades II and III [134]. A complete response represented by the complete regression of neoplastic lesions, was seen in 104 (92.8%) of treated women. The authors suggest that PDT with

Fotolon® is an alternative approach for the treatment of cervical intraepithelial neoplasia, which can be recommended for women of childbearing age. Fotolon® has also been selectively administered both intravenously and intravesically followed by PDT as a bladder sparing therapy to treat non-muscle invasive bladder cancer refractory to intravesical Bacillus Calmette–Guérin (BCG) therapy [229]. Despite this being a small study, it showed intravesical PDT using Fotolon® can be used to treat non-muscle invasive bladder carcinoma in selected individuals. PDT with chlorin e6 photosensitiser has been administered to 14 patients with skin metastases from melanoma. All skin melanoma metastases that received PDT showed complete regression with no recurrence. There were no significant changes in blood cell counts that would indicate systemic chlorin e6 toxic effects. Blood chemistry and urinalysis did not show any evidence of chlorin e6 induced renal and hepatic injury [232].

A.4 Why is a trial needed?

A systematic review of PDT by the NIHR Health Technology Assessment programme looked at the clinical effectiveness and safety of PDT, either as an adjunctive or sole treatment for different cancers [233]. The key points outlined were:

- There is a paucity of well-conducted randomised controlled trials in PDT
- Quality of life outcomes were under reported in these studies
- No PDT trials were found on treatment of early lung cancer
- Further research to determine the role of PDT is needed

Individuals with pre-invasive disease are at high risk of developing synchronous and metachronous lung cancers and HGLs throughout the airways. The frequent occurrence of new invasive cancers elsewhere in the lung means radical treatments may be harmful in the long-term, hence the need for tissue-sparing therapies.

Photodynamic therapy combines tissue specificity with efficacy and repeatability, and these characteristics make it ideal to treat recurrent airway HGLs. A trial was therefore designed to investigate the effect of PDT on the progression of pre-invasive disease to lung cancer, making it the first clinical trial in this area. If effective, this will be the first evidence-based endoscopic treatment for the prevention of lung cancer. Finally, from our increased understanding of the molecular pathology it is becoming apparent that the respiratory epithelium accumulates progressive genetic and epigenetic insults in response to carcinogens. Still, little is known about how to predict those ‘at risk’ of progression. This trial will also map the molecular signatures, which in the future may underpin prediction models of developing invasive lung cancer based on some of the work already done on the ‘high-risk’ cohort in chapter 2 [124].

A.5 Hypothesis

The trial will test the alternative hypothesis that photodynamic treatment of high-grade pre-invasive lesions in the airway will not change the progression to invasive cancer when compared to surveillance alone.

A.6 Study population

Patients with ≥1 histologically confirmed high-grade airway lesion (defined as severe dysplasia or carcinoma in situ) will be eligible for trial entry. Patients will be at least 18 years of age and fit enough to undergo bronchoscopy and endobronchial photodynamic therapy (ECOG performance score 0-2). No upper age limit has been set, but it is expected patients will have a life expectancy of at least 3 years. Exclusion criteria are significant concurrent malignancy or finding of (micro-) invasive lung carcinoma. Individuals with hypersensitivity to porphyrins or photosensitivity will also be excluded.

Patients from known cohorts or through new referrals will be recruited and first undergo clinical, radiological and bronchoscopic evaluation prior to randomisation. Clinical assessment will include examination, lung function testing and CT Thorax with contrast. Smoking status will be recorded and all current smokers will be offered smoking cessation advice. Quality of life questionnaires (EQ-5D, EORTC LC-13, SF-36) will be completed.

Patients will then undergo examination with autofluorescence bronchoscopy (AFB) with either the D-Light autofluorescence bronchoscope (Karl Stortz, Germany), or

Olympus autofluorescence AFI-Lucera (Olympus, Tokyo, Japan). All bronchoscopies will be performed under intravenous sedation according to the British Thoracic Society guidelines[137]. The bronchial tree will be inspected first under white light and then under blue light. All abnormal areas will initially be documented and only sampled when the bronchoscopic examination has been completed. The patient will be recruited once histological diagnosis of ≥1 HGLs in the airway, defined as severe dysplasia or carcinoma in-situ, has been confirmed by two expert pulmonary pathologists.

A.7 Trial design

This is a Phase III multicentre, international randomised controlled trial, with an incorporated phase II (pilot) component. Patients will be randomised in a 2:1 design to either intervention with photodynamic therapy treatment followed by surveillance with CT and AFB or surveillance with CT and AFB alone. Telephone randomisation using permuted blocks of four generated by computer will be employed. Randomisation will be stratified for the number of synchronous high-grade lesions (≤2 vs >2), lesion length (≤1cm vs >1cm), length of time HGL present (≤1 vs >1 year), COPD, and previously surgically treated non-small cell lung cancer or radically treated head and neck squamous cell cancer.

The trial centres will be University College London Hospital, Wythenshaw Hospital in Manchester, Papworth Hospital in Cambridge, and VU Medical Centre in Amsterdam. Following the informed consent process, University College London Clinical Trials Centre (UCL CTC) will co-ordinate the randomisation process. Due to the nature of the intervention, blinding the participants and investigators is not possible. However, the histopathologists reading the biopsies will be blinded to the

intervention. Data will be collected on paper case record forms and entered (using double-data entry) by an independent data clerk onto a secured trial database on a dedicated trial computer.

A.8 Study protocol

A.8.1 Surveillance arm

All patients will attend clinic after randomisation at 12 monthly intervals for a total of 3 years of follow-up. Patients randomised into the surveillance arm will be managed according to the schema in Figure 3-1. Clinical follow-up and assessment will include recording of smoking status, completion of quality of life questionnaires, lung function testing and collection of a patient diary of their healthcare use. Participants will also undergo an annual CT Thorax with contrast. All patients will undergo surveillance AFB according to the most severe grade lesion in the patient's airway. The procedure will be performed as described in chapter 2.

A.8.1.1 When pre-invasive lesions are identified

AFB will be repeated at intervals according to the grade of pre-invasive lesion in the patient's airway:

- For high-grade lesions bronchoscopy will be performed as near as possible to 6 months
- For low-grade lesions or normal epithelium bronchoscopy will be performed as near as possible to 12 months

- The index and/or treated lesion(s) will be biopsied on each occasion, as will any other lesion previously known to harbour pre-invasive pathology.
- Any newly identified lesions will be biopsied and kept under surveillance for the trial period

A.8.1.2 When invasive lesions are identified

- The patient will be managed as directed by a lung cancer multi-disciplinary meeting.
- Surveillance with AFB and CT will continue after treatment for the detected cancer has been completed.

A.8.1.3 When the bronchoscopy is normal

- Biopsies will be taken from the area of the index and/or treated lesion(s) and a control normal site in the airway.

A.8.2 Intervention (PDT) arm

Participants randomised to the intervention arm will undergo two photodynamic therapy treatments to all index HGLs in the airway, followed by surveillance with AFB and CT. Patients will receive Fotolon® (1mg/kg IV) photosensitiser prior to their PDT treatments. The first treatment will occur within 28 days of randomisation. Thereafter, the second treatment will be given between week 6-10. If HGLs are present in both lungs, treatment of each side will need to be undertaken sequentially. Treatment of both lungs must be completed within 2 weeks (Table 4-2).

Week	1		6 (+4 weeks)		8 weeks post treatment
	Day 1	Day 2	Day 1	Day 2	
Fotolon® 1mg/kg IV[a]	•		•		
Photodynamic therapy (PDT) 3-4 hours after Fotolon® administration	•		•		
Autofluorescence bronchoscopy (AFB)	•		•		•
Bronchoscopy for removal of tissue debris		•		•	

Appendix A 2: Summary of drug administration and photodynamic therapy in intervention arm

A.8.2.1 Light dosimetry for PDT

PDT treatment must occur 3-4 hours after Fotolon® administration as optimal uptake of Fotolon® is expected at this time. For the light treatment the patient will undergo flexible bronchoscopy as per BTS guidelines[137]. All index high grade lesions will be identified by AFB and then photodiagnosis and illuminated with light at 665nm (± 5nm). The delivery device used is a 600 micron diameter quartz fibres inserted through the working channel of the bronchoscope – either a cylindrical diffuser or microlens fibre (CeramOptec, Bonn, Germany). The size and topography of the lesion will inform the operator of the length of time that the treatment will need to be applied. The dose is specified in J/cm length of fibre. The bronchial lesions will be treated at 150J/cm length of diffuser at a rate of 400mW/cm of diffuser over a period of 375s. In the case a cylindrical fibre cannot be used, e.g., access to a particular lesion is difficult to cover with cylindrical illumination; a microlens fibre can be utilised with a fluence of 100 mW/cm^2 given over 700s, giving a dose of 70 J/cm^2. Light must be applied to the entire surface of the endobronchial lesion as far as technically feasible. Each field is to be illuminated

once only at each treatment. Multiple non-overlapping fields may be illuminated during one treatment session.

A.8.2.2 Following PDT

The patient will undergo a second bronchoscopy the day following treatment to remove any necrotic airway debris from the treatment. On completion of PDT treatment, as part of the phase II trial component, patients will undergo a bronchoscopy and biopsy assessment post treatment. The end-point for phase II is regression of a HGL to a LGL or normal epithelium at an 8-week AFB assessment. Following this surveillance will resume as per the surveillance arm:

When an HGL lesion is identified

- When a HGL is present in the airway, either the index (treated) lesion(s) or any metachronous lesions(s) that develop over the course of follow-up in the trial will be managed with 6-monthly AFB and 12-monthly CT thorax surveillance as in the control arm.

When an LGL lesion is identified

- When histological response is detected after PDT, a repeat AFB and CT will be performed near as possible to 12 months
- Surveillance will otherwise continue as per the control arm

When invasive lesions are identified

- The patient will be managed as directed by a lung cancer multi-disciplinary meeting
- Surveillance with AFB and CT will continue after treatment for the cancer has been completed as per the control arm

A.9 Trial endpoints

A.9.1 Histology definitions & quality assurance

The definitions for endpoints in the trial will be determined by changes based on histological grade assessed by a blinded pathologist using WHO/IASLC criteria [12].

- Regression: refers to CIS or severe dysplastic lesions (HGLs) reverting to either mild/moderate dysplasia, metaplasia and hyperplasia (LGLs), or to normal epithelium
- Stable: refers to HGLs remaining unchanged over the testing period
- Progression: refers to the site-specific progression of HGLs to invasive disease, where breach of the epithelial basement membrane has occurred.

Histopathologists with a dedicated interest in pulmonary pathology will examine multiple sections from all biopsies. A central pathology review will be employed for all specimens from the index HGL and all other multifocal lesions that develop during surveillance. They will be first reported by the pathologist at the host site and subsequently reviewed by a second at a different recruiting site and verified. Where there is disagreement, the diagnosis will be verified by a reference pathologist who will review the same slides. The diagnosis of the reference pathologist will be accepted as final.

A.9.2 Phase II

The primary endpoint for the phase II component of the trial is the response to photodynamic therapy as measured by regression of a high-grade lesion to low-grade or normal epithelium. The secondary endpoint will look at the efficacy of the

treatment and following review by the independent data monitoring committee will determine whether to continue into the phase III part of the trial.

A.9.3 Phase III

The primary endpoint for the phase III trial is the time interval for site-specific HGL progression to invasive lung cancer within a 3-year follow-up. Pre-specified secondary endpoints are (i) evaluation of site-specific lesion dimension effect on pathological response, (ii) number of metachronous endobronchial lung cancers and pre-invasive lesions developing at remote sites in the central airways as detected by AFB, (iii) cumulative risk of developing lung cancer detected on AFB and CT, (iv) overall and cancer-specific survival from date of randomisation, (v) changes in Health-Related Quality of Life (HRQoL) over time using the EQ-5D-5L, EORTC LC-13 and SF-36, (vi) resource collection to determine cost-effectiveness of photodynamic therapy, (vii) measure difference in spirometry (FEV1 & FVC), and (viii) to evaluate adverse events of Fotolon® and PDT.

A.10 Trial statistics

A.10.1 Summary

Trial Design	**Phase II**	**Phase III**
Design	Single arm Phase II	RCT with control arm
Randomisation	2:1	2:1
End point definition	Regression to LGL or normal	Time to progression to cancer
End point measured	8 weeks	3 years
Patient no. (PDT)	21	62 (67)*
Patient no. (control)	11	31 (33)*
Patient no. (total)	32	93 (100)*
Expected response	>20% response in PDT group 3 out of 21 PDT patients	PDT: 30% progression to cancer Control: 55% progression to cancer
Power	10% one-sided significance and 80% power	5% two-sided statistical significance and 80% power
Reference	A'Hern et al & Machin et al	Machin et al

Appendix A 3: Summary of sample size calculations for the PEARL trial

A.10.2 Sample size calculation

A.10.2.1 Phase II

In the lung and other organ systems, the regression of a treated pre-invasive lesion is accepted as a standard primary endpoint in endoscopic treatment studies [219,223,234]. This outcome has been validated and used in patients undergoing ablative therapy for Barrett's Oesophagus, and correlates with rate of change to invasive disease and long-term outcome [219]. The same methodology has been adopted in head and neck disease; in an open-label randomised controlled trial, Hopper et al showed 85% [CI 79-92%] of patients with CIS or early oropharyngeal cancer completely responded to PDT by 12 weeks. Complete responders (including CIS subgroup) maintained regression of disease in 85% of cases at 1-year follow-up [220]. Photodynamic therapy trials in early central lung cancer are summarised in Table 4-4.

The independent data monitoring committee will be asked to look for a target regression (response) rate of at least 20% (with a lower bound of 5%, because we expect very few untreated control patients to regress in the short assessment time) in the PDT group, as evidence of an efficacy signal for continuing the trial. Using a single arm phase II design [235], at least 3 out of 21 PDT patients should have a response (10% one-sided statistical significance, and 80% power), as evidence of an efficacy signal to continue with the study.

Investigators	Lesions (n)	Outcome of complete response (%)	Comments
Kubota et al (1992) [89]	29	72%	CR 89% in lesions <10mm
Furuse (1993) [90]	59	CIS: 100% INV: 80%	CR in lesions <1cm: 98% CR in lesions >1cm: 43%
Imamura et al (1994) [91]	39	64%	CR in superficial lesions: 76% CR in nodular lesions: 43%
Kato et al (1996) [92]	95	83%	CR in lesions <1cm: 94% CR in lesions 1-2cm: 54%
Kawaguchi et al (2000) [93]	59	73%	53% had no recurrence at 2-year assessment
Miyazu et al (2002) [94]	18	100%	EBUS to identify superficial lesions (9 lesions treated)
Furukawa et al (2005) [95]	114	Lesion <1cm: 93% Lesion >1cm: 58%	5-year survival (<1cm): 58% 5-year survival (>1cm): 59%
Kato et al (2006) [96]	264	85%	Local recurrence in 12% of cases
Corti et al (2007) [106]	50	CIS: 73% INV: 69%	Overall survival (CIS): 120months Overall survival (INV): 36months
Endo et al (2009) [97]	48	94%	Tumour length <10mm Overall 5-year survival: 81%
Usuda et al (2010) [98]	28	100%	PDT used to treat 11 individuals with multifocal disease
Jung et al (2011) [99]	39	100%	PDT used in central airway lesions in individuals with multifocal disease

Appendix A 4: Summary of photodynamic treatment studies in early central lung cancer

A.10.2.2 Phase III

The primary outcome in the main trial is the time for lesion progression to cancer. The progression of severe dysplasia and CIS to invasion is estimated at 43%–87% [18,48,51,52,57]. We have demonstrated in our surveillance cohort around 50% of HGLs progress into invasive lung cancer (Chapter 2). Banerjee et al in their review concluded that 34% of CIS lesions progressed despite treatment[53] and from our review lesion progression appears to occur in 18–34% of lesions despite endobronchial treatment when kept under long-term surveillance [17,48,51,57].

In the oropharynx, Hopper et al treated 95 lesions of severe dysplasia and CIS with PDT with a complete response maintained at 3-year assessment[19]. In a similar study HGLs treated with PDT had a response rate of 79.5% [CI 68%-88%] in a trial of 73 lesions, where patients maintained a disease-free interval for 66 months[31]. Finally, in the gut, PDT has been used to treat 166 patients with preinvasive disease, demonstrating a complete eradication in 83% of cases, with 32% of patients developing local recurrence[17].

We therefore expect a 3-year progression rate of 30% in the intervention arm compared to 55% in the control arm. Using time-to-event analyses (and exponential survival), we would need 93 (62 PDT: 31 controls) patients to show this difference with 80% power and two-sided 5% statistical significance. Because this trial aims to prevent progression, we are ultimately interested in the risk of not progressing which is 70% PDT versus 45% controls. To allow for an approximate 5% dropout rate, we will aim to recruit 100 patients.

A.11 Trial analysis

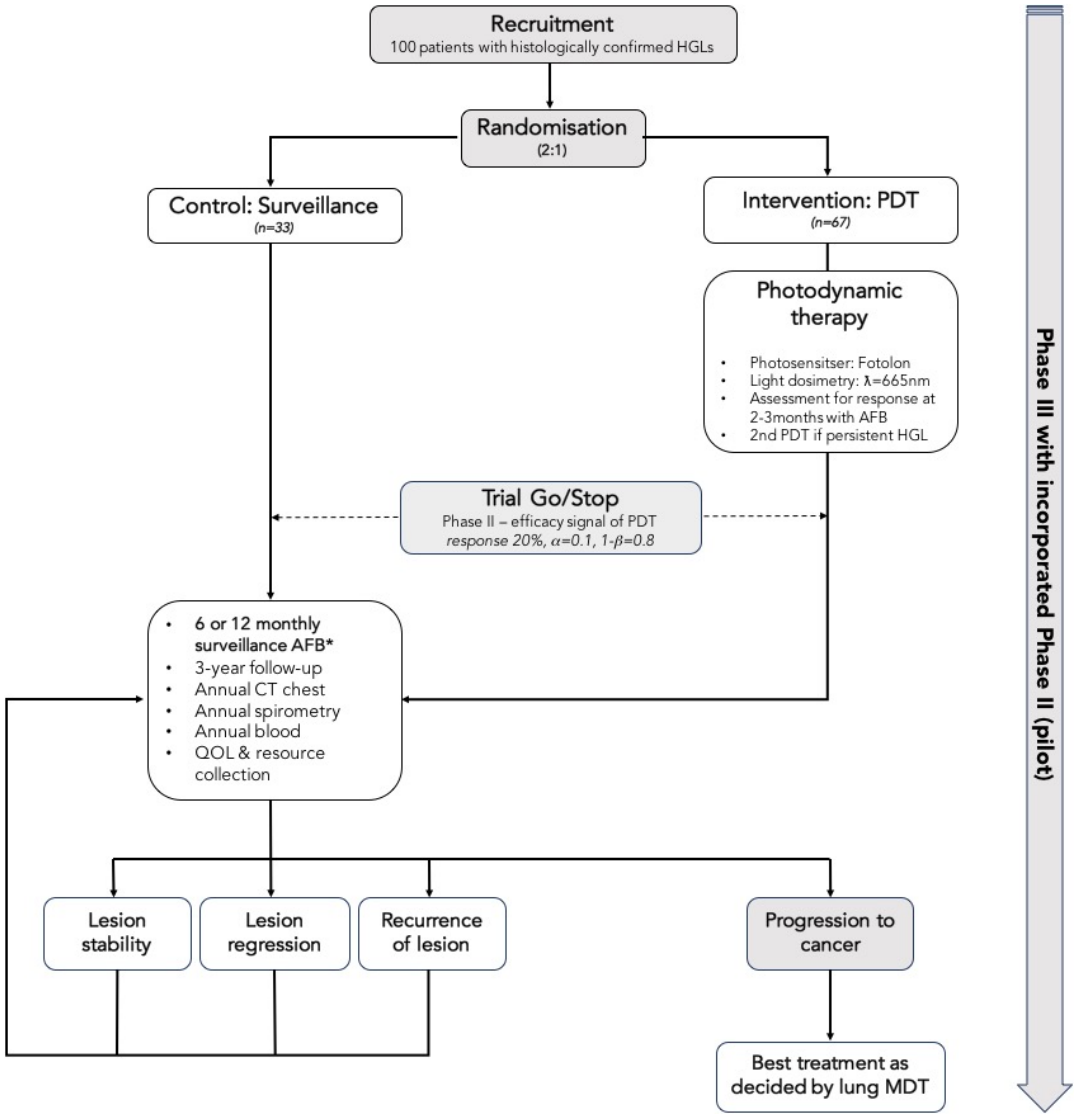
For the primary endpoint, the 3-year progression rate along with the 95% confidence interval will be estimated using Kaplan-Meier life tables in each group, and the hazard ratio for not progressing estimated using Cox regression analysis. Regression analyses will also be used to adjust for key baseline characteristics such as age, smoking status, COPD, lesion number, grade and size. Subgroup analyses using the randomisation stratification factors will be explored. The above analyses will be repeated for patients who started PDT and for control patients who did not have PDT (per protocol analysis). All secondary endpoints will use the (i) ITT population and (ii) the per protocol analysis as defined above. For continuous measures, generalised linear models will be used. For time to event measures, Kaplan Meier and Cox regression methods will be used. Categorical data will be summarised using frequencies and counts, with chi-square or Fishers exact test.

A.12 Economic analysis

Economic analyses will consist of patient level simulation. PEARL will allow a detailed analysis to be undertaken of the cost and cost-effectiveness of bronchoscopic photodynamic treatment of HGLs and surveillance compared with surveillance bronchoscopy alone. The analysis will conform to accepted economic evaluation methods [236]. Over the course of the study, data (notably, health service use, surveillance bronchoscopic costs and treatment costs when progression to cancer occurs) will be collected. Resource data collection will occur across the phase II & phase III components during episodes of contact. We will determine cost and cost-effectiveness for this “within-trial‟ period (3-years). Costs will be assessed from the perspective of the NHS and personal social services (PSS).

Detailed bottom-up costing will be carried out including annuitised capital costs plus consumables. Further, resource use on overall hospital stay, outpatient attendances, and medications will be included. The volume of resource use for each cost component will be measured directly in the trial from patient records and using patient diaries; unit costs will be taken from standard published sources.

The cost-effectiveness measures in the 3-year model will be the incremental cost per % reduction in progression to invasive cancer and the incremental cost per quality-adjusted life year (QALY) gained. Quality of life will be assessed as a secondary outcome in the trial and QALYs will be calculated based on the health-related quality of life (HRQL) and mortality data collected during the trial. HRQL will be measured according to the EQ-5D-5L (http://www.euroqol.org) and LC13, which we will collect at baseline, 6 m, 12 m, 18 m, 24 m, and 36m. This will enable more accurate straight-line relation in the final analysis. The 3month time point in particular is important, as this will be immediately after PDT treatment. Patient-specific utility profiles will be constructed assuming a straight-line relationship between each of the patient's EQ-5D scores at each follow-up point. The QALYs experienced by each patient from baseline to 3 years will be calculated as the area underneath this profile. Multiple imputations by chained equations will be used to deal with missing EQ-5D values. Subsequent analyses of imputed data will include variance correction factors to account for additional variability introduced into parameter values as a result of the imputation process. Non-parametric methods for calculating confidence intervals around the incremental cost effectiveness ratio based on bootstrapped estimates of the mean cost and QALY differences will be used [237]. The bootstrap replications will also be used to construct a cost-effectiveness acceptability curve, which will show the probability that use of the photodynamic therapy is cost-effective at 3-years for different values of the NHS' willingness to pay for an additional QALY.



Appendix A 5 – The PEARL Trial Schema

A.13 Conclusion

A cohort of patients at high-risk of lung cancer are presented in chapter 2, who have the propensity to develop multiple lung cancers over their life-time. In particular, those individuals with high-grade dysplasia are at particular risk. Using these individuals as the study population, a trial using endobronchial photodynamic therapy (the PEARL trial) is presented in this chapter.

Over the course of this thesis, successful funding was obtained from Cancer Research UK with an aim of commencing the trial in early 2020. The trial will open at University College London Hospital first and the other centers will open by 2021.

Appendix B PUBLICATIONS

Cancer Treatment Reviews 58 (2017) 77–90

Contents lists available at ScienceDirect

Cancer Treatment Reviews

journal homepage: www.elsevierhealth.com/journals/ctrv

Tumour Review

Preinvasive disease of the airway

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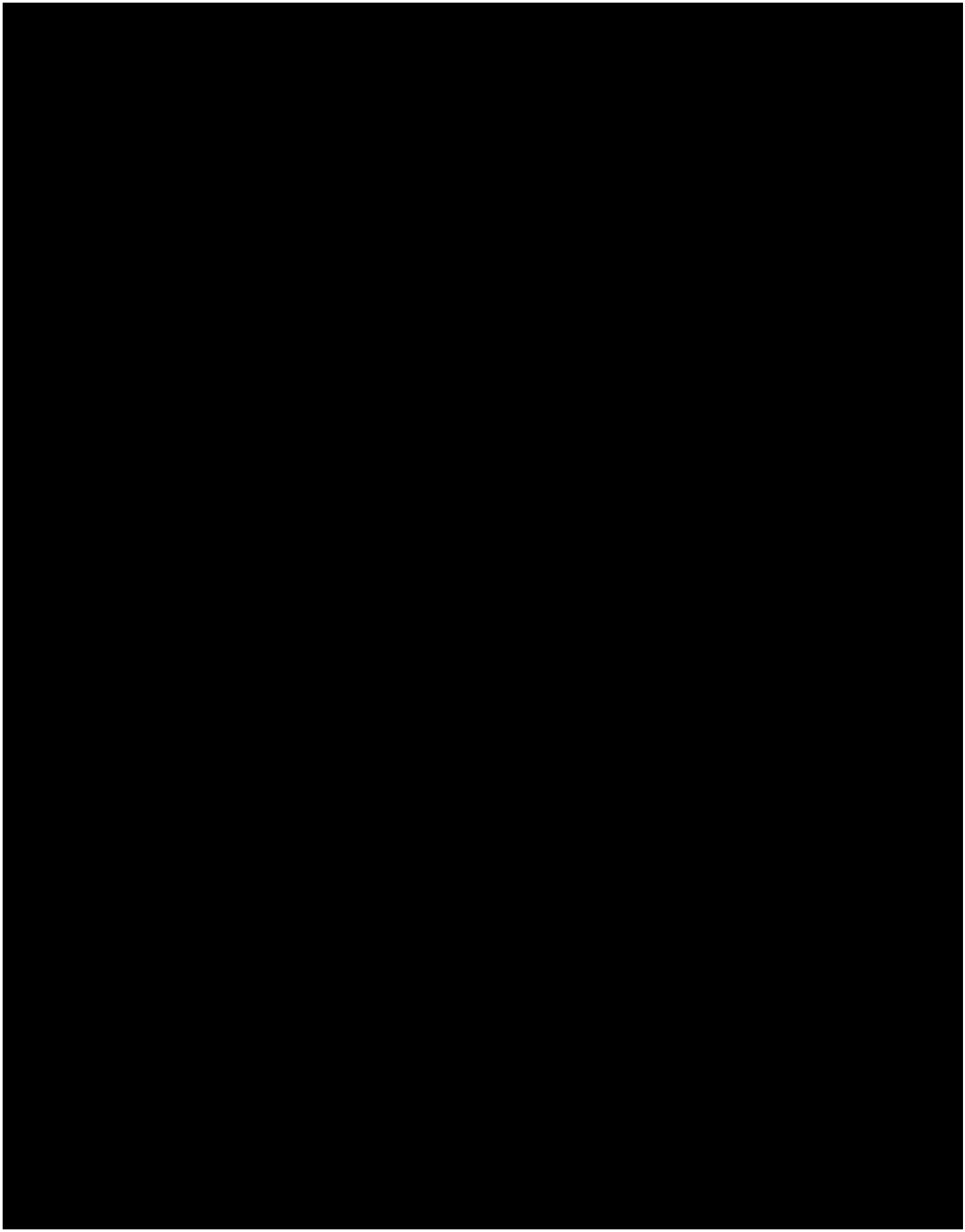
ABSTRACT

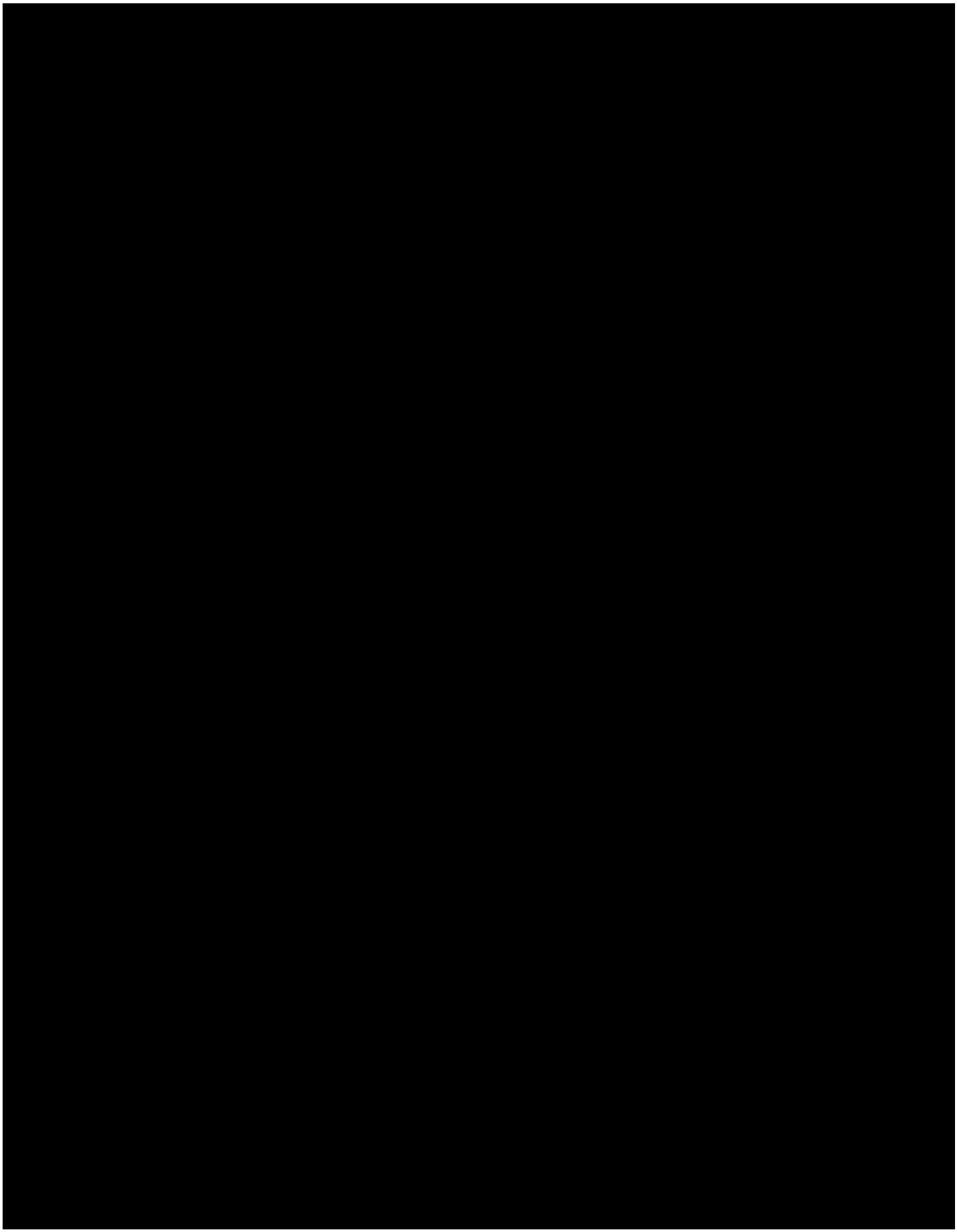
Squamous cell carcinoma of the lung arises from preinvasive progenitors in the central airways. The archetypal model appears to be a stepwise morphological progression until there is invasion of the basement membrane. However, not every lesion appears to follow this course and many individuals can have stable disease, or indeed regress to normal epithelium. From our increased understanding of the molecular pathology it is becoming apparent that the respiratory epithelium accumulates progressive genetic and epigenetic insults in response to carcinogens. Still, little is known about how to predict those 'at risk' of progression, and it is likely that in the future molecular signatures will underpin prediction models of developing invasive lung cancer. Currently, autofluorescence bronchoscopy gives us the ability to follow the natural history of these lesions, with the prospect that detecting and treating lesions early may improve survival. However, treatment remains controversial, and radical therapies are offered to individuals with carcinoma in situ who may never develop invasive cancer. This has paved the way for the use of minimally invasive bronchoscopic treatments, which, while apparently effective, have not been tested in randomised controlled trials. In this paper we describe the known biology and natural history of preinvasive lesions and review the current treatment strategies.

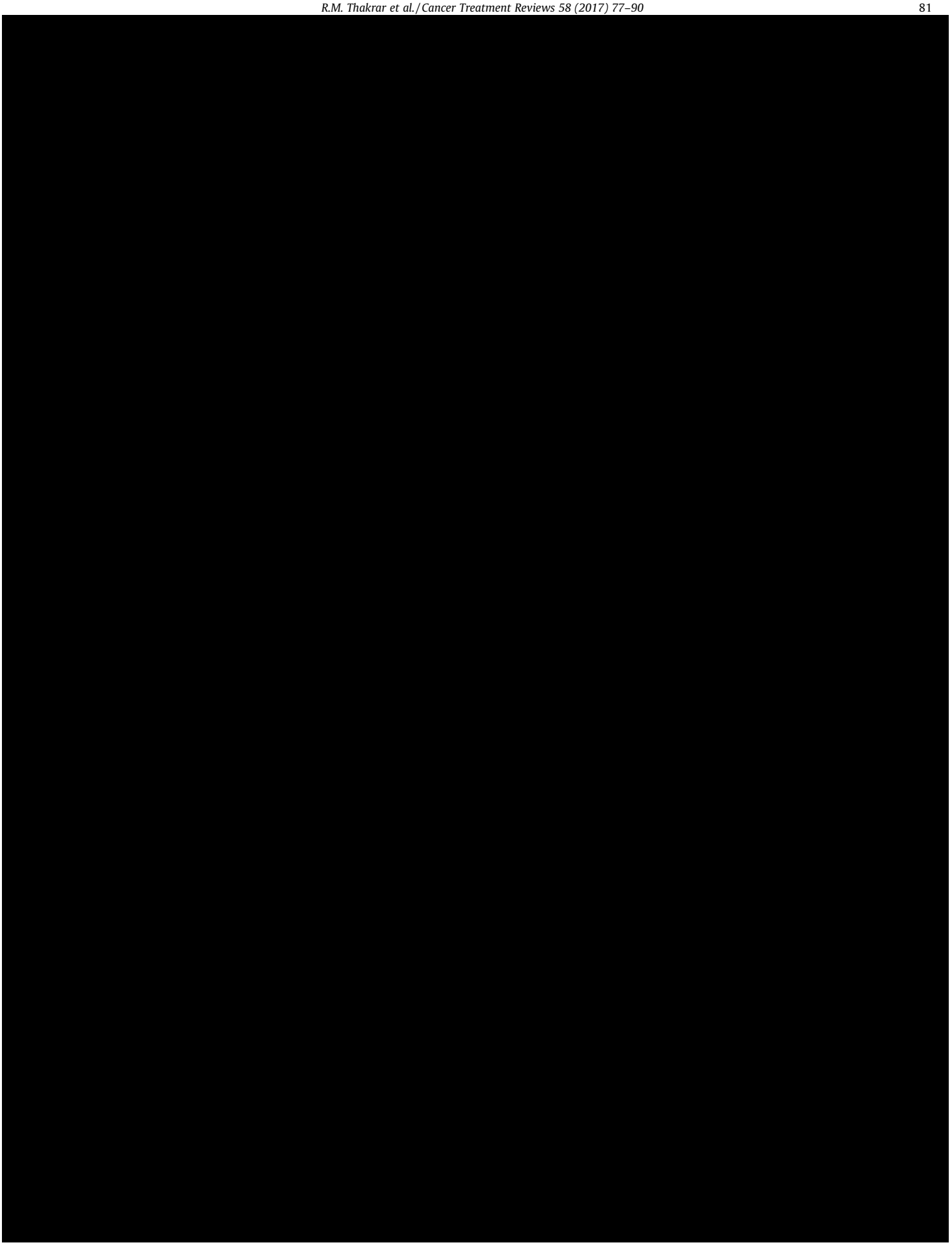
Introduction

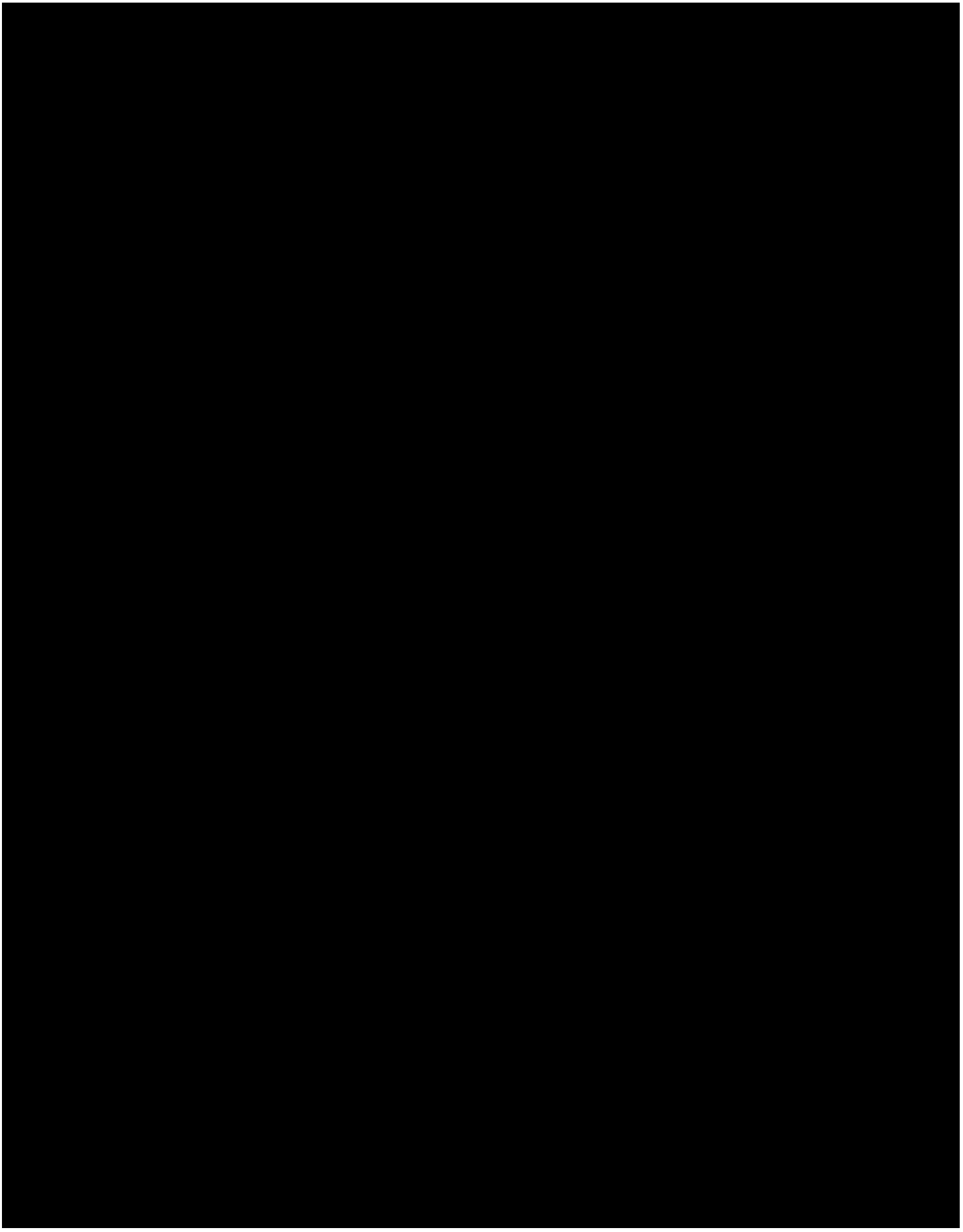
detect small central airway cancers or preinvasive airway disease. Preinvasive lesions are precursors of squamous cell carcinoma aris-

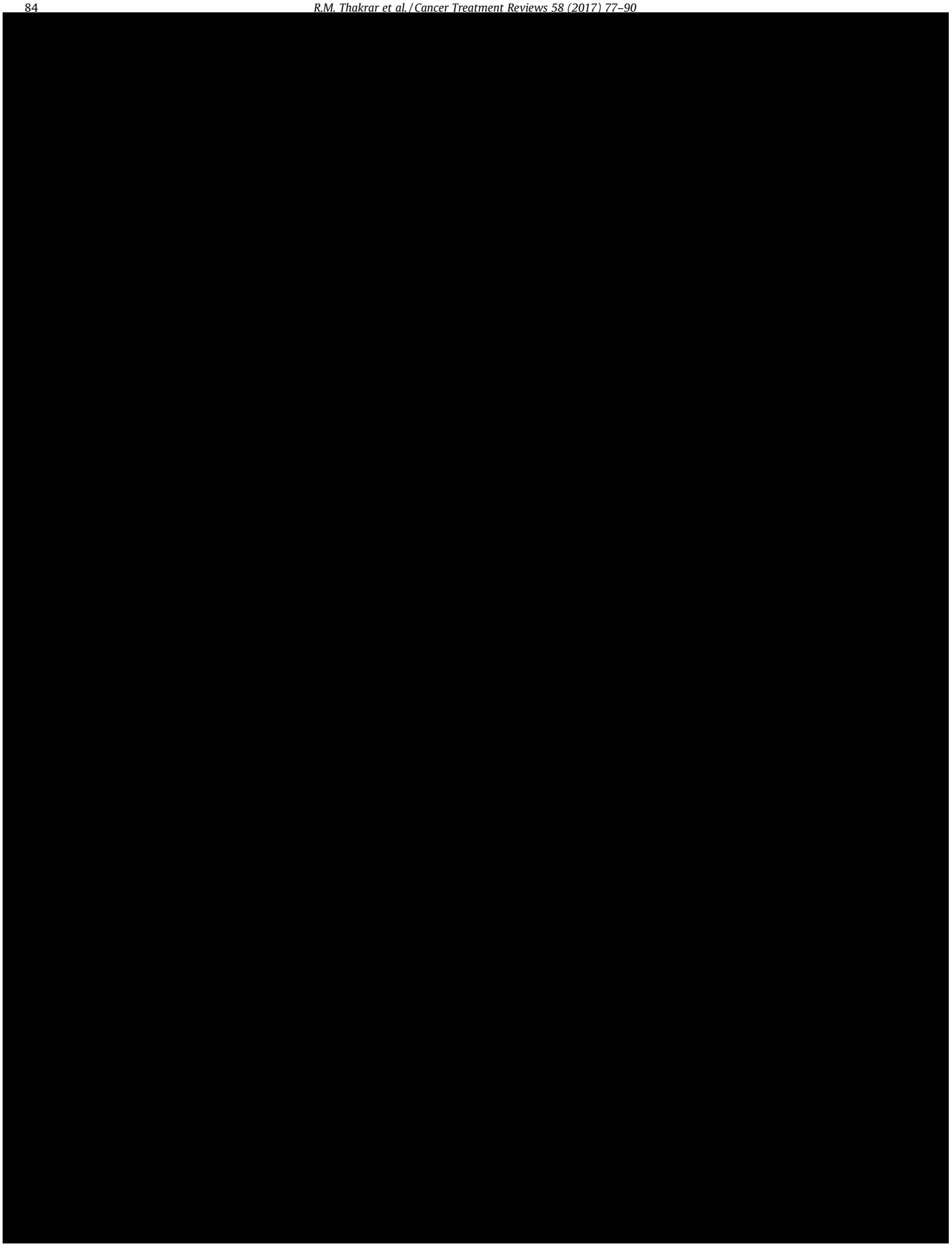
http://dx.doi.org/10.1016/j.ctrv.2017.05.009
0305-7372/

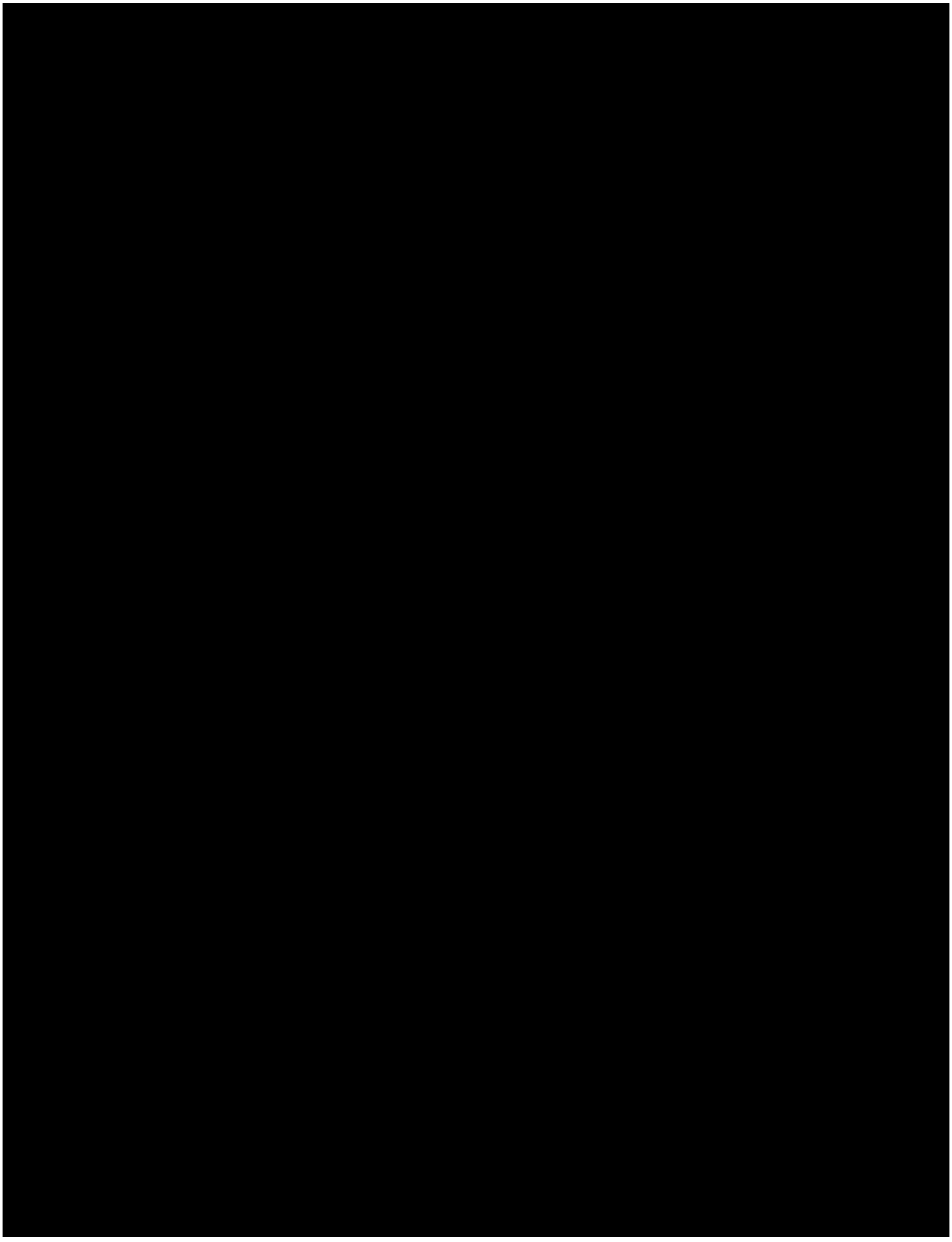


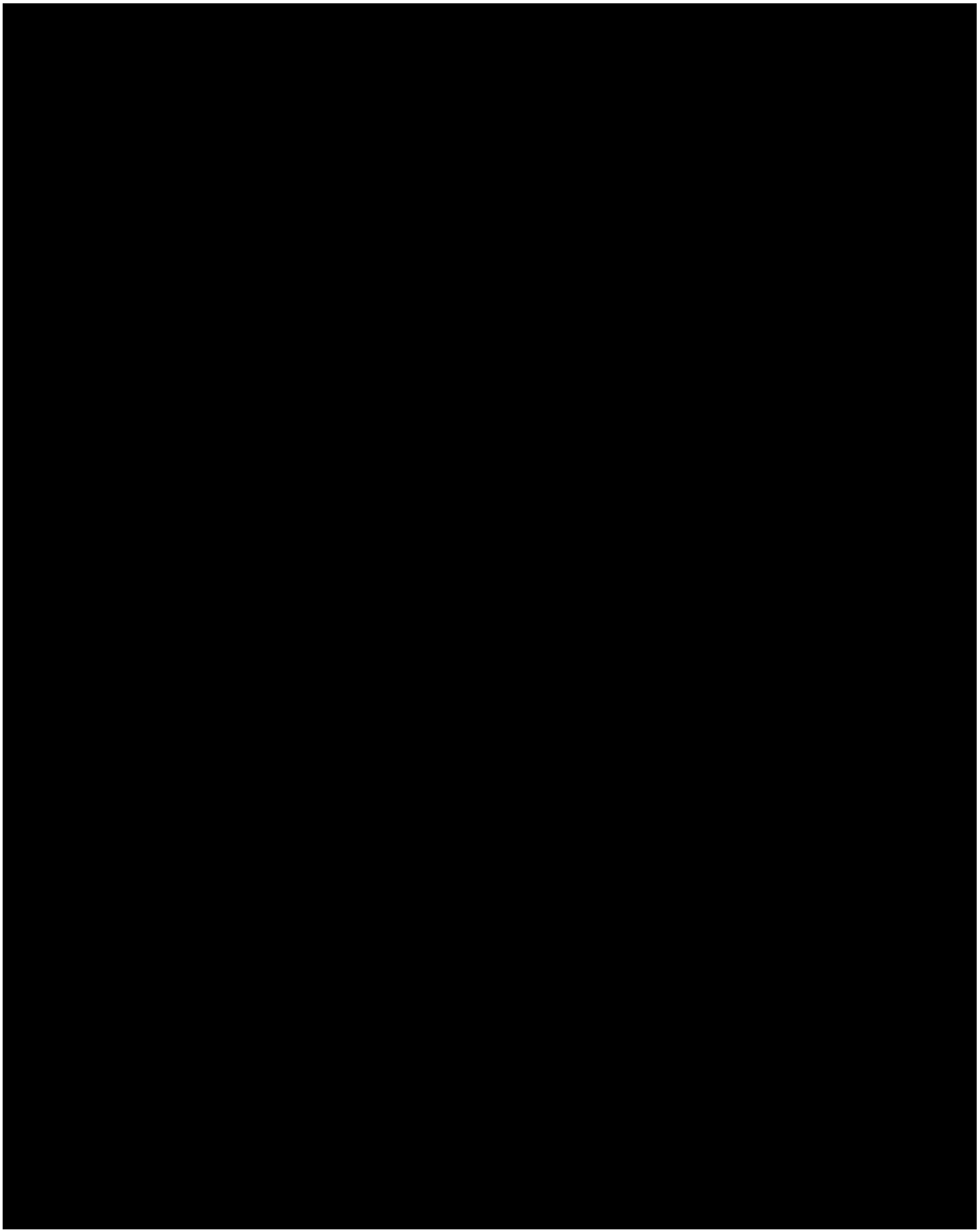


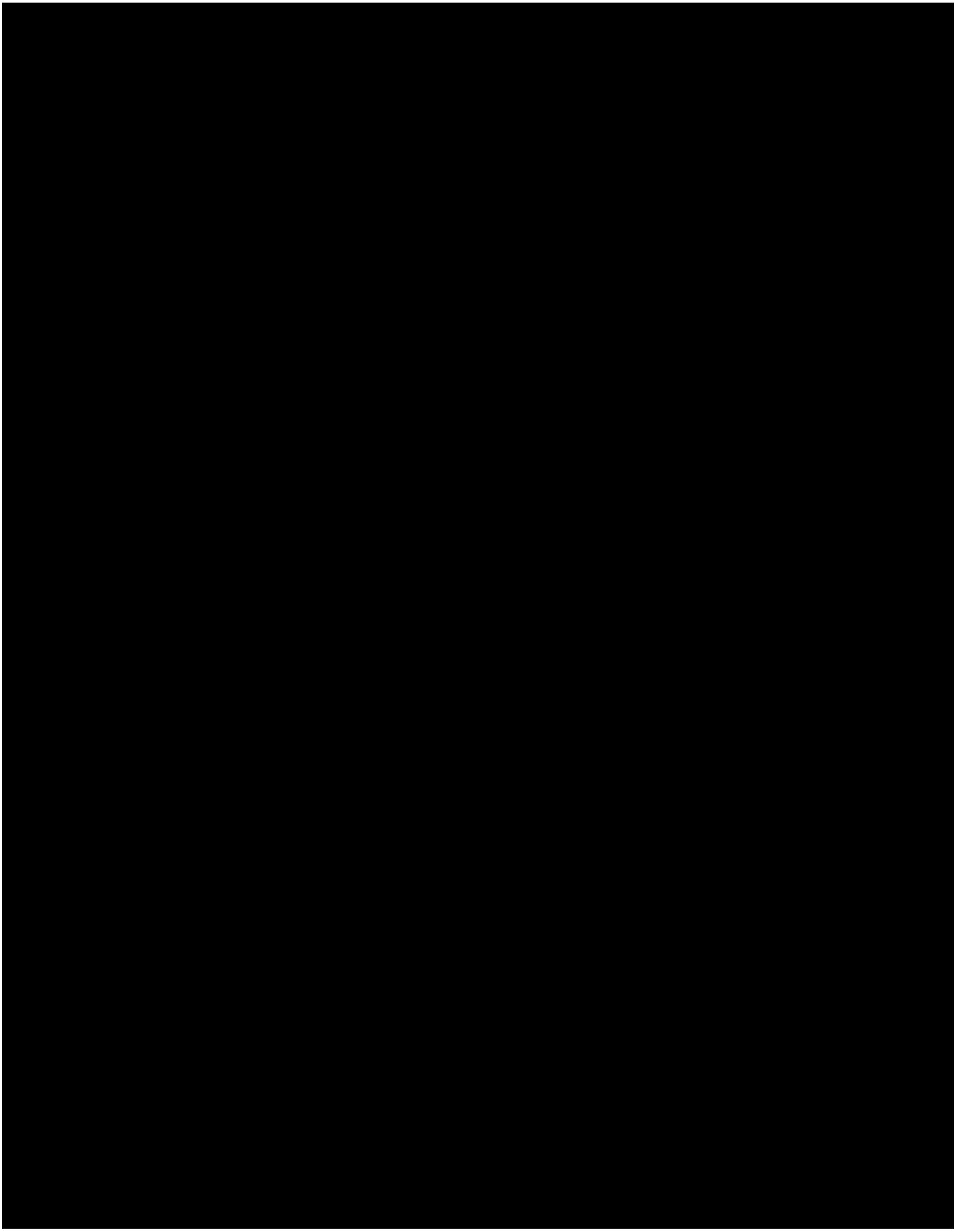


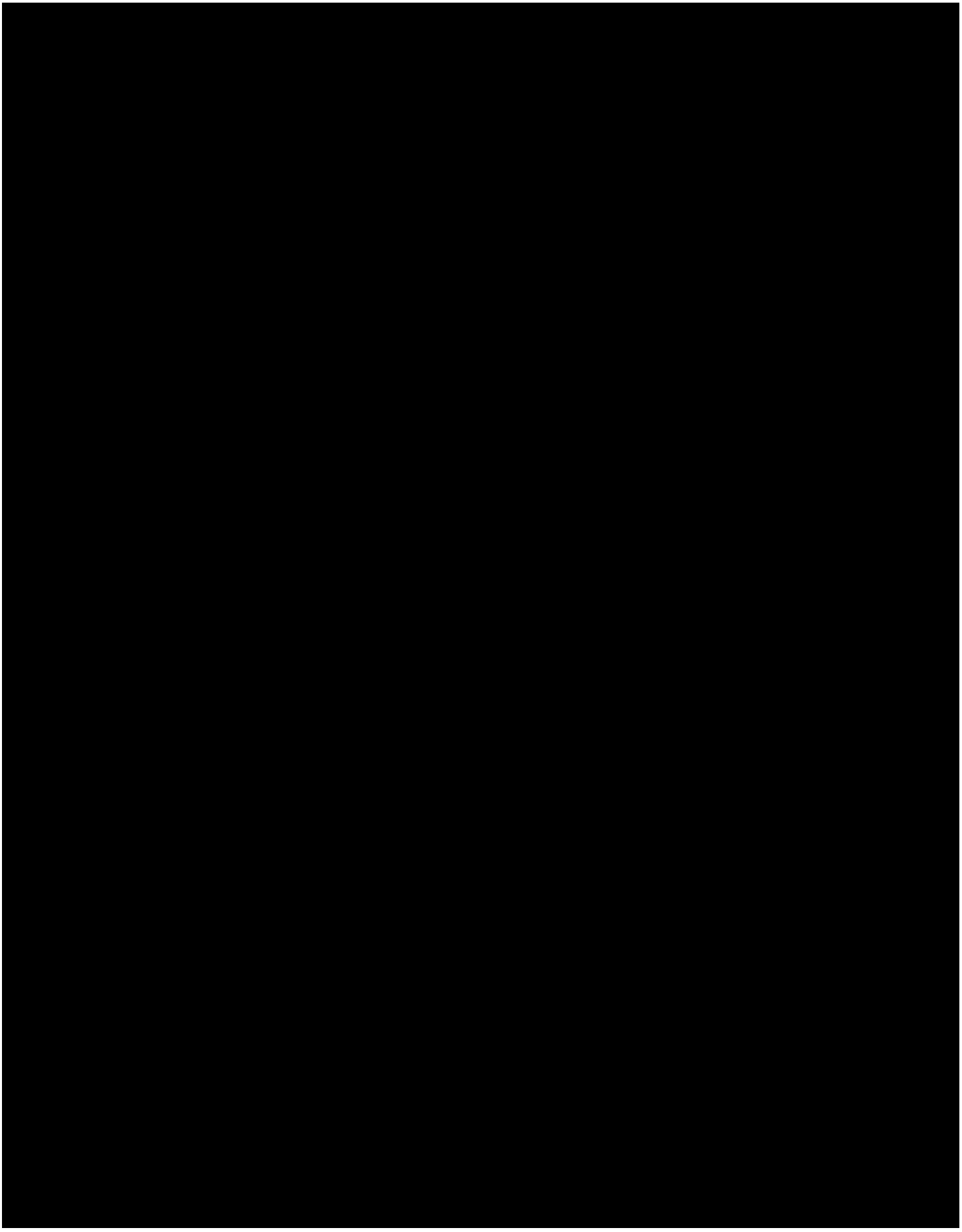


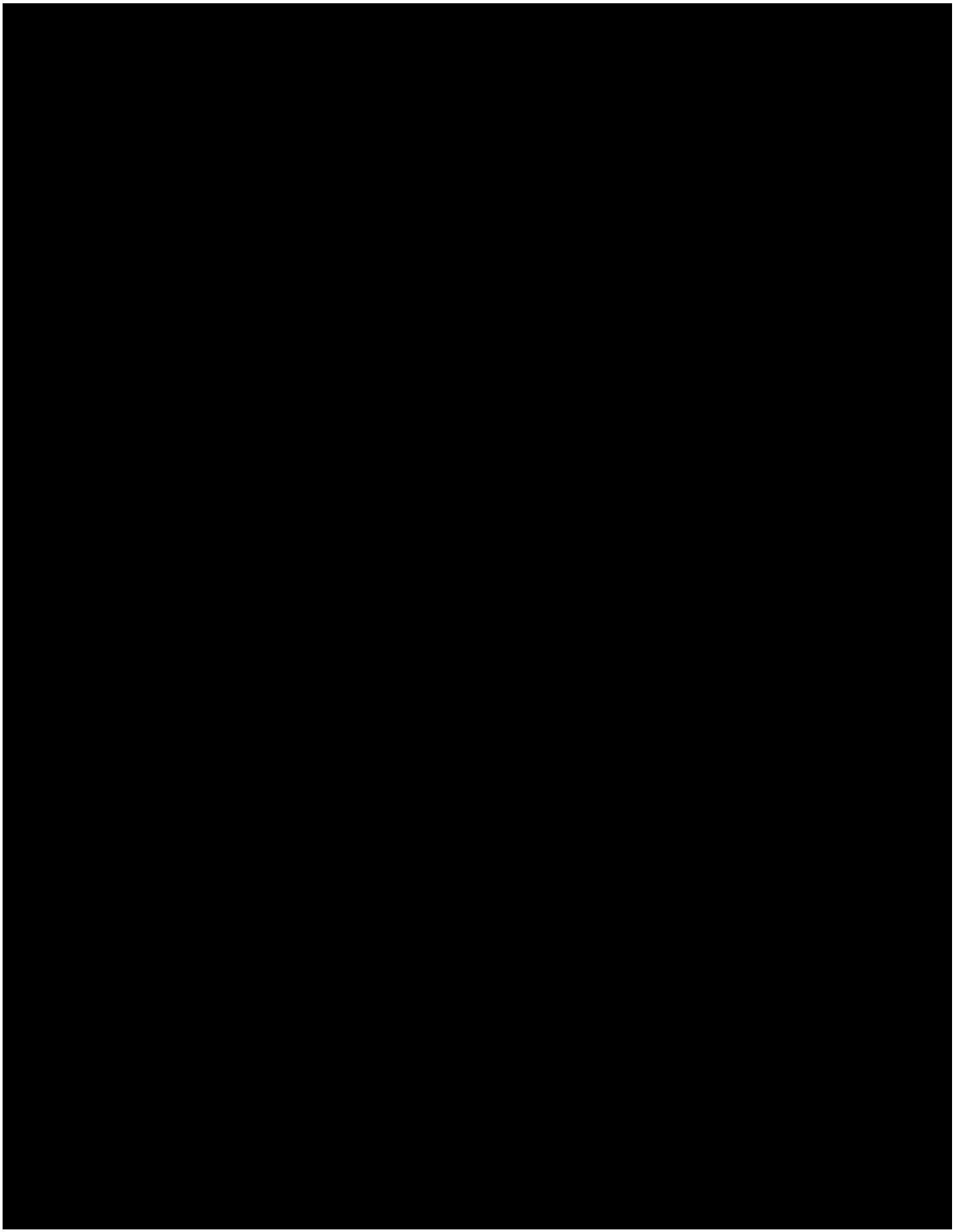












nature medicine

FOCUS | RESOURCE

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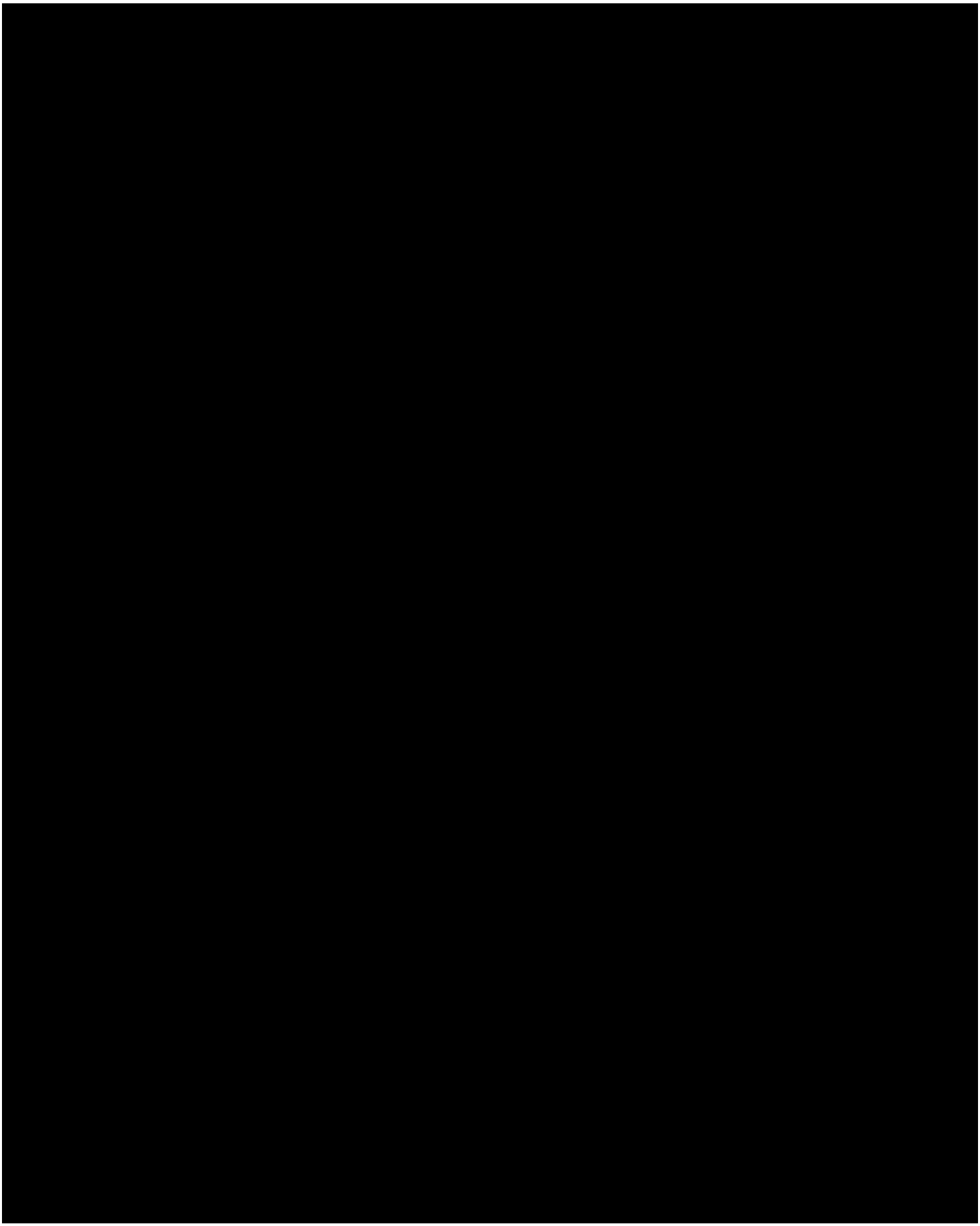
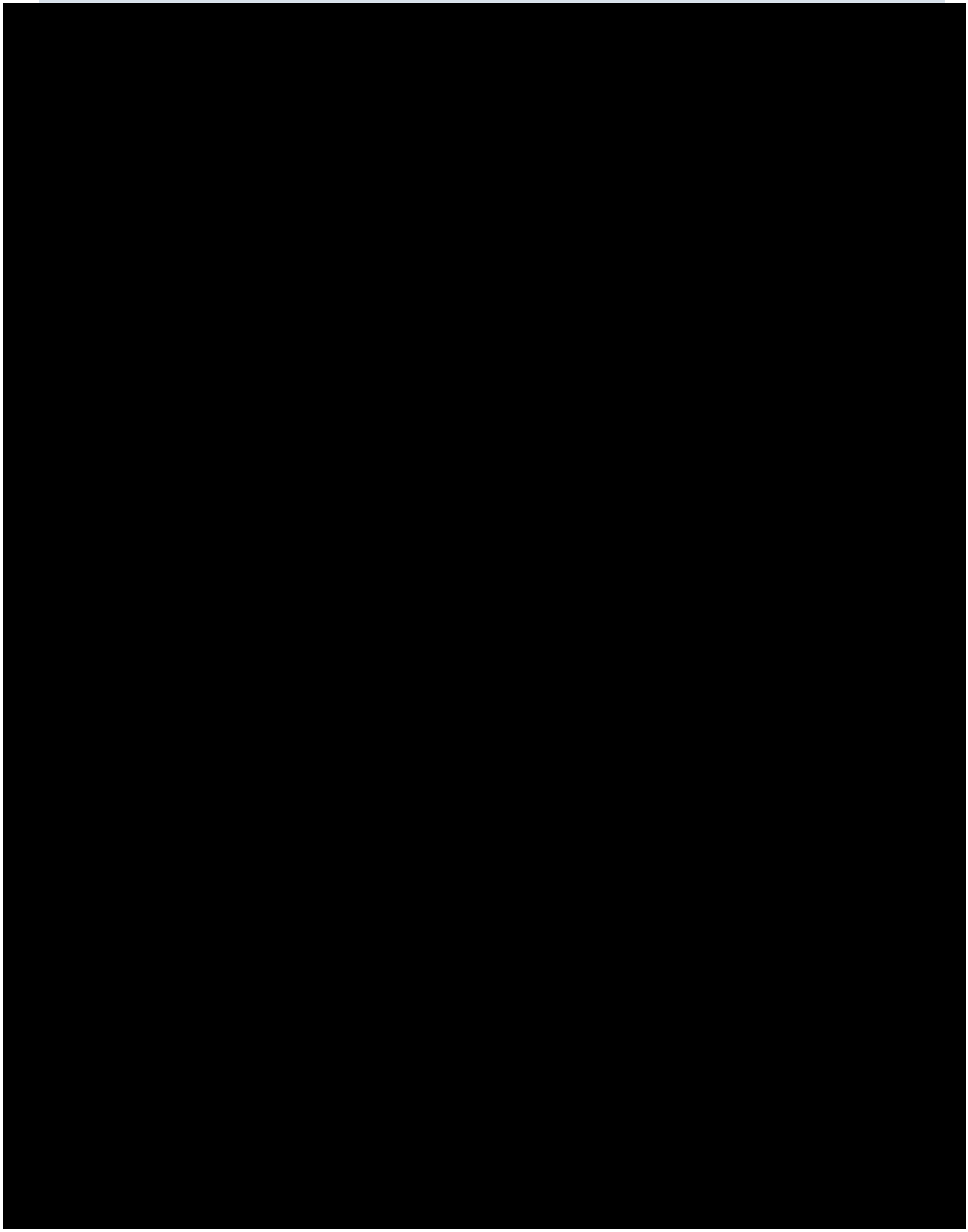
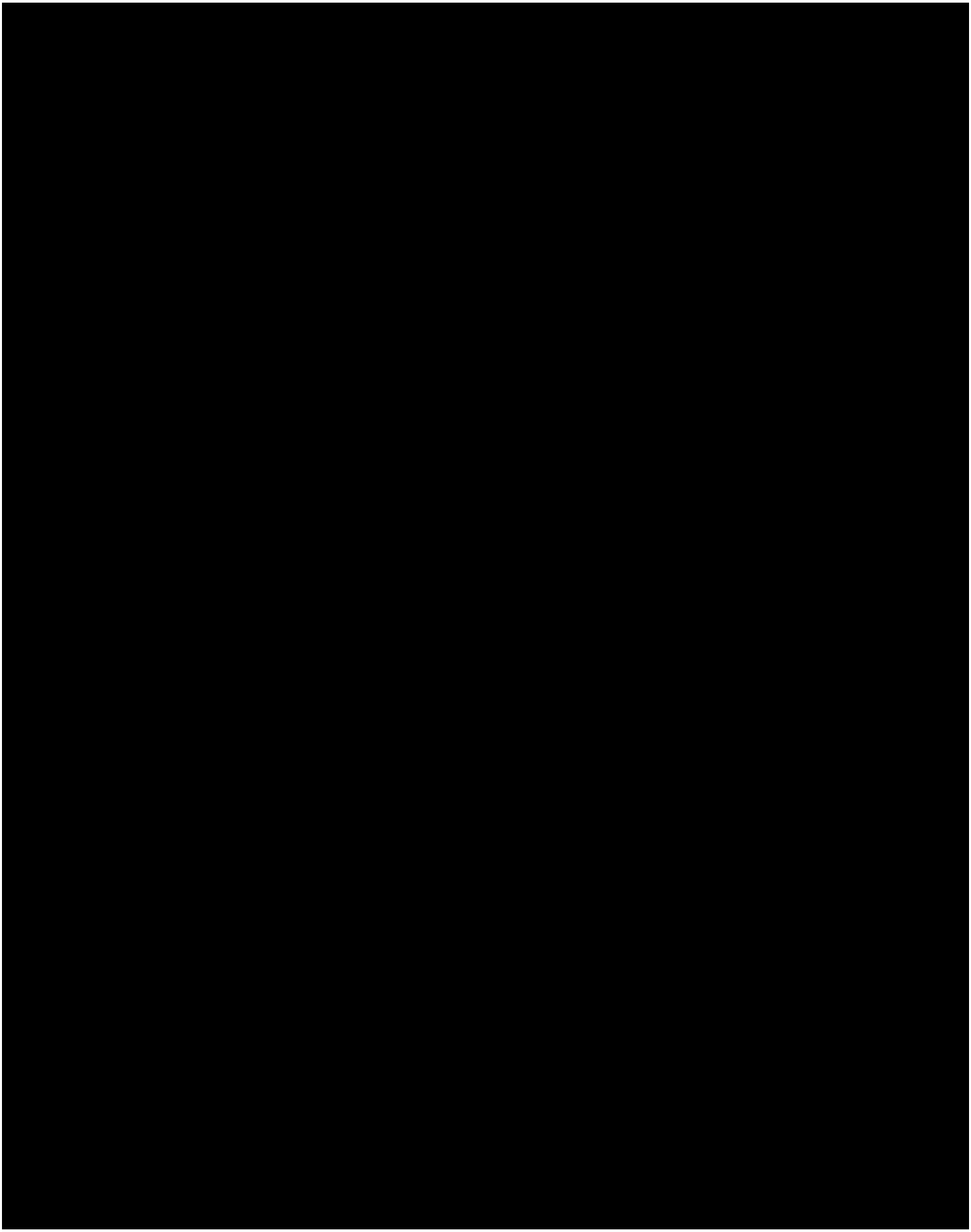
Deciphering the genomic, epigenomic, and transcriptomic landscapes of pre-invasive lung cancer lesions

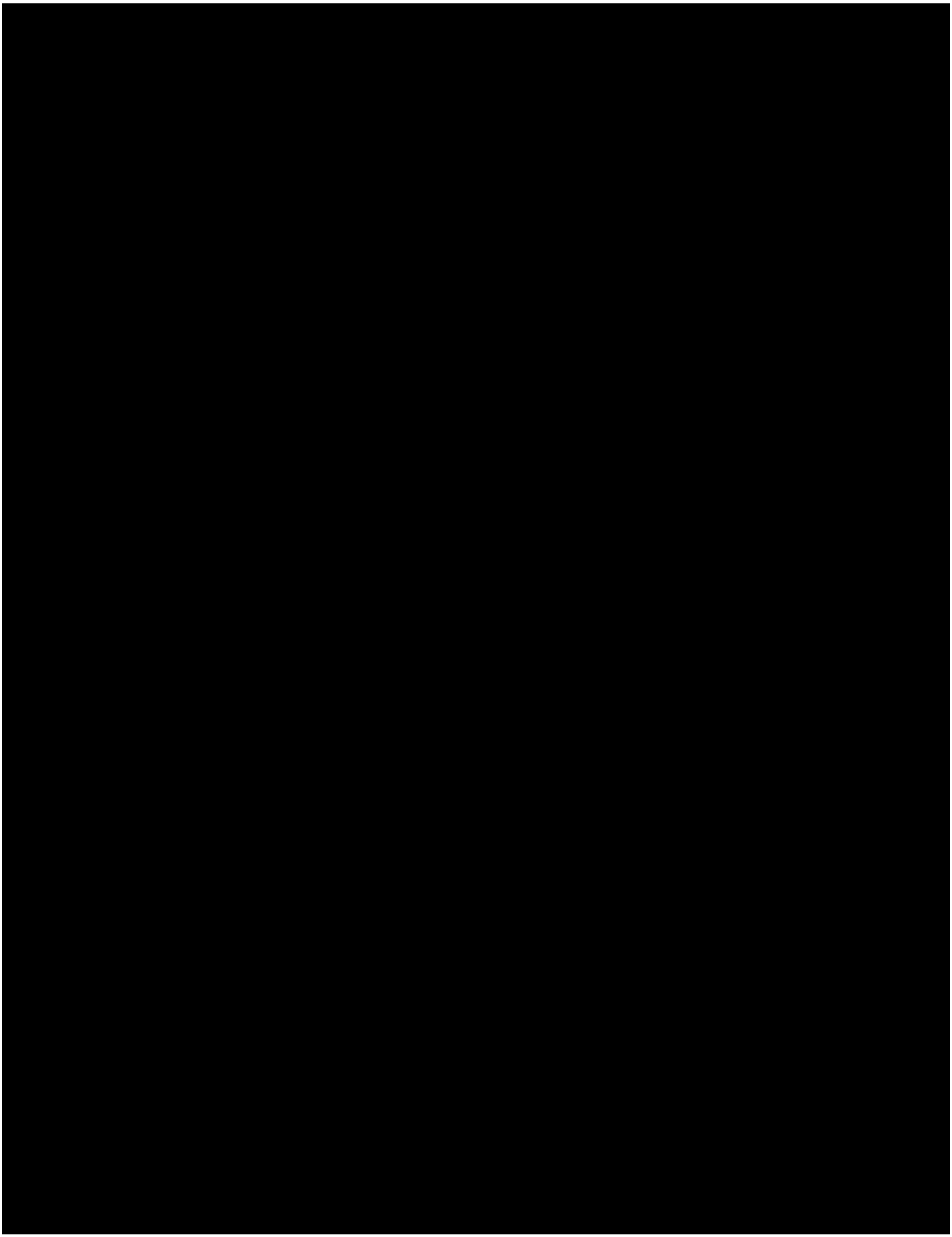
Vitor H. Teixeira[1,13], Christodoulos P. Pipinikas[1,2,13], Adam Pennycuick[1,13], Henry Lee-Six[3], Deepak Chandrasekharan[1], Jennifer Beane[4], Tiffany J. Morris[2], Anna Karpathakis[2], Andrew Feber[2], Charles E. Breeze[2], Paschalis Ntolios[1], Robert E. Hynds[1,5,6], Mary Falzon[7], Arrigo Capitanio[7], Bernadette Carroll[8], Pascal F. Durrenberger[9], Georgia Hardavella[8], James M. Brown[1], Andy G. Lynch[10,11], Henry Farmery[10], Dirk S. Paul[2], Rachel C. Chambers[9], Nicholas McGranahan[5], Neal Navani[1,8], Ricky M. Thakrar[1,8], Charles Swanton[5,6], Stephan Beck[2], Phillip Jeremy George[8], Avrum Spira[4,12], Peter J. Campbell[3], Christina Thirlwell[2] and Sam M. Janes[1,8*]

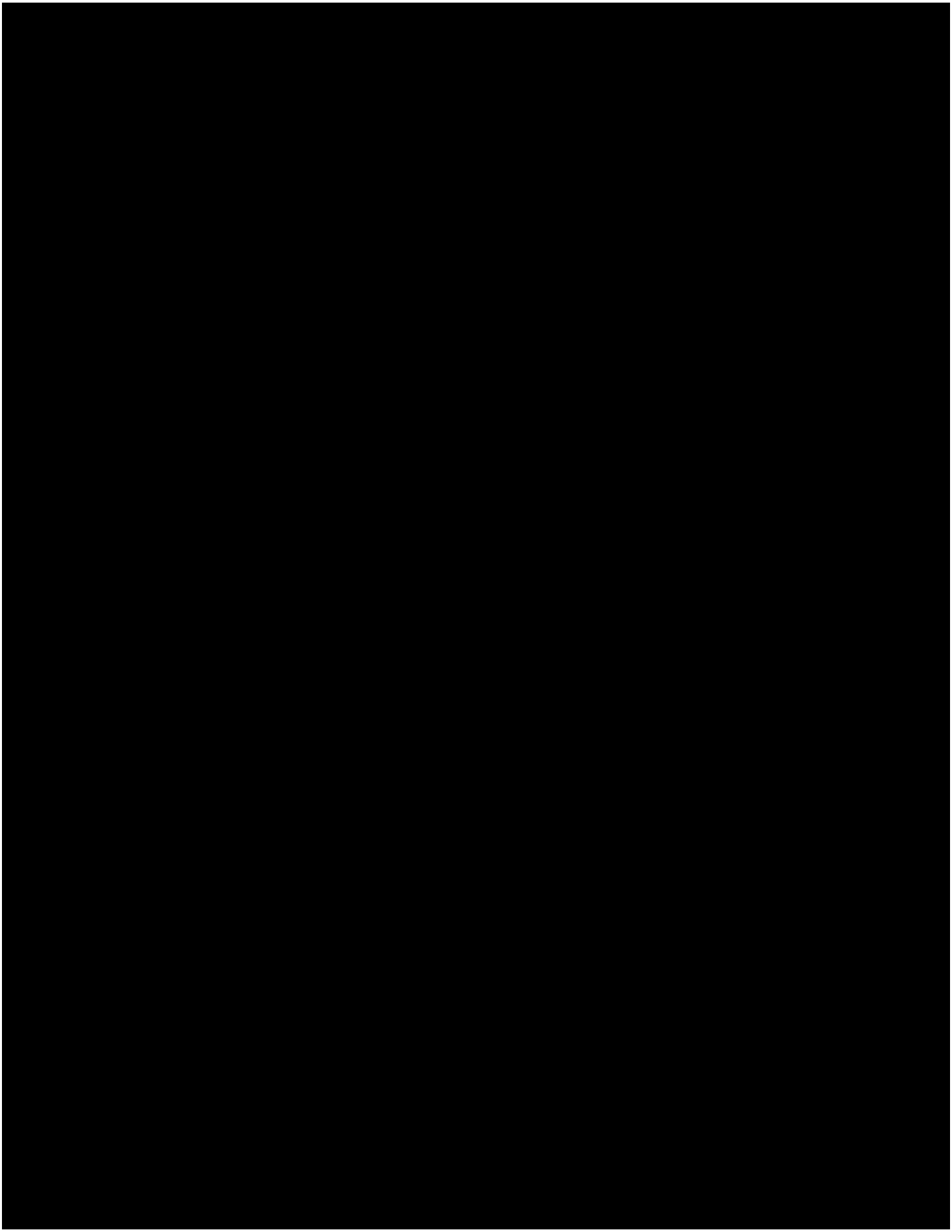
The molecular alterations that occur in cells before cancer is manifest are largely uncharted. Lung carcinoma in situ (CIS) lesions are the pre-invasive precursor to squamous cell carcinoma. Although microscopically identical, their future is in equipoise, with half progressing to invasive cancer and half regressing or remaining static. The cellular basis of this clinical observation is unknown. Here, we profile the genomic, transcriptomic, and epigenomic landscape of CIS in a unique patient cohort with longitudinally monitored pre-invasive disease. Predictive modeling identifies which lesions will progress with remarkable accuracy. We identify progression-specific methylation changes on a background of widespread heterogeneity, alongside a strong chromosomal instability signature. We observed mutations and copy number changes characteristic of cancer and chart their emergence, offering a window into early carcinogenesis. We anticipate that this new understanding of cancer precursor biology will improve early detection, reduce overtreatment, and foster preventative therapies targeting early clonal events in lung cancer.

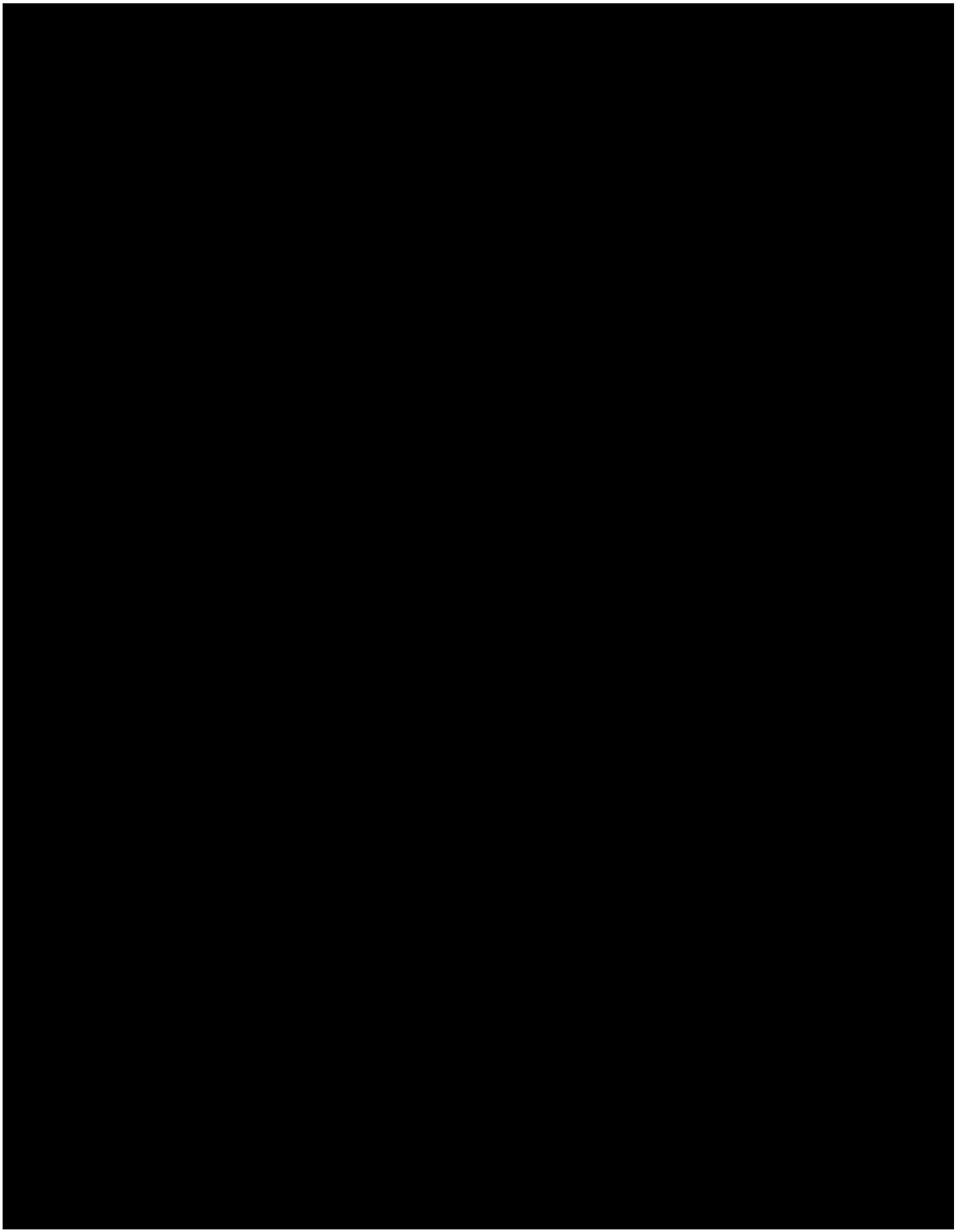


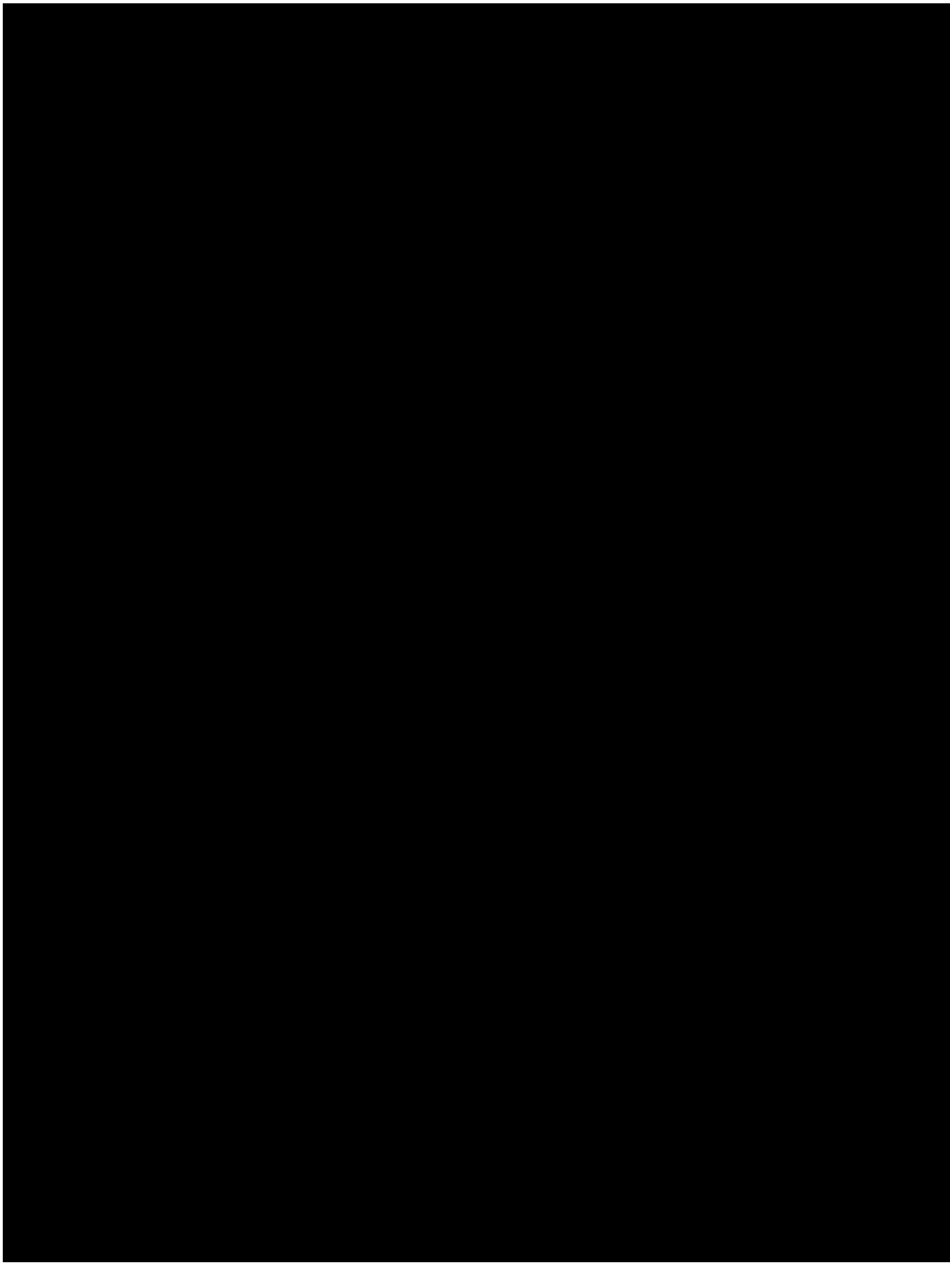












Article

Tobacco smoking and somatic mutations in human bronchial epithelium

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Tobacco smoking causes lung cancer[1–3], a process that is driven by more than 60 carcinogens in cigarette smoke that directly damage and mutate DNA[4,5]. The profound effects of tobacco on the genome of lung cancer cells are well-documented[6–10], but equivalent data for normal bronchial cells are lacking. Here we sequenced whole genomes of 632 colonies derived from single bronchial epithelial cells across 16 subjects. Tobacco smoking was the major influence on mutational burden, typically adding from 1,000 to 10,000 mutations per cell; massively increasing the variance both within and between subjects; and generating several distinct mutational signatures of substitutions and of insertions and deletions. A population of cells in individuals with a history of smoking had mutational burdens that were equivalent to those expected for people who had never smoked: these cells had less damage from tobacco-specific mutational processes, were fourfold more frequent in ex-smokers than current smokers and had considerably longer telomeres than their more-mutated counterparts. Driver mutations increased in frequency with age, affecting 4–14% of cells in middle-aged subjects who had never smoked. In current smokers, at least 25% of cells carried driver mutations and 0–6% of cells had two or even three drivers. Thus, tobacco smoking increases mutational burden, cell-to-cell heterogeneity and driver mutations, but quitting promotes replenishment of the bronchial epithelium from mitotically quiescent cells that have avoided tobacco mutagenesis.