Recent Advances in Histopathology



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Massimo Pignatelli • Patrick Gallagher

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Chapter 3

Classification and treatment of non-small-cell lung carcinoma

Golda Shelley-Fraser, Nidhi Bhatt, Adam Dangoor and Matthew Sephton

INTRODUCTION

Lung cancer mortality remains a major health issue causing over a million deaths worldwide in 2000 according to WHO data [1]. Cancer trends have been steadily changing in recent times. Whilst lung cancer in wosmen remains low in developing countries, incidence and mortality in women have almost doubled in developed countries over a 30-year period. Latest data from WHO GLOBOCAN 2008 [2] shows that lung cancer has surpassed breast cancer as the leading cause of cancer death in women. Alternatively, incidence in men has plateaued in developed countries. Changing lung cancer trends have been related to increasing incidence of smoking in women, particularly of cigarettes with lower tar and nicotine that are also reportedly associated with increase in adenocarcinoma incidence.

Investigative studies into vast survival differences in adenocarcinoma subtypes (as in 2004 WHO classification) in last few years have resulted in major advancements in the management of non-small-cell lung carcinoma (NSCLC).

ADENOCARCINOMA

In 2011, the International Association for the Study of Lung Cancer, American Thoracic Society and European Respiratory Society (IASLC/ATS/ERS) jointly developed a multidisciplinary approach to classification and management of adenocarcinoma [3]. In essence, a diagnostic approach for former bronchioloalveolar carcinoma (BAC) has been developed (**Figure 3.1**). There is emphasis on approach to small specimens for diagnosis as well as to optimise tissue for molecular tests.

Preinvasive neoplasia

Atypical adenomatous hyperplasia (AAH)

This is defined as a ≤5 mm area of atypical pneumocyte proliferation lining centriacinar alveoli with minimal septal widening. The lesional cells are believed to be type 2

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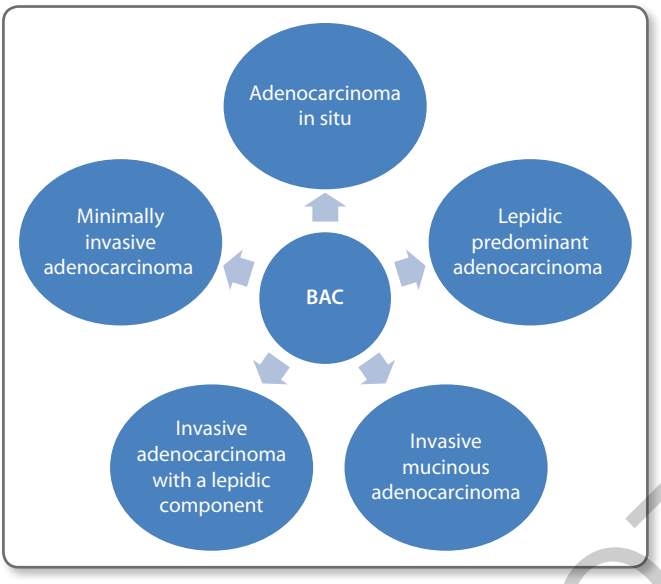


Figure 3.1 Peripheral circles illustrate newly defined and more accurate categories in the 2011 classification which replace the former confusing term of bronchioloalveolar carcinoma (BAC).

pneumocytes and/or Clara cells. They have a 'hobnail' appearance and a high nuclear: cytoplasmic ratio.

AAH can be multifocal and is often an incidental finding in the background of NSCLCs. It may be difficult to differentiate from type 2 pneumocyte hyperplasia, but AAH is discrete unlike the ill-defined and patchy nature of the latter.

Adenocarcinoma in situ (AIS)

AIS replaces former BAC. It is morphologically similar to AAH but larger (>5 mm). It is defined as a localised (≤3 cm) neoplastic lesion growing along existing alveolar walls in a lepidic growth pattern. Alveolar septal widening is usually present. There must be no evidence of parenchymal, pleural or lymphovascular invasion. Diagnosis should be made only after the entire lesion has been examined histologically. If strict diagnostic criteria are used, AIS has a 100% 5-year disease-free survival (DFS).

AIS may be nonmucinous or mucinous – most are, however, nonmucinous. Nonmucinous AIS has a hobnail appearance and discrete borders. Monotonous morphology and diffuse strong p53 expression differentiates it from type 2 pneumocyte hyperplasia or bronchiolar metaplasia. Mucinous AIS is composed of columnar mucinous epithelium with foveolar/goblet cell morphology and may be difficult to differentiate from upper gastrointestinal metastases as thyroid transcription factor 1 (TTF-1) is often not expressed.

Invasive neoplasia

Minimally invasive adenocarcinoma (MIA)

MIA is a small (≤3 cm) adenocarcinoma with lepidic growth pattern and ≥1 foci of invasion measuring ≤5 mm. Invasion is defined by the presence of angulated tumour nests or acini within a desmoplastic or myofibroblastic stroma. By definition, it lacks pleural and lymphovascular invasion and shows no evidence of tumour necrosis. As for AIS, diagnosis

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should be made only after the entire lesion has been examined pathologically. If strict diagnostic criteria are used, MIA has a 100% 5-year DFS.

Invasive nonmucinous adenocarcinoma (INA)

As predominant histological subtypes have been shown to predict prognosis and correlate with molecular data, the 2011 classification recommends recording the percentages of various subtypes within an adenocarcinoma (**Figure 3.2**).

Lepidic predominant adenocarcinoma (LPA)

Larger (>3 cm) adenocarcinomas that comprise mainly lepidic growth but also display invasion are classified as LPAs. Also included are ≤3 cm INAs that show tumour necrosis, lymphovascular invasion or pleural invasion. Stage 1 LPA reportedly shows 90% 5-year DFS.

Acinar predominant adenocarcinoma (APA)

These are invasive adenocarcinomas displaying glandular structures with visible lumina. They may be closely packed or back-to-back or may infiltrate desmoplastic stroma. APA is the commonest invasive pattern seen in a MIA. Cribriform growth pattern is also included.

Papillary predominant adenocarcinoma (PPA)

These are invasive adenocarcinomas showing true luminal papillary structures with fibrovascular cores. Demonstration of desmoplastic stromal reaction is not a prerequisite. This growth pattern has prognosis similar to acinar subtype.

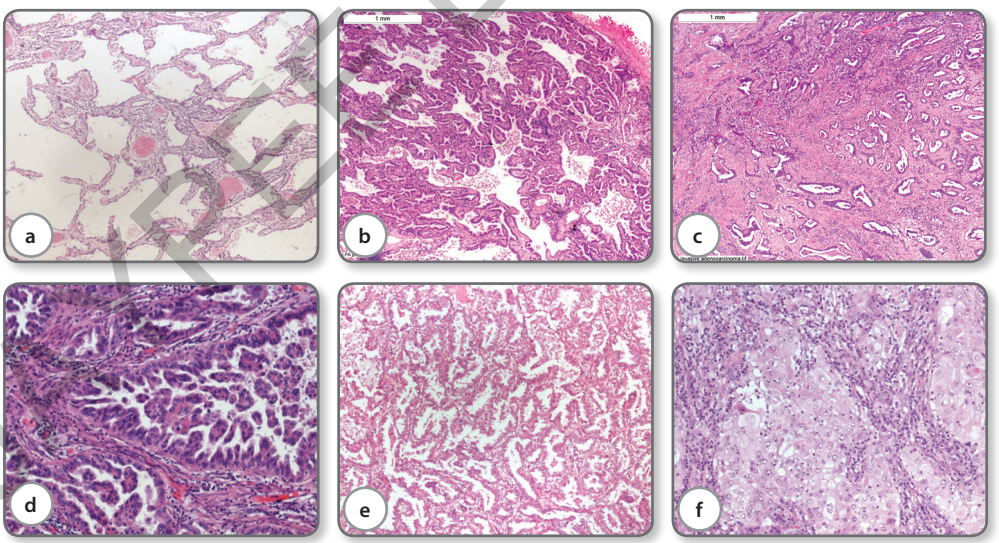


Figure 3.2 Subtypes of invasive nonmucinous adenocarcinoma. (a) Lepidic component of a lepidic predominant adenocarcinoma seen confined to the alveolar lining with no evidence of stromal invasion. (b) Lepidic predominant with possible acinar component. (c) Acinar predominant adenocarcinoma showing glandular profiles invading desmoplastic stroma. (d) Papillary predominant adenocarcinoma showing intra-alveolar tumour papillae with fibrovascular cores. (e) Micropapillary predominant adenocarcinoma showing intra-alveolar tufts with no stromal cores. (f) Solid adenocarcinoma showing no morphological evidence of glandular differentiation.

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Micropapillary predominant adenocarcinoma (MPA)

This subtype is characterised by intra-alveolar detached papillae, tufts or rings of neoplastic cells lacking fibrovascular cores. Tumour groups readily infiltrate the lung parenchyma with minimal stromal response. Lymphovascular invasion is often widespread at the time of diagnosis. Micropapillary and solid subtypes have been shown to have the worst prognosis [4].

Solid predominant adenocarcinoma (SPA)

Solid subtype is characterised by nests and cords of tumour cells that show minimal morphological evidence of glandular differentiation. Mucin stains and immunophenotyping are often needed to demonstrate their glandular nature. Pure forms may be mistaken for nonkeratinising squamous cell carcinoma (SCC) or large cell carcinoma (LCC).

Invasive mucinous adenocarcinoma (IMA)

This term replaces former mucinous BAC. IMAs are now recognised as being morphologically, genetically and clinically distinct from nonmucinous adenocarcinomas. Most common genetic abnormalities are KRAS mutations and EML4-ALK translocation. They usually present at a higher stage and are often mistaken for metastatic disease or multifocal consolidation on imaging due to multifocal involvement probably resulting from aerogenous spread.

IMA comprises mucinous columnar or goblet cell epithelium and shows intracytoplasmic mucin production. The tumour cells show minimal cytological atypia and maintain nuclear polarity. They may display architectural patterns similar to INA, but this is not known to be prognostically significant.

In the 2011 classification, colloid adenocarcinoma and fetal adenocarcinoma subtypes have been retained but mucinous cystadenocarcinoma, signet ring carcinoma and clear cell carcinoma have been abolished as their pure forms are rare and overlap with the existing morphological variants (**Figure 3.3**).

Colloid adenocarcinoma

This is characterised by extracellular mucin lakes that expand and/or destroy existing alveolar spaces and contain groups of malignant mucinous epithelium, which are often difficult to visualise. The mucinous epithelium is of goblet cell or columnar type.

Fetal adenocarcinoma

This is characterised by cribriform and tubular structures composed of columnar cells with clear cytoplasm that recapitulate fetal lung tubules and resemble early secretory phase endometrium. Morular metaplasia, as in endometrioid adenocarcinoma, is also present.

Pure variants are usually seen in younger age groups. They are associated with β-catenin mutations and show nuclear (as opposed to membranous) β-catenin [5]. In the authors' experience, they are often seen as a minor component in otherwise conventional adenocarcinomas; these have normal membranous β-catenin expression.

Enteric adenocarcinoma

This variant is defined as the presence of enteric differentiation (acinar/cribriform architecture and intra-acinar necrosis) in at least 50% of the tumour. Presence of other

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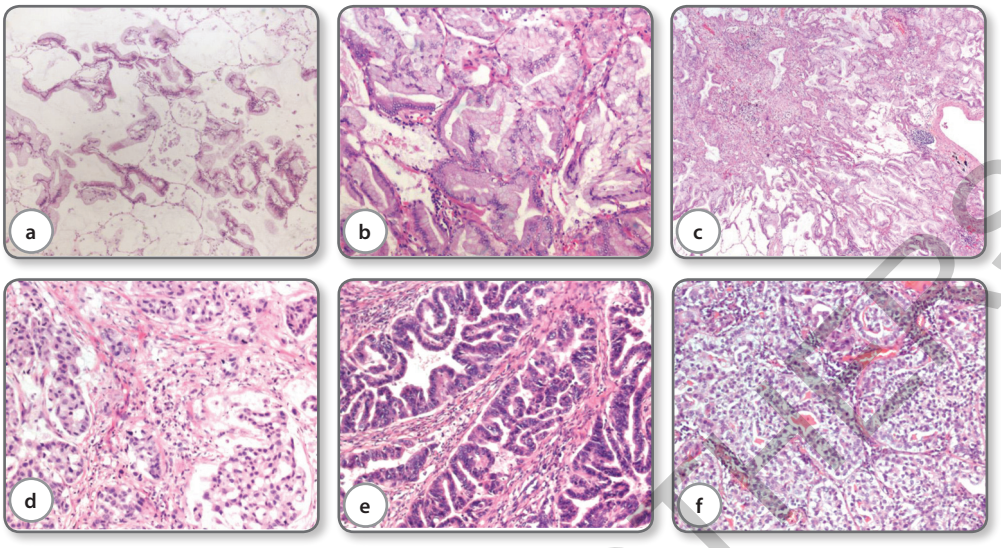


Figure 3.3 Mucinous lung neoplasia. (a) Mucinous atypical adenomatous hyperplasia. (b) Mucinous adenocarcinoma in situ. (c) Mucinous minimally invasive adenocarcinoma. (d) Invasive mucinous adenocarcinoma. (e) Enteric adenocarcinoma. (f) Fetal adenocarcinoma.

subtypes of primary lung adenocarcinomas facilitates distinction from metastatic colorectal adenocarcinoma.

SQUAMOUS CELL CARCINOMA

According to WHO criteria, SCC is an epithelial tumour that shows evidence of keratinisation and /or intracellular bridges arising from bronchial epithelium. SCC represents approximately 30% of NSCLCs. They have a strong association with cigarette smoking but due to changes in smoking behaviour, the incidence is decreasing unlike adenocarcinoma [1].

SCC usually presents as a central tumour close to the hilum. They tend to produce symptoms earlier than adenocarcinoma and commonly show direct spread into adjacent structures, in particular hilar lymph nodes. However, they are late to metastasis compared with adenocarcinoma. SCC often grows to a large size, becomes necrotic and cavitates; they are commonly associated with obstructive pneumonitis downstream.

The current WHO classification mentions four subtypes: papillary, clear cell, small cell and basaloid. SCC expresses high molecular weight keratins, CK5/6 and shows nuclear expression with p63; very few express CK7 or TTF-1.

LARGE CELL CARCINOMA

LCCs are undifferentiated carcinomas lacking squamous and glandular differentiation. They represent approximately 10% of NSCLCs and have a strong association with cigarette smoking. They commonly present as large peripheral tumours that macroscopically show a fleshy, necrotic cut surface without cavitation.

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This is a diagnosis of exclusion that can only be accurately made on a surgical specimen rather than biopsy due to the heterogeneity of lung carcinomas. WHO has separated LCC into subtypes: large cell neuroendocrine carcinoma, large cell undifferentiated carcinoma, basaloid carcinoma, lymphoepithelioma-like carcinoma, clear cell carcinoma and LCC with rhabdoid differentiation.

Large cell neuroendocrine carcinomas express neuroendocrine markers including synaptophysin, chromogranin and CD56, although only expression of one is required for diagnosis. Large cell undifferentiated carcinoma usually expresses cytokeratins and approximately half express TTF-1; neuroendocrine markers are negative. Basaloid carcinoma is rare, resembling its cutaneous counterpart. Lymphoepithelial-like carcinoma of the lung shows a syncytial growth pattern and a prominent lymphocytic infiltrate; there is an association with Epstein–Barr virus. Clear cell carcinoma shows prominent glycogen containing clear cytoplasm and should be distinguished from metastatic renal cell carcinoma. In LCC with rhabdoid differentiation, at least 10% of tumour cells should show rhabdoid differentiation.

ADENOSQUAMOUS CARCINOMA

Adenosquamous carcinoma is defined as carcinoma showing both squamous and glandular differentiation, with each component comprising at least 10%, according to WHO criteria. They represent between 0.4% and 4% of NSCLCs and are associated with cigarette smoking. They often present as a peripheral mass and can show central scar formation.

Histologically, they must show both squamous and glandular differentiation. The two components may be separate or may mingle. Diagnosis on biopsy is dependent on sampling; definitive diagnosis is best made on the surgical specimen. On immunohistochemistry, the adenocarcinoma component should express TTF-1 and CK7, whilst the squamous component is usually decorated by p63 and CK5/6.

SARCOMATOID CARCINOMA

Sarcomatoid carcinomas are a group of poorly differentiated non-small cell carcinomas. They represent approximately 1% of non-small cell carcinomas and are strongly associated with tobacco smoking. WHO divides this subtype into 5: pleomorphic carcinoma, spindle cell carcinoma, giant cell carcinoma, carcinosarcoma and pulmonary blastoma.

They commonly arise centrally, although pleomorphic carcinomas tend to be peripheral showing a predilection for chest wall involvement. Pleomorphic carcinoma is composed of non-small cell carcinoma either SCC, adenocarcinoma or LCC admixed with a population of malignant spindle or giant cells, the latter occupying at least 10% of the total tumour volume.

Spindle cell carcinoma is composed wholly of malignant spindle cells, and likewise giant cell carcinoma consists solely of malignant giant cells. Carcinosarcoma is a biphasic tumour composed of both epithelial and sarcomatoid elements; the carcinoma component is generally SCC. The sarcomatoid component may display chondroid, rhabdoid or osteoid differentiation. Pulmonary blastoma is a primitive tumour composed of epithelial and mesenchymal foci. The epithelial component may resemble fetal adenocarcinoma; squamous morulae may be included. The stromal cell component is undifferentiated and

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resembles blastema. The epithelial elements usually express cytokeratins and the blastema cells express vimentin and smooth muscle actin.

STAGING – TNM 7TH EDITION

Tumour size

TNM 7th edition includes the additional size cut-offs of 2, 5 and 7 cm further to 3cm. T1 tumours are classified as T1a if ≤2 cm or T1b if >2 but <3 cm. T2 tumours are classified as T2a if ≤5 cm or as T2b if >5 cm but <7 cm [6,7].

Multiple tumours

Multiple tumours can present challenges. All tumours must be identified macroscopically, measured accurately and their relationship to one another established. The tumours need to be compared microscopically to determine whether they have similar histological appearances. If similar they are likely to represent intrapulmonary metastases, if not synchronous primaries are favoured (**Table 3.1**) [8,9].

Pleural invasion

TNM 7th edition made recommendations for evaluation of visceral pleural invasion. If there is no suggestion of pleura involvement, the tumour is classified as PL0. If the tumour invades beyond the outer elastic layer, it is classified as PL1. If tumour extends to the pleural surface, it is PL2. The tumour is classified as PL3 when there is parietal pleural involvement. The use of elastic stains is advocated. PL1 or PL2 upstages an otherwise T1 tumour to T2 and PL3 upstages a tumour to T3 (**Figure 3.4**) [10].

Table 3.1 Comparison of TNM 6th edition classification with the changes in TNM 7th edition

Description	6th edition TNM	7th edition TNM
≤2 cm	T1	T1a
>2–3 cm	T1	T1b
≤5 cm	T2	T2a
>5–7 cm	T2	T2b
>7 cm	T2	T3
Invasion of chest wall, diaphragm, phrenic nerve, parietal pleura, mediastinal pleura, parietal pericardium	T3	T3
Separate tumour nodules in the same lobe	T4	T3
Invasion of heart, great vessels, trachea, oesophagus, vertebral body, carina	T4	T4
Separate tumour nodules in an ipsilateral lobe	M1	T4
Malignant pleural or pericardial effusion	T4	M1a
Separate tumour nodules in a contralateral lobe	M1	M1a
Distant metastases	M1	M1b

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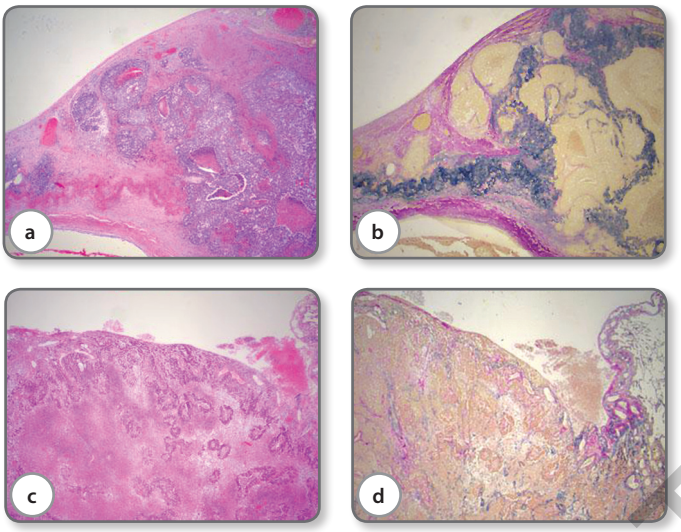


Figure 3.4 Demonstration of visceral pleural invasion. (a) The pleura appears to be intact without invasion on haematoxylin and eosin staining; the elastic stain (EVG) demonstrates invasion of tumour beyond the outer elastic layer (PL1). (b) Tumour extending to the visceral pleural surface (PL2).

PROBLEMS IN DIAGNOSIS

Adenocarcinoma

Interobserver reproducibility in subtypes

Several recent studies have investigated interobserver variability amongst experts in diagnosing the newly described INA subtypes. Whilst these have reported high level of agreement (approaching 90%) in identifying a single pattern as the predominant pattern [11,12], similar agreement is not achievable in identifying all patterns and even less so in determining the percentage proportion of each subtype (authors' experience). The prognostic significance of the latter is debatable but may be useful in distinguishing between a metachronous/synchronous primary and a metastasis.

Assessment of invasion

Distinguishing between AIS and LPA is usually straightforward but separating AIS and MIA & MIA and LPA may be quite challenging even after the entire lesion has been examined histologically [1]. An elastin stain has been recommended to differentiate alveolar collapse from alveolar destruction by invasion, but this is often difficult to interpret.

Squamous cell carcinoma

Cross-cut squamous dysplasia which is growing along mucinous structures can be mistaken for invasion and misdiagnosed as SCC. The other differential diagnoses are solid adenocarcinoma and the small cell variant of SCC which may mimic small cell carcinoma. Clarification can be sought with immunostaining – a solid adenocarcinoma should express TTF-1 and be negative for p63 (**Figure 3.5**). It is worth noting that SCC can show focal mucin deposition. Small cell carcinoma will express neuroendocrine markers and TTF-1, whilst a squame will not.

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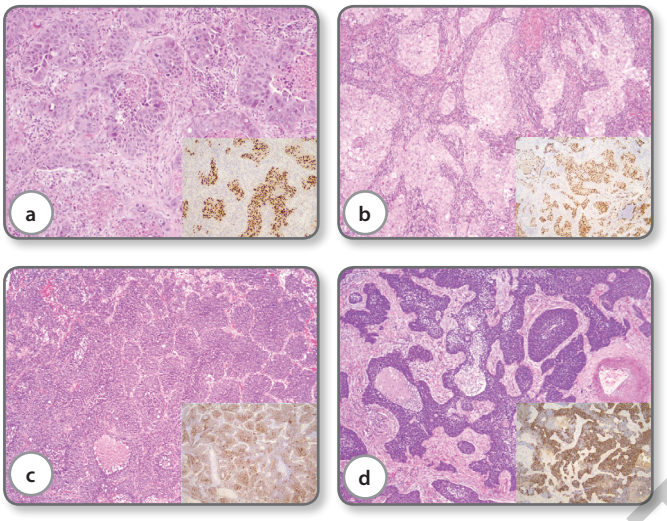


Figure 3.5 Subtypes of non-small cell lung cancer with similar morphologies. (a) Squamous cell carcinoma (with positive nuclear expression of p63). (b) Solid subtype adenocarcinoma (with positive nuclear expression of thyroid transcription factor 1 (TTF-1). (c) Large cell neuroendocrine carcinoma (with positive expression of CD56). (d) Large cell carcinoma undifferentiated (with positive pan cytokeratin staining).

Differentiating between a metastatic SCC in the lung from a primary tumour can be difficult. Immunohistochemistry is not particularly helpful in this matter, although a metastasis from a head and neck tumour may express p16 and/or HPV if HPV related. Otherwise, architectural features such as vascular invasion, a well-circumscribed lesion and the lack of an in situ component favour a metastasis over a primary lesion.

Large cell carcinoma

Large cell neuroendocrine carcinoma often shows cytological features suggestive of neuroendocrine differentiation such as fine chromatin without nucleoli. Differentiation from an atypical carcinoid is based on morphological criteria such as >10 mitoses per 10 high-power field and from small cell carcinoma on cell size, morphology and Ki67 proliferation index.

Adenosquamous carcinoma

Common pitfalls in the diagnosis of adenosquamous carcinoma are entrapped benign bronchial glands within a SCC and squamous dysplasia in an adenocarcinoma. Diagnosis is straight forward if squamous differentiation is associated with acinar, papillary or lepidic growth patterns of adenocarcinoma. The diagnosis is more made difficult when the adenocarcinoma component shows a solid growth pattern. Mucin droplets can be seen in SCC and so in this situation more than five mucin droplets per high-power field are required for a diagnosis [1].

The main differential diagnosis is mucoepidermoid carcinoma, in particular a high-grade mucoepidermoid carcinoma. Low-grade mucoepidermoid carcinomas are generally easy to separate from adenosquamous carcinoma as they lack significant cytological atypia. Also, mucoepidermoid carcinomas tend to be centrally located, whereas adenosquamous carcinomas are generally peripheral.

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TREATMENT OF NSCLC

First-line chemotherapy

The management of metastatic NSCLC has grown in complexity in the past 5 years, guided by the increasing influence from histological and molecular analyses. In the past, it was considered sufficient to have obtained histological confirmation of the diagnosis of lung cancer and having simply differentiated between NSCLC and small cell lung cancer. This was commonly possible on bronchial washings and brushings. Small cell lung cancer is traditionally treated with a platinum agent, cisplatin or carboplatin, in combination with a second agent, such as etoposide. For the first-line treatment of patients with metastatic NSCLC, the platinum agents have commonly been combined with one other agent such as gemcitabine, paclitaxel or vinorelbine in a platinum doublet, regardless of histological subtype.

These regimens have been shown in phase III studies to have comparable efficacy with varying side effect profiles [13–15]. Until 2008-2009, patients in the United Kingdom were commonly treated with a platinum and gemcitabine combination for all histological subtypes of NSCLC (**Figure 3.6**), with median overall survival and progression-free survival in the ranges of 9.5–9.9 months and 4.6–5.5 months respectively [14]. With respect to whether cisplatin or carboplatin should be the platinum-containing agent of choice, the British Thoracic Oncology Group (BTOG) trial BTOG2 showed that carboplatin and gemcitabine were not inferior to the higher dose of cisplatin (80 mg/m^2) used in combination with gemcitabine in one of the trial arms in terms of median overall survival [16] and therefore carboplatin/gemcitabine remained a popular treatment option for metastatic NSCLC until 2009.

In 2008, the importance of identifying the histological subtype of NSCLC in guiding the management was shown. Scagliotti et al. [17] identified that overall survival in patients with NSCLC receiving cisplatin/pemextrexed was noninferior to cisplatin/gemcitabine (median survival 10.3 vs. 10.3 months respectively). However, the study identified that patients with adenocarcinoma subtype had a 1.7 month improved overall survival if they received cisplatin /pemetrexed, rather than cisplatin/gemcitabine (12.6 vs. 10.9 months respectively). In the case of large-cell carcinoma histology, the improvement in

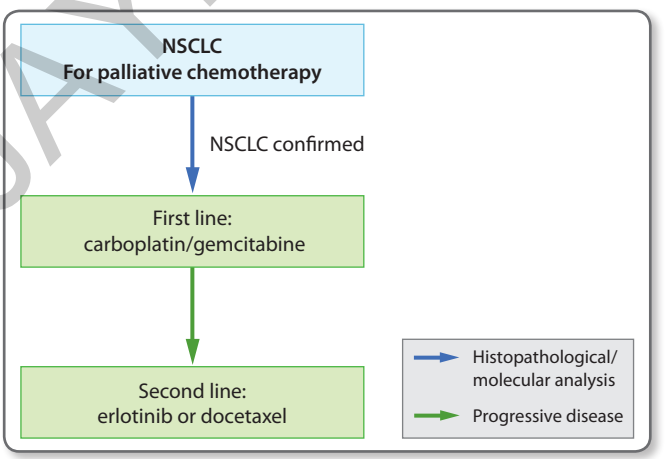


Figure 3.6 Typical management of metastatic non-small cell lung cancer (NSCLC) in 2009. Once histopathological confirmation of the diagnosis had been made, further pathological or molecular analyses played no further role in altering the management. This is a typical situation and is by no means extensive.

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overall survival with cisplatin/pemetrexed was even more marked compared to cisplatin/gemcitabine (10.4 vs. 6.7 months respectively). In contrast, the reverse situation was seen in patients with squamous cell histology, where there was significantly worse survival with cisplatin/pemetrexed versus cisplatin/gemcitabine (9.4 vs. 10.8 months respectively).

This was the first prospective phase III study in NSCLC to show survival differences based on histological subtype. It is thought that the reason for this difference in efficacy of chemotherapy regimens is based on significantly increased levels of thymidylate synthetase (TS) in squamous cell histology. Increased levels of TS have been shown in preclinical data to correlate with reduced sensitivity to pemetrexed. The work from Scagliotti et al. shifted the need from simply diagnosing NSCLC, to the need to identify the histological subtype, in order for the optimal chemotherapy regimen to be recommended for the patient. The National Institute for Health and Clinical Excellence (NICE) in the United Kingdom approved the use of pemetrexed in combination with cisplatin for the first-line treatment of patients with locally advanced or metastatic NSCLC with non-squamous histology [18].

Epidermal growth factor receptor tyrosine kinase inhibitors

The epidermal growth factor receptor (EGFR) has been used as a therapeutic target in NSCLC for a number of years. In early trials, EGFR detection by immunohistochemistry was used as a marker for EGFR expression, but this did not correlate well with responses to EGFR tyrosine kinase inhibitors (TKIs). It was the identification of certain activating mutations in EGFR, present in a proportion of patients with NSCLC, which correlated better with response to EGFR TKIs [19]. Prior to 2010, EGFR TKIs were commonly used in predominantly second-line treatment of NSCLC after disease progression despite first-line cytotoxic chemotherapy. At this time, activating mutations in EGFR were commonly not assessed. The BR21 trial showed that the median overall survival for patients receiving erlotinib, used in the second- and third-line settings for NSCLC, regardless for EGFR mutation status, was 6.7 months compared with 4.7 months for placebo [20]. As a result of an indirect comparison of trials comparing docetaxel and erlotinib in the second-line setting for NSCLC, NICE approved the use of erlotinib as an alternative to docetaxel in this setting in 2008 [21] (**Figure 3.6**)

Shortly after this, interest of the use of EGFR TKIs in the first-line setting developed and in 2009 the results of the IressaPan-Asia Study (IPASS) were published [22]. This study compared gefitinib (Iressa) with carboplatin/paclitaxel in the first-line setting in patients with advanced pulmonary adenocarcinoma in East Asia, who were nonsmokers or former light smokers. The 12-month rates of progression-free survival were 24.9% with gefitinib and 6.7% with carboplatin/paclitaxel, confirming the noninferiority and, in fact, superiority of gefitinib compared with carboplatin/paclitaxel with respect to progression-free survival in the intention-to-treat population. In the subgroup of patients who were positive for an EGFR gene mutation, progression-free survival was significantly longer for those patients who received gefitinib than those who received carboplatin/paclitaxel (Hazard ratio (HR) for progression, 0.48; 95% CI, 0.36–0.64; $P<0.001$).

Objective response rates were also remarkably high in patients with a mutation treated with gefitinib, compared to chemotherapy (71.2% vs. 47.3% respectively). In contrast, in patients who were negative for an EGFR mutation, progression-free survival was significantly longer amongst those who had received carboplatin/paclitaxel (HR for progression with gefitinib, 2.85; 95% CI, 2.05–3.98; $P<0.001$), and the use of gefitinib in this subgroup of patients was associated with a significantly lower objective response rate compared with chemotherapy (1.1% vs. 23.5% with carboplatin/paclitaxel). The IPASS

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study was performed in a population known to have high rates of EGFR gene mutations (Asian patients, non-smokers or former light smokers), but the subsequent EURTAC study demonstrated similar results in the European population using a different EGFR TKI, erlotinib (Tarceva), where median progression-free survival was 9.7 months with erlotinib versus 5.2 months with chemotherapy in EGFR mutation positive patients [23].

In addition to IPASS and EURTAC, other studies have confirmed the additional benefit of using first-line EGFR TKIs in patients with EGFR mutations [24]. It has also been shown that this treatment is commonly better tolerated than cytotoxic chemotherapy and therefore knowing the EGFR mutation status of a patient with metastatic NSCLC is of key importance in guiding the appropriate treatment, in addition to knowing the histological subtype of NSCLC. Rates of EGFR mutations are higher in nonsquamous subtypes (approximately 10% in adenocarcinomas [25] versus 3.4% in squamous cell subtypes [26]), and therefore it is common tumours of the nonsquamous subtype that are sent for EGFR mutation analysis. The importance of testing appropriate samples for EGFR mutation analysis has implications for the need of larger sample sizes during diagnostic procedures.

Anaplastic lymphoma kinase pathway

In addition to the EGFR pathway, other potential therapeutic targets have been studied in NSCLC. In 2007, rearrangements in the gene coding anaplastic lymphoma kinase (ALK), leading to ALK fusion proteins, were found in patients with NSCLC [27]. These fusion proteins appeared to play a critical role in tumourigenesis. The most common ALK fusion protein in NSCLC is the echinoderm microtubule-associated protein-like 4 (EML4-ALK), although other transforming ALK fusion proteins have been described. Further research has indicated that ALK-positive tumours account for approximately 3–5% of NSCLC cases [28,29], mostly in patients who are nonsmokers (or ≤10 pack years) of a younger age than the average for lung cancer, and of adenocarcinoma histology [16].

Preclinical studies of the dual ALK and mesenchymal-epithelial transition factor TKI, crizotinib (Xalkori), were rapidly undertaken and the promising significant clinical responses using crizotinib in ALK-positive patients with NSCLC in phase I and phase II studies led to a prospective, randomised phase III study, PROFILE 1007. This study, comparing second-line crizotinib with pemetrexed or docetaxel chemotherapy in patients with ALK-positive NSCLC, who had received previous platinum-based chemotherapy, showed an improvement in progression-free survival with crizotinib compared to chemotherapy (7.7 months vs. 3.0 months respectively) and an improvement in objective response rate (65.3% with crizotinib vs. 19.5% with chemotherapy) [30]. Crizotinib is therefore indicated for the treatment of previously treated ALK-positive advanced NSCLC and has been referred for review by NICE. Additional confirmatory randomised phase III studies, including those in the first-line setting, are underway.

The development of crizotinib, and the speed of progress from early phase trials to clinical use, illustrates how rapid this process can now be when an appropriate target and effective agent can be identified. Crizotinib is an example of an agent that has been developed to provide personalised medicine for patients with ALK-positive NSCLC. Tailoring a treatment based on histological and molecular analyses is an expanding area not only in the treatment of lung cancer but also in oncology in general. The Cancer Research UK Stratified Medicine Programme is an example of this drive, with the aim of making genetic testing more readily available, therefore improving the access of targeted therapies available for patients (**Table 3.2**).

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