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Abstract

Our increasing understanding of the molecular pathogenesis of non-small cell lung cancer (NSCLC), particularly adenocarcinomas, has opened the door to 'personalised medicine' and the advent of new therapeutic strategies. Over the last few years new drugs, or classes of drugs, have been licensed and entered clinical practice for use in advanced NSCLC. The activity of these drugs is dependent on the presence of specific molecular or protein changes in cancer cells which are usually identified using 'companion diagnostic tests' specifically designed for this purpose. Pathologists and Pathology Departments have had to forge new links with Clinical Scientists in order to facilitate these additional investigations on the, often limited, tissue obtained for diagnosis. This collaboration plays a critical role in providing the link that allows integration of the traditional morphological diagnosis with the results of these new 'companion diagnostic' tests to guide patient management.

Key words

Non-small cell lung cancer, adenocarcinoma, pathology, molecular pathology, *EGFR*, *ALK*, *ROS1*, PD-L1.

Introduction

Lung cancer is the leading cause of cancer-related death worldwide and, despite significant advances in public health policy, delineation of disease pathogenesis and novel therapeutic strategies, mortality rates remains dismal. Historically, histopathological classification has divided tumours into two separate categories, small cell and non-small cell lung carcinoma (SCLC, NSCLC respectively) with the latter accounting for approximately 80% of all malignant primary lung tumours. Further morphological and immunohistochemical subclassification divides NSCLC into several entities of which the most common are adenocarcinoma, squamous cell carcinoma and large cell carcinoma. Previously, the distinction in tumour type beyond NSCLC had little influence on clinical management as chemotherapeutic agents and regimens were limited. Improved understanding of the underlying disease mechanisms and somatic driver mutations has, however, highlighted the need for detailed, definitive phenotypic and genotypic characterisation to establish the correct diagnosis, inform prognosis and guide therapeutic decisions. The advent of personalised medicine in lung cancer management and the central place of the histopathologist in this process serves as a paradigm for the future role of pathology within the multidisciplinary teams throughout all cancer fields.

Molecular Pathogenesis in NSCLC

The development of NSCLC occurs due to numerous distinct somatic mutations occurring in a heterogeneous population of tumour progenitor cells. Adenocarcinomas typically arise from epithelial cells in the terminal respiratory unit and display variable morphological appearances including acinar, lepidic and papillary patterns commonly

expressing thyroid transcription factor 1 (TTF1) and cytokeratin 7. Squamous cell carcinomas are characteristically more central lesions arising from basal bronchial epithelial cells and often arise in the context of squamous metaplasia. Morphologically these lesions typically demonstrate keratinisation and intercellular bridge formation with concomitant p40 and p63 expression. Whilst most lung cancers are associated with tobacco smoke and other inhaled carcinogens there is a group of “never-smokers” who develop adenocarcinomas which often have a distinct molecular phenotype (1).

The advent of next generation sequencing (NGS) and other high throughput modalities has highlighted the complex interplay of genetic and epigenetic factors as well as the tumour microenvironment in determining tumour development. This has lead to the identification of ‘early driver mutations’ in key oncogenes which are believed to be important in tumour development and growth. Oncogenes associated with adenocarcinoma include activating mutations in the epidermal growth factor receptor (*EGFR*) gene and translocations of the anaplastic lymphoma kinase (*ALK*) and *ROS1* gene as well as *KRAS*, hepatocyte growth factor receptor (*MET*), *RET* and human epidermal growth factor receptor 2 (*HER2*) mutations (2). Interestingly adenocarcinomas in ‘non-smokers’ show a significantly higher frequency of mutations in *EGFR*, *ALK* and *ROS* while smokers show a high frequency of *KRAS* mutations suggesting potentially different pathogenetic mechanisms of tumour development in smokers and non-smokers (1). The frequency of these abnormalities is, however, also influenced by geographic / ethnic factors with *EGFR* and *ALK* driver abnormalities seen in 10-15% of white European patients but 60% of those of Asian origin (1,3). Somatic driver mutations in squamous cell carcinoma include discoidin domain-containing receptor 2 (*DDR2*), the fibroblast growth factor receptors *FGFR1-3, TP53*

and PIK3CA (2). In small cell carcinoma inactivation of the tumour suppressor genes *TP53* and Retinoblastoma 1 (*RB1*) are a pre-requisite in its development with further associations with *MYC* translocations described (4).

As adenocarcinomas have seen the greatest advances in the application of therapy-defining tumour profiling these tumours will be main focus of this article. The key molecular features of these tumours will be discussed with regards to their biological and therapeutic relevance.

Key Molecular Targets

EGFR

EGFR is a transmembrane receptor tyrosine kinase (RTK) which, in the context of oncogenesis, is constitutively activated by a variety of somatic mutations. These occur in the tyrosine kinase domain of the *EGFR* gene on chromosome 7 with the most frequent activating mutations being the exon 19 deletions (p.Glu746_Ala750), and exon 18 (p.Gly719Cys, p.Gly719Ser, p.Gly719Ala) and exon 21 (p.Leu858Arg also known as L858R) substitutions. Subsequent activation of EGFR triggers several downstream signalling pathways including *RAS, PI3K*, and *STAT3*. Together, they regulate gene transcription, cell differentiation, proliferation, migration and apoptosis.

EGFR was the first of the molecular targets to be characterised as a therapeutic target in lung adenocarcinoma with the introduction of Gefitinib (EGFR-tyrosine kinase inhibitor (TKI)) which demonstrated the need to define somatic driver mutations in the context of molecular therapies. Early trials of Gefitinib showed that the presence of an activating *EGFR* mutation significantly improved overall progression free survival

whilst treatment in patients without an *EGFR* mutation caused dramatically worse progression free survival (5). EGFR-TKIs are therefore superior to standard platinum-based chemotherapies as first line treatment in this correct patient cohort with response rates ranging from 56-86%. Importantly, not all *EGFR* mutations are amenable to therapy with exon 20 insertions failing to respond. Additionally, resistance can emerge following TKI treatment due to a pThr790Met (also known as T790M) mutation in exon 20 altering the ATP-binding affinity of the kinase and leading to reduced binding of first generation TKIs. Development of third line TKIs which selectively target T790M are currently entering clinical practice (3).

ALK

The ALK protein is a member of the insulin receptor subfamily of RTKs. *ALK* gene rearrangements occur in <5% of adenocarcinomas and are often associated with young, non-smokers with clinically advanced disease. Typically, rearrangements occur as a short inversion of the gene on chromosome 2p in which intron 10 fuses with the intron 13 of the upstream echinoderm microtubule associated protein-like 4 (*EML4*). Less frequently, *ALK* binds to kinesin family member 5B (*KIF5B*), *TFG*, and *KLC-1*. ALK-positive lung cancers are highly responsive to ATP-competitive kinase inhibition by ALK-TKI therapies which specifically target human ALK and human growth factor (HGF) receptors (6). Response rates are approximately 60% with a median progression-free survival of 8.1 months. Crizotinib is therefore approved for first line and subsequent line treatments. Drug resistance however typically develops after 11 months of treatment due to further alterations in *ALK* or activation of bypass signalling pathways including EGFR, KRAS and KIT. Development and application of second line therapies including Ceritinib is therefore ongoing.

ROS-1

The *ROS1* gene (chromosome 6q) also encodes an RTK within the insulin receptor subfamily and shares many similarities to *ALK***.** Various gene rearrangements of this proto-oncogene can occur with fusion partners including *CD74*, *SLC34A2*/*NaPi2b*, and *FIG* all of which result in constitutive activation of the receptor. Importantly, the nature of the *ROS1*-binding partner does not appear to be important in determining response to therapy (7). Downstream signalling pathways are similar to other receptor tyrosine kinases with comparable effects on cellular function. *ROS1*-associated fusions are present in approximately 2% of all adenocarcinomas and, as with *ALK* rearrangements, are more prevalent in young, non-smoking patients. Treatment of *ROS1*-positive tumours with Crizotinib results in a progression-free survival of 19.2 months with a response rate of 72%.

PD-L1

Although not specific to NSCLC the role of programmed death 1 / programmed death ligand 1 (PD-1/PD-L1) as an immunotherapeutic target has highlighted the influence of the tumour microenvironment both on tumour development and as an avenue for therapeutic intervention. Under physiological conditions immune checkpoint receptors such as PD1 and cytotoxic T-lymphocyte–associated protein 4 (CTLA4) are essential in the process of discriminating “self” from “non-self” antigens in the development of immune tolerance.

Certain sub-types of solid tumours (including some lung adenocarcinoma, squamous cell carcinoma and small cell carcinoma) express PD-L1 on their cell surface. Tumour

antigens are presented on major histocompatibility complexes (MHCs) which, upon binding to T cell receptors, allows T cell activation and generation of an appropriate immune response. However, when PD-L1 is concurrently expressed on the tumour cell surface it binds to PD1 present on $CD8^+$ T cells inhibiting T cell proliferation and clonal expansion thereby preventing identification of “non-self” antigens. By inducing this state of immune tolerance tumour cells can evade immune surveillance and grow in a protected and permissive environment. Anti-PD-L1 and anti-PD-1 monoclonal antibodies block the inhibitory interaction between PD-1 and PD-L1 allowing T-cell recognition of tumour cell antigens and generation of an appropriate immune response and subsequent tumour cell death.

As the therapeutic target is tumour-associated immunity the use of PD-L1 inhibitors is not limited by specific tumour types or driver mutations but rather cell surface protein expression. Both squamous cell carcinomas and adenocarcinomas that express PD-L1 are therefore amenable to therapy with improved overall survival relative to conventional chemotherapy in NSCLC (8). Ongoing studies are, at present, evaluating the potential efficacy in small cell carcinoma.

Laboratory Aspects of Molecular Testing in NSCLC

Molecular testing in lung cancer presents a number of process issues for diagnostic histology / cytology labs which have been reviewed elsewhere (9). Some are generic to molecular testing in general eg. optimisation of fixation and avoidance of cross contamination during processing and sectioning. In order to minimise this it is particularly important that block sectioning for DNA or RNA extraction are carried out in a ‘clean’ area of the laboratory designated for this purpose by appropriately trained

staff and not simply carried out at the same time as routine sections for histology and immunohistochemistry (IHC).

In addition, there are issues more specific to testing in NSCLC. The vast majority of patients with NSCLC are diagnosed on small bronchoscopic biopsies, percutaneous biopsies or a variety of cytology specimens (bronchial cytology, sputum cytology, pleural aspiration or increasingly lymph node FNAs from the mediastinum and neck). These specimens often contain limited numbers of tumour cells. This requires attention as to how these specimens are handled in order to optimise the tissue available for both diagnosis and molecular testing. Strategies that can be employed include clipping up core biopsies individually in separate cassettes, cutting spares for IHC at the time of initial sectioning to reduce tissue loss as blocks are 'faced-up' on the microtome and the use of double immunohistochemical staining (eg CK7 + TTF1) to reduce the number of sections required. Cytology specimens should be processed in a manner that allows the production of cell blocks which can then be used for IHC and molecular testing.

Fixation with both formalin or alcohol based fixatives appears to be equally good for molecular testing for *EGFR* mutations as well as IHC / FISH (fluorescent in-situ hybridisation) detection of *ALK*/*ROS1* translocations. PD-L1 testing by IHC is, however, currently only validated in formalin fixed biopsy specimens and therefore alcohol fixed cytology specimens may not be suitable for assessment; although this is very much an area in evolution.

The role of the pathologist

While there has been considerable focus on molecular testing of NSCLC it is important to recognise that the pathologist's primary responsibility is to make a diagnosis and secondly to determine, where possible, a cell type. It is however important that this is achieved in a manner that does not 'waste' tissue that may be required for molecular testing following the pathological diagnosis. When IHC is undertaken the antibodies used should be carefully selected and the indiscriminate use of broad panels should be avoided (10).

In cases where molecular testing is required sections from the case (biopsies or cell blocks) need to be reviewed by the pathologist and the percentage of tumour cells present assessed. In biopsy specimens it is often possible to increase this by identifying areas that can be macroscopically dissected from the slide either by positively selecting an area of high tumour concentration or by removing fragments containing little or no tumour. The minimum percentage tumour cells required varies depending on the technology used and a decision on which cases are insufficient to test requires discussion with the molecular laboratory.

Which specimens to test and when to test them

Current guidance is that molecular testing for *EGFR* and *ALK* / *ROS1* should be carried out on adenocarcinomas, NSCLC - not otherwise specified (NOS) and in cases of squamous carcinoma where there is any suspicion that the histology may not be entirely representative eg. squamous carcinomas in non-smokers or cases where there is co-incidental mucin noted on special stains (11). Initially studies suggested that *EGFR* mutations and *ALK*/*ROS1* translocations were found in non-smoking

female patients but it is now recognised that they are encountered in males and current smokers albeit at a lower frequency. There is therefore no indication to select cases for testing on the basis of sex or smoking history. There is some data which suggests that in Caucasian patients the absence of expression of TTF1 by the tumour cells has a strong negative predictive value for activating EGFR mutations in adenocarcinomas although this is less clear for ALK/ROS1(12)..

The issue of when to test is more contentious. Some centres have adopted a reflex testing model where the pathologist routinely requests testing in all appropriate cases at the time of diagnosis. This, it is argued, reduces the risk of missing cases that should be tested and expedites the availability of the result. Tyrosine kinase inhibitors for *EGFR* mutations and *ALK*/*ROS1* translocations are, however, prescribed to patients with advanced stage disease. They are not indicated in patients with more limited disease who will be managed surgically or with radical chemotherapy/radiotherapy with ‘curative intent’. Similarly, there is no role for these drugs as post-operative adjuvant therapy. Reflex testing is therefore wasteful of resources. The alternative approach is a ‘request’ or ‘on-demand model’ when testing is only undertaken following discussion of the case with an oncologist where the presence or absence of a mutation will immediately direct management. This approach is more efficient in terms of resource use but has the potential to delay the availability of the result, particularly when cases need to be sent to another institution for testing. Individual centres need to agree a model which suits their clinical requirements but we have adopted a pragmatic approach where we reflexively test samples in patients where we know from the nature of the specimen (eg. liver biopsies, pleura aspirates) that the patient has advanced stage disease or the history accompanying the request states

this. As all other cases are discussed at the MDT, testing can be requested by the oncologist at that stage, or at a later date, when the presence of a mutation is relevant to the patient's management.

Assessment of PD-L1 testing is relevant to all subtypes of NSCLC. In the second line setting it can be performed at the time of relapse and there is evidence to suggest that assessment on a contemporaneous biopsy is better as expression levels can be altered by chemotherapy. First line therapy with PD-L1 inhibitors is again primarily indicated in patients with advanced disease and the approaches to patient selection for testing is similar to that used for *EGFR* and *ALK*/*ROS1*.

Methodologies for Molecular Testing in NSCLC

General considerations

It is well known that solid tumour specimens contain a proportion of non-tumour cells, including immune and stromal cells, which do not carry the mutation of interest and dilute tumour DNA. Careful histological assessment of each specimen is required to estimate the proportion of tumour cells and determine if a sample is suitable for molecular analysis (11, 13). It has been recommended that mutation detection methods should have a minimum limit of detection (the minimum proportion of mutated DNA which must be present for reliable detection) of 10% mutated DNA; equivalent at least 20% tumour cells (13), although gene amplification or polysomy may permit the detection of mutations in less tumour-rich tissues. Accurate and reproducible assessment of tumour percentage in tissue sections is, however, difficult and subject to large inter-observer variation which may affect the validity of molecular testing

results (13). To address this UK NEQAS have launched a pilot EQA scheme (info@ukneqas-molgen.org.uk) for tissue assessment allowing individual assessors to benchmark their results against their peers.

Lung cancer specimens are routinely formalin fixed and paraffin embedded (FFPE) and therefore yield poor quality and fragmented DNA; in addition, formalin treatment causes adducts to the DNA which can produce false positive mutations (14). Cytological preparations, which typically undergo primary fixation in alcohol, frequently yield better quality DNA although depending on the anatomical site of the specimen they may also contain a high proportion of non-neoplastic cells.

EGFR

Any somatic *EGFR* mutation detection method should be able to detect all clinically relevant mutations present in greater than 1% of the target population (13) although accurate estimates of mutation incidence can be difficult to attain and are biased by the method chosen in the reference cohort. Although antibodies have been developed for the detection of mutated EGFR protein by IHC they are only designed to bind to the two most common mutations. This means that their overall analytical sensitivity is not sufficient for routine clinical use (15). Currently, nucleic acid based techniques offer the only realistic methods for *EGFR* mutation detection in NSCLC (13). Clinically relevant *EGFR* mutations are concentrated in exons 18, 19, 20 and 21 and Sanger sequencing of these exons is achievable in most lung tissues. However successful mutation detection by Sanger sequencing requires at least 20% of the DNA to be mutated, equivalent to a sample with at least 40% tumour cells. For many lung cancer samples (over 50% in our experience) this is not achievable without laser capture

microdissection; something which is not routinely available in clinical laboratories. Techniques with a lower limit of detection which are more appropriate for lung specimens include allele specific PCR, fragment analysis of PCR products (for small insertions and deletions) and more sensitive sequencing technologies such as pyrosequencing and next generation sequencing (NGS). There are also several commercially available kits for the detection of somatic *EGFR* mutations including the COBAS® EGFR mutation test (Roche Diagnostics), the *therascreen*® EGFR RGQ PCR and EGFR Pyro kits (Qiagen) and the PNAClamp™ EGFR Mutation Detection kit (Panagene). Such kits, with CE IVD status (indicating compliance with European Union directives and standards for *in-vitro* diagnostics), only require verification rather than a full validation and represent a rapid way for laboratories to implement *EGFR* analysis. Molecular pathology techniques for the analysis of NSCLC have been reviewed in more detail elsewhere (16).

ALK

Initial clinical trials investigating ALK inhibitors for the treatment of lung cancer relied on FISH with break-apart probes to detect re-arrangements in the *ALK* gene (17). However the implementation of this costly analysis into routine clinical use has proved challenging due to the large patient population and very low incidence of the *ALK* re-arrangement. More recently the development of IHC assays, designed to detect increased expression of the ALK protein caused by re-arrangement of the *ALK* gene, has allowed for rapid and low cost screening prior to the confirmation of positive results by FISH (figure 1). This has proved controversial, with some groups claiming to find IHC negative FISH positive cases. Despite this several antibodies are now available [5A4 (Novocastra) and D5F3 (Ventana)] which are generally accepted to show robust performance in combination with FISH or as a stand-alone test in unequivocal cases

(18). The significance of IHC-positive, FISH-negative cases remain unclear but there is some evidence to suggest that these cases may respond to ALK targeted TKI therapy and as such these should be reported.

Some laboratories have developed multiplexed reverse transcription PCR (RT-PCR) assays for the detection of *ALK* gene re-arrangements. These methods use PCR primers that have been designed to flank potential breakpoints in mRNA derived from tumour tissues (19); however, large numbers of primers would be required to detect all possible ALK fusion partners. NGS has also been applied to the detection of *ALK,* and other, gene re-arrangements in lung cancer with some success (20) but the reliance of these assays on RNA isolated from FFPE tissues may, however, restrict their use in poorer quality samples.

ROS1

The detection of rearrangements in the *ROS1* gene is in the process of entering routine testing in the UK and is widely performed elsewhere. Similarly to ALK, trials showing benefit from crizotinib in patients with *ROS1* rearranged lung cancer have depended on analysis of the gene using break-apart FISH (21). Given the rarity of the ROS1 rearrangement it is hoped that a similar IHC/FISH algorithm will prove to be effective in detecting this additional group of patients. Commercially available NGS panels to detect fusion genes in lung cancer have been designed to include *ROS1* rearrangements.

KRAS

Although clinical analyses are commonly restricted to biomarkers with direct therapeutic application, other molecular characteristics may show utility. *KRAS*

mutations are common in NSCLC and are widely accepted to be mutually exclusive with *EGFR* mutations and *ALK* translocations. In addition they have been shown to have a strong negative predictive value for response to *EGFR* TKIs (22). Considering the low incidence of *EGFR* or *ALK* aberrations in Caucasian NSCLC patients the detection of a *KRAS* mutation can reduce the number of 'insufficient' reports for the large number of samples with sub-optimal neoplastic cell content and may prevent the need for some repeat biopsies. Depending on the detection method selected *KRAS* mutation analysis could be rapid, low cost and fit easily into a routine testing algorithm.

PD-L1

Assessment of PD-L1 expression by NSCLC is carried out by IHC (figure 2). This is however a very complex field with different drugs developed by different pharmaceutical companies having specific testing kits with different primary antibodies and different scoring criteria (23). The situation has been further complicated by licensing authorities in the USA and Europe requiring tumour testing by IHC for some PD-L1 inhibitors but not others. Assessment of expression, when required, should be carried out on FFPE tissues. Alcohol fixation is believed to affect the level of staining and the use of these assays on cytology specimens has not been validated. Expression of PDL1 is highly variable and needs to be carried out according to the criteria set out for each of the different IHC primary antibodies / kits. Care needs to be taken not to over interpret non-specific staining of degenerate tumour cells, 'edge effect' staining in free lying cells and apparent staining due to expression on the surface of admixed macrophages. Reports must state the primary antibody / kit used and the percentage of tumour cells expressing PD-L1.

Clinical Reporting of Results

It is now widely accepted and recommended as good practice to report molecular pathology test results in an integrated way (9). This approach ensures clinicians receive a timely and accurate report on which clinical management decisions can be made. The molecular pathology report should be short and concise but at the same time it must contain important details such as: patient's demographics, sample details and the reason for testing. In addition the report should detail all the technical information required to correctly interpret the result and there should be an unambiguous clinical statement to guide therapeutic decision making. This should describe what the result means for the patient and the class of drugs the patient may or may not be suitable for (Table 1).

Future Developments

Next generation sequencing (NGS)

NGS is an attractive platform that offers high sensitivity testing at a relatively low cost. As NGS platforms evolve they require a small amount of DNA, compared to performing multiple single gene tests, and provide results for multiple oncogenic targets of interest. However, in order to maintain low costs, batching would be advisable and this could potentially impact on turn-around times. The addition of fusion panels to NGS technology to detect *ALK* or *ROS1* rearrangements makes it even more attractive and reduces both costs and hands-on technical time required for FISH (24). A recent report from USA has however highlighted the issue of potential poor reproducibility that may be encountered using NGS panels (25).

Circulating tumour DNA

Patients with advanced NSCLC with an *EGFR* mutation are likely to respond well to TKI therapy and benefit from an improved quality of life, but for the majority TKI resistance and disease progression occurs. In 50-60% of patients this has been associated with the overgrowth of tumour cell clones harbouring an additional EGFR T790M mutation. These patients can now be treated with new third generation TKIs, such as osimertinib which specifically targets the T790M mutation in tumours. The need for repeat tissue sampling to detect this mutation has, however, been seen as a limiting factor. In recent years a number of studies have demonstrated the potential for using circulating cell-free DNA (cfDNA) as a non-invasive 'liquid biopsy' for the analysis of actionable mutations in a number of cancer types; including advanced NSCLC. The use of cfDNA to detect the presence of a T790M mutation in patients treated with EGFR TKIs is now the test of choice to identify patients eligible for third generation TKIs such as osimertinib (26). It is important, however, to also test the sample for the initial known mutation as well as the T790M as this provides an internal positive control for the presence of tumour DNA. In cases where the initial mutation is not detected the sample may contain insufficient tumour DNA and these patients should be considered for repeat tissue biopsy and traditional tissue based testing if this is clinically indicated. In addition *EGFR* cfDNA analysis has the potential to be used for therapy stratification if a tumour sample is not evaluable for primary testing.

<u>Other biomarkers</u>

In addition to *EGFR*, *ALK, ROS1* and *KRAS* several other potential therapeutic targets have been identified such as *BRAF, PIK3CA, c-MET, HER2, RET, DDR2*, *FGFR.* These targets are already linked to active agents approved for other indications or tumour types and diagnostic test are already in use. Of these, *DDR2* and *FGFR* are

directed at squamous cell carcinoma, a tumour type not currently tested in the molecular laboratories due to the lack of therapeutic targets available. It is likely over the coming years that therapeutic agents against some, or all, of these targets (and others) will enter clinical practice resulting in the need for a more extended panel of testing on a routine basis in all forms of lung cancer. While the use of NGS panels will help to deliver this the availability of sufficient good quality tumour tissue for testing may remain an issue.

Conclusion

The prognosis for patients with advanced stage lung cancer is poor. While the application of molecular technologies and an understanding of immunotherapy provides potential new therapeutic options we need to recognise that only a small proportion of patients will have tumours suitable for these agents and that they remain palliative in nature. As we identify new mutations and targets the proportion of patients who may benefit from ‘personalised medicine’ will hopefully increase. Developments in this area present new challenges for pathology departments, in the way laboratories are organised, as well as pathologists who must now think beyond the ‘diagnosis’. Lung cancer reports must now integrate the traditional morphological aspects of pathology with molecular features which will guide clinical decision making placing the pathologist at the centre of lung cancer patient management.

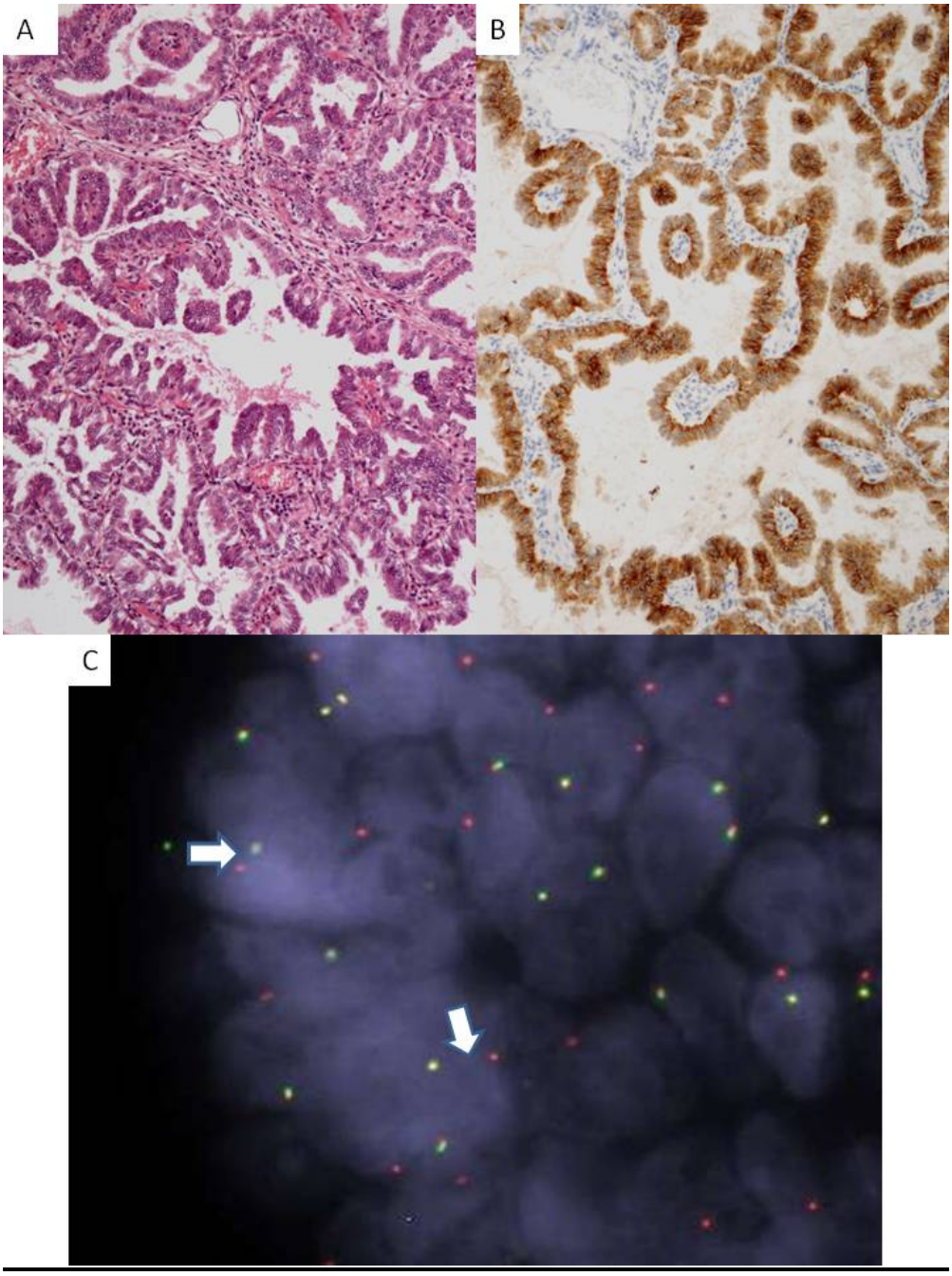

Figure 1.

Pulmonary adenocarcinoma with a non-mucinous lepidic and papillary pattern (A). Molecular testing showed wild type KRAS and EGFR. Immunohistochemistry for ALK protein using the D5F3 monoclonal antibody shows strong cytoplasmic staining with a rather granular appearance (B). FISH performed using Vysis break apart probes demonstrates splitting of the red and green markers (white arrows) in keeping with ALK rearrangement.

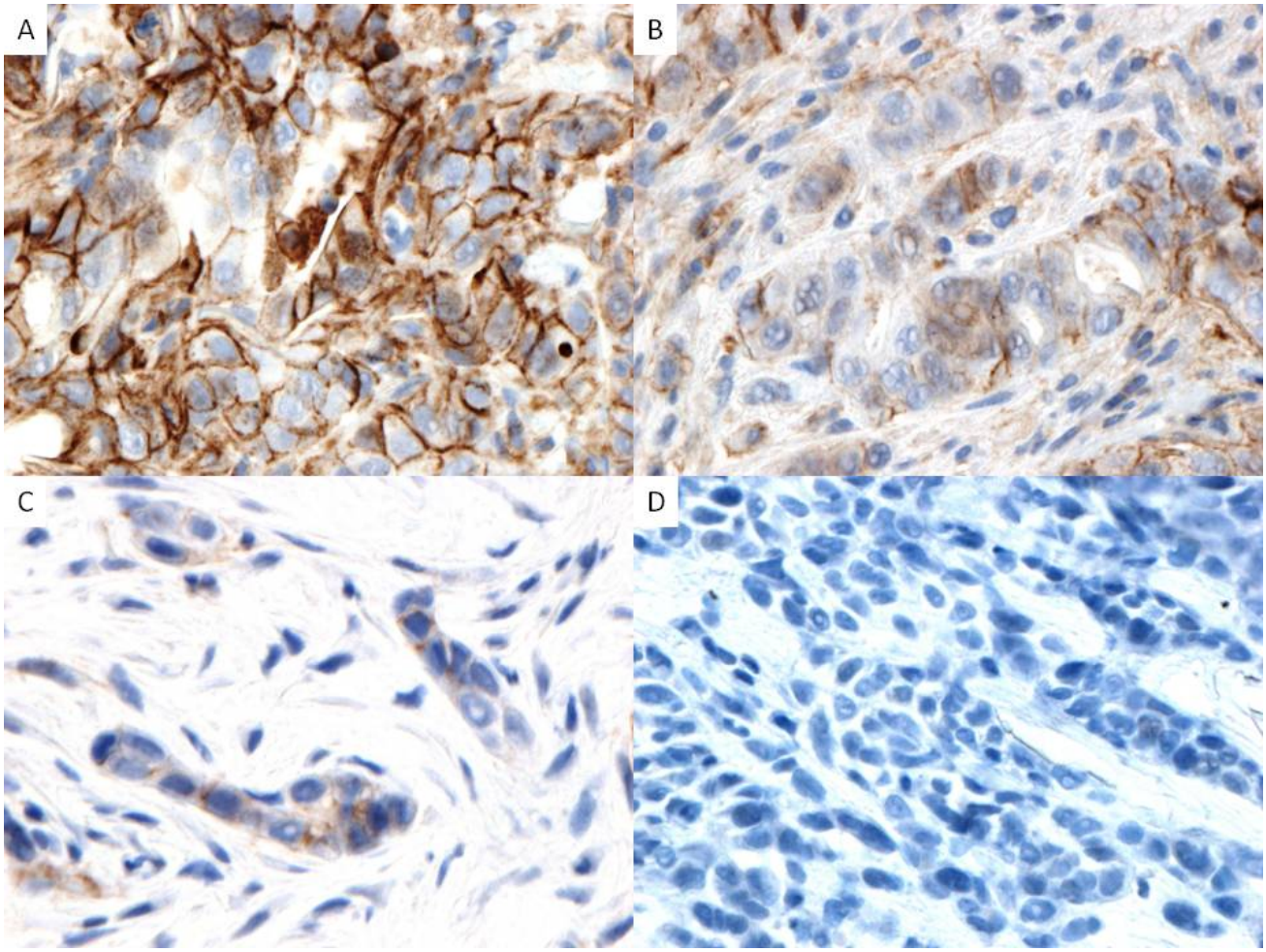

Figure 2

Immunohistochemistry for PD-L1 using the Dako 22C3 pharmaDx kit. (A&B) Areas of an adenocarcinoma which showed high levels of expression but with different intensity. The overall expression of this tumour was assessed at around 80%. (C&D) Sections from a poorly differentiated squamous carcinoma which showed only very focal PD-L1 expression (C) which was weak and only present in a few cells. The majority of the tumour cells were negative (D) and overall expression was assessed as <1%.

Table 1

Summary of molecular targets which are currently routinely available in non-small cell lung cancer

Target	Tumour type	Mutation(s)	Method(s)	Examples of drug(s) that may be indicated[$]
EGFR	Non-squamous, NSCLC	Exon 18 mutations	PCR	gefitinib, erlotinib, afatinib
		Exon 19 deletions[#]	PCR	
		Exon 21 eg L858R	PCR	
		Exon 20 eg T790M*	PCR	osimertinib
ALK	Non-squamous, NSCLC	Translocation	IHC, FISH, RT-PCR	crizotinib, seritinib
ROS1	Non-squamous, NSCLC	Translocation	IHC, FISH, RT-PCR	crizotinib
PDL-1 expression	All NSCLC	None	IHC	nivolumab, pembrolizumab
KRAS		Point mutations codons 12, 13, 61	PCR	None available

PCR – polymerase chain reaction, RT-PCR – reverse transcriptase polymerase chain reaction, IHC – immunohistochemistry, FISH – fluorescent in-situ hybridisation

[$] This list of drugs is provided for illustrative purposes and is not exhaustive. For further information referral should be made to appropriate local formularies.

[#] afatinib may be preferred to other TKIs in patients with exon 19 deletions

* mutation associated with resistance to first and second line tyrosine kinase inhibitors

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Practice Points

1. Molecular testing of advanced stage non-small cell lung carcinoma, especially adenocarcinoma, is now standard practice.
2. Pathologists have a critical role in ensuring small diagnostic specimens are carefully handled to ensure that sufficient good quality material is available, where ever possible, for testing after the traditional diagnostic process is completed.
3. Testing uses a variety of methodologies including PCR, FISH and IHC to detect specific genetic alterations, translocations and protein expression in tumour cells to predict response to specific classes of drugs.
4. Currently only a minority of patients have tumours with specific features that allow use of these new drugs but this is likely to change and the range of tests that require to be carried out will expand.

Conflict of Interest Statement

DAD - MSD funded attendance at a training session for assessment of PD-L1 IHC.

KW - None

AO – Honoraria from Pfizer and Merck Serono for contributions to educational meetings

WAW - Honoraria from Bristol Myers Squibb and Pfizer for attending advisory board meetings and contributions to education meetings. MSD funded attendance at a training session for assessment of PD-L1 IHC.