Preinvasive disease of the airway

Ricky M Thakrar[1, 2], Adam Pennycuick[1], Elaine Borg[3], Sam M Janes[1, 2 *]

[1] Lungs for Living Research Centre, UCL Respiratory, University College London, Rayne Institute, London, 5 University Street, London, WC1E 6JF, UK.
[2] Department of Thoracic Medicine, University College London Hospital, 235 Euston Road, London, NW1 2BU, UK
[3] Department of Pathology, University College London Hospitals NHS Trust, Rockefeller Building, University Street, London, WC1E 6JJ, UK.

***Corresponding Author:**

Sam M Janes
Address: Lungs for Living Research Centre, UCL Respiratory, University College London, 5 University Street, London, WC1E 6JF, UK.
Phone: +44 (0) 207 679 6926 **Fax:** +44 (0) 207 679 6973
E-mail: s.janes@ucl.ac.uk

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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, 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.

Keywords
Preinvasive disease, squamous cell carcinoma, lung cancer, carcinoma in situ, autofluorescence bronchoscopy, bronchoscopic treatment

1. Introduction

Lung cancer is the leading cause of cancer-related death. It accounts for nearly 1.4 million deaths worldwide every year, with a five-year survival rate of just 6%. 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 preinvasive 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 preinvasive airway disease. Preinvasive lesions are precursors of squamous cell carcinoma arising in the bronchial epithelium where the basement membrane remains intact [10]. They are readily accessible to bronchoscopic assessment and the development of autofluorescence bronchoscopy has provided a sensitive way of detecting these lesions in the airway [11].

Early detection and treatment of these lesions is critical to improving survival. Since these 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 preinvasive 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. Therefore, tissue-sparing bronchoscopic therapies such as photodynamic therapy (PDT) and other ablative techniques have been used to treat these lesions. An improved understanding of the natural history of preinvasive disease will however be crucial for effective risk stratification and patient selection.

In this review we describe the natural history of preinvasive disease of the airway and its detection. We review the current understanding of molecular changes in preinvasive disease and their role as predictive biomarkers. Finally, we look at treatment approaches to preinvasive disease and current studies that are underway looking at the prevention of lung cancer.

2. Characteristics of preinvasive lung cancer

Qu. What pathological changes define pre-cancerous lesions of the airway?
Qu. How are preinvasive lesions classified?

Preinvasive bronchial epithelial 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 [12]. 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 in 1999 [10]. These were later revised in 2004, where the various grades of dysplasia and CIS were more clearly distinguished [13]. The pathological grades are summarised as per the latest edition in both figure and table 1 [14]. 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 maturation is absent. 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 [15]. 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 preinvasive lesions, even amongst experienced pulmonary pathologists [16,17]. 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 [18–20] 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.

[Insert Figure 1 near here]
[Insert Table 1 near here]

3. Diagnosis and screening for preinvasive lesions and early cancers in the central airway

3.1 Sputum cytology

Qu. Can sputum cytology be useful in detection and screening of preinvasive lesions?

The potential of sputum cytology as a non-invasive test for lung cancer was first raised by the longitudinal studies of Saccomanno et al [21]. 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 [21]. When precursor lesions form throughout the respiratory epithelium as a consequence of carcinogenic exposure, exfoliated cells are consequently detected in the sputum. 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 [22]. Three large randomised controlled trials have evaluated screening with sputum cytology; despite increased detection of early stage lung cancers they failed to show improvement in overall survival [23–25]. However, there remains ongoing interest in this field in screening high-risk populations. 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% 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 [26]. Ongoing screening trials in these high-risk subjects have combined sputum cytometry with bronchoscopy examination and are soon to report their findings [27].

3.2 Autofluorescence Bronchoscopy

Qu. What is value of autofluorescence over white light bronchoscopy?

The precise localisation of microinvasive carcinomas and preinvasive lesions is difficult as they are not easily visualized with conventional white light bronchoscopy (WLB) [28]. Autofluorescence bronchoscopy (AFB) improves visibility of these lesions by exploiting differences in the fluorescence and light absorption properties of normal and abnormal bronchial epithelium [29]. As preinvasive 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 [30]. Optical systems are designed to detect a combination of these fluorescence and reflectance changes from the airway epithelium (figure 2). The most well known device is the LIFE (Lung Imaging Fluorescence Endoscopy, Xillix Technologies) system, designed by Lam et al in Vancouver [31]. This system uses optical filters and was originally designed for use with fibreoptic bronchoscopes. The Pentax SAFE-3000 system (Pentax Corp., Tokyo, Japan) [32] and the Olympus autofluorescence, AFI-Lucera (Olympus, Tokyo, Japan) [33] are based on similar principles and are configured to work with video bronchoscopes.

The development of autofluorescence bronchoscopy has led to a significant increase in diagnostic sensitivity for detecting preinvasive disease, demonstrating a 1.4–6.3 fold improvement in detection over white-light bronchoscopy (WLB) alone [31–40]. A recent meta-analysis reported a pooled sensitivity for detection of preinvasive disease of 85% for WLB with AFB, compared to 43% using WLB alone (relative sensitivity 2.04, 95% CI 1.56-11.55) [41]. However, AFB can detect a large number of false positive lesions, which maybe inflammatory making its specificity poor [34–36]. The same analysis by Sun et al reported a pooled specificity of 61% [41]. However, recently there has been research into improving AFB specificity using a quantitative scoring system based on red-green reflectance [42], or by combining AFB with narrow-band imaging (NBI) [43], which may in the future reduce the number of sites sampled during a procedure.

AFB has been used to assess prevalence of preinvasive 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 [44]. 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 preinvasive 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 [45]. Further data comes from groups evaluating patients with positive sputum cytology [28,35,40,46], and AFB in this patient cohort can identify a large number of preinvasive and invasive lesions. Chhajed et al looked at 151 patients at a high risk of lung cancer with moderate dysplasia or worse identified on sputum cytology mass screening. Of 343 lesions, WLB & AFB showed mild, moderate or severe dysplasia and CIS in 14%, 15%, 2%, and 1% of the lesions detected, respectively [28]. 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.

With the increasing prevalence of lung adenocarcinoma, the advent of CT screening is set to improve early detection of lung cancer. However, screening for lung cancer with low dose computerized tomography (CT) is often unable to detect occult airway lesions that would be otherwise have been detectable by AFB. Tremblay et al set out to answer this question, utilizing AFB in the setting of CT screening of lung cancer in their Pan-Canadian Early detection of Lung Cancer Study. The group demonstrated, the addition of AFB to CT screening detected an additional occult carcinoma in 0.15% (95% CI, 0.0-0.6%) of cases, making its use in a screening program difficult to justify [47].

[Insert Figure 2 near here]

3.3 Narrow band imaging

Qu. Does narrow band imaging have a role in detection of preinvasive disease?

NBI (Olympus Optical Co., Japan) is an optical imaging technology designed to improve visualization of microvascular structures in the mucosal and submucosal layers [48]. 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 [49]. It is potentially useful for detection of preinvasive disease as angiogenesis has been shown to occur early on in these lesions [50]. Only one study has directly compared NBI and AFB prospectively in preinvasive disease [43]. 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.

3.4 Optical Coherence Tomography

Qu. Can OCT be used as an adjunct to AFB?

Optical coherence tomography (OCT) is an optical imaging technique that employs near infra-red light to visualize 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 [51–53]. 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 microinvasive carcinoma [51,54]. Although not currently employed in routine clinical practice, OCT may have a role as an adjunct to AFB, particular in confirming whether there is breach of the basement membrane in cases with equivocal pathology.

3.5 Other imaging techniques

Qu. Which other imaging techniques can distinguish preinvasive vs invasive disease?

3.5.1 Endobronchial ultrasound (EBUS) advances have enabled miniaturization of a probe that passes down the working channel of a bronchoscope and houses a 20- or 30-MHz rotating transducer, providing 360° images of the airway wall and surrounding structures. Kurimoto et al performed the seminal study comparing the ultrasound and histopathological findings of cancers in the airway [55]. They showed that radial EBUS was able to accurately predict the depth of invasion in 23 of 24 cases. Other investigators have since shown radial EBUS to be accurate in detecting tumour invasion of the basement membrane [56]. EBUS is more sensitive and specific than CT in assessing depth of tumour invasion [57], and more specific than AFB alone for predicting invasion in AFB-positive lesions [58].

3.5.2 Computerised tomography (CT) and positron emission tomography (PET) have a role in detection of parenchymal lesions, staging, and identification of metastatic disease. A high-grade preinvasive 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 [18,19]. 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 [59]. PET remains in its infancy in assessment of preinvasive disease, however a pilot study of 20 lesions certainly looked promising [60]. 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 [61]. 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.

4. The natural history of preinvasive lesions

Qu. What is the natural history of low-grade vs high-grade preinvasive disease?
Qu. Is there evidence of a 'field-change' effect on the epithelium?

Longitudinal studies using AFB-guided biopsy of lesions in the central airways have provided some insight into how preinvasive disease behaves over time [18–20,62–66]. However, developing statistical models of which lesions progress or regress is not straightforward, especially in severe dysplasia and CIS [67]. There are problems with intra- and inter-observer variability in clearly defining the severity of the dysplasias [16,17], interpreted on small bronchoscopic mucosal biopsies. Comparison of specific studies is also difficult [45,67,68]. Most studies enroll 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 it as progression to severe dysplasia, CIS or invasion, or combine the outcomes [69]. Finally, with the exception one study [18], CIS is often treated with concerns 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, summarised in table 2.

Progression rates to invasive carcinoma can vary depending on the initial grade of lesion [19,20,63] and it is generally accepted that high-grade lesions are more likely to progress to invasive cancer than low-grade lesions [18–20,45,63,69]. 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 [18]. 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%) [63]. Similarly, other authors have reported low rates of progression of low-grade lesions to invasive cancer [20,64,70]. 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 [19]. 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, authors have described progression of CIS to invasion as high as 43%–87% [20,62,65,66,71]. However, these many lesions were assessed at short time intervals, and the persistence of stable CIS or invasion were both considered 'progression' and subsequently treated [20,62,65,71], 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 [18]. Interestingly, this is a similar incidence of progression to cancer as seen in treated high-grade lesions [19,71].

Importantly, the data from these studies show the presence and development of synchronous and metachronous lesions, both preinvasive and of lung cancer [18,19,65,70,72]. Van Boerdonk and colleagues performed a longitudinal study with AFB and CT in 164 patients (80 with high-grade lesions) [19]. They detected 61 cancers in 55 patients with a median time-to-event of 16.5months. 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 preinvasive lesions is similar to that seen by other authors [18,70,73]. 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 [18]. These studies support the theory of 'field carcinogenesis', and suggest preinvasive 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 progenitor will progress to invasive disease will determine whether intervention is necessary.

[Insert Table 2 near here]

5. Molecular hallmarks of preinvasive airway lesions

Qu. Which molecular changes occur early in the pathogenesis of squamous cell carcinoma?

The paradigm of preinvasive disease undergoing step-wise progression in severity likely results from the accumulation of genetic and epigenetic insults. Ongoing investigation has demonstrated a sequence of changes occurs as seen in other epithelial cancers, which may aid the discovery of potential molecular biomarkers. Hanahan & Weinberg initially described six hallmarks of development of cancer, updating these principles again in 2011 [74]. Gazdar et al then presented how the pre-cancerous changes of non-small cell lung cancer encompass these hallmarks [75]. We similarly discuss the molecular changes that occur in preinvasive disease of the central airway.

5.1 Growth signaling

Oncogene mutation leads to dysregulation of cell proliferation signals; a mechanism well described in lung cancer. Oncogenic mutations or gains have been described in preinvasive disease [76–78]. Franklin et al investigated chromosomal aneusomy in preinvasive disease using 4 FISH probes. They included common oncogenes detecting chromosome 6 centromere, 5p15.2, 7p12 (epidermal growth factor receptor), and 8q24 (MYC) sequences, in cases of dysplasia and CIS [76]. Chromosomal aneusomy (2/4 FISH probes) was found 41.8% of cases and the proportion increased from moderate- (22.2%) and severe- (41.7%) dysplasia to CIS (75%). In a cohort of patients, McCaughan et al also showed amplification of 3q region is consistently observed in high-grade, but not low-grade preinvasive lesions. The group further showed the focus of this amplification is likely SOX-2, a transcription factor essential for maintaining self-renewal, or pluripotency, of undifferentiated embryonic stem cells [77]. While all these were in small numbers of individuals, the detection of gain at 3q in low grade lesions by other authors [79,80], show that this genomic aberration seems to be critical for disease progression and may imply that an incremental amplification of 3q promotes malignancy.

5.2 Evading growth suppression

Lung cancer harbors distinct genetic changes that enable it to circumvent the processes that regulate proliferation, normally governed by tumour suppressor genes (TSGs) [81]. Loss of heterozygosity (LOH), where there is chromosomal or allelic alteration, is one such cause widely described in preinvasive lung cancer [82–88]. Allelic losses at chromosome 3p have been shown to occur most frequently, and have even been detected in histologically normal epithelium in smokers [88–90]. LOH at 3p occurs more frequently, and demonstrates progressive chromosome alteration with advancing grade of preinvasive lesion [83,88]. In contrast, LOH at 17p13 (P53), 9p21 ($p16^{INK4}$), and 5q are seen infrequently in early preinvasive lesions, but occur more often in cases of high-grade preinvasive and invasive lesions [85,87,90]. Promoter hypermethylation, an epigenetic process, is also able to silence the expression of TSGs in lung cancer. In a cohort of 70 preinvasive lesions, Lamy et al showed aberrant methylation of p16INK4, a retinoblastoma regulating protein, occurred in 19% of lesions, with the frequency of silencing increasing with the histologic grade of the lesion [91]. Hypermethylation of p16 can also be detected in exfoliated cells of smokers [92] and it has been suggested that this process may occur early in preinvasive disease [75].

5.3 Resisting cell death

The equilibrium of anti- (Bcl2) and pro- (Bax) apoptotic factors in cells determines susceptibility to cellular death [93]. The overexpression of Bcl2 has been demonstrated in lung cancer [94] and dysregulation of tumour-suppressor genes will affect Bax transcription [95]. Jeanmart et al assessed

these in preinvasive lesions, showing the ratio of Bcl2:Bax was increasingly dysregulated with severity of the preinvasive lesion, in favour of overexpressed Bcl2 [96]. Given the disparity of expression between preinvasive lesions and invasive lung cancer, it has been proposed that the Bcl2:Bax ratio may play a key role in clonal selection of preinvasive lesions [97].

5.4 Immortalisation

A telomere is a nucleotide sequence at each end of a chromosome and it is truncated during cellular division, thus regulating cellular senescence. Telomerase controls this process and is dysregulated in lung cancer, conferring cellular immortality [98]. In preinvasive disease, Lantuejoul et al demonstrated increasing telomerase expression from normal epithelium to CIS in a cohort of 106 lesions [99]. They also observed correlation of telomerase expression with p53 and Bcl:Bax ratio, conferring proliferation and resistance to apoptosis. These data suggest that immortalisation occurs as an early process in preinvasive disease.

5.5 Angiogenesis

Lung cancer is inherently reliant on forming new vasculature in order to survive and grow. This process is commonly driven by the expression of vascular endothelial growth factor (VEGF) [100]. To determine whether this process occurs in preinvasive disease, Lantuejoul et al measured expression of VEGF and their receptors (NP1 & NP2) in 50 lesions. They demonstrated that VEGF expression increases from low-grade to high-grade lesions, correlating with overexpression of its receptors [101].

5.6 Invasion & migration

Invasion and metastasis are not features of preinvasive disease. However, investigation into the 'field cancerisation' theory [102] has shed new light onto how migration may be responsible for the presence of multi-focal preinvasive histological changes. It has previously been proposed that the precancerous field is monoclonal in origin [103,104]. 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 [105]. By detecting a rare somatic TP53 mutation we demonstrated multi-focal high grade lesions were derived from a common clonal ancestor; and since neighboring mucosa was normal (p53-wild type), propose that clonal migration occurs across the airway epithelium. We have also identified β-catenin signaling as a possible mechanism of reduced intracellular adhesion and cellular migration [106].

The presence of these hallmarks puts together a picture of SQCC pathogenesis of the lung. Hanahan et al described genetic instability and mutation as a tumour characteristic, which as already described is the cornerstone of preinvasive disease, enabling acquisition of the core hallmarks. Certainly, a succession of genetic changes is likely needed before a select subclone develops the characteristics that enable morphological progression. Further work will shed light as to whether preinvasive lesions exhibit the 'emerging hallmarks', evasion of immune destruction and dysregulated cellular energetics. Little is yet known about the former, however dysregulation of cell energy is likely driven by oncogenic mutations which may explain some of the properties exhibited by these lesions, such as abnormal fluorescence and FDG avidity. A summary of molecular changes occurring in preinvasive disease are shown in figure 3.

[Insert Figure 3 near here]

6. Predictive risk factors

Qu. Are there clinical or molecular biomarkers that predict the malignant potential of preinvasive lesions?
Qu. Can these biomarkers be used as an adjunct or replace histopathological reporting of lesions?
Qu. Can these biomarkers identify individuals that need treatment?

6.1 Clinical risk factors

Clinical risk factors and risk prediction tools have been extensively studied as decision aids in management of suspicious parenchymal lesions [107,108]. Although there are no validated models for preinvasive disease there are some recognised associations. Active smoking, presence of synchronous lung cancer, number of baseline preinvasive lesions, previous head and neck cancer and exposure to carcinogens including asbestos have all been shown to be risk factors for harboring high-grade lesions in the airway [18–20,35,72,109]. 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 [72]. However, these causative risk factors such as COPD, previous head and neck or lung cancer and smoking behavior do not appear to consistently correlate with progression of preinvasive disease [20,63,65,70]. 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. Diagnosis of new severe dysplastic lesions 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 [110]. Despite the study combining the end-points it demonstrates the importance of carcinogen exposure to the 'field' in predicting lung cancer risk. Using the same principle, Pasic et al showed in a cohort of 46 individuals that the number of suspicious lesions at baseline bronchoscopy correlated with ultimate development of the invasive cancer using AFB scoring. With detection of either one, two, or three suspicious lesions on AFB, cancer developed in 8%, 50% and 100% of cases, respectively [109]. This describes a higher distribution of high-grade lesions in those individuals with multifocal airway lesions. 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. The presence of multi-focal, high-grade lesions in high-risk patients does appear to increase the overall risk of lung cancer [18,19,62], however it is likely that increased understanding of molecular alterations in these lesions will play a far more important role for risk prediction.

6.2 Molecular risk factors

Genetic & epigenetic changes are likely to long precede the morphological transformation of preinvasive lesions, with carcinogenesis ensuing following accumulation of successive molecular abnormalities, resulting in selection of clonal cells capable of invasion. 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 LOH and presence of more than one site of LOH were associated with increased risk of progression to lung cancer [71]. 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 [77]. 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 [111]. 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 [84]. 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 7 high-grade baseline lesions) and 24 cases remained 'cancer or CIS-free'[84]. 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 CNA-based classifier system as an objective determinant for progression of preinvasive disease and likely a determinant for developing cancer.

Investigators have also utilised immunohistochemistry and other methods to detect markers may predict progression of preinvasive disease. These include p53 [64,96], Ki-67 labelling index [64,112], telomerase activity [64,99], apoptotic proteins [96], C-reactive protein [113] and proteomic signatures [114]. 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 preinvasive disease appears hold far greater promise.~~

Detection of genetic alterations appear to hold the greatest promise in identifying those individuals at risk. In those individuals with preinvasive disease, a process affecting the central airways, bronchoscopy will likely continue to play a role in the early detection of cancer. However, this can come at the expense of repeated procedures and biopsies. Recently, Silvestri et al validated a novel 23 gene expression classifier in morphologically normal epithelium of the bronchial airways in 939 patients as a means of improving the diagnostic performance of bronchoscopy for lung nodules. The test has 88% sensitivity and a 94% negative predictive value for lung cancer [115]. The use of this gene classifier to help re-stratify patients with non-diagnostic bronchoscopy results into low, intermediate, and high-risk subgroups could potentially lead to the performance of fewer unnecessary and potentially harmful procedures. While the classifier had much more profound effect on the sensitivity of peripheral adenocarcinoma lesions, the classifier had a sensitivity of 90% for squamous cell carcinoma diagnoses. This concept has vast implications in the dawn of lung cancer screening, and future work in preinvasive disease is needed to see whether individuals at risk of developing cancer can be identified.

There are other novel techniques on the horizon that may become complementary to bronchoscopy or even alternative approaches for the early diagnosis of invasive cancer. Several biomarkers in the blood (free circulating nucleic acids, proteins, and circulating tumour cells) have been investigated and found to complement CT screening for lung cancer [116]. Further, the identification of volatile organic compound signatures in exhaled breath are under investigation and may become clinically useful for the early detection of cancer [117]. It is likely that a combination of bronchoscopic, histological evaluation complemented with assessment with epigenetic and genetic signatures will provide a better understanding of carcinogenesis, and form the basis of biomarkers that guide treatment decisions and indicate prognosis. However, pioneering techniques of liquid biopsy and exhaled breath analysis should be investigated in preinvasive disease as these non-invasive tests may in the future become the framework for surveillance through early detection of the 'high-risk' individual and through the early detection of lung cancer.

~~Assessment with epigenetic and genetic signatures in the future will not only provide a better understanding of carcinogenesis, but are likely to form the basis of biomarkers that guide treatment decisions and indicate prognosis.~~

7. Treatment of preinvasive lesions in the airway

Qu. Should we be treating high-grade preinvasive lesions?
Qu. Should surgical resection be considered best practice?
Qu. Which bronchoscopic treatment modalities are available?

The American College of Physicians and other authors advocate surgical treatment for CIS and early lung cancers in the airway [4,6,7,118]. 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%) [18]. Bota et al made a treatment decision after 3 months of follow-up for 32 CIS and 27 severe dysplasia cases [20]. 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 [67–69]. 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 [67]. 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 [7,67,69]. Since none of the studies have included a control arm [62,65,69,119,120], 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 3.

[Insert Table 3 near here]

7.1 Photodynamic Therapy

Photodynamic therapy (PDT) has a proven track record of successful tumour ablation [121]. 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 [122–133]. In an early phase II study, Furuse et al treated 59 early cancers with photofrin, a first-generation photosensitizer [123]. 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% [134]. 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 [127,128]. Furukawa et al used PDT as the definitive treatment in 114 stratified lesions (<1cm or >1cm), with long-term follow-up [128]. 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 [127], 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).

7.2 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 [135]. 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 [136]. 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% [137]. Other groups have reported equally good outcomes, with CR rates ranging from 83-96% and 2 or 3-year survival between 45–92% [138–141]. 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 [142]. 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% [143]. However, longer-term complications were observed, with bronchial stenosis occurring in 12 of 33 (36%) individuals.

7.3 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 [120]. 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.

7.4 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) [119]. 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. 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 [144]. 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 preinvasive lesions [145].

8. Chemoprevention

Qu. Is there any role for chemoprevention of lung cancer?

The use of a chemotherapeutic agent given systemically is based on the principle of 'field carcinogenesis', whereby the whole airway affected by carcinogen exposure is 'at risk'. The pathogenesis of squamous cell carcinoma is complex with no specific known targetable mutations. As a result, investigators are searching for targets in pre-clinical and clinical studies to prevent the progression of preinvasive disease and thereby reduce the burden from lung cancer [146,147]. Although there are many studies ranging from the pre-clinical setting to phase III studies in chemoprevention of lung cancer [146], we will focus on those studies enrolling patients with preinvasive squamous cell carcinoma.

Kelly and colleagues investigated the effect of 13-cis retinoic acid (RA) in a phase II randomised controlled trial in 'high-risk' individuals defined by the presence of sputum atypia who underwent follow-up with bronchoscopy [148]. A 12-month treatment of 13-cis RA produced no change in bronchial histology. Another phase II [149] and phase III trial [150] with the same agent were also negative. Lam et al investigated inhaled budesonide in smokers with airway dysplasia in a phase II study [151]. On AFB surveillance, no difference in site-specific progression of lesions was seen. Another target that has been investigated is phosphatidylinositol 3-kinase (PI3K), which is expressed in lung cancer and dysplastic lesions [152]. Myoinositol, an inhibitor of PI3K, was used in a phase I study of 36 individuals with preinvasive disease [153]. Although many individuals had low-grade lesions, the results looked promising with high rates of regression when compared to a historical control. The results of a follow-up phase IIb trial were recently presented showing a mixed response [154]. When accounting for the severity of dysplasia, response rates were similar in the moderate/severe dysplasia, with more patients in the intervention arm appearing to have lesion progression [154]. Only two other chemoprevention agents have have reached their primary or secondary end-points in phase II studies [155,156]. Keith et al used a prostacyclin analogue, Illoprost, in a phase II trial of 152 individuals with a histology primary end-point [156]. A bronchoscopy was performed before and 6 months after administration of oral Illoprost. Although no effect was seen in current smokers, an improvement of histological grade and dysplasia index was seen in former smokers.

Many of these chemoprevention studies have included individuals with low-grade lesions which are likely to spontaneously regress [151,153,156] or who have had short follow-up after intervention [155,156]. Currently, no agents have been shown to be efficacious at preventing progression of tracheobronchial preinvasive disease to lung cancer. This may be explained by the heterogeneity of preinvasive disease; and as focus turns to understanding the molecular mechanisms of preinvasive disease and its hallmarks, novel treatments for chemoprevention will likely come to light.

9. Conclusion

Carcinogens, in particular tobacco exposure, leads to development of synchronous and metachronous preinvasive lesions in the bronchial epithelium. Autofluorescence bronchoscopy techniques can now accurately locate these, which raises the hope of detecting and eradicating preinvasive bronchial lesions before they have progressed to invasive carcinoma. However, our understanding of the natural history of preinvasive lesions remains incomplete and their management controversial. Use of sensitive biological markers has come some way to help our understanding of the hallmarks of preinvasive lesions. However, detection of epigenetic and genetic changes in longitudinal studies of these lesions is likely to hold the most promise of predicting which lesions will progress, streamlining those that need treatment into randomised controlled trials of minimally invasive endobronchial therapies. Finally, the future may hold promise in novel non-invasive techniques with liquid biopsies and exhaled breath in both the early diagnosis of lung cancer and screening for individuals at risk.

Acknowledgements & Declaration

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Tables

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

Title: Summary of pathological changes occurring in preinvasive lesions of the airway.
Description: Categorising lesion as either low- or high- grade lesions enables distinction of two groups of individuals that have noticeably different malignant potentials.
Footnotes: Summary of pathological descriptions based on Histological Typing of Lung and Pleural Tumours by Travis et al [13].

Investigators	Baseline histology & lesion (no.)	Median follow-up	Lesions treated	Lesion end point	Comments
High Grade Lesions					
Venmans et al (2000) [62]	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) [120]	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) [20]	SD (27) CIS (31)	3–24	Yes	SD→CIS: 37% CIS→CIS: 87%	HGLs had 3mo assessment prior to treatment decision
Hoshino et al (2004) [64]	SD (11)	7 (5–17)	No	SD→INV: 2%	
Moro-Sibilot et al (2004) [65]	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) [63]	SD (25)	NS	Yes	SD→LGL: 52% SD→SD: 16% SD→CIS: 32%	Progression to invasion not stated
George et al (2007) [18]	HGL (36)	21 (1–72)	No	HGL→LGL: 19% HGL→HGL: 64% HGL→INV: 17%	Time to progression 4-17months
Salaun et al (2008)[$] [66]	SD (23) CIS (31)	68 (19–117)	Yes	SD→INV: 0% CIS→LGL: 13% (untreated) CIS→LGL: 32% (treated) CIS→CIS: 32% CIS→INV: 23%	HGLs had 3mo assessment prior to treatment decision
Van Boerdonk (2015) [19]	HGL (80) CIS:14	30 (4–152)	Yes	HGL→INV: 18% (site specific progression)	Cumulative 5-year lung cancer risk 39% in HGLs
Low Grade Lesions					
Bota et al (2001) [20]	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) [63]	MET (45) LGD (64)	NS	No	MET→CIS: 9% LGD→CIS: 9%	Progression to invasion not stated
Hoshino et al (2004) [64]	MiD (32) MoD (56)	7 (5–17)	No	MiD→INV: 0% MoD→INV: 2%	
George et al (2007) [18]	LGD (17)	21 (1–72)	No	LGD→INV: 0%	
Van Boerdonk (2015) [19]	LGL (84)	30 (4–152)	Yes	LGL→INV: 12% (site specific progression)	Metaplasia included in definition of low-grade lesion

Table 2

Title: Natural history of preinvasive disease of the airway.

Footnotes: [$]Of this cohort of high-grade lesions, 37 were from a nest cohort (Bota et al [20]).

HGL = high-grade lesions, LGL = low grade lesion, LGD = low-grade dysplasia, CIS = carcinoma in situ, SD = severe dysplasia, MoD – moderate dysplasia, MiD = mild dysplasia, MET = metaplasia, SqM = normal epithelium, INV = invasion, NS=not stated.

Investigators	Lesions (n)	Outcome of complete response (%)	Comments
Photodynamic Therapy			
Kubota et al (1992) [122]	29	72%	CR 89% in lesions <10mm
Furuse (1993) [123]	59	CIS: 100% INV: 80%	CR in lesions <1cm: 98% CR in lesions >1cm: 43%
Imamura et al (1994) [124]	39	64%	CR in superficial lesions: 76% CR in nodular lesions: 43%
Kato et al (1996) [125]	95	83%	CR in lesions <1cm: 94% CR in lesions 1-2cm: 54%
Kawaguchi et al (2000) [126]	59	73%	53% had no recurrence at 2-year assessment
Miyazu et al (2002) [127]	18	100%	EBUS to identify superficial lesions (9 lesions treated)
Furukawa et al (2005) [128]	114	Lesion <1cm: 93% Lesion >1cm: 58%	5-year survival (<1cm): 58% 5-year survival (>1cm): 59%
Kato et al (2006) [129]	264	85%	Local recurrence in 12% of cases
Corti et al (2007) [130]	50	CIS: 73% INV: 69%	Overall survival (CIS): 120months Overall survival (INV): 36months
Endo et al (2009) [131]	48	94%	Tumour length <10mm Overall 5-year survival: 81%
Usuda et al (2010) [132]	28	100%	PDT used to treat 11 individuals with multifocal disease
Jung et al (2011) [133]	39	100%	PDT used in central airway lesions in individuals with multifocal disease
Brachytherapy			
Perol et al (1997) [138]	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) [140]	22	96%	Brachytherapy schedule: 7-10Gy x 3-5 fr. Survival (median): 17months
Marsiglia et al (2000) [139]	34	94%	Brachytherapy schedule: 5Gy x 6 fr. Local control in 85% at 2-year median
Lorchel et al (2003) [143]	35	86%	Brachytherapy schedule: 5Gy x 6 fr. Bronchial stenosis observed in 36% of cases
Hennequin et al (2007) [137]	106	59%	Brachytherapy schedule: 7Gy x 5-6 fr. 5-year survival 48%
Electrocautery			
Van Boxem et al (1998) [119]	15	77%	30 watts power applied until visible necrosis
Cryotherapy			
Deygas et al (2000) [120]	35 (CIS only)	91%	28% local recurrence (1 year) Disease free-interval 13-45months

Table 3

Title: Summary of bronchoscopic treatment studies.

Description: Authors have used different treatment modalities through the working channel of the bronchoscope to ablate lesions of both high-grade dysplasia and early microinvasive carcinomas of the airway.

Footnotes: Table adapted from Wisnivesky et al – American College of Chest Physicians guidelines in the diagnosis and treatment of bronchial intraepithelial neoplasia and early lung cancer of the central airways [7].

Survival figures represent median survival unless otherwise stated.

CIS=carcinoma in situ, INV=invasive disease, CR=complete response, Gy=gray, fr.=fraction.

Figures

Figure 1: Step-wise progression of preinvasive lesions to squamous cell carcinoma. Preinvasive lesions change through a sequence of progressively worsening cytological atypia, loss of maturation and increased epithelial involvement until breach of basement membrane occurs, indicating 'invasive' disease.

Figure 2: Bronchoscopic detection of preinvasive disease. Severe dysplasia on carina between right middle lobe and right lower lobe basal segments undetectable on (a) white light bronchoscopy, but visible on (b) autofluorescence bronchoscopy. Images from an Olympus autofluorescence bronchoscope (BF-F260), AFI-Lucera (Olympus, Tokyo, Japan).

Figure 3: Molecular changes in the progression of preinvasive 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 [75–77,82–92].

Figure based on the originally proposed hallmarks of cancer by Hanahan et al [74].

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=neuropilin